Fungal Biology

Ahmed M. Abdel-Azeem
Ajar Nath Yadav
Neelam Yadav
Minaxi Sharma *Editors*

Industrially Important Fungi for Sustainable Development

Volume 2: Bioprospecting for Biomolecules

Springer

Fungal Biology

Series Editors

Vijai Kumar Gupta
Biorefining and Advanced Materials Research Center
Scotland's Rural College (SRUC), SRUC Barony Campus, Parkgate
Dumfries, Scotland
United Kingdom

Maria G. Tuohy
School of Natural Sciences
National University of Ireland Galway
Galway, Ireland

Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and non-living is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of "one pot" microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

More information about this series at http://www.springer.com/series/11224

Ahmed M. Abdel-Azeem
Ajar Nath Yadav • Neelam Yadav
Minaxi Sharma
Editors

Industrially Important Fungi for Sustainable Development

Volume 2: Bioprospecting for Biomolecules

Springer

Editors
Ahmed M. Abdel-Azeem
Botany and Microbiology Department
Suez Canal University
Ismailia, Egypt

Ajar Nath Yadav
Department of Biotechnology
Eternal University
Sirmour, Himachal Pradesh, India

Neelam Yadav
Gopi Nath PG College
Veer Bahadur Singh Purvanchal University
Ghazipur, Uttar Pradesh, India

Minaxi Sharma
Chemistry and Biotechnology Department
Tallinn University of Technology
Tallinn, Estonia

ISSN 2198-7777 ISSN 2198-7785 (electronic)
Fungal Biology
ISBN 978-3-030-85602-1 ISBN 978-3-030-85603-8 (eBook)
https://doi.org/10.1007/978-3-030-85603-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

Natural products are the new interest of the current era worldwide over synthetic ones, as sustainability is now on everyone's mind. Various natural products have been obtained from the different sources including animals, plants, and microbes, which have great value in industries. Microbes, especially fungi, represent an incredibly rich reservoir of natural products or biomolecules. Fungi, the highly diverse clade of eukaryotes, are known to produce various biomolecules, such as enzymes, organic acids, fatty acids, pigments, secondary metabolites, and bioactive compounds. These biomolecules have been explored in various industries, including cosmetic, food, tannery, and textiles, as coloring agents, pH adjuster, and catalyst for various biochemical reactions for the production of goods. Fungal biomolecules do comprise various benefits, such as low production cost of natural products and ease to obtain. In comparison to the synthetic products, these products have no detrimental effects on the environment. This volume clearly describes the emerging industrial application of biomolecules obtained from the fungal communities.

I recommend ***Industrially Important Fungi for Sustainable Development, Volume 2: Bioprospecting for Biomolecules*** to researchers and students working in this emerging and fascinating field of mycology. The book will advance the knowledge to a greater extent in these areas with significant broader research on fungal communities. The editors of this book deserve credit for such a splendid and innovative contribution to mycological research.

Davinder Singh,
Eternal University, Baru Sahib
Baru Sahib, Himachal Pradesh, India

Foreword

Today, fungal communities offer important advances in global industries due to their mind-blowing potential in medical, agriculture, and pharmaceutical industries; food and feed processing; and environment for sustainable development. Fungi have been obtained from different sources including plants, soil, and water, which have great value in pharmaceutical industries and are used in several fermentative processes like production of enzymes, vitamins, pigments, lipids, glycolipids, polysaccharides, and polyhydric alcohols. The unique characteristics of fungi hold important promise for the production of various biomolecules, such as organic acid, pigments, secondary metabolites, and bioactive compounds. These biomolecules have been explored in several industries such as synthetic pigments are used as additives, antioxidants, colorants, and color intensifiers, in many aspects including the textile for the coloring agent, pharmaceutical, cosmetic, painting, food, and beverage industries, tannery and catalysts for various biochemical reactions for the goods production. Fungal secondary metabolites as structurally different compounds show a variety of biological activities like antimicrobial, antitumor, antiparasitic, antioxidant, and immunosuppressant activities, and they can also act as plant growth stimulators, pesticides, molluscicides, anthelmintics, and nematicides, leading to industrial scale production of enzyme alkaloids, acids, detergents, and bio-surfactants. Fungi are being used as high-cost food due to their high protein and low color value. This book clearly mentions the evolving industrial applications of biomolecules obtained from the fungal communities.

This volume on ***Industrially Important Fungi for Sustainable Development, Volume 2: Bioprospecting for Biomolecules*** is a very timely publication, which provides state-of-the-art information in the area of mycology, broadly involving fungi and fungus-based products for sustainable development in the industry. The book volume comprises 23 chapters. The first chapter by Nouh et al. describes the bioprospecting for biomolecules from different fungal communities. Nahas et al.

highlight fungi as a gold mine of antioxidants in Chap. 2. Chapter 3 by Abdel-Azeem et al. describes endophytic fungi as a source of new pharmaceutical biomolecules. Chapter 4 by Gezaf et al. highlights fungal communities from different habitats for tannins in industry. Nouh et al. describe recent advances in fungal antimicrobial molecules in Chap. 5. In Chap. 6, Nahas et al. have given the details of fungal laccases to where and where. Ghosh et al. highlight the current research and future challenges of fungal cellulases in Chap. 7.

In Chap. 8, Marwa Tamim A. Abdel-Wareth describes the current research, commercial aspects, and applications of fungal secondary metabolites. Balbool et al. highlight bioprospecting of thermophilic fungal enzymes and potential applications in Chap. 9. Berde et al. highlight bioactive secondary metabolites from psychrophilic fungi and their industrial importance in Chap. 10. Fungal amylases and their industrial applications have been described by Patil et al. in Chap. 11. Chapter 12 by Parsa Mahmood Dar describes current research and applications of fungal phytases in the food industry. Darwish et al. highlight insights into molecular structures and biotechnological applications of fungal lipases in the medicine and dairy industry in Chap. 13. Dar and Dar discuss fungal xylanases for different industrial applications in Chap. 14. Fungal pigments for the food industry are discussed in Chap. 15 by Soliman et al. Dikkala et al. describe the fungal production of vitamins and their food industrial applications in Chap. 16. Jagadish et al. describe the nutraceutical potential of wild edible mushroom *Hygrocybe alwisii* in Chap. 17. Current research, production, and potential applications of fungal biopharmaceuticals have been discussed in Chap. 18 by Askari et al. Chapter 19 by Ashok et al. describes natural pigments from filamentous fungi and their applications. Kour et al. describe the bioprospecting of industrially important mushrooms in Chap. 20. Bioactive attributes of *Xylaria* species from the scrub jungles of southwest India have been described by Jagadish et al. in Chap. 21. Current research and future challenges of fungicide as potential vaccine are discussed in Chap. 22 by Verma et al. Finally, the conclusion and future prospects of bioprospecting for biomolecules from industrially important fungi have been described by the editors and co-authors in the last chapter.

Overall, great efforts have been carried out by the editorial team and scientists from different countries to compile this book as a highly unique and up-to-date source on ***Industrially Important Fungi for Sustainable Development, Volume 2: Bioprospecting for Biomolecules*** for students, researchers, scientists, and academicians. I hope that the readers will find this book highly useful and interesting during their pursuit of mycology.

Amrik Singh Ahluwalia
Eternal University, Baru Sahib
Baru Sahib, Himachal Pradesh, India

Preface

Fungi are an essential, fascinating, and biotechnologically useful group of organisms with an incredible biotechnological potential for industrial exploitation. Knowledge of the world's fungal diversity and its use is still incomplete and fragmented. There are many opportunities to accelerate the process of filling knowledge gaps in these areas. The worldwide interest of the current era is to increase the tendency to use natural substances instead of synthetic ones. The increasing urge in society for natural ingredients has compelled biotechnologists to explore novel bioresources, which can be exploited in the industrial sector. Fungi, due to their unique attributes and broad range of biological activities, hold great promise for their applications in biotechnology and industry. Fungi are an efficient source of antioxidants, enzymes, pigments, and many other secondary metabolites.

Industrially Important Fungi for Sustainable Development, Volume 2: Bioprospecting for Biomolecules covers major aspects of industrially important fungi. The book focuses on fungal communities from diverse niches and habitats as potential source of industrially important compounds. The increasing use and exploration of novel bioactive compounds from fungi solve countless problems mankind faces in today's constantly changing scenario, such as emergence of life-threatening viruses and drug-resistant bacteria and increasing incidences of fungal and bacterial infections. The large-scale production of fungal pigments and their utility provide natural coloration without creating harmful effects on entering the environment, a safer alternative to synthetic colorants. Fungal enzymes can be exploited in a wide range of industries, such as food, detergent, and paper, and also for removal of toxic waste. Thus, this book will surely serve as a valuable reference to current state of knowledge and a stepping-stone for unexplored novel compounds from fungi. The book will be extremely useful for researchers, students, microbiologists, and scientists especially working in the field of mycology. Each chapter has been contributed by internationally recognized researchers and scientists with their firm viewpoints and experiences in the field of mycology. This book will serve as a valuable source of information as well as will provide new directions to researchers to conduct novel research in the field of mycology.

Industrially Important Fungi for Sustainable Development, Volume 2: Bioprospecting for Biomolecules provides a discussion of fungal communities from diverse habitats and their industrial applications for future sustainability. This volume encompasses advanced research of fungal communities and their potential biotechnological applications in industry and allied sectors. The book will be useful to scientists, researchers, and students working in microbiology, biotechnology, agriculture, molecular biology, environmental biology, and related subjects.

Ismailia, Egypt — Ahmed M. Abdel-Azeem
Sirmour, Himachal Pradesh, India — Ajar Nath Yadav
Ghazipur, Uttar Pradesh, India — Neelam Yadav
Tallinn, Estonia — Minaxi Sharma

Contents

Contributors

Ahmed M. Abdel-Azeem Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Mohamed A. Abdel-Azeem Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Industries, Sinai University, Al Arish, North Sinai, Egypt

Marwa Tamim A. Abdel-Wareth Environmental Research and Medical Malacology Department, Theodor Bilharz Research Institute, Giza, Egypt

Hebatallah H. Abo Nahas Zoology Department (Physiology), Faculty of Science, Suez Canal University, Ismailia, Egypt

Yousef H. Abo Nahas Microbiology Department, Faculty of Medicine, Helwan University, Cairo, Egypt

Fatma Ahmed Abo Nouh Botany and Microbiology Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt

Conrad C. Achilonu Department of Genetics, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa

Amrik Singh Ahluwalia Department of Botany, Akal College of Basic Sciences, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

S. Aishwarya School of Basic and Applied Sciences, Department of Biological Sciences, Dayananda Sagar University, Bengaluru, Karnataka, India

Haider Alrafas Department of Microbiology and Immunology, College of Veterinary Medicine, University of Basrah, Northern Campus of Qarmat Ali, Basrah, Iraq

Kenneth Yongabi Anchang Faculty of Health Sciences, Department of Public Health, Imo State University, Owerri, Nigeria

Ganapathy Ashok National Institute of Pharmaceutical Education and Research, Kolkata, West Bengal, India

Amir Hosein Askari Pour Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Department of Basic Sciences, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

Bassem A. Balbool Faculty of Biotechnology, October University for Modern Sciences and Arts, Giza, Egypt

Chanda Vikrant Berde Marine Microbiology, School of Earth, Ocean & Atmospheric Sciences, Goa University, Taleigao Plateau, Goa, India

Vikrant Balkrishna Berde Department of Zoology, Arts, Commerce and Science College, Lanja, Maharashtra, India

Atul Bhargava Department of Botany, Mahatma Gandhi Central University, Motihari, India

Meganathan Boominathan Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

Farial Mehmood Dar District Headquarter Hospital, Jhelum, Punjab, Pakistan

Parsa Mahmood Dar Institute of Chinese Medical Sciences, Avenida da Universidade, Taipa, Macau, China

Amira Mohamed Galal Darwish Department of Food Technology, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SRTACity), Alexandria, Egypt

Hosamane Ramesh Dattaraj Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

Rubee Devi Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

Praveen Kumar Dikkala Department of Food Science and Technology, Punjab Agriculture University, Ludhiana, Punjab, India

Roshan Dsouza J. Craig Venter Institute, Rockville, MD, USA

Wedad M. El-Kholy Department of Dairy Research, Food Technology Research Institute (A.R.C.), Alexandria, Egypt

Yousra A. El-Maradny Microbiology Department, High Institute of Public Health, Alexandria University, Alexandria, Egypt

Mervet. A. Elsayed Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Sara Atef Gezaf Botany and Microbiology Department, Faculty of Science, Arish University, North Sinai, Egypt

Mohammadhassan Gholami-Shabani Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Soumya Ghosh Department of Genetics, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa

Asha Giriyan Fellow, Coastal Ecology and Marine Resource Center, The Energy and Resource Institute, Goa, India

Liliana Godoy Department of Fruit and Oenology, Faculty of Agronomy and Forestry, Pontifical Catholic University of Chile, Santiago, Chile

Marieka Gryzenhout Department of Genetics, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa

Vijai Kumar Gupta Biorefining and Advanced Biomaterials Research Center, Scotland's Rural College (SRUC), Kings Buildings, Edinburgh, UK

Ravish Huchegowda Department of Neurochemistry, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, Karnataka, India

S. P. Hudeda Department of Dravyaguna, JSS Ayurvedic Medical College, Lalithadripura, Mysuru, Karnataka, India

Bijavara Ramakrishnappa Jagadish Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

Fatemehsadat Jamzivar Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Tanvir Kaur Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

Yasmin H. Korra Faculty of Pharmacy, Pharos University, Alexandria, Egypt

K. Kounaina Post Graduate Department of Dravyaguna Vignana, Alva's Ayurveda Medical College, Dakshina Kannada, Karnataka, India

Divjot Kour Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

Harpreet Kour Department of Botany, University of Jammu, Jammu, India

Satwinder Kour Department of Botany, University of Jammu, Jammu, India

Bimlesh Kumar School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

Shiv Kumar Department of Food Science and Technology (Hotel Management), Maharishi Markandeshwar University, Mullana, Ambala, Haryana, India

Daniela Landa-Acuña Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Peru

Professional Career in Environmental Engineering, Faculty of Engineering, Private University of the North, Lima, Peru

Bernabe Luis-Alaya Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Perú

Professional Career in Environmental Engineering, Faculty of Engineering, Private University of the North, Lima, Perú

J. Madhavi Department of Physics, Indian Institute of Science, Bengaluru, Karnataka, India

Shivannegowda Mahadevakumar Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India

Safaa A. Mansour Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Akram H. Mohamed Microbial Genetic Resources Department, National Gene Bank, Agriculture Research Center, Giza, Egypt

Nagabhushana Chandramohana Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore, Karnataka, India

Utpal Mohan National Institute of Pharmaceutical Education and Research, Kolkata, West Bengal, India

Miguel Angel Nieto-Taype Department of Chemical, Biological and Environmental Engineering, School of Engineering, Universitat Autònoma de Barcelona, Bellaterra, Spain

Ahmed Noah Badr Toxicology and Food Contaminants Department, National Research Centre, Dokki, Cairo, Egypt

Alaa A. Osman Department of Pharmaceutical Chemistry, Faculty of Pharmacy, New Giza University, Cairo, Egypt

Abdelrahman M. Othman Faculty of Pharmacy and Drug Manufacturing, Pharos University, Alexandria, Egypt

Subrahmanya Padyana Post Graduate Department of Dravyaguna Vignana, Alva's Ayurveda Medical College, Dakshina Kannada, Karnataka, India

Roshan Pais PathoGutOmic Laboratory, Department of Biochemistry, School of Basic and Applied Sciences, Department of Biological Sciences, Dayananda Sagar University, Shavige Malleshwara Hills, Bengaluru, Karnataka, India

Anirudh G. Patil PathoGutOmic Laboratory, Department of Biochemistry, School of Basic and Applied Sciences, Department of Biological Sciences, Dayananda Sagar University, Bengaluru, Karnataka, India

Anjanapura V. Raghu Department of Chemistry, School of Engineering and Technology, Jain Global Campus, Jain University, Bengaluru, Karnataka, India

Fatemeh Rahmani Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Velayutham Ravichandiran National Institute of Pharmaceutical Education and Research, Kolkata, West Bengal, India

Kakarla Raghava Reddy School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, NSW, Australia

Mehdi Razzaghi-Abyaneh Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Maria Reyes-Córdova Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Perú

Essa M. Saied Chemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Institute for Chemistry, Humboldt Universität zu Berlin, Berlin, Germany

Venugopal Senthilkumar Pondicherry Centre for Biological Sciences, Puducherry, India

Masoomeh Shams-Ghahfarokhi Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Amarish Kumar Sharma Department of Bioengineering & Biosciences, Lovely Professional University, Phagwara, Punjab, India

Isha Sharma Department of Microbiology, Shoolini University, Vill-Bhajhol Solan, Himachal Pradesh, India

Minaxi Sharma Department of Applied Biology, University of Science & Technology, Techno City, India

Yashpal Sharma Department of Botany, University of Jammu, Jammu, India

Shaveta Singh Department of Microbiology, Shoolini University, Vill-Bhajhol Solan, Himachal Pradesh, India

Tarek Nour Soliman Dairy Department, Food Industries and Nutrition Research Division, National Research Centre, Cairo, Egypt

Richard A. Solorzano Acosta Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Peru

Faculty of Pharmacy and Biochemistry, Norbert Wiener Private University, Lima, Peru

Kandikere Ramaiah Sridhar Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

Centre for Environmental Studies, Yenepoya (Deemed to be University), Mangalore, Karnataka, India

Zeba Usmani Department of Applied Biology, University of Science and Technology, Meghalaya, India

Celia Vargas-De-La-Cruz Faculty of Pharmacy and Biochemistry, Academic Department of Pharmacology, Bromatology and Toxicology, Latin American Center for Teaching and Research in Food Bacteriology (CLEIBA), Research Group Biotechnology and Omics in LifeSciences, Universidad Nacional Mayor de San Marcos, Lima, Perú

Garima Verma Department of Bioengineering & Biosciences, Lovely Professional University, Phagwara, Punjab, India

Chandran Viswanathan Department of Biotechnology, Sree Narayana Guru College, Coimbatore, Tamil Nadu, India

Marwa I. Wahba Chemistry of Natural and Microbial Products Department, National Research Centre, Giza, Egypt

Centre of Scientific Excellence-Group of Advanced Materials and Nanotechnology, National Research Centre, Giza, Egypt

Ajar Nath Yadav Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

Ashok Yadav Department of Botany, Institute of Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

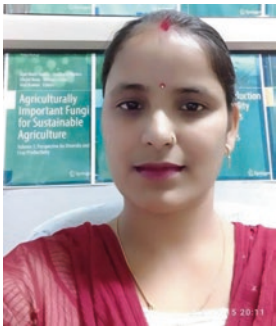
Neelam Yadav Gopi Nath P.G. College, Veer Bahadur Singh Purvanchal University, Ghazipur, Uttar Pradesh, India

Farhan Zameer PathoGutOmic Laboratory, Department of Biochemistry, School of Basic and Applied Sciences, Department of Biological Sciences, Dayananda Sagar University, Bengaluru, Karnataka, India

About the Editors

Ahmed M. Abdel-Azeem is currently working as a mycosystematist and professor of mycology at the Botany and Microbiology Department, Faculty of Science, Suez Canal University. Abdel-Azeem's research includes isolation, identification, and taxonomic assessments of fungi with particular emphasis on those which produce bioactive materials from different ecological habitats. Most recently, his interests have been on the effects of climate change on fungi and fungal conservation. He is the founder of Arab Society for Fungal Conservation and executive committee member of IMA (2018–2022). He is a founder member of the International Society for Fungal Conservation, member in the European Mycological Association, and member in the Mycological Society of America. Abdel-Azeem founded Egypt's National Fungus Day and many mycologists' networks like Egyptian, Iraqi, Algerian, and Pakistani networks. He is the founder of Pan Arab Mycologists. Abdel-Azeem has particular interest in the ecology, taxonomy, biology, and conservation of fungi and his specialist interest is in members of the phylum Ascomycota. He is a member of the IUCN Species Survival Commission Specialist Group for Cup Fungi, Truffles, and their Allies. He is the editor in chief of Microbial Biosystems (MB) and a reviewer of more than 15 international journals. He has published more than 75 research paper journals, 40 book chapters in the books published by international publishers, and 9 books.

Ajar Nath Yadav is an Assistant Professor (Senior Scale) and Assistant Controller of Examinations at Eternal University, Baru Sahib, Himachal Pradesh, India. He has 8 years of teaching and 12 years of research experience in the fields of microbial biotechnology, microbial diversity, fungal biology, and plant-microbe interactions. Dr. Yadav obtained a doctorate in Microbial Biotechnology, jointly from the Division of Microbiology, ICAR-Indian Agricultural Research Institute (IARI), Pusa, New Delhi, and Birla Institute of Technology, Mesra, Ranchi, India; M.Sc. (Biotechnology) from Bundelkhand University, Jhansi, Uttar Pradesh; and B.Sc. (CBZ) from the University of Allahabad (A Central University), Uttar Pradesh, India.Dr. Yadav has 251 publications, with h-index of 56, i10-index of 152, and 9100 citations (Google Scholar, on 07/07/2020). Dr. Yadav is the editor of 20 books in Springer Nature; 5 in CRC Press, Taylor & Francis; 5 in Elsevier; and 1 in Wiley-Blackwell. In his credit, there is one granted patent "Insecticidal formulation of novel strain of Bacillus thuringiensis AK 47." Dr. Yadav has got 12 Best Paper Presentation Awards and 1 Young Scientist Award (NASI-Swarna Jayanti Puraskar). Dr. Yadav received the "Outstanding Teacher Award" in the 6th Annual Convocation 2018 by Eternal University, Baru Sahib, Himachal Pradesh. Dr. Yadav is currently handling two projects, one funded by the Department of Environments, Science and Technology (DEST), Shimla, and one by HP Council for Science, Technology and Environment (HIMCOSTE). Dr. Yadav has guided two Ph.D. and one M.Sc. scholars, and presently he is guiding four scholars for Ph.D. and one for M.Sc. degree. In his credit, there are 8700 microbes (archaea, bacteria, and fungi) isolated from diverse sources and 550 potential and efficient microbes deposited at the culture collection of ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh, India. He has deposited 2423 nucleotide sequences, 3 whole-genome sequences (*Bacillus thuringiensis* AKS47, *Arthrobacter agilis* L77, and *Halolamina pelagica* CDK2), and 2 transcriptomes to NCBI GenBank databases: in the public domain. The niche-specific microbes from extreme environments were reported as specific bio-inoculants (biofertilizers) for crops growing in normal and diverse abiotic stress conditions. Dr. Yadav and group have developed technology for screening of archaea for phosphorus solubilization for the first time. He has been serving as reviewer for 50 national and international peer-reviewed journals. Dr. Yadav is Editor in Chief for the Journal of Applied Biology and Biotechnology and Editor/Associate Editor for Annals of Microbiology (Springer Nature), BMC Microbiology (Springer Nature), and Current Microbiology (Springer Nature). He has lifetime membership of the Association of Microbiologists in India and Indian Science Congress Council, India. Please visit https://sites.google.com/view/ajarnathyadav/ for more details.



Neelam Yadav is currently working on microbial diversity from diverse sources and their biotechnological applications in agriculture and allied sectors. She obtained her postgraduation degree from Veer Bahadur Singh Purvanchal University, Uttar Pradesh, India. She has a research interest in the areas of microbial biotechnology, fungal biology, beneficial microbiomes, and their biotechnological application in agriculture, medicine environment, and allied sectors. In her credit, there are 79 publications in different reputed international and national journals and publishers with h-index 31, i10-index 52, and 2559 citations (Google Scholar, on 07/07/2020). She is the editor of 3 books in Elsevier; 5 in CRC Press, Taylor & Francis; and 11 in Springer Nature.She has published 21 research communications in different conferences/symposiums/workshops. She got the two Best Paper Presentation Awards and two Certificate of Excellence in Reviewing Awards. In her credit, there are 1700 microbes (archaea, bacteria, and fungi) isolated from diverse sources and 115 potential and efficient microbes deposited at the culture collection. She has deposited 295 nucleotide sequences to NCBI GenBank databases: in the public domain. She is editor/associate editor/reviewer of different international and national journals including Plos One, Extremophiles, Annals of Microbiology, Journal of Basic Microbiology, and Advances in Microbiology and Biotechnology. She has the lifetime membership of the Association of Microbiologists in India; Indian Science Congress Council, India; and National Academy of Sciences, India. Please visit https://sites.google.com/site/neelamanyadav/ for more details.

Minaxi Sharma is currently working as Senior Research Scientist at ERA Chair VALORTECH, Estonian University of Life Sciences (EMU), Tartu, Estonia. Her research at EMU is focused on the processing technologies of various food raw materials/products to produce bioactive components and development of advanced technologies for increasing nutritional value of various food products, including different encapsulation methods, technologies improving final product texture, sensory properties, and nutritive value and ensuring stability of these ingredients during manufacturing, etc.She also worked as Assistant Professor with teaching and research responsibilities in the Department of Food Technology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India. Dr. Sharma has 11 years of extensive teaching and research experience in the area of applications of antimicrobial nanoencapsulation of plant essential oils in dairy foods, probiotic Indian dairy products, development of functional foods using micro/nano-colloidal delivery systems of potential plant bioactive components, and food waste valorization for the extraction of valuable plant bioactive nutrients.

Recently, she has been awarded with the "Young Scientist Award" of Society for Bioinformatics and Biological Sciences (SBBS), India (2020). She is the editor of few international journals, including Medicinal Plants - International Journal of Phytomedicines and Related Industries (ISSN: 0975-4261), India; Microbial Biosystems (ISSN 2357-0334), Egypt; and EUREKA: Life Sciences (ISSN 2504-5695), Estonia. She is also the member of the Society for Bioinformatics and Biological Sciences (SBBS). Dr. Sharma is Visiting Assistant Professor in the Department of Applied Biology (Food Science and Technology) under the School of Biological Sciences, University of Science and Technology, Meghalaya (USTM) (March 2021–); Visiting Assistant Professor, Department of Botany at Sri Avadh Raj Singh Smarak Degree College, Bishunpur Bairiya, Gonda (affiliated to Dr. RML Avadh University, Ayodhya, UP, India (July 2019); and Visiting Faculty, Department of Food Technology at Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India (February 2020). She is a well-recognized researcher and has Web of Science Researcher ID: L-9405-2019; ORCID: https://orcid.org/0000-0001-6493-5217. She has published 38 research/review papers in reputed journals, 8 book chapters in the books published by international publishers, and 1 book in Springer Nature. She has a few upcoming books in the current year with reputed publishing houses of CRC Press, Wiley, and Springer.

Chapter 1
Bioprospecting for Biomolecules from Different Fungal Communities: An Introduction

Fatma Ahmed Abo Nouh, Sara Atef Gezaf, Hebatallah H. Abo Nahas, Yousef H. Abo Nahas, Celia Vargas-De-La-Cruz, Richard A. Solorzano Acosta, Daniela Landa-Acuña, Bernabe Luis-Alaya, and Ahmed M. Abdel-Azeem

F. A. Abo Nouh (✉) · A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt
e-mail: fatma.nouh@science.suez.edu.eg

S. A. Gezaf
Botany and Microbiology Department, Faculty of Science, Arish University, North Sinai, Egypt

H. H. Abo Nahas
Zoology Department (Physiology), Faculty of Science, Suez Canal University, Ismailia, Egypt

Y. H. Abo Nahas
Microbiology Department, Faculty of Medicine, Helwan University, Cairo, Egypt

C. Vargas-De-La-Cruz
Faculty of Pharmacy and Biochemistry, Academic Department of Pharmacology, Bromatology and Toxicology, Latin American Center for Teaching and Research in Food Bacteriology (CLEIBA), Research Group Biotechnology and Omics in LifeSciences, Universidad Nacional Mayor de San Marcos, Lima, Peru

R. A. Solorzano Acosta
Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Peru

Faculty of Pharmacy and Biochemistry, Norbert Wiener Private University, Lima, Peru

D. Landa-Acuña · B. Luis-Alaya
Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Peru

Professional Career in Environmental Engineering, Faculty of Engineering, Private University of the North, Lima, Peru

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_1

Contents

1.1 Introduction

Approximately 1.5 million species of fungi colonize the Earth and invariably use chemical language to interact with each other and/or with other organisms or the niche environment (Hawksworth 1991). Fungi are among the most important

organisms in the world, because of not only their vital roles in ecosystem functions but also their influence on humans and human-related activities (Devi et al. 2020b). Low-molecular-weight organic compounds (biomicromolecules or biomolecules) found in living organisms can be broadly classified into two major categories: (1) primary metabolites, which include sugars, amino acids, fatty acids, and nucleotides and (2) secondary metabolites, such as alkaloids, flavonoids, quinone, phenols steroids, terpenoids (Guo et al. 2000), coumarins, glycosides, peptide (Selim et al. 2016), and volatile organic compounds (Arora and Ramawat 2017). Secondary metabolites are defined as low-molecular-weight compounds that are not required for the organism's growth vice versa for primary metabolites. They are characterized by a great diversity of chemical structures and variations in different environmental conditions, conferring a selective advantage to the producer organism (Butler and Buss 2006; Rastegari et al. 2019a, b). Fungal secondary metabolites may be considered a large and heterogeneous group of small molecules not directly essential for growth but having an important role in signaling, development, and interactions with other organisms (Mukherjee et al. 2012). As with actinomycetes and plants, fungi are a rich source of a wide array of secondary metabolites. Several studies demonstrated that endophytic fungi are capable of producing a number of important bioactive secondary metabolites (Priti et al. 2009; Rana et al. 2019a).

Endophytes could have directly provided their hosts with these metabolites, thereby contributing to their chemical defense, or they might have transferred the corresponding genes to the host genome or vice versa (Wink 2008). Generally, the fungal secondary metabolites are classified as polyketides (PKs) (e.g., aflatoxins), nonribosomal peptides (e.g., penicillin), terpenes (e.g., gibberellins), and prenylated tryptophan derivatives (e.g., ergot and indole alkaloids) (Hoffmeister and Keller 2007; Devi et al. 2020a). The production of secondary metabolites varies according to the particular compound, the species and the presence of other microorganisms, and the balance between the biosynthesis of elicitors and the rate of biotransformation (Degenkolb and Bruckner 2008; Yadav et al. 2019a). In addition, general environmental factors, such as carbon and nitrogen sources, temperature, light, and pH, as well as competitive forces, host plant interaction and communication, have further influence on fungal secondary metabolite production. Environmental effects are typically transmitted through Cys2His2 zinc-finger proteins (Shwab and Keller 2008), such as CreA for carbon signaling (Dowzer and Kelly 1989), AreA for nitrogen signaling, and PacC for pH signaling (Tilburn et al. 1995). These proteins may either positively or negatively regulate metabolite production, for example, CreA positively regulates penicillin production, while PacC has a negative regulatory effect (Martin 2000).

Fungi offer a rich and diverse source of biomolecules that continue to be explored for food, health, and environmental applications through research and development of both public and private sectors (Kumar et al. 2021; Yadav 2018). In sum, the chapter contains fascinating and essential information on various fungal metabolites and their applications.

1.2 Different Ecological Groups of Fungi

1.2.1 Thermotolerant and Thermophilic

Thermophiles are heat-loving organisms, which not only tolerate high temperatures but also usually require these for their growth and survival (Maheshwari et al. 2000; Kumar et al. 2014; Verma et al. 2019). In general, thermophilic organisms can be classified as either moderate thermophilic or hyperthermophilic. The former exhibits growth temperatures ranging from a minimum of 20 °C up to a maximum of 60 °C and with optimal growth above 40 °C. These moderate thermophiles include species from the domains Bacteria and Archaea and representatives from Eukarya (mostly filamentous fungi), whose maximum temperature limit has been recorded to be 62 °C (Tansey and Brock 1972; Saxena et al. 2016; Yadav et al. 2020c). On the other hand, the hyperthermophiles are organisms able to grow at temperatures between 65 and 110 °C. They contain several representatives from the domains Bacteria and Archaea but do not include organisms from the domain Eukarya (Vieille and Zeikus 2001; Sahay et al. 2017). Thermophilic fungi are those with a maximum growth at 50 °C or above and a minimum growth temperature at 20 °C or above, separating them from the thermotolerant fungi, which could grow up to 50 °C and below 20 °C, but authors found several exceptions that do not fit to these criteria. As an example, *Aspergillus fumigatus* is a thermotolerant fungus able to grow at temperatures above 50 °C and below 20 °C (Mouchacca 2000).

Thermophilic and thermotolerant fungi also can be isolated from bird nests, alligator nests, coal tips, and volcanic hot springs. It is interesting that thermophilic and thermotolerant fungi are ubiquitous in soils wherever the sun can heat the soil to temperatures that are suitable for germination and growth (Abdullah and Al-Bader 1990) but are also found in temperate grasslands, forests, and agricultural fields (Mouchacca 1995). Natural habitats that harbor a considerable variety of thermophilic microorganisms include terrestrial geothermal and volcanic areas and deep-sea hydrothermal vents (submarine hydrothermal vents) (Mehta and Satyanarayana 2013; Yadav et al. 2020b). Most of the currently known extreme thermophiles and hyperthermophiles have been recovered from these regions. Geothermal and volcanic areas include terrestrial fumaroles (e.g., solfataras), terrestrial hot springs, and geysers. Other natural habitats include geothermally heated oil and petroleum reservoirs and sun-heated soils/sediments (Greene et al. 1997).

During the last four decades, many species of thermophilic fungi sporulating at 45 °C have been reported by Abdullah and Zora (1993).Thermophilic fungi are the only eukaryotic organisms that can grow at temperatures above 45 °C. Various genera of this group include the *Phycomycetes*, *Ascomycetes*, *Fungi Imperfecti*, and *Mycelia sterilia* (Mouchacca 1997). Abdullah and Al-Bader (1990) reported 35 thermotolerant and thermophilic fungal species were isolated from soil temperatures ranging from 37 to 44 °C in Iraq. Fungi isolated have been divided in four groups according to their percentage of occurrence: *Aspergillus terreus*, *A.*

fumigatus, and *A. niger* were present with frequencies of occurrence of 70%, 68%, and 60% respectively.

Olagoke (2014) reported a total of seven species of thermophilic fungi isolated from three locations in Ibadan, Nigeria. These were *Absidia corymbifera*, *Gilmaniella humicola*, *Talaromyces helicus*, *Chaetomium elatum*, *Chaetomium* sp., *Humicola* sp., and *Rhizomucor pusillus*, respectively. The fungi still grow up to 45 °C. Unal (2015) isolated thermophilic fungi *Aspergillus niger*, *Aspergillus oryzae*, and *Aspergillus terreus* from water and soil samples from the area where the thermal springs range from 55 to 90 °C around Afyon and Eskisehir in Turkey. Eukaryotes can grow only up to 62 °C, and growth at this temperature is represented by a small number of fungal species. Species of *Thermomyces*, *Chaetomium*, and *Mycothermus* are the most exist genera in different environments. Among them, *Thermomyces lanuginosus* and *Mycothermus thermophilus* are found as the most abundant (Powell et al. 2012). *Curvularia protuberata* from *Dichanthelium lanuginosum* is isolated from geothermal soils at temperature ranging between 38 and 65 ° of Lassen Volcanic and Yellowstone National Parks (Ali et al. 2018).

1.2.2 Psychrotolerant and Psychrophilic

Approximately, 85% of Earth is cold with temperatures ranging below 5 °C, permanently or seasonally (Hoshino and Matsumoto 2012). Cold habitats range from deep sea to high mountains and from Antarctica to Arctic region. Around 71% of the biosphere is occupied by oceans and provides temperature from −1 to 4 °C, the snow covers 35% of total terrestrial environment, frozen ground 24% of terrestrial environment, sea ice 13% of the earth surface, and glaciers 10% of terrestrial environment, providing a temperature of about −5 °C along with some other low-temperature environments comprising cold soils, lakes, caves, and cold deserts (Singh et al. 2006; Yadav et al. 2017, 2018). The living entities that have adapted to and live in cold environments are termed as psychrophiles and psychrotrophs. Psychrophiles and psychrotrophs are defined as the organisms that can grow at or near 0 °C. More specifically, the optimum and maximum temperature for the growth of psychrophiles is 15 and 20 °C, respectively. Psychrotrophs grow well above 15 °C (Cavicchioli et al. 2002; Yadav et al. 2019b). Obligate psychrophiles require 15 °C for their optimal growth, 20 °C for their maximal growth, and 0 °C or lower for their minimum growth (Turchetti et al. 2008), and facultative psychrophiles grow well below 0 °C (Raspor and Zupan 2006).

True psychrophilic fungi are restricted to permanently cold habitats, such as oceans, polar areas, alpine soils and lakes, snow and ice fields, and caves (Hassan et al. 2016). Psychrophilic and psychrotolerant fungi are found in genera such as *Alternaria*, *Cladosporium*, *Keratinomyces*, *Leptomitus*, *Mucor*, *Penicillium*, *Rhizopus*, and *Typhula* (Gounot 1986). Snow molds are low-temperature–tolerant soil-borne fungi that can damage and kill grasses, cereals, and other plants (Nelson and Sturges 1982). The name “snow mold” is based on the fact that these fungi can

grow at the bases of snow-covered plants. The most common species of "snow molds" are *Coprinus psychromorbidus* (Traquair et al. 1987), *Microdochium nivale*, *Myriosclerotina borealis* (Saito 1998), and *Typhula ishikariensis* and other *Typhula* species (Hsiang et al. 1999).

Arenz et al. (2006) studied various filamentous Ascomycetes (New Harbor), Basidiomycetes (Allan Hills), Ascomycete yeasts, *Geomyces* sp. (Mt Fleming), and Zygomycetes (Lake Fryxell Basin). Duncan et al. (2006) isolated filamentous fungi from Terra Nova Hut which were mainly cold-active and grow at 4 and 25 °C. The *Geomyces* sp. and *Cadophora* sp. are widely present in Antarctica, playing significant role in the decaying and nutrient recycling (Arenz and Blanchette 2009). Blanchette et al. (2010) isolated 69 filamentous fungi from Nimrod Hut, Cape Royds, Antarctica, which included the genera *Cadophora*, followed by *Thielavia*, and *Geomyces*. Franz Joseph Land is a high-arctic desert or semi desert archipelago. The average air temperature during summer, ranges from 0.2 to 1.3 °C, with an average for the year as 14.1 °C. Bergero et al. (1999) have isolated fungal species of *Geomyces*, *Phialophora*, *Phoma*, *Acremonium*, *Thelebolus*, and *Mortierella* from Franz Joseph Land.

The filamentous *Penicillium species* have been investigated in three different polythermal glaciers of Arctic region (Svalbard, Norway). The most predominant species was *Penicillium crustosum* (Sonjak et al. 2006). The Arbuscular mycorrhizal fungal communities have also been found in Arctic ecosystems (Allen et al. 2006). Ectomycorrhizal fungi are widely distributed in arctic and alpine habitats on all continents. Some widely distributed Ectomycorrhizal fungi genera include Inocybe, Cortinarius, Hebeloma, Russula, Thelephora, Tomentella, Cenococcum, and Laccaria (Deslippe et al. 2011). *Aspergillus* sp., *Fusarium* sp., *Curvularia* sp., *Penicillium* sp., and *Cladosporium* sp. have been isolated at 5 °C from deep-sea sediment core of the Indian Ocean (Raghukumar et al. 2004).

1.2.3 Halotolerant and Halophilic

Halophiles are salt-loving organisms that inhabit hypersaline environments. They can be loosely classified according to their salt requirement into three categories: slight halophiles, grow optimally at 0.2–0.85 mol/l (2–5%) NaCl; moderate halophiles grow optimally at 0.85–3.4 mol/l (5–20%) NaCl; and extreme halophiles grow optimally above 3.4–5.1 mol/l (20–30%) NaCl. Halotolerant organisms can grow both in high salinity and in the absence of a high concentration of salts (Buchalo et al. 1999; Yadav et al. 2020a, 2015). Fungi have been mainly isolated from brine and in some cases also from agar baits, biofilms on the surface of crystallization ponds, wood immersed in hypersaline waters, and more recently, microbial mats (Gunde-Cimerman et al. 2009). The mycobiota in hypersaline waters around the world comprise meristematic melanized yeast-like fungi and different related species of the genus *Cladosporium* (Gunde-Cimerman et al. 2000), non-melanized yeasts (Butinar et al. 2005b), filamentous genera *Wallemia*, *Scopulariopsis,* and

Alternaria (Zalar et al. 2005), and different species of the genera *Aspergillus* and *Penicillium*, with their teleomorphic stages (Eurotium, Emericella, and Petromyces) (Butinar et al. 2005a).

Halotolerant fungi, including species in the genera *Alternaria*, *Aspergillus*, *Penicillium*, *Myrothecium*, *Stachybotrys*, and *Trichoderma*, are commonly isolated from arid soils. Halotolerant fungi, such as *Basipetospora halophila*, *Hortaea werneckii*, and *Aspergillus penicilloides*, often are isolated from salted or dried fish. Those species have been found to grow optimally at salinities of 2.5 M NaCl (Blomberg and Adler 1993). However, these fungi will also grow on more dilute media. *Basipetospora halophila* and *H. werneckii* are considered halophilic because they grow at higher rates on NaCl media than on media with other osmoticants (e.g., glucose/fructose and glycerol) (Andrews and Pitt 1987). The species *Hortaea werneckii* is one of the most halotolerant fungi, with a broad growth optimum from 1.0 to 3.0 M NaCl, and it can grow in nearly saturated salt solutions as well as without sodium chloride (Gunde-Cimerman et al. 2000). The species *Candida glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* were isolated for the first time from the hypersaline water of the Dead Sea. Among these, only *C. parapsilosis* was known previously as food-borne halotolerant yeast, while the others were not known for their halotolerance (Butinar et al. 2005b).

The Dead Sea, a typical high-salt habitat for microorganisms, contains 340 g/l of dissolved salt; a variety of filamentous fungi have been isolated from the Dead Sea. Guiraud et al. (1995) reported that no strictly halophilic fungi (only halotolerant fungi) were isolated from localities along the Dead Sea valley. *Gymnascella marismortui* is a remarkable salt-tolerant fungus that has been isolated from the surface water down to a depth of 300 m in the Dead Sea (Buchalo et al. 1998). *G. marismortui* grows optimally at NaCl concentrations between 0.5 and 2 M (Buchalo et al. 2000). Among 476 fungal isolates from the Dead Sea, *Aspergillus terreus*, *Aspergillus sydowii*, *Aspergillus versicolor*, *Eurotium herbariorum*, *Penicillium westlingii*, *Cladosporium cladosporoides*, and *Cladosporium sphaerospermum* were isolated consistently and probably form the stable core of the fungal community (Kis-Papo et al. 2003), and approximately 43% of fungal isolates from the Dead Sea were found to belong to the genera *Eurotium* and *Aspergillus* (Yan et al. 2005b).

The dominant halophilic species are represented by *Hortaea werneckii*, *Phaetotheca triangularis*, and *Trimmatostroma salinum* (Gunde-Cimerman et al. 2009). In naturally hypersaline environments, such as salt flats, saltpans, and brine pools, only a few fungi are considered to be obligate halophiles, and some fungi grow better in the presence of salt than when it is absent and thus have a competitive advantage over less salt-tolerant competitors (Hocking 1993). *Aspergillus halophilicus* and *Scopulariopsis halophilica* are halophilic species that have been isolated from various saline and arid-region soils (Abdel-Hafez et al. 1989). Melanized fungi are a new group of eukaryotic halophiles isolated from hypersaline waters, represented by black, yeast-like hyphomycetes: *Hortaea werneckii*, *Phaeotheca triangularis*, *Trimmatostroma salinum*, and *Aureobasidium pullulans*, together with

phylogenetically closely related *Cladosporium* species, all belong to the order Dothideales (De Hoog et al. 1999).

Hortaea werneckii, *Phaeotheca triangularis*, and *Aureobasidium pullulans* of the Dothideales and *Trimmatostroma salinum* of the Chaetothyriales are black, yeast-like fungi, which are associated with hypersaline saltpans of 15–30% salinity (Gunde-Cimerman et al. 2000). Multi-pond solar salterns that provide a full range of salinities have always been popular environments for studies on halophilic microorganisms. Of particular interest, there are the crystallization ponds, where NaCl saturation is reached. Recent studies have shown that precrystallization and crystallization ponds also harbor a surprisingly rich diversity and abundance of halophilic and halotolerant fungi (Zalar et al. 2007, 2008). Species of *Wallemia ichthyophaga*, *Wallemia muriae*, *Phialosimplex salinarum*, *Aspergillus baarnensis*, *Aspergillus salisburgensis, and Aspergillus atacamensis* are obligate halophilic fungi that strictly require NaCl from 5% to 10% (Martinelli et al. 2017). Also, *Gymnascella marismortui* (Buchalo et al. 1998), *Trichosporium* spp. (Elmeleigy et al. 2010), *Aspergillus unguis* (Nazareth et al. 2012), and *Aspergillus penicillioides* (Nazareth and Gonsalves 2014) have also been reported to be obligate halophiles according to their minimum saline requirement.

1.2.4 Osmotolerant and Osmophilic

An important characteristic of water in any environment is not the amount present but its availability for biological activity. Life exists over water-activity values that range from 0.60 to 1.0 aw, which is defined as the water availability range for biological activity. Most microorganisms are restricted to substrata with aw values of 0.95 and higher (Pitt 1981). Osmophiles are microorganisms adapted to environments with high osmotic pressures, such as high sugar concentrations. Osmophiles are similar to halophiles because a critical aspect of both types of environment is their low water activity (Fadl-Allah and Sayed 1991). Soil fungi in Egypt are generally osmophilic or osmotolerant (recovered on up to 50% sucrose agar) and halophilic or halotolerant (recovered on 5% or more sodium chloride agar) (Moubasher et al. 1990). In environments where salt concentrations are naturally high, such as salt brines or some soils, a fungus must cope with both high ionic strengths and little available water. High sugar concentrations represent a growth-limiting factor for many microorganisms, yet osmophiles protect themselves against this high osmotic pressure by the synthesis of osmoprotectants such as alcohols and amino acids (Moubasher et al. 2015).

Abdel-Hafez et al. (1977) reported that *Aspergillus*, *Cladosporium*, *and Penicillium* were the most frequent osmophilic genera of Egyptian salt marshes recovered on 60% sucrose. Moustafa and Al-Musallam (1975) reported that *Aspergillus repens*, *A. amstelodami*, and *A. restrictus* were highly osmophilic fungi recovered on 60% sucrose. EL-Sharouny et al. (1988) reported that *Aspergillus niger*, *A. ruber*, and *A. amstelodami* fungi recovered on 60% sucrose isolated from

dry dates and osmotolerant fungi that showed nearly equal growth at 20% and 1% glucose could also grow at 40% and 60% sucrose. This group included *Fusarium moniliforme*, *F. equiseti*, *Ulocladium botrytis*, *Rhizopus stolonifer*, *Chaetomium globosum*, *Alternaria alternata*, and *Drechslerabi septata.* Foods with low water activities as a result of high sugar or salt concentrations that are subject to spoilage by fungi include salted fish, jams, jellies, and candy. A wide variety of mitosporic fungi can be isolated from these materials, for example, *Aspergillus*, *Aureobasidium*, *Chrysosporium*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Myrothecium*, *Oidiodendron*, *Paecilomyces*, *Penicillium*, *Rhizopus*, *Scopulariopsis*, *Trichoderma*, *Wallemia*, and *Xeromyces* (Pitt 1981).

Important osmophilic yeasts include *Debaryomyces hansenii* and *Saccharomyces rouxii* (Jennings 1984). Interestingly, the number of colonies of osmophilic fungi isolated per gram of desert soil in Saudi Arabia was low, but species richness was high. *Aspergillus* was the most common genus isolated (Abdel-Hafez 1982). Hamada and Yamada (1991) recorded *Wallemiasebi*, *Aspergillus restrictus*, and *Eurotium* spp. as the most abundant osmophilic mycobiota of house dust. Confectionery, jams, conserves, honey, syrups, dried fruit, fruit cakes, and similar products are susceptible to spoilage by xerophilic fungi particularly adapted to high sugar environments. These products have water activity values between 0.60 and 0.70. In a study by Moubasher et al. (2015), *Emericella* and *Eurotium* genera were isolated from soil of high osmotic stress have grown in media containing 40% sucrose. *Xeromyces bisporus* is often associated with spoilage of confectionery products, covering the substrate in a fine white overgrowth of mycelium (Pettersson and Leong 2011). The majority of *X. bisporus* strains have been isolated from high-sugar foods, including dried fruits, and thus, wizened berries and fruits are likely to be the natural habitat for this fungus (Aguilera and González-Toril 2019).

1.2.5 Xerotolerant and Xerophilic

Xerotolerant and xerophilic fungi are designated as such for their abilities to grow on substrata in which the low matrix potential, rather than osmotic stress from sugars and salts, accounts for low substratum water activity. Xerophilic fungus could grow at a water activity value below 0.85 under at least one set of environmental conditions. Most fungi able to tolerate water activities below 0.90 aw are ascomycetes or their anamorphs. Fungi in this ecological group most frequently are isolated from arid and semi-arid ecosystems and most often are associated with stored cereal grains and dried foods (Magan and Lacey 1984a). The genus *Aspergillus* is collectively the most xerotolerant group of fungi and contains many of the most xerotolerant forms. *Aspergillus* species are better competitors than *Penicillium* species at high temperatures and low water activities (Magan and Lacey 1984b). Desert ecosystems also have a higher number of species than would be predicted based solely on abiotic conditions (Wicklow 1981).

Several researchers have isolated the genus *Aspergillus* from desert soils in Argentina, Chile, and Mexico. *Aspergillus flavus* and *A. fumigatus* were reported from desert soils worldwide (Abdel-Azeem et al. 2019), and *A. carneus* recorded exclusively from desert soils in the Middle East (El-Said and Saleem 2008) were missing in the Atacama soil. Moderate xerophiles include species within *Aspergillus*, *Penicillium*, and *Eurotium* (Pettersson and Leong 2011). Only a few Penicillium species are capable of growth below 0.80 aw: Among these are *Penicillium brevicompactum*, *P. chrysogenum*, *P. implicatum*, and *Eupenicillium cinnamopurpureum* (Hocking and Pitt 1979). The most xerophilic of the aspergilli are species in the section Restricti (Peterson 2008), particularly *Aspergillus restrictus* and *Aspergillus penicillioides*, both of which produce blue-green conidia. *A. penicillioides* is perhaps best regarded as an extreme xerophile, as it grows very slowly or not at all at high aw, grows optimally at 0.91–0.93 aw, and is capable of growth down to at least 0.73 aw in experimental systems (Andrews and Pitt 1987).

Xeromyces bisporus is an ascomycete filamentous fungus that has the unique trait of being, arguably, the most xerophilic organism discovered to date. *X. bisporus* actively grows in conditions of decreased water availability and has an optimal water activity for growth around 0.85 (Grant 2004). Xerotolerant fungi are best known as storage fungi, the spoilage microorganisms of agricultural products and dried foods in the store. The principal fungal contaminants of seeds, cereals, and hay at harvest are field fungi, *Cladosporium* species, and the other inhabitants of plant phylloplanes, together with some specific parasites in the case of seeds and cereals (*Fusarium*, *Drechslera* species, etc.). Field fungi require minimum water activities of at least 0.85 aw for growth (Magan and Lacey 1984a). *Basipetospora halophila* has a minimum aw for growth of 0.73. All known isolates of *B. halophila* have come from salty environments: salted, dried, or cured fish and dried food-grade seaweed (Pettersson and Leong 2011).

1.2.6 Acidophilic and Alkalophilic

Organisms that thrive at the extremes of pH are classified as either acidophiles, which exhibit optimal growth below pH 3, or alkaliphiles, which grow optimally at pH greater than 9 (Rothschild and Mancinelli 2001). Acidophiles and alkaliphiles have been discovered in habitats all over the world. Acidophiles thrive in sites of acid mine drainage, solfataric fields, acidothermal hot springs and fumaroles, coal spoils, and bioreactors. These environments feature low pH values, temperatures ranging from 25 °C to over 90 °C, pressures up to 5 MPa, low salinity, some heavy metals, and either anaerobic or aerobic conditions (Hallberg et al. 2010).

Among acidophilic eukaryotic organisms, algae and protozoans have received more attention than fungi and yeasts. Generally, fungi are detected in acid habitats like volcanic springs, acid mine drainage, or acid industrial wastewaters (Gross and Robbins 2000). Most aquatic acidic habitats have two major origins, one associated with volcanic activities and the other with metal and coal mining (Johnson 1998).

Two classes of fungi, Dothideomycetes and Eurotiomycetes, have been isolated from the highly acidic (pH 0.8) and metal-rich acid mine drainage from Richmond Iron Mountain (Baker et al. 2009). Many other filamentous fungi have been shown to be able to grow at very low pH values. Many *Aspergillus*, *Eurotium*, *Fusarium* and *Penicillium* spp. can grow down to pH values of 2.0 but also have pH optima of up to 10 (Magan 2007).

Acontium pullulans was isolated at pH 2.5 from acidic coal waste and acid streams by Belly and Brock (1974). Studies have attempted to examine metabolically active eukaryotic communities in acidic mine drainage systems, and these studies have demonstrated that species such as *Dothideomycetes* and *Eurotiomycetes* and a number of other ascomycete fungi are present in acid mine drainage (Baker et al. 2004). Species related to Opisthokonta fungi, mainly affiliated within the Ascomycota *Helotiales* and *Dothideomycetes*, have been identified in Los Rueldos, an abandoned mercury underground mine, as well as the genus *Paramicrosporidium* and the Zygomycota genus *Mucoromycotina* (Mesa et al. 2017). Among the ascomycetes, species belonging to the Hemiascomycetes and Euascomycetes have been described in the Iberian Pyrite Belt (*Glomerella* sp. and *Lecythophora* sp.) as well as basidiomycetous yeasts distributed in the classes Hymenomycetes and Urediniomycetes (Gadanho et al. 2006).

These species have also been described in natural acidic geothermal areas (Russo et al. 2008). Most yeasts grow optimally at pH 5.5–6; some, including *Candidakrusei*, *Rhodotorula mucilaginosa* and *Saccharomyces exigua*, can grow at pH 1.5–2.0 (Battley and Bartlett 1966). Three novels asexual basidiomycetous yeast species have been recently described in the Iberian Pyrite Belt (*Cryptococcus aciditolerans* sp. nov., *Cryptococcus ibericus* sp. nov., and *Cryptococcus metallitolerans* sp. nov.). These *Cryptococcus* species are apparently specialists of acidic aquatic environments since they require low pH for growth (Gadanho and Sampaio 2009).

Approximately 30% of the fungi isolated from slightly alkaline forest soils or from limestone caves are alkaline tolerant or alkalophilic (Nagai et al. 1998). Fungi whose pH optimum for growth exceed pH 8, usually falling between 9 and 10, are defined as alkalophiles (Grant et al. 1990). Species of *Acremonium* and *Fusarium* are the fungi most frequently isolated from these soils. Alkaline soda lakes and soda deserts, low Ca environments with pH values >10, are distributed widely in tropical and subtropical regions. They are characterized by high concentrations of $NaCO_3$ resulting from evaporation (Grant et al. 1990). *Paecillomyces lilacimus* was described as being alkaliphilic and able to grow very well between pH 7.5 and 9.0. True alkaliphilic *Chrysosporium* spp. were isolated and described from bird nests having a pH of 11 maximum for growth (Magan 2007).

Goto et al. (1981) identified *Exophiala alcalophila*, a pleomorphic species of a black yeast-like member of the Herpotrichiellaceae. It was growing in the soil at pH 10.4 with its accompanying teleomorph stage, *Phaeococcomyces alcalophilus. Exophila alcaliphila*, *Candida pseudotropicalis*, and *Saccharomyces fragilis* have been described as being alkalotolerant (Magan 2007). Commercial processes such as cement manufacture and casting, electroplating, paper manufacturing, and the lye treatment of potatoes and animal hides generate alkaline conditions in soils

(Haase et al. 1999). Alkaliphilic fungi have also been isolated from industrial effluents. For example, *Aspergillus nidulans* KK-99 (isolated from the industrial effluents of Shreyans Paper Industry Limited, India) is adapted for growth in an alkalescent environment (pH 10.0) (Taneja et al. 2002). Another alkaliphilic fungus, *Myrothecium* sp. IMER1, also grows well under alkali conditions (pH 9.0) (Zhang et al. 2007). Among the alkaliphilic/strong alkalitolerant fungi, the *Sodiomyces* species (*Plectosphaerellaceae*), *Acrostalagmus luteoalbus* (*Plectosphaerellaceae*), *Emericellopsis* alkaline (*Hypocreales*), *Thielavia* sp. (*Chaetomiaceae*), and *Alternaria sect. Soda* (*Pleosporaceae*) grow best at high ambient pH, but the pH tolerance of *Chordomyces antarcticum*, *Acrostalagmus luteoalbus*, and some other species was largely affected by the presence of extra Na ion in the growth medium, further suggesting that the frequency of alkaliphilic fungi is low, while alkalitolerants seem to be far more widespread in soil (Grum-Grzhimaylo et al. 2016).

1.2.7 Rock Inhabiting

Rocks that are exposed to high solar radiation, high temperatures, low nutrient availability, high electrolyte concentration, and low relative humidity, where moisture is available only sporadically, can be considered extreme environments and are colonized by unique species of stress-tolerant fungi (Gorbushina et al. 1993). Rock-inhabiting fungi can be split into two ecological groups: (1) hyphomycetes of soil and epiphytic origin (De Leo et al. 1996) and (2) black microscopic fungi forming compact restricted microcolonies and known to be typical inhabitants of rock surfaces (Ruibal et al. 2005). Black yeasts and dematiaceous mycelial fungi have been isolated from various rock surfaces and crevices in rock surfaces and stone monuments (Sterflinger and Krumbein 1997). Those fungi are phylogenetically diverse, are nonlichenized, and grow on bare surfaces of diverse rocks types in different climates and are termed microcolonial fungi MCF (Staley et al. 1982). Typical adaptational features of MCF are melanized cell walls (Gorbushina et al. 1993) and optimal surface-to-volume ratio both for their round yeast-like cells as well as for the whole microcolonies (Wollenzien et al. 1995).

Melanized microcolonial fungi in the rock surface habitat are known to penetrate rock crevices. Such fungi share high melanin production; resistance to high temperatures and ultraviolet (UV) radiation; and restricted, meristematic growth morphology (Wollenzien et al. 1997). Rock surfaces in arid regions sometimes are coated by "desert varnish," which is a brown, black, or orange coating, often rich in oxides of manganese and iron and in clay minerals (Staley et al. 1992). Some filamentous fungi that occur on rock surfaces are associated with yeasts and bacteria that produce a layer of extracellular slime that protects the hyphae from desiccation. The fungi and bacteria that grow within desert varnishes are capable of enriching the substrata with iron and manganese, resulting in precipitation and accumulation of the dense black layers on the rock surfaces. For both the microcolonial and the rock varnish fungi, production of a dark pigment or a dark layer on rock surfaces

appears to be an adaptation to reduce exposure to extended periods of high UV irradiation (Urzì et al. 1995).

Many fungal species have been isolated from the stone monuments such as *Alternaria*, *Aspergillus*, *Arthobotrys*, *Helminthosporium*, *Phoma*, *Auerobasidium*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Trichothecium*, and *Trichoderma* have been reported as widespread fungi involved in biodeterioration of stone monuments. The growth of these fungal genera on stone monuments was a cause of discoloring and structural decay of stone material of these monuments (Farooq et al. 2015). Species-colonizing monuments are *Coniosporiuma pollinis* from marble monuments in Greece and Italy (Sterflinger et al. 1997) and *C. uncinatum* in France (De Leo et al. 1999). Some species, particularly recurrent on monuments as *Sarcinomyces petricola*, are isolated from marble and calcarenite in Greece and Italy (Wollenzien et al. 1997). A number of *Capnobotryella* species were isolated from Turkish marble monuments (Sert and Sterflinger 2010). *Pseudotaeniolina globosa* was recently isolated on statues of the Boyl's Palace in Cagliari (Onofri et al. 2012). Egidi et al. (2014) show that many of the rock-inhabiting fungi, isolated from both mild and extreme climates, are found in the family Teratosphaeriaceae (Capnodiales), such as *Friedmanniomyces endolithicus*, *Elasticomyces elasticus*, and *Recurvomyces mirabilis*.

1.2.8 Heavy Metal Tolerant

Heavy metals represent a highly abundant group of toxic compounds in the soil that can be found naturally in the environment (e.g., cadmium, copper, lead, nickel, mercury, silver, zinc). Fungal responses to naturally occurring heavy and human-generated metals are wide-ranging. In most cases, species richness declines, and community structure changes. Nevertheless, some heavy metal–tolerant fungi can be isolated from most metal-contaminated sites (Brown and Hall 1989; Kumar et al. 2019a, b). Contamination at toxic levels to organisms is mainly a consequence of technological development and industrial activities that have deposited waste metals into soils and waterways (Baldrian 2010; Kour et al. 2021; Yadav 2020). High concentrations of heavy metals can create a selective pressure on soil microbiota (Joshi et al. 2011), but little is known about natural heavy metal–resistant fungal communities in the soil, despite natural environments representing an important reservoir of microbial diversity. Although a higher percentage of metal-tolerant fungi can be isolated from metal-contaminated sites, copper-tolerant fungi have also been isolated from locations without high concentrations of the metal (Arnebrant et al. 1987).

Likewise, lead sensitivities of *Aureobasidium pullulans* isolates from contaminated and noncontaminated sites did not differ (Mowll and Gadd 1985). Yamamoto et al. (1985) reported similar findings for fungi from copper-contaminated soil. Species of *Aspergillus*, *Alternaria*, *Geotrichum*, *Fusarium*, *Trichoderma*, *Rhizopus*, *Monilia*, and *Penicillium* have been isolated from Agricultural field soil treated with

municipal wastewater and industrial effluents containing Pb, Cd, Cr, Zn, Fe, and Ni in India (Zafar et al. 2007). Species of *Aspergillus*, *Alternaria*, *Geotrichum*, *Fusarium*, *and Penicillium* have been isolated from Water and sediment contaminated with municipal wastewaters and untreated industrial effluents containing Pb, Cr, Cu, Zn, and Cd in Morocco (Ezzouhri et al. 2009). Species of *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus* have been isolated from Soil from peri-urban agricultural areas contaminated with industrial and sewage polluted water containing Zn, Pb, Ni, and Cd in Pakistan (Iram et al. 2009). Species of *Aspergillus* and *Trichoderma* have been isolated from Sewage, sludge, and industrial effluents from sewage treatment plants and electroplating industry containing Pb, Ni, Cr, and Cd in India (Joshi et al. 2011).

Abdel-Azeem et al. (2007) studied the effects of long-term heavy metal contamination on a diversity of terricolous fungi and nematodes in an agroecosystem in Egypt as a case study. They collected 100 soil samples in a randomized way to represent different stages of land reclamation during the period from September (2004) to February (2005). These profiles represented different land-use periods of 0–20 years. Isolated species belonged to 21 genera. The prevailing genera were *Aspergillus* (12 species including anamorph stages of one *Emericella* and one *Eurotium* species; 52.63% of the total isolates). They found that the most abundant species were: *Aspergillus niger* var. niger, (21.15% of the totalisolate number), *Trichoderma pseudokoningii* (12.65%), *A. flavus* (9.4%), and *A. fumigatus* (8.63%).

Abdel-Azeem et al. (2015) studied the occurrence and diversity of mycobiota in heavy metal-contaminated sediments of a Mediterranean coastal lagoon, El-Manzala, Egypt. They found that the prevailing genera were Aspergillus (11 species including anamorph stages of 2 Emericella species; 36.66% of the total isolates), *Penicillium* (4 species including anamorph of Talaromyces; 13.33%), and the remaining taxa were represented only by two to one species each. *Aspergillus niger*, *A. flavus*, and *A. terreus* showed the highest percentage of frequency of occurrence.

1.2.9 Phoenicoid

The term phoenicoid fungi meaning arising from the ashes. Phoenicoid fungi are a specialized group of primarily ascomycetes and basidiomycetes that fruit on soil; plant material; burned wood following a forest fire, prairie fire, or fireplace fire; and volcanic eruptions (Carpenter and Trappe 1985). The terms pyrophilous, anthracophilous, and carbonicolous have also been used to refer to fungi that fruit on heat-treated substrata. Although a habitat that has burned is not a stressed environment in the classical sense, forest fires usually lead to a 3- to 5-unit increase in pH because of ash deposition. The plant community in which a fire has occurred and the depths of burn play major roles in determining the types of fungi that will appear following the fire (Pandit and Maheshwari 1996).

The phoenicoid fungi have been arranged into four groups in relation to the incidence of fruiting following burning according to Dix and Webster (1995).

- **Group 1**: Species that (under favorable climatic conditions) appear on bonfire sites as early as 7 weeks after burnin, but which do not fruit later than 80 weeks after burning, for example, *Anthracobia* spp., *Pyronema* spp., and *Trichophaea abundans.*
- **Group 2**: Heterogeneous group of species that fruit 10–15 weeks after burning. The duration of fruiting is very variable: for *Peziza trachycarpa* not later than 100 weeks after burning, for *P. praetervisa* and*Tephrocybe carbonaria* up to 150 weeks after burning, and for *Pholiota carbonaria* 190 weeks after burning.
- **Group 3**: Comprises only *Trichophaea hemisphaerioides* and *Peziza endocarpoides*, which take 20–50 weeks to appear and persist for 130–200 weeks.
- **Group 4**: Heterogeneous group of species and includes species that do not usually appear until 50 weeks after burning and may not disappear for 150 weeks, for example, *Ripartites tricholoma*, or 200 weeks, for example, *Myxomphalia maura.*

Volcanic eruptions and subsequent depositions of hot ash, as occurred on Mount St. Helens in the state of Washington (United States), can also lead to abundant fruiting of phoenicoid fungi. Six weeks after the eruption, apothecia of *Anthracobia melaloma* and perithecia of *Gelasinospora reticulospora* appeared within the tephra (Carpenter et al. 1987). Petersen (1971) noted that following a natural forest fire in Denmark, the fructifications of *Anthracobia maurilabra* occurred in hollows in the ground surrounding the trunks of charred *Picea. Geopyxis carbonaria* and *Rhizina undulata* are generally restricted to coniferous forests. *Rhizina undulata* is a good example and is especially common at bonfire sites in pine plantations growing on acid soil (Ginns 1974).

1.2.10 Oligotrophic

Oligotrophy is defined as the ability of microbes to grow when concentrations of nutrients are very low or absent. Oligotrophic habitats are generally regarded with a nutrient flux from 1 to 15 mg of carbon per liter (Poindexter 1981), and a medium containing a carbon concentration of 10 mg per liter was widely suggested to cultivate oligotrophic fungi (Martin and MacLeod 1984). Oligotrophic microorganisms are classified on the basis of the trace nutrients that they scavenge; thus, one can distinguish between oligocarbotroph and oligonitrotrophs, organisms that respectively grow by utilizing traces of carbon and nitrogen. This terminology could be extended to include, for example, oligophosphotrophs and oligoferrotrophs, that is, organisms that grow in the presence of trace amounts of phosphate and iron (Wainwright et al. 1993).

That ability is important for survival in some low-nutrient environments, which mycologists generally consider to be stressful. Carbon plays an important role in fungal community dynamics and taxonomic diversity, particularly in soil. Wood decomposing fungi, such as *Phanerochaete chrysosporium*, and several

mycorrhizal species cannot grow on a carbon-free substratum. It is interesting that numerous zygomycetes and mitosporic ascomycetes are able to grow oligocarbotrophically on nutrient-free silica gel (Wainwright et al. 1992). Inoue (1988) reported the growth of fungi on aluminum, plastics, and electronic components and isolated various fungi from printed circuits and PVC covering electric cables. Fungi, including *Aureobasidium pullulans*, *Cladosporium,* and *Phoma* spp., can also grow on the painted surface without degrading the paint itself.

The ability of fungi to grow oligotrophically by scavenging nutrients from the air explains why materials capable of passing standard fungal resistance tests still occasionally become rapidly contaminated with fungal growth (Smith and Nadim 1983). Caves have been generally regarded as typical oligotrophic environments (Bastian et al. 2009), distinctly characterized by constantly low temperature, high humidity, scarcity of organic matter, and darkness (Gabriel and Northup 2013). Species of *Cephalotrichum*, *C. stemonitis* from Slovakia and Spain and *C. verrucisporum* from Japan, were reported from caves environments (wall and ceiling, rodent feces, and rhizomorphs) (Kuzmina et al. 2012). In a study by Jiang et al. (2017), 169 strains belonging to at least 84 taxa were isolated from carbonate caves in China, using oligotrophic carbon-free silica gel medium (SGM). The most common genera obtained in this study include *Cephalotrichum*, *Plectosphaerella*, *Clonostachys*, *Cladosporium*, and *Fusarium*. The most common species *include Plectosphaerella cucumerina*, *Clonostachys rosea*, *Cephalotrichum oligotriphicum*, and *C. guizhouense.*

1.3 Biomolecules from Endophytic Fungi

Endophytic fungi are microfungi that internally infect living plant tissues without causing disease or any harm to plant and live in a mutualistic association with plants for at least a part of their life cycle (Abo Nouh 2019; Rana et al. 2019b). Endophytic microbes are able to provide survival strategies and provide protection of their host plants including production of chemically different and secondary metabolites (structurally) also drug conduct showing the layout of pharmacological activities or biological, namely, anticancer, antimicrobial, antiviral, insecticidal, antioxidants, antimalarial, antidiabetic, antiparasitics, and immunosuppressants (Strobel and Daisy 2003; Rana et al. 2019c; Yadav et al. 2019d).

Endophytic fungi have protection activity, most notably endophytic fungi protect their host from pathogenic microflora through disallowing the pathogens to expand a systemic connection including the host plant (Jalgaonwalal et al. 2011; Rana et al. 2020), and are controlling plant disease via practice as a new approach. In other words, a different range of metabolites have been separated from endophytic fungi giving special importance to their ecological system or role. However, the evaluation made, since 2003, mentioned 4000 bioactive compounds from a few fungal species, specifically *Penicillium*, *Fusarium*, and *Acremonium*, playing a functional or react role on various perspectives; nevertheless, some of the studies exist from

Table 1.1 Biomolecules compound and their activity

Name of the fungi	Biomolecules compound	Activity	Reference
Acremonium zeae	Pyrrocidines A and B	Antifungal activity against *A. flavus* and *F. verticillioides*	Gao et al. (2020)
Aspergillus fumigatus CY018	Asperfumoid, fumigaclavine C, fumitremorgin C, physcion and helvolic acid	Restrict *Candida albicans*	Pimentel et al. (2011)
Cephalosporium sp. IFB-E001	Graphislactone A	Free radical scavenging	Zhou et al. (2018)
Cephalotheca faveolata	Sclerotiorin	Antibacterial	Zhang et al. (2019)
Chaetomium globosum	Chaetoglobosins A and C	Inhibit *Mucor miehei*	Chen et al. (2020)
Cladosporium sp.	Brefeldin A	Antifungal activity	Wang et al. (2007)
Talaromyces sp.	7-Epiaustdiol	Inhibit multidrug-resistant opportunistic pathogen *P. aeruginosa*	Li et al. (2010)
Xylaria sp.	Griseofulvin	To treat human and animal mycotic diseases	Meshram et al. (2016)

endophytes (Laxmipriya et al. 2013). Bioactive compounds produced by endophytic fungi exhibit their activities (Table 1.1).

1.3.1 Anticancer Agents from Fungal Endophytes

According to World Health Organization (2017a), in 2015, 8.8 million deaths were due to causes of cancer; this disease is demonstrated through uncontrolled or uncontrolled division of abnormal cells in a part of the body that consequent in uncontrolled division cell multiplication or abnormal tissue growth or proliferation in the human body (Deshmukh et al. 2019). Plant origin compounds, for example, taxol like *Taxus baccata*, *T. canadensis*, *and also T. brevifola*, vincristine (*Catharanthus roseus*) and vinblastine, camptothecin, and its preparation, namely, topotecan as well irinotecan, and etoposide derived from epipodophyllotoxin, have played a significant role in the development of useful anticancer drugs clinically (Debbab et al. 2011). Some examples of bioactive compounds produced by endophytic fungi exhibiting anticancer activity are shown (Table 1.2).

Table 1.2 Endophytic fungi producing anticancer activity

Name of the endophyte	Plant source	Compound name	Reference
Phyllosticta spinarum, *Bartalinia robillardoides*	*Taxus baccata*	Taxol	Gangadevi and Muthumary (2008)
Pestalotiopsis microspore	*Torreya taxifolia*	Torreyanic acid	Lee et al. (1996)
Fusarium solani	*Camptotheca acuminate*	Camptothecin	Kusari et al. (2009)
Aspergillus fumigatus	*Podophyllum peltatum*	Podophyllotoxin	Eyberger et al. (2006)
Unidentified fungus XG8D	*Xylocarpus granatum*	Merulin A and C	Chokpaiboon et al. (2010)

1.3.2 Antimicrobial Compounds from Fungal Endophytes

Antimicrobial metabolites manufactured through endophytes are low-molecular-weight extro-lites not inevitable for growth and yield at low concentrations against pathogenic invasion (Guo et al. 2006). Therefore, endophytes are a potential resource of antimicrobial compounds to prevent the major threat from human drug-resistant and plant pathogens (Tan and Zou 2001). Furthermore, based on the literature report, endophytes yield structurally various diseases of antimicrobial compounds, for example, alkaloids, phenols, terpenoids, quinines, steroids, and flavonoids (Guo et al. 2000). *Penicillium* sp. produced the monoterpene preaustinoids A1, A2, and B1, endophytic to Melia azedarac, which exhibited mild bacteriostatic activity against *E. coli*, *P. aeruginosa*, *S. aureus as well Bacillus* sp. (Geris dos Santos and Rodrigues-Fo 2002). Likewise, preaustinoid B2, austinolide, preaustinoid A3, and isoaustinone those are monoterpenes, and four monoterpenes were identified that were produced owing to α-ketol rearrangements also Bayer-Villiger oxidations related to in the biosynthetic system/process (Fill et al. 2007). Bioactive compounds produced by endophytic fungi exhibiting antimicrobial activity are shown (Table 1.3).

1.3.3 Antioxidants from Fungal Endophytes

It goes without saying that in medicinal plants are present natural antioxidants and also in fruits and vegetables; despite that metabolites produced through endophytes are studied to show naturally produced antioxidants. Finally, and most importantly, microbial polysaccharides are additionally reported as natural antioxidants (Huang et al. 2007). Different ROS associated with diseases, namely, atherosclerosis, hypertension, cancer, most notably antioxidants exhibited administration (therapeutic) promise for therapy of varied ROS (Valko et al. 2007). Particularly, endophytic fungus *Pestalotiopsis microspora* produced pestacin and isopestacin, which

Table 1.3 Endophytic fungi producing antimicrobial compounds

Name of the endophyte	Plant source	Biomolecules compound	Reference
Cladosporium sp.	*Quercus variabilis*	Brefeldin	Wang et al. (2007)
Xylaria sp. *YX-28*	*Ginkgo biloba*	7-Amino-4-methylcoumarin	Liu et al. (2008)
Xylaria sp. *F0010*	*Abies holophylla*	Griseofulvin	Park et al. (2005)
Pestalotiopsis adusta	Unidentified plant	Pestalachloride A	Li et al. (2008)
		Pestalachloride B	
Phomopsis cassia	*Cassia spectabilis*	Ethyl 2,4-dihydroxy-5,6-dimethylbenzoate	Silva et al. (2005)
		Phomopsilactone	
Phomopsis sp.	*Erythrina crista*	Phomol	Weber et al. (2004)
Curvularia lunata	*Niphates olemda*	Cytoskyrin A	Jadulco et al. (2014)

Table 1.4 Endophytic fungi exhibiting antioxidant activity

Name of the endophyte	Plant source	Biomolecules compound	Reference
Pestalotiopsis microspora	*Terminalia morobensis*	Pestacin, Isopestasin	Harper et al. (2003)
Cephalosporium sp. IFB-E001	*Trachelospermun jasminoides*	Graphislactone A	Hormazabal et al. (2005)
Microphaeropsis olivacea	*Pilgerodendron uviferum*		
Corynespora cassiicola L36	*Lindenbergia philippensis* (Cham) Benth	Corynesidone B	Chomcheon et al. (2009)
Corynespora cassiicola L36	*Lindenbergia philippensis* (Cham) Benth	Corynesidones A	
Corynespora cassiicola L36	*Lindenbergia philippensis* (Cham) Benth	Corynether A	

displayed potent antioxidant activity. Therefore, pestacin's proposed antioxidant activity is owing to the crack-up of reactive C-H bond and via OH abstraction. There is no doubt that antioxidant activity is 10-fold greater than Trolox (Harper et al. 2003). Bioactive compounds produced by endophytic fungi exhibit antioxidant activity (Table 1.4).

Table 1.5 Bioactive compounds produced by endophytic fungi exhibiting insecticidal activity

Name of the fungi	Plant source	Biomolecules compound	Reference
Nodulisporium sp.	*Bontia daphnoides*	Nodulisporic acid	Demain (2000)
Penicillium sp.	*Acanthus ilicifolius*	Penicinoline	Shao et al. (2010)
Muscodor vitigenus	*Paullinia paullinioides*	Naphthalene	Daisy et al. (2002)
Eupenicillium sp.	*Murraya paniculata*	Alantrypinene	Fábio et al. (2005)

1.3.4 Insecticidal Activities from Fungal Endophytes

Foliar *Clavicipetalean* grass and systemic endophytes produced toxic alkaloids; however, it protected the hosts athwart vertebrate herbivores and insects. Mechanisms concerning the manufacturing of toxic repellent compounds through endophytic fungus have been studied (Shi et al. 2013). A multitude of endophytic nematophagous and entomo-pathogenic fungi are generally thought to be biocontrol agents that assist nematicides or insecticides. Endophytic *Phomopsis* sp. produced phomopsolids that prohibit the repellent and feeding activities in elm bark beetles (Grove 1985). Bioactive compounds produced by endophytic fungi exhibit insecticidal activity (Table 1.5).

1.4 Biomolecules from Mangrove Fungi

Mangroves are salt-tolerant forest habitats restricted to intertidal areas of flooded beaches, estuaries, tidal creeks, backwaters, lagoons, marshes, and tropical and subtropical latitudinal mudflats. Such important ecosystems are a complex ecotone of great ecological, economic, and social importance between the terrestrial and the marine environments (Gopal and Chauhan 2006). Nearly 90% of mangroves are allocated in South-East Asia, America, and Africa. The diversity of mangrove species is well known among animals and plants including microorganisms (Shearer et al. 2007). The related mangrove fungi in mangroves are also called manglicolous fungi, which mainly include marine fungi; however, a select minority of terrestrial fungi also occurs in mangrove area. Because aerial parts in mangroves are subjected to salt spray (Kohlmeyer and Kohlmeyer 2013), these fungi can be shown to have salt tolerances. The latest estimate of marine fungi is 1500 species, which excludes lichens, and many of them are new or inadequately described species (Hyde et al. 1998). Jones and Alias (1997) estimated that there are 269 species of higher marine fungi from mangrove, while Schmit and Shearer (2003) listed 280 fungi (198 ascomycetes, 78 mitosporic and 4 basidiomycetes) worldwide. Fungi in mangrove environment represent bioactive metabolites that enjoy renewed attention for providing novel and interesting chemical scaffold and an essential ecological role in organic matter decomposition through the development of a variety of extracellular

degrading enzymes, such as cellulase, xylanase, pectinase, amylase, and so on (Kobayashi and Tsuda 2004).

1.4.1 Bioactive Compounds from Mangrove Fungi

1.4.1.1 Cytotoxic Compounds

Phomoxanthone A is a dimeric natural product of tetrahydroxanthone that has gained considerable attention because of its cytotoxic, antibacterial, and antifungal effects. This interesting metabolite was obtained from the *Phomopsis longicolla* endophyte derived from the *Sonneratia caesolaris* mangrove plant, harvested on Hainan Island, South China (Frank et al. 2015). The *Aspergillus versicolor* HDN1009 fungal strain obtained from mangrove soil collected in Guangzhou, China, yielded six rare heterogeneous dimers, versixanthones A–F. These compounds were evaluated for cytotoxicity to the cell lines HL-60, K562 (myelogenous leukemia), A549, H1975, MGC-803 (human gastric cancer), HO8910 (ovarian cancer), and HCT-116 (colorectal carcinoma). Compounds versixanthone A, B, and C exhibited activity against at least two types of cells with IC50 values ranging from 2.6 to 25.6 μM and the derivatives versixanthone D, E, and F and secalonic acid D demonstrated cytotoxicity against at least five cancer lines with IC50 values ranging from 0.7 to 21 μM (Wu et al. 2015).

Annulohypoxylon sp. endophyte CA-2013, derived from the mangrove plant *Rhizophora racemosa*, collected in Cameroon, resulted in the characterization of new congeners based on benzofluoranthene, daldinones H–J. Daldinon demonstrated high to moderate cytotoxicity to adult lymphoblastic T cells (Jurkat J16) and Burkitt's lymphoma B lymphocytes with 14.1 and 6.6 μM IC50 values, respectively (Liu et al. 2017). Research on the fungal strain *Rhytidhysteron rufulum* AS21B, obtained from *Azima sarmentosa* collected from a mangrove field in the province of Samutsakhon, Thailand, provided a series of spirobisnaphthalene analogs displaying cytotoxic and nitric oxide (NO) production inhibitory activities. Cultivation of the fungus under mildly acidic conditions (pH 5) resulted in a distinct improvement in its metabolic parameters, with the addition of 2 new spirobisnaphthalenes, rhytidenones G and H, and 11 recognized compounds including rhytidenones E and F (Siridechakorn et al. 2017).

1.4.1.2 Antimicrobial Compounds

A significant number of reports focused on antimicrobial metabolites isolated from mangrove saprophytic fungi, for example, Auranticins, which are antimicrobial depsidones, and the epimeric δ-lactones, helicascolide A and B, were obtained from fungi isolated from mangroves (Poch and Gloer 1991). Aigialomycins A–E, new 14-membered resorcylic macrolides, were isolated together with a known

hypothemycin from the mangrove fungus, *Aigialus parvus* BCC 5311 (Isaka et al. 2002). Hypothemycin and aigialomycin D exhibited in vitro antimalarial activity with IC50 values of 2.2 and 6.6 μg/mL, respectively, while other analogues were inactive. Hypothemycin is reported to exhibit moderate antibiotic activity against the protozoan, *Tetrahymena furgasoni*, and the plant pathogenic fungi *Ustilago maydis* and *Botrytis allii* (Nair and Carey 1980). Christophersen et al. (1999) have also investigated some ubiquitous genera, such as *Aspergillus* and *Penicillium* isolated from lagoons and mangroves in Venezuelan waters, expected to furnish optimal conditions for the discovery of new metabolites. Among the total 227 isolated fungi, 61 strains of *Penicillium citrinum* antibacterial activity and 30 isolates of *Penicillium steckii* produced very similar profiles of secondary metabolites that had activity against either *Vibrio parahaemolyticus* or *Staphylococcus aureus* or both. Cytosporone B shows broad activities against fungi, of which MIC against *Aspergillus niger*, *Trichodema* sp., and *Fusarium* sp. were 0.125 mg/mL, 62.5 μg/mL, and 62.5 μg/mL, respectively, and was purified from the fermentation broth of an endophytic fungus, *Dothiorella* sp., isolated from mangrove plant *Avicennia marina* at the esturary of Jiulong River, Fujian Province, China (Yan et al. 2005a). The fungal extract of *P. brocae* MA-231, grown on the PDB medium, permitted the characterization of five new derivatives of sulfide diketopiperazine, namely, penicibrocazines A–E, and one established analog, phomazine B. All compounds were checked against several human- or plant-pathogenic. The penicibrocazines B–D and phomazine B showed activity against *S. aureus* with MIC values of 82, 0.55, 17.6, and 0.55 μM, respectively. Penicibrocazine C demonstrated good inhibitory activity against *Micrococcus luteus* with an MIC value of 0.55 μM (Meng et al. 2015).

1.4.1.3 Other Bioactive Compounds

β-Carboline, adenosine, and 8-hydroxyl-3,5-dimethyl-isochroman-1-one were isolated from mangrove fungus K32. The interaction of β-carboline with calf thymus DNA was investigated by UV–vis and fluorescence spectra, resulting in the occurrence of the binding reaction, which was proposed to be one possible mechanism of the antitumor activity of β-carboline (Song et al. 2004). Eight new indole triterpenes named shearinines D–K, along with shearinine A, paspalitrem A, and paspaline, have been isolated from the mangrove endophytic fungus *Penicillium* sp. Shearinines D, E, and G exhibit significant in vitro blacking activity on large-conductance calcium-activated potassium channels (Xu et al. 2007). The endophyte *R. rufulum* AS21B produced six new derivatives of spirobisnaphthalene, namely, rhytidenones A–F. Among the isolated compounds, rhytidenone C in lipopolysaccharide (LPS)-stimulated J774.A1 macrophages with an IC50 value of 0.31 μM demonstrated the most potent inhibitory effect on NO output (Pudhom and Teerawatananond 2014). Chromatographic workup of sediment-derived *Penicillium pinophilum* H608 (obtained from the Xiamen coastline, China) extract led to the isolation of a series of phenolic compounds that were assessed for their inhibitory activity against oleic acid-related lipid aggregation in HepG2 cells. Eight compounds were reported to

prevent lipid aggregation at a dosage of 10 μM, with no major cytotoxicity (IC50 > 50 μM) as a result of this bioactivity screening (Wu et al. 2016).

1.4.1.4 Enzymes from Mangrove Fungi

The great biodiversity of mangrove fungi can produce enzymes possessing better physiological characteristics in relation to temperature, pressure, pH, and salinity of medium (Burtseva et al. 2003). A study by Wu (1993) identified 15 genera (42 strains) of fungi from mangroves in the Tansui Estuary near Taipei, Taiwan, and found that most of the ascomycetes were able to secrete a wide range of enzymes potentially capable of decomposing mangrove litter. Raghukumar et al. (2004) reported that a mangrove fungus, *Aspergillus niger*, can produce thermostable, cellulose-free alkaline xylanase activity in bio bleaching of paper pulp, and the crude enzyme of its crude culture filtrate, with high xylanase activity, cellulose-free and unique properties containing 580 Ul-1 xylanase, could bring about bleaching of sugarcane bagasse pulp by a 60-min treatment at 55 °C. D'Souza et al. (2006) reported that a mangrove white-rot basidiomycetous fungus, NIOCC#2a, is able to produce laccase to decolorize colored effluents and synthetic dyes. The efficiency of this fungus in decolorization of various effluents with laccase that is active at pH 3.0–6.0 and 60 °C in the presence of seawater has great potential in bioremediation of industrial effluent. Another example is that a marine hypersaline-tolerant white-rot fungus, *Phlebia* sp. MG-60, screened from mangrove stands (Li et al. 2002), has shown excellent lignin degradation ability and selectivity. It can degrade more than 50% of lignin incubated with whole sugarcane bagasse, but less than 10% of holocellulose was lost, and after biopulping with this strain, the whole sugarcane bagasse might be used to produce animal feed after fermentation (Li et al. 2003).

1.5 Biomolecules from Aquatic Habitat Fungi

Seventy-one percent of our planet's surface comprise of water, yet just 0.6% are lotic and lentic freshwater habitats. Often taken for granted, freshwaters are widely diverse habitats and host >10% of all animal and >35% of all vertebrate species worldwide. Nevertheless, no other main components of global biodiversity are decreasing as rapidly and dramatically as freshwater ecosystem. While there is considerable evidence that freshwater fungal diversity is huge, the study of freshwater (aquatic) fungi biodiversity is still in its infancy (Webster 1976). Hyde et al. (2007) have estimated that there are around 1.5 million fungal taxa on earth. Of these, only about 3000 species are recorded to be associated with aquatic habitats and only 465 species occur in marine waters (Shearer et al. 2007). This small proportion of aquatic fungal taxa is outstanding as the aquatic habitats are a potentially perfect habitat for many species. Based on this notion, we believe that the "true" number of aquatic fungi is much larger than 3000 and includes a broad range of hitherto

undescribed species with unknown ecological function. Aquatic fungi are typically microscopic species, which do not produce visible fruiting bodies but grow asexually (anamorphic fungi). Water-associated fungi have been known traditionally and classified as "phycomycetes," a functionally defined group consisting of "true fungi" (Eumycota) (Webster 1976).

Aquatic and aquatic-derived fungi have been widely studied for their bioactive metabolites and have proven to be a promising source of novel anticancer, antiplasmodial, antiviral agents, antibacterial, and anti-inflammatory (Hernández-Carlos and Gamboa-Angulo 2011). Aquatic-derived fungi were also used as biosorbents of heavy metals and radionuclides from polluted groundwater (Abdel-Azeem and Gab Allah 2011). Several years ago, only a few antibacterial agents have received approval by the U.S. Food and Drug Administration. Thus, there is an urgent need for new antimicrobial agents and other effective pharmaceutical agents. Fungi have a magnificent record in producing bioactive compounds, and several of these currently have significant medicinal and other uses (Lazaro and Hyde 2001).

1.5.1 Bioactive Secondary Metabolites of Aquatic Fungi in Pharmaceutical Applications

Aquatic Fungi are known for their potentiality to produce a wide diversity of bioactive secondary metabolites. The first compound of medical and economic importance derived from an aquatic fungus is the Cephalosporin C, a broad-spectrum antibiotic isolated from *Acremonium chrysogenum* (Abraham 1979). Recently, more than 1000 compounds derived from aquatic fungi were discovered, with possible applications for treating cardiovascular diseases, diabetes, cancer, immune suppressants, antibiotics, antioxidants, and antivirals, among others (Wang et al. 2015). Fungi producing those compounds were isolated from freshwaters, considering environments such as the mangroves or the associations with macroalgae and other organisms. Aquatic fungi turned into a significant reservoir of new chemical structures and bioactive natural compounds (Qin et al. 2015). It is important to highlight that there is still an overwhelming knowledge gap of bioactive compounds isolated from aquatic fungi when compared to their terrestrial counterparts.

1.5.1.1 Bioactive Compounds as Antioxidants and Antimicrobial Activities

Abdel-Wareth and Ghareeb (2018) isolated *Penicillium implicatum* and *Aspergillus niveus* as promising freshwater-derived fungus producing a number of phenolic compounds, among them methyl gallate and *p*-coumaric acid. These two compounds were found to have antibacterial effects and miracicidal and cercaricidal activities (Tables 1.6 and 1.7). Methyl gallate is a derivative of gallic acid a powerful

Table 1.6 The phenolic compounds identified in *Penicillium implicatum* filtrate by reverse phase HPLC

Peak No.	R.T (min)	Area %	Identified compound	µg/100 mL
1	7.26	0.32	Gallic acid	53.74
2	7.37	0.12	Pyrogallol	833.58
3	7.93	0.30	4-Amino-benzoic acid	18.66
4	8.65	0.18	Protocatechuic acid	80.70
5	8.69	0.10	Catechin	56.69
6	9.21	0.23	Chorogenic acid	57.86
7	9.53	1.12	Methyl gallate	285.00
8	9.69	0.10	Epicatechin	23.83
9	10.24	0.25	Caffeic acid	19.19
10	10.35	0.17	Vanilic acid	63.48
11	11.75	0.75	*p*-Coumaric acid	89.24
12	11.86	0.13	Ferulic acid	19.90
13	12.08	0.40	Iso-ferulic acid	68.51
14	12.63	0.14	Reversetrol	7.65
15	13.03	0.35	Ellagic acid	932.51
16	13.10	2.14	*e*-Vanilic acid	8021.09
17	13.28	0.26	α-Coumaric acid	42.48
18	13.43	0.30	Benzoic acid	516.93
19	13.92	0.78	3,4,5-Methoxy cinnamic acid	11.73
20	14.04	0.19	Coumarin	23.41
21	14.39	0.19	Salicylic acid	131.51
22	15.15	0.20	Cinnamic acid	12.08

Source: Abdel-Wareth and Ghareeb (2018)

compound. It was previously isolated from plants showing medicinal properties like antioxidant, antiproliferative, and anticancer abilities (Chaudhuri et al. 2015). On the other hand, *p*-coumaric acid, stated to have biological activities and many physiological functions, is one of the most significant phenolic acids, *p*-coumaric acid (4-hydroxycinnamic acid), classified as a nutraceutical and phytochemical, is identified at significant levels in many fruits and vegetables as well as cereals; *p*-coumaric acid was effective against *Bacillus cereus* as compared with Kanamycin (Abdel-Wareth and Ghareeb 2018). There are more than 600 taxa of freshwater fungi between mitosporic and ascomycetes fungi, where a greater number are known from temperate, in contrast to tropical, regions (Wong et al. 1998).

1.5.1.2 Bioactive Compounds as Nematicidal

As for the search of nematicidal metabolites produced by freshwater fungi micromycetes, the contributions found in the literature corresponded to the research conducted by Dong's group. These were made for alternatives to control *Bursaphelen chusxylophilus*, a very economically important nematode devastating pine wood.

Table 1.7 The phenolic compounds identified in *Aspergillus niveus* filtrate by reverse phase HPLC

Peak No.	R.T (min)	Area %	Identified compound	µg/100 mL
1	7.29	0.13	Gallic acid	12.04
2	7.34	0.27	Pyrogallol	970.06
3	7.93	0.66	4-Amino-benzoic acid	22.21
4	8.64	0.84	Protocatechuic acid	202.17
5	8.68	0.20	Catechin	56.79
6	9.24	0.63	Chorogenic acid	86.84
7	9.55	0.98	Methyl gallate	135.72
8	9.72	0.28	Epicatechin	34.73
9	10.27	0.58	Caffeic acid	24.26
10	10.38	0.54	Vanilic acid	108.31
11	10.64	1.35	*p*-Coumaric acid	87.07
12	11.88	0.32	Ferulic acid	26.99
13	12.20	0.58	Iso-ferulic acid	54.33
14	12.61	0.11	Reversetrol	3.08
15	13.01	0.03	Ellagic acid	57.26
16	13.04	0.06	*e*-Vanilic acid	124.44
17	13.28	0.22	α-Coumaric acid	20.38
18	13.52	0.61	Benzoic acid	573.71
19	13.90	0.04	3,4,5-Methoxy cinnamic acid	3.19
20	13.97	0.10	Coumarin	7.02
21	14.35	0.15	Salicylic acid	56.31
22	15.20	0.12	Cinnamic acid	3.89

Source: Abdel-Wareth and Ghareeb (2018)

During this search, the fungus *Caryospora callicarpa* was detected which produces seven compounds belonging to two groups of chemical families, macrolactons (caryospomycins A–C) and naphthalenes. All exhibited moderate activity against *B. xylophilus* (LC50 = 100–229 lg/mL) (Dong et al. 2007). Also, fungus *Paraniesslia* sp. was able to produce two glycosphingolipids, (2*S*,20*R*,3*R*,30*E*,4*E*,8*E*)-1-*O*-(b-D-glucopyranosyl)-3-hydroxyl-2-[*N*-20-hydroxyl30eicosadecenoyl]amino-9-methyl-4,8octadecadiene and cerebroside C both showed weaker (LC50 = 110 lg/mL) nematicidal activities against *B. xylophilus* (Dong et al. 2005). A compilation of freshwater species of fungi have been explored in terms of their chemical content and biological properties, which belong to different fungal genus. These include *Anguillospora*, *Annulatascus*, *Astrosphaerilla*, *Camposporium*, *Caryospora*, *Clavariopsis*, *Decaisnella*, *Dendrospora*, *Glarea*, *Helicodendron*, *Helicoon*, *Kirshchsteiniothelia*, *Massarina*, *Mortierella*, *Ophioceras*, *Paraniesslia*, *Pseudohalonectria*, *Stachybotrys*, and *Vaginatispora* genera (Hernández-Carlos and Gamboa-Angulo 2011). Some of species producing antimicrobial and nematicidal agents are shown (Table 1.8).

Table 1.8 Metabolites with pesticidal properties isolated from fungi found in freshwater systems

Fungal species	Metabolite	Type of compound	Activity	Reference
Stachybotrys sp. (CS-710-1)	Stachybotrins A and B	Alkaloid	Antimicrobial	Xu et al. (1992)
Anguillospora longissima	Anguillosporal	Polyketide	Antimicrobial	Harrigan et al. (1995)
Helicodendron giganteum	Heliconols A–C	Polyketide	Antimicrobial	Mudur et al. (2006)
Decaisnella thyridioides	Decaspirones A–E	Spirodioxynaphthalenes	Antimicrobial	Jiao et al. (2006)
Paraniesslia sp. YMF1.01400	(2*S*,20*R*,3*R*,30*E*,4*E*,8*E*)-1-*O*-(b-ᴅGlucopyranosyl)-3-hydroxyl-2-[*N*-20-hydroxyl-30 Eicosadecenoyl] amino-9-methyl-4,8-octadecadiene	Sphingolipid	Nematicidal	Bills et al. (1999)
Pseudohalonectria adversaria YMF1.01019	Pseudohalonectrin A–B	Pyrone-quinone	Nematicidal	Dong et al. (2006)
Caryospora callicarpa YMF1.01026	Caryospomycins A–C	Macrolactone	Nematicidal	Dong et al. (2007)
	4,8-Dihydroxy-3,4-dihydronaphthalen-1(2*H*)-one 4,6-Dihydroxy-3,4-dihydronaphthalen-1(2H)-one 4,6,8-Trihydroxy-3,4-dihydronaphthalen-1(2*H*)-one 3,4,6,8-Tetrahydroxy-3.4-dihydronaphthalen-1(2*H*)-one(*cis*-4-hydroxyscytalone)	Naphtahlene	Nematicidal	Zhu et al. (2008)

1.5.2 Aquatic Fungi: An Effective Tool in Bioremediation

Mycoremediation is a type of bioremediation in which fungi have been used to decontaminate the area because it is the cheapest option for remediation. Fungi are unique microorganisms because they secrete extracellular enzymes. Decomposition of lignocellulose is classified as the most significant degradative event in the carbon cycle of the earth (Bennett and Faison 1997; Yadav et al. 2019c, e). Mycoremediation

is the determination of the right fungal species to target a particular pollutant. The purpose of fungi in the ecosystem is the decomposition and conversion of organic and inorganic substrates (Kshirsagar et al. 2012). Cooke (1976) encouraged the use of fungi in the treatment of wastewater as fungi tended to exhibit higher rates of degradation of organic matter. In addition to the extracellular enzyme production, the fungal cell walls and their components play a significant role in the biosorption of toxic substances during the treatment of wastewater. Aksu (2005) recorded biosorption of organic contaminants by different types of fungal taxa. Denizli et al. (2004) discovered that fungal biomass eliminates large quantities of organic contaminants from aqueous solution by adsorption. Aquatic fungi also play a role as natural environmental remediators (Hasija 1994). Kshirsagar et al. (2012) particularly focus on elimination of contaminants from wastewater during wastewater treatment.

Essential parameters such as pH, BOD, COD, phosphate, and nitrate were used to understand the function of aquatic fungi in remediation. Aquatic fungi were isolated from water from river Mula, Pune. This study was carried out using four species of fungi. The most dominant species were *Aspergillus terreus*, *Aspergillus niger*, and *Cunninghamella* sp. (Table 1.9) (Kshirsagar and Gunale 2011; Kshirsagar 2012) and were used as test organisms for the treatment of wastewater. Fungal Medium and culture were used for isolation of fungi from the polluted water from the Mula river. The wastewater samples were tested for nitrate, phosphate, COD, BOD, and pH. The following approach was used to research the role of fungi in wastewater treatment. (1) Wastewater treated with *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans*, and *Cunninghamella* sp. and (2) Wastewater treated without *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans*, and *Cunninghamella* sp. (Control). Wastewater was tested for pH, BOD, COD nitrate, and phosphate before and after treatment with fungi. *Aspergillus terreus* was shown to have the highest nitrate removal from wastewater. By order of elimination of nitrate, *Aspergillus terreus* > *Aspergillus niger* > *Rhizopus nigricans* > *Cunninghamella* sp. > Control. *Aspergillus niger* effectively eliminated nitrogen and could be maintained as dominant.

Reduction of BOD in wastewater by *Aspergillus terreus* and *Aspergillus niger* demonstrates the highest reduction potential of BOD during wastewater treatment (Preetha and Viruthagiri 2005). *Rhizopus* sp. is reported to be essential adsorbents

Table 1.9 Analysis of pH from wastewater using aquatic *A. terreus*, *A. niger*, *R. nigricans* and *Cunninghamella* sp.

Initial pH 7.41 ± 0.10					
Days	Control	*A. terreus*	*A. niger*	*R. nigricans*	*Cunninghamella*
5	7.69 ± 0.07	6.70 ± 0.16	7.02 ± 0.12	7.09 ± 0.10	7.33 ± 0.18
10	7.08 ± 0.06	7.15 ± 0.12	7.61 ± 0.07	7.28 ± 0.07	7.59 ± 0.05
15	7.66 ± 0.14	7.99 ± 0.21	7.48 ± 0.08	7.63 ± 0.16	7.17 ± 0.06
20	7.73 ± 0.06	7.81 ± 0.22	7.65 ± 0.14	7.89 ± 0.10	7.68 ± 0.15

Values represent the mean ± S.E of the three replica

for the removal of metals and organic matter from urban and industrial wastewater. *Rhizopus* sp. was reported to have reduced by 23.74% of phosphate. When used *Cunninghamella* sp. were 50.83%, 52.57%, 47.48%, and 42.40% respectively, but *Aspergillus niger* resulted in the highest—52.57% of phosphate elimination. So, the percentage of phosphate reduction order in wastewater were *Aspergillus niger* > *Aspergillus terreus* > *Rhizopus nigricans* > *Cunninghamella* sp. > Control. Pedro et al. (2007) report *Aspergillus terreus* and *Aspergillus niger* demonstrated excellent ability to extract contaminants. While *Aspergillus terreus* has demonstrated the best result for elimination of nitrate and BOD, *Aspergillus niger* showed best removal of phosphate and COD from wastewater.

1.6 Biomolecules from Soil Fungi (Desert–Agricultural–Salt Marshes)

The soil is probably home to the most diverse biome among the resources of our planet (Jansson and Hofmockel 2020) due to its heterogeneous nature in physical and chemical factors that condition this means of life, where microorganisms such as fungi have adapted developing survival strategies (Neifar et al. 2015), which involves, among others, the production of antimicrobials, which is a very important characteristic for obtaining drugs for the preservation of human and veterinary health (Chandra and Kumar 2017). Fungi are resistant to stress, able to grow in water scarcity, and tolerate drought through spore formation (Sterflinger et al. 2012). According to the functions that their metabolic capacity establishes on the soil, they can be classified into three functional groups: biological controllers, ecosystem regulators, and decomposers of organic matter and other compounds such as pesticides and heavy metals (Verbruggen et al. 2012).

1.6.1 Biomolecules from Soil of Desert Fungi

Desert soils are limited in water and nutrients; additionally, they present adverse conditions for common life such as extreme temperatures between day and night or high ultraviolet (UV) radiations (Makhalanyane et al. 2015). In this type of soil, the fertility with respect to the addition of phosphate is limited by the solubility of phosphate (Ameen et al. 2019). This is how species of phosphate-solubilizing fungi (mostly organic acid producers) are mentioned, such as *Aspergillus nidulans*, *A. niger*, *A flavus*, *Penicillium lilacinum*, *P. frequentans*, *Fusarium moniliforme*, and others, have the ability to degrade cellulose and lignin, for example, *Aspergillus niger* and *Humicola fuscoatra* (El-Gindy and Saad 1990). The fungi that have adapted to grow in desert conditions can be classified into earth fungi, fungi

associated with plants, hyphomycetes, yeasts, and microcolonial fungi (Sterflinger et al. 2012).

Among the metabolites produced by desert fungi, we can mention sterigmatocystin, produced by *Emericella nidulans* (imperfect phase of *Aspergillus nidulans*) and *Emericella rugulosa* (imperfect phase of *Aspergillus rugulovalvus*) (Klich et al. 2001). Sterigmatocystin (STC) is a widespread mycotoxin produced by *Aspergillus* fungi, with hepatotoxic and carcinogenic properties (Huţanaşu et al. 2011). *Aspergillus nidulans* is also capable of producing norsolorinic acid, which exhibits antiproliferative activity in MCF-7 cells of human breast adenocarcinoma (Wang et al. 2008). *A. flavus* produces aflatoxins, which are the most toxic and potent natural hepatocarcinogenic compounds known and are the second leading cause of invasive aspergillosis (Hedayati et al. 2007). However, these substances are dependent on the strain and the culture conditions that regulate its metabolome, in biofilm conditions, for example, other strains stand out for the absence of carcinogenic aflatoxins that would allow a greater valorization of *A. flavus* to develop biopesticides. Among the metabolites produced by this species can be mentioned aspergillic acid, beta-cyclopiazonic acid, cyclopiazonic acid, ferrineospergiline, flavacol, and spermadin A (Francis et al. 2020). The peptide-like antibiotic leucinostatin was isolated from *P. lilacinum*, and this compound showed activity against some gram-positive bacteria and a wide range of fungi and showed some inhibitory effect on the EHRLICH subcutaneous solid tumor (Arai et al. 1973). Currently, its enzymes are used, such as amylases, in food and other biotechnological applications because they maintain their activities even under severe pH and temperature conditions (Kashif et al. 2018).

P. frequentans produces pectinases and cellulases that, according to some in vitro and in vivo tests, would be correlated with their antagonistic capacity against some pathogens such as *Cercospora beticola* (El-Fawy et al. 2018) and *Monilinia* spp. (Guijarro et al. 2017), where the probability of risks to human and animal health is considered very low. *F. moniliforme* has a wide capacity for the production of metabolites of scientific interest, from some mycotoxic substances, such as moniliformin, fusaric acid, fusarin C, and fusariocin C, to enzymes such as renin, cellulolytic, and amylolytic enzymes, pectinases, and phenol-degrading enzymes (Brückner et al. 1989). This species is also a contaminant of the corn crop and produces metabolites called fumonisins, which have been shown to be carcinogenic in laboratory rats and cause acute toxicity in domestic animals such as pulmonary edema in pigs and are associated with an incidence of esophageal cancer. in humans (Norred 1993). *H. fuscoatra* is a mycoparasitic fungus, originally isolated as a colonist from sclerotia of *Aspergillus flavus*, and known metabolites include 7-deoxyisterigmatocystin, sterigmatocystin, isosclerone, and decarestrictins A and I, among other triterpenoid glycosides with activity in antifungal assays against *A. flavus* (Joshi et al. 2002). This fungus also produces cellulolytic enzymes capable of improving fermentation processes in the use of agro-industrial waste such as rice straw to obtain other economically important products (Kaur et al. 2015).

On the other hand, there are the desert truffles that are species of hypogeal fungi that establish mycorrhizal associations with diverse plants, many of them endemic.

According to the hymenium, which is the layer formed by the spore-producing elements, some genera can be grouped into *Exothecia* (*Ruhlandiella*, *Sphaerozone*), *Ptychotecia* (*Hydnocystis*, *Genea*, *Balsamia*, *Choiromyces*), and *Estereothecia* (*Tuber*, *Loculotuber*) (Moreno et al. 2014). Desert truffles have nutritional value due to their proteins, carbohydrates, fats, and fibers; they also produce phenols, carotenoids, anthocyanins, ascorbic acid, flavonoids, tannins, glycosides, and ergosterol, which confer very diverse pharmacological activities: immunomodulatory, hepatoprotective, antidepressant, antibacterial, antifungal, antiviral, and antioxidant (Owaid 2018).

1.6.2 Biomolecules from Soil of Agricultural Fungi

Agricultural activity changes and restricts many of the initial soil conditions where the fungal microbiota develops; this alteration is reflected in later disorders on pests and the nutrient cycle (Thiele-Bruhn et al. 2012). In soils dedicated to agriculture, Mycoflora depends on the type and content of organic matter in the soil, with some species such as *Penicillium*, *Trichoderma*, *Aspergillus*, *Fusarium*, and *Mucor* becoming predominant due to its ability to produce a wide variety of extracellular enzymes that mineralize organic matter and complex minerals (Žifčáková et al. 2016). In soils where agriculture is practiced, there are also other species of fungi such as those that establish symbiotic relationships with plants, arbuscular mycorrhizae, which by colonizing the plant root produce biomolecules that stimulate growth and immunity in plants; among them can be mentioned auxins, cytokinins, ethylene and gibberellins (Contreras-Cornejo et al. 2015); in addition, inoculation with arbuscular mycorrhizal fungi can increase the production of secondary metabolites of plants that have medicinal or nutritional potential (Pedone-Bonfim et al. 2015). Many species of fungi also have the ability to remedy toxic metals such as cadmium, copper, mercury, lead, and zinc, accumulating them in their fruiting bodies (Baldrian 2003).

Penicillium oxalicum and *Aspergillus niger*, isolated from soil with excessive application of phosphate fertilizer for decades in China, showed the ability to excrete various organic acids with phosphate-solubilizing activity that, applied as a seed treatment, also increased the fresh mass of corn (Yin et al. 2015). *Trichoderma* spp. could be an important tool for soil management due to its ability to solubilize nutrients and reduce the load of phytopathogenic fungi in the soil, thus promoting a greater generation of crop biomass (Zhang et al. 2018). *Mucor* spp. and *Aspergillus parasiticus*, on the other hand, show antimicrobial activity on gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*), and even on other fungi (*Candida albicans*) that show us the already known potential of fungi from the soil to obtain drug-producing strains (Mensah et al. 2016).

1.6.3 Biomolecules from Soil of Salt Marshes Fungi

Saline soils are widespread throughout the world and represent 7–8% of the Earth's surface (Artiola et al. 2019), and fungi are also found there, particularly in marshes, where they have been found in greater occurrence to *Aspergillus fumigatus*, *Aspergillus niger*, *Cladosporium herbarum and Alternatia alternata*, *Aspergillus terreus*, *Curvularia spicifera and Penicillium notatum*; in moderate occurrence: *Penicillium*, *Fusarium*, *Curvularia*, *Rhizopus*, *Stachybotrys* and *Chaetomium*; and in low occurrence to: *Paecilomyces*, *Cephalosporium*, *Epicoccum*, *Mucor* and *Myrothecium*, all of them with the ability to produce enzymes that degrade cellulose and lignin (Abdel-Hafez et al. 1978), in addition to these halophilic and halo-tolerant fungi, their resistance to high ultraviolet (UV) radiation and extreme pH values (Gunde-Cimerman et al. 2000).

Fungi have also been found in solar salines (Gunde-Cimerman et al. 2000), and many of these species had previously been reported as contaminants in foods preserved with salt (Zalar et al. 2005) and today they have become a source of new metabolites with antibiotic and anticancer properties. Its use is also considered an attractive strategy to meet the needs of contaminated ecosystems (Naranjo-Briceno et al. 2013). Species such as *Debaryomyces hansenii*, *Hortaea werneckii*, and *Wallemia ichthyophaga* have also been reported as fungal strains isolated from hypersaline environments (Gunde-Cimerman et al. 2009). In this group, halophilic and halo-tolerant black yeasts, *Hortaea werneckii*, *Phaeotheca triangularis*, *Aureobasidium pullulans* and *Trimmatostroma salinum*, and others such as *Cladosporium*, *Aspergillus* and *Penicillium*, have become valuable biological resources with potential for biotechnological applications (Chung et al. 2019). As large areas around the world are exposed to salinization, a better understanding of the Extremophilic fungi, their metabolism, and their genes will help us to adapt to changes in the adverse future (Gunde-Cimerman 2009). A promising species is *Aspergillus* that, in addition to growing in salinity conditions (0–6.34 M), stands out from other fungi because some species can grow in temperature ranges (10–50 °C), pH (2–11), as well as degrading and metabolizing polycyclic aromatic hydrocarbons (Atagana 2009). In *Wallemia* spp., a total of approximately 25 metabolites have been isolated and identified among them: wallimidione, walleminone, walleminol, and UCA. Wallimidione is the most toxic of Wallemia's metabolites reported to date. Walleminol and walleminone are sesquiterpene caryophylenes. UCA1064-A and UCA1064-B are metabolites with known antitumor activities (Jančič et al. 2016).

There are different solutes that accumulate in large quantities when the fungus is in a saline environment; thus, the accumulation of polyols in yeasts is common (Brown and Simpson 1972). Also, glycerol has been found in a large number of fungi (Adler et al. 1985) as is the case of yeast *Debaryomyces hansenii* tolerant to salt, which was subjected to saline stress, produced glycerol, and the internal level

of glycerol increased linearly in proportion to the increases in external salinity (Adler et al. 1985); in addition, this species has a high biotechnological potential, particularly in the food industry (Prista et al. 2016), where it contributes directly and indirectly to the flavor of the foods in which they grow by adding a full range of different volatile compounds or altering their composition with their particular proteolytic enzymes (Gori et al. 2012).

The ascomycotic black yeast *Hortaea werneckii* was identified as a potential source of melanin. Melanin has different applications in many fields, such as the cosmetic and pharmaceutical industry. Furthermore, the melanin extracted from this species showed antimicrobial activity against different pathogens such as *Staphylococcus* sp., *Streptococcus pyogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, and *Erwinia carotovora* (Saleh et al. 2018).

Different strains of *Aspergillus pullulans* can produce amylase, proteinase, lipase, cellulase, xylanase, mannanase, transferases, pullulan, siderophore, and unicellular protein, and the genes encoding proteinase, lipase, cellulase, xylanase, and siderophore have been cloned and characterized (Chi et al. 2009). In *Aspergillus versicolor*, four xanthones, eight anthraquinones, and five alkaloids were isolated, including a new xanthone, oxisterigmatocystin, and a new alkaloid, aspergillusin (Wu et al. 2018).

Special mention should be made of the fungi from which pigments are extracted, among which the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Paecilomyces*, *Epicoccum*, *Lecanicillium*, *Monascus*, and *Trichoderma*. Pigments commonly produced by fungi are carotenoids, quinones, flavins, ankaflavin, anthraquinone, naphthoquinone, and carotene. Pigments produced by soil fungi have a valuable use in medicines, textile dyes, food dyes, and cosmetics (Kumar et al. 2019a, b).

1.7 Biomolecules from Coprophilous Fungi

The conformation of dung is very complex due to the number of compounds it contains; some reports mention that the carbon and nitrogen ratio is the most important likewise organic matter content (Frank et al. 2017). On the other hand, volatile organic compounds (VOCs) have a special influence on the diversity of living things, such as coleoptera insects, and there are unique patterns in composition and ubiquitous components such as *p*-cresol (Frank et al. 2018). Dung of herbivores animal represents a diverse and nutrient-rich ecosystem and thus supports growth and development of different types of saprotrophic microbial communities (Jain et al. 2020). Within these microbial communities, common inhabitants are the dung fungi, also known as coprophilous fungi of fimicolous fungi.

1.7.1 Classification of Coprophilous Fungi

Coprophilous fungi are an artificial taxonomic group encompassing fungi with physiological and ecological adaptations at different levels, adapted to grow on the dung of a diverse, mainly herbivorous animals (Doveri 2004). Studies reported the confirmed diversity of coprophilous fungi to over 225 species until 2017 (Calaça et al. 2017). Coprophilous fungi are a taxonomically broad fungal group classified in two denominations, in accordance with its life history and substrate preference, namely, the *true dung fungi* and *the fimicolous fungi* (Doveri 2004). The true dung fungi are all fungi whose spores must pass through the animal gut as a decisive step to initiate germination of spores and subsequent growth on dung after defecation. The fimicolous fungi on the other hand do not require a passage through the animal digestion system to grow in dung. For fimicolous fungi, dung is just one of the possible suitable substrates where it can complete its life cycle (Abdel-Azeem and Salem 2015; Simões Calaça et al. 2020). Despite being a taxonomically diverse and not necessarily related group of fungi, authors confirmed several genera and species as being commonly present in the dung of various herbivores (Table 1.10).

Table 1.10 Some common genera found in coprophilous fungi

Authors	Richardson (2001)	Melo et al. (2016)	Jain et al. (2020)
Genera			
Ascobolus	+	–	–
Cheilymenia	–	–	+
Coniocheta	+	–	–
Coprinellus	–	+	–
Coprinopsis	–	+	–
Coprinus	+	–	+
Cyathusstercoreus	–	–	+
Deconica	–	+	–
Iodophanus	+	–	–
Lasiobolus	+	–	–
Paneolus	–	+	+
Pilobolus	–	–	+
Podospora	+	–	–
Poroniaerici	–	–	+
Psylocybe	–	+	+
Saccobolus	+	–	–
Schizothecium	+	–	–
Sporomiella	+	–	–
Thelebolus	+	–	–

1.7.2 Biomolecules from Coprophilous Fungi

The importance of coprophilous fungi may be used also as an indicator of habitat diversity (Richardson 2001). Overall, coprophilous fungi play a significant role in the decomposition of organic matter, nutrient cycling, and maintenance of the ecological balance on the Earth (Amandeep et al. 2015). Coprophilous fungi have a high potential for the production of various bioactive compounds. With the rich and diverse substrates, and high population density of microbial communities, there is a need for the production of various biomolecules that ensure the survival and use of available nutrients. Among common biomolecules produced by coprophilous fungi are antibiotics, growth stimulators, biopesticides, and various oxidative enzymes that have a potential to (partially) decompose various xenobiotics (Jain et al. 2020).

1.7.2.1 Biomolecules from Coprophilous Fungi in the Pharmaceutical Industry

An example of a true coprophilous fungus with high antibiotic resistance is *Cryptococcus neoformans* var. *neoformans* which was repeatedly isolated from feces of pigeons in recreational parks in Perú, with all isolates showing an unidentified biomolecules-based resistance to fluconazole and itraconazole (Huamán et al. 2018). The diversity of dung fungi offers accessible systems for dissecting the function of antibiotics and for exploring fungal genomes for new antibiotics, their potential for antibiotic discovery is evidenced by a high frequency of antifungal antibiotics and bioactive secondary metabolites from limited prior efforts and from mapping biosynthetic pathways in the genomes of the coprophilous fungi *Podospora anserina* and *Sordaria macrospora* (Bills et al. 2013). On the other hand, Krug et al. (2004) consider the myxobacteria within the taxon of fungi coprophilous because they have cells aggregating into fruiting bodies.

They present high biotechnological potential, as is associated with the production capacity of crocacin, which constitutes a class of metabolites of the dipeptide type derived from glycine and a 6-aminoexadienic or 6-aminoexenoic acid, which presents moderate to high antibiotic potential inhibiting the growth of gram-positive bacteria and some species of fungi (Oliveira et al. 2008). The most commonly reported biological activity associated with these biomolecules so far has been antifungal activity, for example, *Stilbella fimetaria* produced peptaibol antibiotics (antiamoebins) in rabbit dung pellets at concentrations well above concentrations needed for inhibiting most competing coprophilous fungi and other fungi and bacteria. The fungus also coproduced the diterpene myrocin B, but the levels measured in colonized dung were below the predicted antifungal thresholds (Lehr et al. 2006). *Sordaria araneosa* produce a biomolecule called Sordarin a diterpene glycoside, and its aglycone, the effects of sordarins on fungal protein biosynthesis via interaction of elongation factor 2 at the ribosomal P-protein have been reviewed extensively (Dominguez and Martín 2005).

1.7.2.2 Other Biomolecules of Coprophilous Fungi of Industrial Importance

Several bioactive compounds from coprophilous fungi were already brought to biotechnological use, for example, metabolites from *Chaetomium* spp. were used as a biocontrol of various plant pathogens, and the further development is directed of producing an efficient and eco-friendly nanomaterial-based carrier to be subsequently loaded with efficient biomolecules from *Chaetomium*. Functional use of such applications is expected in various biological and agricultural applications (Abdel-Azeem 2020). The development of applications with bioactive molecules use only is highly appreciated in *Chaetomium*, as releasing of spores or whole mycelia is discouraged due to its high inhalant allergen potential, already recorded or an indoor environment (Wang et al. 2016).

Conocybe is a fimicolous coprophilous genus with high biomolecules production potential. Opposite to *Chaetomium*, *Conocybe* spp. form macroscopic, easy to spot and record fruiting bodies. Thus, its wide distribution and diversity among different regions, dung types, seasons, and growing habits are well recorded (Amandeep et al. 2015). *Conocybe* spp. produces a range of biomolecules, such as cyclic peptides, rare biomolecules that were found in several poisonous species as *Conocybe*, 22 distinct cyclopeptide toxins have been identified in this fungi that are chemically classified into four major groups: amatoxins (bicyclic octapeptides), phallotoxins (bicyclic heptapeptides), virotoxins (monocyclic heptapeptides), and the nontoxic cycloamanides, all having several known uses in biotechnology applications (Pomilio et al. 2006). Another fimicolous fungus *Agrocybe pediades* can also grow on cattle dung as its main substrate (Calaça et al. 2020).

This species was confirmed to contain flavonoids, ascorbic acid, phenol, β-carotene, lycopene, and other components with antioxidant potential, proven radical scavenging activity (EC_{50} value of 1.03 mg/mL), and a total antioxidant capacity of 11.71 μg ascorbic acid equivalents (Acharya et al. 2017). *Agrocybe pediades* may be a good source of bioactive molecules and suggest their purification approach as helpful in authentication of the biomolecules and quality control approach for raw fungal material testing. Some other bioactive biomolecules reported from coprophilous fungi include preussomerins, flutimide, australifungin, sordarins, zaragozic acid B, antiamoebins, talaroderxines, coniochaetones, communiols, curvicollides, podosporins, bombardolides, terezines, sporminarins, and petriellins (Bills et al. 2013).

1.8 Biomolecules from Marine Fungi

Marine fungi are a well-defined taxonomic group of microorganisms, ecologically divided into two groups, according to their ability to grow in marine environments: obligate marine fungi and optional marine fungi (Agrawal et al. 2018). This capacity could be explained by the possible evolutionary origin of the superior marine

fungi, adapting to live in different tissues of organisms or other marine sources, such as sponges, algae, sediments, tunicates, coral mollusks, algae, fish, wood, and marine macrophyte debris (Bugni and Ireland 2004). These symbiotic associations position fungi in marine ecosystems as the main decomposers of woody and herbaceous substrates in addition to being important pathogens of marine plants and animals (Hyde et al. 1998). It should be mentioned that although we know well how marine fungi can produce a set of diverse and complex metabolites, their synthesis depends on certain conditions (ecological, physical, and biological) (Pejin and Maja 2017).

1.8.1 Diversity of Marine Fungi

The diversity of marine fungi is determined by two important factors: the geographical distribution and the temperature of the sea (Jones 2000). Sea temperature is considered the most important and determining factor in the geographical distribution of marine fungi (Booth and Kenkel 1986). Likewise, psychrotolerant marine fungi have been isolated from antarctic, arctic, and desert marine environments associated with sea urchins (*Cryptococcus laurentii*) and a macralga *Palmaria decipiens* from *Geomyces* sp. (Murray et al. 2013). Likewise, deep-sea fungi have been reported, and where environmental characteristics are extreme, still, a wide diversity of fungi in these environments is indicated (Mahé et al. 2013). The discovery of marine fungi has been slow in coming. By 1958, only 100 species had been described (Jones 2011). Gradually, with further research, this number has increased to about 530 obligate marine fungi, divided into 321 genera that include 424 *Ascomycota* (251 genera), 94 Mitosporic fungi (61 genera), and 12 *Basidiomycota* (9 genera) (Jones et al. 2009). However, it was in tropical waters and mangrove ecosystems that the number increased to 290 taxa, including 537 genera (Jones 2011). It is worth mentioning that the number of fungi has been estimated in 1500 species (Jones and Mitchell 1996); however, many of them have been inadequately described or are synonyms of existing taxa (Jones 2011).

1.8.2 Biomolecules of Marine Fungi in the Pharmaceutical Industry

Various marine fungi biomolecules are used for the development of new pharmaceutical, nutraceutical, and cosmeceutical products. However, their potential has been partially evaluated (Agrawal et al. 2018) because only a small amount could be isolated and used as cosmetic ingredients (Corinaldesi et al. 2017). This leaves an incredible potential in marine fungi still to be discovered and studied, mainly when it is known that marine fungi have developed specific metabolic pathways that are

not present in terrestrial fungi (Abdel-Lateff 2008). By 2011, more than 1000 marine fungal compounds had been isolated, including alkaloids, terpenoids, lipids, polyethylene, and non-ribosomal peptides (NRP) (Rateb and Ebel 2011). Thus, *Cephalosporium* sp., a marine fungus that produces cephalosporin C, has been one of the most important discoveries so far (secondary metabolite of a non-ribosomal peptide) (Rateb and Ebel 2011). Marine fungi are also an important source of metabolites with photoprotective capacity, among them micosporins produced mainly by *Phaeotheca triangularis*, *Trimmatostroma salinum*, *Hortaea werneckii*, *Aureobasidium pullulans* and *Cryptococcus liquefaciens*, specifically micosporin-glutaminol-glucoside, and micosporin-glutamicol-glucoside (Kogej et al. 2006). Among metabolites derived from marine fungi that serve as UV filters are micosporine-like amino acids such as scytonemine (Pallela et al. 2010), carotenoids coming from the genera *Rhodotorula*, *Phaffia*, and *Xanthophyllomyces* (Rastogi et al. 2010), and benzodiazepine alkaloids (circundatin I, C and G) found in a marine fungus of the genus *Exophiala* with high UV detection levels (Zhang et al. 2008).

Also, hydroquinone derivatives with significant antioxidant activity, produced by *Acremonium* sp., are used today for their preventive effect on aging (Abdel-Lateff et al. 2002). On the other hand, some species of the genus *Myrothecium* (3-amino-5-ethylcyclopentenones, microtenones A and B) have the capacity to produce tyrosinase inhibitors (Li et al. 2005), a glycoprotein that catalyzes the first two reactions of melanin synthesis, which causes darkening of the skin (Fais et al. 2009). In addition, 6-[(*E*)-Hept-1-enyl]-α-pyrone (α-Pyrone Derivative) from *Botrytis* sp. (Zhang et al. 2007), Homothallin-II from *T. viride* H1–7 (Tsuchiya et al. 2008), 1β,5α,6α,14-tetraacetoxy-9α-benzoyloxy-7β*H*-eudesman-2β11-diol and 4α,5α-diacetoxy-9α-benzoyloxy-7β*H*-eudesman-1β,2β,11,14-tetraol from *Pestalotiopsis* sp. Z233 (Wu et al. 2013) have been reported.

Studies about the activity of marine fungal extracts have also been reported, such as *Trichoderma viride* 4MCMC1 and *Penicillium citrinum* 4MCMC4, which demonstrated antifungal efficiency on *Candida albicans* ATCC 1023 with maximum inhibition halos of 3.08 mm and 2.52 mm, respectively. Within the group of ascomycetes with antimicrobial capacity, there is *Amycolatopsis alba* that produces Pyridinium, *Salinospora arenicola* whose cyclomarazins have inhibitory properties against *Entrococcus faecium* and *Staphylococcus aureus* (Jayaprakashvel 2012). Regarding the development within the pharmaceutical industry, marine yeasts such as *Yarrowia* sp. and *Candida* sp. are recognized as capable of synthesizing diverse pharmaceutical products, although, unlike terrestrial yeasts, these are still in their initial stages for application in the pharmaceutical industry (Abdelrahman et al. 2014), and a strain of *Rhodotorula glutinis* YS-185 is capable of producing astaxanthin (He et al. 2011), *Aureobasidium pullulans* producing siderophores (Wang et al. 2009), *Candida membranifaciens* subsp. *flavinogenie* synthesizing riboflavin (Wang et al. 2008), and *Aureobasidium* sp. and *Pichia* sp. producing insulin (Chi et al. 2011).

1.8.3 Other Biomolecules of Marine Fungi of Industrial Importance

The metabolic versatility of marine fungi gives them the ability to produce diverse biomolecules, including xylanases, lignin peroxidases, manganese peroxidases, and laccases (Parte et al. 2017). Among the main ones are alkaline proteases (*Scopulariopsis* spp.) used in the formulation of detergents (Niyonzima and More 2014), amylase, cellulase, chitinase, gelatinase, lipases (*Penicillium*, *Aspergillus*, *Scopulariopsis*, *Cephalosporium*, *Humicola*, *Gymnoascus*, *Endomysis*, *Zygorhynchus*, *Trichoderma*, *Zalerion*, *Pleospora*, *Chaetomium* and Phoma, *Botryphialophora*) involved in mineralization processes (Smitha et al. 2014), Tannase (acyl hydrolase tannin) (*Aspergillus awamori* BTMFW032) useful in the food industry as an antioxidant, and catechin gallate applied in the manufacture of instant tea, coffee flavored drinks, juice clarification, improves the taste of alcoholic beverages either wine or beer (Beena 2010). Enzymes such as cellulases, xylanases, and pectinases are isolated from *Alternaria alternata*, *Aspergillus terreus*, *Cladosporium cladosporioides*, *Emericella nidulans*, *Fusarium solani*, and *Cochliobolus australiensis*; their use is not only limited to the food industry but, on the contrary, they are also of great importance in the textile, paper, and chemical industries (Moubasher et al. 2016). Meanwhile, marine fungi isolated from Arctic, Antarctic, and desert environments have been found to be notable lipolytic (lipase) producers of the genus *Basidiomycota* (Murray et al. 2013).

Some genera of marine yeasts have been reported as *Rhodotorula*, *Rhodosporidium*, *Candida*, *Debaryomyces*, *Cryptococcus*, *Yarrowia*, *Aureobasidium*, *Metschnikowia*, *Torulopsis*, *Pichia*, *Kluyveromyces*, *Saccharomyces*, *Williopsis*, *Pseudozyma*, *Hansenula*, *Trichosporon*, *Filobasidium*, and *Leucosporidium* (Chi et al. 2016). Some marine yeasts have been identified as producers of surfactants. Yasan, an emulsifier isolated from *Y. lipolytica* IMUFRJ50682 (Amaral et al. 2006), in the same way, has been reported as a producer of citric acid (Liu et al. 2013) and *Williopsis saturnus*, a producer of xylitol (Kamat et al. 2013). With respect to ethanol production, *Pichia salicaria* (Kathiresan et al. 2011) and *Candida albicans* (Senthilraja et al. 2011) have been reported as ethanol producers, producing 12.3 ± 0.8 g/l and 47.3 ± 3.1 g/l of bioethanol, respectively. Likewise, terrestrial and marine strains of *Saccharomyces cerevisiae* showed that the marine strain of *S. cerevisiae* obtained the highest bioethanol production with 25.1 g/l higher than the terrestrial strain (Saravanakumar et al. 2013).

1.9 Biomolecules from Yeast and Yeast-Like Fungi

Bioactive compounds are naturally occurring in small quantities in plants, microorganisms, and food products (Kris-Etherton et al. 2002). Most bioactive compounds include secondary metabolites, such as antibiotics, mycotoxins, alkaloids, plant

growth factors, and phenolic compounds (Nigam 2009). Secondary metabolites are widely used in industrial products, such as pharmaceuticals; microorganisms are one of the widely employed strategies used as a host for the production of secondary metabolites, especially yeast (Siddiqui et al. 2012). Yeast has been used to synthesize various biomolecules that have various important biological roles such as defense against biotic and abiotic stresses, antimicrobial activity, and signal transduction (Rahmat and Kang 2020). Yeast is a single-celled eukaryotic microorganism that belongs to the fungal kingdom. Yeast species represented a percentage of all identified fungal members (Kurtzman and Piškur 2006); approximately 1500 yeast species have been identified until now (Hoffman et al. 2015). Some yeast species, likes *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, is used as a model for eukaryotic organisms and used for the construction of powerful standard biotechnological techniques (Brückner et al. 2009). Secondary metabolites can be produced or synthesized naturally from yeast cells (native) or artificially from other organisms (non-native) such as plant and bacteria. Yeast can produce many biomolecular compounds, such as terpenoids, alkaloids, flavonoids, non-ribosomal peptides, and polyketides (Rahmat and Kang 2020).

1.9.1 Secondary Metabolite Production in Yeast

1.9.1.1 Alkaloids

Alkaloids are a class of secondary metabolites that include nitrogen rings as part of their structure and exhibit strong bioactivity (Siddiqui et al. 2012). There are many drugs belonging to alkaloids, including noscapine (antitussive agent), vincristine (anticancer agents), codeine (analgesic), and sedative. The earliest example of engineering yeast to express plant enzymes to synthesize alkaloid molecules showed the production of cathenamine, an indole alkaloid that serves as an intermediate for the biosynthesis of other alkaloids (Geerlings et al. 2001). In this two-enzyme system was strictosidine synthase (STR) (Brown et al. 2015). Strictosidine is obtained from *Catharanthus roseus* where about 14 genes of *Catharanthus roseus* were expressed to produce strictosidine in the engineered yeast. Strictosidine serves as an intermediate for the biosynthesis of terpenoid indole alkaloids (TIAs), which include vincristine (anticancer) and ajmalicine (anti- hypertensive) (Nam et al. 2007). Benzylisoquinoline alkaloid (BIA) biosynthetic pathway constructed in yeast can be divided into two parts: the production of the key branchpoint metabolite (S)-reticuline and the downstream modification (Trenchard et al. 2015). Reticuline is an important intermediate and precursor to many BIAs of medicinal interest including the powerful analgesics codeine and morphine (Hawkins and Smolke 2008). *S. cerevisiae* has been engineered for the synthesis of (S)-reticuline, which is an important intermediate in the biosynthetic pathway of various alkaloids, including the baine and morphine, which are known as opioids (Trenchard et al. 2015).

1.9.1.2 Polyketides

Polyketides comprise a huge group of secondary metabolites assembled from acetyl-CoA, malonyl-CoA, or other CoA-activated subunits (Siddiqui et al. 2012). Polyketides have many biological compounds, encompassing various drugs, such as antitumor and antibiotic drugs. Antibiotics e.g. such as tetracycline and erythromycin. The polyketides chain is synthesized by fungi and bacteria using activity enzymes called polyketide synthases (PKS). The functional type III PKS enzymes in yeast are the expression of the production of flavonoids and stilbenes. Several efforts have been taken up to synthesize polyketide in the engineered *S. cerevisiae*, such as the production of 6-methylsalicylic acid (6-MSA) (Kealey et al. 1998), methylmalonyl-CoA (Mutka et al. 2006), and simvastatin (Bond and Tang 2019). Simvastatin is a semisynthetic drug that was produced in engineered yeast by entering six heterologous biosynthetic genes and the acyl-donor dimethylbutyryl-*S*-methyl mercaptopropionate (DMB-SMMP) into the *S. cerevisiae* genome. Simvastatin was used to decrease the cholesterol level and has a large market demand as the world's top-selling statins. Various strategies were applied to increase the production yield (Bond and Tang 2019). Eichenberger et al. (2017) reported the de novo production of dihydrochalcones (DHCs) by expressing the ScTSC13 gene in the engineered *S. cerevisiae*. After the production of phloretin, various derivatives of DHCs, such as phlorizin (antidiabetic), nothofagin (antioxidant), and naringin dihydrochalcone (sweetener compound), were successfully produced by a prolongation pathway including glycosylation or methylation using enzymes (Eichenberger et al. 2017).

1.9.1.3 Non-ribosomal Peptides

Nonribosomal peptides (NRPs) are a type of secondary metabolites that are synthesized by multidomain mega-enzymes named nonribosomal peptide synthetases (NRPSs), without the need for the cell ribosomal machinery and messenger RNAs (Evans et al. 2011). Microorganisms such as bacteria and fungi are naturally synthesized NRPs by the symbionts of higher eukaryotes naturally synthesized NRPs (Dai and Ding 2012). NRPs include a wide range of bioactivities and pharmacological properties. For example, NRP natural products such as antibiotics (e.g., penicillin, cephalosporin, actinomycin, and vancomycin), cytotoxics (e.g., bleomycin), and immunosuppressants (e.g., cyclosporines) have found beneficial applications in human medicine (Hoffmeister and Keller 2007). NRPs also synthesize some pigments such as indigoidine and some harmful toxins such as HC-toxin and AM-toxin phytotoxins (Desriac et al. 2013). Gurmarin is an example of nonribosomal peptide production in engineered yeast which used as a pharmacological agent in sweet-taste transduction studies due to its ability to selectively impede the neural response to sweet substances in rats and mice (Rahmat and Kang 2020). Gurmarin was produced from the *Pichia pastoris*, a methylotrophic yeast (Sigoillot et al. 2012).

1.9.1.4 Phenolics

Phenolic compounds are widely ubiquitously distributed phytochemicals found in plant food. They are secondary metabolites characterized as containing at least one hydroxylated aromatic ring and that are synthesized through the shikimic acid and phenylpropanoid pathways (De la Rosa et al. 2019). Phenolic compounds are important in cell protection ascribed to their capacity of scavenging, transferring electrons to free radicals, activating antioxidant enzymes, and inhibiting oxidase enzymes (Dumitriu et al. 2015) also anti-inflammatory, analgesic, gastroprotective (Lajili et al. 2016), and antimicrobial actions (Cetin-Karaca and Newman 2015). Besides, those compounds have protective action against oxidative stress, which has been suggested as the root cause of aging and human disorders, such as diabetes, arteriosclerosis, cancer, and neurodegenerative diseases (Asadi et al. 2010). Many valuable flavonoids and stilbenoid compounds are found in a limited number of plant species. *Saccharomyces cerevisiae* is well known as a host cell to support the biosynthesis of phenolic compounds. Although yeast does not naturally produce phenylpropanoid phenolics, its central metabolism provides the amino acid precursors that are necessary for an introduced phenolic biosynthesis pathway (Lee et al. 2016).

1.9.1.5 Flavonoids

Flavonoids are secondary metabolites naturally synthesized by plants and microorganisms, such as (fungi) from aromatic amino acids L-phenylalanine and L-tyrosine (Rodriguez et al. 2017). Flavonoids play an important role in the physiology of plants; their main functions comprise UV protection, reduction of oxidative damage in cells, and antibacterial effect (Cushnie and Lamb 2011). Research on human cells showed positive properties of cancer and cardiovascular diseases (Bulzomi et al. 2012). Moreover, some flavonoids such as naringenin, liquiritigenin, and fisetin have neuroprotective, antioxidant properties, antitumor, and antidiabetic activity (Choi 2012). For increasing the supply of flavonoid production, it can be an advantage to engage genetically engineered microbial cell factories such as *S. cerevisiae*. Naringenin is a key intermediate of flavonoids from glucose using an engineered yeast strain which is the first study for flavonoid production in engineered *S. cerevisiae* was reported by Koopman et al. (2012). Moreover, naringenin to engineered cells enable the production of other flavonoids, such as genistein, kaempferol, and quercetin (Trantas et al. 2009).

1.9.1.6 Terpenoids

Terpenoids are the largest class of natural products, which can be resources for pharmaceuticals and with diverse biological functions. Terpenoids were showed a wide range of biological activities including antibacterial, antifungal, antiviral,

antiparasitic, anti-inflammatory, antiallergenic, antihyperglycemic, antispasmodic, antitumoral, and immunomodulatory activities (Wagner and Elmadfa 2003). Terpenoids are manufactured in yeast by engineering the pertinent metabolic pathways. The synthesis of artemisinin precursor of antimalarial (Westfall et al. 2012) is considered as one of the most successful yeast (*Saccharomyces cerevisiae*) synthetic biology and metabolic engineering methods for the production of terpenoids. The first documented on the medicinally useful terpenoid production in yeast was the synthesis of amorphadiene in *S. cerevisiae*. Generally, the strategies that have been used to engineer the biosynthetic pathway of terpenoids in yeast are as follows: overexpressing UPC2-1 and genes involved in the mevalonate pathway (ERG10, ERG13, HMGR, ERG12, ERG8, IDI1, and ERG20) (Siddiqui et al. 2012) to increase the pathway fluxes; inserting HXT1p (glucose inducible promoter), CTR3p (copper repressible promoter), or MET3p (methionine repressible promoter) (Paddon et al. 2013) to substitute the original promoter to downregulate the expression of ERG9 gene; enhancing carotenoid synthesis and mevalonate pathway fluxes by knocking out several regulators (YPL062W, YIL064W, and ROX1) obtained from the YKO Collection (Ozaydin et al. 2012).

1.10 Biomolecules from Wood Degrading Fungi

The wood-decaying fungi use both enzymatic and non-enzymatic systems for the degradation and complete decomposition of wood. In the wood decay process, the wood turns discolored and loses weight, strength, and density by the action of fungi. The wood decaying involves three broader groups, namely, white-rot, brown-rot, and soft-rot fungi (Schwarze 2007). Basidiomycetous white-rot and brown-rot fungi are the most efficient degraders of wood among microorganisms in the environment. White-rot fungi attack all components of wood: cellulose, hemicellulose, and lignin (Goodell et al. 1997). Brown-rot fungi utilize predominantly hemicellulose and cellulose in wood, leaving behind a chemically modified lignin residue. White-rot fungi colonize both hardwoods and softwoods, although they primarily attack hardwood, while brown-rot fungi attack softwood (Messner et al. 2003). Soft-rot-type decay is caused by ascomycetes and mitosporic fungi (Deuteromycetes or Fungi imperfecti). They decompose wood carbohydrates, but some can partially degrade lignin (Kluczek-Turpeinen et al. 2003).

1.10.1 White-Rot Fungi

White-rot and litter-decomposing basidiomycetous fungi are the only microorganisms that are able to efficiently degrade all the components of plant cell walls, both carbohydrates and lignin (Kuhad et al. 1997). Degradation of lignin is more efficient than in the case of brown-rot and soft-rot fungi because they possess a unique ability

to complete mineralization to CO_2. Lignin consumption is mainly accomplished by laccases, manganese-dependent peroxidases (MnP), lignin peroxidases (LiP), and versatile peroxidases (VP) that are the major groups of ligninolytic enzymes produced by the white-rot fungi (Kracher and Ludwig 2016). Based on their main extracellular ligninolytic enzymes, white-rot fungi can be classified into three or four groups: LiP and MnP group (e.g., *Phanerochaete chrysosporium*, *Phlebia radiata*), MnP-laccase group (e.g., *Ceriporiopsis subvermispora*), LiP-laccase group (e.g., *Phlebiaochraceofulva*), and a fourth, the laccase group (*Pycnoporus cinnabarinus*) (Hatakka 1994).

The MnP-laccase-producing fungi are the most common group (Hatakka 2001). They cause two main white-rot types: simultaneous or nonselective and selective white-rot. In selective white-rot lignin and hemicellulose are lost preferentially and in nonselective white-rot, cellulose, hemicellulose, and lignin are degraded more or less simultaneously (Messner et al. 2003). Species of *Phanerochaete chrysosporium* and *Trametes versicolor* are typical nonselective rot-type fungi (Hatakka 2001). But *Pleurotus ostreatus* (Martinez et al. 1994), *Pycnoporus cinnabarinus* (Eggert et al. 1996), *Ceriporiopsis subvermispora* (Hakala et al. 2004), *Phlebia radiata*, *Phlebia tremellosa* (syn. *Merulius tremellosus*) (Fackler et al. 2006), and *Physisporinusrivulosus* (Hildén et al. 2007) represent the selective degraders of wood that remove lignin preferentially to cellulose.

1.10.2 Brown-Rot Fungi

Brown-rot fungi preferentially attack dead coniferous wood, timber, and wooden structures in which they cause destructive types of decay and rapid degradation of cellulose (Goodell 2003). The brown-rot fungi generally reduce the strength of wood up to 75% by decomposing the cell wall polymers such as cellulose and hemicellulose leaving behind the lignin (Yelle et al. 2008). Brown-rot fungi must first penetrate hemicelluloses to access cellulose because cellulose microfibrils are enveloped by hemicellulose. They remove hemicellulosic xylose and mannose before cellulosic glucose. Hemicellulose hydrolysis may provide fungal enzymes with substrates for H_2O_2 production which is necessary for the attack on lignocellulose by brown-rot fungi (Green and Highley 1997). Models for the study of brown-rot fungi are *Gloephyllum trabeum*, *Coniophora puteana*, and *Postia placenta* (Arantes and Goodell 2014). The brown-rot fungi dries out, making wood into powder when crushed, and it is characterized by reddish-brown color and dry, crumbly, and brittle consistency.

Brown rot is often referred to as “dry rot.” *Poria incrassate* is one of the waters conducting brown-rot fungi having specific rhizomorphs based on root-like water-conducting tubes to transport water from the soil to the wood and can be decayed by the fungus (Martínez et al. 2005). Once the brown-rot fungus infected, it can rapidly multiply from side-to-side building and destroying large areas of floor covering and walls in 1 or 2 years. Examples of such wood-decaying brown-rot fungi include

Gloeophyllum trabeum, *Fomitopsislilacino-gilva*, *Laetiporus portentosus*, *Postia placenta*, and *Serpula lacrymans* (Wong 2009).

1.10.3 Soft-Rot Fungi

Soft-rot fungi ascomycetes are usually thought to degrade mainly carbohydrates in soil, forest litter, and compost, but they may also degrade lignin in these environments. Soft-rot fungi otherwise referred to as micro fungi were characterized by cavity formation in the secondary walls of the wood cells (Tuomela et al. 2000). Thus, some ascomycetes/deuteromycetes ("molds") were found to be able to mineralize grass lignins, for example, *Penicillium chrysogenum*, *Fusarium oxysporum*, and *F. solani* (Rodriguez et al. 1996). Generally, soft-rot fungi utilize cellulose and hemicellulose. Soft-rot fungi degrade wood at a slower rate compared to brown-rot fungi and white-rot fungi. Some of these fungi are common decomposers of cellulose in soil, and they are the least specialized wood-decaying fungi. Most of soft-rot fungi belong to genera *Aspergillus* and *Neurospora* (Woiciechowski et al. 2013).

1.10.4 Enzymes Involved in Lignocellulose Degradation

Fungi have two types of degradation systems: intracellular, together with the outer cell envelope layer, and extracellular, important for polysaccharide degradation. Furthermore, the extracellular enzymatic system includes two types of enzymes: hydrolytic, responsible for polysaccharide degradation; and oxidative, which degrade lignin and open phenyl rings. Extracellular enzymes such as cellulases, hemicellulases, MnP (Manganese peroxidase), LiP (Lignin Peroxidase), and Lac (Laccase). These enzymes can be used in the management of environmental pollutants, such as textile effluents, pulp effluents, organochloride agrochemicals, and crude oil residues (Mtui and Nakamura 2004; Kour et al. 2019a).

1.10.4.1 Cellulases

Cellulase hydrolyzes the glycoside bond present between the glucose residues in the organic polymer cellulose. Cellulose can be hydrolyzed by β-1,4-endoglucanases, exoglucanases or 1,4-β-cellobiosidase, and β-glucosidase (Vlasenko et al. 2010; Kour et al. 2019b). Production of the cellulolytic enzyme from aerobic fungi is widespread; among them, species of *Aspergillus*, *Trichoderma*, *Penicillium*, and *Sclerotium* are found as highly cellulolytic and are mainly considered for commercial exploitation (Milala et al. 2005). Immanuel et al. (2009) reported cellulase production by *Aspergillus niger* and *Aspergillus fumigates* and optimized the parameters including pH, inoculums size, temperature, presence of inducers, and so on.

Trichoderma reesei is identified as the efficient cellulase producer by many researchers to degrade cellulose (Ravikumar et al. 2014). In this study, the cellulose produced by the *Aspergillus* sp. was used for the enzymatic saccharification of lignocellulosic agro-waste (Rana and Kaur 2012).

Sohail et al. (2009) investigated the production of cellulases from *A. niger* in an attempt to obtain a sufficient amount of β-glucosidase. Many other ascomycetous fungi such as *Aspergillus nidulans*, *A. fumigatus* and *A. oryzae*, *Magnaporthe grisea*, *Neurospora crassa*, and *Fusarium gramineum* have a higher number of cellulases (Hatakka and Hammel 2010). Similarly, *T. harzianum* and *T. viride* are also used as naturally producing sources of cellulases like *A. niger* (Menedez et al. 2015). One of the most extensively studied aerobic fungi is T. *reesei* which is capable of hydrolyzing native cellulose (Reczey et al. 1996). *T. reesei* possesses two genes encoding for exoglucanase, eight for endoglucanases, and seven for glucosidases (Sukumaran et al. 2005). Cellulase production from *Humicola species* (*H. grisea* and *H. insolens*) is effective under mild alkaline conditions and at elevated temperatures. So, they are mostly used as additives in detergents and washing powders (Uhlig 1998). Two strains of Penicillium were identified from subtropical soils with potentials for the production of cellulase (Picart et al. 2007); *Chaetomium thermophilum*, *Sporotrichum thermophile*, *Talaromyces emersonii*, and *Thermoascus aurantiacus* grew well and decomposed cellulose very rapidly producing thermostable cellulases (Li et al. 2011).

1.10.4.2 Hemicellulases

Hemicellulases are divided according to their action on distinct substrates into two types of enzymes involved in hemicellulose degradation: endo-1,4-β-xylanase and exo-1,4-β-xylosidase; alternative names: xylan β-1,4-xylosidase, 1,4-β-D-xylanxylohydrolase, β-xylosidase, or xylobiase. Hemicellulases such as xylanase hydrolyzes the xylan and are extensively studied and applied on an industrial scale with higher pulp brightness resulting in a lower chemical input (Saha 2003). The chief xylanase producers from fungal genera include *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Pichia* (Kavya and Padmavathi 2009). White-rot fungi have been reported to synthesize extracellular xylanase which can act on a broad range of hemicellulose materials such as the following: *Coriolus versicolor* synthesize mixture of xylanolytic enzyme and *Phanerochaete chrysosporium* synthesize α-glucuronidase in large amounts (El-Nasser et al. 1997). A comparative study of Milagres and Sales (2001) showed that the brown-rot species *Wolfiporia cocos* produced a higher level of xylanases in comparison to the white-rot fungus *Poriamedulapanis*. In a recent study, cold-active xylanase was isolated from a marine fungus, *Cladosporium* sp. (Del-Cid et al. 2014). In addition, the xylanase and endo-xylanase production has been widely studied in fungi such as *Penicillium thomii*, *P. pinophilum*, *A. niger*, and *Ceratocystis paradoxa* (Kantharaj et al. 2017). Among the mesophilic fungi, *Trichoderma* and *Aspergillus* are the two genera that are preeminentin xylanase production (Shah and Madamwar 2005). Xylanase enzyme reported from

various species of thermophilic fungi are reported which include *Thermoascus aurantiacus*, *Thermomyces lanuginosus*, *Talaromyces emersonii*, *Talaromyces byssochlamydoides*, *Paecilomyces variotii*, *Melanocarpus albomyces*, *Humicola grisea*, *Humicola lanuginosa*, *Humicola insolens*, and *Chaetomium thermophile* (Li et al. 2011).

1.10.4.3 Laccase

Laccase is a copper-containing oxidase that utilizes molecular oxygen as an oxidant and also oxidizes phenolic rings to phenoxy radicals (Baldrian 2006). Laccase can also oxidize nonphenolic substrates in the presence of certain auxiliary substrates 94. Laccase can cleave Cα-Cβ bonds in the side chains of syringylglycerol-β-guaiacyl ether, β-1 lignin substructures, and demethoxylatemethoxyl groups (Higuchi 1990). Extracellular laccases are present in most fungal species but most typically in basidiomycetous white-rot fungi and related litter-composting fungi (Steffen et al. 2000). Almost all species of white-rot fungi produce extracellular laccase, for example, *C. subvermispora* and *Phlebia* spp. (Baldrian 2006). Atalla et al. (2010) have isolated *Trematosphaeria mangrovei* from the mangrove ecosystem which produces laccase enzyme in significant quantity. A thermostable, metal-tolerant laccase is reportedly produced by marine-derived fungi, *Cerrena unicolor* (D'Souza-Ticlo et al. 2009). Various researchers have isolated laccase-producing fungi from different sources including *Trichoderma harzianum*, *Trichoderma atroviride* and *Trichoderma longibrachiatum*, *Trametes versicolor*, *Lentinus tigrinus*, *Trametes pubescens*, *Cyathus bulleri*, *Paecilomyces* sp., *P. chrysosporium*, *Lentines edodes* and *Pleurotus ostreatus*, *Ganoderma lucidum*, *Alternaria tenuissima*, and *Trichoderma* sp. (Kantharaj et al. 2017). Some examples of fungi that produce laccase with high activity are *Trametes*, *Coriolus hirsutus*, *Trametes hirsuta*, *Trametes versicolor*, *Pycnoporus cinnabarinus*, *Neurospora crassa*, and *Pleurotus ostreatus* (Andlar et al. 2018).

1.10.4.4 Lignin Peroxidase

Lignin peroxidases are the heme glycoprotein that plays a vital role in lignin degradation, which cleaves C-C bonds and oxidizes benzyl alcohols to aldehydes or ketones (Piontek et al. 2001). Lignin peroxidases act on both phenolic (e.g., syringic acid, guaiacol, catechol, vanillyl alcohol, and acteosyringone) and nonphenolic lignin substrates (Wong 2009). Mostly, basidiomycetes are shown to produce efficient lignin peroxidases (Abbas et al. 2005). Extracellular lignolytic enzymes are prominently produced by *P. chrysosporium* and *P. radiata* (Lee et al. 1997), whereas *Coriolus tersicolor* is capable of producing intracellular lignolytic enzymes (Lobarzewski 1990). Researchers have studied the lignin peoxidase producing ability of different fungi including *P. chrysosporium*, *T. versicolor*, *Pleurotus ostreatus*, *Panus* sp., *P. coccineus*, *Perenniporia medullapanis*, and *P. sanguineus* (Kantharaj

et al. 2017). LiPs evidently arose only once in the Polyporales, which harbors many white-rot taxa, whereas MnPs and VPs are more widespread and may have multiple origins. The phlebioid clade of polyporales includes fungi such as *Phanerochaete chrysosporium* and *Phlebia radiata*, which are well-known producers of LiP (Lundell et al. 2010).

1.10.4.5 Manganese Peroxidase

Manganese peroxidase degrades the lignin mainly by attacking phenolic lignin component (Asgher et al. 2008). In the presence of H_2O_2, manganese peroxidase oxidizes the phenolic structures by converting Mn^{2+} to Mn^{3+}. Oxalate and malonate are the mediators that produce carbon-centered radicals, peroxyl radicals, and superoxide radicals which improve the effective lignin-degrading system (Wong 2009). Manganese peroxidase is an essential component to certain basidiomycetes and some wood-decaying white-rot fungi, which secrete manganese peroxidase in several forms into their environment. Among the basidiomycetes, *Agaricus bisporus* (Lankinen et al. 2001), *Lenzites betulinus* (Hoshino et al. 2002), *Panus tigrinus* (Lisov et al. 2003), and *Nematoloma frowardii* (Hilden et al. 2008) are identified to produce more stable manganese peroxidases. Järvinen et al. (2012) have studied MnP production on selected lignin-degrading organism's *P. chrysosporium*, *Physisporinus rivulosus*, *P. radiata,* and *Bjerkandera* sp. and found *P. chrysosporium* as the best manganese peroxidase producer. Bonugli-Santos et al. (2010) isolated marine fungi, *Mucor racemosus,* which possess the ability to produce salt-tolerant manganese peroxidase. MnP is the most significant ligninolytic enzyme produced by *Ceriporiopsis subvermispora* and *Physisporinus rivulosus*. Laccase is produced by almost all white-rot fungi (Hatakka 2001).

References

Abbas A, Koc H, Liu F, Tien M (2005) Fungal degradation of wood: initial proteomic analysis of extracellular proteins of *Phanerochaete chrysosporium* grown on oak substrate. Curr Genet 47:49–56

Abdel-Azeem AM (2020) Taxonomy and biodiversity of the genus *Chaetomium* in different habitats. In: Abdel-Azeem AM (ed) Recent developments on genus *Chaetomium*, Fungal biology. Springer Nature, Switzerland, pp 3–77. https://doi.org/10.1007/978-3-030-31612-9_1

Abdel-Azeem AM, Gab Allah MM (2011) *Aspergillus niger* and *Spirogyra varians* as new biosorbents of heavy metals and radionuclides from polluted groundwater in South Sinai, Egypt, Assiut Univ J Bot 40(2): 159–193

Abdel-Azeem AM, Salem FM (2015) Fungi fimicola Aegyptiaci: I. Recent investigations and conservation in arid South Sinai. Mycosphere 6(2):174–194. https://doi.org/10.5943/mycosphere/6/2/8

Abdel-Azeem AM, Abdel-Moneim TS, Ibrahim ME, Hassan MAA, Saleh MY (2007) Effect of long-term heavy metal contamination on diversity of terricolous fungi and nematodes in Egypt - a case study. Water Air Soil Pollut 186(1):233–254

Abdel-Azeem AM, El-Morsy EM, Nour El-Dein MM, Rashad HM (2015) Occurrence and diversity of mycobiota in heavy metal contaminated sedimentsof Mediterranean Coastallagoon El-Manzala, Egypt. Mycosphere 6(2):228–240

Abdel-Azeem AM, Abdel-Azeem MA, Abdul-Hadi SY, Darwish AG (2019) *Aspergillus*: biodiversity, ecological significances, and industrial applications. In: Yadav AN et al (eds) Recent advancement in white biotechnology through fungi, Fungal biology. Springer Nature, Switzerland, pp 121–179. https://doi.org/10.1007/978-3-030-10480-1_4

Abdel-Hafez SII (1982) Osmophilic fungi of desert soils in Saudi Arabia. Mycopathologia 80:9–14

Abdel-Hafez SII, Moubasher AH, Abdel-Fattah HM (1977) Studies on mycoflora of salt marshes in Egypt. IV-Osmophilic fungi. Mycopathologia 62(3):143–151

Abdel-Hafez SII, Maubasher AH, Abdel-Fattah HM (1978) Cellulose-decomposing fungi of salt marshes in Egypt. Folia Microbiol (Praha) 23(1):37–44. https://doi.org/10.1007/BF02876594

Abdel-Hafez SII, Zidan MA, Bagy MMK, Abdel-Sater MA (1989) Distribution of two halophilic fungi in the Egyptian soils and glycerol accumulation. Cryptogam Mycol 10:125–133

Abdel-Lateff A (2008) Chaetominedione, a new tyrosine kinase inhibitor isolated from the algicolous marine fungus *Chaetomium* sp. Tetrahedron Lett 49(45):6398–6400

Abdel-Lateff A, König GM, Fisch KM, Höller U, Jones PG, Wright AD (2002) New antioxidant hydroquinone derivatives from the algicolous marine fungus *Acremonium* sp. J Nat Prod 65(11):1605–1611

Abdelrahman SZ, Tucker GA, Daw ZY, Du C (2014) Marine yeast isolation and industrial application. FEMS Yeast Res 14(6):813–825

Abdel-Wareth MT, Ghareeb MA (2018) Bioprospecting certain freshwater-derived fungi for phenolic compounds with special emphasis on antimicrobial and larvicidal activity of methyl gallate and p-coumaric acid. Egypt J Chem 61(5):773–784

Abdullah SK, Al-Bader SM (1990) On the thermophilic and thermotolerant mycoflora of Iraqi soils. Sydowia 42:1–7

Abdullah SK, Zora SE (1993) *Chaetomium mesopotamicum*, a new thermophilic species from Iraqi soil. Cryptogam Bot 3:387–389

Abo Nouh FA (2019) Endophytic fungi for sustainable agriculture. Microb Biosyst 4(1):31–44

Abraham EP (1979) A glimpse of the early history of the cephalosporins. Rev Infect Dis 1(1):99–105

Acharya K, Sikder R, Dutta AK (2017) Physiochemical characterization and antioxidant activity of methanol extract from an edible mushroom *Agrocybe pediades*. Int J ChemTech Res 10(3):204–211

Adler L, Blomberg A, Nilsson A (1985) Glycerol metabolism and osmoregulation in the salt-tolerant yeast Debaryomyces hansenii. J Bacteriol 162:300–306

Agrawal S, Adholeya A, Barrow CJ, Deshmukh SK (2018) Marine fungi: an untapped bioresource for future cosmeceuticals. Phytochem Lett 23:15–20

Aguilera A, González-Toril E (2019) Eukaryotic life in extreme environments: acidophilic fungi. In: Tiquia-Arashiro SM, Grube M (eds) Fungi in extreme environments: ecological role and biotechnological significance. Springer Nature, Switzerland, pp 21–38. https://doi.org/10.1007/978-3-030-19030-9_2

Aksu Z (2005) Application of biosorption for the removal of organic pollutants: a review. Process Biochem 40(3–4):997–1026

Ali AH, Radwan U, El-Zayat S, El-Sayed MA (2018) Desert plant-fungal endophytic association: the beneficial aspects to their hosts. Biol Forum 10(1):138–145

Allen N, Nordlander M, McGonigle T, Basinger J, Kaminskyj S (2006) Arbuscular mycorrhizae on Axel Heiberg Island (801 N) and at Saskatoon (521 N) Canada. Can J Bot 84:1094–1100

Amandeep K, Atri N, Munruchi K (2015) Diversity of species of the genus *Conocybe* (Bolbitiaceae, Agaricales) collected on dung from Punjab, India. Mycosphere 6(1):19–42. https://doi.org/10.5943/mycosphere/6/1/4

Amaral PFF, Da Silva JM, Lehocky BM, Barros-Timmons AMV, Coelho MAZ, Marrucho IM et al (2006) Production and characterization of a bioemulsifier from *Yarrowia lipolytica*. Process Biochem 41(8):1894–1898

Ameen F, Al Yahya SA, Al Nadhari S, Alasmari H, Alhoshani F, Wainwright M (2019) Phosphate solubilizing bacteria and fungi in desert soils: species, limitations and mechanisms. Arch Agron Soil Sci 65(10):1446–1459

Andlar M, Rezic T, Marđetko N, Kracher D, Ludwig R, Santek B (2018) Lignocellulose degradation: an overview of fungi and fungal enzymes involved in lignocellulose degradation. Eng Life Sci 18:768–778

Andrews S, Pitt JI (1987) Further studies on the water relations of xerophilic fungi, including some halophiles. J Gen Microbiol 133:233–238

Arai T, Mikami Y, Fukushima K, Utsumi T, Yazawa K (1973) A new antibiotic, leucinostatin, derived from *Penicillium lilacinum*. J Antibiot (Tokyo) 26(3):157–161. https://doi.org/10.7164/antibiotics.26.157

Arantes V, Goodell B (2014) Current understanding of brown-rot fungal biodegradation mechanisms: a review. In: Schultz TP, Goodell B, Nicholas DD (eds) Deterioration and protection of sustainable biomaterials. American Chemical Society, Mississippi, pp 4–21

Arenz BE, Blanchette RA (2009) Investigations of fungal diversity in wooden structures and soils at historic sites on the Antarctic Peninsula. Can J Microbiol 55:46–56

Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biol Biochem 38:3057–3064

Arnebrant K, Bååth E, Nordgren A (1987) Copper tolerance of microfungi isolated from polluted and unpolluted forest soil. Mycologia 79:890–895

Arora J, Ramawat KG (2017) An introduction to endophytes. In: Maheshwari DK (ed) Endophytes: biology and biotechnology, sustainable development and biodiversity 15. Springer International Publishing, Switzerland, pp 1–16. https://doi.org/10.1007/978-3-319-66541-2_1

Artiola JF, Walworth JL, Say M, Crimmins MA (2019) Soil and land pollution. In: Environmental and pollution science. Academic, New York, pp 219–235

Asadi S, Ahmadiani A, Esmaeili MA, Sonboli A, Ansari N, Khodagholi F (2010) In vitro antioxidant activities and an investigation of neuroprotection by six Salvia species from Iran: a comparative study. Food Chem Toxicol 48(5):1341–1349. https://doi.org/10.1016/j.fct.2010.02.035

Asgher M, Bhatti HN, Ashraf M, Legge RL (2008) Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation 19:771–783

Atagana HI (2009) Biodegradation of HPAs by fungi in contaminated soil containing cadmium and nickel ions. Afr J Biotechnol 8:5780–5789

Atalla MM, Zeinab HK, Eman RH, Amani AY, Abeer AA (2010) Screening of some marine-derived fungal isolates for lignin degrading enzymes (LDEs) production. Agric Biol J N Am 1(4):591–599

Baker BJ, Lutz MA, Dawson SC, Bond PL, Banfield JF (2004) Metabolically active eukaryotic communities in extremely acidic mine drainage. Appl Environ Microbiol 70:6264–6271

Baker BJ, Tyson GW, Goosherst L, Banfield JF (2009) Insights into the diversity of eukaryotes in acid mine drainage biofilm communities. Appl Environ Microbiol 75:2192–2199

Baldrian P (2003) Heavy metal interactions with white rot fungi. Enzym Microb Technol 32:78–91. https://doi.org/10.1016/S0141-0229(02)00245-4

Baldrian P (2006) Fungal laccases - occurrence and properties. FEMS Microbiol Rev 30:215–242

Baldrian P (2010) Soil heavy metals: effect of heavy metals on saprotrophic soil fungi. In: Sherameti I, Varma A (eds) Soil heavy metals, Soil biology. Springer, Berlin, pp 263–279

Bastian F, Alabouvette C, Saiz-Jimenez C (2009) The impact of arthropods on fungal community structure in Lascaux Cave. J Appl Microbiol 106:1456–1462

Battley EM, Bartlett EJ (1966) A convenient pH-gradient method for the determination of the maximum and minimum pH for microbial growth. Antonie Van Leeuwenhoek 32:245–255

Beena PS (2010) Acidophilic tannase from marine Aspergillus awamori BTMFW032. J Microbiol Biotechnol 20:1403–1414
Belly RT, Brock TD (1974) Widespread occurrence of acidophilic strains of *Bacillus coagulans* in hot springs. J Appl Bacteriol 37:175–177
Bennett JW, Faison BD (1997) Use of fungi in biodegradation. In: Hurst CJ (ed) Manual of environmental microbiology. ASM Press, Washington, DC, pp 758–765
Bergero R, Girlanda M, Varese GC, Intili D, Luppi AM (1999) Psychrooligotrophic fungi from arctic soils of Franz Joseph Land. Polar Biol 21:361–368
Bills GF, Platas G, Pelaez F, Masurekari P (1999) Reclassification of a pneumocandin-producing anamorph, *Glarea lozoyensis* gen. et sp. nov., previously identified as *Zalerion arboricola*. Mycol Res 103:179–192
Bills GF, Gloer JB, An Z (2013) Coprophilous fungi: antibiotic discovery and functions in an underexplored arena of microbial defensive mutualism. Curr Opin Microbiol 16:549–565. https://doi.org/10.1016/j.mib.2013.08.001
Blanchette RA, Held BW, Arenz BE et al (2010) An Antarctic hot spot for fungi at Shackleton's historic hut on Cape Royds. Microb Ecol 60:29–38
Blomberg A, Adler L (1993) Tolerance of fungi to NaCl. In: Jennings DH (ed) Stress tolerance of fungi. Marcel Dekker, New York, pp 209–232
Bond CM, Tang Y (2019) Engineering Saccharomyces cerevisiae for production of simvastatin. Metab Eng 51:1–8
Bonugli-Santos RC, Durrant LR, DaSilva M, Sette LD (2010) Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. Enzyme Microb Technol 46:32–37
Booth T, Kenkel N (1986) Ecological studies of lignicolous marine fungi: a distribution model based on ordination and classification. In: Moss ST (ed) The biology of marine fungi. Cambridge University Press, Cambridge, pp 297–310
Brown MT, Hall IR (1989) Metal tolerance in fungi. In: Shaw AJ (ed) Heavy metal tolerance in plants: evolutionary aspects. CRC Press, Boca Raton, FL, pp 95–104
Brown AD, Simpson JR (1972) Water relations of sugar-tolerant yeasts: the role of intracellular polyols. J Gen Microbiol 72:589–591
Brown S, Clastre M, Courdavault V, Connor SEO (2015) De novo production of the plant derived alkaloid strictosidine in yeast. Proc Natl Acad Sci U S A 112:3205–3210
Brückner B, Blechschmidt D, Schubert B (1989) Fusarium moniliforme Sheld. a fungus producing a broad spectrum of bioactive metabolites. Zentralbl Mikrobiol 144(1):3–12. https://doi.org/10.1016/s0232-4393(89)80019-8
Brückner A, Polge C, Lentze N, Auerbach D, Schlattner U (2009) Yeast two-hybrid, a powerful tool for systems biology. Int J Mol Sci 10(6):2763–2788. https://doi.org/10.3390/ijms10062763
Buchalo AS, Nevo E, Wasser SP, Oren A, Molitoris HP (1998) Fungal life in the extremely hypersaline water of the Dead Sea: first records. Proc R Soc Lond B 265:1461–1465
Buchalo AS, Wasser SP, Molitoris HP, Volz PA, Kurchenko I, Lauer I et al (1999) Species diversity and biology of fungal life in the extremely hypersaline water of the Dead Sea. In: Wasser SP (ed) Evolutionary theory and processes: modern perspectives. Kluwer Academic, Dordrecht, pp 293–300
Buchalo AS, Nevo E, Wasser SP, Oren A, Molitoris HP, Volz PA (2000) Fungi discovered in the Dead Sea. Mycol Res News 104:132–133
Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21:143–163
Bulzomi P, Galluzzo P, Bolli A, Leone S, Acconcia F, Marino M (2012) The proapoptotic effect of quercetin in cancer cell lines requires ERb-dependent signals. J Cell Physiol 227:1891–1898. https://doi.org/10.1002/jcp.22917

Burtseva YV, Verigina NS, Sova VV, Pivkin MV, Zvyagintseva TN (2003) Filamentous marine fungi as producers of o-glycosylhydrolases: β-1,3-glucanase from *Chaetomium idicum*. Mar Biotechnol 5:349–359

Butinar L, Zalar P, Frisvad JC, Gunde-Cimerman N (2005a) The genus Eurotium – members of indigenous fungal community in hypersaline waters of salterns. FEMS Microbiol Ecol 51:155–166

Butinar L, Santos S, Spencer-Martins I, Oren A, Gunde-Cimerman N (2005b) Yeast diversity in hypersaline habitats. FEMS Microbiol Lett 244:229–234

Butler MS, Buss AD (2006) Natural products-the future scaffolds for novel antibiotics? Biochem Pharmacol 71:919–929

Calaça FJS, Araújo JC, Xavier-Santos S (2017) The ecological status of coprophilic fungi communities. Pesq Ensino Ci Exatas Nat 1(2):136–143. https://doi.org/10.29215/pecen.v1i2.452

Calaça FJS, Cortez VG, Xavier-Santos S (2020) Dung fungi from Brazil: Agrocybepediades (Fr.) Fayod (Basidiomycota) in Cerrado. Scientia Plena 16:066201. https://doi.org/10.14808/sci.plena.2020.066201

Carpenter SE, Trappe JM (1985) Phoenicoid fungi: a proposed term for fungi that fruit after heat treatment of substrates. Mycotaxon 23:203–206

Carpenter SE, Trappe JM, Ammirati JR (1987) Observations of fungal succession in the Mount St. Helens devastation zone, 1980–1983. Can J Bot 65:716–728

Cavicchioli RK, Siddiqui S, Andrews C, Sowers KR (2002) Low-temperature extremophiles and their application. Curr Opin Biotechnol 13:1–9

Cetin-Karaca H, Newman MC (2015) Antimicrobial efficacy of plant phenolic compounds against *Salmonella* and *Escherichia coli*. Food Biosci 11:8–16. https://doi.org/10.1016/j.fbio.2015.03.002

Chandra N, Kumar S (2017) Antibiotics producing soil microorganisms. In: Antibiotics and antibiotics resistance genes in soils. Springer, Cham, pp 1–18

Chaudhuri D, Ghate NB, Singh SS, Mandal N (2015) Methyl gallate isolated from *Spondias pinnata* exhibits anticancer activity against human glioblastoma by induction of apoptosis and sustained extracellular signal-regulated kinase 1/2 activation. Pharmacogn Mag 11(42):269

Chen R, Yang S, Zhang L, Zhou YJ (2020) Advanced strategies for production of natural products in yeast. iScience 23(3):100879. https://doi.org/10.1016/j.isci.2020.100879

Chi Z, Wang F, Chi Z, Yue L, Liu G, Zhang T (2009) Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. Appl Microbiol Biotechnol 82(5):793–804. https://doi.org/10.1007/s00253-009-1882-2

Chi ZM, Zhang T, Cao TS, Liu XY, Cui W, Zhao CH (2011) Biotechnological potential of inulin for bioprocesses. Bioresour Technol 102:4295–4303

Chi Z, Liu GL, Lu Y, Jiang H, Chi ZM (2016) Bio-products produced by marine yeasts and their potential applications. Bioresour Technol 202:244–252

Choi EM (2012) Liquiritigenin isolated from Glycyrrhiza uralensis stimulates osteoblast function in osteoblastic MC3T3-E1 cells. Int Immunopharmacol 12:139–143. https://doi.org/10.1016/j.intimp.2011.11.003

Chokpaiboon S, Sommit D, Teerawatananond T, Muangsin N, Bunyapaiboonsri T, Pudhom K (2010) Cytotoxic nor-chamigrane and chamigrane endoperoxides from a basidiomycetous fungus. J Nat Prod 73:1005–1007

Chomcheon P, Wiyakrutta S, Sriubolmas N, Ngamrojanavanich N, Kengtong S, Mahidol C, Ruchirawat S, Kittakoop P (2009) Aromatase inhibitory, radical scavenging, and antioxidant activities of depsidones and diaryl ethers from the endophytic fungus *Corynespora cassiicola* L36. Phytochemistry 70:407–413

Christophersen C, Crescente O, Frisvad JC, Gram L, Nielsen J, Nielsen PH, Rahbæk L (1999) Antibacterial activity of marine-derived fungi. Mycopathologia 143:135–138

Chung D, Kim H, Choi HS (2019) Fungi in salterns. J Microbiol 57(9):717–724. https://doi.org/10.1007/s12275-019-9195-3

Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio JS (2015) Fungal biomolecules in plant growth promotion. In: Fungal biomolecules: sources applications and recent developments. Wiley, Hoboken, NJ, pp 345–362

Cooke WB (1976) Fungi in sewage. In: Jones EBG (ed) Recent advances in aquatic mycology. Elek Science, London, pp 389–434

Corinaldesi C, Barone G, Marcellini F, Dell'Anno A, Danovaro R (2017) Marine microbial-derived molecules and their potential use in cosmeceutical and cosmetic products. Mar Drugs 15(4):118

Cushnie TPT, Lamb AJ (2011) Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents 38:99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014

D'Souza DT, Tiwari R, Sah AK, Raghukumar C (2006) Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthetic dyes. Enzym Microb Technol 38:504–511

D'Souza-Ticlo D, Sharma D, Raghukumar CA (2009) Thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus. Mar Biotechnol 11:725–737

Dai LX, Ding K (2012) Organic chemistry: breakthroughs and perspectives. Wiley-VCH, Weinheim. ISBN 9783527333776

Daisy B, Strobel GA, Castillo U, Ezra D, Sears J, Weaver D et al (2002) Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. Microbiology 148:3737–3741

De Hoog GS, Zalar P, Urzı C, de Leo F, Yurlova NA, Sterflinger K (1999) Relationships of dothideaceous black yeasts and meristematic fungi based on 5.8S and ITS2 rDNA sequence comparison. Stud Mycol 43:31–37

De la Rosa AL, Moreno-Escamilla OL, Rodrig-Garcia J, Alvarez-Parrilla E (2019) Phenolic compounds. In: Yahia ME, Garrillo-Lopez A (eds) Postharvest physiology and biochemistry of fruits and vegetables. Elsevier Inc, Amsterdam, pp 253–271

De Leo F, Criseo G, Urzi C (1996) Impact of surrounding vegetation and soil on the colonisation of marble statues by dematiaceous fungi. In: Proceedings of the 8th International congress on deterioration and conservation of stone, Berlin, Germany, vol 2, pp 625–630

De Leo F, Urzı C, de Hoog GS (1999) Two Coniosporium species from rock surfaces. Stud Mycol 43:77–85

Debbab A, Aly AH, Proksch P (2011) Bioactive secondary metabolites from endophytes and associated marine derived fungi. Fungal Divers 49:1–12

Degenkolb T, Bruckner H (2008) Peptaibiomics: towards a myriad of bioactive peptides containing C*a*-Dialkylamino acids? Chem Biodivers 5:1817–1843

Del-Cid A, Ubilla P, Ravanal MC, Medina E, Vaca I, Levicán G, Eyzaguirre J et al (2014) Cold-active xylanase produced by fungi associated with Antarctic marine sponges. Appl Biochem Biotechnol 172:524–532

Demain AL (2000) Microbial natural products: a past with a future. In: Wrigley SK, Hayes MA, Thomas R, Chrystal EJT, Nicholson N (eds) Biodiversity: new leads for pharmaceutical and agrochemical industries. The Royal Society of Chemistry, Cambridge, UK, pp 3–16

Denizli A, Cihangir N, Rad AY, Taner M, Alsancak G (2004) Removal of chlorophenols from synthetic solutions using Phanerochaete chrysosporium. Process Biochem 39(12):2025–2030

Deshmukh SK, Gupta MK, Prakash V, Reddy MS (2019) Fungal endophytes: a novel source of cytotoxic compounds. In: Jha S (ed) Endophytes and secondary metabolites, Reference series in phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-319-90484-9_13

Deslippe JR, Hartmann M, Mohn WW, Simard SW (2011) Long-term experimental manipulation of climate alters the ectomycorrhizal community of Betula nana in Arctic tundra. Glob Change Biol 17:1625–1636

Desriac F, Jégou C, Balnois E, Brillet B, Le Chevalier P, Fleury Y (2013) Antimicrobial peptides from marine proteobacteria. Mar Drugs 11:3632–3660

Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020a) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Devi R, Kaur T, Kour D, Rana KL, Yadav A, Yadav AN (2020b) Beneficial fungal communities from different habitats and their roles in plant growth promotion and soil health. Microb Biosyst 5:21–47. https://doi.org/10.21608/mb.2020.32802.1016

Dix NJ, Webster J (1995) Fungal ecology. Chapman Hall, London, England

Dominguez JM, Martín JJ (2005) Sordarins: inhibitors of fungal elongation factor-2. In: An Z (ed) Handbook of industrial mycology. Marcel Dekker, New York, pp 335–353

Dong J, Ru L, He HP et al (2005) Nematicidal sphingolipids from the freshwater fungus *Paraniesslia sp.* YMF 1.01400. Eur J Lipid Sci Technol 107:779–785

Dong J, Zhou Y, Li R et al (2006) New nematicidalazaphilones from the aquatic fungus Pseudohalonectria adversaria YMF1.01019. FEMS Microbiol Lett 264:65–69

Dong J, Zhu Y, Song H, Li R, He H, Liu H et al (2007) Nematicidalresorcylides from the aquatic fungus Caryospora callicarpa YMF1.01026. J Chem Ecol 33(5):1115–1126

Doveri F (2004) Fungi Fimicoli italici. A guide to the recognition of Basidiomycetes and Ascomycetes living on feacal material. Fondazione Studi Micologici, Trento. 1104 p

Dowzer CE, Kelly JM (1989) Cloning of the CreA gene from Aspergillus nidulans: a gene involved in carbon catabolite repression. Curr Genet 15:457–459

Dumitriu D, Peinado RA, Peinado J, de Lerma NL (2015) Grape pomace extract improves the in vitro and in vivo antioxidant properties of wines from sun light dried Pedro Ximénez grapes. J Funct Foods 17:380–387. https://doi.org/10.1016/j.jff.2015.06.003

Duncan SM, Farrell RL, Thwaites JM et al (2006) Endoglucanase-producing fungi isolated from Cape Evans historic expedition hut on Ross Island, Antarctica. Environ Microbiol 8:1212–1219

Eggert C, Temp U, Dean JFD, Eriksson KEL (1996) A fungal metabolite mediates degradation of non-phenolic lignin structures and synthetic lignin by laccase. FEBS Lett 391:144–148

Egidi E, de Hoog GS, Isola D, Onofri S, Quaedvlieg W, De Vries M et al (2014) Phylogeny and taxonomy of meristematic rock-inhabiting black fungi in the Dothideomycetes based on multilocus phylogenies. Fungal Divers 65:127–165

Eichenberger M, Lehka BJ, Folly C, Fischer D, Martens S, Simón E et al (2017) Metabolic engineering of Saccharomyces cerevisiae for de novo production of dihydrochalcones with known antioxidant, antidiabetic, and sweet tasting properties. Metab Eng 39:80–89

El-Fawy MM, El-Sharkawy RMI, Abo-Elyousr KAM (2018) Evaluation of certain isolates of Penicillium frequentans against Cercospora leaf spot disease of sugar beet. Egypt J Biol Pest Control 28:49

El-Gindy AA, Saad RR (1990) Fungi of virgin and cultivated soil of Salhiah Desert, Egypt. Zentralbl Mikrobiol 145(7):547–551

Elmeleigy MA, Hoseiny EN, Ahmed SA, Alhoseiny AM (2010) Isolation, identification, morphogenesis and ultrastructure of obligate halophilic fungi. J Appl Sci Environ Sanit 5:201–202

El-Nasser NHA, Helmy SM, El-Gammal AA (1997) Formation of enzymes by biodegradation of agricultural wastes with white rot fungi. Polym Degrad Stab 55:249–255

El-Said AHM, Saleem A (2008) Ecological and physiological studies on soil fungi at western region, Libya. Mycobiology 36(1):1–109

El-Sharouny HMM, Moubasher AH, Nassar MS (1988) Mycoflora associated with dry dates in upper Egypt II. Osmophilic fungi and test of osmophilic ability. Qatar Univ Sci Bull 8:59–68

Evans BS, Robinson SJ, Kelleher NL (2011) Surveys of non-ribosomal peptide and polyketide assembly lines in fungi and prospects for their analysis in vitro and in vivo. Fungal Genet Biol 48:49–61

Eyberger L, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produce podo-phyllotoxin. J Nat Prod 69:1121–1124

Ezzouhri L, Castro E, Moya M, Espinola F, Lairini K (2009) Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. Afr J Microbiol Res 3:35–48

Fábio A, Barros P, Filho ER (2005) Four spiroquinazoline alkaloids from *Eupenicillium* sp. isolated as an endophytic fungus from leaves of *Murraya paniculata* (Rutaceae). Biochem Syst Ecol 33:257–268

Fackler K, Gradinger C, Hinterstoisser B, Messner K, Schwanninger M (2006) Lignin degradation by white rot fungi on spruce wood shavings during short-time solid-state fermentations monitored by near infrared spectroscopy. Enzym Microb Technol 39:1476–1483

Fadl-Allah EM, Sayed OH (1991) Proline accumulation and osmoregulation in Aspergillus melleus. Bull Fac Sci Assiut Univ 20:207–215

Fais A, Corda M, Era B, Fadda MB, Matos MJ, Santana L et al (2009) Tyrosinase inhibitor activity of coumarin-resveratrol hybrids. Molecules 14:2514–2520

Farooq M, Hassan M, Gull F (2015) Mycobial deterioration of stone monuments of Dharmarajika, Taxila. J Microbiol Exp 2(1):00036. https://doi.org/10.15406/jmen.2015.02.00036

Fill TP, Pereira GK, Geris Dos Santos RM, Rodrigues-Fo E (2007) Four additional meroterpenes produced by Penicillium sp. found in association with Melia azedarach. Possible biosynthetic intermediates to Austin. Z Naturforsch 62b:1035–1044

Francis F, Druart F, Mavungu JDD, De Boevre M, De Saeger S, Delvigne F (2020) Biofilm mode of cultivation leads to an improvement of the entomotoxic patterns of two *Aspergillus* species. Microorganisms 8(5):705

Frank M, Niemann H, Böhler P, Stork B, Wesselborg S, Lin W et al (2015) Phomoxanthone A-from mangrove forests to anticancer therapy. Curr Med Chem 22(30):3523–3532

Frank K, Brückner A, Hilpert A, Heethoff M, Blüthgen N (2017) Nutrient quality of vertebrate dung as a diet for dung beetles. Sci Rep 7:12147

Frank K, Brückner A, Blüthgen N et al (2018) In search of cues: dung beetle attraction and the significance of volatile composition of dung. Chemoecology 28:145–152. https://doi.org/10.1007/s00049-018-0266-4

Gabriel CR, Northup DE (2013) Microbial ecology: caves as an extreme habitat. Cave microbiomes: a novel resource for drug discovery. Springer, New York, pp 85–108

Gadanho M, Sampaio JP (2009) *Cryptococcus ibericus* sp. nov., *Cryptococcus aciditolerans* sp. nov. and *Cryptococcus metallitolerans* sp. nov., a new ecoclade of anamorphic basidiomycetous yeast species from an extreme environment associated with acid rock drainage in Sao Domingos pyrite mine, Portugal. Int J Syst Evol Microbiol 59:2375–2379

Gadanho M, Libkind D, Sampaio JP (2006) Yeast diversity in the extreme acidic environments of the Iberian Pyrite Belt. Microb Ecol 2:552–563

Gangadevi V, Muthumary J (2008) Isolation of *Colletotrichum gloeosporioides*, a novel endophytic taxol-producing fungus from the leaves of a medicinal plant, Justicia gendarussa. Mycol Balc 5:1–4

Gao M, Glenn AE, Gu X, Mitchell TR, Satterlee T, Duke MV et al (2020) Pyrrocidine, a molecular off switch for fumonisin biosynthesis. PLoS Pathog 16(7):e1008595. https://doi.org/10.1371/journal.ppat.1008595

Geerlings A, Redondo FJ, Contin A, Memelink J, van der Heijden R, Verpoorte R (2001) Biotransformation of tryptamine and secologanin into plant terpenoid indole alkaloids by transgenic yeast. Appl Microbiol Biotechnol 56:420–424

Geris dos Santos RM, Rodrigues-Fo E (2002) Meroterpenes from Penicillium sp. found in association with Melia azedarach. Phytochemistry 61:907–912

Ginns JH (1974) *Rhizina* root rot: severity and distribution in British Columbia. Can J For Res 4: 143–146

Goodell B (2003) Brown-rot fungal degradation of wood: our evolving view. In: Goodell B, Nicholas DD, Schultz TP (eds) Wood deterioration and preservation, ACS Symposium series, vol 845. ACS, Washington DC, pp 97–118

Goodell B, Jellison J, Liu J, Daniel G, Paszczynski A, Fekete F et al (1997) Low molecular weight chelators and phenolic compounds isolated from wood decay fungi and their role in the fungal biodegradation of wood. J Biotechnol 53:133–162

Gopal B, Chauhan M (2006) Biodiversity and its conservation in the Sundarban mangrove ecosystem. Aquat Sci 68(3):338–354

Gorbushina A, Krumbein WE, Panina L, Soukharjevsky S, Wollenzien U, Hamann KH (1993) On the role of black fungi in colour change and biodeterioration of antique marbles. Geomicrobiol J 11:205–221

Gori K, Sørensen LM, Petersen MA, Jespersen L, Arneborg N (2012) *Debaryomyces hansenii* strains differ in their production of flavor compounds in a cheese-surface model. Microbiol Open 1(2):161–168

Goto S, Ano R, Sugiyama J, Horikoshi K (1981) *Exophiala alcalophila*, a new black yeast-like hyphomycete with an accompanying *Phaeococcomyces alcalophilus* morph, and its physiological characteristics. Trans Mycol Soc Jpn 22:429–439

Gounot AM (1986) Psychrophilic and psychrotrophic microorganisms. Experientia 42:1192–1197

Grant WD (2004) Life at low water activity. Philos Trans R Soc Lond B 359:1249–1267

Grant WD, Mwatha WE, Jones BE (1990) Alkaliphiles: ecology, diversity and applications. FEMS Microbiol Rev 75:255–270

Green F, Highley TL (1997) Mechanism for brown-rot decay: paradigm or paradox 1. Int Biodeterior Biodegrad 39:113–124

Greene AD, Patel BKC, Sheehy AJ (1997) Deferribacter thermophilus gen. nov., sp. nov., a novel thermophilic manganese and iron-reducing bacterium isolated from a petroleum reservoir. Int J Syst Bacteriol 47:505–509

Gross S, Robbins EI (2000) Acidophilic and acid-tolerant fungi and yeasts. Hydrobiologia 433:91–109

Grove JF (1985) Metabolic products of Phomopsis oblonga, Part 2. Phomopsolide A and B, tiglic esters of two 6-substituted 5,6-dihydro-5-hydroxypyran-2-ones. J Chem Soc Perkin Trans 1:865–869

Grum-Grzhimaylo AA, Georgieva ML, Bondarenko SA, Debets AJM, Bilanenko EN (2016) On the diversity of fungi from soda soils. Fungal Divers 76:27–74

Guijarro B, Larena I, Melgarejo P, De Cal A (2017) Adaptive conditions and safety of the application of Penicillium frequentans as a biocontrol agent on stone fruit. Int J Food Microbiol 254:25–35. https://doi.org/10.1016/j.ijfoodmicro.2017.05.004

Guiraud P, Steiman R, Seigle-Murandi F, Sage L (1995) Mycoflora of soil around the Dead Sea II—Deuteromycetes (except *Aspergillus* and *Penicillium*). Syst Appl Microbiol 18:318–322

Gunde-Cimerman N, Zalar P, de Hoog S, Plemenitas A (2000). Hypersaline waters in salenters-Naturals ecological niches for halophilic black yeasts. FEMS Microbiol Ecol 32:235–240

Gunde-cimerman N, Ramos J, Plemenitas A 2009. Halotolerant and halophilic fungi mycological research 113.1231–1241

Guo B, Dai JR, Ng S, Huang Y, Leong C, Ong W, Carte BK (2000) Cytonic acids A & B: novel tridepside inhibitors of h CMV protease from the endophytic fungus Cytonaema species. J Nat Prod 63:602–604

Guo BH, Wang YC, Zhou XW, Hu K, Tan F, Miao ZQ, Tang KX (2006) An endophytic taxol producing fungus BT2 isolated from Taxus chinensis var. mairei. Afr J Biotechnol 5:875–877

Haase G, Sonntag L, Melzer-Krick B, de Hoog GS (1999) Phylogenetic inference by SSU-gene analysis of members of the Herpotrichiellaceae with special reference to human pathogenic species. Stud Mycol 43:80–97

Hakala TK, Maijala P, Konn J, Hatakka A (2004) Evaluation of novel wood-rotting polypores and corticioid fungi for the decay and biopulping of norway spruce (*Piceaabies*) wood. Enzym Microb Technol 34:255–263

Hallberg KB, González-Toril E, Johnson KB (2010) Acidithiobacillus ferrivorans, sp. nov.; facultatively anaerobic, psychrotolerant iron-, and sulfur-oxidizing acidophiles isolated from metal mine-impacted environments. Extremophiles 14:9–19

Hamada N, Yamada A (1991) Seasonal changes in the fungal flora of house dust. Trans Mycol Soc Jpn 32:45–53

Harper JK, Arif AM, Ford EJ, Strobel GA, Tomer DP, Grant DM, Porco J, Oneill K (2003) Pestacin: a 1,3-dihydroisobenzofuran from Pestalotiopsis microspora possessing antioxidant and antimycotic activities. Tetrahedron 59:2471–2476

Harrigan G, Armentrout BL, Gloer JB, Shearer CA (1995) Anguillosporal, a new antibacterial and antifungal metabolite from the freshwater fungus Anguillospora longissima. J Nat Prod 58:1467–1469

Hasija SK (1994) Biodegradation by aquatic fungi. Presented at the Fifth International Mycological Congress, 14–21, Vancouver, BC, Canada

Hassan N, Rafiq M, Hayat M, Shah AA, Hasan F (2016) Psychrophilic and psychrotrophic fungi: a comprehensive review. Rev Environ Sci Biotechnol 15:147–172. https://doi.org/10.1007/s11157-016-9395-9

Hatakka AI (1994) Lignin-modifying enzymes from selected white-rot fungi – production and role in lignin degradation. FEMS Microbiol Rev 13:125–135

Hatakka AI (2001) Biodegradation of lignin. In: Hofrichter M, Steinbuchel A (eds) Biopolymers. Lignin, humic substances and coal. Wiley-VCH, Weinheim, Germany, pp 129–180

Hatakka A, Hammel KE (2010) Fungal biodegradation of lignocelluloses. In: Hofrichter M (ed) Industrial applications, The mycota, vol X, 2nd edn. Springer, Berlin, pp 319–340

Hawkins KM, Smolke CD (2008) Production of benzylisoquinoline alkaloids in Saccharomyces cerevisiae. Nat Chem Biol 4:564–573

Hawksworth DL (1991) The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol Res 95:641–655

He L, Liu J, Qin S, Yu J, Sun M (2011) Identification of an astaxanthin-producing marine yeast strain YS-185 and optimization of its fermentation conditions. Prog Fish Sci 32(4):93–101

Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW (2007) Aspergillus flavus: human pathogen, allergen and mycotoxin producer. Microbiology 153(6):1677–1692. https://doi.org/10.1099/mic.0.2007/007641-0

Hernández-Carlos B, Gamboa-Angulo MM (2011) Metabolites from freshwater aquatic microalgae and fungi as potential natural pesticides. Phytochem Rev 10(2):261–286

Higuchi T (1990) Lignin biochemistry: biosynthesis and biodegradation. Wood Sci Technol 24:23–63

Hildén K, Hakala TK, Maijala P, Lundell TK, Hatakka A (2007) Novel thermotolerant laccases produced by the white-rot fungus *Physisporinus rivulosus*. Appl Microbiol Biotechnol 77:301–309

Hilden KS, Bortfeldt R, Hofrichter M, Hatakka A, Lundell TK (2008) Molecular characterization of the basidiomycete isolate *Nematoloma frowardii* b19 and its manganese peroxidase places the fungus in the corticioid genus *Phlebia*. Microbiology 154:2371–2379

Hocking AD (1993) Responses of xerophilic fungi to changes in water activity. In: Jennings DH (ed) Stress tolerance of fungi. Marcel Dekker, New York, pp 233–256

Hocking AD, Pitt JI (1979) Water relations of some Penicillium species at 258°C. Trans Br Mycol Soc 73:141–145

Hoffman CS, Wood V, Fantes PA (2015) An ancient yeast for young geneticists: a primer on the Schizosaccharomyces pombe model system. Genetics 201(2):403–423. https://doi.org/10.1534/genetics.115.181503

Hoffmeister D, Keller NP (2007) Natural products of filamentous fungi: enzymes, genes, and their regulation. Nat Prod Rep 24:393–416

Hormazabal E, Schmeda-Hirschmann G, Astudillo L, Rodriguez J, Theoduloz C (2005) Metabolites from *Microsphae-ropsisolivacea*, an endophytic fungus of *Pilgerodendronuviferum*. Z Naturforsch C 60:11–21

Hoshino T, Matsumoto N (2012) Cryophilic fungi to denote fungi in the cryosphere. Fungal Biol Rev 26:102–105

Hoshino F, Kajino T, Sugiyama H, Asami O, Takahashi H (2002) Thermally stable and hydrogen peroxide tolerant manganese peroxidase (MnP) from *Lenzites betulinus*. FEBS Lett 530:249–252

Hsiang T, Matsumoto N, Millett S (1999) Biology and managemen of *Typhula* snow molds of turfgrass. Plant Dis 83:788–798

Huamán A, Béjar V, Sáez G, Guevara J, Sevilla R, Tapia M et al (2018) Cryptococcus neoformans en heces de palomas (Columba livia) en Lima Metropolitana. Revista Medica Herediana 29(2):85

Huang WY, Cai YZ, Xing J, Corke H, Sun M (2007) Potential antioxidant resource: endophytic fungi isolated from traditional Chinese medicinal plants. Econ Bot 61:14–30

Huţanaşu C, Sfarti C, Trifan A et al (2011) High levels of sterigmatocystin in patients with chronic liver diseases. Rev Med Chir Soc Med Nat Iasi 115(1):33–37

Hyde KD, Jones EBG, Leano E, Pointing SB, Poonyth AD, Vrijmoed LLP (1998) Role of fungi in marine ecosystems. Biodivers Conserv 7:1147–1161

Hyde KD, Soytong K 2007 Understanding microfungi diversity - acirtique, cryptogamie, mycologie 28(4):281–289

Immanuel G, Bhagavath C, Iyappa Raj P, Esakkiraj P, Palavesam A (2009) Production and partial purification of cellulase by *Aspergillus niger* and *A. fumigates* fermented in coir waste and sawdust. Int J Microbiol 3(1):1–11

Inoue M (1988) The study of fungal contamination in the field of electronics. Biodeterioration 4:580–584

Iram S, Ahmad I, Javed B, Yaqoob S, Akhtar K, Kazmi MR et al (2009) Fungal tolerance to heavy metals. Pak J Bot 41:2583–2594

Isaka M, Suyarnsestakorn C, Tanticharoen M (2002) Aigialomycins A–E, new resorcylic macrolides from the marine mangrove fungus *Aigialus parvus*. J Org Chem 67:1561–1566

Jadulco RC, Koch M, Kakule TB, Schmidt EW, Orendt A, He H et al (2014) Isolation of pyrrolocins A–C: cis- and trans-decalin tetramic acid antibiotics from an endophytic fungal-derived pathway. J Nat Prod 77:2537–2544

Jain A, Jain R, Jain S (2020) Isolation of coprophilous fungi (moist chamber method). In: Basic techniques in biochemistry, microbiology and molecular biology, Springer protocols handbooks. Humana, New York. https://doi.org/10.1007/978-1-4939-9861-6_32

Jalgaonwalal RE, Mohite BV, Mahajan RT (2011) Evaluation of endophytes for their antimicrobial activity. Afr J Biotechnol 10:103–107

Jančič S, Frisvad JC, Kocev D, Gostinčar C, Džeroski S, Gunde-Cimerman N (2016) Producción de metabolitos secundarios en ambientes extremos: Wallemiaspp. Producen metabolitos tóxicos en condiciones hipersalinas. PLoS One 11(12):e0169116

Jansson JK, Hofmockel KS (2020) Soil microbiomes and climate change. Nat Rev Microbiol 18(1):35–46

Järvinen J, Taskila S, Isomäki R, Ojamo H (2012) Screening of white-rot fungi manganese peroxidases: a comparison between the specific activities of the enzyme from different native producers. AMB Express 2:62

Jayaprakashvel M (2012) Therapeutically active biomolecules from marine actinomycetes. J Modern Biotechnol 1(1):1–7

Jennings DH (1984) Polyol metabolism in fungi. Adv Microb Physiol 25:149–193

Jiang JR, Cai L, Liu F (2017) Oligotrophic fungi from a carbonate cave, with three new species of Cephalotrichum. Mycology 8:1–14. https://doi.org/10.1080/21501203.2017.1366370

Jiao P, Gloer JB, Campbell J, Shearer CA (2006) Altenuene derivatives from an unidentified freshwater fungus in the family Tubeufiaceae. J Nat Prod 69:612–615

Johnson DB (1998) Biodiversity and ecology of acidophilic microorganisms. FEMS Microb Ecol 27:307–317

Jones EBG (2000) Marine fungi: some factors influencing biodiversity. Fungal Divers 4(193):53–73

Jones EBG (2011) Are there more marine fungi to be described? Bot Mar 54(4):343–354

Jones EBG, Alias SA (1997) Diversity of mangrove fungi. In: Hyde KD (ed) Diversity of tropical microfungi. Hong Kong University Press, Hong Kong, pp 71–91

Jones EBG, Mitchell JL (1996) Biodiversity of marine fungi. In: Cimerman A, Gunde-Cimerman N (eds) Biodiversity: international biodiversity seminar. National Institute of Chemistry and Slovenia National Commission for UNESCO, Ljubljana, Slovenia, pp 31–42

Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL (2009) Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fungal Divers 35(1):187

Joshi BK, Gloer JB, Wicklow DT (2002) Bioactive natural products from a sclerotium-colonizing isolate of Humicola fuscoatra. J Nat Prod 65(11):1734–1737. https://doi.org/10.1021/np020295p

Joshi PK, Swarup A, Maheshwari S, Kumar R, Singh N (2011) Bioremediation of heavy metals in liquid media through fungi isolated from contaminated sources. Indian J Microbiol 51:482–487

Kamat S, Gaikwad S, Kumar RA, Gade WN (2013) Xylitol production by *Cyberlindnera* (Williopsis) *saturnus*, a tropical mangrove yeast from xylose and corn cob hydrolysate. J Appl Microbiol 115(6):1357–1367

Kantharaj P, Boobalan B, Sooriamuthu S, Mani R (2017) Lignocellulose degrading enzymes from fungi and their industrial applications. Int J Curr Res 9(21):1–12

Kashif A, Naz S, Ayub A (2018) Characterization of invertase and alpha amylase from two fungal species, *Penicillium lilacinum* and *Aspergillus niger*. Int J Biol Biotechnol 15(2):351–355

Kathiresan K, Saravanakumar K, Senthilraja PAGS (2011) Producción de bioetanol por levaduras marinas aisladas de sedimentos de manglares costeros. Int Multidiscip Res J 1:19–24

Kaur B, Sy B, Phutela U (2015) Production of cellulases from Humicola fuscoatra MTCC 1409: role of enzymes in the digestion of rice straw. Afr J Res Microbiol 9(9):631–638

Kavya V, Padmavathi T (2009) Optimization of growth conditions for xylanase production by *Aspergillus niger* in solid state fermentation. Pol J Microbiol 58:125–130

Kealey JT, Liu L, Santi DV, Betlach MC, Barr PJ (1998) Production of a polyketide natural product in nonpolyketide-producing prokaryotic and eukaryotic host. Proc Natl Acad Sci U S A 95:505–509

Kis-Papo T, Grishkan I, Oren A, Wasser SP, Nevo E (2003) Survival of filamentous fungi in hypersaline Dead Sea water. Microb Ecol 45:183–190

Klich M, Mendoza C, Mullaney E, Keller N, Bennett JW (2001) A new sterigmatocystin-producing Emericella variant from agricultural desert soils. Syst Appl Microbiol 24(1):131–138. https://doi.org/10.1078/0723-2020-00007

Kluczek-Turpeinen B, Tuomela M, Hatakka A, Hofrichter M (2003) Lignin degradation in a compost environment by the deuteromycete *Paecilomyces inflatus*. Appl Microbiol Biotechnol 61:374–379

Kobayashi J, Tsuda M (2004) Bioactive products from Okinawan marine micro and macroorganisms. Phytochem Rev 3:267–274

Kogej T, Gostinčar C, Volkmann M, Gorbushina AA, Gunde-Cimerman N (2006) Mycosporines in extremophilic fungi-novel complementary osmolytes? Environ Chem 3(2):105–110

Kohlmeyer J, Kohlmeyer E (2013) Marine mycology: the higher fungi. Elsevier, Amsterdam

Koopman F, Beekwilder J, Crimi B, van Houwelingen A, Hall RD, Bosch D et al (2012) De novo production of the flavonoid naringenin in engineered Saccharomyces cerevisiae. Microb Cell Factories 11:155. https://doi.org/10.1186/1475-2859-11-155

Kour D, Rana KL, Kaur T, Singh B, Chauhan VS, Kumar A et al (2019a) Extremophiles for hydrolytic enzymes productions: biodiversity and potential biotechnological applications. In: Molina G, Gupta VK, Singh B, Gathergood N (eds) Bioprocessing for biomolecules production, pp 321–372. https://doi.org/10.1002/9781119434436.ch16

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019b) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kour D, Kaur T, Devi R, Yadav A, Singh M, Joshi D et al (2021) Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: present status and future challenges. Environ Sci Pollut Res 28:24917–24939. https://doi.org/10.1007/s11356-021-13252-7

Kracher D, Ludwig R (2016) Cellobiose dehydrogenase: an essential enzyme for lignocellulose degradation in nature. J Land Manag Food Environ 67:145–163

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88

Krug JC, Benny GL, Keller HW (2004) Coprophilous fungi. In: Mueller G, Bills GF, Foster MS (eds) Biodiversity of fungi, inventory and monitoring methods. Elsevier Academic, London, pp 467–499

Kshirsagar AD, Gunale VR (2011) Pollution status of river Mula (Pune city) Maharashtra, India. J Ecophysiol Occup Health 11(1/2):81

Kshirsagar AD, Ahire ML, Gunale VR (2012) Phytoplankton diversity related to pollution from Mula River at Pune city. Terrest Aquat Environ Toxicol 6(2):136–142

Kuhad RC, Singh A, Eriksson K-E (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57:45–125

Kumar M, Yadav AN, Tiwari R, Prasanna R, Saxena AK (2014) Deciphering the diversity of culturable thermotolerant bacteria from Manikaran hot springs. Ann Microbiol 64:741–751

Kumar A, Prajapati S, Nikhil S, Neogi TG (2019a) Industrially important pigments from different groups of fungi. In: Recent advancement in white biotechnology through fungi. Springer, Cham, pp 285–301

Kumar A, Chaturvedi AK, Yadav K, Arunkumar KP, Malyan SK, Raja P et al (2019b) Fungal phytoremediation of heavy metal-contaminated resources: current scenario and future prospects. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for sustainable environments, vol 3. Springer, Cham, pp 437–461. https://doi.org/10.1007/978-3-030-25506-0_18

Kumar A, Yadav AN, Mondal R, Kour D, Subrahmanyam G, Shabnam AA et al (2021) Myco-remediation: a mechanistic understanding of contaminants alleviation from natural environment and future prospect. Chemosphere 284:131325. https://doi.org/10.1016/j.chemosphere.2021.131325

Kurtzman CP, Piškur J (2006) Taxonomy and phylogenetic diversity among the yeasts. In: Sunnerhagen P, Piskur J (eds) Comparative genomics: using Fungi as models. Springer, Berlin, pp 29–46

Kusari S, Zühlke S, Spiteller M (2009) An endophytic fungus from Camptotheca acuminata that produces camptothecin and analogues. J Nat Prod 72:2–7

Kuzmina L, Galimzianova N, Abdullin S, Ryabova A (2012) Microbiota of the Kinderlinskaya cave (South Urals, Russia). Microbiology 81(2):251–258

Lajili S, Azouaou SA, Turki M, Muller CD, Bouraoui A (2016) Antiinflammatory, analgesic activities and gastro-protective effects of the phenolic contents of the red alga, Laurencia obtusa. Eur J Integr Med 8(3):298–306. https://doi.org/10.1016/j.eujim.2015.12.006

Lankinen VP, Bonnen AM, Anton LH, Wood DA, Kalkkinen N, Hatakka A et al (2001) Characteristics and N-terminal amino acid sequence of manganese peroxidase from solid substrate cultures of *Agaricus bisporus*. Appl Microbiol Biotechnol 55:170–176

Laxmipriya P, Yugal KM, Sujogya KP (2013) Endophytic fungi with great promises: a review. J Adv Pharm Educ Res 3(3):152–170

Lazaro JE, Hyde KD (2001) Fungal diversity research series 6:93–129

Lee JC, Strobel GA, Lobkovsky E, Clardy J (1996) Torreyanic acid: a selectively cytotoxic quinone dimer from the endophytic fungus Pestalotiopsis microspora. J Org Chem 61:3232–3233

Lee S, Ha JK, Kang HS, McAllister T, Cheng KJ (1997) Overview of energy metabolism, substrate utilization and fermentation characteristics of ruminal anaerobic fungi Korean. J Anim Nutr Feedstuffs 21:295–314

Lee SJ, Lee SY, Chung MS, Hur SJ (2016) Development of novel in vitro human digestion systems for screening the bioavailability and digestibility of foods. J Funct Foods 22:113–121. https://doi.org/10.1016/j.jff.2016.01.005

Lehr NA, Meffert A, Antelo L, Sterner O, Anke H, Weber RW (2006) Antiamoebins, myrocin B and the basis of antifungal antibiosis in the coprophilous fungus *Stilbella erythrocephala* (syn. S. fimetaria). FEMS Microbiol Ecol 55:105–112

Li X, Kondo R, Sakai K (2002) Biodegradation of sugarcane bagasse with marine fugus *phlebia* sp. MG-60. J Wood Sci 48:159–162

Li X, Kondo R, Sakai K (2003) Studies on hypersaline-tolerant white-rot fungi IV: Effects of Mn and NH_4 on manganese peroxidase production and Roly R-478 decolorization by the marine isolate *phlebia* sp. MG-60 under saline conditions. J Wood Sci 49:355–360

Li X, Kim MK, Lee U, Kim SK, Kang JS, Choi HD et al (2005) Myrothenones A and B, cyclopentenone derivatives with tyrosinase inhibitory activity from the marine-derived fungus *Myrothecium* sp. Chem Pharm Bull 53(4):453–455

Li E, Jiang L, Guo L, Zhang H, Che Y (2008) Pestalachlorides A–C, antifungal metabolites from the plant endophytic fungus *Pestalotiopsis adusta*. Bioorg Med Chem 16:7894–7899

Li LQ, Yang YG, Zeng Y, Zou C, Zhao PJ (2010) A new Azaphilone, Kasanosin C, from an endophytic *Talaromyces* sp. T1BF. Molecules 15:3993–3997

Li DC, Li AN, Papageorgiou AC (2011) Cellulases from thermophilic fungi: recent insights and biotechnological potential. Enzym Res 2011:308730

Lisov AV, Leontievsky AA, Golovleva LA (2003) Hybrid Mn-peroxidase from the ligninolytic fungus *Panus tigrinus* 8/18. Isolation, substrate specificity, and catalytic cycle. Biochem Mosc 68:1027–1035

Liu X, Dong M, Chen X, Jiang M, Lv X, Zhou J (2008) Antimicrobial activity of an endophytic *Xylaria* sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. Appl Microbiol Biotechnol 78:241–247

Liu XY, Chi Z, Liu GL, Madzak C, Chi ZM (2013) Both decrease in ACL1 gene expression and increase in ICL1 gene expression in marine-derived yeast *Yarrowia lipolytica* expressing INU1 gene enhance citric acid production from inulin. Mar Biotechnol 15(1):26–36

Liu Y, Stuhldreier F, Kurtán T, Mándi A, Arumugam S, Lin W et al (2017) Daldinone derivatives from the mangrove-derived endophytic fungus Annulohypoxylon sp. RSC Adv 7(9):5381–5393

Lobarzewski J (1990) The characteristics and functions of the peroxidases from Trametes versicolor in lignin biotransformation. J Biotechnol 13(2–3):111–117

Lundell T, Makela M, Hilden K (2010) Lignin-modifying enzymes in filamentous basidiomycetes: ecological, functional and phylogenetic review. J Basic Microbiol 50:1–16

Magan N (2007) Fungi in extreme environments. In: Kubicek CP, Druzhinina IS (eds) Environmental and microbial relationships, The mycota IV, 2nd edn. Springer, Berlin, pp 85–103

Magan N, Lacey J (1984a) Effect of temperature and pH on water relations of field and storage fungi. Trans Br Mycol Soc 82:71–81

Magan N, Lacey J (1984b) Effect of water activity, temperature and substrate on interactions between field and storage fungi. Trans Br Mycol Soc 82:83–93

Mahé S, Rédou V, Calvez TL, Vandenkoornhuyse P, Burgaud G (2013) Fungi in deep-sea environments and metagenomics. In: Martin F (ed) The ecological genomics of fungi. Wiley., Hoboken, NJ, pp 325–354

Maheshwari R, Bharadwaj G, Bhat MK (2000) Thermophilic fungi: their physiology and enzymes. Microbiol Mol Biol Rev 64:461–488

Makhalanyane TP, Valverde A, Gunnigle E, Frossard A, Ramond JB, Cowan DA (2015) Ecología microbiana de los sistemas edáficos del desierto caliente. FEMS Microbiol Rev 39(2):203–221

Martin JF (2000) Molecular control of expression of penicillin biosynthesis genes in fungi: regulatory proteins interact with a bidirectional promoter region. J Bacteriol 182:2355–2362

Martin P, MacLeod RA (1984) Observations on the distinction between oligotrophic and eutrophic marine bacteria. Appl Environ Microbiol 47:1017–1022

Martinelli L, Zalar P, Gunde-Cimerman N, Azua-Bustos A, Sterflinger K, Piñar G (2017) Aspergillus atacamensis and A. salisburgensis: two new halophilic species from hypersaline arid habitats with a phialosimplex-like morphology. Extremophiles 21(4):755–773

Martínez AT, Speranza M, Ruiz-Dueñas FJ, Ferreira P, Camarero S, Guillén F et al (2005) Biodegradation of lignocellulosics: microbiological, chemical and enzymatic aspects of fungal attack to lignin. Int Microbiol 8:195–204

Mehta D, Satyanarayana T (2013) Diversity of hot environments and thermophilic microbes. In: Satyanarayana T, Satyanarayana T, Littlechild J, Kawarabayasi Y (eds) Thermophilic microbes in environmental and industrial biotechnology: biotechnology of thermophiles. Springer Science + Business Media, Dordrecht, pp 3–60

Melo RFR, Chikowski RS, Miller AN, Maia LC (2016) Coprophilous Agaricales (Agaricomycetes, Basidiomycota) from Brazil. Phytotaxa 266(1):001–014. https://doi.org/10.11646/phytotaxa.266.1.1

Menedez E, Garcia-Fraile P, Rivas R (2015) Biotechnological applications of bacterial cellulases. AIMS Bioeng 2:163–182

Meng LH, Zhang P, Li XM, Wang BG (2015) Penicibrocazines A–E, five new sulfide diketopiperazines from the marine-derived endophytic fungus Penicillium brocae. Mar Drugs 13(1):276–287

Mensah JK, Kwoseh C, Akuoko Y, Ali RB, Tawiah S, Anamoah C et al (2016) Assessment of the antimicrobial and antioxidant activities of the secondary metabolites produced by pure cultured Curvularialunata, Aspergillus parasiticus and Mucor spp. Curr Sci Perspect 2(4):95–104

Mesa V, Gallego JL, González-Gil R, Lauga B, Sánchez J, Méndez-García C et al (2017) Bacterial, archaeal, and eukaryotic diversity across distinct microhabitats in an acid mine drainage. Front Microbiol 8:1756

Meshram V, Saxena S, Paul K (2016) Xylarinase: a novel clot busting enzyme from an endophytic fungus *Xylariacurta*. J Enzym Inhib Med Chem 31:1–10

Messner K, Fackler K, Lamaipis P, Gindl W, Srebotnik E, Watanabe T (2003) Overview of white-rot research: where we are today. In: Goodell B, Nicholas DD, Schultz TP (eds) Wood deterioration and preservation. ACS, Washington DC, pp 73–96

Milagres AM, Sales RM (2001) Evaluating the basidiomycetes *Pariamedula-panis* and *Woljiporia cocos* for xylanase production. Enzym Microb Technol 28:522–526

Milala M, Shugaba A, Gidado A, Ene A, Wafar J (2005) Studies on the use of agricultural wastes for cellulase enzyme production by Aspergillus niger. Res J Agric Biol Sci 1:325–328

Moreno G, Alvarado P, Manjón JL (2014) Hypogeous desert fungi. In: Desert truffles. Springer, Berlin, pp 3–20

Moubasher AH, Abdel-Hafez SII, Bagy MMK, Abdel-Sater MA (1990) Halophilic and halotolerant fungi in cultivated, desert and salt marsh soils from Egypt. Acta Mycol 27(2):65–81

Moubasher AAH, Ismail MA, Hussein NA, Gouda HA (2015) Osmophilic/osmotolerant and halophilic/halotolerant mycobiota of soil of Wadi El-Natrun region, Egypt. J Basic Appl Mycol (Egypt) 6:27–42

Moubasher AAH, Ismail MA, Hussein NA, Gouda HA (2016) Enzyme producing capabilities of some extremophilic fungal strains isolated from different habitats of Wadi El-Natrun, Egypt. Part 2: Cellulase, xylanase and pectinase. Eur J Biol Res 6:103–111

Mouchacca J (1995) Thermophilic fungi in desert soils: a neglected extreme environment. In: Allsopp D, Colwell RR, Hawksworth DL (eds) Microbial diversity and ecosystem function. CAB International, Wallingford, UK, pp 265–288

Mouchacca J (1997) Thermophilic fungi: biodiversity and taxonomic status. Cryptogam Mycol 18:19–69

Mouchacca J (2000) Thermotolerant fungi erroneously reported in applied research work as possessing thermophilic attributes. World J Microbiol Biotechnol 16:869–880

Moustafa AF, Al-Musallam AA (1975) Contribution to the funal flora of Kuwait. Trans Br Mycol Soc 65(3):547–553

Mowll JL, Gadd GM (1985) Effect of vehicular lead pollution on phylloplane mycoflora. Trans Br Mycol Soc 84:685–689
Mtui G, Nakamura Y (2004) Lignin–degrading enzymes from mycelial cultures of basidiomycetes fungi isolated in Tanzania. J Chem Eng Jpn 37(1):113–118
Mudur SV, Swenson DC, Gloer JB (2006) Heliconols A–C: antimicrobial hemiketals from the freshwater aquatic fungus Helicodendrongiganteum. Org Lett 8:3191–3194
Mukherjee PK, Horwitz BA, Kenerley CM (2012) Secondary metabolism in *Trichoderma* – a genomic perspective. Microbiology 158:35–45
Murray PM, Moane S, Collins C, Beletskaya T, Thomas OP, Duarte AWF et al (2013) Sustainable production of biologically active molecules of marine based origin. New Biotechnol 30(6):839–850
Mutka SC, Bondi SM, Carney JR, Da Silva NA, Kealey JT (2006) Metabolic pathway engineering for complex polyketide biosynthesis in Saccharomyces cerevisiae. FEMS Yeast Res 6:40–47
Nagai K, Suzuki K, Okada G (1998) Studies on the distribution of alkalophilic and alkali-tolerant soil fungi II: Fungal flora in two limestone caves in Japan. Mycoscience 39:293–298
Nair MSR, Carey ST (1980) Metabolites of pyrenomycetes XIII: structure of (+) hypothemycin, an antibiotic macrolide from *Hypomyces trichothecoides*. Tetrahedron Lett 21:2011–2012
Nam KH, Chung HJ, Jeon EJ, Park MK, Yim YH, Liu JR et al (2007) In vitro biosynthesis of strictosidine using Lonicera japonica leaf extracts and recombinant yeast. J Plant Biol 50:315–320
Naranjo-Briceno L, Perniam B, Guerra M (2013) Potential role of oxidative exoenzymes of the extremophilic fungus Pestalotiopsis palmarum BM-04 in biotransformation of extraheavy crude oil. Microb Biotechnol 6:720–730. https://doi.org/10.1111/1751-7915.12067
Nazareth S, Gonsalves V (2014) Aspergillus penicillioides—a true halophile existing in hypersaline and polyhaline econiches. Ann Microbiol 64:397–402
Nazareth S, Gonsalves V, Nayak S (2012) A first record of obligate halophilic aspergilli from the Dead Sea. Indian J Microbiol 52:22–27
Neifar M, Maktouf S, Ghorbel RE, Jaouani A, Cherif A (2015) Extremophiles as source of novel bioactive compounds with industrial potential. In: Gupta VK, Tuohy MG, O'Donovan A, Lohani M (eds) Biotechnology of bioactive compounds: sources and applications. Wiley, Hoboken, NJ, pp 245–268
Nelson DL, Sturges DL (1982) A snowmold disease of mountain big sagebrush *Artemisia tridentatavaseyana*. Phytopathology 72:965
Nigam PS (2009) Production of bioactive secondary metabolites. In: Nigam PS, Pandey A (eds) Biotechnology for agro-industrial residues utilization, 1st edn. Springer, Netherlands, pp 129–145
Niyonzima F, More S (2014) Purification and properties of detergent-compatible extracellular alkaline protease from *Scopulariopsis* spp. Prep Biochem Biotechnol 44(7):738–759
Norred WP (1993) Fumonisins—mycotoxins produced by Fusarium moniliforme. J Toxicol Environ Health 38(3):309–328. https://doi.org/10.1080/15287399309531720
Olagoke OA (2014) Amylase activities of some thermophilic fungi isolated from municipal solid wastes and palm-kernel stack. Am J Microbiol Biotechnol 1:64–70
Oliveira LG, Dias LC, Rosso GB (2008) Total synthesis of crocacins A, C and D: new antibiotics isolated from Chondromycescrocatus and Chondromycespediculatus. Química Nova 31(4):854–871
Onofri S, de la Torre R, de Vera JP, Ott S, Zucconi L, Selbmann L et al (2012) Survival of rock-colonizing organisms after 1.5 years in outer space. Astrobiology 12:508–516
Owaid MN (2018) Bioecology and uses of the desert truffle (Pezizales) in the Middle East. Revista de Ciencia y Tecnología de Walailak (WJST) 15(3):179–188
Ozaydin B, Burd H, Lee TS, Keasling JD (2012) Carotenoid-based phenotypic screen of the yeast deletion collection reveals new genes with roles in isoprenoid production. Metab Eng 15:174–183
Paddon CJ, Westfall PJ, Pitera DJ, Benjamin K, Fisher K, McPhee D et al (2013) High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 496:528–553

Pallela R, Na-Young Y, Kim SK (2010) Anti-photoaging and photoprotective compounds derived from marine organisms. Mar Drugs 8(4):1189–1202

Pandit A, Maheshwari R (1996) Life-history of *Neurospora intermedia* in a sugar cane field. J Biosci 21:57–79

Park JH, Choi GJ, Lee HB, Kim KM, Jung HS, Lee SW et al (2005) Griseofulvin from *Xylaria* sp. strain F0010, an endophytic fungus of *Abies holophylla* and its antifungal activity against plant pathogenic fungi. J Microbiol Biotechnol 15:112–117

Parte S, Sirisha V, D'Souza J (2017) Chapter four-Biotechnological applications of marine enzymes from algae, bacteria, fungi, and sponges. Adv Food Nutr Res 80:75–106

Pedone-Bonfim MVL, da Silva FSB, Maia LC (2015) Production of secondary metabolites by mycorrhizal plants with medicinal or nutritional potential. Acta Physiol Plant 37:27

Pedro D, Ballester A, Munoz JA, Blazquez ML, Gonzalez F, Garcia C (2007) Dephosphoration of an iron ore by a filamentous fungus. Ouro Preto-MG 12(8):285–293

Pejin B, Maja K (2017) Antitumor natural products of marine-derived fungi. In: Mérillon JM, Ramawat KG (eds) Fungal metabolites. Springer International Publishing, Cham, pp 1–28

Petersen PM (1971) The macromycetes in a burnt forest area in Denmark. Botanisk Tiddskrift 66:228–248

Peterson SW (2008) Phylogenetic analysis of Aspergillus species using DNA sequences from four loci. Mycologia 100:205–226

Pettersson OV, Leong SL (2011) Fungal Xerophiles (Osmophiles). In: Enclyclopedia of life sciences. Wiley, Chichester, pp 1–12. https://doi.org/10.1002/9780470015902.a0000376.pub2

Picart P, Diaz P, Pastor FI (2007) Cellulases from two Penicillium sp. strains isolated from subtropical forest soil: production and characterization. Lett Appl Microbiol 45:108–113

Pimentel MR, Molina G, Dionísio AP, Maróstica Junior MR, Pastore GM (2011) The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol Res Int 2011:576286. https://doi.org/10.4061/2011/576286

Piontek K, Smith AT, Blodig W (2001) Lignin peroxidase structure and function. Biochem Soc Trans 29:111–116

Pitt JI (1981) Food spoilage and biodeterioration. In: Cole GT, Kendrick B (eds) Biology of Conidial fungi. Academic, New York, pp 111–142

Poch GK, Gloer JB (1991) Auranticins A and B: two depsidones from a mangrove isolate of the fungus *Preussia aurantiaca*. J Nat Prod 54:213–217

Poindexter JS (1981) Oligotrophy. Adv Microb Ecol 5:63–89

Pomilio AB, Battista ME, Vitale AA (2006) Naturally-occurring cyclopeptides: structures and bioactivity. Curr Org Chem 10(16):2075–2121. http://hdl.handle.net/20.500.12110/paper_13852728_v10_n16_p2075_Pomilio

Powell AJ, Parchert KJ, Bustamante JM, Ricken JB, Hutchinson MI, Natvig DO (2012) Thermophilic fungi in an aridland ecosystem. Mycologia 104:813–825

Preetha B, Viruthagiri T (2005) Biosorption of zinc (II) by Rhizopus arrhizus: equilibrium and kinetic modelling. Afr J Biotechnol 4(6):506–508

Prista C, Michán C, Miranda IM, Ramos J (2016) The halotolerant Debaryomyces hansenii, the Cinderella of non-conventional yeasts. Yeast 33(10):523–533

Priti V, Ramesha BT, Singh S, Ravikanth G, Ganeshaiah KN, Suryanarayanan TS et al (2009) How promising are endophytic fungi as alternative sources of plant secondary metabolites. Curr Sci 97:477–478

Pudhom K, Teerawatananond T (2014) Rhytidenones A–F, spirobisnaphthalenes from Rhytidhysteron sp. AS21B, an endophytic fungus. J Nat Prod 77(8):1962–1966

Qin XY, Yang KL, Li J, Wang CY, Shao CL (2015) Phylogenetic diversity and antibacterial activity of culturable fungi derived from the zoanthid Palythoahaddoni in the South China Sea. Mar Biotechnol 17(1):99–109

Raghukumar C, Muraleedharan U, Gaud VR, Mishra R (2004) Xylanases of marine fungi of potential use of bioleaching of paper pulp. J Ind Microbiol Biotechnol 31:433–441

Rahmat E, Kang Y (2019) Adventitious root culture for secondary metabolite production in medicinal plants: a review. J Plant Biotechnol 46:143–157

Rahmat E, Kang Y (2020) Yeast metabolic engineering for the production of pharmaceutically important secondary metabolites. J Appl Microbiol Biotechnol 104:4659–4674. https://doi.org/10.1007/s00253-020-10587-y

Rana S, Kaur M (2012) Isolation and screening of cellulase producing microorganisms from degraded wood. Int J Pharm Bio Sci 2(1):10–15

Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN et al (2019a) Endophytic fungi: biodiversity, ecological significance and potential industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer, Switzerland, pp 1–62

Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V et al (2019b) Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer International Publishing, Cham, pp 105–144. https://doi.org/10.1007/978-3-030-03589-1_6

Rana KL, Kour D, Yadav AN (2019c) Endophytic microbiomes: biodiversity, ecological significance and biotechnological applications. Res J Biotechnol 14:142–162

Rana KL, Kour D, Kaur T, Devi R, Negi C, Yadav AN et al (2020) Endophytic fungi from medicinal plants: biodiversity and biotechnological applications. In: Kumar A, Radhakrishnan E (eds) Microbial endophytes. Woodhead Publishing, Cambridge, MA, pp 273–305. https://doi.org/10.1016/B978-0-12-819654-0.00011-9

Raspor P, Zupan J (2006) Yeasts in extreme environments. In: Peter G, Rosa C (eds) The yeast handbook. Biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 371–417

Rastegari AA, Yadav AN, Yadav N (2019a) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5

Rastegari AA, Yadav AN, Yadav N, Tataei Sarshari N (2019b) Bioengineering of secondary metabolites. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 55–68. https://doi.org/10.1016/B978-0-444-63504-4.00004-9

Rastogi RP, Sinha RP, Singh SP, Häder DP (2010) Photoprotective compounds from marine organisms. J Ind Microbiol Biotechnol 37(6):537–558

Rateb ME, Ebel R (2011) Secondary metabolites of fungi from marine habitats. Nat Prod Rep 28(2):290–344

Ravikumar M, Bala Kumaran MD, Balashanmugam P (2014) Production of cellulase enzyme by *Trichoderma reesei* Cef19 and its application in the production of bio-ethanol. Pak J Biol Sci 17:735–739

Reczey K, Szengyel Z, Eklund R, Zacchi G (1996) Cellulase production by *Trichoderma reesei*. Bioresour Technol 57:25–30

Richardson MJ (2001) Diversity and occurrence of coprophilous fungi. Mycol Res 105(4):387–402

Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, De La Fuente G et al (1996) Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti. FEMS Microbiol Ecol 21:213–219

Rodriguez A, Strucko T, Stahlhut GS, Kristensen M, Svenssen KD, Forster J et al (2017) Metabolic engineering of yeast for fermentative production of flavonoids. Bioresour Technol 245:1645–1654

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101

Ruibal C, Platas G, Bills GF (2005) Isolation and characterization of melanized fungi from limestone in Mallorca. Mycol Prog 4:23–38

Russo G, Libkind D, Sampaio JP, VanBrock MR (2008) Yeast diversity in the acidic Río Agrio-Lake Caviahue volcanic environment (Patagonia, Argentina). FEMS Microbiol Ecol 65:415–424

Saha BC (2003) Hemicellulose bioconversion. J Ind Microbiol Biotechnol 30:279–291

Sahay H, Yadav AN, Singh AK, Singh S, Kaushik R, Saxena AK (2017) Hot springs of Indian Himalayas: potential sources of microbial diversity and thermostable hydrolytic enzymes. 3 Biotech 7:1–11

Saito I (1998) Non-gramineous hosts of *Myriosclerotinia borealis*. Mycoscience 39:145–153

Saleh H, Abdelrazak A, Elsayed AE, Osman Y (2018) Exploring antimicrobial potentials of melanin from a black yeast strain. J Biol Life Sci 9(2):24–38

Saravanakumar K, Senthilraja P, Kathiresan K (2013) Bioethanol production by mangrove-derived marine yeast, *Sacchromyces cerevisiae*. J King Saud Univ Sci 25(2):121–127

Saxena AK, Yadav AN, Rajawat M, Kaushik R, Kumar R, Kumar M et al (2016) Microbial diversity of extreme regions: an unseen heritage and wealth. Indian J Plant Genet Resour 29:246–248

Schmit JP, Shearer CA (2003) A checklist of mangrove-associated fungi, their geography and known host plants. Mycotaxon 80:423–477

Schwarze F (2007) Wood decay under the microscope. Fungal Biol Rev 21(4):133–170

Selim KA, Nagia MMS, El-Ghwas DE (2016) Endophytic Fungi are multifunctional biosynthesis: ecological and chemical diversity. In: Hughes E (ed) Endophytic fungi: diversity, characterization and biocontrol. Nova Science Publisher, New York, pp 39–91

Senthilraja P, Kathiresan K, Saravanakumar K (2011) Comparative analysis of biotethanol production by different strains of immobilized marine yeast. J Yeast Fung Res 8:113–116

Sert HB, Sterflinger K (2010) A new Coniosporium species from historical marble monuments. Mycol Prog 9:353–359

Shah AR, Madamwar D (2005) Xylanase production by a newly isolated *Aspergillus foetidus* strain and its characterization. Process Biochem 40:1763–1771

Shao CL, Wang CY, Gu YC, Wei MY, Pan JH, Deng DS et al (2010) Penicinoline, a new pyrrolyl 4-quinolinone alkaloid with an unprecedented ring system from an endophytic fungus *Penicillium* sp. Bioorg Med Chem Lett 20:3284–3286

Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanová L, Padgett D et al (2007) Fungal biodiversity in aquatic habitats. Biodivers Conserv 16(1):49–67

Shi Y, Zhang X, Lou K (2013) Isolation, characterization, and insecticidal activity of an endophyte of drunken horse grass, Achnatherum inebrians. J Insect Sci 13:151. https://doi.org/10.1673/031.013.15101

Shwab EK, Keller NP (2008) Regulation of secondary metabolite production in filamentous ascomycetes. Mycol Res 112:225–230

Siddiqui MS, Thodey K, Trenchard I, Smolke CD (2012) Advancing secondary metabolite biosynthesis in yeast with synthetic biology tools. FEMS Yeast Res 12:144–170

Sigoillot M, Brockhoff A, Lescop E, Poirier N, Meyerhof W, Briand L (2012) Optimization of the production of gurmarin, a sweet-taste suppressing protein, secreted by the methylotrophic yeast Pichia pastoris. Appl Microbiol Biotechnol 96:1253–1263

Silva GH, Teles LH, Trevisan CH, Bolzani SV, Young MC, Haddad R et al (2005) New bioactive metabolites produced by *Phomopsis cassiae*, an endophytic fungus in *Cassia spectabilis*. J Braz Chem Soc 16:1463–1466

Simões Calaça FJ, Xavier-Santos S, Abdel-Azeem AM (2020) Recent advances on occurrence of genus Chaetomium on dung. In: Abdel-Azeem AM (ed) Recent developments on genus Chaetomium, Fungal biology. Springer Nature, Switzerland, pp 143–159

Singh SM, Puja G, Bhat DJ (2006) Psychrophilic fungi from Schirmacher Oasis, East Antarctica. Curr Sci 90:1388–1392

Siridechakorn I, Yue Z, Mittraphab Y, Lei X, Pudhom K (2017) Identification of spirobisnaphthalene derivatives with anti-tumor activities from the endophytic fungus Rhytidhysteron rufulum AS21B. Bioorg Med Chem 25(11):2878–2882

Smith RN, Nadim LM (1983) Fungal growth on inert surfaces. Biodeterioration 5:538–547

Smitha SL, Correya NS, Philip R (2014) Marine fungi as a potential source of enzymes and antibiotics. Int J Res Mar Sci 3:5–10

Sohail M, Siddiqi R, Ahmad A, Khan SA (2009) Cellulase production from *Aspergillus niger* MS82: effect of temperature and pH. New Biotechnol 25:437–441

Song XH, Liu XH, Lin YC (2004) Metabolites of mangrove fungus No. K23 and interaction of carboline with DNA. J Trop Oceangr 23(3):66–71
Sonjak S, Frisvad JC, Gunde-Cimerman N (2006) Penicillium mycobiota in Arctic subglacial ice. Microbiol Ecol 52:207–216
Staley JT, Palmer FE, Adams JB (1982) Microcolonial fungi: common inhabitants on desert rocks? Science 215:1093–1095
Staley JT, Adams JB, Palmer FE (1992) Desert varnish: a biological perspective. In: Stotzky G, Bollag JM (eds) . Soil biochemistry, Marcel Dekker, New York, pp 173–195
Steffen KT, Hofrichter M, Hatakka A (2000) Mineralisation of 14C-labelled synthetic lignin and ligninolytic enzyme activities of litter-decomposing basidiomycetous fungi. Appl Microbiol Biotechnol 54:819–825
Sterflinger K, Krumbein WE (1997) Dematiaceous fungi as a major agent for biopitting on Mediterranean marbles and limestone. Geomicrobiol J 14:219–230
Sterflinger K, de Baere R, de Hoog GS, de Wachter R, Krumbein WE, Haase G (1997) Coniosporium perforans and C. apollinis, two new rock-inhabiting fungi isolated from marble in the Sanctuary of Delos (Cyclades, Greece). Antonie Van Leeuwenhoek 72:349–363
Sterflinger K, Tesei D, Zakharova K (2012) Fungi in hot and cold deserts with special reference to microcolonial fungi. Fungal Ecol 5(4):453–462
Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502
Sukumaran RK, Singhania RR, Pandey A (2005) Microbial cellulases production, applications and challenges. J Sci Ind Res 64:832
Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459
Taneja K, Gupta S, Kuhad RC (2002) Properties and application of a partially purified alkaline xylanase from an alkalophilic fungus Aspergillus nidulans KK-99. Bioresour Technol 85:39–42
Tansey MR, Brock TD (1972) The upper temperature limit for eukaryotic organisms. Proc Natl Acad Sci U S A 69:2426–2428
Thiele-Bruhn S, Bloem J, de Vries FT, Kalbitz K, Wagg C (2012) Linking soil biodiversity and agricultural land management. Curr Opin Environ Sustain 4(5):523–528
Tilburn J, Sarkar S, Widdick DA, Espeso EA, Orejas M, Mungroo J et al (1995) The Aspergillus PacC zinc finger transcription factor mediates regulation of both acid- and alkaline-expressed genes by ambient pH. EMBO J 14:779–790
Trantas E, Panopoulos N, Ververidis F (2009) Metabolic engineering of the complete pathway leading to heterologous biosynthesis of various flavonoids and stilbenoids in Saccharomyces cerevisiae. Metab Eng 11:355–366. https://doi.org/10.1016/j.ymben.2009.07.004
Traquair JA, Gaudet DA, Kokko EG (1987) Ultrastructure and influences of temperature on the *in vitro* production of *Coprinus psychromorbidus* sclerotia. Can J Bot 65:124–130
Trenchard IJ, Siddiqui MS, Thodey K, Smolke CD (2015) De novo production of the key branch point benzylisoquinoline alkaloid reticuline in yeast. Metab Eng 31:74–83
Tsuchiya T, Yamada K, Minoura K, Miyamoto K, Usami Y, Kobayashi T et al (2008) Purification and determination of the chemical structure of the tyrosinase inhibitor produced by Trichoderma viride strain H1-7 from a marine environment. Biol Pharm Bull 31:1618–1620
Tuomela M, Vikman M, Hatakka A, Itävaara M (2000) Biodegradation of lignin in a compost environment: a review. Bioresour Technol 72:169–183
Turchetti B, Buzzini P, Goretti M, Branda E, Diolaiuti G, D'Agata C et al (2008) Psychrophilic yeasts in glacial environments of Alpine glaciers. FEMS Microb Ecol 63:73–83
Uhlig H (1998) Industrial enzymes and their applications. Wiley, New York, p 435
Unal A (2015) Production of α-amylase from some thermophilic *Aspergillus* species and optimization of its culture medium and enzyme activity. Afr J Biotechnol 14:3179–3183
Urzì C, Wollenzien U, Criseo G, Krumbein WE (1995) Biodiversity of the rock inhabiting microbiota with special reference to black fungi and black yeasts. In: Allsopp D, Colwell

RR, Hawksworth DL (eds) Microbial diversity and ecosystem function. CAB International, Wallingford, UK, pp 289–302
Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human diseases. Int J Biochem Cell Biol 39(1):44–84
Verbruggen E, Van Der Heijden MG, Weedon JT, Gay K, Röling WF (2012) Community assembly, species richness and nesting of arbuscular mycorrhizal fungi in agricultural soils. Ecol Mol 21(10):2341–2353
Verma P, Yadav AN, Khannam KS, Mishra S, Kumar S, Saxena AK et al (2019) Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. Saudi J Biol Sci 26:1882–1895. https://doi.org/10.1016/j.sjbs.2016.01.042
Vieille C, Zeikus GJ (2001) Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. Microbiol Mol Biol Rev 65:1–43
Vlasenko E, Schulein M, Cherry J, Xu F (2010) Substrate specificity of family 5, 6, 7, 9, 12, and 45 endoglucanases. Bioresour Technol 101(7):2405–2411
Wagner KH, Elmadfa I (2003) Biological relevance of terpenoids: overview focusing on mono-, di- and tetraterpenes. Ann Nutr Metab 47:95–106
Wainwright M, Barakah F, M-Turk I, Ali AT (1992) Oligotrophic organisms in industry, medicine and the environment. Sci Progr 75:313–322
Wainwright M, Ali TA, Barakah F (1993) A review of the role of oligotrophic micro-organisms in biodeterioration. Int Biodeterior Biodegradation 31:1–13
Wang FW, Jiao RH, Cheng AB (2007) Antimicrobial potentials of endophytic fungi residing in Quercus variabilis and brefeldin A obtained from *Cladosporium* sp. World J Microbiol Biotechnol 23:79–83. https://doi.org/10.1007/s11274-006-9195-4
Wang CC, Chiang YM, Kuo PL, Chang JK, Hsu YL (2008) Norsolorinic acid from *Aspergillus nidulans* inhibits the proliferation of human breast adenocarcinoma MCF-7 cells via Fas-mediated pathway. Basic Clin Pharmacol Toxicol 102(6):491–497. https://doi.org/10.1111/j.1742-7843.2008.00237
Wang WL, Chi ZM, Chi Z, Li J, Wang XH (2009) Siderophore production by the marine-derived *Aureobasidium pullulans* and its antimicrobial activity. Bioresour Technol 100:2639–2641
Wang YT, Xue YR, Liu CH (2015) A brief review of bioactive metabolites derived from deep-sea fungi. Mar Drugs 13(8):4594–4616
Wang XW, Houbraken J, Groenewald JZ, Meijer M, Andersen B, Nielsen KF et al (2016) Diversity and taxonomy of *Chaetomium* and chaetomium-like fungi from indoor environments. Stud Mycol 84:145–224. https://doi.org/10.1016/j.simyco.2016.11.005
Weber D, Sterner O, Anke T, Gorzalczancy S, Martin V, Acevedo C (2004) Phomol, a new anti-inflammatory metabolite from an endophyte of the medicinal plant *Erythrina cristagalli*. J Antibiot 57:559–563
Webster J (1976) Aquatic fungi. Nature 261(5555):82–82
Westfall PJ, Pitera DJ, Lenihan JR, Eng D, Woolard FX, Regentin R et al (2012) Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin. Proc Natl Acad Sci U S A 109:111–118
Wicklow DT (1981) Biogeography and conidial fungi. In: Cole GT, Kendrick B (eds) The biology of conidial fungi. Academic, New York, pp 417–447
Wink M (2008) Plant secondary metabolism: diversity, function and its evolution. Nat Prod Commun 3:1205–1216
Woiciechowski AL, Porto de Souza Vandenberghe L, Karp SG et al (2013) The pretreatment step in lignocellulosic biomass conversion: current systems and new biological systems. In: Faraco V (ed) Lignocellulose conversion: enzymatic and microbial tools for bioethanol production. Springer, Berlin
Wollenzien U, de Hoog GS, Krumbein WE, Urzi C (1995) On the isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. Sci Total Environ 167:287–294

Wollenzien U, de Hoog GS, Krumbein W, Uijthof JMJ (1997) *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. Antonie Van Leeuwenhoek 71:281–288

Wong DW (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157:174–209

Wong KM, Goh T, Hodgkiss IJ et al (1998) Role of fungi in freshwater ecosystems. Biodivers Conserv 7:1187–1206

Wu RY (1993) Studies on the microbial ecology of the Tansui Estuary. Bot Bull Acad Sin 34:13–30

Wu B, Wu X, Sun M, Li M (2013) Two novel tyrosinase inhibitory sesquiterpenes induced by $CuCl_2$ from a marine-derived fungus *Pestalotiopsis* sp. Z233. Mar Drugs 11:2713–2721

Wu G, Yu G, Kurtan T, Mandi A, Peng J, Mo X et al (2015) Versixanthones A–F, cytotoxic xanthone–chromanone dimers from the marine-derived fungus *Aspergillus versicolor* HDN1009. J Nat Prod 78(11):2691–2698

Wu C, Zhao Y, Chen R, Liu D, Liu M, Proksch P, Lin W (2016) Phenolic metabolites from mangrove-associated *Penicillium pinophilum* fungus with lipid-lowering effects. RSC Adv 6(26):21969–21978

Wu ZH, Liu D, Xu Y, Chen JL, Lin WH (2018) Antioxidant xanthones and anthraquinones isolated from a marine-derived fungus *Aspergillus versicolor*. Chin J Nat Med 16(3):219–224. https://doi.org/10.1016/S1875-5364(18)30050-50

Xu X, De Guzman FS, Gloer JB, Shearer CA (1992) Stachybotris A and B: novel bioactive metabolites from a brackish water isolate of fungus *Stachybotrys* sp. J Org Chem 57:6700–6703

Xu MJ, Gessner G, Groth I, Lange C, Christner A, Bruhn T, Deng ZW, Li X, Heinemann SH, Grabley S, Bringmann G, Sattler I, Lin WH (2007) Shearing D–K, new indole triterpenoids from an endophytic *Penicillium* sp. (strain HKI0459) with blocking activity on large-conductance calcium-activated potassium channels. Tetrahedron 63:435–444

Yadav AN (2018) Biodiversity and biotechnological applications of host-specific endophytic fungi for sustainable agriculture and allied sectors. Acta Sci Microbiol 1:01–05

Yadav AN (2020) Recent trends in mycological research, Agricultural and medical perspective, vol 1. Springer, Switzerland

Yadav AN, Sharma D, Gulati S, Singh S, Dey R, Pal KK et al (2015) Haloarchaea endowed with phosphorus solubilization attribute implicated in phosphorus cycle. Sci Rep 5:12293. https://doi.org/10.1038/srep12293

Yadav AN, Verma P, Kumar V, Sachan SG, Saxena AK (2017) Extreme cold environments: a suitable niche for selection of novel psychrotrophic microbes for biotechnological applications. Adv Biotechnol Microbiol 2:1–4

Yadav AN, Verma P, Sachan SG, Kaushik R, Saxena AK (2018) Psychrotrophic microbiomes: molecular diversity and beneficial role in plant growth promotion and soil health. In: Panpatte DG, Jhala YK, Shelat HN, Vyas RV (eds) Microorganisms for green revolution, Microbes for sustainable agro-ecosystem, vol 2. Springer, Singapore, pp 197–240. https://doi.org/10.1007/978-981-10-7146-1_11

Yadav AN, Kour D, Rana KL, Yadav N, Singh B, Chauhan VS et al (2019a) Metabolic engineering to synthetic biology of secondary metabolites production. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 279–320. https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Yadav AN, Kour D, Sharma S, Sachan SG, Singh B, Chauhan VS et al (2019b) Psychrotrophic microbes: biodiversity, mechanisms of adaptation, and biotechnological implications in alleviation of cold stress in plants. In: Sayyed RZ, Arora NK, Reddy MS (eds) Plant growth promoting Rhizobacteria for sustainable stress management, Rhizobacteria in abiotic stress management, vol 1. Springer, Singapore, pp 219–253. https://doi.org/10.1007/978-981-13-6536-2_12

Yadav AN, Mishra S, Singh S, Gupta A (2019c) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019d) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019e) Recent advancement in white biotechnology through fungi, Perspective for sustainable environments, vol 3. Springer International Publishing, Cham

Yadav AN, Kaur T, Kour D, Rana KL, Yadav N, Rastegari AA et al (2020a) Saline microbiome: biodiversity, ecological significance and potential role in amelioration of salt stress in plants. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, pp 283–309. https://doi.org/10.1016/B978-0-12-820526-6.00018-X

Yadav AN, Rastegari AA, Yadav N (2020b) Microbiomes of extreme environments, Biotechnological applications in agriculture, environment and industry, vol 2. CRC Press, Taylor & Francis Group, Boca Raton, FL

Yadav AN, Rastegari AA, Yadav N (2020c) Microbiomes of extreme environments: biodiversity and biotechnological applications. CRC Press, Taylor & Francis, Boca Raton, FL

Yamamoto H, Kadzunori T, Uchiwa T (1985) Fungal flora of soil polluted with copper. Soil Biol Biochem 17:785–790

Yan XQ, Jian HY, Hui ZZ, Yang SS (2005a) Purification, elucidation and activities study of cytosporone B. J Xiamen Univ Nat Sci 44(3):425–428

Yan J, Song WN, Nevo E (2005b) A MAPK gene from Dead Sea fungus confers stress tolerance to lithium salt and freezing–thawing: prospects for saline agriculture. Proc Natl Acad Sci U S A 102:18992–18997

Yelle DJ, Ralph J, Lu FC, Hammel KE (2008) Evidence for cleavage of lignin by a brown rot basidiomycete. Environ Microbiol 10:1844–1849

Yin Z, Shi F, Jiang H, Roberts DP, Chen S, Fan B (2015) Phosphate solubilization and promotion of maize growth by *Penicillium oxalicum* P4 and *Aspergillus niger* P85 in a calcareous soil. Can J Microbiol 61(12):913–923. https://doi.org/10.1139/cjm-2015-0358

Zafar S, Aqil F, Ahmad I (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour Technol 98:2257–2561

Zalar P, de Hoog GS, Schroers H-J, Frank JM, Gunde-Cimerman N (2005) Taxonomy and phylogeny of the xerophilic genus Wallemia (Wallemiomycetes and Wallemiales, cl. et ord. nov.). Antonie Van Leeuwenhoek 87:311–328

Zalar P, de Hoog GS, Schroers H-J, Crous J, Groenewald JZ, Gunde-Cimerman N (2007) Phylogeny and ecology of the ubiquitous saprobe *Cladosporium shpaerospermum*, with descriptions of seven new species from hypersaline environments. Stud Mycol 58:157–183

Zalar P, Frisvad JC, Gunde-Cimerman N, Varga J, Samson RA (2008) Four new species of Emericella from the Mediterranean region of Europe. Mycologia 100:779–795

Zhang X, Liu Y, Yan K, Wu H (2007) Decolorization of an anthraquinone-type dye by a bilirubin oxidase-producing nonligninolytic fungus *Myrothecium* sp. IMER1. J Biosci Bioeng 104:104–114

Zhang D, Yang X, Kang JS, Choi HD, Son BW (2008) Circumdatin I, a new ultraviolet-a protecting benzodiazepine alkaloid from a marine isolate of the fungus Exophiala. J Antibiot 61:40

Zhang F, Huo Y, Cobb AB, et al (2018) *Trichoderma* biofertilizer links to altered soil chemistry, altered microbial communities, and improved grassland biomass. Front Microbiol. 9:848. https://doi.org/10.3389/fmicb.2018.00848

Zhang S, Fang H, Yin C, Wei C, Hu J, Zhang Y (2019) Antimicrobial metabolites produced by Penicillium mallochii CCH01 isolated from the gut of Ectropis oblique, cultivated in the presence of a histone deacetylase inhibitor. Front Microbiol 10:2186. https://doi.org/10.3389/fmicb.2019.02186

Zhou J, Diao X, Wang T, Chen G, Lin Q, Yang X et al (2018) Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species

Rhizophora stylosa and R. mucronata in the South China Sea. PLoS One 13(6):e0197359. https://doi.org/10.1371/journal.pone.0197359

Zhu Y, Dong J, Wang L et al (2008) Screening and isolation of antinematodal metabolites against Bursaphelenchus xylophilus produced by fungi. Ann Microbiol 58:375–380

Žifčáková L, Vetrovský T, Howe A, Baldrian P (2016) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environ Microbiol 18(1):288–301

Chapter 2
Fungi as a Gold Mine of Antioxidants

Hebatallah H. Abo Nahas, Amira Mohamed Galal Darwish, Yousef H. Abo Nahas, Mervet. A. Elsayed, Mohamed A. Abdel-Azeem, and Ahmed M. Abdel-Azeem

Contents

H. H. Abo Nahas (✉)
Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

A. M. G. Darwish
Food Technology Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt

Y. H. Abo Nahas
Microbiology Department, Faculty of Medicine, Helwan University, Cairo, Egypt

M. A. Elsayed · A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

M. A. Abdel-Azeem
Faculty of Pharmacy and Pharmaceutical Industries, Sinai University, Al Arish, North Sinai, Egypt

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_2

2.1 Introduction

There has been a current upsurge in the medical implications of free radicals and related species during the past several decades. These chemical species are integral components produced during normal biochemical and physiological processes but lead to oxidative stress when produced in excess and cause potential damage to cells. A wide range of nonenzymatic and enzymatic antioxidant defenses exists to counteract the damaging effects of free radicals. There exist epidemiological evidence correlating higher intake of antioxidant-rich foodstuffs with greater free radical neutralizing potential to lower incidence of several human morbidities or mortalities. Novel biomolecules and the use of functional foods enriched with antioxidants are milestones to newer approaches to reduce free radical damage. So, the putative antioxidants from different sources play a significant role in controlling oxidative stress and reduce the incidence of concerned diseases (Kalam et al. 2012).

One of the exciting moves in microbial sciences has been to refocus and revitalize efforts to mine the secondary fungal metabolome (SMs) and natural products (NPs). The magnitude of biosynthetic gene clusters (BGCs) combined with the historical number of sequenced genomes in a single filamentous fungal genome suggests that the secondary metabolite wealth of filamentous fungi is largely untapped. Mining algorithms and scalable expression platforms have greatly increased access to the chemical repertoire of secondary metabolites derived from fungi (Keller 2019).

Fungi have a long and intimate relationship with the human, especially on the chemical level. The realization that fungi were the source of both dangerous and beneficent compounds was brought to light in the 1960s by the aflatoxin poisoning case Turkey X disease, and the discovery of the first wide-spectrum antibiotic, penicillin, was considered World War II2's wonder drug (Devi et al. 2020; Yadav 2019; Yadav et al. 2018, 2019a). These bioactive molecules may be fungal secondary metabolites or primary metabolite (natural products NPs), these metabolites are produced by specific fungal taxa principally by filamentous fungi belonging to the Pezizomycotina, Ascomycete class, and many Basidiomycete classes (viz., Agaricomycetes and Exobasidiomycetes), additionally by unexpected taxa such as *Kluyveromyces lactis*, in which pulcherrimin gene recently located (Keller 2019; Yadav et al. 2019b).

Secondary metabolites are derived from core metabolic pathways and primary metabolite reservoirs, with acyl-CoAs being the essential initial building blocks that feed into polyketide synthesis (e.g., aflatoxin) and terpene (e.g., carotene) secondary metabolites and amino acids that are used to synthesize non-ribosomal secondary metabolites, for example, penicillin. In contrast to genes that are required for the synthesis of a primary metabolite that is dispersed throughout the fungal genome (Krause et al. 2018; Rastegari et al. 2019a, b). About 1500 compounds have been isolated from fungi between 1993 and 2001. More than half of them exhibited antifungal, antibacterial, or antitumor activity was discovered between 2009 and 2013 confirmed as prospective fungal secondary metabolites (Schueffler and Anke 2014).

Bioprospecting is described as the search for naturally occurring chemical compounds and biological material, especially in extreme harsh habitats under different stressors or biodiversity-rich environments (Abdel-Azeem et al. 2012; Yadav et al. 2020). There are many lines of proof supporting ecological fitness roles for fungal secondary metabolites, involving the assessment of regulatory cascades. These studies showed that many genes that encode secondary metabolites are regulated in a manner congruent with fungal development or in response to stressors (both abiotic and biotic) and that overproduction or loss will alter the specific fungus development and survival (Keller 2019).

Fungi are the most versatile and plentiful group on the planet and since they mimic the animal system, they are an excellent candidate for the development of bioactive metabolites (Kour et al. 2019). Molecular test methods recently suggested there were up to 5.1 million fungal species, of which only around 100,000 have been recorded in the literature. They involve 14,000 mushroom species, 5000 macro fungi, and more than 1800 fungi with medicinal and pharmacological therapeutic properties (Kirk et al. 2001; Abdel-Azeem et al. 2021). The different fungal kingdoms are classified into many phyla, including Ascomycota (sac fungi) and Basidiomycota (club fungi), Zygomycota and Blastocladiomycota and Chytridiomycota. Most pharmacological and therapeutic studies focus solely on Ascomycota and Basidiomycota strains or species (Cui et al. 2015), while Zygomycota is relatively less investigated. However, the majority of those in the diverse, high-antioxidant capacity, the secondary metabolite-rich endophytic community of fungi also belong to the divisions Ascomycota and Basidiomycota, with few members known as *Mucor* and *Umbelopsis* of the genera Zygomycota (Cui et al. 2015). The adaptation and morphogenesis of host plants by protecting against insects, predators, microbial pathogens, and latent pathogens are being played an important role by endophytic fungi. Similarly, Mucorales of Zygomycota is known as the Entomophthoral (pathogens of insects) sister group, Mucorales' pathogenic nature is believed to be caused mainly by endocellular excretions and the development of subtilizers, chitinases, proteinases, and antioxidant proteins (e.g., superoxide dismutase, catalase, and peroxidase) (Freimoser et al. 2003).

2.2 Oxidative Stress: Basic Overview

Oxidation is essential for many living organisms to produce energy to fuel biological processes (Yang et al. 2002). Oxygen is a highly reactive atom that is able to become part of massively damaging molecules commonly called "free radicals". Free radicals are capable of attacking the healthy cells of the body. Cellular destruction is caused by free radicals, in the form of reactive nitrogen species (RNS) or mainly in the form of reactive oxygen species (ROS) during normal metabolic processes in aerobic cells. Free radicals derived from molecular oxygen are the most important class of radical species formulated in living systems (Miller et al. 1990). A free radical is categorized as any atom or molecule with unpaired electrons in the

outer orbit, which are usually unstable and very reactive (Gutteridge and Halliwell 2000). Oxidative stress is known as an imbalance in free radical production, as a result of overproduction of ROS or loss of natural antioxidant defenses. Overproduction can contribute to lipid, DNA, or protein oxidation, and is a major contributor to aging (Barja 2004), degenerative diseases such as cancer (Valko et al. 2006b), cardiovascular disease (Shah and Channon 2004), compromised immune function, inflammation, and renal failure (Valko et al. 2006a). For the past 40 years, oxidative stress has been progressively recognized as a contributing factor in aging and different forms of pathophysiology generally associated with aging (Hybertson et al. 2011).

Oxidant signals interfere in aerobic cells with the otherwise usual natural metabolic activity of ROS, such as; cell proliferation, differentiation, and apoptosis. When such damage occurs, there is cell toxicity leading to health ramifications (Suzuki et al. 2010). Mitochondria are often the first victim of a free radical attack because the lipid membrane is very susceptible to ROS degradation; this attack is called lipid peroxidation. ROS interaction with lipid molecules produces new free radicals; superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydrogen radicals ($OH^{\bullet}$). Then, these radical groups interact cytotoxically with biological systems (Barros et al. 2007). The relationship between antioxidant defense and ROS production is usually a representation of the degree of oxidative stress (Suzuki et al. 2010). Different free radicals involved in oxidative stress have different biological, chemical, and physical properties, including hydroxyl, alkoxyl, peroxyl, superoxide, nitric oxide, sulfur, and nitrogen-centered radicals (Niki 2010). The free radical scavenging capacity is influenced by several factors; the rate and number of radical molecules scavenged and the fate of antioxidant-derived radicals. The fate of this radical is also an important consideration of antioxidant efficacy (Niki and Noguchi 2000). There are many factors that impact the free radical scavenging capacity like, interaction with other antioxidants, concentration, mobility in the environment, and the adsorption, distribution, retention, and metabolism of the antioxidant compounds, the rate and number of radical molecules scavenged (Niki and Noguchi 2000; Niki 2010). When an active radical is scavenged by an antioxidant compound, a stable non-radical product is formed. At the same time the antioxidant yields one antioxidant-derived radical. The fate of this radical is also a significant consideration of antioxidant effectiveness (Niki and Noguchi 2000) (Fig. 2.1).

2.2.1 Oxidative Stress as "Mother" of Many Human Diseases at Strong Clinical Impact

Oxidative stress can be considered "mother" of numerous human diseases, which are life-threatening. Oxidative stress is a condition in which oxidation surpasses the antioxidant reactions, causing an imbalance between oxidative and antioxidant

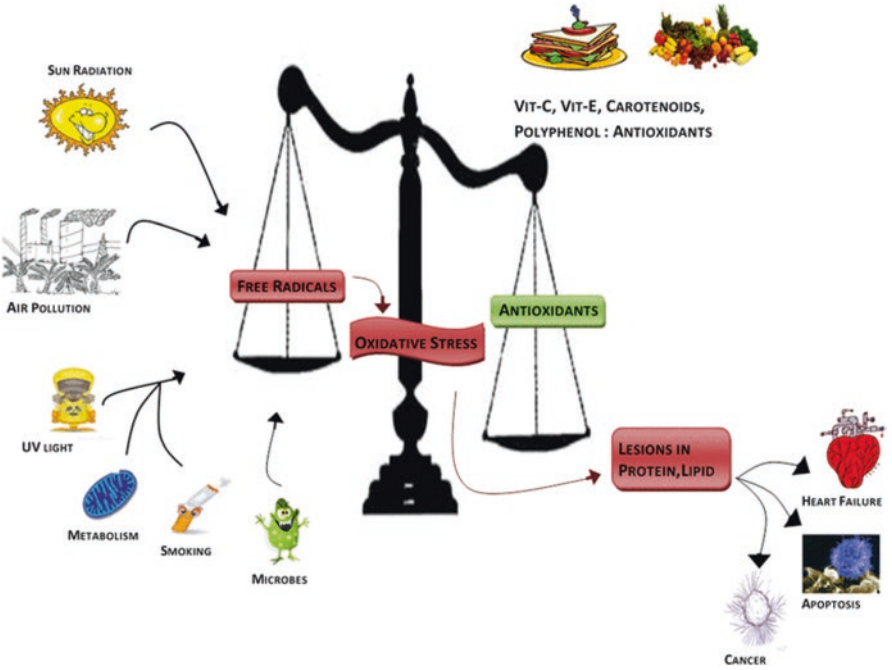


Fig. 2.1 Oxidative modifications of biomolecules. In normal physiological conditions, and how it ultimately causes various diseases. (Adapted from http://robinthomas.biz/so-what-causes-oxidative-stress-anyway/)

systems, with the predominance of reactive oxygen species ROS. Under typical conditions, ROS are kept up at physiological levels by many endogenous antioxidant systems, as superoxide dismutase, glutathione peroxidases, catalase, lactoperoxidases, glutathione reductase, and others. However, if active ROS are massively created, the balance between the formation and the removal of these species is lost. Resulting oxidative damage can be generated from both endogenous and exogenous sources. Endogenous ROS are produced in normal metabolic reactions. Exogenous ROS derive by exposure to environmental pollutants, cigarette smoke, and consumption of alcohol in excess, exposure to ionizing radiations, viral and bacterial infections, and others as mention before. Individual, hereditary factors, and lifestyle are the major determinants of oxidative stress. So, we are going to refer to some pathology favored by detrimental impacts of ROS, responsible for morbidity and death (Cacciapuoti 2016).

Aging is a process characterized by the progressive depletion of organ and tissue function. The oxidative stress theory of aging is based on the hypothesis that age-associated functional losses are due to the accumulation of ROS-induced damages. Simultaneously, oxidative stress is included in many age-related conditions (viz., cardiovascular diseases (CVDs), chronic kidney disease, neurodegenerative

diseases, chronic obstructive pulmonary disease, and cancer), including frailty and sarcopenia. Various types of oxidative stress biomarkers have been recognized and may provide significant information about the effectiveness of the treatment, guiding the selection of the most effective drug for patients and, if especially relevant from a pathophysiological point of view, acting on a particular therapeutic target. Given the significant role of oxidative stress is the pathogenesis of clinical conditions and aging (Liguori et al. 2018). The cancer-induction is a multifactorial process that involves many factors, as physical, chemical, genetic, and environmental factors. Recent knowledge's in ROS biology elucidate that free radicals control different features of tumor development involving inflammation, transformation, survival, proliferation of cancers' cells, invasion, angiogenesis, and metastasis (Waris and Ahsan 2006). Particularly, free radicals directly or indirectly act, via DNA damage, on gene expression and signaling at the cellular levels. In progression, the major impacts of ROS on tumor genesis and some clinical complications (Cacciapuoti 2016).

Youn et al. (2014) hypothesized that ROS produced in vascular smooth muscle cells (VSMC) play a significant role in the development of obesity, causing a condition of overweight due to glucose intolerance, leptin resistance, and inflammation. On the other hand, the expansion of visceral adipose tissue caused by overconsumption of food, increases visceral adipose tissue. As visceral fat stores expand, adipocytes generate increased ROS levels and metabolic syndrome. Therefore, two conditions (oxidative stress and obesity) can be considered correspondingly as cause and impact one of another. Indication loss in nerve structure and function leads to progressive brain damage and neurodegeneration as a complication of neurogenerative diseases. Apart from environmental or genetic factors, oxidative stress mainly contributes to neuro degeneration. Especially, ROS have been implicated in the progression of Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). Cacciapuoti (2016), in the case of Parkinson's disease, felt that majority of cases of PD is idiopathic. Exposure to some substances (e.g., pesticides, toxins, organic solvent) is viral and bacterial (Gomez-Cabrera et al. 2005). Oxidative stress also intervenes in other pathologic conditions commonly occurring among human diseases, such as chronic obstructive pulmonary disease, chronic fatigue syndrome, and skin disease (Cacciapuoti 2016).

2.3 Meet Your Free Radical Surveyors: Antioxidants

Antioxidants are a wide group of compounds that constitute the primary line of defense against free radical stress. They are fundamental for keeping up ideal well-being as defensive operators that can deactivate or stabilize free radicals (Kalam et al. 2012). Free radical damage complication and ramification raises the require

for admissions of antioxidants, free radical hoist due to contamination, cigarette smoke, ailment, and therapeutics operators. Recognizable proof of pharmacologically potential antioxidants expanded staggeringly as they show no side impact for us in preventive medication and food industry.

Antioxidants are a family of compounds considered to be the leading endeavor against a number of age-related issues such as Alzheimer and many diseases. Termed as a wonder element; antioxidants are basic to great health and well-being as the concept of well-being enhancement has ended up a genuine portion of healthcare. The capacity to utilize oxygen has given people the advantage of metabolizing fats, proteins, and carbohydrates for vitality (Percival 1998). Humans have advanced an exceedingly complicated antioxidant defense technique to combat the harmful impacts of free radicals. These incorporate both endogenous and exogenous components, which work with intelligence and synergistically to neutralize free radicals (Liebler 1993). These components are (1) Antioxidant chemicals (Superoxide Dismutase, Glutathione peroxidase, and Catalase), (2) Metal official proteins (Ferritin, Lactoferrin, Egg whites, and Ceruloplasmin), (3) Nutrient-derived antioxidants (Ascorbic corrosive, Tocopherols, Tocotrienols, Carotenoids, Glutathione, and Lipoic corrosive), and (4) Phytonutrients (Flavonoids, Phenolic corrosive, Stilbenes, Tannins, and Carotenoids). The sum of antioxidants is calculated as ORAC values, that is, Oxygen Radical Absorbance Capacity, which could be a degree of the capacity of nourishments to stifle hurtful oxygen free radicals that can harm the human body.

The ORAC values for two glasses of dark tea are proportionate to that of one glass of ruddy wine or seven glasses of orange juice or consuming 20 glasses of apple juice. In addition to, dark chocolate snack within a balanced diet can enhance DNA resistance to oxidative stress in healthy subjects (Spadafranca et al. 2010). Antioxidants are intimately elaborated in the prevention of cellular damage the general pathway for cancer, aging, and different disease. DNA damage and other cellular organelles by free radicals occupy the highest position in the onset and development of diseases. For instance, Atherosclerosis and its complexity most particularly Coronary Heart Disease (CHD) continue to be the main cause of premature death in the developing world. Polyunsaturated fatty acid residues in lipoproteins have a chemical configuration that makes them a particularly vulnerable target for free radical oxidation. Studies have illustrated an inverse relationship between vitamin C intake and cardiovascular disease mortality in humans. In hypercholesterolemic persons, treatment with a combination of vitamin E and slow-release vitamin C slows down atherosclerotic progression.

On the other hand, antioxidants in plant cells mainly include glutathione, tocopherol, ascorbate, betaine, proline, and others, which are also information-rich redox buffers and important redox signaling components that interact with cellular organelles. As an unfortunate ramification of aerobic life for higher plants, ROS are

formed by partial reduction of molecular oxygen. The enzymatic and nonenzymatic antioxidants in higher plant cells can protect their cells from oxidative damage by scavenging ROS. Thus, a special investigation is given to ROS and ROS-antioxidant interaction as a metabolic interface for various types of signals derived from metabolisms and the changing environment. Besides exacerbating cellular damage, ROS act as ubiquitous signal molecules in higher plants as well as a central ingredient in stress responses (Kalam et al. 2012). Other than the health advancing impacts from the utilization of natural antioxidants, avoidance of oxidation is vital in other areas too. Within the nourishment industry, oxidation influences the dietary esteem of food and can cause rancidity of color, flavor, and texture. Microbial decay may be a huge issue for nourishment producers. Customarily salt, sulfite, and antimicrobial compounds have been used to restrain the development of microorganisms in food items; in any case, usually not perfect. Moreover, utilization of synesthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyltoluene (BHT) are presently known to have a negative effect on health (Branen 1975; Larson 1988).

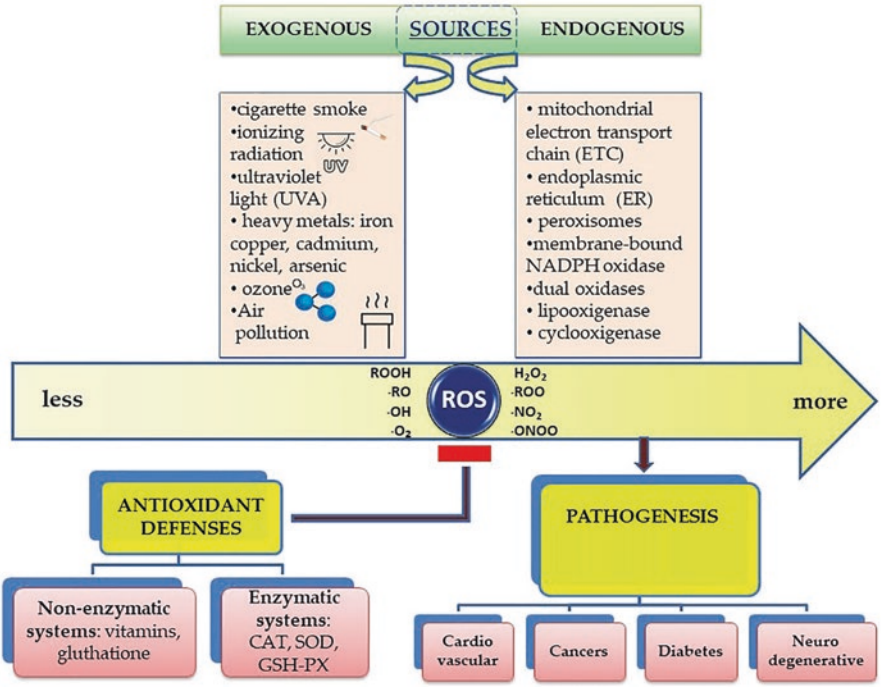


Fig. 2.2 The sources of free radicles and their effects on human body (Kalam et al. 2012)

With expanding consumer awareness of food preparation, controversy has surrounded the use of synthetic antioxidants like, BHT and BHA as food additives, which lead to a particular interest in safe preservatives from natural sources. A wide extent of additives can be utilized alone or as a mix. A mix can create a less costly item without compromising proficiency. For example, tocopherols may be mixed with another prevalent antioxidant, such as rosemary extract. The food preservation market is expected to reach €2 billion by 2018, in spite of the fact that natural preservatives are projected to contribute to the smallest share of the markets. The European market alone is extended from €79 million, recorded in 2011 to €188 million by 2018. With consumer perception with respect to the benefits of natural additives and health hazards related with consumption of foods preserved with the utilization of nourishments protected chemically, natural preservatives, such as antioxidants are in demand (Smith 2014). New trends launched lately depend on using fungi as a potential source of antioxidants that can be utilized to prevent oxidative damage and, as such, can decrease their harmful impacts on humans and animals alike (Smith 2014) (Fig. 2.2).

2.4 Antioxidant Classification

Antioxidants can be classified into two major types based on their source, that is, natural and synthetic antioxidants (schematic representation of the classification of antioxidants is shown in Fig. 2.3).

2.4.1 Natural Antioxidants

Either is synthesized in the human body through a metabolic process or is supplemented from other characteristic sources and their action depends very much upon their physical and chemical properties and component of action. This could be advance separated into two categories, that is, enzymatic antioxidants and nonenzymatic antioxidants (Mamta et al. 2013).

2.4.2 Enzymatic Antioxidants

Enzymatic antioxidants are uniquely produced in the human body and can be subdivided into primary and secondary antioxidants (Mamta et al. 2013) (Fig. 2.4).

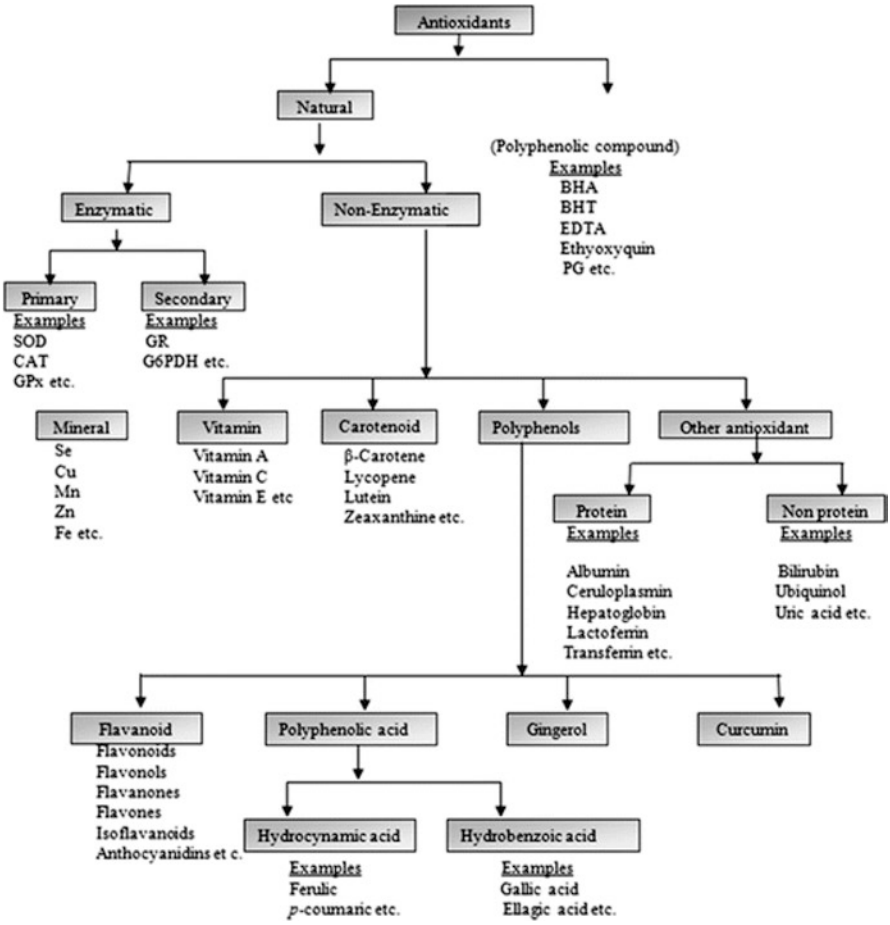


Fig. 2.3 Schematic representation of antioxidants classification (Mamta et al. 2013)

2.4.2.1 Primary Antioxidants

Primary antioxidants basically include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) as portrayed below. Superoxide dismutase (SOD) enzyme is found in both the dermis and the epidermis. It expels the superoxide radical (O_2^-) and repairs the body cells harmed by free radicals. SOD catalyzes the reduction of superoxide anions to hydrogen peroxide. SOD is additionally known to compete with nitric oxide (NO) for superoxide anion, which inactivates NO to create peroxynitrite. Subsequently, scavenging superoxide anions promotes the activity of NO (Chakraborty et al. 2009).

$$2O_2^{??} + 2H \overset{SOD}{?} H_2O + O_2$$

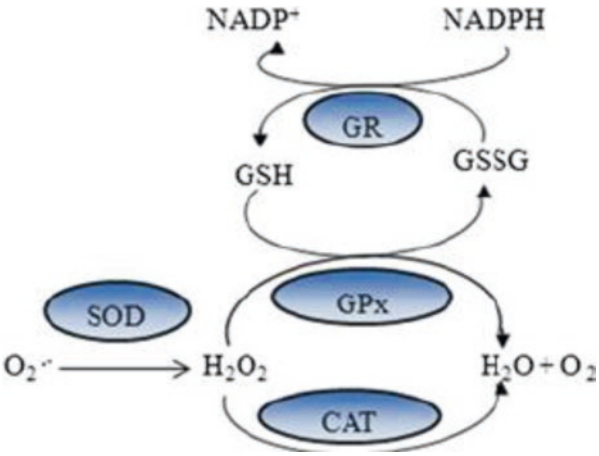


Fig. 2.4 Outline of the mechanism of enzymatic antioxidants in the removal of free radicals (Mamta et al 2013)

Catalase enzyme (CAT) is found within the blood and most of the living cells and breaks down H_2O_2 into water and oxygen. Catalase besides glucose peroxidase is additionally utilized commercially for the conservation of the natural product juices, a cream consisting of egg yolk and serving of mixed greens by evacuating the oxygen (Chakraborty et al. 2009).

$$2H_2O_2 \overset{CAT}{?} 2H_2O + O_2$$

Glutathione peroxidase (GPx) could be a gather of selenium-dependent enzymes, and it comprises cytosolic, plasma, phospholipid hydroperoxide, and gastrointestinal glutathione peroxidase. GPx (cellular and plasma) catalyzes the response of H_2O_2 by decreased glutathione (GSH); as a result, oxidized glutathione (GSSG) is delivered and it is once more restored to its reduced form by glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Chakraborty et al. 2009).

$$2GSH + H_2O_2 \overset{GPx}{?} GSSG + 2H_2O$$

2.4.2.2 Secondary Antioxidant

Secondary antioxidant incorporates glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH). G6PDH creates NADPH. GR is required to reuse the reduced glutathione (GSH) utilizing secondary enzyme GR and NADPH.

$$GSSG + NADP \overset{GR}{?} NADP + 2GSH$$

Glutathione is a cysteine-containing peptide-type antioxidant and is synthesized in the body cells. The thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. An elevated level of glutathione was found within the cells (~3100 μg/g of tissue), kept up within the reduced form (GSH) by the protein GR and in turn decreases other metabolites and enzyme systems, such as ascorbate. Due to its elevated concentration and its part in maintaining the redox state within the cells, it is considered one of the foremost critical cellular antioxidants (Hissin and Hilf 1976).

2.4.3 Nonenzymatic Antioxidants

These are a class of antioxidants that are not normally present in the body, but must be included in the diet for proper metabolism. Vitamins, carotenoids, minerals, polyphenols, and other antioxidants are some of the identified nonenzymatic antioxidants as mentioned below (Veysi et al. 2007).

2.4.3.1 Minerals

Minerals are needed for the proper functioning of the enzymes in the body cells. It is understood that their absence affects the metabolism of several macromolecules. Minerals include selenium, copper, iron, zinc, manganese, and so on. They serve as cofactors for the antioxidant enzymes. Iron (Fe) is the most common trace metal used in the biological system to bind to the protein. The free iron concentration is naturally very small, and small iron-binding protein concentrations promote ROS formation, lipid peroxidation, and oxidative stress (Dabbagh et al. 1994). The intake of iron thus helps to reduce oxidative stress. Magnesium (Mg) is a glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) cofactor associated with pentose cycle that catalyzes NADPH production during glucose metabolism and thus maintains a proper GSH to GSSG ratio and a normal redox state in cells. Magnesium deficiency decreases GR activity and GSSG aren't reduced to GSH, thereby resulting in oxidative cell damage (Fang et al. 2002). Selenium (Se) is also an essential enzymatic antioxidant element. In the existence of selenium (Se), glutathione peroxidase (GPx) provides excellent protection against lipid oxidation and serves to protect the cell membrane and participates in the metabolism of H_2O_2 and hydroxyperoxide of lipids. (Se) thus acts like vitamin E and can be supplemented with vitamin E and is used to reduce cancer risk and cardiovascular disease (Sikora et al. 2008). Copper (Cu), Zinc (Zn), and Manganese (Mn) SOD is an enzyme class consisting of different forms of SODs, based on their metal cofactor including Cu–Zn and Mn. Cu–Zn SOD is located in the cytosol with

Cu and Zn at their active sites that aid in the conduction of protons, while Mn-SOD is located in the mitochondria and has Mn at their active site. Those metals are the source of antioxidant activities of SOD (Mamta et al. 2013).

2.4.3.2 Vitamins

Vitamins are the micronutrient class necessary to act properly in the body's antioxidant enzyme system, such as vitamin A, vitamin C, vitamin E, and vitamin B. They are not synthesizable in our body and therefore have to be replaced in the dietary intake (Rastegari et al. 2020). Vitamin A helps to improve night vision and epithelial cells in the mucous membranes and skin. This also strengthens the immune system because of its antioxidant properties and is present in three primary forms: retinol, 3,4-didehydroretinol, and 3-hydroxyretinol. Sweet potatoes, carrots, milk, egg yolks, and mozzarella cheese are the principal sources of vitamin A. Vitamin C is soluble in water and is also known as ascorbic acid. It is found in fruits (mostly citrus), vegetables, cereals, beef, poultry, and fish, and so on. It helps to avoid some of the DNA damage caused by free radicals, which can lead to the aging process and disease progressions, such as cancer, heart disease, and arthritis. Vitamin E is a vitamin that is soluble in lipids. It consists of eight various forms such as α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol. It is most common in almonds, safflower oil, soybean oils, wheat germ oil, nuts, broccoli, fish oil, and so on, α-tocopherol exhibits the highest bioavailability and is the most significant lipid-soluble antioxidant that reacts with the lipid radical and preserves the membranes from lipid peroxidation; as a result, oxidized α-tocopheroxyl radicals are generated, which can be restored to the reduced form through reduction by many other antioxidants, such as ascorbate and retinol (Mamta et al. 2013).

2.4.3.3 Carotenoid

Carotenoid is formed of β-carotene, lycopene, lutein, and zeaxanthin. They are colored compounds found in fruits and vegetables that are fat-soluble. β-Carotene is mostly found in radish-orange-green foods, including carrots, sweet potatoes, apricots, pumpkin, mangoes and cantaloupe, together with some green and leafy vegetables, such as spinach and kale. In green leafy vegetables such as collard greens, spinach, and kale, lutein is prevalent (Hamid et al. 2010). Lutein is best known to play a role in protecting the retina from dangerous free radical activity and also helps to prevent atherosclerosis (Sikora et al. 2008). While there is no provitamin A production in lycopene, lutein, canthaxanthin, and zeaxanthin, β-carotene is known as a precursor to vitamin A (Fang et al. 2002). Tomatoes are a good source of lycopene and zeaxanthin is a good source of spinach. Lycopene is a powerful antioxidant and is the most effective compound contained in strawberries, watermelon, guava, papaya, apricots, pink grapefruit, and other foods to eliminate singlet oxygen (Mamta et al. 2013).

2.4.3.4 Polyphenols

Polyphenols are a class of phytochemicals with notable antioxidant activity. Their antioxidant activities rely on our physical and chemical characteristics, which depending on their molecular structures, manage the metabolism (Ajila et al. 2011). These include phenolic acids, flavonoids, gingerol, curcumin, and so on (Kunwar and Priyadarsini 2011). Flavonoid is a significant component of polyphenolic compound and is found mainly in vegetables, fruits, grains, seeds, leaves, flowers, bark, and many more. Many of the spices, such as ginger and turmeric, are also excellent sources of polyphenolic compounds, for example, gingerol is derived from ginger rhizomes, whereas curcumin (diferuloylmethane) is the primary bioactive component of turmeric and is considered to have strong antioxidant activity. Curcumin is an outstanding scavenger of ROS, such as $O_2^{\bullet -}$ radicals, lipid peroxyl radicals ($LO_{2^{\cdot}}$), OH radicals and nitrogen dioxide ($NO_{2^{\cdot}}$) radicals that triggered oxidative stress. Curcumin has also been proven to prevent lipid peroxidation and GSH levels have also been shown to rise in epithelial cells leading to lower ROS development (Biswas et al. 2005).

2.4.3.5 Other Antioxidants

Albumin, ceruloplasmin, haptoglobin, and transferrin are the metal-binding transition proteins present in human plasma, binding with transition metals and controlling the development of metal-catalyzed free radicals. Albumin and ceruloplasmin are sequesters of copper ions, haptoglobin is a sequester of hemoglobin, and transferrin serves as the free iron sequester. Nonprotein antioxidants such as bilirubin, uric acids, and ubiquinol are antioxidants that prevent oxidation by scavenging free radicals (Papas 1998). Bilirubin is a catabolism final product of heme. It is a cytotoxic outcome that is lipid-soluble and must be eliminated. Nevertheless, bilirubin effectively scavenges peroxyl radical at in vitro model, micromolar concentrations (Stocker et al. 1987) and is considered the strongest antioxidant against lipid peroxidation. Uric acid is an effective antioxidant and a scavenger of singlet oxygen and radical substances. Urate reduces the oxidant developed by peroxide reaction with hemoglobin and prevents erythrocytes from peroxidative effects. Human plasma urate levels are about 300 μM, making it one of the most important antioxidants in humans (Ames et al. 1981). Coenzyme Q is also known as ubiquinol (Co Q) and is an antioxidant that is soluble in oil. This is generated by monovalent pathways in the body, in the heart, liver, kidney, pancreas, and so on. The action mechanism can occur in two ways. The reduced form of ubiquinol (CoQH) functions as a chain-breaking antioxidant in the first mechanism, reducing peroxyl (ROO) and alcoxyl radicals (LO) (Papas 1998).

$$\mathrm{CoQH} + ? \, \mathit{ROO}\,\mathrm{Q} + \mathrm{ROOH}$$

It interacts with vitamin E radical (TO•) and regenerates vitamin E in the second mechanism.

$$\mathrm{CoQH} + ?\,\mathrm{TO}\,\mathrm{Q}\,\mathrm{R} + OOH$$

2.4.4 Synthetic Antioxidants

Synthetic antioxidants are generated or synthesized artificially, using different techniques. They are mainly polyphenolic compounds that detect the free radicals and stop the chain reactions. Polyphenolic derivatives often involve more than one group of hydroxyls or methoxyes. Ethoxy quinine is the only heterocyclic, N-containing compound recorded to be used in food, especially animal feed, as an antioxidant. Most of the recorded synthetic phenolic antioxidants are *p*-substituted, whereas the *o*-substituted are mainly natural phenolic compounds. Because of their lower toxicity, the *p*-substituted substances are better suited. To boost their solubility in fats and oils and to reduce their toxicity, synthetic phenolic antioxidants are often substituted with alkyl groupings. Such organic compounds with antioxidant activity are widely used as preservatives for cosmetics in pharmaceuticals and for stabilizing food fat, oil, and lipid (Gupta and Sharma 2006). Such recent results about synthetic antioxidants have led the researchers to create new synthetic antioxidants in terms of their water solubility, stability, and nontoxicity. Features of some of the identified synthetic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediaminetetraactic acid (EDTA), 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin), propyl gallate (PG), and tertiary butyl hydroquinone (TBHQ) (Hamid et al. 2010).

2.4.4.1 BHA

This is a monophenolic, lipid-soluble antioxidant and is best used in animal fat for lipid oxidation compared with vegetable oil (Wanasundara and Shahidi 2005).

2.4.4.2 BHT

This is also a monophenolic fat-soluble antioxidant but at high temperatures, it is much more stable than BHA and both function in synergy. Many antioxidant formulations that are currently available incorporate both of those antioxidants. BHA reacts with peroxy radicals to create a radical BHA phenoxy, which can, in turn, detach a hydrogen atom from the BHT hydroxyl group. The hydrogen radical provided by BHT regenerates BHA. The so-formed BHT radicals can interact with a radical peroxide and function as a chain terminator (Wanasundara and Shahidi 2005).

2.4.4.3 EDTA

EDTA is a widely used sequestrant, a water-soluble antioxidant that is applied to food, body care, and home products. It attaches with the trace minerals that may be present in the foodstuff, such as copper, iron, and nickel. If not inhibited, these minerals may result in discoloration, rancidity, and breakdown of the textures. Added as an antioxidant, EDTA helps to prevent oxygen from triggering changes in color and rancidity.

2.4.4.4 Ethoxyquin

It is chiefly used as an antioxidant to prevent carotenoid oxidation all through storage in animal feeds, vegetables, and fruits.

2.4.4.5 PG

This is an ester, created by gallic acid and propanol condensation. It works as an antioxidant that is used as a food additive to preserve primarily oils and fat in the foodstuff.

2.4.4.6 TBHQ

TBHQ is a remarkably efficient antioxidant to the diphenolic system. This is used as a preservative in food for unsaturated vegetable oils and other edible animal fats. Although in the presence of iron, it does not trigger discoloration and does not even alter the flavor or odor of the material to which it is introduced. It is used industrially as a stabilizer to restrict organic peroxide auto-polymerization. It can be used in biodiesel as a corrosion inhibitor. It is used as a fixative in perfumery, to lower the rate of evaporation and increase stability. It is also applied to additives to the varnishes, lacquers, resins, and field oil. It can be used individually or in conjunction with BHA or BHT (Said et al. 2002).

2.4.5 Natural Antioxidant versus Synthetic Antioxidant

Based on complex toxicity studies, synthetic antioxidants have been tested to ensure safety and approval for use in limited concentrations in foods. While synthetic antioxidants have been widely used in most countries, their safety remains in question (Shahidi 2005). An antioxidant should have two requirements to be declared safe:

its LD50 should not be less than 1000 mg/kg body weight and the antioxidant must have no noticeable impact on the growth of the experimental animal in long-term experiments at a level 100 times higher than that indicated for human consumption (Lehman et al. 1951). An antioxidant for use in food often needs comprehensive toxicological studies of its possible mutagenic, teratogenic, and carcinogenic actions (Yanishlieva et al. 2000). It has been shown that high concentrations of antioxidants can share a range of toxic properties (Shahidi 2005). BHT had negative effects on rat's liver, kidney, and lung due to their possible action as carcinogenesis, as per the Lanigan and Yamarik studies (2002). A few studies have shown that BHA and BHT are cytotoxic due to the carcinogenicity of BHA in the rodents' forestomach (Saito et al. 2003; Verhagen et al. 1991), therefore, governments have taken some steps to minimize the use of synthetic antioxidants in foods. It is clear that negative consequences of synthetic antioxidants only occur at elevated concentrations.

Meanwhile, several studies have shown that the amount of synthetic antioxidants commonly used in foods does not only have any harmful effects on humans, but also anticarcinogenic and antimutagenic qualities and other beneficial effects (Whysner et al. 1994; Hirose et al. 1999; Valenzuela et al. 2003; Williams et al. 1999). BHA and BHT show no cancer threat according to Williams et al. (1999) and may be anticarcinogenic at current rates of use. The safety of synthetic antioxidants during long-term consumption is still a controversial issue due to their potentially harmful effects. It seems rational that if there is a slight chance of synthetic antioxidants being toxic, we would seek to substitute them with natural antioxidants, which are more relevant to human nature. Using TG/DTA methodology, Santos et al. (2012) tested the thermal stability of industrial synthetic antioxidants and some natural antioxidants using both dynamic and isothermal (110 °C) research methods. They found that synthetic antioxidants showed thermal resistance in the following order: PG > TBHQ > BHA > BHT and natural antioxidants showed the following stability: α-tocopherol > caffeic acid > ferulic acid > gallic acid. Cruz et al. (2007) studied the thermal stability of three biomass-derived fractions with antioxidant activity (ethyl acetate soluble-fraction of *Eucalyptus globulus* acid hydrolysates, ethyl acetate soluble-fraction of red grape pomace auto-hydrolysis liquors after fermentation and distillation and water washing of the same feedstock) and two synthetic antioxidants in food is BHA and BHT. For assays lasting up to 120 min, the nonvolatile component, antioxidant activity, and the percentage of phenolic recovered during solid phase were assessed at 100, 150, or 200 °C. The tests showed higher thermal stability than BHA or BHT in the ethyl acetate soluble from acid hydrolysates of *Eucalyptus globulus* wood, from auto-hydrolysis and the washing of distilled red grape pomace. Furthermore, after the heating treatment, the naturally derived antioxidants displayed more antioxidant activity. Encapsulation has been proven to improve the thermal stability of natural antioxidants (Taghvaei et al. 2013). The Arabic gum encapsulated OLE improved soybean oil's thermal stability

more than OLEs. The reason for this could be traced back to the protection of natural antioxidants by encapsulation against destructive factors. It can be established that certain natural antioxidants not only have a greater capacity for prevention of oxidation than synthetic antioxidants but also have greater thermal stability and can stay further active after heat treatment compared to synthetic antioxidants.

Taking into consideration the adverse effects of high concentrations of synthetic antioxidants and their poor thermal stability in the heat processing and frying of food products, the substitution of synthetic antioxidants with natural ones seems reasonable. There are plenty of natural antioxidants that can be derived from low-cost sources with more antioxidant activity and thermal stability than synthetic ones in different edible oils, according to several studies done over the past two decades. For instance, green tea extract derived from tea waste may be used in the food industry as the correct natural antioxidants. Additionally, extracts rich in rosemary and β-carotene and tocopherols can be used more often in the future. Several of these studies show that natural antioxidants can be extracted and applied in the edible oil industry, instead of synthetic ones. Both tea extract and rosemary extract have been proposed as an effective alternative for synthetic antioxidants in the research of Yanishlieva et al. (2000). The authors suggested that the health issues need to be resolved in future research for the use of natural antioxidants. Abo Nahas (2019) reported that the food industry is sighted on replacing synthetic antioxidants with natural antioxidants. He studied the utilization of fennel and chamomile extracts, rich in phenolic compounds, as natural antioxidants in biscuits and compared their performance with a synthetic 62 antioxidant vastly used, the butylated hydroxyl anisole (BHA).

The complete nutritional profile, free sugars, fatty acids, and antioxidant activity were estimated after baking immediately, also after 15, 30, 45, and 60 days of storage. The results showed that the incorporation of natural and synthetic additives did not cause significant changes in color or the nutritional value of biscuits when compared with control samples. Both natural and synthetic additives conferred similar antioxidant activity to the biscuits. Therefore, natural additives are a more convenient solution for consumers who prefer foods “free” from synthetic additives. Moreover, natural additives were given by aqueous extraction, an eco-friendly and safe process. The main focus of studies in this field over the past decade has been to add more powerful natural antioxidants, which were mainly from plant resources and were usually considered healthy. When we use olive extract at very low doses, for example, there is no need to examine the harmful effects of such extract on humans. Yet certain unrecognized natural antioxidants have been added, and the potential toxic effects on the human body need to be investigated. It has been shown that animal PHIs do have an adequate antioxidant activity and may be more efficient from plant resources and synthetic antioxidants than certain natural antioxidants. Further studies are required to apply animal PHIs in edible oils and evaluate the long-term stability of the oil, since the antioxidant activity was examined through model systems in most studies in this area (Fig. 2.5).

Fig. 2.5 Natural antioxidant versus synthetic antioxidant (Abo Nahas 2019)

2.5 Fungal Jewels

Fungi are the most diverse and plentiful group on the planet and they are a brilliant candidate for bioactive metabolite production due to their resemblance to the animal system. Recently, molecular screening methods claimed there are as many as 5.1 million species of fungi, of which only approximately 100,000 have been reported in the literature (Kirk et al. 2008; Yadav 2020). Among these fungi, 14,000 species are mushrooms, 5000 are macro fungi, and more than 1800 fungi have been identified as possessing pharmacological, therapeutic, and medicinal features (Kirk et al. 2008).

Higher Basidiomycetes represent a taxonomically, ecologically, and physiologically extremely diverse group of eukaryotic organisms. Recently, extensive research on these fungi has markedly increased mainly due to their potential use in a variety of biotechnological applications, particularly for the production of food, enzymes, dietary supplements, and pharmaceutical compounds (Cohen et al. 2002; Wasser 2002). Many pharmaceutical substances with unique properties were extracted from mushrooms. The cholesterol-lowering, antidiabetic, and immune-modulating compounds are ready for industrial trials and further commercialization, while others are in various stages of development. Some of these substances are not strictly pharmaceutical products (medicines) but rather they represent a novel class of dietary supplements or nutraceuticals. The most important new pharmaceutical products from medicinal mushrooms include polysaccharides, antioxidants, and lectins (Guillot and Konska 1997; Wasser 2002; Ng 2004). In the last few years, there has been significant interest in the use of mushrooms and/or mushroom extracts as dietary supplements based on theories that they enhance immune function and promote health. Endobiotic (endophytic) fungi are micro fungi that host plant tissue intercellular and/or intracellular without any apparent pathological symptoms (Wilson 1995; Das and Varma 2009). To be able to sustain steady symbiosis, endophytes develop chemical substances that enhance the development of plants and benefit them to acclimatize better to the harsh environment (Gouda et al. 2016). As a treasure mine of bioactive metabolites, endophytic fungi are considered a sustainable source of various natural products, namely, quinones, saponins, alkaloids,

steroids, phenolic acids, terpenoids, and tannins that exhibit antimicrobial and anticancer properties (Verma et al. 2009). In the last decade, a "bioprospecting" term was applied to refer to an old practice for searching useful bioactive compounds and other potentially valuable biochemical products from nature (Abdel-Azeem and Salem 2012).

Filamentous fungi create a wide extent of low molecular mass natural products (NPs) often related to unique bioactive properties. Prominent among these fungal secondary metabolites (SMs) and Primary metabolites (PMs) are compounds beneficial to human like antibiotics, antioxidants, fragrances or pharmaceuticals, pigments (Demain 2014). Fungal (SMs) are principally categorized as polyketides, terpenoids, alkaloids, or small non-ribosomal peptides (Keller et al. 2005). Later advances in large-scale DNA sequencing from a wide range of filamentous fungi have led to the discovery that fungal genomes possessing a broad genetic potential to produce (SMs). A remarkably large number of enzymes that produce (SMs) as non-ribosomal peptide synthases (NRPSs), and terpene synthases (TSs) has been described often as part of biosynthetic gene clusters predicted to be responsible for the synthesis of one or more NPs (Keller et al. 2017).

2.5.1 Fungal Communication as an Inducer of Silent Secondary Metabolite

Fungi and their SMs are known as one of the potential resources for novel drugs. Fungi form diverse multispecies communities within the natural habitat. They are subjected to intra- and interspecies interactions, which may result in beneficial or even harmful outputs for the species included. The real triggers leading to the activation of natural product biosynthesis in these communities are as diverse as the products themselves. They range from environmental signals, such as pH, carbon, and nitrogen sources, to organisms living in the same habitat (Yu and Keller 2005).

2.5.2 Wild Mushroom as Treasure of Natural Antioxidant

Ferreira et al. (2009) has proposed that the sensitivity of species to free radicals has contributed to the creation of endogenous defense mechanisms to eradicate them. These defenses were the response of transformation to the inevitable ramification of ROS production under aerobic conditions. Natural products with antioxidant activity can be beneficial to the endogenous defense system. In this context, the antioxidants present in the diet assume significant importance as a potential preventive agent to mitigate oxidative damage. In particular, the antioxidant properties of wild mushrooms have been studied widely and many antioxidant compounds isolated from these sources have been established so far in mushrooms.

In addition to the mechanism of action involved in their antioxidant properties, wild mushrooms can be used directly in diet to improve health, reaping the benefits of the additive and synergistic effects of both bioactive compounds. Mushrooms have become desirable as functional foods and as a source of physiologically advantageous medicines (Chang 1996). Such benefits of using mushrooms over plants as bioactive compound sources are that sometimes the fruiting body can be generated in far less time, the mycelium can also be produced swiftly in liquid culture, and the culture medium can be modified to generate optimum active product amounts as well. Many wild mushroom species had antioxidant activity, primarily associated with their phenolic content (Soares et al. 2009). Mushroom antioxidants are predominantly phenolic compounds (phenolic acids and flavonoids) accompanied by tocopherols, ascorbic acid, and carotenoids. These molecules were quantified mainly from India, Korea, Finland, Poland, Portugal, Taiwan, and Turkey in tens of different species (Table 2.1) These values are valuable in the literature but differently expressed in basis (fresh weight and dry weight extract). India's *Helvella crispa* reported the highest content of phenolic compounds produced per g of extract (34.65 mg/g), while Korea's *Sparassis crispa* reported the lowest dry-weight value (0.76 mg/g). The richest species in tocopherols were *Auricularia fuscosuccinea* (white) from Taiwan (32.46 mg/g extract), *Agaricus silvaticus* (3.23×10^{-3} mg/g dry weight), and *Ramaria botrytis* (2.50×10^{-4} mg/g fresh weight) from Portugal. The highest concentrations of ascorbic acid were found in *Auricularia fuscosuccinea* from Taiwan (11.24 mg/g extract), *Suillus collinitus* from Portugal (3.79 mg/g dry weight), and *Agaricus bisporus* from Poland (0.22 mg/g fresh weight). *Lactarius deliciosus* from Portugal presented the highest contents in β-carotene (0.09 mg/g of extract). The literature includes a few studies concerning the study of the phenolic components of wild mushrooms. High-performance liquid chromatography coupled with photodiode array detector (HPLC-DAD) (Puttaraju et al. 2006; Ribeiro et al. 2006, 2007; Kim et al. 2008; Valentão et al. 2005; Barros et al. 2008), or an ultraviolet detector (Jayakumar et al. 2009), or gas chromatography-mass spectrometry selected ion monitoring (GC-MS SIM) (Mattila et al. 2001).

Tocopherol: Some reports have been published on the tocopherols content of mushrooms. All reported the same methodology including saponification in the extraction process and analysis by HPLC coupled to UV detector. Only Barros et al. (2008) described an extraction process without saponification, adding an antioxidant to avoid tocopherols oxidation, using special precautions to protect the samples from light and heat. Ascorbic acid can also be extracted from many wild mushroom species using HPLC coupled to UV or fluorescence detector or by the spectrophotometer. Carotenoids are nature's most extensive pigments and have also received substantial attention because of both their provitamin and antioxidant roles. Particularly, β-carotene was found in several mushroom species. Carotenoids are synthesized by plants and microorganisms but not animals. Fruits and vegetables constitute the major sources of carotenoids in the human diet. Close to 90% of the carotenoids in the diet and human body are represented by β-carotene, α-carotene, lycopene, lutein, and β-cryptoxanthin (Ferreira et al. 2009).

Table 2.1 Antioxidants quantified from wild mushrooms

Mushroom species	Phenolic compounds	Tocopherols	Ascorbic acid	β-Carotene	Country
Agaricus arvensis	0.17[a]	1.22×10^{-3}[a]	0.02[c]	8.52×10^{-3}[c]	Portugal
Agaricus bisporus (white)	4.32×10^{-3}[a]	–	0.17[a]	–	Finland
Agaricus bisporus (brown)	4.69×10^{-3}[a]	–	0.21[a]	–	Finland
Agaricus bisporus	0.54[a]	–	–	–	Korea
Agaricus bisporus	–	–	0.22[b]	–	Poland
Agaricus bisporus	0.03[a]	2.41×10^{-3}[a]	0.03[c]	1.95×10^{-3}[c]	Portugal
Agaricus bisporus	–	9.20[c]	–	0.04[c]	Turkey
Agaricus blazei	0.70[a]	–	–	–	Korea
Agaricus blazei	–	5.44[c]	–	–	Taiwan
Agaricus romagnesii	0.08[a]	1.29×10^{-3}[a]	0.04[c]	1.32×10^{-3}[c]	Portugal
Agaricus silvaticus	–	3.23×10^{-3}[a]	0.04[c]	5.42×10^{-3}[c]	Portugal
Agaricus silvicola	0.35[a]	1.17×10^{-3}[a]	0.04[c]	3.02×10^{-3}[c]	Portugal
Agrocybe cylindracea	–	5.27[c]	–	–	Taiwan
Amanita caesarea	–	–	2.07[a]	–	Portugal
Amanita rubescens	0.49[a]	–	0.03[a]	–	Portugal
Auricularia mesenterica	–	9.45[c]	1.63[c]	–	Taiwan
Auricularia fuscosuccinea (brown)	–	12.69[c]	11.24[c]	–	Taiwan
Auricularia fuscosuccinea (white)	–	32.46[c]	7.99[c]	–	Taiwan
Auricularia polytricha	3.17[c]	–	–	–	India
Auricularia polytricha	–	23.61[c]	3.28[c]	–	Taiwan
Boletus badius	–	8.80[c]	–	–	Turkey
Boletus edulis	10.19[c]	–	–	–	India
Boletus edulis	–	3.30×10^{-4}[a]	–	2.73×10^{-3}[c]	Portugal
Boletus edulis	–	6.18[c]	–	–	Taiwan
Calocybe gambosa	–	4.00×10^{-4}[a]	0.40[c]	6.41×10^{-3}[c]	Portugal
Calvatia gigantea	–	–	0.15[a]	–	India
Cantharelus cibarius	2.00[c]	3.00×10^{-5}[a]	0.42[a]	–	India
Cantharelus cibarius	7.80×10^{-3} to 2.54×10^{-2}[a]	1.50×10^{-4}[a]	0.48[c]	0.01[c]	Portugal
Cantherallus clavatus	13.22[c]	–	–	–	India
Clavulina cinerea	–	–	0.42[a]	–	India
Craterellus cornucopioides	–	1.87×10^{-3}[a]	0.87[c]	0.01[c]	Portugal
Fistulina hepatica	0.37–0.55[a]	–	2.80[a]	–	Portugal
Flammulina velutipes	0.17[a]	–	–	–	Korea
Ganoderma lucidum	0.16[a]	–	–	–	Korea
Ganoderma lucidum	–	1.19[c]	–	–	Taiwan
Ganoderma tsugae	–	1.07[c]	–	–	Taiwan

(continued)

Table 2.1 (continued)

Mushroom species	Phenolic compounds	Tocopherols	Ascorbic acid	β-Carotene	Country
Geastrum arenarius	4.80[c]	–	–	–	India
Gomphus floccosus	–	–	0.26[a]	–	India
Grifola frondosa	–	0.05, 0.11[c]	0.05, 0.14[c]	–	Taiwan
Helvella crispa	34.65[c]	–	–	–	India
Hericium erinaceus	–	0.06[c]	–	–	Taiwan

Source: Ferreira et al. (2009)

2.5.3 Ganoderma lucidum *A Treasure Trove of Antioxidant*

Ganoderma lucidum (aphyllophoromycetideae from the family Polyporaceae) is a mushroom that has been utilized in customary Chinese medication for a long time. Likewise, *G. lucidum* has been accounted for as a significant wellspring of bioactive compounds, for example, polysaccharides, triterpenoids, and proteins, which are utilized to avert or treat different human diseases, for example, malignancy, immunological issues, neurodegenerative infections, hepatitis, hypertension, ceaseless bronchitis, bronchial asthma, and so on.

Past investigations have detailed that *G. lucidum* performs antioxidant agent, hypoglycemic, antitumor, and immunomodulatory. Also, *G. lucidum* acts to lessen oxidative pressure and advance neuroprotective impacts, prompting neuronal separation and securing against cerebral ischemic injury by hindering apoptosis. Also, *G. lucidum* extract lessens the statements of proinflammatory and cytotoxic elements from the initiated microglia and viably secures the dopaminergic neurons against incendiary and oxidative harm. Zhang et al. (2006) recommended that *G. lucidum* jams the harmed spinal engine neuron articulation levels of the proteins that assume significant jobs in axonal regeneration. These outcomes infer that the polysaccharide disengaged from *G. lucidum* have neural protection and antioxidant properties (Lacin et al. 2019).

An assortment of polysaccharides and triterpenoids developed in many biological activities, for example, *G. lucidum*. The cell dividers of *G. lucidum* spores contain a high number of polysaccharides. An assortment of bioactive polysaccharides segregated from *G. lucidum* has been seen as β-1,3-glucans polysaccharide peptides like peptidoglycan, which interface with the immune system. A few water-soluble polysaccharides have been fractionated and cleaned from the aqueous extraction of *G. lucidum*. More than 140 triterpenoid mixes were found in *G. lucidum*, which can be separated into *Ganoderma* alcohols or *Ganoderma* acids. Some triterpene-rich extracts of *G. lucidum* contain high amounts of lucidenic acids, which can be purified from the extract, and exert immunostimulatory activities. Several nucleotides and nucleobases were qualitatively identified in the mushroom samples. Some investigations show that *G. lucidum* extract elevates the activity of super oxide dismutase and catalase enzymes associated with wiping out harming responsive oxygen reactive species. Zhu et al. (1999) studied the antioxidant activity of *G. lucidum*

mix in vitro. *Ganoderma* crude extract was exposed to boiling water media, then the aqueous extract was separated. Polysaccharide and terpene fractions have been achieved. Both of the fractions were analyzed for their antioxidant activity.

It has been demonstrated that the terpene fraction showed the highest antioxidant activity. In that fraction, ganoderic acids A, B, C, and D, lucidenic acid B reported the highest antioxidant activity (Zhu et al. 1999).

Heleno et al. (2013) concluded that extracts obtained from *G. lucidum* grown on germinated brown rice (GLBR) show important antioxidant activity against several in vitro antioxidant systems. Consumption of GLBR extract could distinctly expand the activity of enzymes like superoxide dismutase, glutathioneperoxidase, and catalase in the sera, liver, and brain of mice (Hasnat et al. 2013).

Ganoderma polysaccharides have been determining their activity as antioxidants by different strategies. Entire polysaccharides complex affirmed the competent radical scavenging capacities. Notwithstanding the extraordinary antioxidants capability of homo-glucans and hetero-glucans from this *G. lucidum* and their mechanism of action have not yet been clarified. Molecular mass, chemical composition, type of glycosidic linkage, and conformation are the fundamental variables influencing the bioactivity of polysaccharides. Among them, the molecular weight was one of the most significant structural features of polysaccharides, as it is identified with the quantity of reductive hydroxyl group terminals (on a per unit mass basis) liable for accepting and wiping reactive oxygen species. Low molecular weight polysaccharides subsequently have relatively higher antioxidant ability (*Ganoderma lucidum* polysaccharides1 GLPL1and *Ganoderma lucidum* polysaccharides2 GLPL2) (Wang et al. 2017).

The scavenging impact against superoxide radicals of low molecular weight chitosan (9 kDa) was more powerful than high molecular weight chitosan (760 kDa) (Xing et al. 2005). Structural analyzes of *G. lucidum* polysaccharides (GL-PSs) show that GL-PSs are heteropolymers, in which glucose is the utmost sugar component, while mannose, galactose xylose, and fucose are present in lower amounts (Wachtel-Galor et al. 2011). Polysaccharides isolated from the fruit bodies of *G. linghzi* indicated also potential antioxidant activity (Zhu et al. 2013). Additionally, Kao et al. (2013) record that β-1,3-glucan (a low-molecular weight glucan) isolated from *G. lucidum* was skillful to elevate (from 40% to 80%) the feasibility of a mouse leukemic monocyte macrophage cell line (RAW 264.7) with H_2O_2—hydrogen peroxide free radical induced oxidative stress, and decrease reactive oxygen species (ROS) formation. It also terminated the activities of neutral and acidic sphingomyelinases (SMases). Mannose-based homo-polysaccharide was able to increase the activity of antioxidant enzymes. Moreover, the antioxidant capability of high-purity polysaccharides, many researches focused on the high radical scavenging impact of polysaccharide conjugates such as polysaccharide-protein complexes and polyphenolic-associated polysaccharides, polysaccharide chelating metal, metal ion-enriched polysaccharides, and polysaccharide mixtures (Wang et al. 2017).

The protein or peptide moiety in polysaccharides and the scavenging effect on superoxide and hydroxyl radicals are elucidated by (Liu et al. 2007) Polysaccharide-protein complexes extracted from *G. lucidum* with lower polysaccharide/protein ratios were more fruitful in the scavenging function. Further investigations indicated that, besides the quantity of peptide or protein molecules, their composition has to be considerable Amino acids, such as tryptophan tyrosine, methionine, lysins, histidine, and tyrosine, are able to donate protons to electron-deficient radicals. The most common polysaccharide isolated from *G. lucidum*, GLP, consists of 14 amino acids. Several polysaccharides (D-rhamnose, D-fructose, D-galactose, D-mannose, D-xylose, and D-glucose) are present as sugars. The molecule has a great potentiality to increase antioxidants, serum insulin levels, and diminish lipid peroxidation. The survival rate of macrophages, and securing the mitochondria against injury by a membrane-permeant oxidant (t-BOOH), which has also demonstrated the high antioxidant activity (Jiang et al. 2012).

Another approach to influencing the antioxidant activity of polysaccharides is a chemical modification by moderating the solubility of water-insoluble polysaccharides. Chen et al. (2005) reported that *G. lucidum* polysaccharides could highly improve antioxidant enzyme activities. Moreover, Liu et al. (2007) recorded that sulfation strongly improved the water solubility and bile acid-binding abilities of a water-insoluble polysaccharide from *G. lucidum* (GLP) (Fig. 2.6).

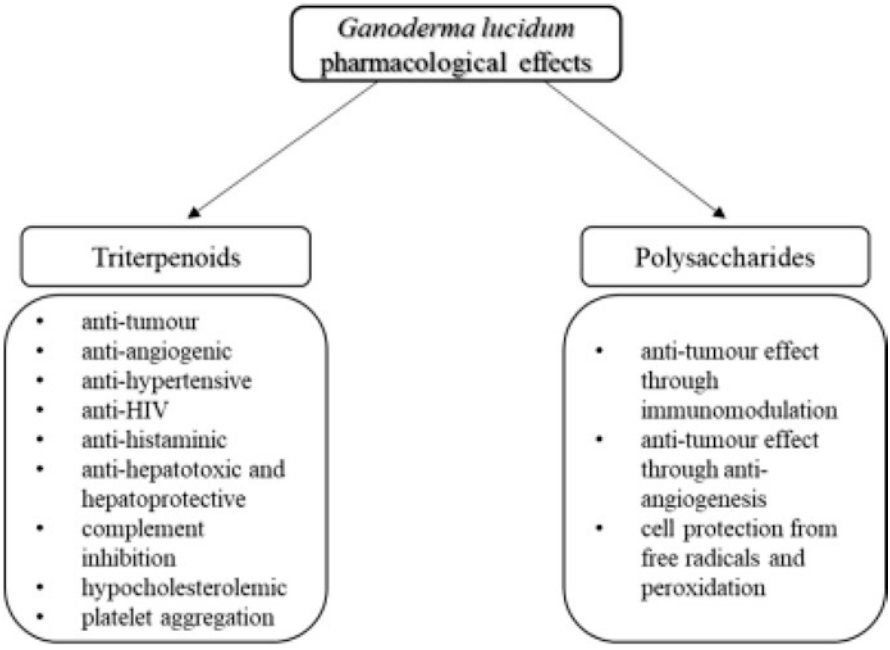


Fig. 2.6 *Ganoderma lucidum* pharmacological effects related to the specific group of biological compounds (Boh et al. 2007)

2.5.4 *Glutathione, Altruistic Metabolite in Filamentous Fungi and Yeast*

Baker's yeast *Saccharomyces cerevisiae* have represented as a biological model system that assisted the unraveling of the role of glutathione in cellular processes. Glutathione was first termed as "philothion" by Rey-Pahlade over 120 years earlier, as a substance that appropriates to reduce elemental sulfur discharging hydrogen sulfide (Meister 1988). This "sulfur-loving" compound was isolated by the English biochemist Frederic Gowland Hopkins and renamed glutathione. Regarding chemistry, glutathione (GSH) was base as a thiol tripeptide with unusual γ-glutamyl linkage (γ-L-glutamyl-Lcysteinyl glycine). Research on the character and function of GSH in animals over the last 40 years have been active and enriched by Alton Meister and his colleagues (Meister and Anderson 1983) Most research concerned with animal GSH is multidisciplinary, comprising biochemical, toxicological, physiological, and clinical aspects of its biological function. In comparison with the biology of GSH in microbial systems has received less publicity, even though it is generally accepted that GSH is physiologically relevant nonprotein thiol (NPT) present in most microorganisms. GSH can make up about 1% of the cellular dry weight in many types of human and animal cells so glutathione is way significant and abundant molecular (Penninckx and Elskens 1993) researches typically concentrate on biochemical "in vivo" Studies, and the metabolism of GSH was investigated as a significant component in cellular processes a (Fig. 2.7).

GSH is a significant multifaceted cellular metabolite in Fungi. Its clear cooperation in the response of suffering cells subjected to stress places GSH in the category of altruistic compounds. The most particular physiological roles of GSH relate to the state of stress. For microorganisms, stress means the response to a chronic or sudden experience of different harmful circumstances like heat, cold, osmotic shock, starvation, alterations in the pH, water potential, or exposure to radiation. These situations, however, refer to cultures grown in laboratory media, which scarcely represent the most desirable ecological state or even may hide unexpected development of stress, for example, the presence of harmful metabolic by-products normally created by general carbon and/or nitrogen cellular metabolism. In this case, GSH plays a significant role. The role of GSH is not limited to excessively stressful conditions at all. A cell completely deprived of GSH is unable to function even under conditions free of stress. One of the main roles of GSH is related to the maintenance of cellular integrity, in particular to the membrane structures, as well as to cellular differentiation and development. To accomplish this essential role, the fungal cell does not need necessarily to use its full potential for the synthesis of GSH; often only a few amounts of the thiol are sufficient.

There are several important enzyme systems accompanied by GSH. However, the chemical reactivity of the tripeptide, which modulates its effect in the cellular

Fig. 2.7 GSH production and consumption machines in the metabolic network of fungi (Pocsi et al. 2004)

redox cycle, should not be ignored! Undoubtedly, this is one of the most significant aspects that demonstrate the usefulness of this compound. So, we must emphasize the deep influence exerted by investigators of mammalian physiology, on the development of GSH research. Research on GSH in microorganisms, particularly in fungi, has exploded in the last 10 years. This can be attributed in part to the enthusiasm of investigators working in very different fields, for example, plant and animal physiology, to use microbial model organisms, particularly yeasts (Pocsi et al. 2004). A brilliant study in 2009 investigated that *Aspergillus nidulansis fungus specific gulthion transferase* (Sato et al. 2009). Finally, in addition to glutathione, several glutathione analogs and precursors are also commercially important, most of the analogs are produced by synthetic chemistry, but extraction of glutathione itself from potential and sustainable sources, like yeast and fungi needs to be investigated with greater intensity.

2.5.5 *Micro Fungi as Source of Antioxidants*

2.5.5.1 *Sanghuangporus sanghuang*

Sanghuangporus sanghuang, as a new species has been discovered that only grows on living mulberry trees. Studies on *Sanghuang* showed that its main components are polysaccharides, flavonoids, and triterpenoids. Triterpenoids are a class of bioactive substances in medicinal fungi, but their antitumor and antioxidant properties have been less studied than those of polysaccharides. They have many functions, such as inhibiting histamine release, lowering blood pressure, and protecting the liver.

The first use of the medicinal fungus *Sanghuang* can be traced back to 2000 years ago in China. According to The Theory of Medicinal, *Sanghuangporus sanghuang* tastes bitter and is used as a traditional Chinese medicine for the treatment of diarrhea, night sweats, metrorrhagia, drench, and stomach pain, prolapse of spilled blood, leucorrhea, and amenorrhoea. *Shennong's* Herbal Classic of Materia Medica stated that long-term use of *Sanghuangporus sanghuang* can prolong life, detoxify, and improve digestion.

To maximize the yield of the antioxidants and active ingredients such as the triterpenoids from authentic *Sanghuangporus sanghuang*. Cai et al. (2019) examined four parameters of the extraction process, including the extraction time, solid–liquid ratio, extraction temperature, and ethanol concentration to optimize the triterpenoid extraction processes of *Sanghuangporus sanghuang* mycelium. The results showed that the optimum conditions of ultrasonic extraction required an 80% ethanol concentration, a 1:20 solid–liquid ratio, a 20-min extraction time, and a 60 °C extraction temperature, to obtain a maximum triterpenoid extraction of 13.30 mg/g. Antioxidant capacity tests showed that the *Sanghuangporus sanghuang* triterpenoids had high clearance capabilities for hydroxyl free radicals, superoxide anions, 2,2-diphenyl-1-picrylhydrazyl free radicals, and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) radicals, indicating that the *Sanghuangporus sanghuang* triterpenoids had high antioxidant activities.

In 1968, Ikekawa et al. (1968) found that the sarcoma cell line S-180 was inhibited by 96.7% in mice when treated with a water extract of the *Sanghuang* fruiting body. The medicinal functions of *Sanghuang* have since been studied by many researchers, who have characterized its antitumor and antioxidant properties. *Sanghuang* is considered one of the most effective anticancer drugs found in higher fungi and has been extensively studied as a medicinal fungus.

2.5.5.2 *Cerrena unicolor*

Investigation and isolation of new natural bioactive substances are important for the food industry due to the growing importance of their antioxidative activity, which is crucial in food preservation processes. Unfortunately, the commonly used synthetic

substances such as hydroxyanisole (BHA) and hydroxytoluene (BHT) are likely to be toxic for living organisms. Interestingly, the physiological life cycle of the white rot *Basidiomycota* is associated with a relatively high concentration of ROS, which might initiate the secondary wood cell wall decay processes. Therefore, these organisms also possess a very efficient antioxidative system consisting of enzymatic (peroxidases, laccase, catalase, and superoxide dismutase) and nonenzymatic elements (phenolic derivatives or polysaccharides). It is known that, besides the polysaccharides, fungi can produce many secondary metabolites with antioxidative activities including a number of phenolic compounds (e.g., hispidin and its dimmers or fungal pigments usually isolated from fruiting bodies) (Jaszek et al. 2013).

Three bioactive fractions, extracellular laccase (ex-LAC), crude endopolysaccharides (c-EPL), and a low molecular subfraction of secondary metabolites (ex-LMS), were isolated from the idiophasic cultures of the white rot fungus *Cerrena unicolor*. The highest reducing capability was found for the ex-LMS 800 μg/mL. A very high prooxidative potential was observed for the ex-LAC probes. They showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Jaszek et al. 2013).

2.5.5.3 *Mucor circinelloides*

Molecular screening methods claimed there are as many as 5.1 million species of fungi, of which only approximately 100,000 have been reported in the literature. Among these fungi, 14,000 species are mushrooms, 5000 are macro fungi, and more than 1800 fungi have been identified as possessing pharmacological, therapeutic, and medicinal features. The pathogenicity of Mucorales is largely believed to be due to endocellular excretions and the production of subtilisins, chitinases, proteinases, and antioxidant proteins (e.g., superoxide dismutase, catalase, and peroxidase). From an industrial point of view, the class Zygomycetes of Zygomycota is more important than its second class Trichomycetes because of its two widely used bioactive compound producing genera: Mucor and Rhizopus. Zygomycetes can produce a wide range of metabolites including enzymes, lipids, ethanol, organic acids, food colorants, amino acids, chitosan, chitin, and proteins.

Genus *Rhizopus*, specifically, has been majorly exploited to produce lactic acid, fumaric acid, amylases, pectinases, steroids, lipases, ureases, and tannases, whereas genus *Mucor* is considered a good source for cellulases, phytases, proteases, ethanol, lipids, and food colorants. Due to the large storage capability of these metabolites in their mycelium/biomass, as well as their nutritional and pharmaceutical importance, there is an increasing interest in utilizing the biomass of zygomycetes as a source of microbial proteins, microbial lipids, microbial ethanol, microbial food colorants, and microbial bioactive components such as essential amino acids, antibiotics and chitosan in food, aquaculture feed, and pharmaceutical industries. Mucor and Rhizopus can be a great source of natural antioxidants and these natural antioxidants can be termed as “microbial antioxidants”.

Mucor circinelloides strains belong to the family Mucoraceae, order Mucorales, subclass Incertaesedis, and class Zygomycetes. These strains are excellent producers of carotene, lycopene, lipids, and bioactive component, for example, Linolenic acid (GLA), and are also easy to handle and produce with the availability of molecular and transformation tools and genome sequence features. Phenolic compounds were detected, especially tannins and flavonoids. Total phenol content was attributed to overall antioxidant capacity. Submerged fermentation with nutritional stress conditions was found to be an excellent way of producing surplus amounts of natural antioxidants/secondary metabolites with their vast potential commercial application in food and pharmaceutical industries from these fungi (Hameed et al. 2017).

2.6 Why Do Plants Synthesize Antioxidants?

Plants have been the vital sources of natural products since the starting of investigation, and it may too be expressed that they are the natural skilled workers of molecules created in infinite orders. Since plants are seated organisms, they experience a number of changes to adjust stress conditions. These changes happen due to the formation of different significant compounds. These compounds are valuable resources of plants since they keep up their age and health. Endophytes and plants have a symbiotic relationship, where the endophytes obtain benefits in the form of nutrition, and in return, synthesize specific compounds that assist the plant in metabolism and protection from stressful conditions. These compounds produced by the endophytes present a hidden range of known and unknown medicinal significance (Darwish et al. 2020). Gave the concept of horizontal gene transfer that reveals that endophytes and the host plant collaborate to the production of bioactive molecules. Some endophytes have been predominating biosynthetic capabilities, owing to their likely gene recombination with the host while dwelling and reproducing inside the healthy plant tissues (Li et al. 2005). Taxol, jesterone, torreyanicacid, pestaloside, ambuic acid, pestalotiopsins, and 2-a-hydroxydimeniol are few examples of such compounds (Strobel and Daisy 2003). These bioactive molecules synthesized by plants can be utilized for the treatment of human diseases. Apart from plants, endophytes, which are in a symbiotic relationship with the plants, are also considered to be a vital source of antioxidants (Huang et al. 2007).

2.6.1 Endophytic Fungi as a Natural Source of Antioxidants

As all higher plants are hosts to one or more endophytic microbe on this earth. Endophytic fungi are microbes that reside in living plant tissues without causing any immediate harm to their host. They are highly diverse microorganisms that are chemical synthesizers inside host plants. Most antioxidants known today are industrially synthesized although being accounted for causing liver damage and

carcinogenesis. In contrast, natural-derived antioxidants, like those produced by endophytes, have not been found to be harmful. Endophytes have the ability to use several substrates, producing a wide array of secondary metabolites. These comprise a large but little explored proportion of fungal diversity. Paclitaxel, a potent anticancer agent isolated from endophytic fungi such as *Taxomyces andreanae* and *Pestalotia* spp., so, endophytes have been recognized as potential new sources of anticancer, antimicrobial, and antimalarial bioactive metabolites, attracting much more attention from researchers. These metabolites include steroids, xanthines, phenols, isocoumarins, quinones, and terpenoids (Caicedo et al. 2019). Fusaria both positively and negatively affect crop cultivation: the harmful effects of pathogens and toxin producers and the beneficial effects of the biological control agents, which can be used as microbial pesticides, are easily understood. In contrast, the effects and potential of endophytes on crop cultivation are poorly understood. Although most endophytes are thought to be nonpathogenic further analyzes of the ecological functions of *Fusarium* endophytes are needed to elucidate their roles in crop cultivation (Imazaki and Kadota 2015).

Several studies have shown that extracellular polysaccharides of endophytic fungi present antioxidant activity (Yadav et al. 2014; Caicedo et al. 2019). Caicedo et al. (2019) reported high antioxidant potential of *Fusarium oxysporum* endophytic fungus isolated from the leaves of *Otoba gracilipes*, a medicinal tree from a tropical rainforest in Colombia.

Chaetomium globosum CDW7, an endophyte from *Ginkgo biloba*, exhibited strong inhibitory antifungal activity against phytopathogens such as *Fusarium graminearum*, *Rhizoctonia solani*, *Magnaporthe grisea*, *Pythium ultimum*, and *Sclerotinia sclerotiorum* both in vitro and in vivo. Extract from *C. globosum* CDW7, which had been deposited in the China General Microbiological Culture Collection Center (CGMCC) with an accession number 6658, has the strongest antioxidant activity among the studied endophytic fungi from *G. biloba* comparable to those of vitamin C and trolox, the well-known antioxidants. *Chaetomium globosum* and *C. cochlioides* are antagonistic to species of *Fusarium* and *Helminthosporium*. Flavipin is considered the major antioxidant component of CDW7's metabolites; it reacts by donating its electrons to the free radicals, leading to SOD and GSH-Px activity improvement and suppression of MDA content. Chaetopyranin also showed antioxidant activity. The azaphilone compounds are produced by different *Chaetomium* species, which display various biological activities such as antioxidant, nematicidal, antimicrobial, antifungal, anticancer, and inflammatory activities. Endophytic fungus *Chaetomium globosum* INFU/Hp/KF/34B isolated from *Hypericum perforatum* has been shown to produce hypericin and emodin of high medicinal value as antioxidants. *C. cupreum* can be a new source of natural antioxidants useful for industrial applications (Darwish et al. 2020).

Yadav et al. (2014) reported the presence of alkaloids, phenols, flavonoids, saponins, and terpenes in 21 endophytic fungi isolated from *Eugenia jambolana*, which can be a potential source of novel natural antioxidant compounds. A significant positive correlation was found between antioxidant activity and TPC in fungal extracts. There are 36% of endophytic extracts having high phenolic content

exhibited potent antioxidant activity. *Chaetomium* sp., *Aspergillus* sp., *Aspergillus peyronelii*, and *Aspergillus niger* strains showed the highest antioxidant activity ranging from 50% to 80% having 58 to 60 mg/g GAE total phenolics. In their work, Darwish et al. (2020) collected all available data concerning antioxidants produced by endophytic Chaetomium. These are explained in the following paragraphs.

2.6.1.1 Flavipin

Flavipin is a well-known natural product that is isolated from endophytes belonging to *Chaetomium* sp. associated with leaves of *Ginkgo biloba* (Ye et al. 2013). Yan et al. (2018) succeeded in isolating bioactive metabolites with antifungal activities from this fungus; the metabolites are flavipin, chaetoglobosins A and D, chaetoglobosins R (4) and T (5), new isocoumarin derivative prochaetoviridin A (1), new indole alkaloid, and chaetoindolin A (2) and chaetoviridin A (3). Flavipin is considered the major antioxidant component of CDW7's metabolites; it reacts by donating its electrons to the free radicals, leading to SOD and GSH-Px activity improvement and suppression of MDA content. This metabolite possesses three phenolic hydroxyl and two aldehyde groups, which are characteristic functional groups with antioxidant activity. When cultured under the optimal condition (25 °C, 100/250 mL flask, 12 discs/flask, 150 rpm, pH 6.5) for 14 days, *Chaetomium globosum* CDW7 was a highly yielded bio-source of antioxidant Flavipin synthesizing a remarkable production of 315.5 mg/L (Ye et al. 2013).

Another endophytic fungus from *Ginkgo biloba*, *Chaetomium* sp. NJZTP21 (GenBank accession number: JN588553), isolated from the healthy leaf of the plant was able to produce Flavipin, which significantly inhibited the growth of several plant pathogenic fungi, especially *Fusarium graminearum*. But the extract from *C. globosum* CDW7, which had been deposited in the China General Microbiological Culture Collection Center (CGMCC) with an accession number 6658, has the strongest antioxidant activity among the studied endophytic fungi from *G. biloba* comparable to those of vitamin C and trolox, the well-known antioxidants (Ye et al. 2013). *Chaetomium globosum* and *C. cochlioides* are antagonistic to species of *Fusarium* and *Helminthosporium*. They exhibited good control over many plant pathogens; seed coating treatments with viable spores of *Chaetomium globosum* were found to exert antagonistic effect controlled *Fusarium roseum* f. sp. *cerealis* "graminearum" in corn; reduced disease incidence of apple scab caused by *Venturia inequalis*; suppressed damping-off of sugar beet caused by *Pythium ultimum*; had an antagonistic effect against *Macrophomina phaseolina*, *Pythium ultimum*, *Bipolaris sorokiniana*, *Rhizoctonia solani*, and *Alternaria brassicicola*; and reduced the quantity of sporulation of *Botrytis cinerea* on dead lily leaves exposed in the field (Biswas et al. 2012; Shternshis et al. 2005).

2.6.1.2 Chaetopyranin

The basic structure of chaetopyranin (I) is chromenol (I) (chromene carrying one or more hydroxyl substituents). It is chemically known as 3,4-dihydro-2*H*-chromene substituted by a hydroxyl group at position 6, a 3-hydroxybut-1-en-1-yl at position 2, a formyl group at position 5, and a prenyl group at position 7 (Wang et al. 2006). These two compounds have been isolated from an endophytic fungus *Chaetomium globosum*, associated with *Polysiphonia urceolata*, and are found to possess antioxidant activity. The former compound also exhibits anticancer activity (Wang et al. 2006). Chaetopyranin also showed antioxidant activity.

2.6.1.3 Azaphilone

The most remarkable and valuable properties of azaphilones include their natural origin, yellow-red spectra, thermostability (in comparison with other natural red pigments), and water solubility. The azaphilone compounds produced by different *Chaetomium* species display various biological activities such as antioxidant, nematicidal, antimicrobial, antifungal, anticancer, and inflammatory activities (Borges et al. 2011).

2.6.1.4 Hypericin and Emodin

Endophytic fungus *Chaetomium globosum* INFU/Hp/KF/34B isolated from *Hypericum perforatum* has been shown to produce hypericin and emodin of high medicinal value as antioxidants. This endophytic fungus has significant scientific and industrial potential to meet the pharmaceutical demands in a cost-effective, easily accessible, and reproducible way (Kusari et al. 2008; Zhao et al. 2011).

2.6.1.5 Mollicellins

Mollicellins O (1) isolated from the endophytic fungus *Chaetomium* sp., Eef-10, which was isolated from *Eucalyptus exserta* by Ouyang et al. (2018), showed antioxidant activity based on DPPH radical scavenging. For more details concerning antioxidants producing fungi in Egypt please check Abdel-Azeem et al. (2018) and Abo Nahas (2019).

2.7 Fungal Antioxidants: Extraction, Estimation, and Biological Assay

The field of free radical chemistry is gaining more attention these days. Free radicals are reactive oxygen and nitrogen species that are generated by various physiological processes in the body. Uncontrolled generation of free radicals leads to attack on membrane lipids, proteins, enzymes, and DNA causing oxidative stress and ultimately cell death. These ROS are responsible for many degenerative human diseases like diabetes mellitus, cancer, neurodegenerative disorders, Alzheimer's disease, Parkinson's disease, atherosclerosis, aging, and inflammatory diseases (Yadav et al. 2014).

Carbohydrate antioxidants are expected to have better applicability as they are easily isolated, purified, water-soluble, and have fewer chances of toxicity towards the cell. Fungal EPSs have several applications in the food, feed, cosmetic, medicine, and pharmaceutical industries. The activities of fungal carbohydrate compounds are dependent on different content and arrangements during the polymerization of its building unit, monosaccharides.

Their composition varies from pure sugars to sugars combined with a second unit such as protein, phosphate, sulfate, or amine. Different types of sugar units were found in fungal EPSs such as glucose, mannose, galactose, xylose, fucose, and rhamnose. It was also noticed that EPSs composed of the same monosaccharide units that were synthesized by different fungi had different molecular weights. This is caused by differing chain lengths or branching patterns that give polysaccharides a great diversity of structure, property, and functions. For instance, the extracellular polysaccharide produced by the mangrove-associated fungus *Fusarium oxysporum* Dzf17 is defined as galactofuranose-containing mannoglucogalactan, consisted of galactose, glucose, and mannose in a molar ratio of 1.33:1.33:1.00, and its molecular weight was about 61.2 kDa (Abdel-Azeem et al. 2019).

Fortunately, not all fungal secondary metabolites are toxic to humankind such as; antibiotics, phytotoxins, enzymes, and antioxidants, which gave great importance to fungi in industrial applications (Darwish 2019). Novel fungi that have the capability to synthesize unique polysaccharides with antioxidant properties became the scientists' target.

References

Abdel-Azeem AM, Salem FM (2012) Biodiversity of laccase producing fungi in Egypt. Mycosphere 3:900–920

Abdel-Azeem AM, Omran MA, Mohamed RA (2018) Evaluation of the curative probability of bioactive metabolites from endophytic fungi isolated from some medicinal plants against paracetamol-induced liver injury in mice. LAP LAMBERT Academic Publishing, Republic of Moldova. ISBN: 978-613-9-89820-6

Abdel-Azeem AM, Abdel-Azeem MA, Khalil WF (2019) Chapter 21 - Endophytic fungi as a new source of antirheumatoid metabolites. In: Watson RR, Preedy VR (eds) Bioactive food as dietary interventions for arthritis and related inflammatory diseases, 2nd edn. Academic, Cambridge, MA, pp 355–384

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abo Nahas HH (2019) Endophytic fungi: a gold mine of antioxidants. Microbial Biosyst 4(1):58–79

Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valero JR (2011) Extraction and analysis of polyphenols: recent trends. Crit Rev Biotechnol 31(3):227–249

Ames BN, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci U S A 78(11):6858–6862

Barja G (2004) Free radicals and aging. Trends Neurosci 27(10):595–600

Barros L, Ferreira MJ, Queiros B, Ferreira IC, Baptista P (2007) Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem 103(2):413–419

Barros L, Dueñas M, Ferreira IC, Baptista P, Santos-Buelga C (2008) Phenolic acids determination by HPLC–DAD–ESI/MS in sixteen different Portuguese wild mushrooms species. Food Chem Toxicol 47(6):1076–1079

Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I (2005) Curcumin induces glutathione biosynthesis and inhibits NF-κB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. Antioxid Redox Signal 7(1–2):32–41

Biswas SK, Aggarwal R, Srivastava KD, Gupta S, Dureja P (2012) Characterization of antifungal metabolites of Chaetomium globosum Kunze and their antagonism against fungal plant pathogens. J Biol Control 26(1):70–74

Boh B, Berovic M, Zhang J, Zhi-Bin L (2007) Ganoderma lucidum and its pharmaceutically active compounds. In: El-Gewely MR (ed) Biotechnology annual review, vol 13. Elsevier, Amsterdam, The Netherlands, pp 265–230

Borges WS, Mancilla G, Guimar-aes DO, Duran-Patron R, Collado IG, Pupo MT (2011) Azaphilones from the endophyte Chaetomium globosum. J Nat Prod 74:1182–1187

Branen AL (1975) Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J Am Oil Chem Soc 52(2):59–63

Cacciapuoti F (2016) Oxidative stress as "mother" of many human diseases at strong clinical impact. J Cardiovasc Med Cardiol 3(1):001–006

Cai C, Ma J, Han C et al (2019) Extraction and antioxidant activity of total triterpenoids in the mycelium of a medicinal fungus, Sanghuangporus sanghuang. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-43886-0

Caicedo NH, Davalos AF, Puente PA et al (2019) Antioxidant activity of exo-metabolites produced by Fusarium oxysporum: an endophytic fungus isolated from leaves of Otoba gracilipes. Microbiologyopen 8:1–7. https://doi.org/10.1002/mbo3.90

Chakraborty P, Kumar S, Dutta D, Gupta V (2009) Role of antioxidants in common health diseases. Res J Pharm Technol 2(2):238–244

Chang R (1996) Functional properties of edible mushrooms. Nutr Rev 54:S91–S93

Chen WQ, Luo SH, Li HZ, Yang H (2005) Effects of Ganoderma lucidum polysaccharides on serum lipids and lipoperoxidation in experimental hyperlipidemic rats. J Chin Mater Med 30:1358–1360

Cohen R, Persky L, Hazan-Eitan Z, Yarden O, Hadar Y (2002) Mn^{2+} alters peroxidase profiles and lignin degradation by the white-rot fungus Pleurotus ostreatus under different nutritional and growth conditions. Appl Biochem Biotechnol 102–103(1–6):15–430

Cruz JM, Conde E, Domínguez H, Parajó JC (2007) Thermal stability of antioxidants obtained from wood and industrial wastes. Food Chem 100(3):1059–1064

Cui JL, Guo TT, Ren ZX, Zhang NS, Wang ML (2015) Diversity and antioxidant activity of culturable endophytic fungi from alpine plants of Rhodiola crenulata, R. angusta, and R. sachalinensis. PLoS One 10(3):e0118204

Dabbagh AJ, Mannion T, Lynch S, Frei B (1994) The effect of iron overload on rat plasma and liver oxidant status in vivo. Biochem J 300(3):799–803

Darwish AMG (2019) Fungal mycotoxins and natural antioxidants: two sides of the same coin and significance in food safety. Microb Biosyst 4:1–16

Darwish AMG, Abdelmotilib NM, Abdel-Azeem AM, Abo Nahas HH, Mohesien MT (2020) Applications of Chaetomium functional metabolites with special reference to antioxidants. In: Abdel-Azeem AM (ed) Recent developments on genus Chaetomium, fungal biology. Springer Nature, Switzerland AG, pp 227–240

Das A, Varma A (2009) Symbiosis: the art of living. In: Varma A, Kharkwal AC (eds) Soil biology symbiotic fungi. Springer, Berlin, pp 1–28

Demain AL (2014) Valuable secondary metabolites from fungi. In: Martin JF, Garcia-Estrada C, Zeilinger S (eds) Biosynthesis and molecular genetics of fungal secondary metabolites, fungal biology. Springer Science+Business Media, New York, pp 1–15

Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18(10):872–879

Ferreira IC, Barros L, Abreu RM (2009) Antioxidants in wild mushrooms. Curr Med Chem 16(12):1543–1560. https://doi.org/10.2174/092986709787909587

Freimoser FM, Screen S, Hu G, Leger RS (2003) EST analysis of genes expressed by the zygomycete pathogen Conidiobolus coronatus during growth on insect cuticle. Microbiology 149(7):1893–1900

Gomez-Cabrera MC, Borrás C, Pallardó FV, Sastre J, Ji LL, Viña J (2005) Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 567(1):113–120

Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:15–38

Guillot J, Konska G (1997) Lectins in higer fungi. Biochem Syst Ecol 25(3):203–230

Gupta VK, Sharma SK (2006) Plants as natural antioxidants. Nat Prod Radiance 5:326–334

Gutteridge JMC, Halliwell B (2000) Free radicals and antioxidants in the year 2000 a historical look to the future. Ann N Y Acad Sci 899:136–147

Hameed A, Hussain SA, Yang J et al (2017) Antioxidants potential of the filamentous fungi (Mucor circinelloides). Nutrients 9:1–20. https://doi.org/10.3390/nu9101101

Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A (2010) Antioxidants: its medicinal and pharmacological applications. Afr J Pure Appl Chem 4(8):142–151

Hasnat MA, Pervin M, Lim BO (2013) Acetylcholinesterase inhibition and in vitro and in vivo antioxidant activities of Ganoderma lucidum grown on germinated brown rice. Molecules 18:6663–6678

Heleno SA, Ferreira ICFR, Esteves AP, Ćirić A, Glamočlija J, Martins A, Soković M, Queiroz MJRP (2013) Antimicrobial and demelanizing activity of Ganoderma lucidum extract, p-hydroxybenzoic and cinnamic acids and their synthetic acetylated glucuronide methyl esters. Food Chem Toxicol 58:95–100

Hirose M, Takahashi S, Ogawa K, Futakuchi M, Shirai T, Shibutani M et al (1999) Chemoprevention of heterocyclic amine-induced carcinogenesis by phenolic compounds in rats. Cancer Lett 143(2):173–178

Hissin PJ, Hilf R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 74(1):214–226

Huang WY, Cai YZ, Xing J, Corke H, Sun MA (2007) Potential antioxidant resource: endophytic fungi from medicinal plants. Econ Bot 61:14–30

Hybertson BM, Gao B, Bose SK, McCord JM (2011) Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. Mol Asp Med 32(4–6):234–246. https://doi.org/10.1016/j.mam.2011.10.006

Imazaki I, Kadota I (2015) Molecular phylogeny and diversity of Fusarium endophytes isolated from tomato stems. FEMS Microbiol Ecol 91:1–16. https://doi.org/10.1093/femsec/fiv098

Jaszek M, Osińska-Jaroszuk M, Janusz G et al (2013) New bioactive fungal molecules with high antioxidant and antimicrobial capacity isolated from Cerrena unicolor idiophasic cultures. Biomed Res Int 2013:497492. https://doi.org/10.1155/2013/497492

Jayakumar T, Thomas PA, Geraldine P (2009) In-vitro antioxidant activities of an ethanolic extract of the oyster mushroom, Pleurotus ostreatus. Innovative Food Sci Emerg Technol 10(2):228–234

Jiang H, Sun P, He J, Shao P (2012) Rapid purification of polysaccharides using novel radial flow ion exchange by response surface methodology from Ganoderma lucidum. Food Bioprod Process 90:1–8

Kalam S, Singh R, Mani A, Patel J, Khan FN, Pandey AK (2012) Antioxidants: elixir of life. Int Multidisc Res J 2(1):18–34

Kao C, Jesuthasan AC, Bishop KS, Glucina MP, Ferguson LR (2013) Anti-cancer activities of Ganoderma lucidum: active ingredients and pathways. Funct Foods Health Dis 3:48–65

Keller NP (2019) Fungal secondary metabolism: regulation, function and drug discovery. Nat Rev Microbiol 17(3):167–180

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3(12):937–947

Keller, N., Palmer, J., Bayram, O., eds. (2017). Fungal Jewels: Secondary Metabolites. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-136-4

Kim MY, Seguin P, Ahn JK, Kim JJ, Chun SC, Kim EH, Ro HM (2008) Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J Agric Food Chem 56(16):7265–7270

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth and Bisby's dictionary of the fungi, 9th edn. CABI Publishing, Wallingford, UK

Kirk PM, Cannon PF, Minter DW, Stalper JA (2008) Dictionary of the fungi. CABI Publishing, Wallingford, UK, p 335

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Krause DJ, Kominek J, Opulente DA, Shen XX, Zhou X, Langdon QK, DeVirgilio J, Hulfachor AB, Kurtzman CP, Rokas A, Hittinger CT (2018) Functional and evolutionary characterization of a secondary metabolite gene cluster in budding yeasts. Proc Natl Acad Sci U S A 115(43):11030–11035. https://doi.org/10.1073/pnas.1806268115

Kunwar A, Priyadarsini KI (2011) Free radicals, oxidative stress and importance of antioxidants in human health. J Med Allied Sci 1(2):53

Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008) An endophytic fungus from Hypericum perforatum that produces hypericin. J Nat Prod 71(2):26–29

Lanigan RS, Yamarik TA (2002) Final report on the safety assessment of BHT (1). Int J Toxicol 21:19

Larson RA (1988) The antioxidants of higher plants. Phytochemistry 27(4):969–978

Lehman AJ, Fitzhugh OG, Nelson AA, Woodard G (1951) The pharmacological evaluation of antioxidants. In: Advances in food research, vol 3. Academic, Cambridge, MA, pp 197–208

Li Y, Song YC, Liu JY, Ma YM, Tan RX (2005) Anti-Helicobacter pylori substances from endophytic fungal cultures. World J Microbiol Biotechnol 21:553–558
Liebler DC (1993) The role of metabolism in the antioxidant function of Vitamin E. Crit Rev Toxicol 23(2):147–169
Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D et al (2018) Oxidative stress, aging, and diseases. Clin Interv Aging 13:757
Liu J, Shimizu K, Konishi F, Kumamoto S, Kondo R (2007) The anti-androgen effect of ganoderol B isolated from the fruiting body of Ganoderma lucidum. Bioorg Med Chem 15:4966–4972
Mamta, Misra K, Dhillon G, Brar S, Verma M (2013) Antioxidants. In: Biotransformation of waste biomass into high value biochemicals. Springer Nature, Switzerland, pp 117–138. https://doi.org/10.1007/978-1-4614-8005-1_6
Mattila P, Könkö K, Eurola M, Pihlava JM, Astola J, Vahteristo L, Piironen V (2001) Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. J Agric Food Chem 49(5):2343–2348
Meister A (1988) On the discovery of glutathione. Trends Biochem Sci 13:185–188
Meister A, Anderson ME (1983) Glutathione. Annu Rev Biochem 52:711–760
Miller DM, Buettner GR, Aust SD (1990) Transition metals as catalysts of "autoxidation" reactions. Free Radic Biol Med 8:95–108
Ng TB (2004) Peptides and proteins from fungi. Peptides 25(6):1055–1073
Niki E (2010) Assessment of antioxidant capacity in vitro and in vivo. Radic Biol Med 49(4):503–515
Niki E, Noguchi N (2000) Evaluation of antioxidant capacity. What capacity is being measured by which method? IUBMB Life 50(4–5):323–329
Ouyang J, Mao Z, Guo H, Xie Y, Cui Z, Sun JWH, Wen X, Wang J, Shan T (2018) Mollicellins O–R, four new depsidones isolated from the endophytic fungus Chaetomium sp. Eef-10. Molecules 23:3218
Papas AM (ed) (1998) Antioxidant status, diet, nutrition, and health, vol 9. CRC Press, Boca Raton, FL
Penninckx MJ, Elskens MT (1993) Metabolism and functions of glutathione in micro-organisms. Adv Microb Physiol 32:239–301
Percival M (1998) Antioxidants. Clinical nutrition insights. NUT031 1/96 Rev. 10/98
Pocsi I, Prade RA, Penninckx MJ (2004) Glutathione, altruistic metabolite in fungi. Adv Microb Physiol 49(1):1–76
Puttaraju NG, Venkateshaiah SU, Dharmesh SM, Urs SMN, Somasundaram R (2006) Antioxidant activity of indigenous edible mushrooms. J Agric Food Chem 54(26):9764–9772
Rastegari AA, Yadav AN, Yadav N (2019a) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5
Rastegari AA, Yadav AN, Yadav N, Tataei Sarshari N (2019b) Bioengineering of secondary metabolites. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 55–68. https://doi.org/10.1016/B978-0-444-63504-4.00004-9
Rastegari AA, Yadav AN, Yadav N (2020) New and future developments in microbial biotechnology and bioengineering: trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam
Ribeiro B, Rangel J, Valentão P, Baptista P, Seabra RM, Andrade PB (2006) Contents of carboxylic acids and two phenolics and antioxidant activity of dried Portuguese wild edible mushrooms. J Agric Food Chem 54(22):8530–8537
Ribeiro B, Valentão P, Baptista P, Seabra RM, Andrade PB (2007) Phenolic compounds, organic acids profiles and antioxidative properties of beefsteak fungus (Fistulina hepatica). Food Chem Toxicol 45(10):1805–1813

Said S, Allam M, Moustafa H, Mohamedz A (2002) A thermal stability of some commercial natural and synthetic antioxidants and their mixtures. J Food Lipids 9:277–293
Saito M, Sakagami H, Fujisawa S (2003) Cytotoxicity and apoptosis induction by butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Anticancer Res 23(6C):4693
Santos NA, Cordeiro AM, Damasceno SS, Aguiar RT, Rosenhaim R, Carvalho Filho JR et al (2012) Commercial antioxidants and thermal stability evaluations. Fuel 97:638–643
Sato I, Shimizu M, Hoshino T, Takaya N (2009) The glutathione system of Aspergillus nidulans involves a fungus-specific glutathione S-transferase. J Biol Chem 284(12):8042–8053. https://doi.org/10.1074/jbc.M807771200
Schueffler A, Anke T (2014) Fungal natural products in research and development. Nat Prod Rep 31:1425–1448
Shah AM, Channon KM (2004) Free radicals and redox signalling in cardiovascular disease. Heart 90:486–487
Shahidi F (2005) Bailey's industrial oil & fats products. Wiley, Hoboken, NJ
Shternshis M, Tomilova O, Shpatova T, Soytong K (2005) Evaluation of Ketomium-mycofungicide on Siberian isolates of phytopathogenic fungi. J Agric Technol 1(2):247–254
Sikora E, Cieślik E, Topolska K (2008) The sources of natural antioxidants. Acta Sci Pol Technol Aliment 7(1):5–17
Smith HA (2014) Production of antimicrobials and antioxidants from filamentous fungi. Doctoral dissertation, National University of Ireland Maynooth
Soares SC, Esteves F, Lundqvist D, Öhman A (2009) Some animal specific fears are more specific than others: evidence from attention and emotion measures. Behav Res Ther 47(12):1032–1042
Spadafranca A, Conesa CM, Sirini S, Testolin G (2010) Effect of dark chocolate on plasma epicatechin levels, DNA resistance to oxidative stress and total antioxidant activity in healthy subjects. Br J Nutr 103(7):1008–1014
Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN (1987) Bilirubin is an antioxidant of possible physiological importance. Science 235(4792):1043–1046
Strobel GA, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Res 67:491–502
Suzuki J, Isobe M, Morishita R, Nagai R (2010) Antioxidation in prevention of cardiovascular diseases – an effect of polyphenols. In: Bondy S, Maiese K (eds) Aging and age-related disorders. Humana, New York, pp 297–307
Taghvaei M, Jafari SM, Mahoonak AS, Nikoo AM, Rahmanian N, Hajitabar J, Meshginfar N (2013) The effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil. LWT-Food Sci Technol 56(1):124–130
Valentão P, Andrade PB, Rangel J, Ribeiro B, Silva BM, Baptista P, Seabra RM (2005) Effect of the conservation procedure on the contents of phenolic compounds and organic acids in chanterelle (Cantharellus cibarius) mushroom. J Agric Food Chem 53(12):4925–4931
Valenzuela A, Sanhueza J, Nieto S (2003) Cholesterol oxidation: health hazard and the role of antioxidants in prevention. Biol Res 36(3–4):291–302
Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2006a) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006b) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 160(1):1–40
Verhagen H, Schilderman PA, Kleinjans JC (1991) Butylated hydroxyanisole in perspective. Chemico Biol Interact 80(2):109–134
Verma V, Kharwar R, Strobel G (2009) Chemical and functional diversity of natural products from plant associated endophytic fungi. Nat Prod Commun 4(11):1934578X0900401
Veysi RA, Rahimi Z, Zahraei M, Nourouzian M, Pourmoteabed A (2007) Association between enzymatic and non-enzymatic antioxidant defense with Alzheimer disease. Acta Med Iran 46:271–276

Wachtel-Galor S, Yuen J, Buswell JA, Benzie IFF (2011) Ganoderma lucidum (Lingzhi or Reishi): a medicinal mushroom. In: Benzie IFF, Wachtel-Galor S (eds) Herbal medicine: biomolecular and clinical aspects. CRC Press/Taylor & Francis, Boca Raton, FL

Wanasundara PKJPD, Shahidi F (2005) Bailey's industrial oil and fat products. Wiley, Hoboken, NJ. https://doi.org/10.1002/047167849X

Wang S, Li XM, Teuscher F, Li DL, Diesel A, Ebel R, Proksch P, Wang BG (2006) Chaetopyranin, a benzaldehyde derivative, and other related metabolites from Chaetomium globosum, an endophytic fungus derived from the marine red alga Polysiphonia urceolata. J Nat Prod 69(11):1622–1625

Wang J, Hu S, Nie S, Yu Q, Xie M (2017) Reviews on mechanisms of in vitro antioxidant activity of polysaccharides. https://www.hindawi.com/journals/omcl/2016/5692852/. Accessed 22 Dec 2017

Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinogenesis 5:14. https://doi.org/10.1186/1477-3163-5-14

Wasser SP (2002) Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 60(3):258–274

Whysner J, Wang CX, Zang E, Iatropoulos MJ, Williams GM (1994) Dose response of promotion by butylated hydroxyanisole in chemically initiated tumours of the rat forestomach. Food Chem Toxicol 32(3):215–222

Williams GM, Iatropoulos MJ, Whysner J (1999) Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food Chem Toxicol 37(9–10):1027–1038

Wilson D (1995) Endophyte: the evolution of a term, and clarification of its use and definition. Oikos 73(2):274–276. https://doi.org/10.2307/3545919

Xing R, Liu S, Guo Z, Yu H, Wang P, Li C, Li Z, Li P (2005) Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities in vitro. Bioorg Med Chem 13:1573–1577

Yadav AN (2019) Fungal white biotechnology: conclusion and future prospects. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for sustainable environments, vol 3. Springer International Publishing, Cham, pp 491–498. https://doi.org/10.1007/978-3-030-25506-0_20

Yadav AN (2020) Recent trends in mycological research, Agricultural and medical perspective, vol 1. Springer, Switzerland

Yadav M, Yadav A, Yadav JP (2014) In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia jambolana Lam. Asian Pac J Trop Med 7:S256–S261. https://doi.org/10.1016/S1995-7645(14)60242-X

Yadav AN, Verma P, Kumar V, Sangwan P, Mishra S, Panjiar N et al (2018) Biodiversity of the genus Penicillium in different habitats. In: Gupta VK, Rodriguez-Couto S (eds) New and future developments in microbial biotechnology and bioengineering, Penicillium system properties and applications. Elsevier, Amsterdam, pp 3–18. https://doi.org/10.1016/B978-0-444-63501-3.00001-6

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N (2020) Microbiomes of extreme environments, Biotechnological applications in agriculture, environment and industry, vol 2. CRC Press, Taylor & Francis Group, Boca Raton, FL

Yang BK, Kim DH, Jeong SC, Das S, Choi YS, Shin JS, Lee SC, Song CH (2002) Hypoglycemic effect of a Lentinusedodesexo-polymer produced from a submerged mycelial culture. Biosci Biotechnol Biochem 66(5):937–942. https://doi.org/10.1271/bbb.66.937-1495

Yanishlieva N, Pokorný J, Gordon M (2000) Antioxidants in food: practical applications. CRC Press, Boca Raton, FL

Ye Y, Xiao Y, Ma L, Li H, Xie Z, Wang M, Liu J (2013) Flavipin in Chaetomium globosum CDW7, an endophytic fungus from Ginkgo biloba, contributes to antioxidant activity. Appl Microbiol Biotechnol 97(16):7131–7139. https://doi.org/10.1007/s00253-013-5013-8

Youn JY, Siu KL, Lob HE, Itani H, Harrison DG, Cai H (2014) Role of vascular oxidative stress in obesity and metabolic syndrome. Diabetes 63(7):2344–2355. https://doi.org/10.2337/db13-0719

Yu JH, Keller N (2005) Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol 43:437–458

Zhang W, Zeng YS, Wang Y, Liu W, Cheng JJ, Chen SJ (2006) Primary study on proteomics about Ganoderma lucidium spores promoting survival and axon regeneration of injured spinal motor neurons in rats. J Chin Integr Med 4(3):298–302

Zhao J, Shan T, Mou Y, Zhou L (2011) Plant-derived bioactive compounds produced by endophytic fungi. Min Rev Med Chem 11:159–168

Zhu M, Chang Q, Wong LK, Chong FS, Li RC (1999) Triterpene antioxidants from Ganoderma lucidum. Phytother Res 13L:529–531

Zhu LN, Luo X, Tang Q, Liu Y, Zhou S, Yang Y, Zhang JS (2013) Isolation, purification, and immunological activities of a low-molecular-weight polysaccharide from the Lingzhi or Reishi medicinal mushroom Ganoderma lucidum (higher basidiomycetes). Int J Med Mushrooms 15:407–414

Lacin, N., İzol, S. B., İpek, F., & Tuncer, M. C. (2019). Ganoderma lucidum, a promising agent possessing antioxidant and anti-inflammatory effects for treating calvarial defects with graft application in rats1. Acta cirurgica brasileira, 34

Ikekawa, T., NAKANISHI, M., UEHARA, N., CHIHARA, G., & FUKUOKA, F. (1968). Antitumor action of some basidiomycetes, especially Phellinus linteus. GANN Japanese Journalof Cancer Research, 59(2), 155-157.)

Yan, W., Cao, L. L., Zhang, Y. Y., Zhao, R., Zhao, S. S., Khan, B., & Ye, Y. H. (2018). New metabolites from endophytic fungus Chaetomium globosum CDW7. Molecules, 23(11), 2873.

Chapter 3
Endophytic Fungi as a Source of New Pharmaceutical Biomolecules

Mohamed A. Abdel-Azeem, Yousra A. El-Maradny, Abdelrahman M. Othman, and Ahmed M. Abdel-Azeem

Contents

M. A. Abdel-Azeem (✉)
Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Industries, Sinai University, Al Arish, North Sinai, Egypt
e-mail: mohamed.abdelazeem@su.edu.eg

Y. A. El-Maradny
Microbiology Department, High Institute of Public Health, Alexandria University, Alexandria, Egypt

A. M. Othman
Faculty of Pharmacy and Drug Manufacturing, Pharos University, Alexandria, Egypt

A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_3

3.1 Introduction

Recently, several studies have led to the discovery of important plant secondary metabolites from endophytic fungi thus raising the prospect of using such organisms as alternative sources of these metabolites (Rana et al. 2019a, 2020). In addition to being alternative sources for secondary metabolites known from plants, endophytes accumulate a wealth of other biologically active and structurally diverse natural products that are unprecedented in nature and are of importance for drug discovery or as lead compounds for agriculture (Tan and Zou 2001; Strobel and Daisy 2003; Zhang et al. 2006; Chandra 2012; Devi et al. 2020). It is hence now generally accepted that endophytes represent an important and largely untapped reservoir of unique chemical structures that have been modified through evolution and are believed to be involved in host plant protection and communication (Gunatilaka 2006).

As a result of these long-held associations, it is possible to imagine that some of these endophytic microbes may have devised genetic systems allowing for the transfer of information between themselves and the higher plant and vice versa (Stierle et al. 1993a, b).

3.2 Biology, Ecology, and Distribution

Roughly, there are almost 300,000 plant species and each different plant is the host to one or more different endophytes, and many of them may inhabit certain hosts. It has been anticipated that there are as many as one million different endophytic fungal taxa proving endophyte's hyper-diversity (Selim et al. 2012). Endophyte's importance was established over an extended period of time as a potential source of pharmaceutical drugs as they were reported to produce secondary metabolites used as anticancer, antibacterial, antiviral, and so many more (Sudha et al. 2016; Kour et al. 2019; Yadav et al. 2019b). The diversity of the endophytic fungi inhabitant plants claims that plants with different atmospheres can inhabit different endophytic fungi producing either similar or different metabolites (Ka et al. 2012; Rana et al. 2019b; Yadav et al. 2019a), for example, Taxol a highly functionalized anti-cancer diterpenoid can be produced by various endophytic fungi from different plants as *Taxomyces andreanae* isolated from *Taxus brevifolia* (Stierle et al. 1993a, b), *Pestalotiopsis microspore* isolated from *Taxus wallichiana* (Strobel et al. 1996), *Pestalotiopsis microspore* isolated from bald cypress in South Carolina (Li et al. 1996), *Fusarium solani* isolated from *Taxus chinensis* and *Aspergillus* isolated from *Ginkgo biloba* (Li et al. 2005; Gu 2009).

Since each plant can give multiple different secondary metabolites isolated from various Endophytic fungi, several criteria must be considered in selecting and isolating the plant and the endophytic fungi, respectively, to target specific endophytes (Strobel and Daisy 2003) as:

1. The place of origin of the plant acts as an important cornerstone in determining the type of the endophytic fungi, as *Rhyncholacis penicillate* which is an aquatic plant that lives in a harsh aquatic environment produces oocydin A isolated from *Serratia marcescens* endophytic fungi to help resists infection by common oomyceteous fungi (Strobel et al. 1999a).
2. Plants that grow in only specific parts of the world or occupied a certain ancient landmass as *Maytenus hookeri* that only distributed in areas of Yunnan, China that produces *Chaetomium globosum* endophytic fungi isolating Chaetoglobosin B showing antituberculosis activity (Ding et al. 2006).
3. Plants bordered by pathogen-infected plants, and displaying no symptoms are more likely to produce endophytic fungi possessing antimicrobial activity (Tuntiwachwuttikul et al. 2008).
4. Younger plants are more appropriate for the isolation of endophytic fungi than older ones (Bacon and White 2018).

Schulz et al. (1999) has claimed that environmentally we can control the isolated endophytes from the plant, as they proved that when two plants from different regions one can produce a bioactive product and the other fails due to the evolution of the endophytic fungi from the harsh environment. For example, the herbicidal activity of the secondary metabolites of endophytic fungi of *Phyllosticta capitalensis* differed with the different places of the plant host from which it was isolated. The environment also can make the secondary metabolite of the endophytic fungi be more aggressive and hostile to the host plant in response to some environmental signs (Hendry et al. 2002) it also can lead to alteration in the metabolite profile, number, and variety. This proves the importance of studying the effect of host plants on endophytic metabolites production and host-endophytes relationships.

3.3 Material and Methods of Preparation and Isolation

The isolation procedure is a critical important step in working with endophytic fungi (Abdel-Azeem 2012; Abdel-Azeem et al. 2012, 2016, 2019; Abo Nahas 2019; Attia et al. 2020; Balbool, and Abdel-Azeem 2020). It should be delicate enough to recover endophytic fungi and at the same time be able to eliminate epiphytes and other organisms (Mckinnon 2016). Therefore, the isolation procedure must be tailored to the respective tissues and microorganisms. The isolation of the endophytic fungi and the extraction of the medicinal drugs undergo several steps. The most common first step used is surface sterilization (Gautam et al. 2012; Paper 2002; Phongpaichit et al. 2006; Mckinnon 2016), this step is where the concerned part of the plant is sterilized using sterilization chemicals as distilled water (Nalini et al. 2014), ethyl alcohol (Tolulope et al. 2015), sodium hypochlorite (Wiyakrutta et al. 2004). The second step is the incubation of the concerned plan part in an appropriate medium to facilitate the fermentation of the endophytic fungi (Mckinnon 2016) the most commonly used medium is the PDA medium (Kjer et al. 2010; Raunsai et al.

2018). The third step is to identify the extracted endophytic fungi using different methods as morphological identification, microscopical identification, or DNA isolation PCR amplification. The final step is to try to extract the drug from the endophytic fungi using the appropriate medium and technique (Mckinnon 2016). Various isolation techniques we summarized in Table 3.1.

3.4 Chemistry of Endophytes

Novel natural secondary metabolites from endophytes play a very important role in various biological applications such as antimicrobial, anticancer, antioxidant, and antidiabetic. The same endophyte strain can produce different bioactive metabolites (Stierle et al. 1993a, b; Zhang et al. 2006). The chemistry and characters of some bacterial, actinomycetes, and fungal endophytes will be illustrated herein Table 3.2.

3.5 Endophytes Applications

Fungal endophytes are isolated from different medicinal and other plant species under different geographical and ecological conditions. They are the prospective source of various bioactive metabolites and novel compounds to be used in a wide fields variety as; medicine, agriculture, pharmaceutical, and industry (Kusari et al. 2011; Gupta et al. 2020; Rastegari et al. 2020). The endophytic fungus is easily produced on large scale for modern medicine using culture systems and fermenters rather than plant harvesting and disturbance of environmental biodiversity, since about 40% of prescription drugs are based on them (Nisa et al. 2015; Rastegari et al. 2020). Natural products and their derivatives contribute about 68% of antimicrobial compounds and 34% of anticancer therapy. Endophytes metabolites could be grouped according to their chemistry into; xanthones, isocoumarins, quinones alkaloids, steroids, terpenoids, flavonoids, glycosides, phenyl propanoids, lignans, aliphatic metabolites, lactones, etc. (Zhang et al. 2006; Newman, and Cragg 2007).

3.5.1 Antimicrobial Compounds

Microorganisms are the source of antimicrobial metabolites which are natural organic substances with low molecular weight and active against other microorganisms (Wani et al. 1971). The antimicrobial activity of the endophytic fungal crude extracts shown activity against bacteria, viruses, pathogenic fungi, cytotoxicity, and antimalarial activity (Liu et al. 2001; Singh and Yadav 2020). Many metabolites from fungal endophytes have low toxicity and promising antimicrobial activity such as piperine, javanicin, artemisinin, asperfumin, asperfumoid, phomol, and

Table 3.1 Methods of isolation and extraction of ten plants covering most of the techniques used

No	Plant	Surface sterilization	Inoculation media/time/temp.	No. of isolate strains with pharmaceutical use	Method of identification	Type of isolated drug	Method of extraction of drug	References
1	*Ricinus communis*	– 0% ethanol for 1 min – Sodium hypochlorite solution for 30 s to 1 min	PDA plate for 5–7 days at 28 ± 10 °C	3/slant	PDA plate for 7 days identified by: morphology	Antibacterial	150 mL of PDB was inoculated with various fungi culture and incubated at 28 ± 1 °C in the incubator	Sandhu et al. (2014)
2	*Taxus mairei*	– 70% ethanol for 1 min	PDA plate for 14 days at 25 °C	58 strains 21 strains	PDA plate identified by phenotypic techniques	Antibacterial Anticancer	Potato dextrose broth for 7 days at 25 °C then centrifuged at 4000 rpm for 5 min	Huang et al. (2001)
3	*Garcinia* *G. atroviridis* *G. dulcis* *G. mangostana* *G. nigrolineata* *G. scortechinii*	– 95% ethanol for 30 s – 5% sodium hypochlorite for 5 min – 95% ethanol for 30 s	Cornmeal agar and incubated at 25 °C then transferred to PDA without anti-bacterial	18 strains 15 strains 34 strains 3 strains 1 strain	PDA using the molecular identification techniques	Antibacterial	Three mycelial agar plugs were inoculated into 500-mL flasks containing 300 mL PDB and incubated at room temperature for 3 weeks then filtrated	Phongpaichit et al. (2007)

(continued)

Table 3.1 (continued)

No	Plant	Surface sterilization	Inoculation media/time/temp.	No. of isolate strains with pharmaceutical use	Method of identification	Type of isolated drug	Method of extraction of drug	References
4	*Coscinium fenestratum*	– 70% ethyl alcohol for 1 min – Immersion in 4% sodium hypochlorite for 3 min – 70% ethyl alcohol for a minute	(PDA) plate. Sealed with parafilm and incubated at 27 ± 2 °C for 15 days under dark conditions	– 36 strains from leaf – 5 strains from stem	PDA, SDA, and MRBA agar media identified by: morphology	Antifungal Antibacterial	–	Goveas et al. (2011)
5	*Pasania edulis*	– 70% ethanol for 1 min – 15% H_2O_2 for 15 min – 1 min in 70% ethanol	2% malt extract agar plate and incubated at room temperature for more than 1 month	21 strains	–	–	–	Paper (2002)
6	*Musa acuminata*	– 36% formaldehyde solution for 1 min – Being rinsed three times in autoclaved distilled water	1.5% potato-dextrose agar (PDA) and 1.5% water after incubation at 26 °C for several days individual hyphal tips of the developing fungal colonies were removed and placed onto PDA, incubated for 8–10 days	– 13 strain from leaves – 10 strains from roots	PDA medium a sterile microscope slide, incubated at 26 °C Identified by: * microscope	Antibacterial	–	Cao et al. (2002)

No	Plant	Surface sterilization	Inoculation media/time/temp.	No. of isolate strains with pharmaceutical use	Method of identification	Type of isolated drug	Method of extraction of drug	References
7	*Cannabis sativa*	– Sterilized distilled water for 1 min – Sodium hypochlorite for 40 s	PDA medium incubated at 27 °C ± 2 °C for 1–2 weeks in BOD incubator	12 strains	Identified by phenotypic and cultural growing parameters	Antifungal	The fungal material was incubated at 27 °C for more than 15 days in 250mL potato dextrose broth in a conical flask in BOD incubator. The resulting extract after filtration was centrifuged 10,000–12,000 rpm for 30 min	Gautam et al. (2012)
8	*Rhizophora mucronata*	– Immersed in 70% ethanol for 60–120 s – 4% NaOCl for 60 s – Rinsed several times in sterile distilled water	Medium A with the final pH adjusted from 7.4 to 7.8. The plates were incubated at 27 °C for 7 days then to medium b (without chloramphenicol) for 7 days	78 strains	By molecular identification	Anti-fungal Antioxidant Anti-bacteria	– Inoculated into a 500 mL Erlenmeyer flask containing 100 mL of Potato Dextrose Broth at pH 6.5 and allowed to grow in a shaking incubator (120 rpm) at 26 °C for 14 days – The filtrate was extracted twice with equal volume of ethyl acetate – The extract was concentrated under reduced pressure at 40 °C	Noraida et al. (2018)

(continued)

No	Plant	Surface sterilization	Inoculation media/time/temp.	No. of isolate strains with pharmaceutical use	Method of identification	Type of isolated drug	Method of extraction of drug	References
9	*Catharanthus roseus*	–	Potato dextrose agar (PDA) slants have optimum growth at pH 7.0 and temperature 27 °C for 7 days	1 strain	PDA medium identified by phenotypic techniques	Anti-cancer	– A two-stage fermentation procedure was made – Culture filtrates and mycelia were lyophilized – Lyophilized culture filtrate was extracted using ethyl acetate – The extraction was MS-MS spectrum either vincristine or vinblastine – Drying using anhydrous sodium sulfate – Small amount of crude extract was dissolved in ethyl acetate and subjected to TLC on silica gel-G	Kumar et al. (2013)

(continued)

Table 3.1 (continued)

No	Plant	Surface sterilization	Inoculation media/time/temp.	No. of isolate strains with pharmaceutical use	Method of identification	Type of isolated drug	Method of extraction of drug	References
10	*Taxus chinensis*	– 70% ethanol (v/v) for 1 min and 0.1% mercuric chloride (v/v) for 8 min	PDA incubated at 28 °C for 2–15 days	39 strains	–	Anti-cancer	– 500-mL Erlenmeyer flasks containing 300 mL of the liquid medium and cultured at 160 rpm at 28 °C for 14 days in a rotary shaker – The fermentation broths and ground mycelia were extracted three times with ethyl acetate at room temperature for 5 h – All extracts were combined and condensed in a rotating evaporator under reduced pressure – The residues were re-dissolved with 10 mL of 100% methanol (v/v)	Liu et al. (2009a, b)

Table 3.2 The chemistry and characters of some bacterial, actinomycetes, and fungal endophytes

Endophyte source	Biomacromolecules	Secondary metabolites	References
Bacteria	*Polysaccharides* – Exopolysaccharides – Lipopolycharrides	*Phytohormones* – Ecomycins – Adenine derivatives	Holland and Polacco (1992), Leigh and Coplin (1992), Ivanova et al. (2001)
	Enzymes and proteins – Chitinase – Pectinase – Pectinolytic enzymes	Salicylic acid *Unusual amino acids* – Homoserine – β-Hydroxyaspartic acid	Pleban et al. (1997), Quadt-Hallmann et al. (1997), Miller et al. (1998), van Loon et al. (1998)
Actinomycetes	– 1-*N*-methylalbonoursin – α-Amylase	– Munumbicins A, B, C and D – Kakadumycin A – Coronamycins (peptides) – Sesquiterpenes – Cyclopentenone	Stamford et al. (2001), Castillo et al. (2002), Guan et al. (2005), Lin et al. (2006)
Fungi	*Polysaccharides*	*Alkaloids* – Amines and amides – Indole derivatives – Pyrrolizidines – Quinazolines	Wilkinson et al. (2000), Tan and Zou (2001), Shen et al. (2006)
	Enzymes and proteins	*Steroids* Ergosterol derivative	
	Lipase and glucoamylase	*Terpenoids* – Sesquiterpenes – Diterpenes – Cytochalasins – Myrothecines (10,13-cyclotrichothecane derived macrolides)	Torres et al. (2003), Krohn et al. (2005)
		Isocoumarin derivatives	Kongsaeree et al. (2003)
		Quinones – Cyclohexanone – Epoxides – Jesterone – Hydroxyjesterone – Ambuic acid	Li and Strobel (2001), Ge et al. (2005)
		Phenylpropanoids and lignans Guignardic acid	Drandarov et al. (2001)
		Phenols and phenolic acids – Pestacin – Isopestacin (dihydroisobenzofuan)	Harper et al. (2003), Bashyal et al. (2005)
		Aliphatic compounds Chaetomellic acid A	Desai and Argade (1997)

(continued)

Table 3.2 (continued)

Endophyte source	Biomacromolecules	Secondary metabolites	References
		Lactones – Phomol – Microcarpalide – Sequoiamonascins A–D – Graphislactones A, G and H	Weber et al. (2004), Prasad et al. (2007)
		Miscellaneous metabolites – Sequoiatones C–F – Asperfumoidand asperfumin – Naphtho-c-pyrones – Rubrofusarin B – Fonsecinone A – Asperprone B – Aurasperone A	Chen et al. (2003), Liu et al. (2004), Campos et al. (2005)

hypericin. *Fusarium* sp. CR377, a pentaketide agent, collected in the Guanacaste Conservation Area of Costa Rica showed potent antifungal activity against *Candida albicans* (Brady and Clardy 2000). Guanacastepene Aditerpenoids, is another metabolite from Costa Rica isolated from CR115 fungi endophytes in *Daphnopsis americana* tree. These metabolites showed strong antibiotic activity against *Staphylococcus aureus* and *Enterococcus faecium* resistant strains (Brady et al. 2001). Rhizotonic acid an enzophenone, purified from *Rhizoctonia* sp., in *Cynodon dactylon*, was found to be active against the gastric ulcer and *Helicobacter pylori* strains (Ma et al. 2004).

Moreover, another antimicrobial metabolite altersetin related to equisetin, isolated from two strains of *Alternaria* sp. P 0506 and P 0535 were endophytes in *Vinca minor* and *Euonymus europaeus*, respectively (Hellwig et al. 2002). *Artemisia annua* is a known plant for its production of artemisinin an antimalarial drug. Liu et al. (2001) isolated 39 endophytes from *A. annua* whereas, showing the most effective in-vitro antifungal activity against *Gaeumannomyces graminis* var. tri-tici, *R. cerealis*, *Helminthosporium sativum*, *Fusarium graminearum*, *Gerlachianivalis,* and *Phytophthora*. Strobel et al. (2001) obtained *Muscodoralbus* a xylariaceaous (non-spore producing) endophytic fungus from *Cinnamomum zeylanicum* (cinnamon tree). The five classes of volatile compounds extracted from the fungus showed mild antifungal and antibacterial activity, but collectively they cause broad-spectrum effects against pathogenic microorganisms. The most biologically active compound was the ester and they state that "mycofumigation" are very beneficial and promising in human and agricultural applications.

The endophyte metabolite phomol from *Phomopsis* sp., active polyketide lactone, was isolated from the medicinal plant *Erythrina cristagalli* and produced antifungal and antibacterial activity (Weber et al. 2004). Liu et al. (2004) recognized for the first time *Aspergillus fumigatus* CY018 endophyte from the leaf of

Cynodondactylon. Twelve metabolites were isolated and showed in-vitro antifungal activity against *Candida albicans*, *Trichophyton rubrum,* and *Aspergillus niger*. Two of them were new isolate asperfumoid and asperfumin with structures of spiro-(3-hydroxyl-2,6-dimethoxyl-2,5-diene-4-cyclohexone-(1,30)-50-methoxyl-70-methyl-(10*H*,20*H*, 40*H*)-quinoline-2040-dione) and 5-hydroxyl-2-(6-hydroxyl-2-methoxyl-4-methylbenzoyl)-3,6-dimethoxyl-benzoic methyl ester, respectively. Another was study done by Maria et al. (2005) isolated 14 endophytic fungi from *Acanthus ilicifolius* and *Acrostichum aureum*, two mangrove plants. Studied endophytes especially *Aspergillus* sp. and *Pestalotiopsis* sp. were inhibited by many bacteria including *Bacillus subtilis*, *Enterococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aerugionsa*, *Salmonella typhi* and *Staphylococcus aureus*, and fungi as *Candida albicans* and *Trichophyton metagrophytes*.

Sette et al. (2006) tested the antimicrobial activity of 37 endophytic filamentous fungi from coffee plants (*Coffea arabica* and *Coffea robusta*). Seventeen fungi inhibited the pathogenic human bacteria including *Salmonella choleraesuis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and four different *Escherichia coli* serotypes. Raviraja et al. (2006) checked the antibacterial and antifungal activity of 15 endophytes from 8 medicinal plant hosts from the Western Ghats of India. They found that the most promising antimicrobial activities were in *Alternaria* sp., *Nigrospora oryzae,* and *Papulospora* sp. against fungi and both gram-positive and gram-negative bacteria. In another study by Tejesvi et al. (2007) they checked the endophyte *Pestalotiopsis* strains from *Azadirachta indica*, *Holarrhena antidysenterica*, *Terminalia arjuna,* and *T. chebula* medicinal plants. The best antifungal activity was found in the *Pestalotiopsis* strains extracted from *Terminalia arjuna* against *Alternaria carthami*, *Fusarium oxysporum*, *Fusarium verticilloides*, *Macrophomina phaseolina*, *Phoma sorghina,* and *Sclerotinia sclerotiorum*.

Fungal endophytes isolated from *Hypericum perforatum* (St. John's wort) producing hypericin a naphthodianthrone derivative, showing strong antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica,* and *Escherichia coli* and antifungal activity against *Aspergillus niger* and *Candida albicans* (Kusari et al. 2008a, b). Zhang et al. (2008) characterize the secondary metabolite caphalosol from *Cephalosporium acremonium* IFB-E007 endophytes that reside in *Trachelospermum jasminoides* (Apocynaceae) the most potent antifungal activity of cephalosol was against *Trichophyton rubrum* and *Candida albicans*. A study on the *coffee arabica* L. screened the antimicrobial activity of 22 isolated endophytic fungi. The higher inhibition zone was found in the crude extract of *Alternaria alternata* against *S. aureus* (Fernandes et al. 2009). Javanicin as $C_{15}H_{14}O_6$ naphthaquinone is a selective antibacterial against *Pseudomonas* sp. and the most biologically active compound extracted from *Chloridium* sp. endophyte inhabitant in *Azadirachta indica* an Indian plant known as (neem or Indian Lilac) (Kharwar et al. 2009).

Another study found that from 16 endophytes extracted from the leaf of *Eucalyptus citriodora* Hook only *Chaetomium* and another 2 unidentified isolates showed strong antifungal activity against *Curvularia lunata* (Kharwar et al. 2010).

Mahapatra and Banerjee (2010) studied the antibacterial activity of *Curvularia* sp., *Aspergillus* sp., and another unidentified fungus isolated from *Alstonia scholaris* against four human pathogenic bacteria; *Escherichia coli*, *Bacillus cereus*, *Vibrio cholerae,* and *Klebsiella pneumoniae*. Jalgaonwala et al. (2010) showed that the spectrum of activity of endophytes was differed according to the type of isolates and the population of endophytes was denser in the aerial parts of plants rather than the underground tissues. One hundred forty-two endophytic fungi were obtained from different Indian medicinal plants, 14 of them showed antibacterial and antifungal activity. The most powerful antibacterial activity was in endophytic fungi HFR4, HFR6 isolated from *Curcuma longa* L. rhizomes against pathogenic *Bacillus subtilis*, *Staphylococcus aureus,* and *Protease vulgaris* and AFR1, AFR4, AFR7 fungal endophytes from roots of *Aloe vera* L. against *S. typhi*. While KTP1 endophytes from roots of *Morrayo konengi* L. and TF5 from leaves of *Osimum sanctum* L. showed the best antifungal activity.

Another study by Zhang et al. (2012) evaluates the antimicrobial activity of 11 isolated endophytes from *Artemisia annua* L. The strongest antibacterial activities against *Escherichia coli*, *Staphylococcus aureus,* and *Trichophyton rubrum* were showed in three strains of *Aspergillus* sp. SPS-02, SPS-04, and SPS-01. Endophytes from *Aspergillus* sp. SPS-02 and *Cephalosporium* sp. SPS-08 had the best activity against plant pathogen *Magnaporthe grisea*. While endophytes *Mucor* sp. SPS-11 showed a pronounced effect against *Rhizoctonia cerealis* another plant pathogen.

The antimicrobial activity of *Cochliobolus intermedius*, *Phomopsis* sp., and two unidentified endophytes isolated from *Sapinduss aponaria* L., a Brazilian tree known as "sabãode-soldado," was carried out by Garcia et al. (2012). They found that G2-20 a metabolite from *C. intermedius* showed the strongest activity against tested pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Micrococcus luteus,* and *Enterococcus hirae*. A work done by Gond et al. (2012) on the *Nyctanthes arbor-tristis* leaf and stem tissues extracted nineteen fungal endophytes. Maximum inhibition against *Shigella* sp. and *Pseudomonas aeruginosa* was by *Nigrospora oryzae* showed. While, *Chaetomium globosum* and *Colletotrichum dematium* showed antibacterial activity against *P. aeruginosa*, *shigella* sp., *Salmonella enteritidis,* and *Salmonella paratyphi*.

In a study carried by Shaaban et al. (2013) ten bioactive secondary metabolites were extracted and identified from *Aspergillus fumigatus* sp. isolate R7 resides in the leaves of sweet potato *Ipomoea batatas*. The crude extract of this endophyte exhibited potent antimicrobial activity against the microalgae *Chlorella vulgaris*, *Chlorella sorokiniana,* and *Scenedesmus subspicatus*and Gram-positive *Streptomyces viridochromogenes* (*Tü 57*) and *Bacillus subtilis*. The further antifungal and antibacterial activity showed in compounds fumiquinazoline-F and fumiquinazoline-D against *Mucor miehi*, *Candida albicans,* and *Staphylococcus aureus*.

Supaphon et al. (2013) extracted 160 fungal endophytes from 3 southern Thailand seagrasses including *Cymodocea serrulata*, *Halophila ovalis,* and *Thalassia hemprichii*. Seven isolates including *Trichoderma* spp. (PSU-ES8 and PSU-ES38), *Hypocreales* sp. PSU-ES26, *Penicillium* sp. PSU-ES43, *Fusarium* sp. PSU-ES73,

Stephanonectria sp. PSU-ES172 and an unidentified endophyte PSU-ES190 showed pronounced antimicrobial activity against ten pathogenic microorganisms. Another endophytic fungi DZY16 belonging to *Nigrospora* and isolated from *Eucommia ulmoides Oliv*. tissues were studied by Ting et al. (2013) and showed a pronounced bioactive antimicrobial activity against *Rhizoctonia solani* and *Gibberella zeae*.

The endophytic fungus *Nodulisporium* sp. PT11 and *Phoma* sp. PT01 isolated from *Mitragyna javanica* were had strong antimicrobial and anticancer activity, respectively (Thirawatthana et al. 2013). Chithra et al. (2014) reported that the fungal endophytes *Colletotrichum gloeosporioides* isolated from *Piper nigrum* had the ability to produce the bioactive alkaloid piperine. Piperine had broad bioactive properties such as bronchodilatation, antimicrobial, anti-inflammatory, and decreasing the toxicity of aflatoxins (Gagini et al. 2010). Musavi and Balakrishnan (2014) isolated the *Fusarium oxysporum NFX06* endophyte from the leaf of *Nothapodytes foetida* in Karnataka and reported the antimicrobial activity of these fungal isolates against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Candida albicans*.

Akpotu et al. (2017) extracted five fungal endophytes CR-LC, CR-MR1B, CR-MR1, CR-MRB2, and CR-MR3 associated with *Catharanthus roseus* leaves. This study suggested that the antimicrobial activity of these isolates against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Aspergillus fumigatus*, and *Candida albicans* because of the presence of bioactive metabolites including acropyrone, nigricinol, questinol, citreoisocoumarin, citreoisocoumarinol, hydroxyemodin, methyl 2-(4-hydroxyphenyl) acetate, and cladosporin. Jin et al. (2017) were observed a remarkable broad-spectrum antibacterial and antifungal activity of the saponins producing endophytes *Fusarium* sp. PN8 and *Aspergillus* sp. PN17 from the Chinese herb *Panax notoginseng*. Three fungal endophytes were extracted from the leaves of *Olea europaea* L. and tested for their antimicrobial potential by Malhadas et al. (2017). They found that *Penicillium canescens and Penicillium commune* were effective in inhibition of *Bacillus cereus*, *Bacillus subtilis and Staphyloccocus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida glabrata, and Candida parapsilosis* when compared with standard control while *Alternaria alternata* showed maximum activity against yeasts.

Arora and Kaur (2019) studied the antimicrobial activity of *Aspergillus fumigatus* (DSE 17) from *Moringa oleifera* against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Candida albican,* and clinical isolate of *methicillin-resistant Staphylococcus aureus*. They found that *A. fumigatus* produced promising non-cytotoxic broad-spectrum antimicrobial metabolites. A recent study on 20 Thai orchid samples reported that CK F05-5 endophytes (identified as *Fusarium oxysporum* KU527806) from *Dendrobium lindleyi* exhibited the best antioxidant and antifungal activity against *Fusarium* sp., *Colletotrichum* sp., and *Curvularia* sp. (Bungtongdee et al. 2019). New upcoming field on medical research and pharmaceutical applications used the myconanotechnology. A work done by Hulikere and Joshi (2019) synthesized silver nanoparticles of the *Cladosporium cladosporioides* endophytes extracted from the

brown algae *Sargassum wightii*. These particles were found to be effective against human pathogenic microorganism's including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermis,* and *Candida albicans*. Another study by (Ganesan et al. 2020) reported that the synthesized ZnO nanoparticles of *Periconium* sp. exhibited a pronounced activity against Gram-positive bacteria more than Gram-negative ones.

Many studies evaluated the antimicrobial activity of different metabolites such as polyoxygenated decalin and dehydroxychlorofusareilin Bfrom *Aspergillus* sp. (Debbab et al. 2010), pestalone from *Pestalotia* sp. and *Penicillium glabrum* (Bugni and Ireland 2004; Zhang et al. 2009b) these endophytes were isolated from the brown algae *Sargassum horneri*, *Rosenvingea* sp., and *Sargassum thunbergii*, respectively. Other bioactive antimicrobial isolates from endophytes that inhabited on the surface of marine algae including 2-deoxy-sohirnone C (Jiang et al. 2018), 20-acetoxy-7-chlorocitreorosein (He et al. 2017), Penicilactone (Debbab et al. 2010), and naphthaquinonejavanicin (Radić and Štrukelj 2012) were extracted from fungal endophytes *Penicillium* sp. *GD6*, *Penicillium citrinum HL-5126*, *Penicillium* sp., and *Chloridium* sp., respectively.

The experimental results showed that CK F05-5 isolated from flowers of *Dendrobium lindleyi* (which was identified as *Fusarium oxysporum*) exhibited the strongest antipathogenic fungal activity against fungal pathogens. This present study reported the colonization of endophytic fungi isolated from 20 orchid samples collected in northern Thailand from 12 genera of orchids. From the experimental results, the extracted chemical components of the endophytes CK F05-5 (identified as *Fusarium oxysporum* KU527806) may be responsible for antifungal, antioxidant, and antimutagenic activities.

3.5.2 Antiviral Compounds

Disease caused by pathogenic viral infections afflicted the whole human race with high mortality rates (Williams et al. 2013) and remained as one of the prime causes of human death worldwide. Human immunodeficiency virus (HIV), influenza, and hepatitis C virus (HCV) are the three most perilous viral diseases which cause maximum human death. Dengue virus (DENV) is an important mosquito-borne pathogen for causing dengue fever (DF) and dengue hemorrhagic fever (DHF). DF is relatively mild, but DHF leads to the life-threatening dengue shock syndrome (Simmons et al. 2012; Yadav 2020).

In this study, we examined the anti-DENV activity of secondary metabolites produced by a fungal strain, *Penicillium* sp. FKI-7127. We isolated and identified brefeldin A (BFA) as a novel antiviral agent against DENVs. Inhibition of BFA on Japanese encephalitis virus (JEV) and Zika virus (ZIKV) was also demonstrated (Raekiansyah et al. 2017). The antiviral drugs inhibit the virus infection either by specifically targeting the viral proteins or the host cellular factors that the viruses exploit for their reproduction (Clercq 2002). For the past 25 years, bioactive

compounds from many traditional medicinal plants have been screened for their antiviral activity by various research groups in Asia, Far East, Europe, and America (Jassim and Naji 2003). Many endophytes were reported as potent viral inhibitors. Recently, it was reported that an endophytic *Streptomyces* sp. strain isolated from the mangrove plant *Bruguiera gymnorrhiza*, produced xiamycin, which exhibits selective anti-HIV activity (Ding et al. 2010). Additionally, the endophytic *Emericella* sp. (HK-ZJ) isolated from another mangrove plant *Aegiceras corniculatum*, produces several bioactive isoindolone compounds, for which two of them showed moderate activity against influenza A virus (H1N1) (Zhang et al. 2011).

However, also already relatively well-known fungi should not be overlooked. Less intensively investigated fungi for their bioactivities include tree-pathogens that also seem a promising source of antiviral agents. A previous study has detected a number of plant pathogenic fungi with various ecological roles (white-rot fungi, soft-rot fungi, blue-stain fungi, and insect-symbionts) having antiviral activities (Mlinaric et al. 2005). Fifteen extracts inhibited viral reproduction (positive signs), and the remaining tested extracts were inactive. On the other hand, the coherence and reproduction of the VSV virus were inhibited by 16 endophytic extracts. The endophytic fungus, Pleosporatarda, associated with medicinal plant *Ephedra aphylla*, and sterile mycelia (w9) from host plant *Ephedra alata*, were the most potent candidates against HSV-2, with inhibitory activity of 40.7% for both. On the other hand, different endophytic metabolites interrupted the attack of VSV to Vero cells, for example, *Acremonium strictum* from *Launeaspinos*, *Penicillium* sp. from *Phlomisaurea*, and *Mucor fuscus* from *Stachys aegyptiaca*. The most active strain to stop the reproduction of the VSV was *Aspergillus* sp. from Galiumsinaicum (Selim et al. 2018).

Some studies have identified substances that inhibit viruses (Bunyapaiboonsri et al. 2010; Phongpaichit et al. 2007), specifically the cytonic acids A and B isolated from endophytic fungi were found to inhibit the human cytomegalovirus protease enzyme (Guo et al. 2000). However, the effect of compounds from endophytic fungi on antiviral activity, especially anti-HIV, has been largely unexplored (Liu et al. 2009a, b; Yu et al. 2008). Isoindolone derivatives distribute broadly in natural products of microbial origin. This family of secondary metabolites displays large diversity in structure and biological activity. Some of them are phytotoxins such as zinnimidine and porritoxin (Horiuchi and Yamada 2003; Suemitsu et al. 1995; Stierle et al. 1993a, b) whereas others, for instance, stachybotrins and staplabin (Minagawa et al. 2002; Shinohara et al. 1996) are lead compounds with antiviral and plasminogen activation activities.

3.5.3 Antiparasitic Compounds

Fungi can produce a wide range of secondary metabolites (Costa et al. 2016) such as peptides, alkaloids, terpenes, polyketides, quinones, sterols, and coumarins (Abdel-Azeem et al. 2021). These metabolites can be used to treat a diversity of diseases (Keller et al. 2005) including neglected tropical diseases caused by

protozoa parasites. The potential for obtaining new antiparasite drugs from macrofungi has been underestimated.

Widely studied medicinal basidiomycetes like *Ganoderma lucidum* are used to treat several health conditions. However, its antiplasmodial activity against *P. falciparum* has been largely overlooked (Adams et al. 2010). In the same context, *Autraeus hygrometricus* is an Indian mushroom used to treat leishmaniosis, and although popular in traditional communities of India, it is not known in the rest of the world (Lai et al. 2012; Mallick et al. 2015). *Pycnoporus sanguineus*, a pantropical white-rot fungus, also showed powerful leishmanicidal activity. Correa et al. (2006) reported the possible action of ergosterol endoperoxide, which could involve reactions that cause the disruption of the *Leishmania panamensis* membrane. *Trametes versicolor* (Polyporaceae) is another basidiomycete that has antileishmanial activity against promastigotes and intracellular amastigotes of *Leishmania amazonensis* (Leliebre-Lara et al. 2016). Sesquiterpenes are widely produced by plants and fungi and are very effective against parasitemia, presenting fast action (Kayser et al. 2003).

Artemisin, a sesquiterpene endoperoxide lactone, is widely used to treat several parasitic diseases. It is a natural compound found in the leaves of *Artemisia annua*, and studies have verified its effectiveness in vitro and in vivo against the parasites *Leishmania major*, *L. donovani*, *L. infantum*, *T. cruzi*, *Trypanosoma brucei*, *T. gondii*, *Neospora caninum*, *Eimeria tenella,* and *Acanthamoeba castellanii* (Loo et al. 2017). Several sesquiterpenic molecules produced by fungi show effects against these species and other parasites. Sesquiterpenic lactones produced by *Acanthospermum hispidum* showed in vivo and in vitro antiparasitic effects against *P. falciparum*, *Leishmania mexicanum*, and *T. brucei* (Ganfon et al. 2012).

Antiparasitic diterpenes produced by plants have been extensively studied and reported in the literature. Fungi also produce diterpenes. However, no research on the application of diterpenes produced by fungi as antiparasitic agents could be found. The fungus *Gibberella fujikuroi* cultivated in a mineral medium produces the diterpene kaurenoic acid (Jennings et al. 1993).

In an extract of the marine fungus *Chromocleista* sp., the diterpene agathic acid has been identified (Park et al. 2006). Kaurenoic acid and agathic acid show antiparasitic activity against *T. cruzi* (Izumi et al. 2012) when extracted from the leaves of *Copaifera oleoresins*.

An extract of *Pestalotiopsis adusta*, an endophytic fungus, provided the diterpene uncinatone. Tests performed with material from the plant *Clerodendrum eriophyllum* containing uncinatone presented antiparasitic activity against *L. donovani* and *P. falciparum* (Xu et al. 2016). Triterpenoids are very common antiparasitic agents. Nyongbela et al. (2013) isolated the triterpenoid ester saponin from the plant *Pittosporum mannii*, which presented antiparasitic activity against *P. falciparum* and *L. donovani*. These molecules are also found in *Cornus florida*, which is used popularly to treat malaria. However, in one study, the terpenes extracted from the bark presented a more potent action against *Leishmania tarentolae*. The most active molecules were the triterpenes ursolic acid, 3β-*O*-acetyl betulinic acid, 3-epideoxyflindissol, and 3β-*O*-*trans*-coumaroyl betulinic acid (Graziose et al. 2012).

3.5.4 Antitubercular Compounds

World Health Organization (2020) reported that in 2018 about 1.5 million people died from tuberculosis and there were 78% had Multidrug-resistant TB (MDR-TB). The finding and development of new antitubercular agents are very important because of spreading the resistant strains of *Mycobacterium tuberculosis*. Endophytic fungi *Phomopsis* sp. from *Garcinia* sp. producing antimycobacterial isolates such as Phomoxanthone A and B, Phomoenamide and Phomonitroester (Isaka et al. 2001; Rukachaisirikul et al. 2008). Other antitubercular secondary metabolites from fungal endophytes including Tenuazonic acid ($C_{10}H_{15}NO_3$) was isolated from *Alternaria alternata* endophyte that inhabited in *Indigoferaennea phylla* L. (Sonaimuthu et al. 2011) and benzopyranonesdiaportheone A and B from *Diaporthe* sp. isolated from *Pandanus amaryllifolius* leaves (Bungihan et al. 2011).

Shah et al. (2016) studied the antibacterial and antimycobacterial activity of endophytes from Indian *Glycyrrhiza glabra* L. They resulted that *Fusarium solani* strain (KT16646) and *Colletotrichum gleosporoides* strain (KT166445) were exhibited the highest activity against *Mycobacterium tuberculosis*. Wijeratne et al. (2013) reported a weak antimycobacterial activity of Phomapyrrolidones B and C alkaloids isolated from *Phoma* sp. NRRL 46751. Two secondary metabolites Heraclemycin C (Liu et al. 2014) and Murayaquinone (Bunbamrung et al. 2020) showed strong activity against *Mycobacterium bovis* bacillus Calmette–Guèrin (BCG). They were extracted from *Streptomyces* sp. strain Y3111 and *Streptomyces* sp. TBRC7642, respectively. Another study isolated *Aspergillus fumigatus* MF029 from marine sponge sample resulted that the quinonoidsemodin (Dey et al. 2014) and trypacidin had a very strong and effective antitubercular activity (Song et al. 2019).

3.5.5 Anticancer

The first isolated compound as a potent anticancer was **taxol** $C_{47}H_{51}NO_{14}$, a diterpenic polyoxygenated pseudoalkaloid (also known as paclitaxel), from the endophytic fungus *Taxomyces andreanae* (Stierle et al. 1993a). In addition, *Seimatoantlerium tepuiense*, *Seimatoantlerium nepalense* was isolated from *Maguireothamnus speciosus* (Strobel et al. 1999b), and *Tubercularia* sp. strain TF5 (Wang et al. 2000), other fungal endophytes reported to produce paclitaxel. Paclitaxel was approved as an anticancer for the treatment of breast and ovarian cancer by the Food and Drug Administration (FDA) (Cremasco et al. 2009). Yousefzadi et al. (2010) reported that Phyton Biotech and Cytoclonal Pharmaceutics companies produced taxol on the industrial level.

Chaetomellic acids A and B and TAN-1813 were isolated from *Chaetomella acutisea* endophytes in *Robinia pseudoacacia* and *Phoma* sp. FL-41510 strain, respectively, were found to be specific inhibitors of Ras-farnesyl-protein transferase (Lingham et al. 1993; Ishii et al. 2000). Similarly, preussomerin N1, palmarumycin

CP4a, and palmarumycin CP5 isolated from *Coniothyrium* sp. endophytes were reported the same mechanism of activity (Tan and Zou 2001). Another potent cytotoxic agent was torreyanic acid $C_{38}H_{44}O_{12}$ a quinone dimer, from *Pestalotiopsis microsporum* endophytes inhabited in *Torreya taxifolia*. The main mechanism of cytotoxicity of torreyanic acid is apoptosis especially in protein kinase C agonists sensitive cell lines (Lee et al. 1996). Vinca alkaloids, Vinblastine, and vincristine $C_{46}H_{56}N_4O_{10}$, are well-known natural alkaloids from *Catharanthus roseus* or *Vinca rosea* for treatment of lymphoma and in acute lymphoblastic leukemia, respectively (Barnett et al. 1978). Recent studies reported the isolation of vinca alkaloids from fungal endophytes in *C. roseus* such as *Alternaria* sp., *Talaromycesradicus,* and *Fusarium oxysporum* (Xianzhi et al. 2004; Kumar et al. 2013; Palem et al. 2015).

Moreover, microcarpalidean alkyl-substituted nonenolide, purified from unidentified fungus endophytes in *Ficus microcarpa* bark, showed weak cytotoxicity and a microfilament disrupting agent (Ratnayake et al. 2001). Cytochalasin compounds were exhibited antitumor and cytotoxic activity. These compounds isolated from fungal endophytes *Rhinocladiella* sp. from *Tripterygium wilfordii* (Wagenaar et al. 2000) and *Chaetomium globosum* IFB-E019 inhabiting *Imperata cylindrica* (Ding et al. 2006). Camptothecin, $C_{20}H_{16}N_2O_4$ pentacyclic quinoline alkaloid, isolated for the first time by (Wall et al. 1966) from *Camptotheca acuminata Decaisne* (Nyssaceae) Chinese plant and then by (Puri et al. 2005) from endophyte *Entrophospora infrequens* inhabiting *Nothapodytes foetida*. Camptothecin showed potent in-vitro and in-vivo broad-spectrum anticancer activity by interfering with eukaryotic DNA. FDA approved the use of two Camptothecin derivatives Irinotecan and Topotecan, against colorectal and ovarian cancers (Shaanker et al. 2008). Camptothecin analogs 9-methoxycamptothecin and 10-hydroxycamptothecin, from the endophytic *Fusarium solani* in *Camptotheca acuminata* was isolated by (Kusari et al. 2009b).

Ergoflavin $C_{30}H_{26}O_{14}$ dimeric xanthene, and secalonic acid D ($C_{32}H_{30}O_{14}$) a mycotoxin, are two potent cytotoxic compounds by cell apoptosis and belonging to the ergochrome class. Ergoflavin and secalonic acid D were extracted from *Ascomycetes* PM0651480 isolated from *Mimosops elengi* (Sapotaceae) (Deshmukh et al. 2009) and mangrove endophytic fungus No. ZSU44, respectively (Zhang et al. 2009a). Another important effective natural product against leukemias, lung, and testicular cancer is podophyllotoxin $C_{22}H_{22}O_8$ aryl tetralin lignans, and its synthetic derivatives etoposide $C_{29}H_{32}O_{13}$ and teniposide $C_{32}H_{32}O_{13}S$ and etopophos phosphate $C_{29}H_{33}O_{16}P$ (Kour et al. 2008; Majumder and Jha 2009). Podophyllotoxin has been reported to be isolated from fungal endophytes such as *Phialocephala fortinii* from *Podophyllum peltatum* (Eyberger et al. 2006), *Trametes hirsute* from *Podophyllum hexandrum* (Puri et al. 2006), *Fusarium oxysporum* isolated from *Juniperus recurva* (Kour et al. 2008), *Aspergillus fumigatus* from *Juniperus communis* (Kusari et al. 2009a) and *Fusarium solani* isolated from the roots of *Podophyllum hexandrum* (Nadeem et al. 2012).

The present study was aimed at evaluating the anticancer activity of the crystallized compound alternariol methyl ether (AME) against hepatocellular carcinoma (HCC) both in vitro and in vivo from an endophytic fungus residing in the medicinal

plant *Vitex negundo*. Palanichamy et al. (2019) evaluate the in-vitro and in-vivo anticancer activity of alternariol methyl ether compound isolated from *Alternaria alternata* endophytes inhabiting in *Vitex negundo* showing promising anti-hepatocellular carcinoma.

One more study by (Yuniati et al. 2019) isolate *N*-(3′-chloro-5′-oxobutyl)-1-methyl-5-phenyl-1*H*-pyrrole3-carboxamide from *Aspergillus* sp. endophytic fungi in *Phyllanthus niruri* and discovered its anticancer activity against the T47D cell line. The use of nanotechnology with endophytic fungi showed potent cytotoxic activity against various types of cancer cell lines and is considered safer and less toxic chemotherapeutic agents. Silver nanoparticles were synthesized from endophytic fungus *Botryosphaeria rhodina* isolated from *Catharanthus roseus* (Akther et al. 2019). Another study synthesized the gold nanoparticles using an endophytic *Fusarium solani* ATLOY-8 isolated from the plant *Chonemorpha fragrans* (Clarance et al. 2020). Few studies were demonstrated the anticancer effect of fungal endophytes from marine plants such as mangrove plants and algae. Kasanosins A and B (azaphilones), Alterporriol (bianthraquinone derivative) were isolated from *Talaromyces* sp. and *Alternaria* sp. ZJ9-6B endophytes in seaweed in Kasai Rinkai Park, Tokyo, Japan, and *Aegiceras corniculatum*, respectively, these compounds were shown promising anticancer activity (Kimura et al. 2008; Huang et al. 2012a).

Lee et al. (2010) found that sterigmatocystin, averantin, methylaverantin, and nidurufin exhibited anticancer activity and purified from *Aspergillus versicolor* inhabited in marine sponge *Petrosia* sp. Another study was isolated seven new cytochalasan derivatives (cytoglobosins A–G) from *Chaetomium globosum* QEN-14 fungal endophytes in the marine green alga *Ulva pertusa* (Ulvaceae) and found that cytoglobosins C and D were potent anticancer agents (Cui et al. 2010).

3.5.6 Antioxidant

Antioxidants or free-radical scavengers are natural or artificial substances that protect and slow the damage on cells caused by free radical molecules. Fungal endophyte metabolites are a novel, safe, and natural source of antioxidant compounds possess as anti-inflammatory, antitumor, anticancer, and against degenerative diseases (Harper et al. 2003; Kawanishi et al. 2005; Huang et al. 2007).

Pestacin 1,3-dihydroisobenzofuran, and Isopestacin $C_{15}H_{12}O_5$ isobenzofuranone, are two potent antioxidant substances (Strobel et al. 2002). Pestacin was isolated from an endophytic fungus *Pestalotiopsis microspore* in *Terminalia morobensis* (Harper et al. 2003) while, Isopestacinand its derivative 4,6-dihydroxy-5-methoxy-7-methylphthalide were obtained from *Cephalosporium* sp. AL031 isolated from *Sinarundinaria nitida* (Huang et al. 2012b). Graphislactone A is another antioxidant compound, it was isolated from fungal endophyte *Cephalosporium* sp. IFB-E001 in *Trachelospermum jasminoides* (Song et al. 2005). Strong antioxidant activity was found in various endophytes due to the presence of flavonoids and phenolic acids. *Xylaria* sp. YX-28 endophytes from *Ginkgo biloba* (Liu et al. 2007), *Chaetomium*

sp. from *Nerium oleander* (Huang et al. 2007), and two endophytic fungi *Chaetomium globosum* T24 and *Creosphaeria* sp. T38 isolated from *Scapania verrucose* (Zeng et al. 2011). In addition, Cui et al. (2015) investigated for the first time the antioxidant activity of different fungal endophytes *Fusarium*, *Alternaria*, *Phoma,* and *Penicillium* isolated from three *Rhodiola* species.

Cajaninstilbene acid $C_{12}H_{22}O_4$, 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid, isolated from *Fusarium* an endophyte of *Pigeon pea* and *Cajanus cajan* was found to exhibit a potent natural antioxidant activity (Zhao et al. 2012a, b).

Caicedo et al. (2019) reported a high antioxidant activity of the crude extract of *Fusarium oxysporum* endophytes in *Otoba gracilipes* a medicinal tree from a tropical rainforest in Colombia. Whereas, *Cladosporium uredinicola* endophytes were isolated from *Calophyllum tomentosum* (Calophyllacace) plant grows in Sri Lanka and India. These endophyte extracts containing alkaloids, phenols, flavonoids, and coumarins and showed in-vitro antioxidant, antidiabetic, and anti-HIV-1 activity (Govindappa et al. 2019). In this study, we isolated an endophytic fungus from the leaves of *Otoba gracilipes*, a medicinal tree from a tropical rainforest in Colombia. Following isolation and cultivation, we evaluated its extracellular crude extract for antioxidant activity. In their work Darwish et al. (2020) collected all available data concerning antioxidants produced by endophytic Chaetomium they were:

3.5.6.1 Flavipin

Flavipin is a well-known natural product that is isolated from endophytes belonging to *Chaetomium* sp. associated with leaves of *Ginkgo biloba* (Ye et al. 2013). Yan et al. (2018) succeeded in isolating bioactive metabolites with antifungal activities from this fungus; the metabolites are flavipin, chaetoglobosins A and D, chaetoglobosins R (4) and T (5), new isocoumarin derivative prochaetoviridin A (1), a new indole alkaloid, and chaetoindolin A (2) and chaetoviridin A (3). Flavipin is considered the major antioxidant component of CDW7's metabolites; it reacts by donating its electrons to the free radicals, leading to SOD and GSH-Px activity improvement and suppression of MDA content. This metabolite possesses three phenolic hydroxyl and two aldehyde groups, which are characteristic functional groups with antioxidant activity. When cultured under the optimal condition (25 °C, 100/250 mL flask, 12 discs/flask, 150 rpm, pH 6.5) for 14 days, *Chaetomium globosum* CDW7 was a highly yielded bio-sourced of antioxidant flavipin synthesizing a remarkable production of 315.5 mg/L (Ye et al. 2013). Another endophytic fungus from *Ginkgo biloba*, *Chaetomium* sp. NJZTP21 (GenBank accession number: JN588553), isolated from the healthy leaf of the plant was able to produce flavipin, which significantly inhibited the growth of several plant pathogenic fungi, especially *Fusarium graminearum*. But the extract from *C. globosum* CDW7, which had been deposited in the China General Microbiological Culture Collection Center (CGMCC) with an accession number 6658, has the strongest antioxidant activity among the studied endophytic fungi from *G. biloba* comparable to those of vitamin C and trolox, the well-known antioxidants (Ye et al. 2013).

Chaetomium globosum and *C. cochlioides* are antagonistic to species of *Fusarium* and *Helminthosporium*. They exhibited good control over many plant pathogens; seed coating treatments with viable spores of *Chaetomium globosum* were found to exert antagonistic effect controlled *Fusarium roseum* f. sp. *cerealis* "graminearum" in corn; reduced disease incidence of apple scab caused by *Venturia inequalis*; suppressed damping-off of sugar beet caused by *Pythium ultimum*; had an antagonistic effect against *Macrophomina phaseolina*, *Pythium ultimum*, *Bipolaris sorokiniana*, *Rhizoctonia solani*, and *Alternaria brassicicola*; and reduced the quantity of sporulation of *Botrytis cinerea* on dead lily leaves exposed in the field (Biswas et al. 2012; Shternshis et al. 2005).

3.5.6.2 Chaetopyranin

The basic structure of chaetopyranin (I) is chromenol (I) (chromene carrying one or more hydroxyl substituents). It is chemically known as 3,4-dihydro-2*H*-chromene substituted by a hydroxyl group at position 6, a 3-hydroxybut-1-en-1-yl at position 2, a formyl group at position 5, and a prenyl group at position 7 (Wang et al. 2006). These two compounds have been isolated from an endophytic fungus *Chaetomium globosum*, associated with *Polysiphonia urceolata*, and are found to possess antioxidant activity. The former compound also exhibits anticancer activity (Wang et al. 2006). Chaetopyranin also showed antioxidant activity.

3.5.6.3 Azaphilone

The most remarkable and valuable properties of azaphilones include their natural origin, yellow-red spectra, thermostability (in comparison with other natural red pigments), and water solubility. The azaphilone compounds produced by different Chaetomium species display various biological activities such as antioxidant, nematicidal, antimicrobial, antifungal, anticancer, and inflammatory activities (Borges et al. 2011).

3.5.6.4 Hypericin and Emodin

Endophytic fungus *Chaetomium globosum* INFU/Hp/KF/34B isolated from *Hypericum perforatum* has been shown to produce hypericin and emodin of high medicinal value as antioxidants. This endophytic fungus has significant scientific and industrial potential to meet the pharmaceutical demands in a cost-effective, easily accessible, and reproducible way (Kusari et al. 2008a, b; Zhao et al. 2011).

3.5.6.5 Mollicellins

Mollicellins O (1) isolated from the endophytic fungus *Chaetomium* sp., Eef-10, which was isolated from *Eucalyptus exserta* by Ouyang et al. (2018), showed antioxidant activity based on DPPH radical scavenging. For more details concerning antioxidants producing fungi in Egypt please check Abo Nahas (2019).

3.5.7 Immunomodulatory Agents

Subglutinols A and B, noncytotoxic diterpene pyrones, purified from *Fusarium subglutinans* endophyte of *Triptergium wilfordii* and exhibited as immunosuppressive agent (Lee et al. 1995).

3.5.8 Insecticidal Agents

Cryptocin is a tetramic acid, showing potent activity against various plant pathogenic fungi and worst plant pests *Pyricularia oryzae* and Cryptocin have been isolated from the endophytic fungus *Cryptosporiopsis quercina* inhabited in *Tripterigeum wilfordii* (Li et al. 2000).

3.5.9 Antidiabetic Agents

Diabetes mellitus, a group of metabolic disorders, around two-third of the population in developing countries suffered from it is one of the major causes of death among all health issues. Antidiabetic drugs regulate diabetes by controlling the amylase enzymes and other enzymes, the same mechanism of endophytic fungi. Fungal metabolite L-783,281, non-peptidal quinine, isolated from *Pseudomassaria* sp. endophyted in African rainforest near Kinshasa in the Democratic Republic of the Congo. It is administrated orally as it was not destroyed by digestive enzymes but it mimics the insulin in its activity (Zhang et al. 1999). Three plants *Mangifera indica*, *Azadirachta indica,* and *Syzygium cumini* (jambolana) were screened for their production of antidiabetic endophytic fungi. Aqueous extract of endophytic fungi isolated from jambolana showed a remarkable natural antidiabetic activity (Khan et al. 2019). Neglecting A novel polyketide-derived metabolite, purified from *Pestalotiopsis neglecta* endophytes in *Kandelia candel*, were found their inhibitory activity to protein tyrosine phosphatases (PTPs) and had an important role in the negative regulation of insulin signaling (Gao et al. 2019). Xanthone α-glucosidase inhibitors are an antihyperglycemic compound isolated from an endophytic *Penicillium canescens*, recovered from fruits of *Juniperus polycarpos* (Malik et al. 2020).

3.5.10 Other Applications

Abdel-Azeem et al. (2016) studied ten medicinal plant species in Wadi Tala, Saint Katherine Protectorate, and arid Sinai, Egypt, and reported the anti-inflammatory, and anti-rheumatoid arthritis, in an AIA rat model, of the methanol extract of *Chaetomium globosum* (KC811080) endophytes in maidenhair fern. Sartorypyrone Ea meroditerpene agent is a secondary metabolite from *Neosartorya fischeri* JS0553 endophytes in *Glehnia littoralis* and is used in stroke healing and as a neuroprotective agent (Bang et al. 2019). Rustamova et al. (2020) reported that *Aspergillus* sp. XJA6 endophytes in *Vernonia anthelmintica* showed pronounced activity in melanin synthesis and against cervical and colon human cancer cell lines.

3.6 Conclusion

Natural products and their derivatives contribute about 60% of the FDA-approved drugs to be used as antimicrobial and anticancer agents. Endophytes and their secondary metabolites are considered as an important bioactive natural source in pharmaceutical applications and the production of new therapeutic compounds. Compounds produced from endophytes are mimicking the chemistry of their hosts with a high level of structural diversity as a result of gene recombination. The sustainability of metabolites from endophytes and their use on a large commercial and industrial-scale as they are easily produced from these fungi. High specificity, low toxicity, best effect, sustainability, all these criteria making endophytes are promising sources of therapeutic agents of various life-threatening diseases in the future. New hosts of fungal endophytes as marine plants and algae could consider new sources of different bioactive compounds. More genetic engineering, clinical and experimental studies on endophyte metabolites should be done to obtain new products and identify their mechanism of action.

References

Abdel-Azeem A (2012) Biodiversity of laccase producing fungi in Egypt. Mycosphere 3:900–920. https://doi.org/10.5943/mycosphere/3/6/4

Abdel-Azeem AM, Salem FM, Mohamed HM et al (2012) (PDF) Bioprospecting of Egyptian fungi for ecological and medical applications

Abdel-Azeem AM, Zaki SM, Khalil WF et al (2016) Anti-rheumatoid activity of secondary metabolites produced by endophytic Chaetomium globosum. Front Microbiol 7:1–11. https://doi.org/10.3389/fmicb.2016.01477

Abdel-Azeem AM, Abdel-Azeem MA, Khalil WF (2019) Endophytic fungi as a new source of antirheumatoid metabolites. In: Bioactive food as dietary interventions for arthritis and related inflammatory diseases. Elsevier, Amsterdam, pp 355–384

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abo Nahas HH (2019) Endophytic fungi: a gold mine of antioxidants. Microb Biosyst 4:58–79. https://doi.org/10.21608/mb.2019.41030

Adams M, Christen M, Plitzko I, Zimmermann S, Brun R, Kaiser M, Hamburger M (2010) Antiplasmodial Lanostanes from the Ganoderma lucidum mushroom. J Nat Prod 73:897–900

Akpotu MO, Eze PM, Abba CC, Umeokoli BO, Nwachukwu CU, Okoye FBEC (2017) Antimicrobial activities of secondary metabolites of endophytic fungi isolated from Catharanthus roseus. J Heal Sci 7:15–22. https://doi.org/10.17532/jhsci.2017.421

Akther T, Mathipi V, Kumar NS et al (2019) Fungal-mediated synthesis of pharmaceutically active silver nanoparticles and anticancer property against A549 cells through apoptosis. Environ Sci Pollut Res 26:13649–13657. https://doi.org/10.1007/s11356-019-04718-w

Arora DS, Kaur N (2019) Antimicrobial potential of fungal endophytes from Moringa oleifera. Appl Biochem Biotechnol 187:628–648. https://doi.org/10.1007/s12010-018-2770-y

Attia EA, Singh BP, Dashora K, Abdel-Azeem AM (2020) A potential antimicrobial, extracellular enzymes, and antioxidants resource: endophytic fungi associated with medicinal plants. Int J Biosci 17:119–132. https://doi.org/10.12692/ijb/17.1.119-132

Bacon CW, White JF (2018) Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton, FL

Balbool BA, Abdel-azeem AM (2020) Diversity of the culturable endophytic fungi producing L-asparaginase in arid Sinai, Egypt. Ital J Mycol 49:8–24. https://doi.org/10.6092/issn.2531-7342/10063

Bang S, Song JH, Lee D, Lee C, Kim S, Kang KS, Lee JH, Shim SH (2019) Neuroprotective secondary metabolite produced by an endophytic fungus, Neosartorya fischeri JS0553, isolated from Glehnia littoralis. J Agric Food Chem 67:1831–1838. https://doi.org/10.1021/acs.jafc.8b05481

Barnett CJ, Cullinan GJ, Gerzon K et al (1978) Structure-activity relationships of dimeric Catharanthus alkaloids. 1. Deacetyl vinblastine amide (vindesine) sulfate. J Med Chem 21:88–96

Bashyal BP, Kithsiri Wijeratne EM, Faeth SH, Gunatilaka AAL (2005) Globosumones A–C, cytotoxic orsellinic acid esters from the sonoran desert endophytic fungus Chaetomium globosum. J Nat Prod 68:724–728. https://doi.org/10.1021/np058014b

Biswas SK, Aggarwal R, Srivastava KD, Gupta S, Dureja P (2012) Characterization of antifungal metabolites of Chaetomium globosum Kunze and their antagonism against fungal plant pathogens. J Biol Control 26(1):70–74

Borges WS, Mancilla G, Guimar-aes DO, Duran-Patron R, Collado IG, Pupo MT (2011) Azaphilones from the endophyte Chaetomium globosum. J Nat Prod 74:1182–1187

Brady SF, Clardy J (2000) CR377, a new pentaketide antifungal agent isolated from an endophytic fungus. J Nat Prod 63:1447–1448. https://doi.org/10.1021/np990568p

Brady SF, Bondi SM, Clardy J (2001) The guanacastepenes: a highly diverse family of secondary metabolites produced by an endophytic fungus. J Am Chem Soc 123:9900–9901. https://doi.org/10.1021/ja016176y

Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21:143–163. https://doi.org/10.1039/b301926h

Bunbamrung N, Intaraudom C, Dramae A, Thawai C, Tadtong S, Auncharoen P, Pittayakhajonwut P (2020) Antibacterial, antitubercular, antimalarial and cytotoxic substances from the endophytic Streptomyces sp. TBRC7642. Phytochemistry 172:112275. https://doi.org/10.1016/j.phytochem.2020.112275

Bungihan ME, Tan MA, Kitajima M et al (2011) Bioactive metabolites of Diaporthe sp. P133, an endophytic fungus isolated from Pandanus amaryllifolius. J Nat Med 65:606–609. https://doi.org/10.1007/s11418-011-0518-x

Bungtongdee N, Sopalun K, Laosripaiboon W, Iamtham S (2019) The chemical composition, antifungal, antioxidant and antimutagenicity properties of bioactive compounds from fungal endophytes associated with Thai orchids. J Phytopathol 167:56–64. https://doi.org/10.1111/jph.12773

Bunyapaiboonsri T, Yoiprommarat S, Srikitikulchai P, Srichomthong K, Lumyong S (2010) Oblongolides from the endophytic fungus Phomopsis sp. BCC 9789. J Nat Prod 73:55–59

Caicedo NH, Davalos AF, Puente PA, Rodríguez AY, Caicedo PA (2019) Antioxidant activity of exo-metabolites produced by Fusarium oxysporum: an endophytic fungus isolated from leaves of Otoba gracilipes. Microbiology 8:1–7. https://doi.org/10.1002/mbo3.903

Campos FR, Barison A, Daolio C et al (2005) Complete 1H and 13C NMR assignments of aurasperone A and fonsecinone A, two bis-naphthopyrones produced by Aspergillus aculeatus. Magn Reson Chem 43:962–965. https://doi.org/10.1002/mrc.1654

Cao LX, You JL, Zhou SN (2002) Endophytic fungi from Musa acuminata leaves and roots in South China. World J Microbiol Biotechnol 18:169–171

Castillo UF, Strobel GA, Ford EJ et al (2002) Munumbicins, wide-spectrum antibiotics produced by Streptomyces NRRL 30562, endophytic on Kennedia nigriscans. Microbiology 148:2675–2685. https://doi.org/10.1099/00221287-148-9-2675

Chandra S (2012) Endophytic fungi: novel sources of anticancer lead molecules. Appl Microbiol Biotechnol 95:47–59. https://doi.org/10.1007/s00253-012-4128-7

Chen G, Lin Y, Wen L et al (2003) Two new metabolites of a marine endophytic fungus (No. 1893) from an estuarine mangrove on the South China Sea coast. Tetrahedron 59:4907–4909. https://doi.org/10.1016/S0040-4020(03)00703-8

Chithra S, Jasim B, Sachidanandan P, Jyothis M, Radhakrishnan EK (2014) Piperine production by endophytic fungus Colletotrichum gloeosporioides isolated from Piper nigrum. Phytomedicine 21:534–540. https://doi.org/10.1016/j.phymed.2013.10.020

Clarance P, Luvankar B, Sales J et al (2020) Green synthesis and characterization of gold nanoparticles using endophytic fungi Fusarium solani and its in-vitro anticancer and biomedical applications. Saudi J Biol Sci 27:706–712. https://doi.org/10.1016/j.sjbs.2019.12.026

Clercq ED (2002) Strategies in the design of antiviral drugs. Nat Rev 1:13–25

Correa E, Cardona D, Quiñones W, Torres F, Franco AE, Vélez ID, Robledo S, Echeverri F (2006) Leishmanicidal activity of Pycnoporus sanguineus. Phytother Res 20:497–499. https://doi.org/10.1002/ptr.1890

Costa TM, Tavares LBB, Oliveira D (2016) Fungi as a source of natural coumarins production. Appl Microbiol Biotechnol 100:6571–6584

Cremasco MA, Hritzko BJ, Linda Wang NH (2009) Experimental purification of paclitaxel from a complex mixture of taxanes using a simulated moving bed. Braz J Chem Eng 26:207–218. https://doi.org/10.1590/S0104-66322009000100020

Cui CM, Li XM, Li CS et al (2010) Cytoglobosins A–G, cytochalasans from a marine-derived endophytic Fungus, chaetomium globosum QEN-14. J Nat Prod 73:729–733. https://doi.org/10.1021/np900569t

Cui JL, Guo TT, Ren ZX, Zhang NS, Wang ML (2015) Diversity and antioxidant activity of culturable endophytic fungi from alpine plants of Rhodiola crenulata, R. angusta, and R. sachalinensis. PLoS One 10:1–16. https://doi.org/10.1371/journal.pone.0118204

Darwish AMG, Abdelmotilib NM, Abdel-Azeem AM, Abo Nahas HH, Mohesien MT (2020) Applications of Chaetomium Functional Metabolites with Special Reference to Antioxidants. In: Abdel-Azeem A (eds) Recent Developments on Genus Chaetomium. Fungal Biology. Springer, Cham. https://doi.org/10.1007/978-3-030-31612-9_8

Debbab A, Aly AH, Lin WH, Proksch P (2010) Bioactive compounds from marine bacteria and fungi: minireview. Microb Biotechnol 3:544–563. https://doi.org/10.1111/j.1751-7915.2010.00179.x

Desai SB, Argade NP (1997) A facile synthesis of ras farnesyl-protein transferase inhibitor chaetomellic acid A. J Org Chem 62:4862–4863. https://doi.org/10.1021/jo9700383

Deshmukh SK, Mishra PD, Kulkarni-Almeida A et al (2009) Anti-inflammatory and anticancer activity of ergoflavin isolated from an endophytic fungus. Chem Biodivers 6:784–789. https://doi.org/10.1002/cbdv.200800103

Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems:

perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Dey D, Ray R, Hazra B (2014) Antitubercular and antibacterial activity of quinonoid natural products against multi-drug resistant clinical isolates. Phyther Res 28:1014–1021. https://doi.org/10.1002/ptr.5090

Ding G, Song YC, Chen JR et al (2006) Chaetoglobosin U, a cytochalasan alkaloid from endophytic Chaetomium globosum IFB-E019. J Nat Prod 69:302–304. https://doi.org/10.1021/np050515+

Ding L, Münch J, Goerls M, Fiebig HH, Lin WH, Hertweck C (2010) Xiamycin, a pentacyclic indolosesquiterpene with selective anti-HIV activity from a bacterial mangrove endophyte. Bioorg Med Chem Lett 20(22):6685–6687

Drandarov K, Hesse M, Werner C, De Janeiro R (2001) Guignardic Acid, a novel type of secondary metabolite produced by the endophytic fungus Guignardia sp.: isolation, structure elucidation, and asymmetric synthesis. Helvetica 84:3766–3772

Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from Podophyllum peltatum produce podophyllotoxin. J Nat Prod 69:1121–1124. https://doi.org/10.1016/S0016-5085(75)80170-3

Fernandes MDRV, Pfenning LH, Costa-Neto CMD, Heinrich TA, Alencar SMD, Lima MAD, Ikegaki M (2009) Biological activities of the fermentation extract of the endophytic fungus Alternaria alternata isolated from Coffea arabica L. Braz J Pharm Sci 45:677–685. https://doi.org/10.1590/S1984-82502009000400010

Gagini TB, Silva RE, Castro IS et al (2010) Oral administration of piperine for the control of aflatoxin intoxication in rats. Braz J Microbiol 41:345–348. https://doi.org/10.1590/S1517-83822010000200013

Ganesan V, Hariram M, Vivekanandhan S, Muthuramkumar S (2020) Periconium sp. (endophytic fungi) extract mediated sol-gel synthesis of ZnO nanoparticles for antimicrobial and antioxidant applications. Mater Sci Semicond Process 105:104739. https://doi.org/10.1016/j.mssp.2019.104739

Ganfon H, Bero J, Tchinda AT, Gbaguidi F, Gbenou J, Moudachirou M, Frédérich M, Quetin-Leclercq J (2012) Antiparasitic activities of two sesquiterpenic lactones isolated from Acanthospermum hispidum D.C. J Ethnopharmacol 141:411–417. https://doi.org/10.1016/j.jep.2012.03.002

Gao WB, Han LP, Xie FX et al (2019) A new polyketide-derived metabolite with PTP1B inhibitory activity from the endophytic fungus Pestalotiopsis neglecta. Chem Nat Compd 55:1056–1058. https://doi.org/10.1007/s10600-019-02892-4

Garcia A, Rhoden SA, Bernardi-Wenzel J, Orlandelli RC, Azevedo JL, Pamphile JA (2012) Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant Sapindus saponaria L. J Appl Pharm Sci 2:35–40. https://doi.org/10.7324/JAPS.2012.21007

Gautam AK, Kant M, Thakur Y (2012) Archives of phytopathology and plant protection isolation of endophytic fungi from Cannabis sativa and study their antifungal potential. Arch Phytopathol Plant Protect 46:37–41. https://doi.org/10.1080/03235408.2012.749696

Ge HM, Song YC, Shan CY et al (2005) New and cytotoxic anthraquinones from Pleosporo sp. IFB-E006, an endophytic fungus in Imperata cylindrical. Planta Med 71:1063–1065. https://doi.org/10.1055/s-2005-864190

Gond SK, Mishra A, Sharma VK, Verma SK, Kumar J, Kharwar RN, Kumar A (2012) Diversity and antimicrobial activity of endophytic fungi isolated from Nyctanthes arbor-tristis, a well-known medicinal plant of India. Mycoscience 53:113–121. https://doi.org/10.1007/s10267-011-0146-z

Goveas SW, Madtha R, Nivas SK, Souza LD (2011) Isolation of endophytic fungi from Coscinium fenestratum - a red listed endangered medicinal plant. Eurasian J Biosci 53:48–53. https://doi.org/10.5053/ejobios.2011.5.0.6

Govindappa M, Thanuja V, Tejashree S et al (2019) In vitro and in silico antioxidant, anti-diabetic, anti-HIV and anti-Alzheimer activity of endophytic fungi, Cladosporium uredinicola phyto-

chemicals. Int J Pharmacol Phytochem Ethnomedicine 13:13–34. https://doi.org/10.18052/www.scipress.com/ijppe.13.13

Graziose R, Rojas-Silva P, Rathinasabapathy T, Dekock C, Grace MH, Poulev A, Lila MA, Smith P, Raskin I (2012) Antiparasitic compounds from Cornusflorida L. with activities against Plasmodium falciparum and Leishmania tarentolae. J Ethnopharmacol 142:456–461. https://doi.org/10.1016/j.jep.2012.05.017

Gu W (2009) Bioactive metabolites from alternaria brassicicola ML-P08, an endophytic fungus residing in Malus halliana. World J Microbiol Biotechnol 25:1677–1683. https://doi.org/10.1007/s11274-009-0062-y

Guan S, Grabley S, Groth I et al (2005) Structure determination of germacrane-type sesquiterpene alcohols from an endophyte Streptomyces griseus subsp. Magn Reson Chem 43:1028–1031. https://doi.org/10.1002/mrc.1710

Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity and implication of their occurrence. J Nat Prod 69:509–526

Guo B, Dai JR, Ng S et al (2000) Cytonic acids A and B: novel tridepsideinhibitors of hCMV protease from the endophytic fungus Cytonaema species. J Nat Prod 63:602–604

Gupta S, Chaturvedi P, Kulkarni MG, Van Staden J (2020) A critical review on exploiting the pharmaceutical potential of plant endophytic fungi. Biotechnol Adv 39:107462. https://doi.org/10.1016/j.biotechadv.2019.107462

Harper JK, Arif AM, Ford EJ, Strobel GA, Porco JA Jr, Tomer DP, Oneill KL, Heider EM, Grant DM (2003) Pestacin: a 1,3-dihydro isobenzofuran from Pestalotiopsis microspora possessing antioxidant and antimycotic activities. Tetrahedron 59:2471–2476. https://doi.org/10.1016/S0040-4020(03)00255-2

He KY, Zhang C, Duan YR et al (2017) New chlorinated xanthone and anthraquinone produced by a mangrove-derived fungus Penicillium citrinum HL-5126. J Antibiot (Tokyo) 70:823–827. https://doi.org/10.1038/ja.2017.52

Hellwig V, Grothe T, Mayer-Bartschmid A et al (2002) Altersetin, a new antibiotic from cultures of endophytic Alternaria spp. Taxonomy, fermentation, isolation, structure elucidation and biological activities. J Antibiot (Tokyo) 55:881–892. https://doi.org/10.7164/antibiotics.55.881

Hendry SJ, Boddy L, Lonsdale D (2002) Abiotic variables effect differential expression of latent infections in beech (Fagus sylvatica). New Phytol 155:449–460. https://doi.org/10.1046/j.1469-8137.2002.00473.x

Holland MA, Polacco JC (1992) Urease-null and hydrogenase-null phenotypes of a phylloplane bacterium reveal altered nickel metabolism in two soybean mutants. Plant Physiol 98:942–948. https://doi.org/10.1104/pp.98.3.942

Horiuchi M, Yamada Y (2003) A novel isoindoline, porritoxin sulfonic acid, from Alternaria porri and the structure–phytotoxicity correlation of its related compounds. Biosci Biotechnol Biochem 67:1580–1583

Huang Y, Wang J, Li G et al (2001) Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants Taxus mairei, Cephalataxus fortunei and Torreya grandis. FEMS Immunol Med Microbiol 31:5–9

Huang WY, Cai YZ, Hyde KD et al (2007) Endophytic fungi from Nerium oleander L (Apocynaceae): main constituents and antioxidant activity. World J Microbiol Biotechnol 23:1253–1263. https://doi.org/10.1007/s11274-007-9357-z

Huang C, Jin H, Song B et al (2012a) The cytotoxicity and anticancer mechanisms of alterporriol L, a marine bianthraquinone, against MCF-7 human breast cancer cells. Appl Microbiol Biotechnol 93:777–785. https://doi.org/10.1007/s00253-011-3463-4

Huang XZ, Zhu Y, Guan XL et al (2012b) A novel antioxidant isobenzofuranone derivative from fungus Cephalosporium sp. AL031. Molecules 17:4219–4224. https://doi.org/10.3390/molecules17044219

Hulikere MM, Joshi CG (2019) Characterization, antioxidant and antimicrobial activity of silver nanoparticles synthesized using marine endophytic fungus - Cladosporium cladosporioides. Process Biochem 82:199–204. https://doi.org/10.1016/j.procbio.2019.04.011

Isaka M, Jaturapat A, Rukseree K, Danwisetkanjana K, Tanticharoen M, Thebtaranonth Y (2001) Phomoxanthones A and B, novel xanthone dimers from the endophytic fungus Phomopsis species. J Nat Prod 64:1015–1018. https://doi.org/10.1021/np010006h

Ishii T, Hayashi K, Hida T et al (2000) TAN-1813, a novel ras-farnesyltransferase inhibitor produced by Phoma sp. Taxonomy, fermentation, isolation and biological activities in vitro and in vivo. J Antibiot (Tokyo) 53:765–778. https://doi.org/10.7164/antibiotics.53.765

Ivanova EG, Doronina NV, Trotsenko YA (2001) Aerobic methylobacteria are capable of synthesizing auxins. Microbiology 70:392–397. https://doi.org/10.1023/A:1010469708107

Izumi E, Nakamura TU, Veiga VF Jr, Pinto AC, Nakamura CV (2012) Terpenes from copaifera demonstrated in vitro antiparasitic and synergic activity. J Med Chem 55:2994–3001. https://doi.org/10.1021/jm201451h

Jalgaonwala RE, Mohite BV, Mahajan RT (2010) Evaluation of endophytes for their antimicrobial activity from indigenous medicinal plants belonging to north Maharashtra region India. Int J Pharm Biomed Res 1:136–141

Jassim SA, Naji MA (2003) Novel antiviral agents: a medicinal plant perspective. J Appl Microbiol 95:412–427. https://doi.org/10.1046/j.1365-2672.2003.02026.x

Jennings JC, Coolbaugh RC, Nakata DA, West CA (1993) Characterization and solubilization of kaurenoic acid hydroxylase from Gibbereh fujikuroi. Plant Physiol 101:925–930. https://doi.org/10.1104/pp.101.3.925

Jiang CS, Zhou ZF, Yang XH, Lan LF, Gu YC, Ye BP, Guo YW (2018) Antibacterial sorbicillin and diketopiperazines from the endogenous fungus Penicillium sp. GD6 associated Chinese mangrove Bruguiera gymnorrhiza. Chin J Nat Med. 16(5):358–365. https://doi.org/10.1016/S1875-5364(18)30068-2. PMID: 29860997

Jin Z, Gao L, Zhang L, Liu T, Yu F, Zhang Z, Guo Q, Wang B (2017) Antimicrobial activity of saponins produced by two novel endophytic fungi from Panax notoginseng. Nat Prod Res 31:2700–2703. https://doi.org/10.1080/14786419.2017.1292265

Ka S, Aa E-B, Tm A-R, Ai E-D (2012) 2012-Biology of endophytic fungi. Curr Res Environ Appl Mycol 2:31–82. https://doi.org/10.5943/cream/2/1/3

Kawanishi S, Oikawa S, Murata M (2005) Evaluation for safety of antioxidant chemopreventive agents. Antioxidants Redox Signal 7:1728–1739. https://doi.org/10.1089/ars.2005.7.1728

Kayser O, Kiderlen AF, Croft SL (2003) Natural products as antiparasitic drugs. Parasitol Res 90:S55–S62. https://doi.org/10.1007/s00436-002-0768-3

Keller NP, Turner G, Bennett JW (2005) Fungal mecondary metabolism—from biochemistry to genomics. Nat Rev Microbiol 3:937–947

Khan R, Naqvi ST, Fatima N, Muhammad SA (2019) Study of antidiabetic activities of endophytic fungi isolated from plants. Pure Appl Biol 8:1287–1295. https://doi.org/10.19045/bspab.2019.80071

Kharwar RN, Verma VC, Kumar A, Gond SK, Harper JK, Hess WM, Lobkovosky E, Ma C, Ren Y, Strobel GA (2009) Javanicin, an antibacterial naphthaquinone from an endophytic fungus of neem, chloridium sp. Curr Microbiol 58:233–238. https://doi.org/10.1007/s00284-008-9313-7

Kharwar RN, Gond SK, Kumar A, Mishra A (2010) A comparative study of endophytic and epiphytic fungal association with leaf of Eucalyptus citriodora Hook., and their antimicrobial activity. World J Microbiol Biotechnol 26:1941–1948. https://doi.org/10.1007/s11274-010-0374-y

Kimura T, Nishida M, Kuramochi K et al (2008) Novel azaphilones, kasanosins A and B, which are specific inhibitors of eukaryotic DNA polymerases β and λ from Talaromyces sp. Bioorg Med Chem 16:4594–4599. https://doi.org/10.1016/j.bmc.2008.02.037

Kjer J, Debbab A, Aly AH, Proksch P (2010) Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. Nat Protoc 5:479–490. https://doi.org/10.1038/nprot.2009.233

Kongsaeree P, Prabpai S, Sriubolmas N et al (2003) Antimalarial dihydroisocoumarins produced by Geotrichum sp., an endophytic fungus of Crassocephalum crepidioides. J Nat Prod 66:709–711. https://doi.org/10.1021/np0205598

Kour A, Shawl AS, Rehman S et al (2008) Isolation and identification of an endophytic strain of Fusarium oxysporum producing podophyllotoxin from Juniperus recurva. World J Microbiol Biotechnol 24:1115–1121. https://doi.org/10.1007/s11274-007-9582-5

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Krohn K, Dai J, Flörke U et al (2005) Botryane metabolites from the fungus Geniculosporium sp. isolated from the marine red alga Polysiphonia. J Nat Prod 68:400–405. https://doi.org/10.1021/np0498206

Kumar A, Patil D, Rajamohanan PR, Ahmad A (2013) Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus Fusarium oxysporum isolated from Catharanthus roseus. PLoS One 8:e71805. https://doi.org/10.1371/journal.pone.0071805

Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008a) An endophytic fungus from Hypericum perforatum that produces hypericin. J Nat Prod 71(2):26–29

Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008b) An endophytic fungus from Hypericum perforatum that produces hypericin. J Nat Prod 71:159–162

Kusari S, Lamshöft M, Spiteller M (2009a) Aspergillus fumigatus Fresenius, an endophytic fungus from Juniperus communis L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. J Appl Microbiol 107:1019–1030. https://doi.org/10.1111/j.1365-2672.2009.04285.x

Kusari S, Zühlke S, Spiteller M (2009b) An endophytic fungus from Camptotheca acuminata that produces camptothecin and analogues. J Nat Prod 72:2–7. https://doi.org/10.1021/np800455b

Kusari S, Košuth J, Čellárová E, Spiteller M (2011) Survival-strategies of endophytic Fusarium solani against indigenous camptothecin biosynthesis. Fungal Ecol 4:219–223. https://doi.org/10.1016/j.funeco.2010.11.002

Lai TK, Biswas G, Chatterjee S, Dutta A, Pal C, Banerji J, Bhuvanesh N, Reibenspies JH, Acharya K (2012) Leishmanicidal and anticandidal activity of constituents of indian edible mushroom Astraeus hygrometricus. Chem Biodivers 9:1517–1524. https://doi.org/10.1002/cbdv.201100272

Lee JC, Emil L, Pliam NB, Strobel G, Clardy J (1995) The Journal of Organic Chemistry 60(22):7076–7077. https://doi.org/10.1021/jo00127a001

Lee JC, Strobel GA, Lobkovsky E, Clardy J (1996) Torreyanic acid: a selectively cytotoxic quinone dimer from the endophytic fungus Pestalotiopsis microspora. J Org Chem 61:3232–3233. https://doi.org/10.1021/jo960471x

Lee YM, Li H, Hong J et al (2010) Bioactive metabolites from the sponge-derived fungus Aspergillus versicolor. Arch Pharm Res 33:231–235. https://doi.org/10.1007/s12272-010-0207-4

Leigh JA, Coplin DL (1992) Exopolysaccharides in plant-bacterial interactions. Annu Rev Microbiol 46:307–346

Leliebre-Lara V, Fidalgo LM, Pferschy-Wenzig EM, Kunert O, Lima CN, Bauer R (2016) In vitro antileishmanial activity of sterols from Trametes versicolor (Bres. Rivarden). Molecules 21(08):1–10. https://doi.org/10.3390/molecules21081045

Li JY, Strobel GA (2001) Jesterone and hydroxy-jesterone antioomycete cyclohexenone epoxides from the endophytic fungus Pestalotiopsis jesteri. Phytochemistry 57:261–265. https://doi.org/10.1016/S0031-9422(01)00021-8

Li JY, Strobel G, Sidhu R et al (1996) Endophytic taxol-producing fungi from bald cypress, Taxodium distichum. Microbiology 142:2223–2226. https://doi.org/10.1099/13500872-142-8-2223

Li JY, Strobel G, Harper J, Lobkovsky E, Clardy J (2000) Cryptocin, a potent tetramic acid antimycotic from the endophytic fungus Cryptosporiopsis cf. quercina. Org Lett 2:767–770. https://doi.org/10.1021/ol000008d

Li H, Qing C, Zhang Y, Zhao Z (2005) Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. World J Microbiol Biotechnol 21:1515–1519. https://doi.org/10.1007/s11274-005-7381-4

Lin W, Li L, Fu H et al (2006) Erratum: New cyclopentenone derivatives from an endophytic Streptomyces sp. isolated from the mangrove plant Aegiceras comiculatum (The Journal of Antibiotics (2005) 58, 9 (594–598)). J Antibiot (Tokyo) 59:594–598

Lingham RB, Silverman KC, Bills GF et al (1993) Chaetomella acutiseta produces chaetomellic acids A and B which are reversible inhibitors of farnesyl-protein transferase. Appl Microbiol Biotechnol 40:370–374. https://doi.org/10.1007/BF00170395

Liu CH, Zou WX, Lu H, Tan RX (2001) Antifungal activity of Artemisia annua endophyte cultures against phytopathogenic fungi. J Biotechnol 88:277–282. https://doi.org/10.1016/S0168-1656(01)00285-1

Liu JY, Song YC, Zhang Z et al (2004) Aspergillus fumigatus CY018, an endophytic fungus in Cynodon dactylon as a versatile producer of new and bioactive metabolites. J Biotechnol 114:279–287. https://doi.org/10.1016/j.jbiotec.2004.07.008

Liu X, Dong M, Chen X et al (2007) Antioxidant activity and phenolics of an endophytic Xylaria sp. from Ginkgo biloba. Food Chem 105:548–554. https://doi.org/10.1016/j.foodchem.2007.04.008

Liu K, Ding X, Deng B (2009a) Isolation and characterization of endophytic taxol-producing fungi from Taxus chinensis. Ann Microbiol 64:1171–1177. https://doi.org/10.1007/s10295-009-0598-8

Liu L, Liu S, Niu S, Guo L, Chen X, Che Y (2009b) Isoprenylatedchromone derivatives from the plant endophytic fungus Pestalotiopsis fici. J Nat Prod 72:1482–1486

Liu M, Abdel-Mageed WM, Ren B, He W, Huang P, Li X, Bolla K, Guo H, Chen C, Song F, Dai H (2014) Endophytic Streptomyces sp Y3111 from traditional Chinese medicine produced antitubercular pluramycins. Appl Microbiol Biotechnol 98:1077–1085. https://doi.org/10.1007/s00253-013-5335-6

Loo CSN, Lam NSK, Yu D, Su XZ, Lu FF (2017) Artemisinin and its derivatives in treating protozoan infections beyond malaria. Pharmacol Res 117:192–217. https://doi.org/10.1016/j.phrs.2016.11.012

Ma YM, Li Y, Liu JY et al (2004) Anti-Helicobacter pylori metabolites from Rhizoctonia sp. Cy064, an endophytic fungus in Cynodon dactylon. Fitoterapia 75:451–456. https://doi.org/10.1016/j.fitote.2004.03.007

Mahapatra S, Banerjee D (2010) Diversity and screening for antimicrobial activity of endophytic fungi from Alstonia scholaris. Acta Microbiol Immunol Hung 57:215–223. https://doi.org/10.1556/AMicr.57.2010.3.6

Majumder A, Jha S (2009) Biotechnological approaches for the production of potential anticancer leads podophyllotoxin and paclitaxel: an overview. J Biol Sci 1:46–69

Malhadas C, Malheiro R, Pereira JA, de Pinho PG, Baptista P (2017) Antimicrobial activity of endophytic fungi from olive tree leaves. World J Microbiol Biotechnol 33:1–12. https://doi.org/10.1007/s11274-017-2216-7

Malik A, Ardalani H, Anam S et al (2020) Antidiabetic xanthones with α-glucosidase inhibitory activities from an endophytic Penicillium canescens. Fitoterapia 142:104522. https://doi.org/10.1016/j.fitote.2020.104522

Mallick S, Dey S, Mandal S, Dutta A, Mukherjee D, Biswas G, Chatterjee S, Mallick S, Lai TK, Acharya K, Pal C (2015) A novel triterpene from Astraeus hygrometricus induces reactive oxygen species leading to death in Leishmania donovani. Future Microbiol 10(5):763–789. https://doi.org/10.2217/fmb.14.149

Maria GL, Sridhar KR, Raviraja NS (2005) Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. J Agric Technol 1:67–80

Mckinnon AC (2016) Plant tissue preparation for the detection of an endophytic fungus in planta. Methods Mol Biol 1477:167–173. https://doi.org/10.1007/978-1-4939-6367-6

Miller CM, Miller RV, Garton-Kenny D et al (1998) Ecomycins, unique antimycotics from Pseudomonas viridiflava. J Appl Microbiol 84:937–944. https://doi.org/10.1046/j.1365-2672.1998.00415.x

Minagawa K, Kouzuki S, Yoshimoto J, Kawamura Y, Tani H, Iwata T, Terui Y, Nakai H, Yagi S, Hattori N, Fujiwara T, Kamigauchi T (2002) Stachyflin and acetylstachyflin, novel anti-influenza A virus substances, produced by Stachybotrys sp. RF-7260. I. Isolation, structure elucidation and biological activities. J Antibiot (Tokyo). 55(2):155–164. https://doi.org/10.7164/antibiotics.55.155. PMID: 12002997.

Mlinaric A, Kac J, Pohleven F (2005) Screening of selected wood-damaging fungi for the HIV-1 reverse transcriptase inhibitors. Acta Pharma 55:69–79

Musavi SF, Balakrishnan RM (2014) A study on the antimicrobial potentials of an endophytic fungus Fusarium oxysporum NFX 06. J Med Bioeng 3:162–166. https://doi.org/10.12720/jomb.3.3.162-166

Nadeem M, Ram M, Alam P, Ahmad MM, Mohammad A, Al-Qurainy F, Khan S, Abdin MZ (2012) Fusarium solani, P1, a new endophytic podophyllotoxin-producing fungus from roots of Podophyllum hexandrum. Afr J Microbiol Res 6:2493–2499. https://doi.org/10.5897/ajmr11.1596

Nalini MS, Sunayana N, Prakash HS (2014) Endophytic fungal diversity in medicinal plants of Western Ghats, India. Int J Biodivers 2014:494213

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. J Nat Prod 70:461–477. https://doi.org/10.1021/np068054v

Nisa H, Kamili AN, Nawchoo IA, Shafi S, Shameem N, Bandh SA (2015) Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. Microb Pathog 82:50–59. https://doi.org/10.1016/j.micpath.2015.04.001

Noraida T, Hamzah T, Lee SY et al (2018) Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, Rhizophora mucronata, and identification of potential antagonists against the soil-borne fungus, Fusarium solani. Front Microbiol 9:1–17. https://doi.org/10.3389/fmicb.2018.01707

Nyongbela KD, Lannang AM, Ayimele GA, Ngemenya MN, Bickle Q, Efange S (2013) Isolation and identification of an antiparasitic triterpenoid estersaponin from the stem bark of Pittosporum mannii (Pittosporaceae). Asian Pac J Trop Dis 3(5):389–392. https://doi.org/10.1016/S2222-1808(13)60089-4

Ouyang J, Mao Z, Guo H, Xie Y, Cui Z, Sun JWH, Wen X, Wang J, Shan T (2018) Mollicellins O–R, four new depsidones isolated from the endophytic fungus Chaetomium sp. Eef-10. Molecules 23:3218

Palanichamy P, Kannan S, Murugan D et al (2019) Purification, crystallization and anticancer activity evaluation of the compound alternariol methyl ether from endophytic fungi Alternaria alternata. J Appl Microbiol 127:1468–1478. https://doi.org/10.1111/jam.14410

Palem PP, Kuriakose GC, Jayabaskaran C (2015) An endophytic fungus, Talaromyces radicus, isolated from Catharanthus roseus, produces vincristine and vinblastine, which induce apoptotic cell death. PLoS One 10:e0144476. https://doi.org/10.1371/journal.pone.0144476

Paper F (2002) Isolation of endophytic fungi from leaves of Pasania edulis and their within-leaf distributions. Mycoscience 43:369–373

Park YC, Gunasekera SP, Lopez JV, McCarthy PJ, Wright AE (2006) Metabolites from the marine-derived fungus Chromocleista sp. isolated from a deep-water sediment sample collected in the Gulf of Mexico. J Nat Prod 69:580–584. https://doi.org/10.1021/np058113p

Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from Garcinia species. FEMS Immunol Med Microbiol 48:367–372. https://doi.org/10.1111/j.1574-695X.2006.00155.x

Phongpaichit S, Nikom J, Rungjindamai N et al (2007) Biological activities of extracts from endophytic fungi isolated from Garcinia plants. FEMS Immunol Med Microbiol 51:517–525. https://doi.org/10.1111/j.1574-695X.2007.00331.x

Pleban S, Chernin L, Chet I (1997) Chitinolytic activity of an endophytic strain of Bacillus cereus. Lett Appl Microbiol 25:284–288. https://doi.org/10.1046/j.1472-765X.1997.00224.x

Prasad KR, Penchalaiah K, Choudhary A, Anbarasan P (2007) Stereoselective synthesis of (-)-microcarpalide. Tetrahedron Lett 48:309–311. https://doi.org/10.1016/j.tetlet.2006.11.027

Puri SC, Verma V, Amna T, Qazi GN, Spiteller M (2005) An endophytic fungus from Nothapodytes foetida that produces camptothecin. J Nat Prod 68:1717–1719. https://doi.org/10.1021/np0502802

Puri SC, Nazir A, Chawla R, Arora R, Riyaz-ul-Hasan S, Amna T, Ahmed B, Verma V, Singh S, Sagar R, Sharma A (2006) The endophytic fungus Trametes hirsuta as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. J Biotechnol 122:494–510. https://doi.org/10.1016/j.jbiotec.2005.10.015

Quadt-Hallmann A, Benhamou N, Kloepper JW (1997) Bacterial endophytes in cotton: mechanisms of entering the plant. Can J Microbiol 43:577–582. https://doi.org/10.1139/m97-081

Radić N, Štrukelj B (2012) Endophytic fungi - the treasure chest of antibacterial substances. Phytomedicine 19:1270–1284. https://doi.org/10.1016/j.phymed.2012.09.007

Raekiansyah M, Mori M, Nonaka K, Agoh M, Shiomi K, Matsumoto A, Morita K (2017) Identification of novel antiviral of fungus-derived brefeldin A against dengue viruses. Tropical medicine and health, 45, 32. https://doi.org/10.1186/s41182-017-0072-7

Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN et al (2019a) Endophytic fungi: biodiversity, ecological significance and potential industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer, Switzerland, pp 1–62

Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V et al (2019b) Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer International Publishing, Cham, pp 105–144. https://doi.org/10.1007/978-3-030-03589-1_6

Rana KL, Kour D, Kaur T, Devi R, Negi C, Yadav AN et al (2020) Endophytic fungi from medicinal plants: biodiversity and biotechnological applications. In: Kumar A, Radhakrishnan E (eds) Microbial endophytes. Woodhead Publishing, Cambridge, MA, pp 273–305. https://doi.org/10.1016/B978-0-12-819654-0.00011-9

Rastegari AA, Yadav AN, Yadav N (2020) New and future developments in microbial biotechnology and bioengineering: trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam

Ratnayake AS, Yoshida WY, Mooberry SL, Hemscheidt T (2001) The structure of microcarpalide, a microfilament disrupting agent from an endophytic fungus. Org Lett 3:3479–3481. https://doi.org/10.1021/ol016515s

Raunsai M, Wulansari D, Fathoni A, Agusta A (2018) Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. J Appl Pharm Sci 8:69–74. https://doi.org/10.7324/JAPS.2018.8811

Raviraja NS, Maria GL, Sridhar KR (2006) Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the western Ghats of India. Eng Life Sci 6:515–520. https://doi.org/10.1002/elsc.200620145

Rukachaisirikul V, Sommart U, Phongpaichit S et al (2008) Metabolites from the endophytic fungus Phomopsis sp. PSU-D15. Phytochemistry 69:783–787. https://doi.org/10.1016/j.phytochem.2007.09.006

Rustamova N, Gao Y, Zhang Y, Yili A (2020) Biological activity of endophytic fungi from the roots of the medicinal plant Vernonia anthelmintica. Microorganisms 8:586. https://doi.org/10.3390/microorganisms8040586

Sandhu SS, Kumar S, Aharwal RP (2014) Isolation and identification of endophytic fungi from Ricinus communis Linn. and their antibacterial activity. Biology 4:611–618

Schulz B, Römmert AK, Dammann U et al (1999) The endophyte-host interaction: a balanced antagonism? Mycol Res 103:1275–1283. https://doi.org/10.1017/S0953756299008540

Selim KA, El-Beih AA, AbdEl-Rahman TM, El-Diwany AI (2012) Biology of eEndophytic fFungi. Curr Res Environm Appl Mycol. 31–82. https://doi.org/10.5943/cream/2/1/3

Selim KA, Elkhateeb WA, Tawila AM, El-Beih AA, Abdel-Rahman TM et al (2018) Fermentation; Basel, 4(3) https://doi.org/10.3390/fermentation4030049

Sette LD, Passarini MRZ, Delarmelina C, Salati F, Duarte MCT (2006) Molecular characterization and antimicrobial activity of endophytic fungi from coffee plants. World J Microbiol Biotechnol 22:1185–1195. https://doi.org/10.1007/s11274-006-9160-2

Shaaban M, Nasr H, Hassan AZ, Asker MS (2013) Bioactive secondary metabolites from endophytic aspergillus fumigatus: structural elucidation and bioactivity studies. Rev Latinoam Quim 41:50–60

Shaanker RU, Ramesha BT, Ravikanth G, Gunaga R, Vasudeva R, Ganeshaiah KN (2008) Chemical profiling of Nothapodytes nimmoniana for camptothecin, an important anticancer alkaloid: towards the development of a sustainable production system. In: Bioactive molecules and medicinal plants. Springer, New York, pp 197–213

Shah A, Rather MA, Shah AM, Mushtaq S, Hussain A, Rasool S, Parry ZA, Qazi PH (2016) Evaluating the in vitro antituberculosis, antibacterial and antioxidant potential of fungal endophytes isolated from Glycyrrhiza glabra L. Ann Phytomed 5:140–146. https://doi.org/10.21276/ap.2016.5.2.19

Shen L, Ye YH, Wang XT et al (2006) Structure and total synthesis of aspernigerin: a novel cytotoxic endophyte metabolite. Chemistry 12:4393–4396. https://doi.org/10.1002/chem.200501423

Shinohara C, Hasumi K, Hatsumi W, Endo A (1996) Staplabin, a novel fungal triprenyl phenol which stimulates the binding of plasminogen to fibrin and U937 cells. J Antibiot (Tokyo) 49(10):961–966

Shternshis M, Tomilova O, Shpatova T, Soytong K (2005) Evaluation of Ketomium-mycofungicide on Siberian isolates of phytopathogenic fungi. J Agric Technol 1(2):247–254

Simmons CP, Farrar JJ, Nguyen VV, Wills B (2012) Dengue. N Engl J Med 366:1423–1432

Singh J, Yadav AN (2020) Natural bioactive products in sustainable agriculture. Springer, Singapore

Sonaimuthu V, Parihar S, Prakash Thakur J et al (2011) Tenuazonic acid: a promising antitubercular principle from Alternaria alternate. Microbiol Res (Pavia) 2:17. https://doi.org/10.4081/mr.2011.e17

Song YC, Huang WY, Sun C et al (2005) Characterization of graphislactone A as the antioxidant and free radical-scavenging substance from the culture of Cephalosporium sp. IFB-E001, an endophytic fungus in Trachelospermum jasminoides. Biol Pharm Bull 28:506–509. https://doi.org/10.1248/bpb.28.506

Song Z, Liu Y, Gao J, Hu J, He H, Dai S, Wang L, Dai H, Zhang L, Song F (2019) Antitubercular metabolites from the marine-derived fungus strain Aspergillus fumigatus MF029. Nat Prod Res:1–8. https://doi.org/10.1080/14786419.2019.1660331

Stamford TLM, Stamford NP, Coelho LCBB, AraĂjo JM (2001) Production and characterization of a thermostable α-amylase from Nocardiopsis sp. endophyte of yam bean. Bioresour Technol 76:137–141. https://doi.org/10.1016/S0960-8524(00)00089-4

Stierle A, Strobel G, Stierle D (1993a) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science 260:214–216. https://doi.org/10.1126/science.8097061

Stierle A, Hershenhorn J, Strobel G (1993b) Zinnimidine and 5-(30,30-dimethylallyloxy)-7-methoxy-6-methylphthalide from Alternaria porri. Phytochemistry 32:1145–1149

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502. https://doi.org/10.1128/mmbr.67.4.491-502.2003

Strobel G, Yang X, Sears J et al (1996) Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana. Microbiology 142:435–440. https://doi.org/10.1099/13500872-142-2-435

Strobel G, Li JY, Sugawara F et al (1999a) Oocydin A, a chlorinated macrocyclic lactone with potent anti-oomycete activity from Serratia marcescens. Microbiology 145:3557–3564. https://doi.org/10.1099/00221287-145-12-3557

Strobel GA, Ford E, Li JY et al (1999b) Seimatoantlerium tepuiense gen. nov., a unique epiphytic fungus producing taxol from the Venezuelan Guyana. Syst Appl Microbiol 22:426–433. https://doi.org/10.1016/S0723-2020(99)80052-6

Strobel GA, Dirkse E, Sears J, Markworth C (2001) Volatile antimicrobials from Muscodor crispans, a novel endophytic fungus. Microbiology 147:2943–2950. https://doi.org/10.1099/mic.0.032540-0

Strobel G, Ford E, Worapong J et al (2002) Isopestacin, an isobenzofuranone from Pestalotiopsis microspora, possessing antifungal and antioxidant activities. Phytochemistry 60:179–183. https://doi.org/10.1016/S0031-9422(02)00062-6

Sudha V, Govindaraj R, Baskar K et al (2016) Biological properties of endophytic fungi. Braz Arch Biol Technol 59:1–7. https://doi.org/10.1590/1678-4324-2016150436

Suemitsu R, Ohnishi K, Morikawa Y, Nagatomo S (1995) Zinniol-relatedphytotoxins from Alternaria cichorii. Phytochemistry 38:495–497

Supaphon P, Phongpaichit S, Rukachaisirikul V, Sakayaroj J (2013) Antimicrobial potential of endophytic fungi derived from three seagrass species: Cymodocea serrulata, Halophila ovalis and Thalassia hemprichii. PLoS One 8:1–9. https://doi.org/10.1371/journal.pone.0072520

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459

Tejesvi MV, Kini KR, Prakash HS, Subbiah V, Shetty HS (2007) Genetic diversity and antifungal activity of species of Pestalotiopsis isolated as endophytes from medicinal plants. Fungal Divers 24:1–18

Thirawatthana P, Tanapat P, Jittra P et al (2013) Antimicrobial and anticancer activities of endophytic fungi from Mitrajyna javanica Koord and Val. Afr J Microbiol Res 7:5565–5572. https://doi.org/10.5897/ajmr12.2352

Ting D, Wei-Wei S, Yong-Xia Q, Hai-Yang J (2013) Studies on some active components and antimicrobial activities of the fermentation broth of endophytic fungi DZY16 Isolated from Eucommia ulmoides Oliv. Afr J Biotechnol 12:1862–1868. https://doi.org/10.5897/ajb11.2638

Tolulope RA, Adeyemi AI, Erute MA, Abiodun TS (2015) Isolation and screening of endophytic fungi from three plants used in traditional medicine in Nigeria for antimicrobial activity. Int J Green Pharm 9:58–62. https://doi.org/10.4103/0973-8258.150929

Torres M, Dolcet MM, Sala N, Canela R (2003) Endophytic fungi associated with Mediterranean plants as a source of mycelium-bound lipases. J Agric Food Chem 51:3328–3333. https://doi.org/10.1021/jf025786u

Tuntiwachwuttikul P, Taechowisan T, Wanbanjob A et al (2008) Lansai A–D, secondary metabolites from Streptomyces sp. SUC1. Tetrahedron 64:7583–7586. https://doi.org/10.1016/j.tet.2008.05.104

van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483. https://doi.org/10.1146/annurev.phyto.36.1.453

Wagenaar MM, Corwin J, Strobel G, Clardy J (2000) Three new cytochalasins produced by an endophytic fungus in the genus Rhinocladiella. J Nat Prod 63:1692–1695. https://doi.org/10.1021/np0002942

Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AASG (1966) Plant antitumor agents. I. The isolation and structure of Camptothecin, a novel alkaloidal leukemia and tumor inhibitor from Camptotheca acuminata. J Am Chem Soc 88:3888–3890. https://doi.org/10.1021/ja00968a057

Wang J, Li G, Lu H et al (2000) Taxol from Tubercularia sp. strain TF5, an endophytic fungus of Taxus mairei. FEMS Microbiol Lett 193:249–253. https://doi.org/10.1016/S0378-1097(00)00491-2

Wang S, Li XM, Teuscher F, Li DL, Diesel A, Ebel R, Proksch P, Wang BG (2006) Chaetopyranin, a benzaldehyde derivative, and other related metabolites from Chaetomium globosum, an endophytic fungus derived from the marine red alga Polysiphonia urceolata. J Nat Prod 69(11):1622–1625

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia 2. J Am Chem Soc 93:2325–2327. https://doi.org/10.1021/ja00738a045

Weber D, Sterner O, Anke T, Gorzalczancy S, Martino V, Acevedo C (2004) Phomol, a new anti-inflammatory metabolite from an endophyte of the medicinal plant Erythrina crista-galli. J Antibiot (Tokyo) 57:559–563. https://doi.org/10.7164/antibiotics.57.559

Wijeratne EK, He H, Franzblau SG, Hoffman AMGAL (2013) Phomapyrrolidones A–C, antitubercular alkaloids from the endophytic fungus Phoma sp. NRRL 46751. J Nat Prod 76:1860–1865. https://doi.org/10.1021/np400391p

Wilkinson HH, Siegel MR, Blankenship JD et al (2000) Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. Mol Plant-Microbe Interact 13:1027–1033. https://doi.org/10.1094/MPMI.2000.13.10.1027

Williams EJ, Embleton ND, Clark JE et al (2013) Viral infections: contributions to late fetal death, stillbirth, and infant death. J Pediatr 163:424–428

Wiyakrutta S, Sriubolmas N, Panphut W et al (2004) Endophytic fungi with anti-microbial, anticancer and anti-malarial activities isolated from Thai medicinal plants. World J Microbiol Biotechnol 20:265–272

World Health Organization (2020) Tuberculosis. https://www.who.int/news-room/fact-sheets/detail/tuberculosis

Xianzhi Y, Lingqi Z, Bo GSG (2004) Preliminary study of a vincristine-proudcing endophytic fungus isolated from leaves of Catharanthus roseus. Chinese Tradit Herb Drugs 35:79–81

Xu M, Jia O, Wang S, Zhu Q (2016) A new bioactive diterpenoid from Pestalotiopsis adusta, an endophytic fungus from Clerodendrum canescens. Nat Prod Res 30(23):2642–2647. https://doi.org/10.1080/14786419.2016.1138297

Yadav AN (2020) Recent trends in mycological research, Agricultural and medical perspective, vol 1. Springer, Switzerland

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yan W, Cao LL, Zhang YY, Zhao R, Zhao SS, Khan B, Ye YH (2018) New Metabolites from Endophytic Fungus Chaetomium globosum CDW7. Molecules (Basel, Switzerland), 23(11):2873. https://doi.org/10.3390/molecules23112873

Ye Y, Xiao Y, Ma L, Li H, Xie Z, Wang M, Liu J (2013) Flavipin in Chaetomium globosum CDW7, an endophytic fungus from Ginkgo biloba, contributes to antioxidant activity. Appl Microbiol Biotechnol 97(16):7131–7139. https://doi.org/10.1007/s00253-013-5013-8

Yousefzadi M, Sharifi M, Behmanesh M et al (2010) Podophyllotoxin: current approaches to its biotechnological production and future challenges. Eng Life Sci 10:281–292. https://doi.org/10.1002/elsc.201000027

Yu BZ, Zhang GH, Du ZZ, Zheng YT, Xu JC, Luo XD (2008) Phomoeuphorbins A–D, azaphilones from the fungus Phomopsis euphorbiae. Phytochemistry 69:2523–2526

Yuniati Y, Yuliati L, Monica E, Rollando R (2019) Discovering anticancer compound of ethyl acetate extract from RL1 code endophytic fungi culture derived by Phyllanthus niruri Linn leaves through cell cycle modulation in T47d cells. IOP Conf Ser Mater Sci Eng 509:48–53. https://doi.org/10.1088/1757-899X/509/1/012157

Zeng PY, Wu JG, Liao LM, Chen TQ, Wu JZ, Wong KH (2011) In vitro antioxidant activities of endophytic fungi isolated from the liverwort Scapania verrucosa. Genet Mol Res 10:3169–3179. https://doi.org/10.4238/2011.December.20.1

Zhang B, Salituro G, Szalkowski D, Li Z, Zhang Y, Royo I, Vilella D, Díez MT, Pelaez F, Ruby C, Kendall RL (1999) Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. Science 284:974–977. https://doi.org/10.1126/science.284.5416.974

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753–771

Zhang HW, Huang WY, Chen JR, Yan WZ, Tan RX (2008) Cephalosol: an antimicrobial metabolite with an unprecedented skeleton from endophytic Cephalosporium acremonium IFB-E007. Chemistry 14:10670–10674. https://doi.org/10.1002/chem.200801000

Zhang JY, Tao LY, Liang YJ et al (2009a) Secalonic acid D induced leukemia cell apoptosis and cell cycle arrest of G1 with involvement of GSK-3β/β-catenin/c-Myc pathway. Cell Cycle 8:2444–2450. https://doi.org/10.4161/cc.8.15.9170

Zhang Y, Mu J, Feng Y et al (2009b) Broad-spectrum antimicrobial epiphytic and endophytic fungi from marine organisms: isolation, bioassay and taxonomy. Mar Drugs 7:97–112. https://doi.org/10.3390/md7020097

Zhang G, Sun S, Zhu T, Lin Z, Gu J, Li D, Gu Q (2011) Antiviral isoindolone derivatives from an endophytic fungus Emericella sp. associated with Aegiceras corniculatum. Phytochemistry 72:1436–1442

Zhang H, Bai X, Wu B (2012) Evaluation of antimicrobial activities of extracts of endophytic fungi from Artemisia annua. Bangladesh J Pharmacol 7:120–123. https://doi.org/10.3329/bjp.v7i2.10951

Zhao J, Shan T, Mou Y, Zhou L (2011) Plant-derived bioactive compounds produced by endophytic fungi. Min Rev Med Chem 11:159–168

Zhao J, Fu Y, Luo M et al (2012a) Endophytic fungi from pigeon pea [Cajanus cajan (L.) Millsp.] produce antioxidant cajaninstilbene acid. J Agric Food Chem 60:4314–4319. https://doi.org/10.1021/jf205097y

Zhao J, Sun W, Shan T, Mou Y, Lou J, Li Y, Wang M, Zhou L (2012b) Antimicrobial metabolites from the endophytic fungus Gliomastix murorum Ppf8 associated with the medicinal plant Paris polyphylla var. yunnanensis. J Med Plants Res 6:2100–2104. https://doi.org/10.5897/jmpr11.893

Chapter 4
Fungal Communities from Different Habitats for Tannins in Industry

Sara Atef Gezaf, Fatma Ahmed Abo Nouh, and Ahmed M. Abdel-Azeem

Contents

S. A. Gezaf (✉)
Botany and Microbiology Department, Faculty of Science, Arish University, North Sinai, Egypt

F. A. Abo Nouh · A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt
e-mail: fatma.nouh@science.suez.edu.eg

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_4

4.1 Introduction

Tannin acyl hydrolase, commonly known as tannase, is a hydrolase enzyme that is induced in the presence of tannic acid (Belmares et al. 2004). It catalyzes the hydrolysis of ester and depside bonds in hydrolyzable tannins such as tannic acid and gallic acid esters releasing glucose and gallic acid (Sharma et al. 2000). This enzyme was accidentally discovered by Tieghem (1867) in an experiment of formation of gallic acid into an aqueous solution of tannins, where two fungal species grew later, identified as *Penicillium glaucum* and *Aspergillus niger* (Lekha and Lonsane 1997).

Tannase enzyme is known to display two different activities. The first one is an esterase activity; by which it can hydrolyze ester bonds of gallic acid esters with glucose (galloyl-glucose) or alcohols (e.g., methyl gallate). The second activity is called depsidase activity; by which it can hydrolyze depside bonds of digallic acid (Saxena and Saxena 2004). Tannase catalyzes the hydrolysis reaction of the ester bonds present in the gallotannins, complex tannins, and gallic acid esters (Vivas et al. 2004). Tannase can be obtained from plants, animals, and microbial sources. Microorganisms are considered the most important and commercial sources of tannase, that is because the produced tannases are more stable than similar ones obtained from the other sources. Moreover, microorganisms can produce tannase in high quantities. Microbial tannase is favored also because the microbes can be subjected to genetic manipulation more readily than plants and animals, resulting in an increase in tannase production (Aguilar and Gutierrez-Sanchez 2001; Purohit et al. 2006). As a result, enzymes of microbial origin are having important applications in many areas of bio-based industries (Barthomeuf et al. 1994). In microbial sources, the *Aspergillus* and *Penicillium* genus, and lactic acid bacteria mostly produce tannase (Zakipour-Molkabadi et al. 2013; Abdel-Azeem et al. 2021).

Tannase is an industrially important enzyme and has several applications in various industries such as foods, animal feeds, cosmetic, pharmaceutical, chemical, and leather industries (Aguilar et al. 2007; Jun et al. 2007; Kour et al. 2019). The major commercial applications of the tannases are elaboration of instantaneous tea or acorn liquor, hydrolyze tea cream in the processing of tea in the production of gallic acid (Lekha and Lonsane 1997), manufacturing of wine, beer, coffee, and fruit juices (Aguilar et al. 2001); cleaning up the leather industry effluents containing the pollutant tannin; and in the reduction of antinutritional effects of tannins in animal feed (Mukherjee and Banerjee 2006). Gallic acid (3,4,5-trihydroxybenzoic acid) and related compounds possess many potential therapeutic properties. The major application of gallic acid is the synthesis of a broad-spectrum antibacterial agent. It has always been a molecule of industrial importance because of its applications in different sectors from healthcare and food to dyes, inks, paints, and photography (Mukherjee and Banerjee 2003; Yadav et al. 2019). The present review shows relevant points related to the fungal tannase. The substrates for tannase production, the tannase producing fungi, production of tannase by fermentation using different methods, like submerged fermentation (SmF), solid-state fermentation (SSF) and

modified solid-state fermentation (MSSF), and ways to produce it, with the goal to contribute to the knowledge for potential applications of fungal tannase in food and other industrial products.

4.2 Tannins: Natural Substrate

Tannins are naturally occurring polyphenolic compounds with varying molecular weights that occur naturally in the plant kingdom (Aguilar et al. 2007). After lignin, tannins are the second most abundant group of plant phenolics (Aguilar et al. 2001). These phenolic compounds of high molecular weight are usually present in plants with molar masses extending from 500 to over 3000 Da and up to 20,000 Da (Rana et al. 2004). In the plant kingdom, these tannins are found in leaves, bark, and wood. Tannins are considered to be the plants' secondary metabolic products because they play no direct role in plant metabolism. Tannins are polymeric phenolic compounds with numerous hydroxyl groups and quite diverse in chemical structure (Hagerman 1992). It detected more than 8000 different tannins (Aguilar et al. 2007). They can be found in many different plants and plant residues such as *Anacardium occidentale* (cashew), *Vitis vinifera* (grape), *Malpighia glabra* (Barbados cherry), and *Hancornia speciosa* (mangaba fruit). One of the best sources of tannins is *Acacia* species, which belong to the family of Leguminosae in the plant kingdom. Such residues, rich in tannins, can be excellent substrates for the production of tannase (De Lima et al. 2014). Hydrolysis of some of the tannins yields the simple, seven-carbon gallic acid and others give ellagic acid or other phenolic acids (Okuda and Ito 2011).

On the basis of their structural characteristics, it is, therefore, possible to divide the tannins into four major groups: gallotannins, ellagitannins, complex tannins, and condensed tannins (Khanbabaee and van Ree 2001). The term "hydrolyzable tannins" includes both the gallotannins and the ellagitannins. Hydrolyzable tannins (HT) are involved in a monosaccharide core, usually, glucose, esterified with gallic acid, developing the gallotannins, or with hexahydrodiphenoic acid, the precursor of ellagic acid, and gallic acid, developing the ellagitannins (Serrano et al. 2009). Hydrolyzable tannins can be hydrolyzed into smaller compounds, for example, gallic and ellagic acid (Bele et al. 2010). Gallotannins are the simplest hydrolyzable tannins, containing polyphenolic and a polyol residue. The plants *Rhus semialata* (Chinese galls), *Quercus infectoria* (Turkish galls), *Caesalpinia spinosa* (Tara pods), *Aver ginnala* (Korean maple, *Acer tannin* leaves), *Rhus coriaria* (Sumac leaves), *Hammelis virginiana* (Hamammelis—hazelnuts) were documented to be rich in gallotannins (Bhat et al. 1998). Gallotannins yield gallic acid and glucose or quinic acid on hydrolysis (Khanbabaee and Van Ree 2001).

Ellagitannins yield ellagic acid, gallic acid, and glucose on hydrolysis. Plants such as *Caesalpinia coriaria* (Divi-Divi), *Caesalpinia brevifolia* (Algarobillatannin) *Terminalia chebula* (myrobalan seeds), *Castanea sativa* (Chestnut), *Eucalyptous sieberiana*, *Schinopsis* sp., *Quercus velonia*, *Q. aegilops* (Valonea tannin), *Quercus*

coccifera (Garouille) were reported to contain ellagitannins in their bark, nuts or leaves (Bhat et al. 1998; Mukherjee and Banerjee 2003). Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit forms acyl bond with pro anthocyanadines such as catechin, for example, tea polyphenols (Khanbabaee and Van Ree 2001). Condensed tannins are referred to proanthocyanidins. These are generally found in plants that possess a woody growth and also in plant gums and exudates (Haslam 1996). Common plants containing condensed tannins are *Acacia* sp. (Wattle tannins), *Schinopsis* spp. and *Loxopterygium* sp. (Querbacho wood), *Pinus* sp. (Pine bark), *Quercus* sp. (Oakwood), and *Aucoumea kleneana* (Gaboon wood) (Bhat et al. 1998). Condensed proanthocyanidins degrade in strong acid to give corresponding anthocyanidin (Falcão and Araújo 2018).

4.3 Different Source of Tannase

Tannase is known to be a ubiquitous enzyme of the microbial world (Murugan et al. 2007). Microorganisms such as bacteria, fungi, and yeasts are known as prominent producers, also, a few animals and plants have been found as a source of tannase. The enzymes are present in tannin-rich vegetables, fruits, leaves, and bark, and also a few of them are present in fruits of *Terminalia chebula*, pods of *Caesalpinia coriaria*, and bark of *Cassia fistula* (Madhavakrishna et al. 1960). Although novel researches showed that the colonizing microorganisms of these animals are the actual producers, not the animals (Belur and Mugeraya 2011).

4.3.1 Plant Source

Tannase is present in tannin-plants such as Turkish gall (*Quercus infectoria*), sumac (*Rhus coriaria*), tara (*Caesalpinia spinosa*), chestnut (*Castanea sativa*, Bhat et al. 1998), gum arabic tree (*Acacia nilotica*, Lal et al. 2012), red gram (*Cajanus cajan*, Kuppusamy et al. 2015) and waste testa (*Anacardium occidentales*, Lenin et al. 2015), English oak (*Quercus robur*), Pendunculate oak (*Quercus rubra*), Karee tree (*Rhus typhina*) leaves. Plants having condensed tannins are babul (*Acacia arabica*), konnam (*Cassia fistula*), avaram (*Cassia auriculata*), and others. Plants synthesize gallic acid, hexahydroxyphenic acid, and chebulinic acid in addition to the amount of sugar. These acids possibly undergo esterification with glucose molecules during the ripening process with the aid of tannase ultimately resulting in the synthesis of tannins.

4.3.2 Animal Source

Microorganisms that live in the gastrointestinal of the cow, goats, sheep, and other animals are known as effective tannase producers such as many species of bacteria isolated from fecal samples of native sheep and goats, which can hydrolyze acorn tannin in the rumen and reduce negative effects of tannin on animals (Mosleh et al. 2014). Examples of Tannase-producing bacteria are *Streptococcus pneumonia* and *Streptococcus*.

4.3.3 Bacterial and Fungal Sources

Microorganisms are producing tannase enzymes in large quantities than other organisms. They are the main source of commercial enzyme production. The microbial enzymes present several advantages over other sources since the microbial enzymes are more stable in comparison to similar enzymes from other sources (Jana et al. 2014). Microorganisms can ease of genetic manipulation of microbial enzymes thus; they are easy to manipulate genetically, which increases and improves the enzymatic activity (Aguilar and Gutierrez-Sanchez 2001; Purohit et al. 2006). The suitable tannase-producing microorganisms reported are fungi and few bacteria and yeast. Fungal tannase has high activity titer in the degradation of hydrolyzable tannins. However, yeast tannases relatively disintegrate tannic acid easily and flaunt a relatively lesser affinity on the other hand in the degradation of natural tannins (Kumar et al. 2019).

4.3.3.1 Yeast Tannase

There are few reports about yeast used as tannase production. Among yeast *Candida* species (Aoki et al. 1976) *Mycotorula japonica* (Belmares et al. 2004) *Pichia* sp. (Deschamps et al. 1983) *Debaryomyoes hansenii* (Deschamps et al. 1983).

4.3.3.2 Bacteria Tannase

Lewis and Starkey (1969) reported first bacterium capable of hydrolyzing gallotannins as a sole energy source was *Achomobacter* sp., also, *Bacillus* and *Lactobacillus* genus represents a group of tannase producing bacteria (Mondal et al. 2001; Sabu et al. 2006; Nishitani et al. 2004). Lactic acid bacteria have an important role in tannin food degradation. Few other bacterial tannase producers comprise *Klebsiella* (Deschamps et al. 1983), *Lonepinella* (Goel et al. 2007), *Citrobacter* (Kumar et al. 1999), *Corynebacterium*, *Pantonea* (Pepi et al. 2010), *Serratia* (Belur et al. 2010),

Table 4.1 Bacterial tannases sources

Lactobacillus plantarum CECT 748 T	Rodríguez et al. (2008)
Lactobacillus plantarum ATCC 1491 T (recombinant)	Iwamoto et al. (2008)
Lactobacillus plantarum (recombinant)	Curiel et al. (2009)
Enterobacter sp.	Sharma and John (2011)
Bacillus licheniformis KBR 6	Mondal and Pati (2000)
Bacillus cereus KBR9	Mondal et al. (2001)
Bacillus sphaericus	Raghuwanshi et al. (2011)
Enterobacter cloacae	Beniwal et al. (2013)
Staphylococcus lugdunensis MTCC 3614 (recombinant)	Chaitanyakumar and Anbalagan (2016)
Enterococcus faecalis	Goel et al. (2011)

Pseudomonas (Selwal et al. 2010), *Selenomonas* (Skene and Brooker 1995), and *Streptococcus* (Jiménez et al. 2014) as shown in Table 4.1.

4.3.3.3 Fungal Tannase

Tannase is now known to be an ever-present enzyme of the microbial world and has widespread occurrence in various fungi. Most of the reported tannase-producing organisms are fungi (Bhat et al. 1998) and only a few are bacteria (Mondal and Pati 2000; Mondal et al. 2001). Fungi have the ability to degrade tannins as a sole carbon source (Aguilar and Gutierrez-Sanchez 2001) as shown in Table 4.2. The genus *Aspergillus* is considered as the best producer, followed by *Penicillium*, both standing out as great decomposers of tannins (Sabu et al. 2005). The main genera of fungi known as producers of tannase are *Aspergillus*, *Penicillium*, *Fusarium*, and *Trichoderma* (De Sena et al. 2014; Rastegari et al. 2020). Other fungi, including *Chaetomium*, *Rhizoctonia*, and *Cylindrocarpon* are capable of degrading tannery waste constituents (Cruz-Hernandez et al. 2005). The common genus used for tannase production either for research purposes or industrial production was *Aspergillus* and the common *Aspergillus* species used for tannase production was *Aspergillus niger* (Manjit et al. 2008). The tannase from *Aspergillus* has been used widely for the production of gallic acid from tannins (Purohit et al. 2006).

4.3.4 Rich Tannin Plants

Different studies have indicated the presence of hydrolyzable tannins in diverse plant species, especially in their leaves, fruits, bark, and branches. Endophytic microorganisms are fungi and bacteria living inside the aerial parts of the plants without, causing any seeming damage to the host (Abo Nouh 2019). According to Reges de Sena et al. (2014) isolated endophytic fungi isolated from Jamun (*Syzygium*

Table 4.2 Filamentous fungi capable of producing tannases and/or of using tannins as sole carbon source

Ascochyta boltshauseri	Lekha and Lonsane (1997)
Ascochyta pisi	Lekha and Lonsane (1997)
Ascochyta viciae	Lekha and Lonsane (1997)
Aspergillus aculeatus	Banerjee et al. (2001)
Aspergillus acidus	Prigione et al. (2018)
Aspergillus aureus	Bajpai and Patil (1997)
Aspergillus avenaceus	De Lima et al. (2014)
Aspergillus awamori	Bradoo et al. (1996)
Aspergillus caespitosum	Batra and Saxena (2005)
Aspergillus carbonarius	De Lima et al. (2014)
Aspergillus carneus	Aguilar et al. (2007)
Aspergillus clavatus	De Lima et al. (2014)
Aspergillus costaricaensis	Prigione et al. (2018)
Aspergillus fischeri	Bajpai and Patil (1997)
Aspergillus flavus	Aguilar et al. (2007)
Aspergillus flavipes	Aguilar et al. (2007)
Aspergillus foetidus	Banerjee et al. (2005)
Aspergillus fumigatus	Batra and Saxena (2005)
Aspergillus gallonyces	Belmares et al. (2004)
Aspergillus granulosus	De Lima et al. (2014)
Aspergillus japonicus	Bradoo et al. (1997)
Aspergillus niger	Rana and Bhat (2005)
Aspergillus ochraceus	De Lima et al. (2014)
Aspergillus oryzae	Bradoo et al. (1996)
Aspergillus parasiticus	De Lima et al. (2014)
Aspergillus ruber	Kumar et al. (2007)
Aspergillus rugulosus	Bradoo et al. (1996)
Aspergillus tamarii	Costa et al. (2008)
Aspergillus terreus	Bajpai and Patil (1997)
Aspergillus tubingensis	Prigione et al. (2018)
Aspergillus ustus	De Lima et al. (2014)
Aspergillus vadensis	Prigione et al. (2018)
Aspergillus versicolor	Batra and Saxena (2005)
Aspergillus viridinutans	De Lima et al. (2014)
Chaetomium globosum	Lekha and Lonsane (1997)
Cryphonectria parasitica	Farias et al. (1994)
Cunninghamella sp.	Bradoo et al. (1996)
Emericella nidulans	Prigione et al. (2018)
Fusarium oxysporium	Bradoo et al. (1996)
Fusarium solani	Bradoo et al. (1996)
Lentinus edodes	Vattem and Shetty (2003)
Lenzites elegans	Ordonez et al. (2011)

(continued)

Table 4.2 (continued)

Neurospora crassa	Bradoo et al. (1996)
Paecilomyces variotii	Mahendran et al. (2005)
Penicillium acrellanum	Bradoo et al. (1996)
Penicillium aurantiogriseum	De Lima et al. (2014)
Penicillium canescens	De Lima et al. (2014)
Penicillium carylophilum	Bradoo et al. (1996)
Penicillium citrinum	Bradoo et al. (1996)
Penicillium charlessi	Batra and Saxena (2005)
Penicillium chrysogenum	Bradoo et al. (1996)
Penicillium crustosum	Batra and Saxena (2005)
Penicillium commune	De Lima et al. (2014)
Penicillium corylophilum	De Lima et al. (2014)
Penicillium digitatum	Bradoo et al. (1996)
Penicillium fellutanum	De Lima et al. (2014)
Penicillium frequentanse	De Lima et al. (2014)
Penicillium glabrum	Van de Lagemaat and Pyle (2005)
Penicillium glaucum	Lekha and Lonsane (1997)
Penicillium islandicum	Aguilar et al. (2007)
Penicillium lanosum	De Lima et al. (2014)
Penicillium lapidosum	De Lima et al. (2014)
Penicillium lividum	De Lima et al. (2014)
Penicillium montanense	De Lima et al. (2014)
Penicillium notatum	Aguilar et al. (2007)
Penicillium purpurogenum	De Lima et al. (2014)
Penicillium restrictum	Batra and Saxena (2005)
Penicillium simplicissimum	De Lima et al. (2014)
Penicillium spinulosum	De Lima et al. (2014)
Penicillium variable	Batra and Saxena (2005)
Penicillium verruculosum	De Lima et al. (2014)
Penicillium zacinthae	De Lima et al. (2014)
Rhizopus oligosporus	Vattem and Shetty (2002)
Rhizopus oryzae	Purohit et al. (2006)
Syncephalastrum racemosum	Bradoo et al. (1996)
Talaromyces subinflatus	Prigione et al. (2018)
Trichoderma hamatum	Bradoo et al. (1996)
Trichoderma harzianum	Bradoo et al. (1996)
Trichoderma viride	Bradoo et al. (1996)
Trichothecium roseum	Lekha and Lonsane (1997)

cumini (L.) Skeels) leaves, and identified as *Pestalotiopsis guepinii* can produce tannase enzymes. Mahapatra and Banerjee (2009) reported endophytic fungi *hyalopus* sp. isolated from medicinal plant *Ocimum sanctum* presented the high production of tannase enzyme. According to a study by Cavalcanti et al. (2017), endophytic

fungi from species *A. niger* and *A. fumigatus* isolated from barks of angico (*Anadenanthera colubrina*) and cajueiro (*Anacardium occidentale*) presented high production of tannase enzyme (Rana et al. 2020).

Cruz-Hernandez et al. (2005) isolated and characterized 11 fungal strains from species of (*Penicillium commune*, *Aspergillus niger*, *Aspergillus rugulosa*, *Aspergillus terricola*, *Aspergillus ornatus*, and *Aspergillus fumigatus*) isolated from soil and tannin-rich plants (damaged tissue from *Quercus* spp., *Carya illinoensis*, *Larrea tridentata* and *Pinus sembroides*) of the Mexican semidesert. These xerophilic fungi were able to produce tannase and degrade high tannin amounts in low humidity conditions. Zakipour-Molkabadi et al. (2013) reported strains of *Penicillium* sp. EZ-ZH190 from moldy tea leave samples can produce tannase enzymes.

4.3.5 Soil

Kasieczka-Burnecka et al. (2007) isolated an Antarctic filamentous fungus from the soil of King George Island (South Shetlands). This strain (identified as *Verticillium* sp.) produced two psychrophilic tannases with an optimal temperature of 20 and 25 °C, respectively. Marco et al. (2009) demonstrated that a novel extracellular tannase from the xerophilic fungus *A. niger* GHI was produced under solid-state conditions. Nalan and Merih (2009) isolates were selected for gallic acid production by tannase species were isolated from forest soil. Species producing tannase enzyme, for example, *A. niger*, *P. canescens*, *P. frequentans*, *P. spinulosum*, *P. purpurogenum*, and *P. zacinthae*. *Rhizopus oryzae* was isolated from soil samples of Indian Institute of Technology campus to show ability for tannase production (Abou-Bakr et al. 2013; Yadav 2021). Liu et al. (2016) reported amount of 35 strains of fungi species isolated from the soil of Caatinga were used for qualitative selection of strains with potential for production of tannase, the promising potential fungi *Aspergillus* spp. *UCP1284* is able to produce tannase using cashew bagasse as a substrate. In another study by Ire and Nwanguma (2020) 15 different soil samples were collected within Lagos (Oshodi), Nigeria for the potential of tannase production by *Lasiodiplodia plurivora* ACN-10 in SmF and SSF using *Terminalia cattapa* (almond leaves) and *Magnifera indica* (mango leaves) as substrates.

4.3.6 Mangroves and Caves

The mangrove ecosystem is very particular in that it harbors several tannins-rich plant species, such as *Rhizophora apiculata*, and *R. mucronata* (Georgei and Ong 2013). *Penicillium digitatum* FETLDS1, isolated from the dumping area of tannin-rich barks of *Rhizophora apiculata* in mangrove areas in Perak, Malaysia can produce extracellular tannase (Sandai et al. 2012). Georgei and Ong (2013) reported

Aspergillus niger FBT1, a local extracellular strain for tannase production, was isolated from soil collected from Mangrove Forest. Neethu and Pradeep (2018) reported *A. niger*, *A. japonicus*, and *A. aculeatus* species isolated from the soil and effluent samples were collected from different mangrove areas that have the ability to produce tannase enzymes.

Caves are an underexplored ecosystem that may reveal microorganisms of industrial and biotechnological application (Barton 2006). A study by De Melo et al. (2013) reported isolates belonging to seven different genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Epicoccum*, *Trichoderma*, and *Cladosporium*. Isolated from the soil of caves, they have the ability for tannase production. The most tannase-producing species are *Aspergillus japonicus*, *A. niger*, *A. tamarii*, *A. foetidus*, *A. tubingensis*, *A. ochraceus*, *Penicillium funiculosum*, *P. oxalicum*, *P. corylophilum*, and *P. citrinum.*

4.3.7 Marine Habitat

The deep-sea includes the deep-sea hypersaline anoxic basins (DHABs), which are depressions of the seafloor found at more than 2 km below sea level. *Aspergillus awamori*, *A. candidus*, *A. fumigatus* (Panno et al. 2013), *Penicillium notatum* (Gayen and Ghosh 2013), and several strains from *Posidonia oceanica* isolated from (DHABs). *Aspergillus awamori* BTMFW032, isolated from seawater, produced acidophilic tannase as an extracellular enzyme (Beena 2010; Verma et al. 2017). In a study by Panno et al. (2011), 88 fungal species are isolated from seagrass *Posidonia oceanica*, species identified by morphological and molecular methods, and the most important genera were *Penicillium*, *Cladosporium*, *and Acremonium*. Farag et al. (2018) reported *Aspergillus nomius* GWA5 was isolated from marine sediment to produce an active tannase for degradation of tannin.

4.3.8 Tannery or Industrial Effluents

Tannery effluent samples were used for isolating tannase-producing fungi and a promising fungus isolated from a tannery soil sample was identified as *Aspergillus niger* (Murugan et al. 2007). Manjit et al. (2008) reported a tannase yielding fungal culture identified as *A. fumigatus* MA, which was isolated from the effluent collected from a local small-scale tannery. The fungal culture produced high yields of extracellular tannase under SSF using different agroforest residues. *A. tamari* 1M138810 (B) a tannic acid degrading funguses was isolated from soil inundated by the effluent of a tannery at Oji River local (Enemuor and Odibo 2009). Hamada et al. (2013) reported tannase production from *Aspergillus niger* isolated from a tannery soil sample, with a maximum hydrolytic clear zone (53 mm ± 0). Brahmbhatt et al. (2014) reported tannase producing fungi belonging to genera *Aspergillus*,

Mucor, *Penicillium*, and *Rhizopus* species from various tea waste dump sites, agro-residue waste sites, and tannery effluents. These soil samples and tannery effluents are rich in tannins and their derivatives (Girdhari and Peshwe 2015). Twenty-nine fungal isolates were screened on the basis of their tannase-producing efficiency under stationary conditions isolated from soil samples of various tea waste dump-sites, agro-residue waste sites, and sites near local tannery industries.

The fungal isolates *Aspergillus fumigatus*, *Aspergillus carbonarius*, *Penicillium lividum*, and *Penicillium citrinum* exhibit maximum tannase activity. Shajitha and Nisha (2018) isolated a number of mycobiota like *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Rhizopus* spp., *Rhizopus stolonifer*, *Geotrichum* spp., *Penicillium* sp., *Trichoderma viride,* from tea industry waste disposal area soil for tannase production, only four fungal strains *Trichoderma viride*, *Aspergillus flavus*, *Rhizopus* spp., *and Aspergillus niger* showed the maximum hydrolyzing zone. A significantly highest hydrolyzing zone was shown by *Trichoderma viride* followed by *Aspergillus flavus*. Farhaan and Patil (2019) reported *Aspergillus* spp. and *Aspergillus flavus* respectively as tannase producers isolated from soil samples of nearby local tannery industries. A study by Al-Mraai et al. (2019) reported fungi of genus *Aspergillus* are active producers of extracellular tannase, *Aspergillus niger* isolated from soil and tea waste-producing tannase enzyme.

4.4 Production of Tannase by Fermentation

Several fermentation systems have been developed for the production of tannase from fungi using various production media. These systems can be divided into liquid surface fermentation (LSF), submerged fermentation (SmF), solid-state fermentation (SSF), and modified solid-state fermentation (MSSF). Belmares et al. (2004) mentioned in a comprehensive review that tannase production has been carried out mainly under submerged (SmF) and solid-state fermentation (SSF) techniques, depending on the strain and culture conditions. Several studies have reported interesting advantages of tannase produced by SSF over that produced by SmF. All of these studies showed that the first advantage of SSF technique is the higher enzyme titers than in SmF, when comparing the production of the same strain and fermentation broth (Aguilar et al. 2002). Microorganisms can be utilized using SSF for conversion of plant materials and agro-industrial wastes that contain tannin-rich substrates while using SSF to produce economic products such as tannase and gallic acid production, while overcoming their accumulation problems in the environment (Paranthaman et al. 2009). Filamentous fungi are suitable for the SSF process compared to other fermentation processes (Aguilar et al. 2002). The fungal species of *Aspergillus* and *Penicillium* are the most active microorganisms known, capable of producing tannase through submerged and solid-state fermentation (Abdel-Nabey et al. 2011).

The conditions for obtaining the maximal production of the enzyme depend on two factors: the system utilized and the source of the enzyme (Rodriguez-Duran

et al. 2011). Medium optimization by single dimensional search is laborious and time-consuming, especially for a large number of variables and it does not ensure desirable conditions. Plackett-Burman design is widely used in screening experiments as the number of experiments run required is very few, leading to saving of time, chemicals, and manpower (Jamal et al. 2009). The powdered plant tannin substrates (leaves, fruits, pods, bark) were usually extracted either with water or with an organic solvent like acetone (Huang et al. 2005). Tannins were documented to be serving as dual purposes as a solid support in SSF and also as a carbon source such as *Larrea tridentates*, *Syzygium cumini*, *Ziziphus mauritiana* (Kumar et al. 2007). Among the various species, *A. niger*, *A. flavus*, *and A. oryzae* were found to be the best tannase producers using tannic acid as a sole source of carbon (Hassan et al. 2018).

4.5 Tannase Applications

Tannase has received a great deal of attention from the discovery, and it is used in a wide range of applications in many industries including in detannification of food (Boadi and Neufeld 2001), preparation of food preservatives, and pharmaceuticals (Belmares et al. 2004). Actually, the main applications of tannase are elaboration of instantaneous tea (Lekha and Lonsane 1997), acorn, and gallic acid production, which is used for the synthesis of trimethoprim (Yu et al. 2004). Also, tannase is used as clarifying agent in fruit juice (Shrivastava and Kar 2009) beer, wines (Bajpai and Patil 2008), and manufacture of coffee flavored (Anwar and Imartika 2007) beverages (Aguilar et al. 2001; Belmares et al. 2004), treatment of green tea to inhibit the carcinogenic and mutagenic effects of *N*-nitrosamines, stabilization of malt polyphenols (Lekha and Lonsane 1997), improved color stability, and additional organoleptic properties. In animal feeding, tannase is used to reduce the antinutritional effects of tannins and improve animal digestibility.

4.5.1 Food Industries

4.5.1.1 Instantaneous Tea Elaboration

After water, tea is the second most highly consumed beverage worldwide (Venditti et al. 2010). It is an infusion obtained from leaves of *Camellia sinensis* and is consumed by two-thirds of the world's population (Łuczaj and Skrzydlewska 2005). Tea drinking is associated with the reduction of serum cholesterol, prevention of low-density lipoprotein oxidation, and decreased risk of cardiovascular disease and cancer (Karak and Bhagat 2010). During the production of tea beverages, hot and clear tea infusions tend to form turbid precipitates after cooling. These precipitates, called tea creams, are formed by a complex mixture of polyphenols. Tea cream

formation is a quality problem and may have antinutritional effects (Lu et al. 2009). Tannase can hydrolyze the ester bonds of catechins to release free Gallic acid and water-soluble compounds with lower molecular weight, reducing turbidity, and increasing solubility of tea beverage in cold water at 4 °C (Aguilar-Zárate et al. 2014). Precipitates are formed by the interaction of phenolic compounds and caffeine called "tea cream." Tea cream formation is a feature that was effected on the storage quality of these products (Li et al. 2017). Thus, tannase has been widely used to hydrolyze tea cream in the processing of tea (Su et al. 2009).

Enzymatic treatment of tea beverages leads to a better color appearance, less cream formation, better taste, mouth feel, and overall acceptance (Lu et al. 2009). Also, the hydrolysis of the main tea phenols epigallocatechin gallate and epicatechin gallate to epigallocatechin and epicatechin, respectively, increases the antioxidant activity of tea beverage (Lu and Chen 2008). Tannase was documented to be used in deesterification of tea polyphenols in non-converted green tea leaves, which enhances the natural levels of gallic acid and epigallochatechin and favors the formation of epitheaflavic acids, which were responsible for bright reddish color and good cold-water solubility (Aguilar and Gutierrez-Sanchez 2001). *N*-nitrosodimethylamine (NDMA) inhibition by tannase-treated green tea is reported, owing to its antioxidant potential (Lu and Chen 2007). Tea infusions contain four types of catechins such as epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Chen et al. 2014). The taste of tea gets increased with increasing concentration of catechin and the taste palatability gets reduced (Zhang et al. 2016). Tea catechins have another disadvantage in that it has poor bioavailability (Fan et al. 2016). Safety evaluation of tannase enzyme that was from *A. oryzae* was conducted and found to be safe for its usage in tea preparation (Kumar et al. 2019).

4.5.1.2 Beverage Clarification

New fruit juices (pomegranate, cranberry, raspberry, etc.) have recently been acclaimed for their health benefits, in particular, for their antioxidant properties. However, the presence of high tannin content in those fruits is responsible for haze and sediment formation, as well as for color, bitterness, and astringency of the juice upon storage (Aguilar et al. 2007). Tannase is utilized to reduce and remove the undesirable bitterness present in fruits juices by enzymatic treatment possessing the advantage of lowering the haze and increasing the quality of the beverages (Rout and Banerjee 2006). When the concentration of tannins in fruits increase, such as in blueberry, pomegranate, and raspberry, leads to the formation of sediment, color, and bitter taste during the storage of the beverages. In these cases, an enzymatic treatment with tannase is recommended (Aguilar et al. 2007). Rout and Banerjee (2006) documented a reduction of 25% of tannin content in pomegranate juice by using a treatment with tannase, while by using tannase and gelatin the tannin content diminishes by 49%. Hydrolysis by immobilized tannase removed up to 73.6% of the tannin present in Indian gooseberry (*Phyllanthus emblica*) juice. This

enzymatic treatment reduced the content of tannin but increased the gallic acid concentration with a minimum reduction in vitamin C (only 2%) (Srivastava and Kar 2009, 2010).

Tannase is used as a clarifying agent in refreshing drinks with coffee flavor, and, recently, a process for the enhancement of the antioxidant properties of coffee by tannase and other enzymes has been patented (Bel-Rhlid et al. 2009). Tannase has also been utilized in grape musts and barley worts as a prefermentative treatment, coupled with conventional fining for stabilizing wine and beer (Cantarelli et al. 1989). Tannase is employed for the elaboration of acorn wine. Its use in this process favors the production of a better beverage with an alcoholic content of 10%, reducing sugars content of 7%, and a pH of 4.0. In this process, tannase produced by an *Aspergillus* strain helps in improving the flavor of the beverage (Aguilar and Gutierrez-Sanchez 2001). Tannase is used to prevent discoloration and haze development during beer storage. It was also used to make acorn wine from Korean acorns (*Quercus* spp.) and to treat grape must and juice along with lactase to remove phenolic substances for stabilizing and increasing the quality of the wine (Lekha and Lonsane 1997).

4.5.1.3 Nutritional Improvement of Legume Flours

Legumes are of major nutritional importance, especially in developing countries. Seed legumes have high protein contents, and this protein is of good biological value. However, they also have several anti-nutritional factors, affecting the digestibility of nutrients. Different thermal and biological processes have been used to reduce the anti-nutritional factors content, increasing their nutritional value. The flours obtained from the processed legumes can be used as ingredients in food preparations. Several studies mention the uses of tannase alone or in combination with other enzymes for the degradation of some antinutritional factors (tannins) present in legume flours. Duenas et al. (2007) studied the effect of the addition of tannase and other enzymes to a lentil (*Lens culinaris*) flour. They found the production of several phenolic compounds after the treatment with tannase, the decrease of other phenolics such as catechin, epicatechin, and catechin 3-*O*-glucose, and significant increment in the antioxidant activity. On the other hand, the application of tannase on pea (*Pisum sativum*) led to a decrease in all the phenolic compounds studied and a reduction in the antioxidant capacity (Duenas et al. 2009). But, in a different experiment, the hydrolysis of pea flour by tannase led to a significant improvement in the daily weight gain of rats (Urbano et al. 2007).

4.5.2 Animal Feeds Industries

It is well known that high levels of dietary tannins have negative effects on animal nutrition. Tannins form a strong complex with enzymes, minerals, and other nutrients. They are also responsible for a bitter taste, which considerably reduces the feed intake (Belmares et al. 2004). The presence of tannins in tannin-rich plants and agro-industrial waste, which are used as animal feed, make them unusable. Tannins are known as anti-nutritional factors because of their interaction with macromolecules such as proteins makes them indigestible (Frutos et al. 2004). Anti-nutritional effect of tannin could be reduced by a treatment with tannase or tannase-producing microorganisms. For example, there are some cultivars of sorghum with high content of tannins. Tannin content could be decreased by enzymatic treatment, and this material could be used as a complement in animal diet (Aguilar and Gutierrez-Sanchez 2001). Nuero and Reyes (2002) reported the production of an enzymatic extract containing tannase from mycelial wastes of *penicillin manufacture*. This preparation was applied to several flours used as animal feed (barley, bran, maize, oat, rye, soya, and wheat flour). The enzymatic extract from mycelia waste released similar amounts of reducing sugars from all flours when compared with a commercial enzymatic additive used in animal feeding. Tannase is applied for the treatment of tannin-rich plants in the production of animal feed. If they are first treated with tannase, tannin content is decreased and this can then be used as a complement in animal diet. Tannase utilization can be carried out in two ways: direct contact of enzymatic extracts with the material to hydrolyze the polyphenols and avoid their unpleasant polymerization, or growing tannase-producing fungal strains on tannin-rich materials, which are degraded to simpler compounds (De Sena et al. 2014).

4.5.3 Gallic Acid Production

Gallic acid (3,4,5-trihydroxybenzoic acid) is a phenolic compound and the monomeric unit of the gallotannins and complex tannins. Gallic acid and related compounds possess many potential therapeutic properties including anticancer and antimicrobial properties (Ow and Stupans 2003). One of the most important applications of tannase is gallic acid production from hydrolyzable tannins (Kar et al. 2002). Gallic acid is commercially important for its applications in several industries for the synthesis of a variety of chemicals used in the food and pharmaceutical industries. Gallic acid is utilized as a precursor for the synthesis of trimethoprim (2,4, diamino 3,4,5 trimethoxy benzyl pyrimidine) is an antibacterial drug (Sittig 1988). A broad-spectrum antioxidant and antibacterial agent, which is bacteriostatic, since it inhibits folic acid metabolism in pathogenic bacteria (Mukherjee and Banerjee 2003).

In combination with sulfonamide, trimethoprim exerts antibacterial effect at low concentrations against *Streptococci* and *Staphylococci* bacteria, *Shigella* sp.,

Corynebacterium diphtheriae, *E. coli*, *Vibrio cholerae*, *Bacillus pertussis*, and *Clostridium welchii*. Choi et al. (2010) reported the potential of gallic acid as an antiviral agent. They have also cytotoxicity against cancer cells (Beniwal et al. 2013). Curiel et al. (2010) reported a process for the enzymatic production of gallic acid. They immobilized a recombinant tannase from *L. plantarum* expressed then utilized the immobilized enzyme for the hydrolysis of commercial tannic acid. At least 95% of tannic acid was transformed into gallic acid, obtaining an almost pure compound.

Tannase is an enzyme characterized for catalyzing the hydrolysis of gallic acid esters. Weetall (1985) reported the enzymatic synthesis of a variety of gallic acid esters. He applied an immobilized tannase from *Aspergillus niger* to a solution of gallic acid in different alcohols (C1–C12) and diols (C3–C6). In the chemical industry, gallic acid is used for forming pyrogallol, and gallate esters find their applications in food industries, cosmetic industries, and so on. Pyrogallol has also been used as a photographic film developer (Banerjee et al. 2007; Yu and Li 2008; Beniwal et al. 2013). It is also used in the leather industry, in manufacturing gallic acid esters, such as propyl gallate, a potent antioxidant utilized as an antioxidant in fats and oils, in the manufacture of pyrogallol, and as a photosensitive resin in semiconductor production (Sariozlu and Kivanc 2009).

4.5.4 Bioremediation of Tannin-Contaminated Wastewaters

Tannins occur commonly in the effluents derived from several agro-industries. The treatment of this kind of wastewaters is usually difficult because tannins are highly soluble and inhibit the growth of many microorganisms (He et al. 2007). Industrial tannins when used in the tanning industry can represent a serious environmental problem on a global level, although vegetable tanning agents are natural materials, they are poorly biodegradable and act as growth inhibitors toward many microorganisms, ultimately affecting the receiving ecosystem (Prigione et al. 2018). The tannase enzyme has potential uses in the treatment of tannin-containing effluents and pre-treatment of tannin-containing animal feed (Belur and Mugeraya 2011). Fungal strains capable of performing the biotransformation of polyphenolic substances contained in tannins could have a certain environmental impact as bioremediation agents. Moreover, biotransformed tannins could have several applications in agriculture, in the animal feed and wine industries, and the tanning process, for example, improving tanning yields or leather quality (Prigione et al. 2018).

Several authors have reported the biodegradation of tannin-containing wastewaters using model systems. Kachouri et al. (2005) studied the biodegradation and decolorization of olive-mill wastewater by *Aspergillus flavus*. The fungi removed 58% of color and 46% of the chemical demand of oxygen of the wastewater after six days of cultivation. More recently, Murugan and Al-Sohaibani (2010) reported the use of immobilized tannase from *Aspergillus candidus* for the removal of tannin and the associated color from tannery effluent. Enzymatic treatment removed about

42% of the tannin content and 20% of the color of tannery wastewater. These findings suggest that tannase or tannase-producing microorganisms could be utilized for pretreatment of tannin-rich wastewaters.

4.6 Conclusion

Tannin acyl hydrolase (tannase) is one of the important hydrolytic microbial enzymes. Fungi are the most producing tannase in other microorganisms with special to genus *Aspergillus*, which is considered as the best producer of tannase. Microbial tannase is more stable than others sources of tannase and can be genetically modified so that the enzyme is considered industrially important and has several applications in various industries such as foods, animal feeds, cosmetics, pharmaceutical, chemical, industries, and so on. The most important application of the tannase enzyme is producing gallic acid and also degradation of industrial tannins because it causes a serious problem in the environment.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abdel-Nabey MA, Sherief AA, El-Tanash AB (2011) Tannin biodegradation and some factors affecting tannase production by two *Aspergillus* sp. Biotechnology 10:149–158

Abo Nouh FA (2019) Endophytic fungi for sustainable agriculture. Microb Biosyst 4:31–44

Abou-Bakr HA, El-Sahn MA, El-Banna AA (2013) Screening of tannase-producing fungi isolated from tannin-rich sources. Int J Agric Food Res 2:1–12

Aguilar CN, Gutierrez-Sanchez G (2001) Review: Sources, properties, applications and potential uses of tannin acyl hydrolase. Food Sci Technol Int 7:375–382

Aguilar CN, Augur C, Favela-Torres E, Viniegra-González G (2001) Production of tannase by *Aspergillus niger* Aa-20 in submerged and solid state fermentation: influence of glucose and tannic acid. J Ind Microbiol Biotechnol 26:296–302

Aguilar CN, Favela-Torres E, Viniegra-Gonzalez G, Augur C (2002) Culture conditions dictate protease and tannase production in submerged and solid-state cultures of *Aspergillus niger* Aa-20. Appl Biochem Biotechnol 103:407–414

Aguilar CN, Rodriguez R, Gutierrez-Sanchez G, Augur C, Favela-Torres E, Prado-Barragan LA et al (2007) Microbial tannases: advances and perspectives. Appl Microbiol Biotechnol 76:47–59

Aguilar-Zárate P, Cruz-Hernández MA, Montañez JC, Belmares-Cerda RE, Aguilar CN (2014) Bacterial tannase: production, properties and applications. Rev Mex Ing Quím 13:63–67

Al-Mraai STY, Al-Fekaiki DF, Abd Al-Manhel AJ (2019) Purification and characterization of tannase from the local isolate of *Aspergillus niger*. J App Biol Biotechnol 7:029–034

Anwar YAS, Imartika H (2007) The production of tannin acyl hydrolase from *Aspergillus niger*. J Microbiol Indonesia 1:91–94

Aoki K, Shinke R, Nishira H (1976) Purification and some properties of yeast tannase. Agric Biol Chem 40:79–85

Bajpai B, Patil S (1997) Introduction of tannin acyl hydrolase (EC 3.1.1.20) activity on some members of fungi imperfecti. Enzym Microb Technol 20:612–614
Bajpai B, Patil S (2008) A new approach to microbial production of gallic acid. Braz J Microbiol 39:708–711
Banerjee D, Mondal K, Bikas R (2001) Production and characterization of extracellular and intracellular tannase from newly isolated *Aspergillus aculeatus* DBF9. J Basic Microbiol 6:313–318
Banerjee R, Mukherjee G, Patra KC (2005) Microbial transformation of tannin-rich substrate to gallic acid through co-culture method. Bioresour Technol 96:949–953
Banerjee D, Mahapatra S, Pati BR (2007) Gallic acid production by submerged fermentation of *Aspergillus aculeatus* DBF9. Res J Microbiol 2:462–468
Barthomeuf C, Regerat F, Pourrat H (1994) Production, purification and characterization of a tannase from *Aspergillus niger* LCF8. J Ferment Bioeng 77:320–323
Barton HA (2006) Introduction to cave geomicrobiology: a review for the nonspecialist. J Cave Karst Stud 68:43–54
Batra A, Saxena RK (2005) Potential tannase producers from the genera *Aspergillus* and *Penicillium*. Process Biochem 40:1553–1557
Beena PS (2010) Production, purification, genetic characterization and application studies of tannase enzyme from marine fungus *Aspergillus awamori*. PhD Thesis, Faculty of Science Cochin University
Bele AA, Jadhav VM, Kadam VJ (2010) Potential of tannins: a review. Asian J Plant Sci 9:209–214
Belmares R, Contreras-Esquivel JC, Rodriguez-Herrera R, Coronel AR, Aguilar CN (2004) Microbial production of tannase: an enzyme with potential use in food industry. Lebensm Wiss Technol 37:857–864
Bel-Rhlid R, Kraehenbuehl K, Cavin C, Raab TW, Page N (2009) Compositions for preparing a coffee beverage comprising hydrolysed chlorogenic acid, World Intellectual Property Organization Patent Application (WO09132887)
Belur PD, Mugeraya G (2011) Microbial production of tannase. Res J Microbiol 6:25–40
Belur PD, Gopal M, Nirmala KR, Basavaraj N (2010) Production of novel cell-associated tannase from newly isolated Serratia ficaria DTC. J Microbiol Biotechnol 20:732–736
Beniwal V, Kumar A, Goel G, Chhokar V (2013) A novel low molecular weight acido-thermophilic tannase from *Enterobacter cloaca*e MTCC 9125. Biocatal Agric Biotechnol 2:132–137
Bhat TK, Singh B, Sharma OP (1998) Microbial degradation of tannins – a current perspective. Biodegradation 9:343–357
Boadi DK, Neufeld RJ (2001) Encapsulation of tannase for the hydrolysis of tea tannins. Enzyme Microb Technol 28:590–595
Bradoo S, Gupta R, Saxena R (1996) Screening of extracellular tannase producing fungi: development of a rapid simple plate assay. J Gen Appl Microbiol 42:325–329
Bradoo S, Gupta R, Saxena RK (1997) Parametric optimization and biochemical regulation of extracellular tannase from *Aspergillus japonicus*. Process Biochem 32:135–139
Brahmbhatt D, Modi HA, Jain NK (2014) Preliminary isolation and screening of tannase producing bacteria and fungi. Int J Curr Microbiol App Sci 311:193–203
Cantarelli C, Brenna O, Giovanelli G, Rossi M (1989) Beverage stabilization through enzymatic removal of phenolics. Food Biotechnol 3:203–213
Cavalcanti RMF, Ornela PHDO, Jorge JA, Guimarães LHS (2017) Screening, selection and optimization of the culture conditions for tannase production by endophytic fungi isolated from Caatinga. J Appl Biol Biotechnol 5:001–009
Chaitanyakumar A, Anbalagan M (2016) Expression, purification and immobilization of tannase from *Staphylococcus lugdunensis* MTCC 3614. AMB Express 6:89
Chen GH, Yang CY, Lee SJ, Wu CC, Tzen JT (2014) Catechin content and the degree of its galloylation in oolong tea are inversely correlated with cultivation altitude. J Food Drug Anal 22:303–309
Choi HJ, Song JH, Bhatt LR, Baek SH (2010) Anti-human rhinovirus activity of gallic acid possessing antioxidant capacity. Phytother Res 24:1292–1296

Costa AM, Ribeiro WX, Kato E, Monteiro ARG, Peralta RM (2008) Production of tannase by *Aspergillus tamarii* in submerged cultures. Braz Arch Biol Technol 51:399–404

Cruz-Hernandez M, Contreras-Esquivel JC, Lara F, Rodrıguez R, Aguilar CN (2005) Isolation and evaluation of tannin-degrading fungal strains from the Mexican desert. Z Naturforsch C J Biosci 60:844–848

Curiel JA, Rodríguez H, Acebrón I, Mancheño JM, De Las RB, Rosario M (2009) Production and physicochemical properties of recombinant *Lactobacillus plantarum* tannase. J Agric Food Chem 57:6224–6230

Curiel JA, Betancor L, de las Rivas B, Munoz R, Guisan JM, Fernandez-Lorente G (2010) Hydrolysis of tannic acid catalyzed by immobilized-stabilized derivatives of tannase from *Lactobacillus plantarum*. J Agric Food Chem 58:6403–6409

De Lima JS, Cruz R, Fonseca JC, de Medeiros EV, Maciel MDHC, Moreira KA, Motta CMDS (2014) Production, characterization of tannase from *Penicillium montanense* URM 6286 under SSF using agroindustrial wastes, and application in the clarification of grape juice (*Vitis vinifera* L.). ScientificWorldJournal 2014:182025

De Melo AG, Souza PNDC, Maia NDC, Thomas AB, da Silva LBR, Batista LR et al (2013) Screening and identification of tannase-producing fungi isolated from Brazilian caves. Afr J Microbiol Res 7:483–487

De Sena AR, dos Santos ACDB, Gouveia MJ, de Mello MRF, Leite TCC, Moreira KA et al (2014) Production, characterization and application of a thermostable tannase from *Pestalotiopsis guepinii* URM 7114. Food Technol Biotechnol 52:459–467

Deschamps AM, Otuk G, Lebeault JM (1983) Production of tannase and degradation of chestnut tannin by bacteria. J Ferment Technol 61:55–59

Duenas M, Hernandez T, Estrella I (2007) Influence of the action of exogenous enzymes on the polyphenolic composition of pea: effect on the antioxidant activity. Eur Food Res Technol 225:493–500

Duenas M, Hernandez T, Estrella I (2009) Changes in the content of bioactive polyphenolic compounds of lentils by the action of exogenous enzymes. Effect on their antioxidant activity. Food Chem 101:90–97

Enemuor SCL, Odibo FJC (2009) Culture conditions for the production of a tannase of *Aspergillus tamarii* IMI388810 (B). Afr J Biotechnol 8:2554–2557

Falcão L, Araújo MEM (2018) Vegetable tannins used in the manufacture of historic leathers. Molecules 23:1–20

Fan FY, Shi M, Nie Y, Zhao Y, Ye JH, Liang YR (2016) Differential behaviors of tea catechins under thermal processing: formation of non-enzymatic oligomers. Food Chem 196:347–354

Farag AM, Hassan SW, El-Says AM, Ghanem KM (2018) Purification, characterization and application of tannase enzyme isolated from marine *Aspergillus nomius* GWA5. J Pure Appl Microbiol 12:1939–1949

Farhaan MM, Patil RC (2019) Screening and characterisation of tannase producing fungi from soil near tannery industry. Eur J Biotechnol Biosci 7:68–70

Farias GM, Gorbea C, Elkins JR, Griffin GJ (1994) Purification characterization and substrate relationships of the tannase from *Cryphonectria parasitica*. Physiol Mol Plant Pathol 44:51–63

Frutos P, Hervas G, Giráldez FJ, Mantecón AR (2004) Tannins and ruminant nutrition. Spanish J Agric Res 2:191–202

Gayen S, Ghosh U (2013) Purification and characterization of tannin acyl hydrolase produced by mixed solid-state fermentation of wheat bran and marigold flower by *Penicillium notatum* NCIM 923. Bio Med Res Int 2013:1–6

Georgei DS, Ong CB (2013) Improvement of tannase production under submerged fermentation by *Aspergillus niger* FBT1 isolated from a mangrove forest. Bio Technol 94:45–456

Girdhari SN, Peshwe SA (2015) Isolation and screening of tannase producing fungi. Int J Curr Microbiol App Sci 4:765–774

Goel G, Puniya AK, Singh K (2007) Phenotypic characterization of tannin–protein complex degrading bacteria from faeces of goat. Small Ruminant Res 69:217–220

Goel G, Kumar A, Beniwal V, Raghav M, Puniya AK, Singh K (2011) Degradation of tannic acid and purification and characterization of tannase from Enterococcus faecalis. Int Biodeterior Biodegradation 65:1061–1065

Hagerman AE (1992) Tannin-protein interactions. ACS Symp Ser 506:236–247

Hamada A, Abou-Bakr, Malak A, El-Sahn, Amr A, El-Banna (2013) Screening of tannase-producing fungi isolated from tannin-rich sources. Int J Agric Food Res 2:1–12

Haslam E (1996) Natural polyphenols (vegetable tannins) as drugs: possible modes of action. J Nat Prod 59:205–215

Hassan SWM, Ghanem KM, El-Shahat AF, El-Says AMI (2018) Production of tannase by free and immobilized marine *Aspergillus nomius* under optimized culture conditions. RJPBCS 9:30–44

He Q, Yao K, Sun D, Shi B (2007) Biodegradability of tannin-containing wastewater from leather industry. Biodegradation 18(4):465–472

Huang W, Ni J, Borthwick AGL (2005) Biosynthesis of valonia tannin hydrolase and hydrolysis of valonia tannin to ellagic acid by *Aspergillus* SHL 6. Process Biochem 40:245–1249

Ire SF, Nwanguma AC (2020) Comparative evaluation on tannase production by *Lasiodiplodia plurivora* ACN-10 under submerged fermentation (SmF) and solid state fermentation (SSF). Asian J Biotechnol and Bioresource Technol 6:39–49. Article no. AJB2T.55864

Iwamoto K, Tsuruta H, Nishitaini Y, Osawa R (2008) Identification and cloning of a gene encoding tannase (tannin acylhydrolase) from *Lactobacillus plantarum* ATCC 14917T. Syst Appl Microbiol 31:269–277

Jamal P, Tompang MF, Alam MZ (2009) Optimization of media composition for the production of bioprotein from pineapple skins by liquid-state bioconversion. J Appl Sci 9:3104–3109

Jana A, Halder SK, Banerjee A, Paul T, Pati BR, Mondal KC, Mohapatra PKD (2014) Biosynthesis, structural architecture and biotechnological potential of bacterial tannase: a molecular advancement. Bioresour Technol 157:327–340

Jiménez N, Barcenilla JM, de Felipe FL, de las Rivas B, Muñoz R (2014) Characterization of a bacterial tannase from *Streptococcus gallolyticus* UCN34 suitable for tannin biodegradation. Appl Microbiol Biotechnol 98:6329–6337

Jun C, Yoo M, Lee W, Kwak K, Bae M, Hwang W, Son D, Chai K (2007) Ester derivatives from tannase-treated prunioside and their anti-inflammatory activities. Bull Kor Chem Soc 28:73–76

Kachouri S, Halaouli S, Lomascolo A, Asther M, Hamdi M (2005) Decolourization of black oxidized olive-mill wastewater by a new tannase-producing *Aspergillus flavus* strain isolated from soil. World J Microbiol Biotechnol 21:1465–1470

Kar B, Banerjee R, Bhattacharyya BC (2002) Optimization of physicochemical parameters of gallic acid production by evolutionary operation-factorial design technique. Process Biochem 37:1395–1140

Karak T, Bhagat RM (2010) Trace elements in tea leaves, made tea and tea infusion: a review. Food Res Int 43:2234–2252

Kasieczka-Burnecka M, Kuc, Kalinowska KH, Knap M, Turkiewicz M (2007) Purification and characterization of two cold-adapted extracellular tannin acyl hydrolases from an Antarctic strain *Verticillium* sp. P9. Appl Microbiol Biotechnol 77:77–89

Khanbabaee K, van Ree T (2001) Tannins: classification and definition. Nat Prod Rep 18:641–649

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kumar RA, Gunasekaran P, Lakshmanan M (1999) Biodegradation of tannic acid by *Citrobacter freundii* isolated from a tannery effluent. J Basic Microbiol Int J Biochem Physiol Genet Morphol Ecol Microorg 39(3):161–168

Kumar R, Sharma J, Singh R (2007) Production of tannase from *Aspergillus* ruber under solid-state fermentation using jamun (Syzygium cumini) leaves. Microbiol Res 162:384–390

Kumar SS, Sreekumar R, Sabu A (2019) Tannase and its applications in food processing. In: Parameswaran B, Varjani S, Raveendran S (eds) Green bio-processes. Energy, environment, and sustainability. Springer, Singapore

Kuppusamy M, Thangavelu V, Kumar A, Chockalingam (2015) Kinetics and modeling of tannase production using *Aspergillus foetidus* in batch fermentation. Int J Pharm Pharm Sci 7:64–67

Lal D, Divya Shrivastava D, Verma HN, Gardne JJ (2012) Production of tannin acyl hydrolase (E.C. 3.1.1.20) from *Aspergillus niger* isolated from bark of Acacia nilotica. J Microbiol Biotechnol Res 4:566–572

Lekha PK, Lonsane BK (1997) Production and application of tannin acyl hydrolase: state of the art. Adv Appl Microbiol 44:215–260

Lenin B, Lokeswari N, Reddy D (2015) Separation and optimization of phenolic component from Anacardium occidentales testa by solvent extraction method. World J Pharm Res 4:870–874

Lewis JA, Starkey RL (1969) Decomposition of plant tannins by some soil microorganisms. Soil Sci 107(4):235–241

Li J, Xiao Q, Huang Y, Ni H, Wu C, Xiao A (2017) Tannase application in secondary enzymatic processing of inferior Tieguanyin oolong tea. Electron J Biotechnol 28:87–94

Liu TPSL, Porto TS, Moreira KA, Takaki GMC, Brandão-Costa R, Herculano PN et al (2016) Tannase production by *Aspergillus* spp. UCP1284 using cashew bagasse under solid state fermentation. Afr J Microbiol Res 10(16):565–571

Lu MJ, Chen C (2007) Enzymatic tannase treatment of green tea increases in vitro inhibitory activity against N-nitrosation of dimethylamine. Process Biochem 42:1285–1290

Lu MJ, Chen C (2008) Enzymatic modification by tannase increases the antioxidant activity of green tea. Food Res Int 41:130–137

Lu MJ, Chu SC, Yan L, Chen C (2009) Effect of tannase treatment on protein-tannin aggregation and sensory attributes of green tea infusion. LWT—Food Sci Technol 42:338–342

Łuczaj W, Skrzydlewska E (2005) Antioxidative properties of black tea. Prev Med 40(6):910–918

Madhavakrishna W, Bose S, Nayudamma Y (1960) Estimation of tannase and certain oxidizing enzymes in Indian vegetable tanstuffs. Bull Cent Leath Res Inst 7(1):11

Mahapatra S, Banerjee D (2009) Extracellular tannase production by endophytic *hyalopus sp*. J Appl Microbiol 55:255–259

Mahendran B, Raman N, Kim D (2005) Purification and characterization of tannase from *Paecilomyces variotii*: hydrolysis of tannic acid using immobilized tannase. Appl Microbiol Biotechnol 70:445–451

Manjit YA, Aggarwal NK, Kumar K, Kumar A (2008) Tannase production by *Aspergillus fumigatus* MA under solid-state fermentation. World J Microbiol Biotechnol 24:3023–3030

Marco MG, Luis VR, Erika LR, Jacqueline R, Mario ACH, Raúl R, Juan C, Cristóbal NA (2009) Anovel tannase from the xerophilic fungus *Aspergillus niger* GH1. J Microbiol Biotechnol 1:1–10

Mondal KC, Pati BR (2000) Studies on the extracellular tannase from newly isolated *Bacillus licheniformis* KBR 6. J Basic Microbiol 40:223–232

Mondal KC, Samanta S, Giri S, Pati BR (2001) Distribution of tannic acid degrading microorganisms in the soil and comparative study of tannase from two fungal strains. Acta Microbiol Pol 50:75–82

Mosleh H, Naghiha A, Keshtkaran AN, Khajavi M (2014) Isolation and identification of tannin-degrading bacteria from native sheep and goat feces in Kohgiloye and Boyer-Ahmad Province. Int J Adv Biol Biomed Res 2:176–180

Mukherjee G, Banerjee R (2003) Production of gallic acid. Biotechnological routes (Part 1). Chim Oggi 21:59–62

Mukherjee G, Banerjee R (2006) Effects of temperature, pH and additives on the activity of tannase produced by a co-culture of *Rhizopus oryzae* and *Aspergillus foetidus*. World J Microbiol Biotechnol 22:207–212

Murugan K, Al-Sohaibani SA (2010) Biocompatible removal of tannin and associated color from tannery effluent using the biomass and tannin acyl hydrolase (E.C.3.1.1.20) enzymes of mango industry solid waste isolate *Aspergillus candidus* MTTC 9628. Res J Microbiol 5:262–271

Murugan K, Saravanababu S, Arunachalam M (2007) Screening of tannin acyl hydrolase (E.C.3.1.1.20) producing tannery effluent fungal isolates using simple agar plate and SmF process. Bioresour Technol 98:946–949

Nalan YS, Merih K (2009) Isolation of gallic acid producing microorganisms and their use in the production of gallic acid from gall nuts and sumac. Afr J Biotechnol 8:1110–1115, 20

Neethu RS, Pradeep S (2018) Isolation and characterization of potential tannase producing fungi from mangroves and tanneries. Indian J Appl Microbiol 21:1–13

Nishitani Y, Sasaki E, Fujisawa T, Osawa R (2004) Genotypic analyses of lactobacilli with a range of tannase activities isolated from human feces and fermented foods. Syst Appl Microbiol 27:109–117

Nuero OM, Reyes F (2002) Enzymes for animal feeding from *Penicillium chrysogenum* mycelial wastes from penicillin manufacture. Lett Appl Microbiol 34(6):413–416

Okuda T, Ito H (2011) Tannins of constant structure in medicinal and food plants-hydrolyzable tannins and polyphenols related to tannins. Molecules 16(3):2191–2217

Ordonez RM, Colombo I, Alberto MR, Isla MI (2011) Production of tannase from wood-degrading fungus using as substrate plant residues: purification and characterization. World J Microbiol Biotechnol 27:2325–2333

Ow YY, Stupans I (2003) Gallic acid and gallic acid derivatives: effects on drug metabolizing enzymes. Curr Drug Metab 4:241–248

Panno L, Voyron S, Anastasi A, MussatSartor R, Varese GC (2011) Biodiversity of marine fungi associated with the seagrass *Posidonia oceanica*: an ecological and biotechnological perspective. Biol Mar Mediterr 18:85–88

Panno L, Bruno M, Voyron S, Anastasi A, Gnavi G, Miserere L et al (2013) Diversity, ecological role and potential biotechnological applications of marine fungi associated to the seagrass *Posidonia oceanica*. New Biotechnol 30:685–694

Paranthaman R, Vidyalakshmi R, Murugesh S, Singaravadivel K (2009) Effects of fungal coculture for the biosynthesis of tannase and gallic acid from grape wastes under solid state fermentation. Glob J Biotechnol Biochem 4:29–36

Pepi M, Lampariello LR, Altieri R, Esposito A, Perra G, Renzi M et al (2010) Tannic acid degradation by bacterial strains Serratia spp. and Pantoea sp. isolated from olive mill waste mixtures. Int Biodeterior Biodegradation 64(1):73–80

Prigione V, Spina F, Tigini V, Giovando S, Varese GC (2018) Biotransformation of industrial tannins by filamentous fungi. Appl Microbiol Biotechnol 102:10361–10375

Purohit JS, Dutta JR, Nanda RK, Banerjee R (2006) Strain improvement for tannase production from co-culture of *Aspergillus foetidus* and *Rhizopus oryzae*. Bioresour Technol 97:795–801

Raghuwanshi S, Dutt K, Gupta P, Misra S, Saxena RK (2011) Bacillus sphaericus: the highest bacterial tannase producer with potential for gallic acid synthesis. J Biosci Bioeng 111(6):635–640

Rana N, Bhat T (2005) Effect of fermentation system on the production and properties of tannase of *Aspergillus niger* van Tieghem MTCC 2425. J Gen Appl Microbiol 51:203–212

Rana G, Katerji N, Introna M, Hammami A (2004) Microclimate and plant water relationship of the "overhead" table grape vineyard managed with three different covering techniques. Sci Hortic 102:105–120

Rana KL, Kour D, Kaur T, Devi R, Yadav AN, Yadav N et al (2020) Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. Antonie Van Leeuwenhoek 113:1075–1107. https://doi.org/10.1007/s10482-020-01429-y

Rastegari AA, Yadav AN, Yadav N (2020) New and future developments in microbial biotechnology and bioengineering: trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam

Reges de Sena A, Santos ACB, Gouveia MJ, Mello MRFM, Leite TCC et al (2014) Production of tannase by *Pestalotiopsis guepinii*. Food Technol Biotechnol 52(4):459–467

Rodríguez H, de las Rivas B, Gómez-Cordovés C, Muñoz R (2008) Characterization of tannase activity in cell-free extracts of *Lactobacillus plantarum* CECT 748T. Int J Food Microbiol 121:92–98

Rodriguez-Duran VL, Valdivia-Urdiales B, Contreras-Esquivel JC, Rodriguez-Herrera R, Aguilar CN (2011) Novel strategies for upstream and downstream processing of tannin acyl hydrolase. Enzyme Res 2011:1–20

Rout S, Banerjee R (2006) Production of tannase under MSSF and its application in fruit juice debittering. Indian J Biotechnol 5:346–350

Sabu A, Pandey A, Daud MJ, Szakacs G (2005) Tamarind seed powder and palm kernel cake: two novel agro residues for the production of tannase under solid state fermentation by *Aspergillus niger* ATCC 16620. Bioresour Technol 96:1223–1228

Sabu A, Augur C, Swati C, Pandey A (2006) Tannase production by *Lactobacillus* sp. ASR-S1 under solid-state fermentation. Process Biochem 41(3):575–580

Sandai D, Ibrahim D, Kassim J (2012) Calcium alginate entrapped cells of *Penicillium digitatum* FETL DS1 for the improvement of tannase production. BTAIJ 6:27–34

Sariozlu NY, Kivanc M (2009) Isolation of gallic acid producing microorganisms and their use in the production of gallic acid from gall nuts and sumac. Afr J Biotechnol 8:1110–1115

Saxena S, Saxena RK (2004) Statistical optimization of tannase production from *Penicillium variable* using fruits (*chebulic myrobalan*) of Terminalia chebula. Biotechnol Appl Biochem 39:99–106

Selwal MK, Yadav A, Selwal KK, Aggarwal NK, Gupta R, Gautam SK (2010) Optimization of cultural conditions for tannase production by *Pseudomonas aeruginosa* IIIB 8914 under submerged fermentation. World J Microbiol Biotechnol 26:599–605

Serrano J, Puupponen-Pimiä R, Dauer A, Aura AM, Saura-Calixto F (2009) Tannins: current knowledge of food sources, intake, bioavailability and biological effects. Mol Nutr Food Res 53:S310–S329

Shajitha G, Nisha MK (2018) Tannase production from agro-wastes as substrate by *Trichoderma viride*. Int J Curr Res Life Sci 07(05):1994–1997

Sharma KP, John PJ (2011) Purification and characterization of tannase and tannase gene from *Enterobacter* sp. Process Biochem 46(1):240–244

Sharma S, Bhat TK, Dawra RK (2000) A spectrophotometric method for assay of tannase using rhodamine. Anal Biochem 279:85–89

Shrivastava A, Kar K (2009) Characterization and application of tannase produced by *Aspergillus niger* ITCC 6514.07 on pomegranate rind. Brazil J Microbiol 40:782–789

Sittig M (1988) Trimethoprim. In: Sittig M (ed) Pharmaceutical manufacturing encylopedia. Noyes Publication, New Jersey, pp 282–284

Skene IK, Brooker JD (1995) Characterization of tannin acylhydrolase activity in the ruminal bacterium *Selenomonas ruminantium*. Anaerobe 1:321–327

Srivastava A, Kar R (2009) Characterization and application of tannase produced by *Aspergillus niger* ITCC 6514.07 on pomegranate rind. Braz J Microbiol 40:782–789

Srivastava A, Kar R (2010) Application of immobilized tannase from *Aspergillus niger* for the removal of tannin from myrobalan juice. Indian J Microbiol 50:46–51

Su E, Xia T, Gao L, Dai Q, Zhang Z (2009) Immobilization and characterization of tannase and its haze-removing. Food Sci Technol Int 15:545–552

Tieghem P (1867) Sur la fermentation gallique. CR Acad Sci Paris 65:1091–1094

Urbano G, Lopez-Jurado M, Porres JM (2007) Effect of treatment with α-galactosidase, tannase or a cell-wall degrading enzyme complex on the nutritive utilisation of protein and carbohydrates from pea (*Pisum sativum* L.) flour. J Sci Food Agric 87:1356–1363

Van de Lagemaat J, Pyle DL (2005) Modelling the uptake and growth kinetics of *Penicillium* glabrum in a tannic acid-containing solid state fermentation for tannase production. Process Biochem 40:1773–1782

Vattem DA, Shetty K (2002) Solid-state production of phenolic antioxidants from cranberry pomace by *Rhizopus oligosporum*. Food Biotechnol 16:189–210

Vattem DA, Shetty K (2003) Ellagic acid production and phenolic antioxidants activity in cranberry pomace (*Vaccinium macrocarpo*) mediated by Lentinus edodes using a solid-state system. Process Biochem 39:367–379

Venditti E, Bacchetti T, Tiano L, Carloni P, Greci L, Damiani E (2010) Hot vs. cold water steeping of different teas: do they affect antioxidant activity? Food Chem 119(4):1597–1604

Verma P, Yadav AN, Kumar V, Singh DP, Saxena AK (2017) Beneficial plant-microbes interactions: biodiversity of microbes from diverse extreme environments and its impact for crop improvement. In: Singh DP, Singh HB, Prabha R (eds) Plant-microbe interactions in agro-ecological perspectives, Microbial interactions and agro-ecological impacts, vol 2. Springer, Singapore, pp 543–580. https://doi.org/10.1007/978-981-10-6593-4_22

Vivas N, Laguerrre M, Pianet de Boissel I, Vivas de Gaulejac N, Nonier MF (2004) Conformational interpretation of vascalagin and castalagin physicochemical properties. J Agric Food Chem 52:2073–2078

Weetall H (1985) Enzymatic synthesis of gallic acid esters. Appl Biochem Biotechnol 11(1):25–28

Yadav AN (2021) Soil microbiomes for sustainable agriculture, Functional annotation, vol 2. Springer, Cham

Yadav AN, Kour D, Rana KL, Yadav N, Singh B, Chauhan VS et al (2019) Metabolic engineering to synthetic biology of secondary metabolites production. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 279–320. https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Yu XW, Li YQ (2008) Expression of *Aspergillus oryzae* tannase in *Pichia pastoris* and its application in the synthesis of propyl gallate in organic solvent. Food Technol nd Biotechnol 46:80–85

Yu X, Li Y, Wang C, Wu D (2004) Immobilization of *Aspergillus niger* tannase by microencapsulation and its kinetic characteristics. Biotechnol Appl Biochem 40:151–155

Zakipour-Molkabadi E, Hamidi-Esfahani Z, Sahari MA, Azizi MH (2013) A new native source of *Tannase* producer, *Penicillium* sp. EZ-ZH190: characterization of the enzyme. Iran J Biotechnol 11(4):244–250

Zhang YN, Yin JF, Chen JX, Wang F, Du QZ, Jiang YW, Xu YQ (2016) Improving the sweet aftertaste of green tea infusion with tannase. Food Chem 192:470–476

Chapter 5
Recent Advances in Fungal Antimicrobial Molecules

Fatma Ahmed Abo Nouh, Sara Atef Gezaf, and Ahmed M. Abdel-Azeem

Contents

F. A. Abo Nouh (✉) · A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt

S. A. Gezaf
Botany Department, Faculty of Science, Arish University, North Sinai, Egypt

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_5

5.1 Introduction

Fungal diversity present on earth ranges from the conservative 1.5 million species to the newer estimates of 5.1 million based on high-throughput sequencing methods, and researchers estimate there are at least 1 million endophytic fungal species (Strobel and Daisy 2003). The unique ecological niche of endophytic fungi, that is, residing or living within healthy plant tissue without apparent disease symptoms increasing interest in it (Rakshith et al. 2013). Studies have shown that nearly 300,000 plant species exist on the earth, and each individual plant is the host to one or more endophytes (Huang et al. 2007). Endophytic fungi are predominantly ascomycetes that appear to be ubiquitous in nature as they have been recovered from plants adapted to a wide range of ecosystems that include hot deserts, Arctic tundra, mangroves, temperate and tropical forests, grasslands and savannahs, and croplands (Lugtenberg et al. 2016). Since the discovery of penicillin from the fungus *Penicillium notatum* it makes more interest in the discovery and application of microbial metabolites from fungi with activity against both plant and human pathogens (Strobel and Daisy 2003), and since the discovery of Taxol from endophytic fungi *Taxomyces adreanae* there are increasing in searching for endophytic fungi associated with promising bioactive molecules and their derivatives (Nicoletti and Fiorentino 2015).

Natural products from microbial origin have been a most significant source of novel lead molecules and recently several endophytes have been shown to possess the potentials to synthesize novel bioactive compounds (Akpotu et al. 2017). Due to the extraordinary biodiversity of fungal endophytes, they provide a large opportunity to discover novel natural products that have been optimized by coevolution with higher plants (Aly et al. 2011). There are a large number of bioactive compounds that have been isolated from endophytic fungi and these bioactive natural products have demonstrated a broad range of biological activities. Endophytic fungi derived natural products showed several interesting biological activities, for example, antioxidant, anticancer, immune modulatory, antiparasitic, antimicrobial, and insecticidal activities (Kaul et al. 2012). About 80% of endophytic fungi produce bioactive compounds with antimicrobial and herbicidal properties (Schulz et al. 2002).

The recent development of screening technologies has revealed the great potential of fungal endophytes for producing novel biologically active compounds with promising medicinal or agricultural applications (Hussain et al. 2014). Fungi are the most major source of new, useful metabolites. The largest part of these metabolites are produced by species of *Acremonium* sp., *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. (Rodrígues et al. 2000); *Chaetomium* sp. (Selim et al. 2016) approximately 8,500 bioactive metabolites of fungal origin have been described to possess biological activities (Arora and Ramawat 2017). So over the last few years, there has been increasing interest in the investigation of fungal endophytes producing biologically active products (Rodrígues et al. 2000); especially antimicrobial

substances (Schulz et al. 2002; Arivudainambi et al. 2011; Rakshith et al. 2013; Hussain et al. 2014; Lugtenberg et al. 2016; Akpotu et al. 2017).

5.2 Fungi Producing Bioactive Metabolites

Antimicrobial metabolites produced by endophytes are low molecular weight not essential for growth and are produced at low concentrations against pathogenic invasion (Kumar and Mongolla 2018). Thus, endophytes are a promising resource of antimicrobial compounds to counteract the serious threat from human drug-resistant and plant pathogens (Tan and Zou 2001). The bioactive natural products with antimicrobial activity provided by endophytic fungi originate from different biosynthetic pathways and belong to diverse structural groups: Alkaloids, Flavonoids, Quinone, Phenols Steroids, Terpenoids (Guo et al. 2000a); Coumarins, Glycosides, peptide (Selim et al. 2016); and Volatile organic compounds (Arora and Ramawat 2017).

5.2.1 Alkaloids

Alkaloids are quite common secondary metabolites in endophytes, and some of them show antimicrobial activities (Yu et al. 2010). Since a long time, the plants were the sole source of alkaloids in this section and some alkaloids were isolated from endophytic fungi (Selim et al. 2016). Azaphilone dimer alkaloids were first isolated from the fungus *Chaetomium globosum* (Ming et al. 2008). Also, endophytic fungus *C. fusiforme* was isolated from liverwort *Scapania verrucosa Heeg* producing Novel azaphilone alkaloid dimers namely chaetofusins A and B that have antifungal activities (Peng et al. 2012). Another three related new chlorinated azaphilone alkaloids were reported from the culture broth of *C. globosum* named chaetomugilides A–C (Li et al. 2013). Chaetoglobosins A and C were produced by an endophytic *C. globosum* originating from the leaves of *Ginkgo biloba* (Yu et al. 2010). Fungal endophyte *Fusarium* sp. Produce Fusapyridons A and B alkaloids and Fusapyridons A displayed antibacterial activity (Tsuchinari et al. 2007). Two new alkaloids, Mycoleptodiscins A and B were identified from endophytic fungus *Mycoleptodiscus* sp. isolated from *Desmotes incomparabilis* (Selim et al. 2016). *Penicillium* sp. an endophytic fungus isolated from the stem of *Quercus variabilis* (Fagaceae) producing Penicidones A–C alkaloids (Ge et al. 2008). A new pyrrolizidine alkaloid, namely Penibruguieramine A, produced by endophytic fungus *Penicillium* sp. *GD6* isolated from Chinese mangrove *Bruguiera gymnorrhiza* (Zhou et al. 2014).

Peramine, a pyrrolopyrazine alkaloid, is produced by *Neotyphodium coenophialum*, *N. lolli*, *Epichloë festucae*, and *E. typhina* in culture as well as *in planta* when associated with stem, and leaves of tall fescue, ryegrass, and other grasses (Dew

et al. 1990). The culture of *Neotyphodium* secretes indole alkaloids such as agroclavine, chanoclavine, and elymoclavine (Powell and Petroski 1992). *Rhinocladiella* sp., a fungal endophyte of perennial vine *Tripterygium wilfordii*, produces cytochalasin E (Wagenaar et al. 2000). A novel metabolite phomopsichalasin is produced by an endophyte *Phomopsis* sp., recovered from twigs of *Salix gracilistyla* var. *melanostachys*, this metabolite possesses antimicrobial activity against various bacterial and fungal species and human pathogenic yeast (Horn et al. 1996). *Phomopsis* sp., isolated from the bark of the living *Cavendishia pubescens* tree produce indole derivatives such as tremorgenic aspalitrems A and C (Anamika et al. 2018). Endophytic fungi *Aureobasidium pullulans*, *Acremonium coenophialum*, *Colletotrichum* sp. and *Epicoccum purpurascens* present in *Artemisiaannua* produce indole-3-acetic acid. And also *Hypoxylon serpens* strains, isolated from tobacco, produce indole-3-acetonitrile (Anamika et al. 2018).

5.2.2 Coumarins

Coumarins are bicyclic aromatic compounds that contain 2*H*-chromen-2-one or any of its derivatives while isocoumarins are isomers of coumarins in which the orientation of the lactone is reversed to be 1*H*-isochromen-1-one. Dihydroisocoumarin is a reduced form of isocoumarin where the double bond between carbon atoms no. 3 and 4 is saturated (Selim et al. 2016). The ethyl acetate extract of an endophytic fungus *Periconia atropurpurea* collected from *Xylopia aromatica* named as 6,8-Dimethoxy-3-(2′-oxo-propyl)-coumarin (Teles et al. 2006). A novel coumarin series, pestalasins A–E was produced by the endophytic fungus *Pestalotiopsis* sp., associated with the leaves of the Chinese mangrove *Rhizophora mucronata* (Xu et al. 2009). another new coumarin, 4,6-dihydroxy-7-formyl-3 methylcoumarin was isolated from the endophyte *Pestalotiopsis versicolor* (Yang et al. 2015). *Xylaria* sp. YX-28 fungal endophytic isolated from *Ginkgo biloba* produced 7-amino-4-methylcoumarin that showed antimicrobial activity against various pathogens (Liu et al. 2008).

A number of isocoumarins and isocoumarin derivatives had been isolated from endophytic fungi *Penicillium* sp., associated with mangrove plants known as (3*R*,4*S*)-6,8-dihydroxy-3,4,7-trimethylisocoumarin (Han et al. 2009). Several isocoumarin derivatives produced by endophyte-colonized plants exhibit biocontrol potential. *Pezicula* sp. produces mellein that is strongly algicidal, fungicidal and herbicidal (Schulz et al. 1995).

The dihydroisocoumarin (3*R*,4*R*)-3,4-dihydro-4,6-dihydroxy-3-methyl-1--oxo-1*H*-isochromene-5-carboxylic acid was isolated from *Xylaria* sp., a fungus associated with *Piper aduncum* (Piperaceae) that have antifungal activities in vitro (Oliveira et al. 2011). Tenuissimasatin is a new dihydroisocoumarin produced by the endophytic fungus *Alternaria tenuissima* isolated from the bark of *Erythrophleum fordii* Oliver (Fang et al. 2012).

5.2.3 Glycosides

Various types of glycosides have been identified from different genera of endophytic fungi. A macrolacton glycoside was produced by the endophytic *Lecythophora* sp. derived from the Indonesian plant *Alyxia reinwardtii*. It was designated 23-methyl-3-(1-*O*-mannosyl)-oxacyclotetracosan-1-one and it exhibited antifungal activities (Sugijanto et al. 2011). Endophytic fungi *Eurotium rubrum* isolated from the stems of marine mangrove plant *Hibiscus tiliaceus* produce a novel anthraquinone glycoside designated 3-*O*-(α Dribofuranosyl)-questinol (Li et al. 2009). Another endophytic *Eurotium cristatum* isolated from the marine algae *Sargassum thunbergii* produce a new anthraquinone glycoside, 3-*O*-(α-Dribofuranosyl) questinol (Du et al. 2014). As an emphasis on the importance of marine mangrove plants, one *Penicillium* sp. was isolated from the plant *Avicennia marina* that produced a novel aurone glycoside. Its structure is identified as (*Z*)-7,4′-dimethoxy-6-hydroxyaurone-4-*O*-β-glucopyranoside (Song et al. 2015). The endophytic fungus *Phomopsis* sp. (ZH76) isolated from the stem of the mangrove tree *Excoecaria agallocha* found in Sea cost at south chine produced a xanthone *O*-glycoside for the first time namely 3-*O*-(6-*O*-α-Larabinopyranosyl)-β-D-glucopyranosyl-1,4-dimethoxyxanthone (Huang et al. 2013).

5.2.4 Flavonoids

Flavonoids constitute the largest group of polyphenols and are considered to be responsible for the color and taste of many fruits and vegetables. Recently, many reviews concerning the antifungal, antibacterial, or antiviral activities of flavonoids have been published (Zheng et al. 2012). An endophytic fungus strain PM0651480 isolated from leaves of *Mimusops elengi* (Sapotaceae family) produced ergoflavin (Deshmukh et al. 2009). Blue grass (*Poa ampla*) infected with an endophyte produces tricin a flavone compound (Ju et al. 1998). 5-Hydroxy-3,7,40-trimethoxyflavone produced by *Biscogniauxia formosana* isolated from *Cinnamomum* sp./bark (Cheng et al. 2012). Endophytic *Eurotium amstelodami* isolated from *Ipomea pes-carprae* L. produced tetrahydroauroglaucin and flavoglaucin which showed antimicrobial activity (Chaipackdee et al. 2013). Kaemferol, a Flavonol compound produced by *Fusarium chlamydosporum* isolated by *Tylophora indica* (Chaturvedi et al. 2014). Endophytic *Fusarium* sp. strain ZZF41, isolated from mangrove tree *Kandelia candel*, produced 5-*O*-methyl-2′-methoxy-3′-methylalpinumisoflavone (Kumar and Mongolla 2018).

5.2.5 Quinones

Altersolanol A, a highly hydroxylated quinone, exhibiting antibacterial properties is secreted by *Alternaria* spp. and *Phoma* sp. when in association with plants (Yang et al. 1994). Tansuwan et al. (2007) identified two novel benzoquinone metabolites: 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione and xylariaquinone A, from endophytic *Xylaria* sp. PBR-30 isolated from Thai medicinal plants. An isolate of an endophytic fungus from the marine brown algae defined as *Aspergillus niger* EN-13 produced a novel antifungal naphthoquinoneimine derivative named 5,7-dihydroxy-2-(1-(4-methoxy-6-oxo-6*H*-pyran-2-yl)-2-phenylethylamino) (1,4) naphthoquinone (Zhang et al. 2007). Cao and Clardy (2011) reported that endophytic fungus *Delitzchia winteri*, isolated in Costa Rica, afforded two new naphthoquinones, delitzchianones A and B. The endophytic fungus *Penicillium restrictum* was isolated from the stems of a milk thistle plant, produces distinct red-colored guttates, identified as new polyhydroxyanthraquinones (Figueroa et al. 2014). The bioactive compounds naphtha-quinones, anhydro-fusarubin, and methyl ether of fusarubin from *Cladosporium* sp. Isolated from *Rauwolfia serpentina* (L.) Benth. showed higher antimicrobial activity (Khan et al. 2016).

5.2.6 Peptides

Many peptides produced by endophytes displayed significant antimicrobial activities (Yu et al. 2010). Leucinostatin A, an oligopeptide with phytotoxic and antifungal properties, originally produced by *Acremonium* sp., a fungal endophyte of *Taxus baccata* (Strobel et al. 1997), and also detected from *Penicillium lilacinum* (Anamika et al. 2018). *Xylaria* sp. as a seed-endophyte of an angiosperm tree found in the South China Sea produced a new cyclic peptide with an allenic ether linkage of an *N*-(*p*-hydroxycinnamoyl) amide (Lin et al. 2001). Epichlicin, a new cyclic peptide, was produced by the endophytic fungus *Epichloe typhina* isolated from *Phleum pratense* L. (Seto et al. 2007). Two new cyclic peptides were identified from the broth of mangrove endophytic fungus isolated from the leaves of *Kandelia candel* growing in Hong Kong (Huang et al. 2007). Cycloaspeptide A is a cyclic peptide isolated from the extracts of the fermentation broth of *Penicillium janczewskii* isolated from the phloem of the *Chileangymnosperm Prumnopitys andina* (Schmeda-Hirschmann et al. 2008). Cyclopeptides echinocandins A, B, D and H are produced by *Aspergillus rugulosus*, *A. nidulans* var. *echinulatus*, *Cryptosporiopsis* sp. and *Pezicula* sp. endophytic on *Pinus sylvestris* and *Fagus sylvatica* (Anamika et al. 2018).

5.2.7 Phenols

Phenol and phenolic acids have often been isolated from some endophytes cultures originating from a variety of host plants. Two new antibiotics, pestalachloride A and B have been isolated from endophytic *Pestalotiopsis adusta*, which displayed significant antifungal activity against some plant pathogens (Li et al. 2008). *Phoma* sp. produces a phenolic compound named 2-hydroxy-6-methylbenzoic acid, a compound with antibacterial activity (Yang et al. 1994). *Pezicula* sp. strain 553, a tree endophyte, produces a phenolic compound named 2-methoxy-4-hydroxy-6--methoxymethyl benzaldehyde, which exhibited antifungal properties against phytopathogens (Schulz et al. 1995). Tyrosol, *p*-hydroxyphenylacetic acid, *p*-hydroxybenzoic acid and *cis* and *trans-p*-coumaric acids are antifungal phenolic acids isolated from stromata of *E. typhina*, an endophyte of *Pezicula pratense* (Anamika et al. 2018). Colletotric acid, an antimicrobial tridepside, is secreted by *Colletotrichum gloeosporioides*, a fungal endophyte of *Artemisia mongolica* (Zou et al. 2000). *Cytonaema* sp., an endophyte recovered from *Quercus* sp., secretes cytonic acids A and B (Guo et al. 2000b).

5.2.8 Steroids

Aspergillus sp. is extensively found to be associated with marine algae. In addition, many steroids have been isolated from different *Aspergillus* sp., 7-Nor-ergosterolide was characterized from *Aspergillus ochraceus* EN-31 an endophytic fungus isolated from the marine brown alga *Sargassum kjellmanianum* (Selim et al. 2016). Another steroid, Aspory-ergosterol was identified from the broth extract of marine red algae *Heterosiphonia japonica* derived *Aspergillus oryzae*. (Qiao et al. 2010). *Aspergillus flavus* isolated from marine red alga *Corallina officinalis* produced a new steroid, namely 3β,4α-dihydroxy-26-methoxyergosta-7,24-dien-6-one (Qiao et al. 2011). Fungus *Colletotrichum* sp., an endophyte of *A. annua*, secretes steroids such as ergosterol, which were antagonistic to crop pathogens (Lu et al. 2000). Culture broth of *Phomopsis* sp. isolated from *Aconitum carmichaeli* contained two novel steroids, namely (14β,22E)-9,14-dihydroxyergosta-4,7,22-triene-3,6-dione and (5α, 6β, 15β, 22E)-6-ethoxy-5,15-dihydroxyergosta-7,22-dien-3-one, they have antifungal activity (Wu et al. 2013).

Penicillium sp. as endophytic fungi found communalistic with higher plants and marine algae are considered an interesting source of new natural compounds and metabolites belonging to different chemical classes among which steroids are of great importance (Selim et al. 2016). Penicisteroids A and B are two novel highly oxygenated steroids contain tetrahydroxy and C-16-acetoxy groups. They were isolated from the *Penicillium chrysogenum* isolated from red algae (Gao et al. 2011). Also, *Penicillium chrysogenum* found associated with the traditional Chinese herb *Huperzia serrata* produce three new steroids namely norcyclocitrinol A,

erythro-11α-hydroxyneocyclocitrinol, and pseudocyclocitrinol A (Ying et al. 2014). Globosterol is a polyhydroxylated C29 steroid. It was firstly reported from *Chaetomium globosum* isolated from *Ginkgo biloba* along with already identified ergosterol derivative (Qin et al. 2009a). Three novel steroids designated as ergosta-5,7,22-trienol, 5α,8α-epidioxyergosta-6,22-dien-3β-ol, and ergosta-7,22-dien-3β,5α,6β-triol were produced by *Pichia guilliermondii* isolated from medicinal plant (Selim et al. 2016).

5.2.9 Terpenoids

Terpenoids are a large group of organic natural products formed by the assembly of a number of C5 isoprene units. Sesquiterpenes, diterpenoids, and triterpenoids are the major terpenoids isolated from endophytes. Arisugacins I and J are two novel compounds belonging to the subgroup meroterpenoids that were isolated for the first time from *Penicillium* sp. SXH-65 (Sun et al. 2014). Another four meroterpenoids compounds were produced by *Guignardia mangiferae* isolated from the medicinal plant *Smilax glabra*, namely guignardones P-S (Sun et al. 2015). An endophytic fungus identified as *Aspergillus* sp. YXf3 isolated from *Ginkgo biloba* produced a C18 norditerpenoid, aspergiloid I. (Selim et al. 2016). *Ulocladium* sp. isolated from the lichen *Everniastrum* sp. from china produced three novel mixed terpenoids, tricycloalternarenes F–H (Wang et al. 2013).

Two new benzofuran-carrying normonoterpene derivatives have been characterized from fungus, endophytic on *Gaultheria procumbens* (Findlay et al. 1997). Pestalotiopsis spp., endophytic fungi associated with *Taxus brevifolia*, produce monoterpenes and C-methylated acetogenins (Pulici et al. 1997). Monoterpene preaustinoids A1, A2, and B1 produced by *Penicillium* sp., endophytic to *Melia azedarach*, showed moderate bacteriostatic activity (Geris dos Santos and Rodrigues-Fo 2002).

Diterpene subglutinol A and B have been produced by *Fusarium subglutinans* endophytic isolated from *Tripterygium wilfordii* (Lee et al. 1995). An unidentified fungus isolated from *Daphnopsis americana* growing in Guanacaste, Costa Rica, produced guanacastepene, a novel diterpenoid that is antibacterial against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* (Singh et al. 2000). Guanancastepene A, guanacastepene, periconicin A, and perieoniein B were four novel diterpenoid antibiotics isolated from endophytes (Kim et al. 2004).

Sesquiterpenoids were repeatedly reported from endophytes associated with plants from different habitats and proved to be antimicrobial compounds. Brasilamides are tricyclic sesquiterpenes produced by endophytic fungus *Paraconiothyrium brasiliense* isolated from the branches of *Acer truncatum* (Liu et al. 2010). Five cadinane sesquiterpenes derivatives were isolated from *Phomopis cassiae*, an endophytic fungus isolated from *Cassia spectabilis*, they have

antifungal activity (Yu et al. 2010). An endophytic fungus *Phleum pratense* isolated from *Epichloë typhina* is reported to produce sesquiterpenes, chokols A–G, which are toxic to *Cladosporium phlei*, a leaf spot disease pathogen (Kumar and Kaushik 2012). *Epichloë typhina*, endophytic isolated from *Phleum pratense*, produce sesquiterpenes chokols A–G, fungi toxic to *Cladosporium phlei*, a leaf spot disease pathogen (Anamika et al. 2018).

5.2.10 Volatile Organic

Fungal volatile organic compounds (VOCs) are derived from both primary and secondary metabolic pathways (Korpi et al. 2009). These are placed in different classes and occur as a mixture of aldehydes, alcohols, cyclohexanes, simple hydrocarbons, heterocycles, indole and their derivatives, ketones, phenols, thioalcohols, thioesters, and benzene derivatives (Ortíz-Castro et al. 2009). Generally, fungi emit a mixture of VOCs. The qualitative and quantitative composition of VOCs is dependent on fungal species and environmental conditions provided for its growth, approximately 250 volatile organic compounds have been identified from fungi (Effmert et al. 2012). VOCs produced by a majority of fungal groups are antibiotics, endophytic fungi belonging to *Ascomycota* lineages, and *Xylariaceae* family are capable of producing VOCs and a few basidiomycetous members also produce VOCs. (Lee et al. 2009).

A number of *Muscodor* species associated with different plant species produce various VOCs like azulene, aromadendrene, β-caryophyllene, 2-methylfuran, naphthalene, tetrahydrofuran, α-phellandrene, β-phellandrene and 2-pentylfuran when growing on plant hosts such as *Actinidia chinensis*, *Ananas ananassoides*, *Ginkgo biloba* and *Myristica fragrans* (Yuan et al. 2012). Truffles produce more than 200 VOCs during different growth stages, that is, presymbiotic, symbiotic (mycelial as well as mycorrhizal) and reproductive stage (Splivallo et al. 2011). A volatile compound, 2-octenal, has been identified in culture filtrates of *Tuber magnatum*, *T. melanosporum*, and *T. borchii.* Other compounds like 2-methylbutanal, dimethyl sulphide, DMDS and 3-methylbutanal have been found in most truffles, whereas 2-methyl,4,5-dihydrothiophene has been found only from fruiting bodies of *T. borchii* (Anamika et al. 2018) In addition, fungus *Ceratocystis fimbriata* isolated from building materials, diseased plants and wood also secretes volatile organic compounds (Hung et al. 2013).

Phaeosphaeria nodorum, a leaf endophytic fungus of plum (*Prunus domestica*), produces several volatiles identified as 2-propyl-1-ol, acetic acid, ethyl acetate, 3-methylbutan-1-ol, and 2-propenenitrile. These volatiles were antagonistic against *Monilinia fructicola* and inhibited the growth of fungus (Pimenta et al. 2012). The major volatile compounds from *Ampelomyces* sp. and *Cladosporium* sp. are *m*-cresol and methyl benzoate, respectively (Naznin et al. 2014). Fungi belonging to genera *Epichloë*, *Puccinia* and *Uromyces* (rust fungi), *Tuber* spp. (truffles), and

Trichoderma sp. that are soil saprophyte *and* mushroom sporocarps secrete volatile compounds (Anamika et al. 2018).

5.3 Discovering Plants with Microbial Bioactive Metabolites

It is important to understand the methods and rationale used to provide the best opportunities to isolate novel endophytic microorganisms (Strobel et al. 2004), discovering plants that harbor microbes capable of producing novel bioactive metabolites are the first step (Zhou et al. 2010). The search for novel secondary metabolites should primarily center on organisms that inhabit unique biotopes. Successful collection of plants harboring endophytes, which produce novel and unique natural bioactive requires the identification of plants (Fig. 5.1) (Lugtenberg et al. 2016); (1) from unique environmental settings and growing in special habitats, especially those with unusual biology, and possessing novel strategies for survival, *Azadirachta indica*, commonly known as the Indian neem or Indian lilac, or 'dogonyaro' in Nigeria.

Neem is a member of the mahogany family, Meliaceae (Atawodi and Atawodi 2009); (2) that have an ethnobotanical history (used by indigenous peoples) and that are related to the specific uses or applications of interest, for example, Camptotheca acuminata plant (Nyssaceae) is a tree, originally found in the mainland of China. The stem wood and the bark are known to contain several alkaloids (Kusari and Spiteller 2012); (3) that are endemic, that have unusual longevity or that have occupied a certain ancient landmass, for example, *Hypericum perforatum* is a very important medicinal plant occupying a significant place in ancient history. From the time of ancient Greece up till now, extracts of *H. perforatum* are effective in treating

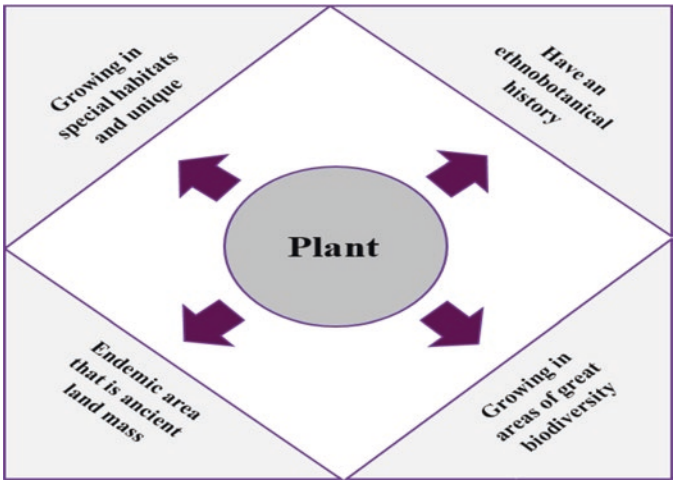


Fig. 5.1 Plants that harbor microbes capable of producing novel bioactive metabolites

mild or moderately severe depression (Cervo et al. 2002). (4) That grow in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity, for example, tropical rainforest such as Amazonian rain forest possessing the greatest biodiversity on the earth led to the discovery of a wide range of secondary metabolites produced by endophytic fungi (Strobel and Strobel 2007) (Fig. 5.1).

There are several alternative strategies that have been adapted to isolate the bioactive compounds from the natural sources, the isolation of endophytic fungal strains from the plants and their parts (leaves, bark, roots, fruits, flowers, stems, etc.) is the source for the isolation of endophytes (Abo Nouh 2019). The plant samples must be collected in sterilized polyethene bags always and processed within a proper time after sampling (Torres et al. 2011). Generally, fresh and clean plant materials should be used for the isolation of endophytes to decrease the chances of contagion. The plant parts must wash in running tap water to eliminate the dirt and debris (Radu and Kqueen 2003). After proper washing, explants will be further processed via surface disinfection under aseptic conditions. Surface disinfection is an essential method by which the exterior surface of the explants is disinfected to ensure that all isolated fungi are endophytic (Jain and Pundir 2017). General route for isolation and purification of bioactive metabolites from endophytic fungi is summarized in Fig. 5.2.

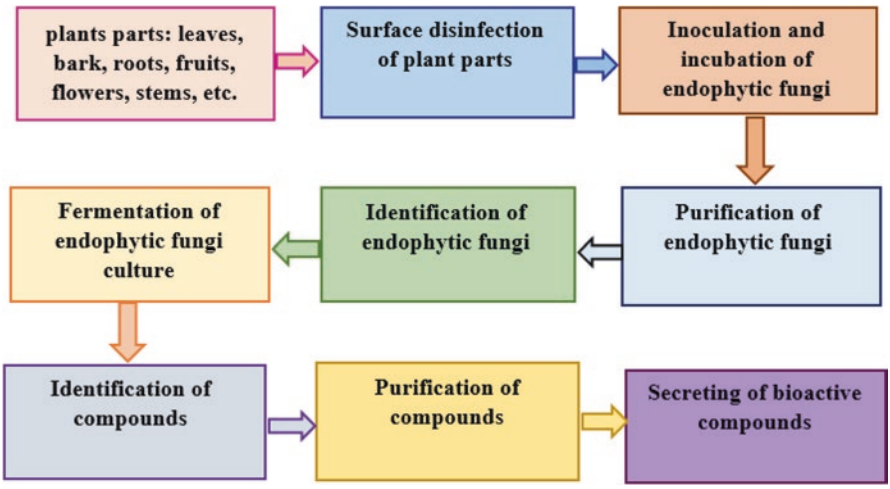


Fig. 5.2 General strategies for isolation and purification of bioactive metabolites from endophytic fungi

5.4 Fungal Metabolites as Antimicrobial

A large number of bioactive compounds have been known to be produced by fungal endophytes, including alkaloids, benzopyranones, depsipeptides, chinones, cytochalasines, enniatines, furandiones, isocumarines, flavonoids, peptides, perylene derivatives, phenols, polyketones, quinols, steroids, terpenoids, tetralones, and xanthones (Elfita et al. 2011; Tenguria et al. 2011). These potential secondary metabolic products act as antimicrobial, antiparasitics, anticancer, neuroprotective, and antioxidant, immunosuppressive (Samuel et al. 2011). Fungal endophyte is known to inhibit fungal growth, bacterial growth and produce effective cytotoxic metabolites (Wang et al. 2007). Tropical plants are usually inhabited by the endophytic fungi belonging to the genus *Xylaria* having a broad-spectrum antimicrobial activity (Liu et al. 2008). Pestacin and isopestacin are two compounds obtained from *Pestalotiopsis microspore* isolated from *Terminalia morobensis*, which also displayed potent antimicrobial activity (Strobel and Daisy 2003).

Compounds such ashypericin and emodin produced by endophytes isolated from Indian medicinal plants have been shown to be having activity against bacterial pathogens, *Klebsiella pneumoniae* ssp. *ozaenae*, *Staphylococcus aureus* ssp. *aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, and fungal pathogens such as *Candida albicans* and *Aspergillus niger* (Kusari et al. 2008). The bioactive compound isolated from the endophytic fungus *Xylaria* sp. isolated from *Ginkgo biloba* L. was named as 7-amino-4-methylcoumarin have been used as broad-spectrum inhibitory activity against several fungal and bacterial food-borne and food spoilage microorganisms and was suggested to be used as a natural preservative in food (Pimentel et al. 2011). *Fusarium* sp. isolated from the *Dendrobium* species showed antagonistic activity against bacterial as well as fungal pathogens. *Phoma* sp. isolated from *Dendrobium devonianum* and *D. thyrsiflorum* showed strong inhibitory activity against different human pathogens namely *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* (Xing et al. 2011).

A naphthodianthrone derived compound, and Emodin (C15H10O5) thought to be the main precursor for the synthesis of hypericin, in an endophytic fungus isolated from a medicinal plant, have antimicrobial activity against a number of bacteria and fungi, like *Staphylococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enteric*, *E. coli* and fungal organisms *Aspergillus niger* and *C. albicans* (Kusari et al. 2012). Fifty-three fungal endophytes isolated from *Dendrobium devonianum* and *D. thyrsiflorum* in which the potential fungal strain *Fusarium tricinctum,* and *Phoma* displayed strong inhibitory activity against pathogens, including *Aspergillus fumigates*, *Bacillus subtilis*, *Candida albicans*, *Cryptococcus neoformans*, *Escherichia coli*, and *Staphylococcus aureus*. *Epicoccum nigrum* exhibited strong antibacterial activity against three pathogens, *S. aureus*, *E. coli*, and *B. subtilis* (Xing et al. 2011). Su et al. (2014) reported 193 endophytic microbes from Chinese medicinal plants *Camptotheca cuminata* Decne, *Gastrodia elata* Blume, and *Pinellia ternata.* Fungal isolates have been found to belong to

Ascomycota, Basidiomycota, and Mucoromycotina. Using submerged culture, fungal strains have been screened for the production of bioactive compounds and have been found to produce camptothecin, 10-hydroxycamptothecin, gastrodin, and ephedrine hydrochloride as bioactive compounds.

5.4.1 Fungal Metabolites as Antibacterial

Penicillin was the first and most important discovery which provides to have an effective action against gram-positive bacteria. The crude extract of *Aspergillus ochraceus* and *Penicillium citrinum* showed wide spectral antibacterial properties, inhibiting developing germs, especially *Pseudomonas aeruginosa* (Demain and Sanchez 2009). *Phomopsis* spp. endophytes from different host plants produce several chemically diverse bioactive compounds. *Phomopsis longicolla*, associated with mint plant *Dicerandra frutescens*, produce dicerandrol A, B, and C with antimicrobial activity exhibiting zones of inhibition of 11, 9.5, and 8.0 mm against *B. subtilis* and 10.8, 9.5, and 7.0 mm against *S. aureus.* Also, *Phomopsis longicolla* strain C81, associated with a seaweed *Bostrychia radicans*, produced dicerandrol C active against *S. aureus* and *Staphylococcus saprophyticus* (Wagenaar and Clardy 2001). Fungal endophytes have been isolated from leaves and branches of five different species of *Garcinia* plants.

The fungal endophyte *Phomopsis* sp. and *Botryosphaeria* sp. showed antibacterial activity against *Staphylococcus aureus* (Phongpaichit et al. 2006). The known compounds 6-*O*-methylalaternin and altersolanol A are nature products producing by endophytic fungi *Ampelomyces* sp. that have been isolated from the medicinal plant *Urospermum picroides* (Asteraceae) displayed as antimicrobial activity against the gram-positive pathogens, *Staphylococcus aureus*, *S. epidermidis* and *Enterococcus faecalis* (Aly et al. 2008). Three steroids, namely 5a,8a-epidioxyergosta-6, ergosta-5,7,22-trienol,22-dien-3b-ol, ergosta-7,22-dien-3b,5a,6b-triol, and one triterpenoid helvolic acid, were separated from *Pichia guilliermondii* an endophytic fungal strain from Paris polyphylla var. Yunnanensis showing the strongest antibacterial activity against many bacteria (Jianglin et al. 2010). Isofusidienol A–D were four bioactive compounds that have been isolated from the endophytic fungus *Chalara* sp. that have been obtained *from Artemisia vulgaris* (Asteraceae) exhibited antibacterial activity against *B. subtilis* (Lösgen et al. 2008; Aly et al. 2010).

The fungal endophyte *Ampelomyces* sp. isolated from *Urospermum picroides* produces an array of bioactive compounds that exhibit significant antimicrobial activity against bacterial pathogens such as *Staphylococcus aureus*, *S. epidermidis*, and *Enterococcus faecalis* (Aly et al. 2008). *Pestalotiopsis* sp., an endophyte of *Rhizophora mucronata*, produced pestalotiopen A exhibiting activity against *Enterococcus faecalis* (Hemberger et al. 2013). A novel phenolic compound was isolated from *Pestalotiopsis mangiferae* associated with *Mangifera indica* exhibits activity against *Bacillus subtilis* and *K. pneumoniae* (MICs 0.039μg/ml), *E. coli* and

Micrococcus luteus (MICs 1.25μg/ml), and *P. aeruginosa* (MIC 5.0μg/ml) (Subban et al. 2013). Two compounds named xanalteric acids I and II metabolites producing from the fungus *Alternaria* sp. (Pleosporaceae) that isolated from leaves of the Chinese Mangrove plant *Sonneratia alba* (Sonneratiaceae) (Kjer et al. 2009). The endophytic fungus *Cryptosporiosis* sp. isolated from *Clidemia hirta* produces an array of bioactive metabolites and exhibits antimicrobial properties against *Bacillus cereus*, *S. aureus*, *Escherichia coli*, and *Pseudomonas fluorescens* (Zilla et al. 2013).

Species belonging to the genera *Phyllosticta*, *Nodulisporium*, and *Xylaria* isolated from *Dipterocarpus* trees are found to have antimicrobial activity against microbial pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli* (Sutjaritvorakul et al. 2011). *Trichophaea abundans*, *Diaporthe phaseolorum*, and *Fusarium redolens* endophytes from the hosts' *Pinus* sp., *Picrorhiza* sp., and *Artemisia* sp., respectively, inhibited growth of pathogenic bacterium *S. aureus*. Similarly, extracts of *Chaetomium globosum* from *Artemisia* sp. and *Phomopsis* sp. from *Nothapodytes* sp. have been found to be effective against *E. coli* and *S. aureus* with IC50 value of 50μg/ml (Qadri et al. 2013).

The endophytic fungus *Fusarium oxysporum* NFX06 isolated from the leaf of *Nothapodytes foetida* of Agumbe forest, Karnataka, showed good activity against all the four test pathogenic strains, viz. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) (Musavi and Balakrishnan 2014). Two endophytes, *Penicillium chrysogenum* Pc_25 and *Alternaria alternata* Aa_27, isolated from the medicinal plant of *Asclepias sinaica* produce extracellular enzymes such as cellulase, gelatinase, and xylanase and exhibited significant antimicrobial potential against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Salmonella typhimurium*, and *C. albicans* (Fouda et al. 2015).

A tetramic acid derivative, Equisetin from endophytic *Fusarium* sp., showed excellent antimicrobial activity against *B. subtilis*, *Staphylococcus aureus* and MRSA at 8 and 16μg/mL minimum inhibitory concentrations, respectively (Ratnaweera et al. 2015). The bioactive compound furano-polyene 3-epi-aureonitol isolated from *Chaetomium* sp. that is obtained from the leaves of *Sapium ellipticum* exhibited antibacterial activity (Akone et al. 2016). The bioactive compounds naphtha-quinones, anhydro-fusarubin, and methyl ether of fusarubin from *Cladosporium* sp. linked with *Rauwolfia serpentina* showed higher antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *Bacillus megaterium* (Khan et al. 2016). Antimicrobial activity varied from species to species in endophytic fungi isolated from the same sources such as *Penicillium commune*, *P. canescens*, and *Alternaria alternata* associated with *Olea europaea* L. tree exhibited antimicrobial activity against gram-positive/negative bacteria (Malhadas et al. 2017). Pinheiro et al. (2017) isolated the polyketide monocerin from the endophytic fungus *Exserohilum rostratum* found in a typical Amazonian plant, *Bauhinia guianensis.*

The potential of the bioactive polyketide monocerin was investigated against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 6633) and *S. typhimurium* (ATCC14028). Intracellular and extracellular extracts of the fungal endophyte *Aspergillus flavus* exhibited a broad

spectrum of antibacterial activity against human pathogenic bacteria (*Lactococcus lactis* NCTC 497, *Bacillus subtilis* 168, *Staphylococcus aureus* SG511, *Staphylococcus carnosus* TM300, *Bacillus pseudomycoides* DSM 12442, and *Escherichia coli* BL21 (DE3)) (Wulandari and Suryantini 2018). The fungal endophyte *Ampelomyces* sp. isolated from *Urospermum picroides* produces an array of bioactive compounds that showed antimicrobial activity against bacterial pathogens such as *S. aureus*, *S. epidermidis*, and *Enterococcus faecalis* (Paramanantham et al. 2019).

5.4.2 Fungal Metabolites as Antifungal

Fungal endophytes are one of the best resources for new bioactive metabolites as antifungal which plays an important role against pathogenic fungi (Pongcharoen et al. 2008). Two antifungal compounds named fusapyrone and deoxyfusapyrone are isolated from endophytic fungi *Fusarium semitectum* against many pathogenic or mycotoxogenic fungi, for example, *Alternaria alternata*, *Aspergillus flavus*, *Cladosporum cucumerinum*, *Phoma tracheiphila*, and *Penicilllium verrucosum* (Altomare et al. 2000). Amphotericin B, nystatin, and natamycin are the main polyenes, which are extensively used for the cure of diseases like *coccidiodal meningitis*, cutaneous dermatophytes, and histoplasmosis and in the treatment of mycotic disease (Gupte et al. 2002; Gohel et al. 2006). Isobenzofuranone as Isopestacin, obtained from the fungal endophyte *Pestalotiopsis microspore* possesses antifungal and antioxidant activity (Strobel et al. 2002).

Wiyakrutta et al. (2004) reported that many isolates isolated from 81 Thai medicinal plant species inhibited *Mycobacterium tuberculosis* when tested using microplate Alamar blue assay. *Aspergillus fumigatus* CY018, a leaf endophytic fungus of *Cynodon dactylon*, produce several metabolites such as asperfumin, fumigaclavine C, asperfumoid, fumitremorgin C, helvolic acid, and physcion, which have antifungal activity against *Candida albicans* (Liu et al. 2004). Strains of *Pestalotiopsis* and *Bartalinia robillardoides* isolated from the medicinal plant *Terminalia arjuna* exhibited antifungal activity. The ethyl acetate extracts of *Pestalotiopsis* showed greater antifungal activity than those isolated from other medicinal plants against six test organisms, namely, *Alternaria carthami*, *Fusarium oxysporum*, *F. verticilloides*, *Macrophomina phaseolina*, *Phoma sorghina*, and *Sclerotinia sclerotiorum* (Gangadevi and Muthumary 2008).

Cycloepoxytriol B and cycloepoxylactone are metabolites extract from fungal endophyte *Phomopsis* sp. that have been isolated from leaves of *Laurus azorica* (Lauraceae) showed antifungal activities against *Microbotryum violaceum* (Hussain et al. 2009). The new nitronaphthalenes and ergosterol, usually present in fungal cultures, were isolated from another Coniothyrium species, an endophyte in the shrub Sideritis chamaedryfolia, from an arid habitat. Compounds showed very good antifungal activity against *M. violaceum* (Krohn et al. 2008). Derivatives of naphthalenes were isolated as dimers of 8-methoxy-naphthol from the endophytic

fungus *Nodulisporium* sp. from *Juniperus cedre* from Gomera. The new dimers showed antifungal activity against *M. violaceum* (Dai et al. 2009). A number of epoxidone derivatives were found in the culture extract of an endophytic *Phoma* sp., isolated from the plant *Salsola oppositifolia*. One new derivative was named epoxydine B has antifungal against *M. violaceum* (Qin et al. 2009b).

Four new pyrenocines, phomopsinones were isolated from an endophytic strain of *Phomopsis* sp., which had colonized the halotolerant plant, *Santolina chamaecyparissus*, from Sardinia Compounds have antifungal activity toward *Botrytis cinerea*, *Pyricularia oryzae*, and *Septoria tritici* (Hussain et al. 2011), *Phytophthora infestans*, *M. violaceum* (Hussain et al. 2012). The endophytic fungus *Lewia infectoria* producing Pyrrocidine C, a novel bioactive compound exhibited potential antifungal activity against *Candida albicans* (Casella et al. 2013). The genus *Xylaria* is an important endophytic fungus inhabiting diverse medicinal plants and produces a wide range of bioactive compounds, for example, multiplolides, glucoside derivatives, xylarosides A, xylarosides B, and sordaricin isolated from *Xylaria* sp. exhibited biocidal activity against *Candida albicans* (Pongcharoen et al. 2008; Paramanantham et al. 2019).

Pyrrocidine C, a novel bioactive compound isolated from *Lewia infectoria* SNB-GTC2402, exhibited potential antifungal activity against *C. albicans* (ATCC10213) (Casella et al. 2013). The presence of bioactive compounds such as viridicatol, tenuazonic acid, alternariol, and alternariol monomethyl ether in *Eleusine coracana* showed antimicrobial activity against *Fusarium graminearum* (Mousa et al. 2015). Recently, *Phaeoacremonium* sp. isolated from the leaves of *Senna spectabilis* produced a wide range of lactone derivatives such as isoaigialones A, B, and C andaigialone. These lactone derivatives showed potent antifungal activity against *Cladosporium cladosporioides* and *C. sphaerospermum* (Silva et al. 2017). Chaetomugilin A and D have been isolated from an endophytic fungus *Chaetomium globosum*, which obtain from *Ginkgo biloba* with antifungal activities. Cytosporone B and C were isolated from a mangrove endophytic fungus *Phomopsis* sp. They inhibited two fungi *C. albicans* and *F. oxysporum* (Pimentel et al. 2011). Two new antifungal and cytotoxic components from the secondary metabolites isolated from *Dendrobium officinale* have antifungal activity against *Cryptococcus neoformans*, *Candida albicans*, *Aspergillus fumigatus* and *Trichophyton rubrum* (Sandhu et al. 2017).

5.4.3 Fungal Metabolites as Antimycobacterial

Fusarium sp. BCC 14842, isolated from bamboo leaves in Thailand, was found to produce javanicin, javanicin (181), 3-*O*-methylfusarubin, and 5-hydroxy-3-methoxydihydrofusarubin A, which showed antimycobacterial activity (Kornsakulkarn et al. 2011). A *Phomopsis* sp. from leaves of *Tectona grandis* L., in Northern Thailand, produce compounds phomoxanthones A and B that showed significant “in vitro” antitubercular activities when compared to the existing drugs

such as isoniazid and kanamycin sulfate (Isaka et al. 2001). 3-Nitropropionic acid compound was obtained from a species of *Phomopsis* belonging to six Thai medicinal plants. This compound is known to modulate the virulence and fatty acid catabolism of *Mycobacterium* and exhibited effective inhibition against *Mycobacterium tuberculosis* with MIC of 3.3μM (Chomcheon et al. 2005). Compounds such as 4-methoxycinnamaldehyde, biscogniazaphilones A and B, 5-hydroxy-3,7,4-trimeth oxyflavone, *N-trans*-feruloyl-3-*O*-methyldopamine, 4-methoxy-*trans*-cinnamic acid, and methyl 3,4-methylene dioxycinnamate isolated from *Biscogniauxia formosana*, residing inside *Cinnamomum* sp., have been shown to be having antimycobacterial activities against *M. tuberculosis* strain H37Rv in vitro (Cheng et al. 2012). *Coniothyrium cereale*, an endophytic fungus, from marine green alga *Enteromorpha* sp., was found to produce trypethelone, which is effective against *Mycobacterium phlei* (Elsebai et al. 2011). *Chaetomium globosum* strain IFB-E036, isolated from *Cynodon dactylon*, secrete chaetoglobosins A and B, having activity against *Micrococcus luteus* and *Mycobacterium smegmatis* (Ge et al. 2011).

Attia et al. (2020) studied the production of antimicrobial, extracellular enzymes and antioxidants by endophytic teleomorphic Ascomycota associated with medicinal plants. A total of 11 teleomorphic species were isolated from four medicinal plant species in Saint Katherine Protectorate in Egypt. *Chaetomium grande* and *Sordaria fimicola* were the most frequently isolated species and represented by 12 (Chg1–Chg12) and 7 (Sf1–Sf7) isolates respectively. The minimum inhibitory concentration (MIC) of all the isolates was determined against nine reference strains of bacteria and fungi. Effectiveness of 100–300μg/mL DEMSO then in H_2O of the ethyl acetate fractions of the most effective two isolates Chg5 and Sf3 on the tested reference strains revealed different inhibitory effects.

Saturated disc of Streptomycin and Rifampin (0.165 mg/mL) was used for bacteria and amphotericin B and fluconazole were used for yeasts and fungi as a positive control. Enzymatically, Chg5 isolates are considered a resource of amylase, cellulase, protease, lipase, and chitinase. However, Sf3 isolates are considered a resource of amylase, laccase, and chitinase out of six screened enzymes. Total phenolics (TP), total flavonoids (TF), and antioxidant activity of the Sf3 and Chg5 extracts were measured. The TP values were expressed as milligram gallic acid equivalents per gram of dry extract of Sf3 and Chg5, which equal to 53.9 ± 0.35 and 97.9 ± 0.48 respectively. TF present in both Sf3 and Chg5 isolates extracts with values equal to 2.44 ± 0.01 and 7 ± 0.05 respectively expressed as routine equivalents. In vitro, the antioxidant activity of the extracts was investigated using DPPH radical-scavenging assay, and equal to 0.06% and 0.39% respectively in the extract of both taxa.

5.4.4 *Fungal Metabolites as Antiviral*

Recently, secondary metabolites of endophytic fungi utilized for inhibition of viruses.The antiviral compound wickerol A isolated from *Trichoderma atroviride* FKI-3737 fungi showed an effective antiviral action against H1N1 flu virus (A/PR/8/34 and A/WSN/33 strains) (Obuchi et al. 1990). The antiviral compound wickerol B diterpene compounds with a novel fused 6-5-6-6 ring skeleton that has been isolated from Endophytic fungus *T. atroviride FKI-3849* founded as an effective antiviral action against the H1N1 flu virus and provides a possibility of being lead compounds to make the development of novel anti-influenza, antiviral drugs with novel structure (Fukami et al. 2000). The extracts of endophytic fungi from local medicinal plants have shown antiviral activity against herpes simplex virus type I (Wiyakrutta et al. 2004). Cytonic acids A and B have been isolated from endophytic fungus *Cytonaema* sp. obtained from *Quercus* sp. (Guo et al. 2008; Jalgaonwala et al. 2011); these compounds are used to be an inhibitor of human cytomegalovirus protease (Sandhu et al. 2017). Emerimidine, emeriphenolicins, aspernidine, austin, austinol, dehydroaustin, and acetoxydehydroaustin have been isolated from endophytic fungus *Emericella* sp. obtained from *Aegiceras corniculatum* these compounds used against Influenza A (H1N1) (Zhang et al. 2011).

The endophytic fungal *Pestalotiopsis theae* that were obtained from an unidentified tree from Jianfeng Mountain and Chinese have the ability to produce an antiviral compound called Pestalotheol C against anti-HIV properties (Li et al. 2008). Altertoxin I, II, III and V compounds have been isolated from *Alternaria tenuissima* endophytic fungus obtained from *Quercus emoryi* used as antiviral compounds against HIV-1 virus (Bashyal et al. 2014; Chetia et al. 2019). The endophytic fungus *Pleospora tarda* obtained from *Ephedra aphylla* and host plant *Ephedra alata* were the most potent candidates against the HSV-2 virus (Selim et al. 2018). Some compounds reported as antiviral activity, isolated from *Fusarium* sp., for example, Cyclo (L-Pro-L-Val) and griseoxanthone C have good potency against HCV NS3/4A protease while, ω-hydroxyemodin and cyclo (L-Tyr-L-Pro) were potent HCVPR inhibitors (Hawas et al. 2016; Toghueo 2020).

5.5 Conclusions

The fungal endophytes are a rich source of novel secondary metabolites. In recent years, there are great achievements in the production of metabolically active compounds from endophytic fungi. These organisms have tremendous sources of metabolically active compounds that may be used in pharmaceutical, medical, agriculture, and industries. Endophytic fungus offers a broad variety of secondary metabolites with their unique structures like flavonoids, terpenoids, alkaloids, phenolic acid, and so on. Such bioactive metabolites have antimicrobial activity, for example, antifungal, antibacterial, antiviral. Research on endophytic fungi facilitates the

discovery of bioactive natural compounds and provides better knowledge about secondary metabolites producing microorganisms.

References

Abo Nouh FA (2019) Endophytic fungi for sustainable agriculture. Microb Biosyst 4:31–44

Akone SH, Mandi A, Kurtan T, Hartmann R, Lin W, Daletos G, Proksch P (2016) Inducing secondary metabolite production by the endophytic fungus *Chaetomium* sp. through fungalebacterial co-culture and epigenetic modification. Tetrahedron 72:6340–6347

Akpotu MO, Eze PM, Abba CC, Umeokoli BO, Nwachukwu CU, Okoye FB, Esimone CO (2017) Antimicrobial activities of secondary metabolites of endophytic fungi isolated from *Catharanthus roseus*. J Health Sci 7(1):15–22

Altomare C, Perrone G, Zonno MC, Evidente A, Pengue R, Fanti F et al (2000) Biological characterization of fusapyrone and deoxyfusapyrone two bioactive secondary metabolites of Fusarium semitectum. J Nat Prod 63(8):1131–1135

Aly AH, Edrada-Ebel RA, Wray V, Muller WEG, Kozytska S, Hentschel U, Proksch P, Ebel R (2008) Bioactive metabolites from the endophytic fungus *Ampelomyces* sp. isolated from the medicinal plant *Urospermum picroides*. Phytochemistry 69:1716–1725

Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41:1–16. https://doi.org/10.1007/s13225-010-0034-4

Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90:1829–1845

Anamika, Joshi S, Sahgal M, Sahu S, Prakash A (2018) Fungal endophytes and their secondary metabolites: role in sustainable agriculture. In: Gehlot P, Singh J (eds) Fungi and their role in sustainable development. Springer Nature Pte Ltd, Singapore, pp 121–146. https://doi.org/10.1007/978-981-13-0393-7_8

Arivudainambi USE, Anand TD, Shanmugaiah V, Karunakaran C, Rajendran A (2011) Novel bioactive metabolites producing endophytic fungus Colletotrichum gloeosporioides against multidrug-resistant Staphylococcus aureus. FEMS Immunol Med Microbiol 61:340–345

Arora J, Ramawat KG (2017) An introduction to endophytes. In: Maheshwari DK (ed) Endophytes: biology and biotechnology, sustainable development and biodiversity, vol 15. Springer International Publishing AG, Swizterland, pp 1–16. https://doi.org/10.1007/978-3-319-66541-2_1

Atawodi SE, Atawodi JC (2009) *Azadirachta indica* (neem): a plant of multiple biological and pharmacological activities. Phytochem Rev 80:601–620

Attia EA, Singh BP, Dashora K, Abdel-Azeem AM (2020) A potential antimicrobial, extracellular enzymes, and antioxidants resource: endophytic fungi associated with medicinal plants. Int J Biosci 17(1):119–132

Bashyal BP, Wellensiek BP, Ramakrishnan R, Faeth SH, Ahmad N, Gunatilaka AAL (2014) Altertoxins with potent anti-HIV activity from *Alternaria tenuissima* QUE1Se, a fungal endophyte of *Quercus emoryi*. Bioorg Med Chem 22(21):6112–6116

Cao S, Clardy J (2011) New naphthoquinones and a new δ-lactone produced by endophytic fungi from Costa Rica. Tetrahedron Lett 52(17):2206–2208

Casella TM, Eparvier V, Mandavid H, Bendelac A, Odonne G, Dayan L, Duplais C, Espindola LS, Stien D (2013) Antimicrobial and cytotoxic secondary metabolites from tropical leaf endophytes: isolation of antibacterial agent pyrrocidine C from *Lewia infectoria* SNB-GTC2402. Phytochemistry 96:370–377

Cervo L, Rozio M, Guiso G, Morazzoni P, Caccia S (2002) Role of hyperforin in the antidepressant-like activity of *Hypericum perforatum* extracts. Psychopharmacology 164:423–428

Chaipackdee V, Pornpakakul S, Chokratin P (2013) Bioactive compounds produced by endophytic fungi isolated from beach morning glory (*Ipomoea pes-caprae* Linn.). Asian J Microbiol Biotechnol Environ Sci 15:479–482

Chaturvedi P, Gajbhiye S, Roy S, Dudhale R, Chowdhary A (2014) Determination of kaemferol in extracts of Fusarium chlamydosporum, an endophytic fungi of Tylophora indica (Asclepeadaceae) and its anti-microbial activity. IOSR J Pharm Biol Sci 9:51–55

Cheng MJ, Wu MD, Yanai H, Su YS, Chen IS, Yuang GF (2012) Secondary metabolites from the endophytic fungus *Biscogniauxia formosana* and their antimycobacterial activity. Phytochem Lett 5:467–447

Chetia H, Kabiraj D, Bharali B, Ojha S, Barkataki MP, Saikia D, Singh T, Mosahari PV, Sharma P, Bora U (2019) Exploring the benefits of endophytic fungi via omics. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer, New York, pp 51–81. https://doi.org/10.1007/978-3-030-03589-1

Chomcheon P, Wiyakrutta S, Sriubolmas N, Ngamrojanavanich N, Isarangkul D, Kittakoop P (2005) 3-Nitropropionic acid (3-NPA), a potent antimycobacterial agent from endophytic fungi: is 3-NPA in some plants produced by endophytes? J Nat Prod 68:1103–1105

Dai J, Krohn K, Draeger S, Schulz B (2009) New naphthalen-chroman coupling products from the endophytic fungus Nodulisporium sp. from Erica arborea. Eur J Org Chem 2009:1564–1569

Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot 24:5–16

Deshmukh SK, Mishra PD, Kulkarni-Almeida A, Verekar S, Sahoo MR, Periyasamy G, Goswami H, Khanna A, Balakrishnan A, Vishwakarma R (2009) Anti-inflammatory and anticancer activity of ergoflavin isolated from an endophytic fungus. Chem Biodivers 6:784–789

Dew RK, Boissonneault GA, Gay N, Boling JA, Cross RJ, Cohen DA (1990) The effect of the endophyte (Acremonium coenophialum) and associated toxin(s) of tall fescue on serum titer response to immunization and spleen cell flow cytometry analysis and response to mitogens. Vet Immunol Immunopathol 26(3):285–295

Du FY, Li XM, Song JY, Li CS, Wang BG (2014) Anthraquinone derivatives and an orsellinic acid ester from the marine alga-derived endophytic fungus Eurotium cristatum EN-220. Helv Chim Acta 97(7):973–978

Effmert U, Kalderás J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38(6):665–703

Elfita E, Muharni M, Munawar M, Legasari L, Darwati D (2011) Antimalarial compounds from endophytic fungi of Brotowali (*Tinaspora crispa* L). Indo J Chem 11:53–58

Elsebai MF, Natesan L, Kehraus S, Mohamed IE, Schnakenburg G, Sasse F, Shaaban S, Gütschow M, König GM (2011) HLE-inhibitory alkaloids with a polyketide skeleton from the marine-derived fungus *Coniothyrium cereale*. J Nat Prod 74:2282–2285

Fang ZF, Yu SS, Zhou WQ, Chen XG, Ma SG, Li Y, Qu J (2012) A new isocoumarin from metabolites of the endophytic fungus Alternaria tenuissima Wiltshire. Chin Chem Lett 23(3):317–320

Figueroa M, Jarmusch AK, Raja HA, El-Elimat T, Kavanaugh JS, Horswill AR, Cooks RG, Cech NB, Oberlies NH (2014) Polyhydroxyanthraquinones as quorum sensing inhibitors from the guttates of Penicillium restrictum and their analysis by desorption electrospray ionization mass spectrometry. J Nat Prod 77(6):1351–1358

Findlay JA, Buthelezi S, Li GQ, Seveck M, Miller JD (1997) Insect toxins from an endophytic fungus from wintergreen. J Nat Prod 60:1214–1215

Fouda AH, Hassan SE, Eid AM, Ewais EE (2015) Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). Ann Agric Sci 60(1):95–104

Fukami A, Nakamura T, Kim YP, Shiomi K, Hayashi M, Nagai T et al (2000) A new anti-influenza virus antibiotic-10-norparvulenone from Microsphaeropsis sp. FO-5050. J Antibiot 53:1215–1218

Gangadevi V, Muthumary J (2008) Taxol, an anticancer drug produced by an endophytic fungus Bartalinia robillardoides Tassi, isolated from a medicinal plant, Aegle marmelos Correa ex Roxb. World J Microbiol Biotechnol 24:717–724

Gao SS, Li XM, Li CS, Proksch P, Wang BG (2011) Penicisteroids A and B, antifungal and cytotoxic polyoxygenated steroids from the marine alga-derived endophytic fungus Penicillium chrysogenum QEN-24S. Bioorg Med Chem Lett 21(10):2894–2897

Ge HM, Shen Y, Zhu CH, Tan SH, Ding H, Song YC, Tan RX (2008) Penicidones A–C, three cytotoxic alkaloidal metabolites of an endophytic Penicillium sp. Phytochemistry 69(2):571–576

Ge HM, Zhang Q, Xu SH, Guo ZK, Song YC, Huang WY, Tan RX (2011) Chaetoglocins A–D, four new metabolites from the endophytic fungus *Chaetomium globosum*. Planta Med 77:277–280

Geris dos Santos RM, Rodrigues-Fo E (2002) Meroterpenes from Penicillium sp. found in association with Melia azedarach. Phytochemistry 61:907–912

Gohel V, Singh A, Vimal M, Ashwini P, Chhatpar HS (2006) Bio-prospecting and antifungal potential of chitinolytic microorganisms. Afr J Biotechnol 5:54–72

Guo LD, Hyde KD, Liew ECY (2000a) Identification of endophytic fungi from Livistona chinensis based on morphology and rDNA sequences. New Phytol 147(3):617–630

Guo B, Dai JR, Ng S, Huang Y, Leong C, Ong W, Carte BK (2000b) Cytonic acids A & B: novel tridepside inhibitors of h CMV protease from the endophytic fungus Cytonaema species. J Nat Prod 63:602–604

Guo B, Wang Y, Sun X, Tang K (2008) Bioactive natural products from endophytes: a review. J Appl Biochem Microbiol 44(2):136–142. https://doi.org/10.1134/S0003683808020026

Gupte M, Kulkarni P, Ganguli BN (2002) Antifungal antibiotics. Appl Microbiol Biotechnol 58:46–57

Han Z, Mei W, Zhao Y, Deng Y, Dai H (2009) A new cytotoxic isocoumarin from endophytic fungus *Penicillium sp*. 091402 of the mangrove plant *Bruguiera sexangula*. Chem Nat Compd 45(6):805–807

Hawas WU, Al-Farawati R, El-Kassem LTA, Adnan J, Turk AJ (2016) Different culture metabolites of the Red Sea fungus Fusarium equiseti optimize the inhibition of hepatitis C virus NS3/4A protease (HCV PR). Mar Drugs 14:190

Hemberger Y, Xu J, Wray V, Proksch P, Wu J, Bringmann G (2013) Pestalotiopens A and B: stereochemically challenging flexible sesquiterpene-cyclopaldic acid hybrids from *Pestalotiopsis* sp. Chem Eur J 19:15556–15564

Horn WS, Simmonds MS, Schwartz RE, Blaney WM (1996) Variation in production of Phomodiol and Phomopsolide B by Phomopsis spp. Mycologia 88:588–595

Huang H, She Z, Lin Y, Vrijmoed LLP, Lin W (2007) Cyclic peptides from an endophytic fungus obtained from a mangrove leaf (Kandelia candel). J Nat Prod 70(11):1696–1699

Huang Z, Yang J, Lei F, She Z, Lin Y (2013) A new xanthone Oglycoside from the mangrove endophytic fungus Phomopsis sp. Chem Nat Compd 49(1):27–30

Hung R, Lee S, Bennett JW (2013) Arabidopsis thaliana as a model system for testing the effect of Trichoderma volatile organic compounds. Fungal Ecol 6(1):19–26

Hussain H, Akhtar N, Draeger S, Schulz B, Pescitelli G, Salvadori P, Antus S, Kurtán T, Krohn K (2009) New bioactive 2,3-epoxycyclohexenes and isocoumarins from the endophytic fungus Phomopsis sp. from Laurus azorica. Eur J Org Chem 2009:749–756

Hussain H, Krohn K, Ahmed I, Draeger S, Schulz B, Di Pietro S, Pescitelli G (2011) Phomopsinones A–D: four new pyranocines from endophytic fungus Phomopsis sp. J Nat Prod 74:365–373

Hussain H, Ahmed I, Schulz B, Draeger S, Krohn K (2012) Four new antimicrobial pyranocines from the endophytic fungus, Phomopsis sp. Fitoterapia 83:523–526

Hussain H, Kliche-Spory C, Al-Harrasi A, Al-Rawahi A, Abbas G, Green IR, Schulz B, Krohn K, Shah (2014) A antimicrobial constituent from three endophytic fungi. Asian Pac J Trop Med 7(Suppl 1):S224–S227

Isaka M, Jaturapat A, Rukseree K, Danwisetkanjana K, Tanticharoen M, Thebtaranonth Y (2001) Phomoxanthones A and B, novel xanthone dimers from the endophytic fungus *Phomopsis* species. J Nat Prod 64:1015–1018

Jalgaonwala RE, Mohite BV, Mahajan RT (2011) A review: Natural products from plant associated endophytic fungi. J Microbiol Biotechnol Res 1(2):21–32

Jianglin Y, Mou Y, Shan T, Li L, Zhou L, Wang M et al (2010) Antimicrobial metabolites from the endophytic fungus Pichia guilliermondii isolated from Paris polyphylla var. yunnanensis. Molecules 15:7961–7970

Ju Y, Sacalis JN, Still CC (1998) Bioactive flavonoids from endophyte-infected blue grass (Poa ampla). J Agric Food Chem 46:3785–3788

Jain P, Pundir RK (2017) Potential role of endophytes in sustainable agriculture-recent developments and future prospects. In: Maheshwari DK (ed) Endophytes: Biology and biotechnology, sustainable development and biodiversity. Springer International Publishing AG, Swizterland, 15, pp 145–160. https://doi.org/10.1007/978-3-319-66541-2_1

Kaul S, Gupta S, Ahmed M, Dhar MK (2012) Endophytic fungi from medicinal plants: a treasure hunt for bioactive metabolites. Phytochem Rev 11:487–505

Khan MIH, Sohrab MH, Rony SR, Tareq FS, Hasan CM, Mazid MA (2016) Cytotoxic and antibacterial naphthoquinones from an endophytic fungus, *Cladosporium* sp. Toxicol Rep 3:861–865

Kim S, Shin DS, Lee T, Oh KB (2004) Periconicins two new fusicoccane diterpenes produced by an endophytic fungus Periconia sp. with antibacterial activity. J Nat Prod 67:448–450

Kjer J, Wray V, Edrada-Ebel RA, Ebel R, Pretsch A, Lin WH, Proksch P (2009) Xanalteric acids I and II and related phenolic compounds from an endophytic Alternaria sp. isolated from the mangrove plant Sonneratia alba. J Nat Prod 72:2053–2057

Kornsakulkarn J, Dolsophon K, Boonyuen N, Boonruangprapa T, Rachtawee P, Prabpai S, Kongsaeree P, Thongpanchang C (2011) Dihydronaphthalenones from endophytic fungus *Fusarium* sp. BCC14842. Tetrahedron 67:7540–7547

Korpi A, Jarnberg J, Pasanen A-L (2009) Microbial volatile organic compounds. Crit Rev Toxicol 39:139–193

Krohn K, Kouam SF, Cludius-Brandt S, Draeger S, Schulz B (2008) Bioactive nitronapthalenes from an endophytic fungus, Coniothyrium sp. and their chemical synthesis. Eur J Org Chem 2008:3615–3618

Kumar S, Kaushik N (2012) Metabolites of endophytic fungi as novel source of biofungicide: a review. Phytochem Rev 11(4):507–522

Kumar CG, Mongolla P (2018) Bioprospecting of endophytic fungi for bioactive compounds. In: Gehlot P, Singh J (eds) Fungi and their role in sustainable development. Springer Nature Pte Ltd., Singapore, pp 651–697. https://doi.org/10.1007/978-981-13-0393-7_8

Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008) An endophytic fungus from *Hypericum perforatum* that produces hypericin. J Nat Prod 71:159–162

Kusari S, Spiteller M (2012) Metabolomics of endophytic fungi producing associated plant secondary metabolites: Progress, challenges and opportunities. In: Ute Roessner (ed) Metabolomics. InTech, Rijeka, Croatia, pp 241–266

Kusari S, Verma VC, Lamshoeft M, Spiteller M (2012) An endophytic fungus from Azadirachta indica A. Juss. that produces azadirachtin. World J Microbiol Biotechnol 28(3):1287–1294

Lee JC, Lobokovsky NB, Strobel GA, Clardy JC (1995) Subglutinol A and B: immunosuppressive compounds from the endophytic fungus Fusarium subglutinans. J Org Chem 60:7076–7077

Lee SO, Kim HY, Choi GJ, Lee HB, Jang KS, Choi YH, Kim JC (2009) Mycofumigation with Oxyporus latemarginatus EF069 for control of postharvest apple decay and Rhizoctonia root rot on moth orchid. J Appl Microbiol 106(4):1213–1219

Li EW, Jiang LH, Guo LD, Zhang H, Che YS (2008) Pestalachlorides A–C, antifungal metabolites from the plant endophytic fungus Pestalotiopsis adusta. Bioorg Med Chem 16:7894–7899

Li DL, Li XM, Wang BG (2009) Natural anthraquinone derivatives from a marine mangrove plant-derived endophytic fungus Eurotium rubrum: structural elucidation and DPPH radical scavenging activity. J Microbiol Biotechnol 19(7):675–680

Li X, Tian Y, Yang SX, Zhang YM, Qin JC (2013) Cytotoxic azaphilone alkaloids from Chaetomium globosum TY1. Bioorg Med Chem Lett 23(10):2945–2947

Lin Y, Wu X, Feng S, Jiang G, Zhou S, Vrijmoed LLP, Jones EBG (2001) A novel N-cinnamoylcyclopeptide containing an allenic ether from the fungus Xylaria sp. (strain 2508) from the South China Sea. Tetrahedron Lett 42(3):449–451

Liu JY, Song YC, Zhang Z, Wang L, Guo ZJ, Zou WX, Tan RX (2004) *Aspergillus fumigatus* CY018, an endophytic fungus in *Cynodon dactylon* as a versatile producer of new and bioactive metabolites. J Biotechnol 114:279–287

Liu X, Dong M, Chen X, Jiang MLX, Zhou J (2008) Antimicrobial activity of an endophytic Xylaria sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. Appl Microbiol Biotechnol 78:241–247

Liu L, Gao H, Chen X, Cai X, Yang L, Guo L, Yao X, Che Y (2010) Brasilamides A–D: sesquiterpenoids from the plant endophytic fungus Paraconiothyrium brasiliense. Eur J Org Chem 17:3302–3306

Lösgen S, Magull J, Schulz B, Draeger S, Zeeck A (2008) Isofusidienols: novel chromone-3-oxepines produced by the endophytic fungus Chalara sp. Eur J Org Chem 2008:698–703

Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by Colletotrichum sp., an endophytic fungus in Artemisia annua. Plant Sci 151(1):67–73

Lugtenberg BJJ, Caradus JR, Johnson LJ (2016) Fungal endophytes for sustainable crop production. FEMS Microbiol Ecol 92(12):1–17

Malhadas C, Malheiro R, Pereira JP, Pinho PG, Baptista P (2017) Antimicrobial activity of endophytic fungi from olive tree leaves. World J Microbiol Biotechnol 33:46

Ming, Ge H, Zhang YW, Ding G, Saparpakorn P, Song CY, Hannongbua S, Tan XR (2008) Chaetoglobins A and B, two unusual alkaloids from endophytic Chaetomium globosum culture. Chem Commun 45:5978–5980

Mousa WK, Schwan A, Davidson J, Strange P, Liu H, Zhou T, Auzanneau FI, Raizada MN (2015) An endophytic fungus isolated from finger millet (*Eleusine coracana*) produces anti-fungal natural products. Front Microbiol 6:1157

Musavi SF, Balakrishnan RM (2014) A study on the antimicrobial potentials of an endophytic fungus Fusarium oxysporum NFX 06. J Med Bioeng 3(3):162–166

Naznin HA, Kiyohara D, Kimura M, Miyazawa M, Shimizu M, Hyakumachi M (2014) Systemic resistance induced by volatile organic compounds emitted by plant growth-promoting fungi in Arabidopsis thaliana. PLoS One 9(1):e86882

Nicoletti R, Fiorentino A (2015) Plant bioactive metabolites and drugs produced by endophytic fungi of spermatophyta. Agriculture 5:918–970

Obuchi T, Kondoh H, Omura S, Yang JS, Liang XT (1990) Armillaric acid, a new antibiotic produced by Armillaria mellea. Plants Med 56:198–201

Oliveira CM, Regasini LO, Silva GH, Pfenning LH, Young MCM, Berlinck RGS, Bolzani VS, Araujo AR (2011) Dihydroisocoumarins produced by *Xylaria sp. and Penicillium sp*., endophytic fungi associated with Piper aduncum and Alibertia macrophylla. Phytochem Lett 4(2):93–96

Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4(8):701–712

Paramanantham P, Pattnaik S, Siddhardha B (2019) Natural products from endophytic fungi: synthesis and application. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer, New York, pp 83–103. https://doi.org/10.1007/978-3-030-03589-1

Peng W, Guo L, Zheng CJ, Zhang QY, Jia M, Jiang YP, Han T, Qin LP (2012) Two new azaphilone alkaloids dimers from endophyte Chaetomium fusiforme of the liverwort Scapania verrucosa Heeg. Biochem Syst Ecol 45:124–126

Phongpaichit S, Rungjindamai N, Rukachaisirikul V, Sakayaroj J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. FEMS Immunol Med Microbiol 48:367–372

Pimenta RS, Moreira da Silva JF, Buyer JS, Janisiewicz WJ (2012) Endophytic fungi from plums (Prunus domestica) and their antifungal activity against Monilinia fructicola. J Food Prot 75(10):1883–1889

Pimentel MR, Molina G, Dionísio AP, Maróstica MR Jr, Pastore GM (2011) The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotechnol Res Int 2011:576286

Pinheiro EAA, Pina JRA, Feitosa AO, Carvalho JM, Borges FC, Marinho PSB, Marinho AMR (2017) Bioprospecting of antimicrobial activity of extracts of endophytic fungi from *Bauhinia guianensis*. Rev Argent Microbiol 49(1):3–6

Pongcharoen W, Rukachaisirikul V, Phongpaichit S, Kuhn T, Pelzing M, Sakayaroj J, Taylor WC (2008) Metabolites from the endophytic fungus *Xylaria* sp. PSU-D14. Phytochemistry 69:1900–1902

Powell RG, Petroski RJ (1992) Alkaloid toxins in endophyte-infected grasses. Nat Toxins 1(3):163–170

Pulici M, Sugawara F, Koshino H, Okada G, Esumi Y, Uzawa J, Yoshida S (1997) Metabolites of Pestalotiopsis spp., endophytic fungi of Taxus brevifolia. Phytochemistry 46(2):313–319

Qadri M, Johri S, Shah BA, Khajuria A, Sidiq T, Lattoo SK, Abdin MZ, Riyaz-Ul-Hassan S (2013) Identification and bioactive potential of endophytic fungi isolated from selected plants of the Western Himalayas. Springerplus 2:1

Qiao MF, Ji NY, Liu XH, Li F, Xue QZ (2010) Asporyergosterol, a new steroid from an algicolous isolate of Aspergillus oryzae. Nat Prod Commun 5(10):1575–1578

Qiao MF, Ji NY, Miao FP, Yin XL (2011) Steroids and an oxylipin from an algicolous isolate of Aspergillus flavus. Magn Reson Chem 49(6):366–369

Qin JC, Gao JM, Zhang YM, Yang SX, Bai MS, Ma YT, Laatsch H (2009a) Polyhydroxylated steroids from an endophytic fungus Chaetomium globosum ZY-22 isolated from Ginkgo biloba. Steroids 74(9):786–790

Qin S, Krohn K, Schulz B (2009b) Two new metabolites, epoxydine A and B, from Phoma sp. Helv Chim Acta 2009:169–174

Radu S, Kqueen CY (2003) Preliminary screening of endophytic fungi from medicinal plants in Malaysia for antimicrobial and antitumour activity. Malaysia J Med Sci 93:23–33

Rakshith D, Santosh P, Satish S (2013) Isolation and characterization of antimicrobial metabolite producing endophytic Phomopsis sp. from Ficus pumila Linn. (Moraceae). Int J Chem Anal Sci 4:156–160

Ratnaweera PB, Silva ED, Williams DE, Andersen RJ (2015) Antimicrobial activities of endophytic fungi obtained from the arid zone invasive plant *Opuntia dillenii* and the isolation of equisetin, from endophytic *Fusarium sp*. BMC Complement Altern Med 15:220

Rodrigues KF, Hesse M, Werner C (2000) Antimicrobial activities of secondary metabolites produced by endophytic fungi from Spondias mombin. J Basic Microbiol 40(4):261–267

Samuel P, Prince L, Prabakaran A (2011) Antibacterial activity of marine derived fungi collected from South East Coast of Tamilnadu, India. J Microbiol Biotechnol Res 1(4):86–94

Sandhu SS, Kumar S, Aharwal RP, Nozawa M (2017) Endophytic fungi: eco friendly future resource for novel bioactive compounds. In: Maheshwari DK (ed) Endophytes: biology and biotechnology. Springer, New York, pp 303–331. https://doi.org/10.1007/978-3-319-66541-2

Schmeda-Hirschmann G, Hormazabal E, Rodriguez JA, Theoduloz C (2008) Cycloaspeptide A and pseurotin A from the endophytic fungus Penicillium janczewskii. Z Naturforsch C J Biosci 63(5/6):383–388

Schulz B, Sucker J, Aust HJ, Krohn K, Ludewig K, Jones PG, Döring D (1995) Biologically active secondary metabolites of endophytic Pezicula species. Mycol Res 99(8):1007–1015

Schulz B, Boyle C, Draeger S, Rommert A-K, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106:996–1004

Selim KA, Nagia MMS, El-Ghwas DE (2016) Endophytic fungi are multifunctional biosynthesis: ecological and chemical diversity. In: Hughes E (ed) Endophytic fungi: diversity, characterization and biocontrol. Nova Science Publisher, New York, pp 39–91

Selim K, Elkhateeb W, Tawila A, El-Beih A, Abdel-Rahman T, El-Diwany A, Ahmed E (2018) Antiviral and antioxidant potential of fungal endophytes of Egyptian medicinal plants. Fermentation 4(3):49

Seto Y, Takahashi K, Matsuura H, Kogami Y, Yada H, Yoshihara T, Nabeta K (2007) Novel cyclic peptide, epichlicin, from the endophytic fungus Epichloe typhina. Biosci Biotechnol Biochem 71(6):1470–1475

Silva GH, Zeraik ML, de Oliveira CM, Teles HL, Trevisan HC, Pfenning LH, Nicolli CP, Young MCM, Mascarenhas YP, Abreu LM, Saraiva AC, Medeiros AI, Bolzani VS, Araujo AR (2017) Lactone derivatives produced by a *Phaeoacremonium* sp., an endophytic fungus from *Senna spectabilis*. J Nat Prod 80:1674–1678

Singh MP, Janso JE, Luckman SW, Brady SF, Clardy J, Greenstein M, Maiese WM (2000) Biological activity of guanacastepene, a novel diterpenoid antibiotic produced by an unidentified fungus CR115. J Antibiot 53(3):256–261

Song YX, Ma Q, Li J (2015) A new aurone glycoside with antifungal activity from marine derived fungus Penicillium sp. FJ-1. Zhongguo Zhongyao Zazhi 40(6):1097–1101

Splivallo R, Ottonello S, Mello A, Karlovsky P (2011) Truffle volatiles: from chemical ecology to aroma biosynthesis. New Phytol 189:688–699

Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502

Strobel SA, Strobel GA (2007) Plant endophytes as a platform for discovery-based undergraduate science education. Nat Chem Biol 3:356–359

Strobel GA, Torczynski R, Bollon A (1997) Acremonium sp.—a leucinostatin A producing endophyte of European yew (Taxus baccata). Plant Sci 128(1):97–108

Strobel G, Ford E, Worapong J, Harper JK, Arif AM, Grant DM, Fung PC, Chau RMW (2002) Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. Phytochemistry 60:179–183

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67:257–268

Su H, Kang J, Cao J, Mo L, Hyde KD (2014) Medicinal plant endophytes produce analogous bioactive compounds. Chiang Mai J Sci 41:1–13

Subban K, Subramani R, Johnpaul M (2013) A novel antibacterial and antifungal phenolic compound from the endophytic fungus *Pestalotiopsis mangiferae*. Nat Prod Res 27:1445–1449

Sugijanto NE, Diesel A, Rateb M, Pretsch A, Gogalic S, Zaini NC, Ebel R, Indrayanto G (2011) Lecythomycin, a new macrolactone glycoside from the endophytic fungus Lecythophora sp. Nat Prod Commun 6(5):677–678

Sun X, Kong X, Gao H, Zhu T, Wu G, Gu Q, Li D (2014) Two new meroterpenoids produced by the endophytic fungus Penicillium sp. SXH-65. Arch Pharm Res 37(8):978–982

Sun ZH, Liang FL, Wu W, Chen YC, Pan QL, Li HH, Ye W, Liu HX, Li SN, Tan GH, Zhang WM (2015) Guignardones PS, new meroterpenoids from the endophytic fungus Guignardia mangiferae A348 derived from the medicinal plant Smilax glabra. Molecules 20(12):22900–22907

Sutjaritvorakul T, Whalley AJS, Sihanonth P, Roengsumran S (2011) Antimicrobial activity from endophytic fungi isolated from plant leaves in Dipterocarpus forest at Wiang Sa district Nan province, Thailand. J Agric Technol 7:115–121

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459

Tansuwan S, Pornpakakul S, Roengsumran S, Petsom A, Muangsin N, Sihanonta P (2007) Antimalarial benzoquinones from an endophytic fungus, *Xylaria* sp. J Nat Prod 70:1620–1623

Teles HL, Sordi R, Silva GH, Castro-Gamboa I, Bolzani VS, Pfenning LH, Magalhaes de Abreu L, Costa-Neto CM, Young MCM, Araujo AR (2006) Aromatic compounds produced by Periconia atropurpurea, an endophytic fungus associated with Xylopia aromatica. Phytochemistry 67(24):2686–2690

Tenguria RK, Khan FN, Quereshi S (2011) Endophytes-mines of pharmacological therapeutics. World J Sci Technol 1:127–149

Toghueo RMK (2020) Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. Mycology 11(1):1–21

Torres M, Tadych M, White JF, Bills GF (2011) Isolation and identification of fungal endophytes. In: Pirttila AM, Sorvari SI (eds) Prospects and applications for plant associated microbes: a laboratory manual, Part B: Fungi. BBI (Biobien Innovations), Turku, Finland, pp 153–164

Tsuchinari M, Shimanuki K, Hiramatsu F, Muratama T, Koseki T, Shiono Y (2007) Fusapyridons A and B, novel pyridone alkaloids from an endophytic fungus, Fusarium sp. YG-45. Z Naturforsch B Chem Sci 62(9):1203–1207

Wagenaar MM, Clardy J (2001) Dicerandrols, new antibiotic and cytotoxic dimers produced by the fungus *Phomopsis longicolla* isolated from an endangered mint. J Nat Prod 64:1006–1009

Wagenaar MM, Corwin J, Strobel G, Clardy J (2000) Three new cytochalasins produced by an endophytic fungus in the genus Rhinocladiella. J Nat Prod 63(12):1692–1695

Wang FW, Jiao RH, Cheng AB, Tan SH, Song YC (2007) Antimicrobial potentials of endophytic fungi residing in *Quercus variabilis* and brefeldin A obtained from *Cladosporium* sp. World J Microbiol Biotechnol 23(1):79–83

Wang QX, Bao L, Yang XL, Guo H, Ren B, Guo LD, Song FH, Wang WZ, Liu HW, Zhang LX (2013) Tricycloalternarenes F–H: three new mixed terpenoids produced by an endolichenic fungus Ulocladium sp. using OSMAC method. Fitoterapia 85:8–13

Wiyakrutta S, Sriubolmas N, Panphut W, Thongon N, Danwisetkanjana K, Ruangrungsi N, Meevootisom V (2004) Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. World J Microbiol Biotechnol 20:265–272

Wu SH, Huang R, Miao CP, Chen YW (2013) Two new steroids from an endophytic fungus Phomopsis sp. Chem Biodivers 10(7):1276–1283

Wulandari RS, Suryantini R (2018) Growth of Albizia in vitro: endophytic fungi as plant growth promote of Albizia. Int Sch Sci Res Inn 12(1):8

Xing Y-M, Chen J, Cui J-L, Chen X-M, Guo S-X (2011) Antimicrobial activity and biodiversity of endophytic fungi in *Dendrobium devonianum* and *Dendrobium thyrsiflorum* from Vietman. Curr Microbiol 62:1218–1224

Xu J, Kjer J, Sendker J, Wray V, Guan H, Edrada R, Mueller WEG, Bayer M, Lin W, Wu J, Proksch P (2009) Cytosporones, coumarins and an alkaloid from the endophytic fungus Pestalotiopsis sp. isolated from the Chinese mangrove plant Rhizophora mucronata. Bioorg Med Chem 17(20):7362–7367

Yang X, Strobel G, Stierle A, Hess WM, Lee J, Clardy J (1994) A fungal endophyte-tree relationship: Phoma sp. in Taxus wallichiana. Plant Sci 102(1):1–9

Yang XL, Huang L, Li HY, Yang DF, Li ZZ (2015) Two new compounds from the plant endophytic fungus Pestalotiopsis versicolor. J Asian Nat Prod Res 17(4):333–337

Ying YM, Zheng Z, Zhang ZLW, Shan WG, Wang JW, Zhan ZJ (2014) Rare C25 steroids produced by Penicillium chrysogenum P1X, a fungal endophyte of Huperzia serrata. Helv Chim Acta 97(1):95–101

Yu H, Zhang L, Li L, Zheng C, Guo L, Li W, Sun P, Qin L (2010) Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiol Res 165:437–449

Yuan J, Raza W, Shen Q, Huang Q (2012) Antifungal activity of Bacillus amyloliquefaciens NJN-6 volatile compounds against Fusarium oxysporum f. sp. cubense. Appl Environ Microbiol 78(16):5942–5944

Zhang Y, Li XM, Wang CY, Wang BG (2007) A new naphthoquinoneimine derivative from the marine algal-derived endophytic fungus Aspergillus niger EN-13. Chin Chem Lett 18(8):951–953

Zhang G, Sun S, Zhu T, Lin Z, Gu J, Li D, Gu Q (2011) Antiviral isoindolone derivatives from an endophytic fungus Emericella sp. associated with Aegiceras corniculatum. Phytochemistry 72(11–12):1436–1442

Zheng N, Wang Z, Shi Y, Lin J (2012) Evaluation of the antifungal activity of total flavonoids extract from Patrinia villosa Juss and optimization by response surface methodology. Afr J Microbiol Res 6(3):586–593

Zhou X, Zhu H, Liu L, Lin J, Tang K (2010) A review: recent advances and future prospects of taxol-producing endophytic fungi. Appl Microbiol Biotechnol 86:1707–1717

Zhou ZF, Kurtan T, Yang XH, Mandi A, Geng MY, Ye BP, Taglialatela-Scafati O, Guo YW (2014) Penibruguieramine A, a novel pyrrolizidine alkaloid from the endophytic fungus Penicillium sp. GD6 associated with Chinese mangrove Bruguiera gymnorrhiza. Org Lett 16(5):1390–1393

Zilla MK, Qadri M, Pathania AS, Strobel GA, Nalli Y, Kumar S, Guru SK, Bhushan S, Singh SK, Vishwakarma RA, Riyaz-Ul-Hassan S, Ali A (2013) Bioactive metabolites from an endophytic *Cryptosporiopsis* sp. inhabiting *Clidemia hirta*. Phytochemistry 95:291–297

Zou WX, Meng JC, Lu H, Chen GX, Shi GX, Zhang TY, Tan RX (2000) Metabolites of Colletotrichum gloeosporioides, an endophytic fungus in Artemisia mongolica. J Nat Prod 63(11):1529–1530

Chapter 6
Fungal Laccases to Where and Where?

Hebatallah H. Abo Nahas, Safaa A. Mansour, Fatma Ahmed Abo Nouh, Daniela Landa-Acuña, Yousef H. Abo Nahas, Miguel Angel Nieto-Taype, and Ahmed M. Abdel-Azeem

Contents

H. H. Abo Nahas (✉)
Zoology Department (Physiology), Faculty of Science, Suez Canal University, Ismailia, Egypt

S. A. Mansour · F. A. Abo Nouh · A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

D. Landa-Acuña
Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Peru

Professional Career in Environmental Engineering, Faculty of Engineering, Private University of the North, Lima, Peru

Y. H. Abo Nahas
Microbiology Department, Faculty of Medicine, Helwan University, Cairo, Egypt

M. A. Nieto-Taype
Department of Chemical, Biological and Environmental Engineering, School of Engineering, Universitat Autònoma de Barcelona, Bellaterra, Spain

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_6

6.1 Introduction

Laccase is one of the exceptional enzymes that has been reported since the end of the nineteenth century. It was first revealed in the exudates of *Rhus vernicifera*, the Japanese lacquer tree (Yoshida 1883; Kour et al. 2019b). The enzyme laccase has drawn much attention for its outstanding features. Laccases are found in some higher plants and also in certain bacteria (Claus 2003). However, ligninolytic basidiomycetes were the sources of the studied laccases (Abdel-Azeem et al. 2021). Laccases are blue multicopper enzymes (EC 1.10.3.2, benzenediol:oxygen oxidoreductase) that belong to a family of multicopper enzymes, which include ascorbic oxidase and ceruloplasmin (Wong 2009). They catalyze the elimination of a single electron from phenolic hydroxylic groups as syringaldehyde or vanillyl glycol with the consequent reduction of molecular oxygen to water and the formation of phenoxy radicals. These radicals undergo further chemical reactions that eventually lead to oxidized quinones and coupled oligomeric products. The study of metal ion contents of the laccase from *Trametes hirsuta* the presence of copper and manganese at a 3:1 ratio was shown (Giardina et al. 2010).

However, many "nonblue" fungal laccases have also been identified, namely, POXAW1w (a white laccase from *Pleurotus ostreatus*), which contains only one copper atom instead of the standard four, along with two zinc atoms and one iron atom in each protein molecule. Other so-called white laccases have been purified from *Trametes hirsuta*, *Pycnoporus sanguineus*, and *Phlebia radiata* (Kubicek et al. 2011). Laccases have many roles in fungi, involving the synthesis of melanin and other pigments and the formation of morphogenesis reproduction such as conidia and fruiting bodies (Baldrian 2006; Giardina et al. 2010). Nevertheless, their role in the decomposition of lignin, although demonstrated in vitro, is doubtful because laccases are present in almost all fungi, even those unable to degrade lignin (namely, several ascomycetes; Baldrian 2006). On the other hand, some white-rot fungi (e.g., *Pycnoporus cinnabarinus*) only produce laccases (Eggert et al. 1996a).

Fungal laccases are generalists' biocatalysts with prospective applications that range from bioremediation to novel green processes. Fueled by molecular oxygen, these enzymes can act on dozens of molecules of different chemical nature, and with the aid of redox mediators, their range of oxidizable substrates is further pushed toward xenobiotic compounds (industrial dyes, pesticides, and PAHs), biopolymers (starch, lignin, and cellulose), and other complex molecules (Kumar et al. 2021; Kour et al. 2021). Recently, extraordinary attempts have been made to engineer fungal laccases by directed evolution and semi-rational approaches to enhance their functional expression or stability (Maté et al. 2011). Due to strong catalytic capacities, there has been directed evolution of fungal laccases. The interest initially stemmed from the greater redox potential of the enzyme, which enables the oxidation of a broader variety of substrates.

In addition, there are many white-rot fungi (involved in lignin combustion) laccases (Yadav et al. 2019a, b). Because of the higher redox potential, fungal laccases are used in many biotechnological applications, especially in the degradation of lignin and elimination of potentially toxic phenols arising during lignin degradation. One of the most significant enzymes in the bio palpation process in paper and pulp industry is laccases. It also has the potentiality to depolymerize lignin and delineate wood pulps, kraft pulp, and chlorine-free fibers (Abdel-Azeem and Salem 2012). Laccase enzymes also have a significant role in physiological processes related to pathogenesis (Edens et al. 1999) and cell detoxification (Bollag et al. 1988). Laccases from fungi have been used in the food and beverage industry enhancement or modification (Ghindilis 2000; Rodríguez Couto and Toca Herrera 2006; Minussi et al. 2002, 2007a; Selinheimo et al. 2006).

6.2 Laccases: A Never-Ending Story

Laccases are common enzymes in nature and are commonly present in plants, fungi, and some bacteria and insects (Minussi et al. 2007b; Kour et al. 2019a). These biocatalyst's physiological functions, which may be secreted or intracellular, differ in the various organisms, but they all catalyze processes of polymerization or depolymerization (Riva 2006). The enzyme was characterized as a metal-containing oxidase (Bertrand 1985). Moreover, it becomes one of the earliest enzymes ever identified. Laccases have subsequently been discovered from numerous other plants (Ranocha et al. 1999), but it is still difficult to detect and purify plant laccases since crude plant extracts contain a large number of oxidative enzymes with diverse substrate specificities (Ranocha et al. 1999), which is possibly the reason why detailed knowledge on the biochemical properties of plant laccase is restricted. *Rhus vernicifera* laccase, however, is an exception and has been extensively studied, especially with respect to its spectroscopic properties (Woolery et al. 1984).

R. vernicifera laccase was also commonly used in laccase general reaction mechanism investigations (Battistuzzi et al. 2003; Johnson et al. 2003). Plant laccases are present in the xylem, where monolignols are probably oxidized in the early stages

of lignification (Gavnholt and Larsen 2002) and also participate in the radical processes of lignin polymer formation (Hoopes and Dean 2004). Furthermore, it has been shown that laccases are involved in the first steps of healing in wounded leaves (De Marco and Roubelakis-Angelakis 1997). However, the occurrence of laccases tends to be much more limited in higher plants than in fungi (Mayer and Staples 2002; Moin and Omar 2013). The first bacterial laccase was identified in the plant root-associated "*Azospirillum lipoferum*" bacterium (Givaudan et al. 1993), where melanin formation was shown to be involved (Faure et al. 1994).

A typical laccase-containing six putative copper-binding sites have been discovered from Marinomonas Mediterranean, but this enzyme has not been assigned a functional role (Sanchez-Amat et al. 2001). *Bacillus subtilis* makes a thermostable CotA laccase that participates in endospore coat pigment production (Martins et al. 2002). Laccases have also been found from *Streptomyces cyaneus* (Arias et al. 2003) and *Streptomyces lavendulae* (Suzuki et al. 2003). However, there are some other records of laccase activity in bacteria as well; it does not appear likely that laccases are specific enzymes from certain prokaryotic groups (Claus 2003). Proteins like bacterial laccase are intracellular or periplasmic proteins (Baldrian 2006). *B. licheniformis* is a novel, soil-isolated, melanogenic soil bacterium that protects strain against UV light and oxidants (Dalfard et al. 2006). It is included in phenolic acid dimerization (Koschorreck et al. 2008). The laccase-producing bacillus endospores were isolated from soil and the enzyme involved in phenol degradation (Singh et al. 2011; Naclerio et al. 2010).

Fungal laccases are involved in the delignification of lignocellulosic material, protection against toxic compounds, the development of the fruiting body, sporulation, fungal morphogenesis, (Dwivedi et al. 2011), and the synthesis of virulent activity molecules (i.e. melanin) causing fungal diseases (Riva 2006). There are vast numbers of fungal laccase producers (Rodríguez Couto and Toca Herrera 2006; Shumakovich et al. 2007) that belong to the basidiomycetes and ascomycetes. Laccase activity in the lower fungi that belong to the zygomycete (glomeromycete) and chytridiomycete has never been recorded.

6.3 Biodiversity of Laccase-Producing Fungi

Laccase (EC 1.10.3.2) is a benzenediol: oxygen oxidoreductase (a multicopper enzyme) that is capable of oxidizing phenolic compounds. It does not contain heme as the cofactor but copper as opposed to peroxidases; nor does it require H_2O_2 as the co-substrate but rather molecular oxygen (Baldrian 2006). So, it is one of the oldest enzymes ever reported. In several fungal species, laccase activity was demonstrated leading to the concept that laccase is produced mostly by the fungi. Nevertheless, this cannot be generalized, since there are other physiological classes of fungi that do not appear to produce laccase. The development of laccase has never been shown in lower fungi, that is, *Zygomycetes* and *Chytridiomycetes* (Couto and Toca-Herrera 2007), except by a *Mucor* genus *Zygomycete* (Bonugli-santos et al. 2010).

Many fungal species belonging to ascomycetes and basidiomycetes have shown laccase activity, and the enzyme has already been extracted from other species. *Ascomycetes* have many records for the production of laccase. Phytopathogenic ascomycetes such as *Melanocarpus albomyces* (D'Souza et al. 1996), *Cerrena unicolor* (Lee et al. 2004), *Magnaporthe grisea* (Schlosser and Höfer 2002), and *Trichoderma reesei* (Levasseur et al. 2010) are examples for the production and purification of laccases. In addition, laccase development of certain soil ascomycete species from the genera *Aspergillus*, *Curvularia*, and *Penicillium* (Scherer and Fischer 1998), as well as some freshwater ascomycetes (Junghanns et al. 2005), was also recorded in plant pathogenic species. Yeasts are a class of both ascomycetes and basidiomycetes that are physiologically different. Until now only the human yeast pathogen *Cryptococcus* (Filobasidiella) *neoformans* had purified laccase. This yeast develops true laccase that can oxidize phenols and aminophenols and are unable to oxidize tyrosine (De Jesus et al. 2008) The enzyme is strongly bound to the cell wall and leads to fungicide resistance (Ikeda et al. 2003). Numerous efforts were made to discover ligninocytic enzymes, including laccases from ectomycorrhizal (ECM) fungi (Cairney and Burke 1998; Burke and Cairney 2002). Many isolates of ECM species, such as *Cortinarius*, *Amanita*, *Hebeloma*, *Lactarius*, *Paxillus*, *Piloderma*, *Russula*, *Tylospora*, and *Xerocomus*, showed gene fragments closely similar to the laccase from wood-rotting fungi (Luis et al. 2004; Chen et al. 2003a). A few fungal-forming ectomycorrhizae have been purified with Laccases: *Cantharellus cibarius* (Ng and Wang 2004), *Lactarius piperatus* (Iwasaki et al. 1967), *Russula delica* (Matsubara and Iwasaki 1972), *Thelephora terestris* (Kanunfre and Zancan 1998), or orchideoid mycorrhiza: Armil-lariamellea (Rehman and Thurston 1992a, b; Billal and Thurston 1996; Curir et al. 1997) as well as from the species of genera which contain both saprotrophic and mycorrhizal fungi, *Marasmius*, *Agaricus*, *Volvariella*, and *Tricholoma*.

To date, the function of some other ligninolytic enzyme, Mn-peroxidase, has only been confirmed in *Tylospora fibrillosa*, a species that also contains a putative laccase sequence (Chambers et al. 1999; Chen et al. 2003b), and probably lignin peroxidase, which has shown that rotting wood ascomycetes such as *Botryosphaeria* and *Trichoderma* also have some laccase activity. While *Botryosphaeria* produces a dimethoxyphenol-oxidizing enzyme that may be a true laccase (Vasconcelos et al. 2000), only certain strains of *Trichoderma* exhibit low-level development of syringaldazine are oxidizing enzymes (Assavanig et al. 1992).

Among physiological fungal classes, laccases are characteristic of wood-rotting basidiomycetes that cause white rot, and a similar group of litter-decomposing saprotrophic fungi, the ones that cause lignin degradation. Nearly, all white-rot fungi species were reported to produce laccase in varying degrees, and the enzyme was extracted from several species (Hofrichter and Steinbüchel 2001). Brown-rot fungi, on the other hand, are usually not considered to have capabilities in the production of laccase. In *Gloeophyllum trabeum*, a DNA sequence with fairly high similarity to that of laccase was found that was capable of oxidizing ABTS (Kiiskinen et al. 2002). However, no laccase protein was extracted from brown-rot species, syringaldazine oxidation was recently detected in *Coniophora puteana* brown-rot fungi

("Purification and Characterization of a Laccase from *Cerrena unicolor* and Its Reactivity in Lignin Degradation," 2002), and ABTS oxidation was recorded in *Laetiporus sulphureus* (Iyer and Chattoo 2003). Laccase being a lignin-degrading enzyme (LDE) must have a nonspecific kind of cleavage, since lignin itself is an amorphous polymer with no particular molecular structure. This laccase nonspecificity for the reduction substrate is their greatest selling point since this laccase activity can be applied to a number of substrates. Sometimes, the organism develops more than one laccase isozyme at any given time (Urzua et al. 1995). The types of isozymes produced often differ according to varying growth and/or nutritional conditions (D'Souza-Ticlo et al. 2006a, b).

These classes of laccases enhance their activity under varying temperature, pH, and substrate type conditions. Laccase also frequently sports a high degree of glycosylation which gives a degree of resistance to protease attacks (Yoshitake et al. 1993) which the enzyme also finds in the wild. Nevertheless, the redox potential varying between different laccase isozymes is not as high as that of peroxidases, especially MnP. The presence of mediators increases laccases' effective substrate range to include non-phenolics by reducing the effective capacity for redox. Unlike the other LDEs, however, laccase does not have the exclusive need for their presence, and the production of laccase should not be subject to strict limits on carbon or nitrogen (as is often seen in the idiophase). This is essential because one cannot always expect to encounter these strict conditions in nature. In the presence of many inducers, the most common of which is copper, laccase development can be greatly increased (Wesenberg et al. 2003).

Abdel-Azeem and Salem (2012) studied the biodiversity of laccase-producing fungi in Egypt. They screened different sources, for example, soil, wood, seaweeds, sponge, ascidia, drifted decaying wood, plants, and miscellaneous materials. They encountered as many as 60 species belonging to 33 genera. Zygomycota is represented by six species (10.16% of the total species number), teleomorphic Ascomycota (9 species, 15.25%), anamorphic Ascomycota (44 species, 74.57%), and Basidiomycota (1 species, 1.69%). Soil showed the highest Simpson's species diversity index of 0.83, while contaminated wax samples and *Adiantum capillus-veneris* showed the lowest value (0). All isolated taxa were tested for laccase production using a qualitative plate assay method by using guaiacol as a color indicator. Sixteen isolates showed positive reaction indicating a lignin-degrading potentiality and out of them, eight measured the highest zone diameter with a high oxidation scale. The most promising taxa were endophytic, namely, *Chaetomium globosum*, *Phoma exigua*, *Thanatephorus cucumeris*, and *Sordaria fimicola*; pH 7; incubation temperature 30 °C; and 1% maltose and 0.3% peptone supported the highest biomass and laccase production for *Chaetomium globosum*.

In 2020, Attia et al. studied the production of antimicrobial, extracellular enzymes and antioxidants by endophytic teleomorphic Ascomycota associated with medicinal plants in Saint Katherine Protectorate in Egypt. They isolated 11 teleomorphic species from four medicinal plant species. *Chaetomium grande* and *Sordaria fimicola* were the most frequently isolated species and represented by 12 (Chg1–Chg12) and 7 (Sf1–Sf7) isolates, respectively. Enzymatically, Chg5 isolate is considered a

resource of amylase, cellulase, protease, lipase, and chitinase. However, Sf3 isolates are considered as a resource of amylase, laccase, and chitinase out of six screened enzymes.

6.4 Laccases: A Structural–Chemical Clarification of Fungal Activity

Laccases (EC 1.10.3.2, *p*-diphenol: dioxygen oxidoreductase) are a group of multi-copper-containing enzymes that catalyze one-electron oxidation of phenolic compounds with concomitant reduction of oxygen to water and whose active site is similar to that of ascorbatoxidase, cerulo-plasmin, and bilirubin oxidase. Fungi belonging to the basidiomycetes group which cause white rot in wood are the best-studied laccases to date (Baldrian 2006). Laccases are mainly extracellular glycoproteins (Heinzkill et al. 1998) and are multinuclear enzymes (Gayazov and Rodakiewicz-Nowak 1996) with molecular weights ranging from 60 to 80 kDa and are multinuclear enzymes (Gayazov and Rodakiewicz-Nowak 1996). Atypical fungal laccases have also been identified, however; for example, a Podospora anserine 390 kDa laccase (Thurston 1994), while *Botrytis cinerea* laccase was previously reported as small as 38 and 36 kDa. All fungal laccases are glycoproteins (Mayer and Staples 2002). These biomolecules can exhibit different glycosylation levels, typically between 10% and 30% (Baldrian 2006). In secretion, proteolytic stability (Bertrand 2010), copper retention power, and thermal stability, glycosylation play an important role (Thurston 1994).

Generally, catalytic activity and stability may differ from one enzyme to another depending on the origin, pH, temperature, and culture medium used for its processing. Laccases at acid pH (3–6) are stable (Nyanhongo et al. 2002), but usually pH 3 is ideal for laccases action. Laccases can be active at a wide range of temperatures (20–55 °C) at an optimal temperature of 55 °C. Thermostable laccases (60–70 °C) were also purified and defined (Saraiva et al. 2012). The existence of isoforms was reported depending on the phase of the fungal growth, the existence of inducers, and the production process conditions. Laccases occur in different forms; they can be monomeric, homotetrameric, heterodimeric, and multimeric. Based on the organism, their molecular weight varies from 50 to 130 kDa (Jaiswal et al. 2015).

The mature protein is commonly a holoenzyme, which can be monomeric, dimeric, or tetrameric in its active form, with four copper atoms for each monomer (Imran et al. 2012). There are currently more than 40 three-dimensional laccase structures accessible via GenBank and NCBI; the majorities are white-rot fungal laccase (Benson et al. 2012). The laccase holoenzyme can exist as a monomer, dimer, or even as a tetramer. Each monomer contains four copper atoms that are divided into three redox sites and are known as copper type 1 (T1), type 2 (T2), and type 3 (T3). Type 1 and T2 contain one atom of copper each, while T3 contains two atoms of copper. The trinuclear cluster is formed together by the T2 and T3 copper

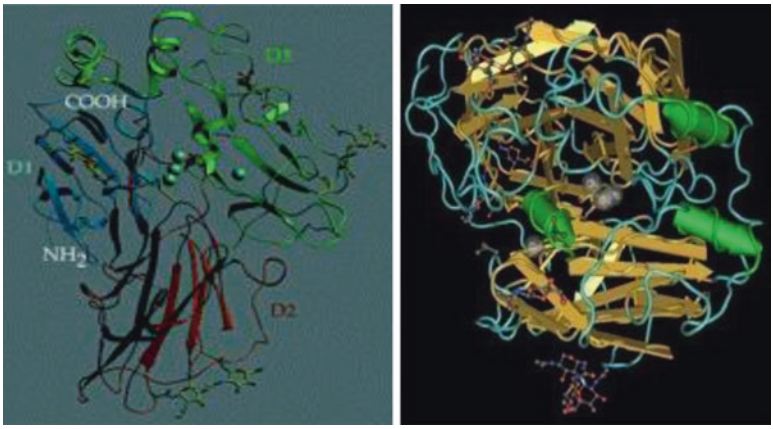


Fig. 6.1 Ribbon diagram of TvL. The arrangement of the domain structure is depicted in different colors (D1–D3). Copper ions are drawn as blue spheres. Carbohydrates and disulfide bonds are included as stick models. (Source: Piontek et al. 2002)

(Farver and Pecht 1984). The apoprotein consists of three domains with architecture similar to that of the ù-barrel type. T1 copper is found in the holoenzyme in domain 3, while the trinuclear cluster is located between domains 1 and 3, with both domains having residues to coordinate the coppers. Figure 6.1 illustrates the structure of laccase (Piontek et al. 2002).

Type 1 copper is paramagnetic with an Electron Paramagnetic Resonance (EPR) signal that is well characterized. Intense electronic absorption induced by a covalent copper–cysteine bond gives rise to the blue color attributed to laccases, observed at 610 nm (in the oxidized state). T1 copper is the site where substrate oxidation takes place, leading to its high redox potential of ~790 mV. Type 2 copper is also paramagnetic with an EPR signal that is well characterized, that is, non-blue copper, though. This is positioned strategically close to the copper pair T3. Type 2 coordinates copper with two histidines. Type 3 is a diamagnetic spin-coupled copper–copper pair, forming a binuclear core with an oxidized state maximum absorbance of 330 nm. The copper pair's antiferromagnetic coupling is the reason behind the absence of an EPR signal (Claus 2004).

Strong anion binding can therefore activate an EPR signal (Gianfreda et al. 1999). A hydroxyl bridge holds the tight bonding between the two copper atoms. The copper center T3 is also a typical characteristic of another superfamily of proteins including tyrosinases and hemocyanins. Copper type 3 is coordinated by 6 histidines (all residues conserved). Throughout the trinuclear cluster, the reduction of molecular oxygen and the release of molecular oxygen occur. The total number of 11 preserved residues, 10 histidine and 1 cysteine, make a laccase of blue multicopper oxidase, along with the residues (AA's) that coordinate the four copper atoms. T1 copper has trigonal coordination, with two histidines and cysteine as preserved equatorial ligands, and the normal variable is the axial ligand. This axial

ligand in fungal laccases is phenylalanine (F) or leucine (L) and methionine (M) in the bacterial (Cot A).

The essential residue is shown in bold reflecting part of the essential pentapeptide found by abox, present across kingdoms. The E° of a *Trametes villosa* laccase was substantially reduced by a mutation from phenylalanine to methionine (Kumar et al. 2003). It has been widely argued that this axial ligand position affects the enzyme's E° significantly, potentially providing the mechanism to control its activity (Claus 2004). The theory is confirmed by the *Trametes versicolor* laccase (TvL) crystal structure. The E° of laccase is a description of a variety of factors, one of which is the coplanar trigonal coordination of copper T1. The T1 copper is in a distorted tetrahedral (fourfold coordinated) structure in most of the blue copper proteins, while the T1 copper core in laccase is trigonal coplanar-coordinated (threefold coordinated). The involvement of ligands brings about this coordination of copper. The ligands are supplied by a cysteine's S-atom (sulfur), and two histidines' N81 nitrogen. The T1 core has an additional axial ligand in the other blue proteins, which is contributed by the S-atom of a methionine, while in laccase methionine, as seen in TvL and Coprinus cinereus laccase (CcL), respectively, it is replaced by either F or L at this position. None of these amino acids is involved in the coordination, resulting in this additional ligand being absent. As a result of this structure, the copper ion lies almost within the plane created by the one S and two N (nitrogen) ligands, while in other copper proteins, the copper lies above the plane toward the additional S ligand due to the presence of an additional axial ligand. Thus, T1 copper coordination in laccase is distinct from those found in blue copper proteins, such as ascorbate oxidase, plastocyanine, and azurine, which provide an additional axial ligand.

The lack of this axial ligand attributes a moderate elevation of the redox potential in the laccase, which is present in other blue copper proteins (Piontek et al. 2002). The E° varies within the laccases too; the presence of a longer Cu1-N82 (His458) bond distance has been shown to correspond with an increased E° of laccase (Piontek et al. 2002). It has been shown that the *Trametes versicolor* laccase (TvL) has a high E° while the CcL has low E°. A comparison of these two structures can account for the difference in the redox potential. A small a-helix (residues 455–461) carrying the T1 copper-ligating His458 in TvL shows a higher displacement from the Type I copper atom, when compared to its corresponding CcL position. An elongated Cu–N bond influences the redox potential as the contribution of the free electron pair from the N to the copper decreased, making the copper more deficient in electron. That would lead to a destabilization of the higher oxidation states, that is, may raise the redox potential of the T1 copper. An H-bond formed between Glu460 and Ser113 is the explanation for such a displacement of the small Į-helix carrying the T1 copper-ligating His458 in TvL. The Ser113 is located in the opposite domain, domain 1 (D-1), and is one of three residues responsible for this H-bond formation. This H-bond would possibly force the Į-helix into an inappropriate conformation of the main chain.

As a consequence of this attractive H-bond formation, the whole helix, containing the His458, is pulled toward D-1, thereby increasing the Cu-N gap, thus giving

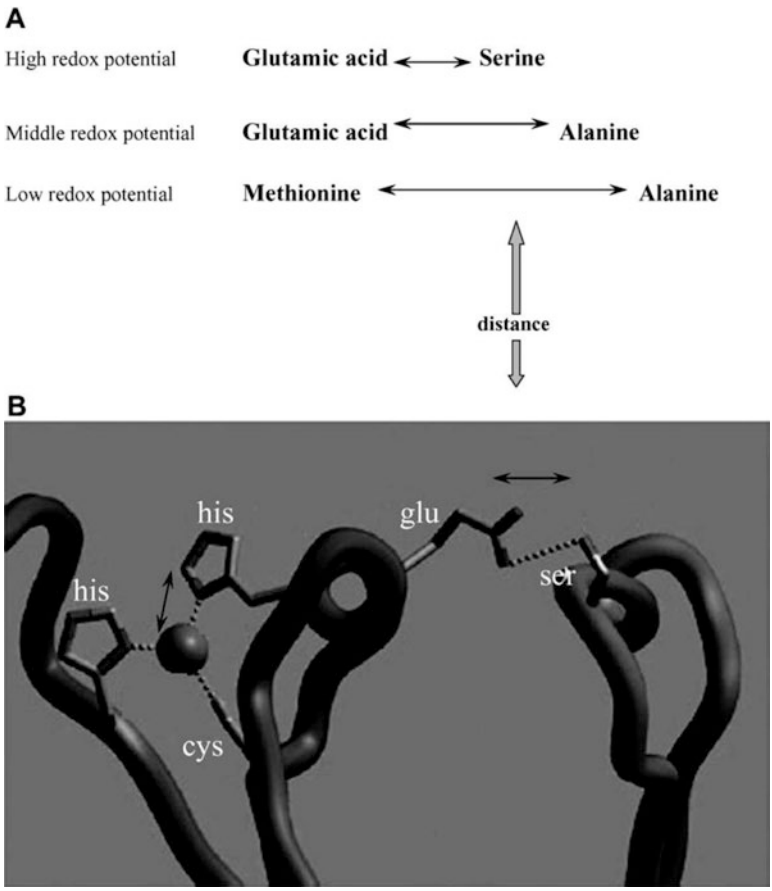


Fig. 6.2 Schematic drawing illustrating (**a**) the distance between significant amino acids for redox potentials of laccases, and (**b**) the movement of a helical segment in the enzymes. (According to Piontek et al. 2002)

rise to an elongated Cu-N bond (Fig. 6.2). In CcL, a methionine replaces Glu460, and a glycine replaces the position corresponding to Ser113. Consequently, an H-bond cannot be formed due to the lack of a suitable H-bond donor and acceptor (Piontek et al. 2002). Continue to say that the redox potential can be increased by more than 200 mV by reducing the contribution of electron density to the metal cation (Cu) by expanding the bond between the ligating amino acid and the metal. This movement may be induced by an effective hydrogen bond that results in the polypeptide segment being displaced, which carries the coordinating amino acid (Piontek et al. 2002).

6.5 Bioinformatic Analysis Revealed High Structural Diversity of Fungal Laccases

Ordinarily, laccases are monomeric extracellular glycoproteins with different molecular weights, extending between 50 and 140 kDa, with an incredible difference both in size (10–45% of the whole weight) and in glycosylation size (Claus 2004). Most fungal laccases contain a total of 520–550 amino acids, not counting the signaling peptide arrangement (~20 buildups) (Thurston 1994). Figure 6.3 represents the ordinary collapsing in Basidiomycete's laccase. The three cupredoxin-like domains, organized in a sequential manner (domains 1, 2, and 3 are depicted in green, yellow, and red, respectively). Each one of them has a β-barrel topology well known to all members of the MCOs (Medicaid managed care organizations) family (Giardina et al. 2010). Copper type 1 (Cu T1) is found in domain 3, whereas the

Fig. 6.3 Laccase's structure cupredoxin-like domains. The sequence shown corresponds to the 2QT6 laccase structure in *Lentinus tigrinus.* Domain one is represented in green, yellow for domain two, and red for domain three. (The sequences used to generate the figure were retrieved from the Protein Data Bank (www.pdb.org) and Classification of Protein Structure Database CATH (www.cathdb.info))

trinuclear center coordinates between spaces 1 and 3; both spaces give buildups for copper coordination. The structure is stabilized by two disulfide bonds; the primary bond found between domains one and three and the other one between domains one and two (Zhukova et al. 2010). Different alignment studies, more than 100 laccase sequences, have explained their tertiary structure. Their redox sites and copper coordination are exceedingly preserved (Kumar et al. 2003).

The eight ligands of His in the trinuclear cluster T2/T3 show an exceedingly conserved pattern with four His-X-His motifs. The X motif is a Cys that binds Cu T1, while the adjacent His bind each a Cu T3 site. At 35–75 amino acid distances, there is another His-X-His motif, and near to the amino terminal, there are two other motifs, separated by 35–60 amino acid residues (Solomon et al. 2008). Twelve amino acids act as ligands for four Cu atoms and are found within four segments of separate sequences with a length of 8 and 24 residues (L1–L4). This feature recognizes laccase from other blue multicopper oxidases. Moreover, laccases have an intraprotein homology between L1 and L3 as well as L2 and L4. This recommends that in laccase's evolution, a duplication event happened. The impressive active location conservation of numerous copper oxidases recommends that the activity related to the three diverse Cu sites was a early evolutionary event (Zumárraga et al. 2007). To illustrate the exceedingly preserved 3D structure, we conducted an alignment with out of 38 sequences of fungal laccase structures from Ascomycetes and Basidiomycetes (Table 6.1).

All sequences were obtained from Protein Data Bank (PDB) with already detailed crystallography structures. Alignment does not involve sequences with induced mutations and duplicate sequences and sequences with no available citation (Fig. 6.4). Since the first report conducted on laccase enzymes from fungal sources (Ducros et al. 1998), few crystallography laccases structures have been established. Up till now, 66 structures of laccases can be obtained from PDB. Thirty-eight are of fungal sources; 26 of them come from 12 taxa of Basidiomycetes, *Coprinopsis cinerea*, *Coriolus zonatus*, *Cerrena maxima*, *Coriolopsis gallica*, *Lentinus tigrinus*,

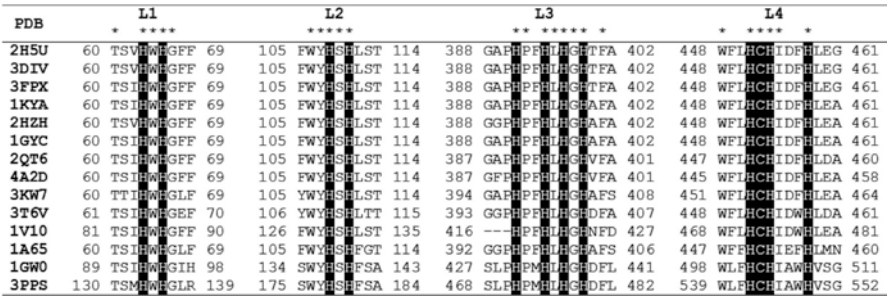


Fig. 6.4 Laccase ClustalW2 alignment. All sequences had determined crystallography structures. Identical positions are marked with an asterisk and joining sites between ligands (amino acids) with copper atoms indicated in black. L1–L4 represents four conserved regions of fungal laccases. The dashes represent gaps in the alignment; the numbers refer to the amino acid sequence Larkin et al. (2007)

Table 6.1 Laccase structures in Basidiomycetes and Ascomycetes fungi respectively

Fungi	Organism	Molecule	PDB	Resolution	Length	Chains	Weight
Basidiomycetes	*Coprinopsis cinerea*	Laccase	1A65	2.23	504	A	55,080.08
		Laccase-1	1HFU	1.68	503		55,367.24
	Trametes versicolor	Laccase	1KYA	2.40	499	A-B-C-D	221,719.74
		Laccase-2	1GYC	1.90		A	55,989.82
	Rigidoporus lignosus	Laccase	1V10	1.70	521		55,858.68
	Cerrena maxima		2H5U	1.90	499		55,670.18
	Coriolus zonatus		2HZH	2.60			54,122.88
	Trametes trogii		2HRH		496		54,247.49
			2HRG	1.58			55,104.45
	Lentinus tigrinus		2QT6	1.50	498	A-B	112,749.96
	Trametes hirsuta		3FPX	1.80	499	A	56,465.33
	Cerrena maxima		3DIV	1.76			56,220.02
	Trametes sp. AH28-2	Laccase B	3KW7	3.44	502	A-B	110,887.03
	Trametes hirsuta	Laccase	3PXL	1.20	499	A	56,354.35
	Coriolopsis gallica		4A2H	2.30	496		54,464.58
			4A2E	1.80			53,906.09
			4A2D	2.30			54,383.02
	Pycnoporus cinnabarinus		2XYB	1.75	497		57,999.61
	Trametes hirsuta		3V9C	2.00	499		56,402.35
	Steccherinum ochraceum		3T6W	2.15	495	A-B-C	163,303.82
			3T6V	2.00			163,207.82
			3T6X	2.15			163,303.82
			3T6Z				162,875.51
			3T71				163,207.82
	Coriolopsis gallica	Laccase	4A2G	1.80	496	A	53779.00
			4A2F	1.90			

(continued)

Table 6.1 (continued)

Fungi	Organism	Molecule	PDB	Resolution	Length	Chains	Weight
Ascomycetes	*Melanocarpus albomyces*	Laccase-1	1GW0	2.40	559	A-B	132,161.81
			2IH9	2.00			129,934.94
			2IH8				130,864.68
			2Q9O	1.30			131,889.56
			3DKH	2.40			130,716.04
			3FU9	2.00			128,050.20
			3FU8	1.80			130,976.90
			3FU7	1.67			131,164.01
			3QPK	1.90			129,328.47
	Thielavia arenaria	Laccase	3PPS	2.50	604	A-B-C-D	276,674.96
	Botrytis aclada		3SQR	1.67	580	A	66,902.22
			3V9E	1.70			66,429.93

These structures were recorded in Protein Data Bank (PDB) gotten by Crystallography and X-ray diffraction, resolution units are Å, weights are in Da and lengths are in amino acid (AA). Proteins with two or more chains are homooligomeric Fungi Organism Molecule PDB Resolution Length Chains Structure weight (Rivera-Hoyos et al. 2013)

Rigidoporus lignosus, *Steccherinum ochraceum*, *P. cinnabarinus*, *T. hirsuta*, *Trametes* sp., *T. versicolor* and *Trametes trogii*; and the remaining 12 structures come from 3 taxa of Ascomycetes, *Botrytis aclada*, *Melanocarpus albomyces,* and *Thielavia arenaria*. Many crystallography resolutions extending from 1.20 to 3.34 Å have been utilized to describe laccase 3D structures (Andberg et al. 2009; Bertrand et al. 2002; De la Mora et al. 2012; Ducros et al. 1998; Kallio et al. 2009, 2011a, b; Polyakov et al. 2009; Zhukova et al. 2010). Many contrasts have been noted as a consequence of the natural differences in molecules, protein extraction conditions, crystallography procedures resolution, or depending on X-rays doses. For illustration, 12 different crystallographic structures of laccases have been obtained from Ascomycetes. Two correspond to laccase from *B. aclada*, nine to laccase-1 from *M. albomyces*, and only one corresponds to a laccase from *T. arenaria* (Kallio et al. 2011a). They achieved structure 3PPS with a 2.5 Å resolution. This structure, 3PPS Asn, is the amino acid residue created for catalytic proton transfer in contrast to Gln in laccase-1 from *M. albomyces*. Additionally, the loops in the region of copper type 1 site shape the pocket where the substrate binds (Kallio et al. 2011a).

Ferraroni et al. (2007) acquired structure 2QT6 from a laccase enzyme in *L. tigrinus* solved at 1.5 Å of resolution. This structure revealed an asymmetric unit (quaternary St.) consisting of two molecules of laccase A and B. De la Mora et al. (2012) recorded five various structures for the same laccase from *C. gallica:* 4A2D, solved at 2.0 Å resolution; 4A2E, solved at 1.8 Å resolution; 4A2G, solved at 1.8 Å resolution, 4A2F, solved at 1.9 Å resolution; and 4A2H, solved at 2.3 Å resolution. Their differences depend on the truth they were extracted at various pH (4.5, 5.5, and 7.0, respectively) during the fugal isolation process. Structural alters were caused by

crystallography resolution variations and radiolysis and radiation-induced reduction. On the other side, the crystal structure of a blue laccase from *S. ochraceum* has been solved at 2.0 Å resolution, using classic data collection from a single crystal (3T6V). The main structural features are characteristic of this class of enzymes; moreover, distances within the trinuclear copper cluster are indicative of a reduction of the metal centers generated by free electrons produced during X-ray data collection. Besides, for this study, the authors obtained four additional structures with various X-ray dosages (3T6W, 3T6X, 3T6Z, and 3T71; all of them solved at 2.15 Å resolution).

Results obtained at progressively increased X-ray doses are aligned with reduction of copper centers which permits the binding of the oxygen; the dioxygen is reduced to peroxide, and as a result, an extra reduction is detected resulting in its splitting in two oxide/hydroxide ions (Ferraroni et al. 2012). Structural data obtained from crystallography are important for understanding the protein's properties, especially the catalytic site. To date, the number of laccase structures created by crystallography represents only a small fraction when compared to the broad variety of laccase isoforms recorded. The deficiency of data can be explained by complications, mainly related to isoform separation during laccase's crystallization process (Piontek et al. 2002). These difficulties result in data from structural identification that might not be consistent with facts. While it is important to high emphasize the efforts seeking to understand laccase's structures, despite the fact that the rest of the molecule is different among various laccases, a high degree of conservation is evident in domains related to copper resulting in what some authors have named "signature sequence" (Alcalde 2007) and have characterized laccases in a unique way.

6.6 Electron Transfer and Reaction Mechanism of Laccases

Laccase catalysis occurs with the reduction of one oxygen molecule to water followed by one-electron oxidation of a wide variety of aromatic compounds, including methoxy-substituted monophenols, polyphenols (Bourbonnais and Paice 1990), and aromatic amines (Bourbonnais et al. 1995). This oxidation results in the formation of free radicals based on oxygen which can be converted into quinone in a second catalyzed enzyme reaction (Gianfreda et al. 1999). Figure 6.5 illustrates the mechanism of laccase catalysis, modified from Baldrian (2006). The catalysis of laccase is viewed as consisting of three major steps (Gianfreda et al. 1999).

6.6.1 Mononuclear Copper Center Reduction

The reducing substrate (commonly phenolic compounds) loses an electron to laccase (Gianfreda et al. 1999). This electron reduces the T1 copper (at the mononuclear copper center) located just below the binding site of the substrate (Piontek

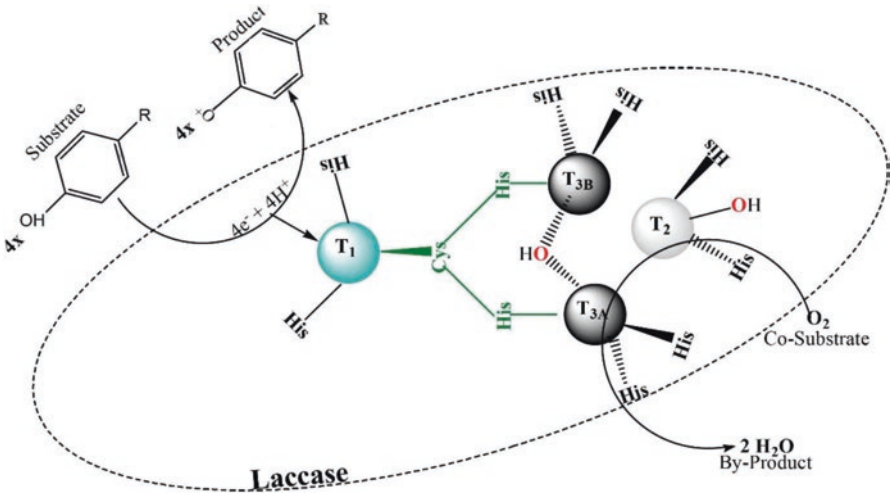


Fig. 6.5 Mechanism of laccase catalysis. (Modified from Baldrian 2006)

et al. 2002). The oxidized substrate has become a radical that can either donate the second electron to the T1 copper and become a quinone or participate directly in any nonenzymatic reactions that result in either polymerization or depolymerization. By passing the electron to the trinuclear copper cluster, the reduced T1 copper oxidizes itself. Thus, there are four such monoelectronic T1 copper reductions that occur sequentially (Gianfreda et al. 1999).

6.6.2 Internal Electron Transfer from the Mononuclear Copper to the Trinuclear Copper Center

A concept based on experimental evidence that the O_2 molecule first binds to the T2 and any of the T3 copper atoms has been suggested. This then undergoes asymmetric activation that results in the formation of four O-H bonds during the generation of two water molecules. The oxygen-binding pocket, in addition to molecular oxygen, appears to limit the entry to oxidizing agents that may account for the exclusivity of laccase for the substrate oxidation. that is molecular oxygen (Gianfreda et al. 1999), as contrasted to its low affinity for the substrate reduction.

6.6.3 *Reduction of Molecular Oxygen at the Trinuclear Copper Center*

The reduction occurs with the concomitant release of water at the trinuclear cluster (Claus 2004). Because of the direction that the nonenzymatic reactions take, this fundamental catalyzed laccase reaction has two very divergent fates (Mayer and Staples 2002). They could also result in polymerization by the cross-linking of monomers or depolymerization of existing polymers (Claus 2004). The destiny of the reaction depends on the type of laccase that catalyzes the reaction as well as the reaction's direct microenvironment. Ascomycete laccases like the *Melanocarpus albomyces* laccase (MaL) have different structures than basidiomycete laccases such as TvL. The oxygen attaches here with a novel geometry; the cosubstrate oxygen enters the trinuclear cluster via a tunnel that is completely open in basidiomycete laccase while in MaL, the C-terminus forms a mobile plug that can obstruct this entry.

So, it may be captured by this plug after the oxygen has entered, resulting in stabilized binding of dioxygen. This C-terminal blockage of ascomycete laccase significantly decreases the rate of the free inflow of O_2 and the release of water molecules, thus providing the oxidized free radicals in the surrounding atmosphere a chance to polymerize, while in the case of basidiomycete laccase, the rapid exchange of O_2 and water does not enable free radicals to build up in the microenvironment thus avoiding polymerization. This blockage of the C-terminal is probably characteristic of ascomycete laccase and may clarify why ascomycete laccase is usually involved in polymerization reactions, whereas basidiomycete laccase is involved in depolymerization reactions (Hakulinen et al. 2002). Therefore, laccase catalyzes two opposite reaction types—depolymerization and polymerization reactions using one single action mechanism, free radical formation. Both laccase systems are a by-product of such two opposing reactions.

6.7 Laccase Cultivation

Fungi, especially filamentous fungi, are used for the large-scale production of laccase enzymes in different cultivation techniques. Laccase enzyme can be produced by fungal species under both solid-state and submerged fermentation using natural and synthetic media for enhancing the production yield (Sharma and Kuhad 2008). Laccase enzyme produced by both solid-state and submerged fermentation is higher in the case of rice bran than other substrates. The rice bran inductive capability is based on phenolic compounds such as vanillic acid and ferulic acid which induces laccase production (Munoz et al. 1997). Many agricultural wastes such as grape stalks, grape seeds, barley bran (Lorenzo et al. 2002), cotton stalk, molasses waste water (Kahraman and Gurdal 2002) bran, and wheat (Souza et al. 2002) are also used as a substrate for laccase production.

6.7.1 Qualitative Assays and Quantitative Determinations of Laccases

Recent studies have reported that ligninolytic enzymes can be produced using plant raw materials as substrates. Production of ligninolytic enzymes, particularly the production of Laccases, should be enhanced for optimum utilization. Optimization of laccase production includes searching for new microbial strains and modifying the physicochemical needs of the potential microbial strains like WRF (white Rot Fungi). For instance, WRF which were isolated and identified from Dagaga-Gambo natural and plantation forests were qualitatively assayed for laccase productions and then laccases of the potential WRF were characterized and their productions were optimized.

6.7.2 Qualitative Assays of WRF for Laccase Production Potential

WRF were qualitatively screened utilizing the lignin-modifying fungal enzymes basal medium (LBM) supplemented with tannic acid (Pointing 1999). The ligninolytic activity was examined for 10 days, and the appearance of brown oxidation zone below and around cultures was recorded. The culture diameter and colored zone

$$E1 = \frac{\text{Diameter of hydrolysis zone}}{\text{Diameter of colony}}$$

6.7.2.1 Quantitative Estimation of Fungal Laccases

6.7.2.1.1 Inoculum Preparation

Inocula of the chosen ligninolytic fungi were prepared using a standard medium of Altaf et al. (2010). Four disks (Ø 0.5 mm) of each isolate were inoculated and grown in 100 mL of the standard media in 250 mL flask at 150 rpm and room temperature. After 6 days of incubation, the mycelial pellets were harvested, homogenized, and utilized as inocula for submerged and solid-state fermentations in the quantification of Laccases.

6.7.2.1.2 Submerged Fermentation

SmF included the growing of microorganisms in high oxygen concentrated liquid nutrient medium. But viscosity of broth is a major problem associated with the fungal submerged fermentations. So different strategies have been involved to recover this problem. Bioreactor operates in a continuous manner for obtaining high efficiency. Species of *Trametes versicolor* is employed which decolorizes the synthetic dye, and for this purpose, pulsed system has been developed (Blanquez et al. 2007). Broth viscosity, oxygen, and mass transfer problems are solved by cell immobilization. A study by Luke and Burton (2001) reported that continuous laccase production takes place without enzyme deactivation for a period of 4 months due to the immobilization of *Neurospora crassa* on the membrane. For bioremediation of pentachlorophenol (PCP) and 2,4-dichlorophenol (2,4 DCP), nylon mesh is used for comparing the free cell culture of *T. versicolor* with immobilized cultures. At the same time, Schliephake et al. (2000) produced laccase by *Pycnoporus cinnabarinus* immobilized on cubes of nylon sponge in a 10-L packed bed bioreactor operated in a batch mode. Also, Park et al. (2006) found that immobilization of the white-rot fungus Funaliatrogii in Na-alginate beads allowed the efficient decoloration of dye Acid Black 52. Another factor affecting laccase production is agitation.

This study investigated that in fixed bed bioreactors, stainless steel showed the highest laccase activity among different synthetic materials that were used as carriers for the immobilization of *Trametes hirsute* (Sedarati et al. 2003). Also, Hess et al. (2002) found that laccase production by *Trametes multicolor* decreased considerably when the fungus was grown in stirred tank reactor, presumably because of damage to mycelia. A study by Mohorcic et al. (2004) found that it was possible to cultivate the white-rot fungus *Bjerkandera adusta* in a stirred tank reactor after its immobilization on a plastic net although very low activities were attained. Tavares et al. (2006) observed that agitation did not play an important role in laccase production by *T. versicolor*. Fed-batch mode of operation is shown to be an effective way of producing laccase. Also, Galhaup et al. (2002) found that operating in fed batch increased the laccase production of *T. pubescens* by twofold and obtained a higher laccase activity. The laccase enzyme was extracted from *Pleurotus ostreatus* PVCRSP-7 species through submerged fermentation method by using statistically optimized media components, which had shown higher laccase production as well as higher enzyme activity up to 2.5-fold compared to the normal production method (Chiranjeevi et al. 2014).

6.7.2.1.3 Solid-State Fermentation

SSF is defined as a fermentation process occurring in the absence or slight absence of free liquid, employing an inert substrate (synthetic materials) or a natural substrate (organic materials) as solid support (Pandey et al. 1999). SSF is shown to be suitable for the production of enzymes by filamentous fungi because they mimic the conditions under which the fungi grow naturally (Kumar and Mishra 2011), so SSF

is considered one of the best methods for the culture of filamentous fungi and the production of ligninolytic enzymes because they are grown under conditions that emulate their natural habitat and as a result can produce certain enzymes at high levels, higher than in submerged fermentation conditions (Kumar and Mishra 2011).

The lignin, cellulose, and hemicelluloses are rich in sugar and promote fungal growth in the fermentor and make the process more economical (Couto and Toca-Herrera 2007). Gomez et al. (2005) report the use of lignocellulosic wastes as substrate, resulting in a 25-fold increase in the production of laccase using the fungus *Coriolopsis rigida* using SSF. Also, Moldes et al. (2003) used grape seeds as a lignocellulosic source to increase the activity of *T. hirsute* tenfold. The major drawback is the bioreactor design in which heat and mass transfer are limited. The major disadvantage with SSF is the lack of any established bioreactor designs. So different bioreactor configurations have been studied for laccase production such as immersion configuration. Couto et al. (2003) tested three bioreactor configurations immersion, expanded bed and tray for laccase production by *T. versicolor* using, and inert (nylon) and noninert support (barley bran). They found that the tray configuration led to the best laccase production. Couto et al. (2006) make comparison between tray and immersion configurations for the production of laccase by *T. hirsuta* using grape seeds as substrate, resulting in tray configuration gave the best results. Also, a study by Rosales et al. (2007) reports tray configuration produced higher laccase activity in *T. hirsuta* cultures raised on orange peels.

6.7.3 Optimization of Fungal Laccases Production

Laccases were generally produced during the secondary metabolism of different fungi growing on a natural substrate or in submerged culture. Various cultivation parameters such as carbon source (sugars or lignocellulosic residues), nitrogen source, pH, temperature, Aromatic Compounds, microelements, and biological interaction influence laccase production (Missall et al. 2005; Lorenzo et al. 2006). Gayazov and Rodakiewicz-Nowak (1996) reported faster laccase production under semi-continuous production with high aeration and culture mixing compared to static conditions. It was found that the production of high titers of laccase was not dependent on high biomass yields. Subsequent sections delineate the role of different process parameters in laccase production.

6.7.3.1 Influence of Carbon on Laccase Production

Breakdown of carbon sources liberates energy that is utilized by all organisms for growth and development. Glucose is the most readily available carbon source used by white-rot fungi (Levin et al. 2002). The use of excessive concentrations of glucose as a carbon source in the cultivation of laccase-producing fungal strains has an inhibitory effect on laccase production. An excess of sucrose or glucose reduces the

production of laccase, as these components allow constitutive production of the enzyme. A simple but effective way to overcome this problem is the use of cellulose as a carbon source during cultivation (Eggert et al. 1996b). Glucose at (5 g/L) in the liquid medium supported laccase production by *Trametes gallica* (Buswell et al. 1995). Collins and Dobson (1995) reported that glucose at (10 g/L) enhanced the growth and laccase production by *Coriolus versicolor*. In *Ganoderma lucidum*, glucose at (20 g/L) increased the mycelial growth but at (10 g/L) favored for expression of the enzyme (Perumal 1997). In *Trametes versicolor*, the amount of glucose at higher concentration (20 g/L) is favored for laccase production (Minussi et al. 2007b). The maximum titers of extracellular laccase in cultures of *Lentinula edodes* and *Grifola frondosa* were grown in a liquid medium with (10 g/L) glucose (Cavallazzi et al. 2005). Maximum laccase production was obtained using response surface methodology with glucose (15.21 g/L) as the carbon source for *Pleurotus florida* NCIM 1243 (Palvannan and Sathishkumar 2010).

Among several carbon sources tested, the malt extract was found to be the best carbon source in the medium for pronounced laccase production by *Phlebia floridensis*, *P. brevispora*, *P. radiata*, and *P. fascicularia* (Da Cunha et al. 2003). A study by D'Souza-Ticlo et al. (2006a, b) screened different carbon sources for maximum laccase production by *Botryosphaeria* sp. They have screened glucose, fructose, galactose, galacturonic acid, xylose, lactose, sucrose, mannitol, pectin, and inulin and found increased laccase production with most carbon sources studied except inulin and galacturonic acid.

6.7.3.2 Influence of Nitrogen Sources on Laccase Production

Laccases of white-rot fungi are mainly activated during the secondary metabolic phase of the fungus and are often triggered by nitrogen depletion (Leatham and Kent Kirk 1983). Fungal laccases are often triggered by nitrogen depletion, but it was also found that in some strains, nitrogen concentration had no effect on the enzyme activity (Leatham and Kent Kirk 1983). In a study by Buswell et al. (1995) it was found that laccases were produced at high nitrogen concentrations although it is generally accepted that a high carbon to nitrogen ratio is required for laccase production. Laccase was also produced earlier when the fungus was cultivated in nitrogen-rich media rather than the nitrogen-limited media (Heinzkill et al. 1998). A study by Chen et al. (2003b) reported a rise in nitrogen concentration from (0.25 to 2.0 g/L) enhanced laccase synthesis yield. Higher nitrogen levels are often required to enhance laccase production (Baldrian and Gabriel 2002) but with certain fungi, nitrogen-limited culture conditions simulate the formation of the laccase enzyme (Lo et al. 2002). The optimum nitrogen concentration for obtaining the highest laccase activity from *Pycnoporus sanguineus* (820 mU/mL) is provided by a sucrose-asparagine medium containing five times as much asparagine as Kirk's medium (Sharma and Arora 2010).

The peptone considered as greatest nitrogen source improved laccase production (1.8-fold increase) (Strong 2011). Revankar and Lele (2006) obtained the highest

laccase activities by *Trametes versicolor* MTCC 138 by using a complex nitrogen source (yeast extract). Leatham and Kent Kirk (1983) screened different nitrogen sources, namely, KNO_3, glutamic acid, beef extract, glycine, and corn steep liquor, which resulted in glutamic acid with low concentration yielding higher amounts of laccase.

6.7.3.3 Influence of pH on Laccase Production

The pH of the culture medium is critical and plays a significant role in the growth and laccase production of the organism. The information about the influence of pH on laccase production is little available, but when fungi are grown in a medium with pH 5.0, laccase will be produced in excess (Thurston 1994). Most reports indicated initial pH levels set between pH 4 and 6 prior to inoculation, but the levels were not controlled during most cultivations (Thurston 1994). The optimum pH of laccase production, as reported in many fungi, falls between 5 and 6 (Papinutti et al. 2003). In white-rot basidiomycetes *Fomessclero dermeus* show maximum titers of laccase and biomass were observed in the medium adjusted to pH 6, the optimal range for the laccase isoforms secreted by *Trametes pubescens* fungal strain has been reported between pH 3 and 4.5 (Strong 2011). Laccases from fungi have been found in wide applications ranging from the pharmaceutical sector to the pulp and paper industry.

6.7.3.4 Influence of Temperature on Laccase Production

The temperature always plays a vital role in the growth and laccase production of the organism. It has been found that the optimal temperature for fruiting body formation and laccase production is 25 °C in the presence of light but 30 °C for laccase production when the cultures are incubated in the dark (Thurston 1994). In general, the fungi have been cultivated at temperatures between 25 and 30 °C for obtaining optimal laccase production (Pointing et al. 2000). But in other reports, laccase-producing fungi cultivated at temperatures higher than 30 °C, the activity of laccase was reduced (Zadrazil et al. 1999). The wood-decaying basidiomycete *Steccherinum ochraceum* isolate 1833 was reported to produce three highly thermostable laccase isoforms with maximum activities in the region 75–80 °C (Hilden et al. 2009). So, the optimum production of laccase can differ greatly from one strain to another under different temperatures.

6.7.3.5 Influence of Metal Ions on Laccase Production

The induction of laccase by metal ions can be explained in terms of defense from toxic stress, as the laccase enzyme is involved in the synthesis of pigments to prevent metal uptake (Lorenzo et al. 2006). The effect of copper on laccase synthesis was also effective for several other basidiomycetes and hence could be used as a

simple method to improve the production of the enzyme (Huber and Lerch 1987). Different isoforms exhibit different characteristics with respect to copper in *P. ostreatus* (Tinoco et al. 2001). The addition of copper sulfate (150 μM) to the medium culture resulted in an increase in the production up to 500-fold of certain laccase isoforms, as in the case of laccase isoform POXA1b, while isoforms were not affected, such as POXA1w (Palmieri et al. 2000). Copper has been reported to be a strong laccase inducer in several species, for example, *Grifola frondosa*, *Lentinula edodes*, *Neurospora crassa*, *Shiraiabam busicola strain* GZ11K2, *Phanerochaete chrysosporium*, *Pleurotus sajorcaju*, *Panus osteratus*, *Pleurotus florida* NCIM1243, *Peniophora* sp., *Paecilomyces* sp. WSHL07, *Trametes versicolor*, *Trametes trogii*, and *Volvariella volvacea* (Viswanath et al. 2014). The metal concentration added to the culture medium affected the LacI/LacII ratio. The presence of Mn^{2+}, Cd^{2+}, or Zn^{2+} in the culture medium increased the LacI/LacII proportion by nearly 100% in comparison to the control cultures. The highest LacI/LacII activity ratio approximately (0.51) was obtained from cultures with (5 mM) copper sulfate, attaining values 360% and 155% higher than those obtained from cultures with (2–3.5 mM) copper sulfate, respectively (Lorenzo et al. 2006).

Pleurotus ostreatus produces four different laccases in potato-dextrose medium supplemented with yeast extract and $CuSO_4$ (Palmieri et al. 2000). Also, this study demonstrated that copper increases the transcription of the laccase genes poxc and poxa 1b of *P. ostreatus* (Faraco et al. 2003). In other studies, Klonowska et al. (2001) reported that *Marasmius quercophilus* produces only one laccase (LacI) in a liquid medium with malt extract. The medium supplemented with $CuSO_4$ permits the induction of three other isoforms, increasing the total activity ten times. Additionally, cultures supplemented with $CuSO_4$ and *p*-hydrobenzoic acid exhibited a 30-fold increase in total activity compared to basal production. Palmieri et al. (2000) found that the addition of (150 μM) copper sulfate to the cultivation media can result in 15-fold increase in laccase activity compared to a basal medium. Huber and Lerch (1987) reported that *Trametes pubescens* grown at (2.0 mM) $CuSO_4$ exhibited high laccase activity (65 U/mL).

6.7.3.6 Influence of Biological Interaction on Laccase Production

Laccases are important to the virulence of many fungal pathogens or as a defense mechanism in ligninolytic fungi, so study the effects of changes in extracellular enzymatic activities during fungal co-cultures is important (Missall et al. 2005). Laccase induction and production have been examined in many cultures of *Lentinula edodes*, *Trametes versicolor*, and *Pleurotus ostreatus* infected with *Trichoderma* sp. Savoie and Mata (1998) reported the induction of laccase from *Lentinula edodes* in liquid cultures challenged with *Trichoderma* sp. Hatvani et al. (2002) also studied the existence of laccase repression in Lentinula during coculture with different strains of *Trichoderma* and induction with their supernatants. A study by Baldrian (2006) reported an increase in production in solid-state laccase cultures by 18 strains of ligninolytic fungi in response to a strain of *Trichoderma harzianum*. The

production and profile of laccase isoforms from *Agaricus bisporus* and *Pleurotus ostreatus* as a function of the type of infection with *Trichoderma* sp. was studied by Flores et al. (2009). In their studies, solid cultures were used to evaluate the effects of the interaction with extracellular metabolites. These results revealed an increase in the production of laccase in in vitro cultures (in a solid medium) of *Agaricus bisporus* and *Pleurotus ostreatus* infected with non-laccase producing strains of *Trichoderma*.

6.7.3.7 Influence of Aromatic Compounds on Laccase Production

Laccase production by ligninolytic fungi can be considerably stimulated by a wide variety of aromatic compounds related to lignin and its derivatives (Marqués de Souza et al. 2004). Aromatic compounds that are structurally related to lignin, such as xylidine, ferulic acid, and veratric acid, are routinely added to fungal cultures to increase laccase production (Arora and Rampal 2002). Lu et al. (1996) found that the addition of xylidine as an inducer had the most pronounced effect on laccase production. The addition of (10 μM) xylidine after 24 h of cultivation gave the highest induction of laccase activity and increased laccase activity by ninefold. At higher concentrations, the xylidine had a reduced effect probably due to toxicity. Xylidine is known to increase laccase transcription in *Trametes villosa*, *Trametes versicolor*, and *P. sajorcaju* (Viswanath et al. 2014). A dark precipitate was observed in xylidine-induced cultures of *T. versicolor* and has been suggested that it may represent a laccase polymerized form of aromatic compounds (Soden and Dobson 2001). Rodrıguez-Couto et al. (2002) reported the production of laccase in *Trametes versicolor* in semi-solid-state fermentation and proved that the addition of xylidine caused laccase activity to reach approximately (1700 U/L). Xylidine is the most widely reported inducer of laccase production and enhanced laccase specific production by fourfolds in *Coriolopsis polyzona* (Jaouani et al. 2006).

Earlier studies on white-rot fungi have shown that methylation of lignin-related aromatics inhibits fungal growth only at higher concentrations (5 and 10 mM) with stimulation occurring at (1 mM) concentration (Barbosa et al. 1996). The type and composition of the medium culture and the use of inducers play important roles in the productivity and profile of the laccases obtained, for example, in *Trametes* sp., the genes lcc1 and lcc2 were induced in cultures where veratrilic acid was added, while the gene lcc3 was not induced by this compound and was repressed by glucose (Mansur et al. 1998). The use of ferulic acid and vanillin increased laccase production ten times in submerged cultures of *Pleurotus pulmonaris* (Marqués de Souza et al. 2004). These authors demonstrated that while LacI and LacII were produced in non-induced cultures, the cultures supplied with vanillin and ferulic acid only produced lacII and lacIII. Veratryl (3,4-dimethoxybenzyl) alcohol is an aromatic compound known to play an important role in the synthesis and degradation of lignin. The addition of veratryl alcohol to the cultivation media of many white-rot fungi has resulted in an increase in laccase production (Lee et al. 1999). Some of these compounds affect the metabolism or growth rate, while others, such

as ethanol, indirectly trigger laccase production (Dhawan and Kuhad 2002). There are many reports describing the different effects of aromatic compounds on laccase activity. Also, the highest laccase activity was observed in *Botryosphaeria rhodina* when veratryl alcohol was added to the nutrient medium at the beginning of fermentation (Cambria et al. 2011).

6.8 Purification and Biochemical Properties of Laccases

Production of extracellular laccase is a common feature of many fungi, particularly those associated with wood decay or the terminal stages of decomposition of leaf litter. Several purification steps are required to obtain a preparation free of both pigment and other contaminant proteins. Multiple steps like ultrafiltration, precipitation using ammonium sulfate or organic solvents, and ion exchange and size exclusion chromatography have been used for the purification of laccases from the culture filtrate. Typical fungal laccase is a protein of approximately (60–70 kDa) with an acidic isoelectric point around 4.0 (Baldrian 2006). Several laccase isoenzymes have been detected in many fungal species. More than one isoenzyme is produced in most white-rot fungi (Palmieri et al. 1997). This has been demonstrated by *p*-phenylenediamine staining the laccase activity in all tested wood rot fungi. All tested species exhibited the production of more than one isoenzyme typically with p*I* in the range of pH (3–5), for example, *Coprinus plicatilis*, *Fomes fomentarius*, *Kuehneromyces mutabilis*, *Heterobasidio nannosum*, *Hypholoma fasciculare*, *Leptoporus litschaueri*, *Stereum hirsutum*, *Panus stipticus*, *Phellinus igniarius*, *Pleurotus corticatus*, *P. ostreatus*, *Polyporus brumalis*, *Trametes gibbosa*, *T. hirsuta*, and *T. versicolor* (Baldrian 2006).

Many researchers have found much more efficient methodologies such as protein precipitation by ammonium sulfate, anion exchange chromatography, desalt/buffer exchange of protein, and gel filtration chromatography. Single-step laccase purification from *Neurospora crassa* takes place by using celite chromatography and 54-fold purification was obtained with a specific activity of (333 U/mg) (Grotewold et al. 1998). Laccase from *T. versicolor* is purified by using ethanol precipitation, DEAE-Sepharose, Phenyl-Sepharose, and Sephadex G-100 chromatography which is a single monomeric laccase with a specific activity of (91,443 U/mg) (Hess et al. 2002). Laccase from *T. versicolor* is purified with Ion Exchange chromatography followed by gel filtration with a specific activity of (101 U/mL) and 34.8-fold purification (Cordi et al. 2007). Laccase from *Stereum ostrea* is purified with ammonium sulfate followed by Sephadex G-100 column chromatography with 70-fold purification (Viswanath et al. 2008).

Laccase from fruiting bodies is purified with ammonium sulfate precipitation with 40–70% saturation and DEAE cellulose chromatography then 1.34- and 3.07-fold purification is obtained, respectively (Khammuang and Sarnthima 2009). The catalytic action of an enzyme is quantitatively described by the Michaelis constant KM and the catalytic efficiency constant *k*cat. The KM values of laccases are

generally in the range of (2.5 μM) depending on the enzyme source and the reducing substrate. The comparison of KM values also shows that laccases from different source organisms have different substrate preferences (Yaver et al. 1999). On the other way, the *k*cat values for a single laccase do not generally differ more than two- to tenfold between different substrates, which reflects the fact that *k*cat describes the rate of the electron-transfer reactions taking place inside the enzyme after substrate binding (Xu 1997).

6.9 Lacasse and Chemical Mediators: Mimicking Nature

The high pertinence of laccases is attributed to their wide substrate specificity to the utilization of molecular oxygen as electron acceptor and the generation of water as the sole reaction by-product. Nevertheless, these green biocatalysts have a quite low redox potential (≤0.8 V) that restricts their oxidation of phenolic compounds (Rivera-Hoyos et al. 2013) although nonphenolic substrates cannot be oxidized by laccases instantly. At all intents and purposes, substrates I a is quite large to penetrate into the enzyme active site or characterized by high redox potential cannot be oxidized. However, laccases are considered to play a main role in the lignin degradation process by white-rot fungi (Thurston 1994). Appropriate compounds, that called mediators, can act as electrons shuttles, empowering laccases to indirectly oxidize large molecules and even nonphenolic substrates (Bourbonnais et al. 1997). Once oxidized by the enzyme, mediators diffuse far away from the enzymatic pocket and can oxidize target compounds that in the rule are not substrates of laccase by different mechanisms from the enzymatic one. A potential redox mediator should be a small-sized compound able to produce stable radicals that do not inactivate the enzyme, its oxidized radical form should have a half-life long enough to permit its diffusion toward the substrate, and its reactivity would permit recycling without degeneration. For example, lignin is a core component of the plant cell. It is a complex and amorphous aromatic polymer with 20–30% of the dry weight of wood (Ralph et al. 2007).

Monomers forming lignin are conyferil, *p*-coumaril, and sinapyl alcohols; they differ from one another by the methoxylation degree. These monomers produce phydroxyphenyl, guaiacyl, and syringyl phenylpropanoid units, which are able to generate electron delocalized radicals that couple at different sites (Ralph et al. 2007). Although laccases are part of the lignin synthesizing system in plant wood tissues, the function of laccases in white-rot fungi is to depolymerize and mineralize lignin. Laccase is a large molecule (Rodgers et al. 2010) that cannot deeply penetrate the wood as it has a low redox potential (≤0.8 V) in comparison to ligninolytic peroxidases (>1 V), laccase can only oxidize phenolic lignin fragments. Nevertheless, the number and form of substrates oxidized by laccase can be expanded by a process the participation of redox mediators. These mediators are low-molecular-weight compounds that can be readily oxidized by laccase, creating highly reactive and

unstable cationic radicals. However, at once, these cationic radicals could oxidize complex compounds (not involving phenolic substrates) before returning to their original state (Torres et al. 2003).

As mentioned before, this mechanism enables mediators to act as diffuse electron transporters, letting the indirect oxidation of polymeric substrates, such as lignin, to penetrate even to less accessible areas of its structure. In addition, because of mediator use, laccases are capable of oxidizing compounds with a higher redox potential than their own; an instance of this is the oxidation mediated by polycyclic aromatic hydrocarbons or PAHs (Riva 2006). Since Bourbonnais has shown that mediator inclusion has increased the catalytic activity of laccase to nonphenolic substrates (Bourbonnais and Paice 1990), more than 100 various mediators have been characterized with ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and the more widely used HBT (1-hydroxybenzotriazole) (Rivera-Hoyos et al. 2013). This laccase-mediator system can be applied to pulp and paper bleaching technology (Bourbonnais et al. 1997; Call and Mücke 1997) as well as bioremediation of xenobiotic compounds such as PAHs (Alcalde et al. 2002; Bourbonnais et al. 1997; Call and Mücke 1997). Additionally, it has been established that the hybrid of two or more mediators (namely, ABTS and HBT) can evolve a synergistic effect on oxidative activity (Pickard et al. 1999). However, the increase in commercial costs of chemical mediators, their high toxicity, and insufficiency studies on derivative impacts, in addition to inactivation caused by their cationic radical exertion on laccases the implementation of laccase mediator system is still limited.

For these reasons, the employment of natural mediators may have environmental and economic advantages. Several compounds included in the natural degradation of lignin can act as mediators in an efficient manner. Such is the case for lignin degradation compounds derived from oxidized lignin units, or those secreted by white-rot fungi. Similarly, the natural mediators 4-hydroxybenzoic acid, 4-hydroxybenzilic alcohol, veratrilic alcohol, syringaldehyde, vanillin, acetosyringone, and *p*-coumaric acid, between others, have been tested with various laccases with Close outcomes to those reached with artificial mediators. A clear instance is the recently PAHs described degradation of benzo[a]pyrene, pyrene, and anthracene using a laccase-mediator system from *p*-coumaric acid and *P. cinnabarinus* (Cañas and Camarero 2010) be assembled to acquire a quaternary structure. Data reached by some authors suggest that these enzymes may also act as monomers (Giardina et al. 2010).

A few laccases that display a homodimeric structure have been isolated. Such is the case for those obtained from Basidiomycetes *T. villosa* (Yaver et al. 1996), *Phellinus ribis* (Min et al. 2001), phytopathogenic Ascomycetes, *R. solani* (Wahleithner et al. 1996), *G. graminis* (Edens et al. 1999), and also for the Ascomycete *Phoma* sp. UHH 5-1-03. It is recognized that some of these enzymes show a pH-dependent dimerization as seen in *Phoma* sp. UHH 5-1-03, where the predominant dimeric state happened at a pH range of between 5.0 and 8.0 (Junghanns et al. 2009). Dimer formation is essential for the proper functioning of the enzyme as seen in homodimeric laccases with two subunit domains extracted from Pleurotuspulmonarius, *P. eryngii* (Wang and Ng 2006), and mycorrhizal fungus

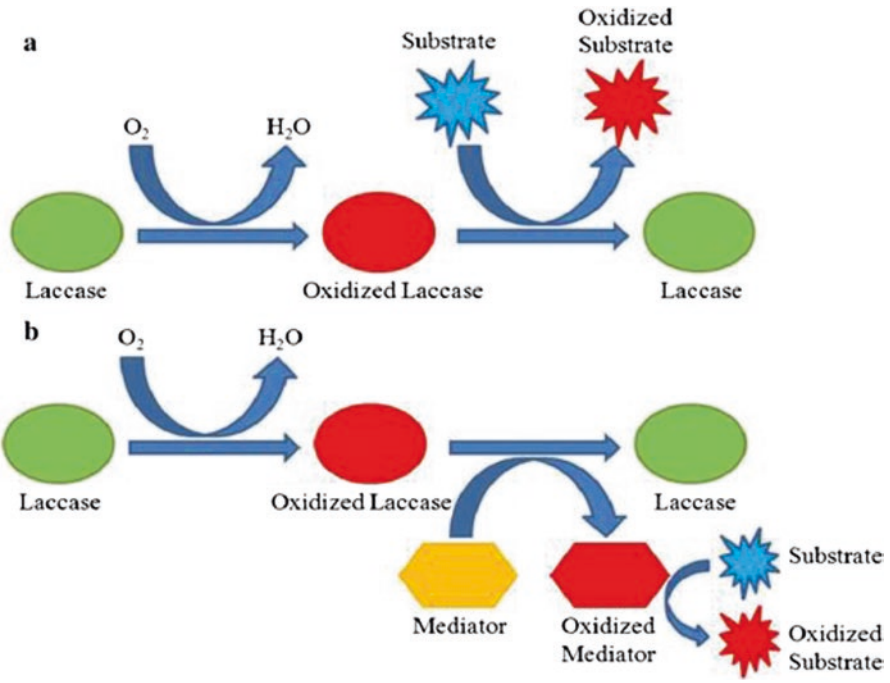


Fig. 6.6 Schematic representation of the reaction catalyzed by laccase; (**a**) direct oxidation: the substrate is oxidized to corresponding radical as a result of direct interaction and (**b**) in-direct oxidation: the substrate is oxidized in the presence of a mediator (Agrawal et al. 2018)

Cantharellus cibarius (Ng and Wang 2004). As mentioned before, laccases contain four copper atoms detected at a wavelength peak close to 600 nm. Even enzymes capable of oxidizing polyphenols, methoxy-substituted phenols, aromatic diamines, and a broad variety of other compounds not containing tyrosine, "yellow" and "white," laccases should be known (Fig. 6.6).

6.10 Laccases-Producing Fungi from Extreme Habitats

Fungi living in extreme habitats like low or high temperatures (psychrophiles and thermophiles), high salinity (halophiles), acidic or alkaline pH values (acidophiles and alkaliphiles, respectively), high pressures (barophiles), anoxygenic conditions (anaerobic fungi), etc. are known as extremophilic fungi (Yadav 2020; Yadav et al. 2020a; Kumar et al. 2019) (Fig. 6.6b). Many reports are available on extracellular phenol oxidase by fungi from various habitats, but scarcely studies on laccase-producing fungi from extreme habitats such as marine, hot springs, and soda lakes are available (Prakash et al. 2019).

6.10.1 Alkaline-Tolerant Laccases from Alkaliphilic Fungi

The hyperalkaline habitat has both industrial and ecological importance as in high alkaline conditions very few fungi can grow. It has been recorded that most of the metropolitan wastewater treatment plants and effluents from industries have high alkalinity and high concentration of metal particles. Consequently, fungi surviving in such conditions and with laccase-producing capacity can work as great bioinoculant for bioaugmentation-based bioremediation.

Functional metagenomic studies of Soda Lake have revealed that many uncultured fungi have laccases-like Cu-oxidase encoded with potential in degradation of phenolic compounds (Vavourakis et al. 2016). Sharma et al. (2016) isolated 104 fungal strains from Lonar lake, a hyperalkaline environment, and 14 were positive for enzyme production in primary screening using 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as substrate. It included *Cladosporium oxysporum*, *Fusarium equiseti*, *Curvularia lonarensis*, *Cladosporium funiculosum*, *Cladosporium halotolerans*, *Aspergillus niger*, a probable novel *Cladorrhinum* species.

Among these fungi, *Fusarium* sp. MEF008, *Curvularia lonarensis* MEF018, *Cladorrhinum* sp. MEF109 and *Cladosporium* sp. MEF135C, and alkaliphilic fungus with potential to be exploited industrially produced laccases at 40 °C, pH 12–14, and at a salinity of 3%. While working on Lonar Lake (Prakash et al. 2019), alkaline-tolerant and thermostable laccase are preferred by the industries, but due to restricted information, its application has not been feasible commercially. However, these limitations can be overcome by the use of different immobilization techniques which can help to improve the stability of laccase (Agrawal et al. 2018). Alkaline-tolerant laccases have their significance in a number of industrial applications, but their application is not conceivable at a commercial scale as few laccases have been recognized till date, for example, in hair-coloring industries, alkaline-tolerant laccase is more favored. Within the work done by Saito et al. (2012), laccase was utilized for the improvement in hair coloring items that was most powerful at pH 9. Additionally, Singh et al. (2007) isolated fungal taxa which may tolerate pH range of 4–10 and was utilized for the degradation of indigo carmine dye. In any case, more investigation is required for the effective isolation and identification of chemicals that can tolerate different pH and have its application within the industrial division.

Laccases display extraordinary industrial potential, but due to restrictions such as less tolerance to high temperatures, less stability, expensive stimulators, and purification processes, their application is limited. The activated thermostable enzyme was reported by (Coll et al. 1993) and from different studies carried out, it was found that packing of protein increases helical fold content, the density of H-bonds, salt bridges, proportions of amino acids, distribution of charged residues, and glycosylation. These factors are responsible for improving the thermostability of the enzyme (Kumar and Nussinov 2001). However, intense study is needed which could help in the identification of thermostable laccase for its utilization in the industrial sector.

6.10.2 Laccase from Marine Fungi

The applications of laccases in the degradation of xenobiotics by aquatic, obligate marine (and marine-derived) fungi have been noted (Martin et al. 2009; Junghanns et al. 2009). These marine fungi produce unique secondary metabolites and enzymes not recorded from fungi dwelling in terrestrial habitats (Jensen and Fenical 2002). D'Souza-Ticlo et al. (2009a, b) recorded that a marine isolate of Cerrena unicolor MTCC 5159 produces halotolerant laccase and moreover degrades raw textile mill effluents (Verma et al. 2010). In general, marine fungi are capable of growing on decaying lignocelluloses substrates such as leaves, branches, and woods of mangroves which include most of Ascomycetes species and with few exceptions of species of Basidiomycetes (Hyde and Jones 1988). Marine fungi play a significant function in the degradation of mangrove leaves, wood pieces, and wooden debris on the shores, thus forming detritus. These fungi play a noteworthy role in the mineralization in the tropical marine system. Nevertheless, the data related to marine laccase is still adequate and requires more work on the characterization of the sort of lignin-modifying enzymes present in marine ecosystems.

Raghukumar et al. (1994) recorded 17 fungi from marine environments, out of which 12 were laccase positive which involved *Gliocladium* sp., *Zalerionvarium*, *Sordaria flmicola*, *Aigialus grandis*, *Halosarpheiarat nagiriensis*, *Gongronella* sp., *Verruculin aenalia*, *Cirrenalia pygmea*, and *Hypoxylo noceanicum*. Jaouani et al. (2014) have discovered the fungal diversity of SebkhaEl Melah, a Saharan salt flat located in southern Tunisia and isolated 21 modestly halotolerant fungi. It involved 15 species related to 6 genera of Ascomycota, such as *Aspergillus* sp., *Cladosporium* sp., *Alternaria* sp., *Penicillium* sp., *Ulocladium* sp., *and Engyodontium* sp. Three species out of 15 indicated laccase activities at 10% NaCl, such as, *Cladosporium sphaerospermum*, *Cladosporium halotolerans*, and *Penicillium canescens*. Laccase production at 10% salt by these strains is of biotechnological interest, especially in the bioremediation of organic pollutants in high salt contaminated environments.

6.10.3 Laccase from Thermophilic and Psychrophilic Fungi

We are aware that life can breathe in highly extreme habitats, and molecular studies related to their survival mechanisms in extreme conditions potting shed new insight about their survival mechanisms in extreme habitats (Yadav et al. 2018, 2020b). The stabilization of processes due to thermal stress is due to several reasons and involves DNA, Necrosomes, proteins, and enzymes (Poli et al. 2017). Thermophilic fungi had obtained colossal attention due to their capacity to produce enzymes appropriate for industrial applications. Species related to genus Corynascus (Myceliopthora) have been of interest to mycologist because it produces thermostable enzymes. For illustration, *Coryascus thermophilus* (axionym: Thiel aviathermophile) produced

thermostable laccases with the high potentiality to express in different hosts (Berka et al. 1997; Bulter et al. 2003; Babot et al. 2011).

Laccases produced by *C. thermophilus* ATCC 42464 are totally characterized, patented genome sequences (Badhan et al. 2007; Beeson et al. 2011). Nevertheless, there is no other record of any thermophilic fungi which is so broadly studied for laccase production. It shows the shortage of thermophilic laccase-producing strains available so far. Many fungi are able to produce extremozymes at different temperatures, pHs, and salt ranges. It is known that they play a significant function in biodegradation in low-temperature habitats. Dhakar and Pandey (2013) and Dhakar et al. (2014) studied the production of laccases by thermotolerant *Penicillium pinophilum* (MCC 1049) and *Trametes hirsuta* (MTCC 11397) isolated from a glacial site in Indian Himalayan Region (IHR). Such features make the strains effective for degradation in extreme conditions. However, as per the literature survey, exceptionally few studies have been done on psychrophilic fungal laccases.

6.11 Bioinformatics Approaches Applied on Fungal Laccases

Since the arrival of post-genomic era, bioinformatics has turned on a strong tool employed at different omic levels (e.g. genomics, transcriptomics, and proteomics). Thus, through its different computational applications, it has demonstrated a great usefulness in a wide range of research fields. Concretely, on fungal laccases, research has been widely reported for different purposes which are summarized below.

Laccases are considered enzymes widely distributed in nature with special emphasis on bacteria and fungi with several biotechnological applications (e.g. bioremediation, textiles, pulp and paper, biofuel cells and biosensors, etc) (Janusz et al. 2020; Mate and Alcalde 2017). The presence of a great laccase diversity on fungi (Arregui et al. 2019) has attracted the interest in looking for new laccases as well as in the characterization or classification. In this context, bioinformatics has been helpful to identify laccase sequences by using different approaches. A clear example of (Copete et al. 2015) could identify partial sequences of four putative laccase genes (Lac1–Lac4) from *Leptosphaerulina* sp. using sequence alignments and phylogenetic analysis. The usage of these two basic bioinformatic approaches was crucial to define Lac 2 as the only related with ferroxidases/laccases proteins whereas the other ones with ascomycete laccase-like proteins. Among them, Lac3 finally could be determined as *sensu-stricto* laccase as it was most closely related to ascomycete laccases genes that was supported by biochemical characterization. A similar strategy for acquiring the genetic sequence was performed by Fonseca et al. (2018) to perform bioinformatic for phylogenetic analysis (T.N.T program).

On the other hand, genome mining emerges as an alternative by using bioinformatic tools such as protein BLAST (https://blast.ncbi.nlm.nih.gov). Therefore, using a reported laccase as a template, it can be detected new homologous sequences as was reported for *Sordaria macrospora k-hell* (Yang et al. 2020). A laccase

detected shows 60% of homology with protein accession from *Myceliophthora thermophila* used as template. Then, the coding gene was retrieved from the database to be recombinantly expressed and characterized. This approach has a scope limitation that is restricted for the organism which has been previously sequenced and registered on databases. This could be supported by Feng et al. (2015) whose work presents a phylogenetic analysis of laccases from 20 plant pathogen (bacterial, fungal, and oomycetes) genomes reported on available databases.

With the development of new generation sequencing technologies, whole genome and transcriptome analysis have been feasible. On one hand, genome analysis has been mainly used for laccase gene prediction (Wang et al. 2015) and phylogenetic studies (Cázares-García et al. 2013), whereas transcriptome analysis provided a window of new opportunities to identify the cDNA of new laccases (Daroch et al. 2014; Vats and Mishra 2018) and get further information of the physiological mechanisms involved/related to laccase biosynthesis under determined conditions. Some examples of transcriptome analysis were performed to elucidate the effect of Cu^{2+} concentration on *Ganoderma lucidum* laccase transcription regulation (Jain et al. 2020). Also, it was employed to determine the synergy of a co-culture *Pleurotus eryngii* with *Rhodotorula mucilaginosa* on laccase gene expression (Zhang et al. 2020).

Moreover, computational tools in association with experimental data (X-ray crystallography, NMR spectroscopy, and electron microscopy) have enabled a detailed determination of protein structural traits (De Salas et al. 2019). Currently, more than 180 laccases are reported on Protein Data Bank (PDB, http://www.rcsb.org/) (Zerva et al. 2019) which includes several fungal laccase structures.

Although it can be considered only descriptive data, it was crucial for laccase protein development of novel improved enzymes with higher activity and/or stability. Thus, three main approaches have been described for this purpose: Based on prior structure information reported which is harnessed for punctual mutations (rational approach), considering saturation mutagenesis on hot-spot residues detected by rational analysis of directed evolution (semi-rational approach) and directed evolution and hybrid approaches (Mate and Alcalde 2015). Some examples are described below for different applications of these strategies on fungal laccase research.

Regarding rational design, the knowledge gathered from protein structure has exerted a critical effect on the selection of target mutations. A clear example of this approach is presented by Madzak et al. (2006) and Galli et al. (2011) during the improvement of catalytic properties of a *Trametes versicolor* through the modification of aminoacidic residues located in substrate interaction areas. Consequently, the results obtained elsewhere (Madzak et al. 2006) were used for basidiomycete PM1 laccase engineering to optimize the optimal pH activity for blood tolerant conditions (Mate et al. 2013).

Semi-rational approach was used also for basidiomycete PM1 laccase, saturation mutagenesis on the thermolabile structural region was accomplished with the aim of enhancing the thermostability of this high-redox potential laccase (Vicente et al. 2020). This strategy was also employed by Zumárraga et al. (2007) to improve the

performance of MtLT2 laccase from *Myceliophthora thermophila* on high concentrations of organic cosolvents.

Although directed evolution does not require a starting structural information to be implemented, the resulting laccases could be characterized by using computational modeling tools as it can be observed in several studies (Mateljak et al. 2019; Vicente et al. 2020). Structural characterization of new clones could be extended also for rational (Andberg et al. 2009) and semi-rational approaches (Zumárraga et al. 2007).

In general terms, bioinformatics could be considered a strong tool that is evolving continuously. For laccases studies, it has been demonstrated its usefulness for genomic, transcriptomic, and proteomic analysis. It should be expected in the next years an increase its usage for basic and applied research.

6.11.1 Laccase Potential Exploitation and Biotechnology Application

Many laccases are candidates for commercialization in several industrial fields. Biodegradable and energy-saving, laccase-based biocatalysts are appropriate for the advancement of highly efficient, economical, and eco-friendly industries. However, only a few of them are basically present in the market for textiles, food, and other industries. Since then laccases are produced by various microorganisms (Giardina and Sannia 2015), especially by bacteria and fungi. With various biological functions such as degradation of complex polymers (lignin, humic acid), lignification, detoxification, pathogenicity, morphogenesis, sporulation, melanin polymerization, spore layer resistance (Strong and Claus 2011), biodegradation, and homeostasis of copper and iron (O'Malley et al. 1993). Thus, laccases (benzenodiol: oxygen oxidoreductases, EC 1.10.3.2) are highly relevant enzymes due to their wide range of substrates (Giardina and Sannia 2015). Likewise, laccases are considered ecological, because to carry out the catalysis, they only require molecular oxygen as a cosubstrate (Mayolo-Deloisa et al. 2020), which is reduced to give rise to the formation of water as the only by-product (Hautphenne et al. 2016).

Market and Emerging Trends are apparent that fungal laccases have a promising future for industrial purposes, offering a green substitute to many current methods that are ecologically unfriendly and unspecific. Till the last decade, this potential was not exploited due to many issues: producing large expensive of the enzyme, specific activity and stability for a specific industrial process, and eventually, the eco-friendly and costs of mediators, if needed. Recent efforts to abundantly produce these enzymes in heterologous hosts offer a valuable alternative to elevate enzyme output in native hosts, whereas utilizing immobilization techniques can enhance enzyme recoverability together with activity and stability. Recently, these approaches resulted in the scaling-up processes from bench scale to pilot and industry levels. The first commercial items containing the laccase enzyme were launched in 1996

by Novozyme (Novo Nordisk, Denmark) (Table 6.2). Denilite ITM is the first industrial laccase and the first bleaching enzyme acting with the help of a redox mediator. The product exhibited good implementation, but treating characteristics were not perfect. So, in 1999, Novozyme North America Inc. (USA) launched DeniLiteIITM (Table 6.2) based on a modern type of laccase with greater activity than that of DeniliteITM. In 2001, the company Zytex Pvt. Ltd. (India) developed Zylite (Table 6.2), a formulation based on LMS able to degrading indigo dye in a very specific technique.

Today, the increasing requirement of specific and eco-friendly treatments for garment manufactures, together with the enhanced methods for natural and recombinant laccase production, have induced the creation of novel small companies that offer customized formulations of laccases to aim specific garment wet process conditions. For instance, Chemicals Dyestuffs Ltd. (Hong Kong) launched a formulated laccase enzyme preparation named Bleach-cut 3S (Table 6.2): different doses and/or treatment time, it is possible to get almost all shades, even the lightest ones. This preparation, containing laccase enzyme buffer system and redox mediator, is greatly efficient in the decolorization of indigo dyes. Bleach-cut 3S may also be utilized for clean-up of back staining and improvement of denim abrasion. Many other examples can surely be found within the textile sector. Most of the new companies are located in the Asian continent, where, although global crises, the textile industry is extending maybe also due to the less bureaucratic constraints for industrial production and lower wages of laborers in this area. Alternately, a few food-grade laccases are accessible on the market: Flavoustar, Suberase, and Laccase M120 (Table 6.2) are produced by a huge company that can invest in good manufacturing processing and product safety, certifying the enzymatic treatment of beverages and food. Much integration of food-grade laccase formulations have begun to utilize in various bread preparations in North America and Northern Europe, although the products are not available on the global market.

Despite the large potential in delignification processing, fewer enzymatic formulations are available for pulp and paper industries. This basically depends on two fundamental factors: the enzyme is costly and its stability which results in making it prohibitive at a cost-effective process and the destitute available data that can be advertised for a correct and genuine integration in an “ancient” and consolidated process flow, such as paper production. The remaining supply is related to enzymes appropriate for specific treatments that are not elucidated and require purified and well-characterized laccases for laboratory or pilot-scale testing. In this field, new companies, such as Metgen and BioPox, are really focused on offering not only a generic product for all customers but also to develop and produce laccases tailored to the industrial customer requirements. Through recombinant expression systems along with enzyme mutagenesis, specific parameters could be moved forward, such as substrate specificity, resistance to temperature, or other environmental factors. Nevertheless, the biodiversity of different environments as well as laboratory evolution may provide new laccases, more potential and specific, to be used in future applications (Piscitelli et al. 2016).

Table 6.2 Commercially accessible laccases

Company	Product	Application
AB Enzymes	Ecostone LCC10	Denim finishing
Advanced Enzyme Technologies Ltd. (India)	Flavourstar	Brewing
Alfa Kimya Company (Turkey)	Novalite IIS	Denim finishing
Amano Enzyme USA Co. Ltd.	LACCASE M120	Food additive
Americos Industries Inc. (India)	Americos Laccase P	Denim bleaching
	Americos Laccase LTC	Denim bleaching
Apollo Chemical Company (USA)	APCOZYME II-S	Denim finishing and bleaching
BioPox (Italy)	Poxa1b from *P. ostreatus*	Not specified
	PoxA3 from *P. ostreatus*	Not specified
	PoxC from *P. ostreatus*	Not specified
	Mix from *P. ostreatus*	Not specified
	Tailored laccase on demand	Not specified
BioSapien (United Arab Emirates)	Recombinant Not specified laccase expressed in *Aspergillus oryizae*	Denim bleaching and finishing
Chemicals Dyestuffs Ltd. (Hong Kong)	Bleach-cut 3S	Denim bleaching and finishing
CHEMOS GmbH (Germany)	Laccase from *Agaricus bisporus*	Not specified
CHT/BEZEMA (Italy)	DENIMCOL LAC	Denim bleaching
Colotex Biotechnology Co. Ltd. (Hong Kong)	Cololacc BB	Denim bleaching and finishing
Condor Speciality Products (USA)	Hypozyme	Denim deiking
ENZYMES NAVEEN (India)	Not specified laccase	Not specified
FENKIM KIMYA SANAYI VE TICARET LTD.STI. (Turkey)	Not specified laccase	Denim bleaching
Genencor Inc. (USA)	IndiStarTM Active	Denim finishing
	PrimagreenEcofade LT100	Denim bleaching and shading
HUT (Vietnam)	Not specified laccase	Not specified laccase
Jena Bioscience GmbH (Germany)	Laccase from *T. versicolor*	Not specified
Julich Chiral Solutions GmbH, A Codexis Company (Germany)	Laccase 001	Not specified
Lignozym GmbH (Germany)	Lignozym-process	Pulp bleaching
Metgen (Finland)	Tailored bacterial laccase	Not specified
Nanjing Chemlin Chemical Industry Co., Ltd. (China)	Not specified laccase	Not specified
Novozyme (Denmark/USA)	DeniliteIITM	Denim finishing
	Novoprime base 268	Denim finishing
	Novozym 51003	Pulp and paper delignification
	Suberase	Cork treatment

(continued)

Table 6.2 (continued)

Company	Product	Application
Prochimica group (Italy)	Easystone E.DUAL/E.TOP/E. TP5	Denim bleaching
Proenzimas Ltda. (Colombia)	LacasaUltratex	Indigo decolourization
Puridet Asia Ltd. (Hong Kong)	Purizyme	Denim bleaching
Season Chemicals Dyestuffs Ltd. (Hong Kong)	Bleach-cut 3S	Denim bleaching
Sigma Aldrich (USA)	Laccase from *A. bisporus*	Not specified
	Laccase from *P. ostreatus*	Not specified
	Laccase from *Rhus vernificera*	Not specified
	Laccase from *T. versicolor*	Not specified
Sunson Industry Group Co., Ltd. (China)	Prozyme LAC	Denim bleaching
Tri-Tex Co. Inc. (Canada)	Trilite II	Indigo decolorization
	Trilite Plus	Indigo decolorization
United States Biological (USA)	Recombinant laccase from *T. versicolor*	Not specified
ZA Biotech (South Africa)	Not specified laccase	Not specified
Zytex Pvt. Ltd. (India)	Zylite	Denim bleaching

Source: Piscitelli et al. (2016)

The functional versatility of laccases allows them to be applied in various fields, such as the paper industry (pulp and paper), bioremediation, the textile industry (textile finishing), pharmaceuticals, painting, and printing (Shanmugapriya et al. 2019), among other transformation reactions such as the oxidation of functional groups for heteromolecular coupling that allows the production of new antibiotic derivatives or intervene in the synthesis of complex natural products (Xenakis et al. 2016).

6.11.2 Fungal Laccases and Its Implication in Bioremediation

Laccases have the ability to transform different xenobiotics, including phenolic pesticides, either by polymerization or oxidation reactions (Majeau et al. 2010), in addition to substrate structure, temperature, and treatment pH as important factors in their activity (Zille et al. 2004; Omar 2008). This makes laccases an essential biotechnological tool in bioremediation processes.

The use of these enzymes in the discoloration and/or degradation of synthetic dyes present in industrial effluents has become an unconventional method for the treatment of these effluents, especially in industries involved in the dyeing of textiles, paper, leather, and plastics (Abo-Farah 2010). *Trametes villosa*, a fungus of the *Polyporaceae* family, generates laccases with activity on benzene sulfonic acid

[3-(4 dimethyl amino-1 phenylazo)] (Zille et al. 2004), *Cladosporium cladosporioides* laccases that degrade the blue azo dye 193 by up to 47% in 08 h (Vijaykumar et al. 2006). It should be mentioned that azo dyes are widely used worldwide, with a presence of approximately 50% in textile effluents (Zille et al. 2004). Laccases can be applied as free, immobilized enzymes and cells containing laccases (Mugdha and Usha 2012).

Laccases from *Trametes versicolor*, immobilized in microfiber supports by encapsulation, degrade synthetic dyes (Dai et al. 2010). On the other hand, laccases from this fungus are also effective by adsorption methods on ZnO/SiO_2 nanocomposite supports over Brilliant, Blue B, and Acid Blue 25 remazol (Li et al. 2015), while the free enzymes show activity in humic acids of previously treated industrial effluents from food plants (Zahmatkesh et al. 2017) as well as phenol removal (Liu et al. 2012). Of equal importance are the laccases of *Coriolopsis gallica*, with R action (Daâssi et al. 2014), Cyathus on Remazol Brilliant, Blue R, Reactive Black 5 and Bismarck Brown bulleri which discolor the red azo dye Acid 27 (Chhabra et al. 2015), *Paraconiothyrium variabile* whose laccases with stable temperature and pH allow it to discolor Acid Blue 25 and Acid Orange 7 (Mirzadeh et al. 2014). On the other hand, laccases of *Bjerkandera adusta* and *Phanerochaete chrysosporium* have demonstrated their capacity to degrade lignin present in synthetic wastewater with a range of 97% and 74% of degradation, respectively, and industrial effluents of the paper pulp industry reached a 100% delignification capacity within 8–10 days (Costa et al. 2017).

As for the Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs), it should be mentioned that they are aromatic hydrocarbons that possess two or more benzene rings fused in various structural configurations with a wide diversity of physical, chemical, and toxicological characteristics (Lawal 2017). These and their derivatives are highly toxic to humans and carcinogenic to living beings, in addition to remaining for long periods of time in soil, water, and air (Ihssen et al. 2015). *Trametes sanguineus* laccases, heterologically expressed in *Trichoderma atroviride,* effectively eliminated phenolic compounds present in wastewater and Biphenol A in culture media (Balcázar-López et al. 2016).

Also, Laccases with manganese peroxidase and lignin peroxidase are a sort of lignin-modifying enzyme (LME). Laccases are utilized as catalysts for the bioremediation of industrial wastes with a broad substrate range. The simple requirements of laccase catalysis (presence of substrate and O_2) as well as its clear stability and deficiency of inhibition (as has been observed with H_2O_2 for peroxidase) make this enzyme more appropriate and interesting for several applications. The applications of laccase enzyme in various fields are presented in (Table 6.3) (Senthivelan et al. 2016).

Table 6.3 Biotechnological applications of fungal laccase

SN	Source of laccaze enzyme	Applications	References
1.	*Botrytis cinerea*	Processing aid for the food industry	Li et al. (1999)
2.	*Pleurotus ostreatus*	Degradation of polycyclic aromatic hydrocarbons in the presence of a synthetic mediator	Pozdnyakova et al. (2006)
3.	*Aspergillus oryzae*	Biosensor and gold nanoparticle	Brondani et al. (2013)
4.	*Pichia pastoris*	Engineered to improve the efficiency of particular bioremediation processes	Dhawan and Kuhad (2002)
5.	*Coriolopsis gallica*	Oxidized recalcitrant polycyclic heterocycles compounds carbozole, *N*-ethylcarbozole, fluorine, and dibenzothiophene present in coal tar and crude oilin presence of 1-hydroxybenzotriazole and 2,20-azinobis (3-ethylbenzthiazoline)-6-sulfonic acid as free radical mediators	Dec and Bollag (2000)
6.	*Coriolus versicolor*	Increased brightness of hardwood kraft pulp	Livernoche et al. (1983)
7.	*Pleurotus florida NCIM 1243*	Preparation of Nanofiber	Jang et al. (2002)
8.	*Trametes pubescens*	Bioremediation of a mixture of pentachlorophenol (PCP), 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), and 2,4,6-trichlorophenol (2,4,6-TCP)	Medina et al. (2011)
9.	*Paraconiothyrium variabile*	Biosynthesis of gold nanoparticles	Faramarzi and Forootanfar (2011)
10.	*Coriolus versicolor*	Degradation of textile dyes	Sanghi et al. (2009)
11.	*Phaenerochaaete chrysosporium*	Dechlorination and decolorization of pulp and paper effluent	Eaton et al. (1980)
12.	*Coriolopsis gallica*	Beer factory wastewater treatment	Madhavi and Lele (2009)
13.	*Trametes versicolor*	Development of microbial fuel cells (MFC) cathode	Luo et al. (2010)
14.	*Pleurotus eryngii*	Lignin and organopollutant degradation, as well as to improve the bioremediation potential	
15.	Yeast *Yarrowia lipolytica*	Removal of Phenolic compounds	Lee et al. (2012)
16.	*Phanerochaete chrysosporium*	Decolorize commercially used reactive textile dyes; reactive orange 96, reactive violet 5, reactive black 5, and reactive blue 38	Heinfling et al. (1997)
17.	*Fusarium incarnatum UC-14*	Bioremediation of Bisphenol A	Chhaya and Gupte (2013)
18.	*Trametes* sp.	Development of bioactive hydrogel dressing	Rocasalbas et al. (2013)
19.	*Pycnoporus cinnabarinus*	Decolorizing of pigment plant effluent	Schliephake et al. (1993)

(continued)

Table 6.3 (continued)

SN	Source of laccaze enzyme	Applications	References
20.	*Schizophyllum commune*	Decolorizing wastewater released from a bagasse-pulping plant	Belsare and Prasad (1988)
21.	*Myceliophthora thermophila*	Dough conditioner	Renzetti et al. (2010)
22.	*Trametes versicolor (ATCC 32745)*	Biosensors	Ardhaoui et al. (2013)
23.	*Aspergillus flavus*	Decolorization of Malachite green dye	Ali et al. (2009)
24.	*Lentinula edodes*	Biodegradation of Polyaromatic hydrocarbon	Wong et al. (2012)
25.	*Trametes versicolor*	Paper biosensor for the detection of phenolic compounds	Oktem et al. (2012)
26.	*Marasmius quercophilus*	Biotransformation of alkylphenols	Farnet et al. (2000)
27.	*Streptomyces cyaneus*	Decolorize and detoxify azo dyes	Moya et al. (2010)

6.11.3 Fungal Laccases in the Pharmaceutical Industry

Laccases have been used within the pharmaceutical industry for the manufacture of various medicines and pharmaceutical products due to their high oxidation potential (Senthivelan et al. 2016). Actinocine is the main drug with anticancer capacity, produced with the help of laccases from 4-methyl-3-hydroxyanthranilic acid (Bourton 2003; Arora and Sharma 2010b). Trials using 43-kDa molecular mass laccases from *Tricholoma giganteum* were shown to inhibit HIV from reverse transcriptase (Wang and Ng 2004). On the other hand, antibiotics are the most widely used drugs worldwide; these components, not being degraded, remain in the environment (Larsson 2014). Therefore, laccases of *T. versicolor* are used for studies of antibiotic degradation as well as with laccases of basidiomycetes *Cerrena* sp. HYB07, *Echinodontium taxodii*, *Perenniporia* strain TFRI707, and *P. sanguineus*, of ascomycetes *Phoma* sp. and *Myceliophthora thermophila* (recombinantly expressed in *Aspergillus oryzae*) (Yang et al. 2017). Recent cosmetic products for skin lightening have been created which are laccase-based hair colors that could be less irritant and more secure than current hair colors. Protein-engineered laccase may be utilized as deodorants, mouthwash, toothpaste, and diapers with decreased allergenicity (Pannu and Kapoor 2014).

6.11.4 Fungal Laccases in the Food Industry

Laccases are also involved in the food industry because of the wide variety of phenolic (Polak and Jarosz-Wilkolazka 2012) and nonphenolic substrates they can use. Their activity on nonphenolic compounds is possible due to the cooperation they establish with compounds called mediators (Chio et al. 2019). Important characteristics for their introduction to the food industry. In beverage processing, laccases favor color stabilization and prevent darkening caused by the presence of polyphenols (Brijwani et al. 2010). With respect to the stability of the characteristic taste of wines and symbols of quality, the use of laccases is an alternative that prevents the loss of taste and color (Osma et al. 2010), especially in those wines with long storage periods (Minussi et al. 2007a). Therefore, laccases can be applied to must and fruit juices (Arora and Sharma 2010a), an example of which is *Trametes versicolor* laccases, which remove phenolic compounds efficiently (Minussi et al. 2002). While it is true that among the laccase-producing fungi are ascomycete, basidiomycetes, and deuteromycetes, white-rot basidiomycetes are the most efficient lignin degraders and laccase producers reported (Arora and Sharma 2010a; Yang et al. 2017).

The cultivation of comestible fungi with the capacity to produce laccases is an advantage for the recovery of laccases from the residual compost and whose action has been studied in different investigations: *Agaricus bisporus* secretes large quantities of laccases (Mayolo-Deloisa et al. 2009) when grown on compost based on horse manure and/or added straw accompanied by nitrogen sources such as ammonium nitrate, poultry manure, gypsum and urea (Kertesz and Thai 2018). The same applies to *Pleurotus ostreatus*, whose residual compost is used in the production of bioethanol, since due to the action of the laccases produced by this fungus; the residual compost is rich in glucose and xylose (Grimm and Wösten 2018).

6.11.5 Fungal Laccases in the Paper Industry

Paper industries discharge effluents with high lignin contents from paper pulp due to conventional bleaching techniques that seek to eliminate it. As part of the chemical bleaching process, chlorine is used which, when it binds to the lignin, forms organochlorine compounds (chlorolignins, chlorophenols, chloroacols, and chloraliphates) that generate highly toxic and dangerous effluents. The application of laccases not only improves the bleaching of the pulp but also optimizes the bleaching and capacity of the recycled paper by eliminating the lignin components (Nyman and Hakala 2011; Xu et al. 2009). The alternative method, mediated by laccases, is a safe and less polluting process than chlorine-based chemical treatment (Gianfreda et al. 1999). *Trametes versicolor* laccases discolor the effluents of these factories to a light-yellow color and thus reduce the toxic compounds present (Karimi et al. 2010), and in turn, their use on the paper pulp demethylyzes and delignifies the pulp

efficiently (Bourbonnais et al. 1992). In turn, the mechanism mediated by laccases conditions this molecule to better penetrate between the fibers (Calafell et al. 2007).

6.11.6 Laccases and Biosensor Development

The immobilization of laccases is aimed at multiple biotechnological applications, by trapping, adsorption, covalent bonding, self-immobilization techniques, or by combinations of all the above, whose enzymatic activity will depend on various conditions, from the type of enzyme to the stabilization parameters (Yang et al. 2017). This immobilization would lead to improved catalytic activity (Kumar et al. 2014) as well as being able to be reused, storage tolerance, and high temperatures (Fernández-Fernández et al. 2013). At the beginning, the response of laccase to different bonding surfaces was studied. *T. versicolor* laccases were successfully bonded to porous glass beads, with a high capacity to degrade phenolic compounds even after several incubations (Leonowicz et al. 1988).

The same happened with *Rhizoctonia praticola*, immobilized by covalent coupling to celite (Shuttleworth and Bollag 1986). Enzymes immobilized to the surface of electrodes by various methods allow the development of enzymatic biosensors (enzyme electrodes). Investigations in biosensors with the use of laccases have been reported, raw enzymatic extract of *Pleurotus ostreatus* as a source of laccases was immobilized in carbon paste obtained a 95% confidence in the detection of catecholamines in pharmaceutical formulations (Leite et al. 2003), the same support was used for the immobilization of laccases produced by *Aspergillus oryzae* for the determination of L-cysteine in pharmaceutical formulations (Santhiago and Vieira 2007). On the other hand, fungal laccases immobilized in electrodes by different techniques of physical adsorption, glutaraldehyde, carbodiimide, and carbodiimide/glutaraldehyde, this last technique presented the highest response, with an optimal glutaraldehyde percentage of 10% (m/v) (Freire et al. 2001), laccases from *Rigidoporus lignosus* were used to manufacture a flow biosensor based on a monomolecular layer of the enzyme on gold support to detect phenols (Vianello et al. 2004) (Fig. 6.7).

6.11.7 Laccases in Organic Synthesis

Due to the wide range of substrates that laccases have, they emerge as an alternative for organic synthesis as they have the ability to convert these substrates into free radicals by polymerization or hydration (non-enzymatic reactions) (Kunamneni et al. 2008). This would reduce the high production costs, numerous intermediate reactions, and generation of toxic compounds (Yaropolov et al. 1994). Likewise, Trametes versicolor laccases are applied to the synthesis of aromatic aldehydes (Fritz-Langhals and Kunath 1998), substituted imidazoles and dimerization

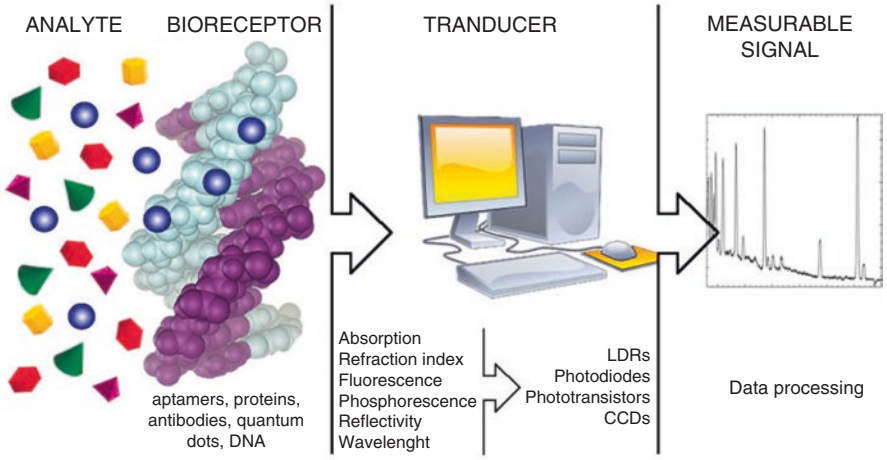


Fig. 6.7 Essential components of a biosensor: bioreceptor, electrochemical transducer, electric pathway in the production of the biosensor response, and typical electrical response of the laccase-based electrode biosensors (Rodríguez-Delgado and Ornelas-Soto 2017)

products (Schäfer et al. 2001), and synthesis of 3,4-dihydro-7,8-dihydroxy-2*H*-dibenzofuran-1-ones, as well as *Agaricus bisporus* (Hajdok et al. 2007). *Coriolus hirsutus* laccases has the ability to synthesize an indamine dye (Baker et al. 1996). On the other hand, laccases from *Curvularia senegalensis* have been reported to have the capacity to degrade polyurethane (Pointing 2001).

References

Abdel-Azeem AM, Salem FM (2012) Biodiversity of laccase producing fungi in Egypt. Mycosphere 3(5):900–920

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abo-Farah SA (2010) Comparative study of oxidation of some azo dyes by different advanced oxidation processes, fenton, fenton-like, photo-fenton and photo-fenton-like. Am J Sci 6(10):128–142

Agrawal K, Chaturvedi V, Verma P (2018) Fungal laccase discovered but yet undiscovered. Bioresour Bioprocess 5(1):1–12

Alcalde M (2007) Laccases: biological functions, molecular structure and industrial applications. In: Industrial enzymes. Springer, Dordrecht, pp 461–476

Alcalde M, Bulter T, Arnold FH (2002) Colorimetric assays for biodegradation of polycyclic aromatic hydrocarbons by fungal laccases. J Biomol Screen 7:547–553

Ali H, Ahmad W, Haq T (2009) Decolorization and degradation of Malachite green by Aspergillus flavus and Alternaria solani. Afr J Biotechnol 8:1574–1576

Altaf SA, Umar DM, Muhammad MS (2010) Production of xylanase enzyme by Pleurotus eryngii and Flamulina velutipes grown on different carbon sources under submerged fermentation. World Appl Sci J 8:47–49

Andberg M, Hakulinen N, Auer S, Saloheimo M, Koivula A, Rouvinen J, Kruus K (2009) Essential role of the C-terminus in Melanocarpus albomyces laccase for enzyme production, catalytic properties and structure. FEBS J 276(21):6285–6300

Ardhaoui M, Bhatt S, Zheng M, Dowling D, Jolivalt C, Khonsari FA (2013) Biosensor based on laccase immobilized on plasma polymerized allylamine/carbon electrode. Mat Sci Eng C 33:3197–3205

Arias M, Arenas M, Rodríguez J, Soliveri J, Ball A, Hernández M (2003) Kraft pulp biobleaching and mediated oxidation of a nonphenolic substrate by laccase from Streptomyces cyaneus CECT 3335. Appl Environ Microbiol 69(4):1953–1958. https://doi.org/10.1128/aem.69.4.1953-1958.2003

Arora DS, Rampal P (2002) Laccase production by some Phlebia species. J Basic Microbiol 42:295–301

Arora DS, Sharma RK (2010a) Lacasas fúngicas ligninolíticas y sus aplicaciones biotecnológicas. Appl Biochem Biotechnol 160:1760–1788

Arora DS, Sharma RK (2010b) Ligninolytic fungal laccases and their biotechnological applications. Appl Biochem Biotechnol 160(6):1760–1788

Arregui L, Ayala M, Gómez-Gil X, Gutiérrez-Soto G, Hernández-Luna CE, de los Santos MH, Trujillo-Roldán MA (2019) Laccases: structure, function, and potential application in water bioremediation. Microb Cell Fact 18(1):200. https://doi.org/10.1186/s12934-019-1248-0

Assavanig A, Amornikitticharoen B, Ekpaisal N, Meevootisom V, Flegel T (1992) Isolation, characterization and function of laccase from Trichoderma. Appl Microbiol Biotechnol 38(2):198–202. https://doi.org/10.1007/bf00174468

Attia EA, Singh BP, Dashora K, Abdel-Azeem AM (2020) A potential antimicrobial, extracellular enzymes, and antioxidants resource: endophytic fungi associated with medicinal plants. Int J Biosci 17(1):119–132

Babot ED, Rico A, Rencoret J, Kalum L, Lund H, Romero J, José C, Martínez ÁT, Gutiérrez A (2011) Towards industrially-feasible delignification and pitch removal by treating paper pulp with Myceliophthora thermophila laccase and a phenolic mediator. Bioresour Technol 102(12):6717–67227

Badhan AK, Chadha BS, Kaur J, Saini HS, Bhat MK (2007) Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus Myceliophthora sp. IMI 387099. Bioresour Technol 98(3):504–510

Baker WL, Sabapathy K, Vibat M, Lonergan G (1996) Laccase catalyzes formation of an indamine dye between 3-methyl-2-benzothiazolinone hydrazone and 3-dimethylaminobenzoic acid. Enzyme Microb Technol 18(2):90–94

Balcázar-López E, Méndez-Lorenzo LH, Batista-García RA, Esquivel-Naranjo U, Ayala M, Kumar VV, Savary O, Cabana H, Herrera-Estrella A, Folch-Mallol JL (2016) Xenobiotic compounds degradation by heterologous expression of a Trametes sanguineus laccase in Trichoderma atroviride. PLoS One 11(2):0147997

Baldrian P (2006) Fungal laccases-occurrence and properties. FEMS Microbiol Rev 30(2):215–242

Baldrian P, Gabriel J (2002) Copper and cadmium increase laccase activity in Pleurotus ostreatus. FEMS Microbiol Lett 206(1):69–74

Barbosa AM, Dekker RFH, Hardy GS (1996) Veratryl alcohol as an inducer of laccase by an ascomycete, Botryosphaeria sp., when screened on the polymeric dye Poly R-478. Lett Appl Microbiol 23(2):93–96

Battistuzzi G, Di Rocco G, Leonardi A, Sola M (2003) 1H NMR of native and azide-inhibited laccase from Rhus vernicifera. J Inorg Biochem 96(4):503–506. https://doi.org/10.1016/s0162-0134(03)00277-0

Beeson WT, Iavarone AT, Hausmann CD, Cate JH, Marletta MA (2011) Extracellular aldonolactonase from Myceliophthora thermophila. Appl Environ Microbiol 77(2):650–656

Belsare DK, Prasad DY (1988) Decolorization of effluent from the bagasse-based pulp mills by the white rot fungus Schikophyllum commune. Appl Microbiol Biotechnol 28:301–306

Benson AD, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW (2012) GenBank. Nucleic Acid Res 40:D48–D53

Berka RM, Schneider P, Golightly EJ, Brown SH, Madden M, Brown KM, Halkier T, Mondorf K, Xu F (1997) Characterization of the gene encoding an extracellular laccase of Myceliophthora thermophila and analysis of the recombinant enzyme expressed in Aspergillus oryzae. Appl Environ Microbiol 63(8):3151–3157

Bertrand G (1985) Sur la laccase et sur le pouvoir oxydant de cette diastase. C R Acad Sci (Paris) 120:266

Bertrand B (2010) Aislamiento y caracterización bioquımica de una lacasa inducida de Trametes versicolor. Tesis de Licenciatura, Facultad de Ciencias Biologicas (FCB), Universidad del Estado de Morelos (UAEM)

Bertrand T, Jolivalt C, Briozzo P, Caminade E, Joly N, Madzak C, Mougin C (2002) Crystal structure of a four-copper laccase complexed with an arylamine: insights into substrate recognition and correlation with kinetics. Biochemistry 41(23):7325–7333

Billal F, Thurston CF (1996) Purification of laccase II from Armillaria mellea and comparison of its properties with those of laccase I. Mycol Res 100:1099–1105

Blanquez P, Caminal G, Sarra M, Vicent T (2007) The effect of HRT on the decolourisation of the grey lanaset G textile dye by Trametes versicolor. Chem Eng J 126(2–3):163–169

Bollag JM, Shuttleworth KL, Anderson DH (1988) Laccase-mediated detoxification of phenolic compounds. Appl Environ Microbiol 54(12):3086–3091

Bonugli-Santos R, Durrant L, da Silva M, Sette L (2010) Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. Enzym Microb Technol 46(1):32–37. https://doi.org/10.1016/j.enzmictec.2009.07.014

Bourbonnais R, Paice MG (1990) Oxidation of non-phenolic substrates: an expanded role for laccase in lignin biodegradation. FEBS Lett 267(1):99–102

Bourbonnais R, Paice MG, Reid ID (1992) Lignin oxidation and pulp delignification by laccase of Trametes versicolor in the presence of ABTS. In: Proceedings of the fifth international conference on biotechnology in pulp and paper industry. Kyoto Japan Uni Publishers Co., Ltd., Tokyo, Japan

Bourbonnais R, Paice M, Reid I, Lanthier P, Yaguchi M (1995) Lignin oxidation by laccase isozymes from Trametes versicolor and role of the mediator 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) in kraft lignin depolymerization. Appl Environ Microbiol 61(5):1876–1880. https://doi.org/10.1128/aem.61.5.1876-1880.1995

Bourbonnais R, Paice MG, Freiermuth B, Bodie E, Borneman S (1997) Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. Appl Environ Microbiol 63(12):4627–4632

Bourton S (2003) Laccases and phenol oxidases in organic synthesis. Curr Org Chem 7:1317–1331

Brijwani K, Rigdon A, Vadlani PV (2010) Fungal laccases: production, function, and applications in food processing. Enzyme Res 2010:149748

Brondani D, Souza BD, Souza BS, Neves A, Vieira LC (2013) PEI-coated gold nanoparticles decorated with laccase: a new platform for direct electrochemistry of enzymes and biosensing applications. Biosens Bioelectron 42:242–247

Bulter T, Alcalde M, Sieber V, Meinhold P, Schlachtbauer C, Arnold FH (2003) Functional expression of a fungal laccase in Saccharomyces cerevisiae by directed evolution. Appl Environ Microbiol 69(2):987–995

Burke RM, Cairney JWG (2002) Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza 12:105–116

Buswell JA, Cai Y, Chang ST (1995) Effect of nutrient nitrogen and manganese on manganese peroxidase and laccase production by Lentinula (Lentinus) edodes. FEMS Microbiol Lett 128(1):81–88

Cairney JWG, Burke RM (1998) Do ecto- and ericoid mycorrhizal fungi produce peroxidase activity. Mycorrhiza 8:61–65

Calafell M, Diaz C, Hadziyska H, Gibert JM, Daga JM, Tzanov T (2007) Bio-catalyzed coloration of cellulose fibers. Biocatal Biotransformation 25:336–340

Call HP, Mücke I (1997) History, overview and applications of mediated lignolytic systems, especially laccase-mediator-systems (Lignozym®-process). J Biotechnol 53(2–3):163–202
Cambria MT, Ragusa S, Calabrese V, Cambria A (2011) Enhanced laccase production in white-rot fungus Rigidoporus lignosus by the addition of selected phenolic and aromatic compounds. Appl Biochem Biotechnol 163(3):415–422
Cañas AI, Camarero S (2010) Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. Biotechnol Adv 28(6):694–705
Cavallazzi JRP, Kasuya CM, Soares MA (2005) Screening of inducers for laccase production by Lentinula edodes in liquid medium. Braz J Microbiol 36(4):383–387
Cázares-García SV, Vázquez-Garcidueñas MS, Vázquez-Marrufo G (2013) Structural and phylogenetic analysis of laccases from trichoderma: a bioinformatic approach. PLoS One 8:55295. https://doi.org/10.1371/journal.pone.0055295
Chambers SM, Burke RM, Brooks PR, Cairney JWG (1999) Molecular and biochemical evidence for manganese-dependent peroxidase activity in Tylospora fibrillosa. Mycol Res 103:1098–1102
Chen DM, Bastias BA, Taylor AFS, Cairney JWG (2003a) Identification of laccase-like genes in ectomycorrhizal basidiomycetes and transcriptional regulation by nitrogen in Piloderma byssinum. New Phytol 157:547–554
Chen S, Ma D, Ge W, Buswell JA (2003b) Induction of laccase activity in the edible straw mushroom, Volvariella volvacea. FEMS Microbiol Lett 218(1):143–148
Chhabra M, Mishra S, Sreekrishnan TR (2015) Immobilized laccase mediated dye decolorization and transformation pathway of azo dye acid red 27. J Environ Health Sci Eng 13(1):38
Chhaya U, Gupte A (2013) Possible role of laccase from Fusarium incarnatum UC-14 in bioremediation of Bisphenol A using reverse micelles system. J Hazard Mat 254–255:149–156
Chio C, Sain M, Qin W (2019) Lignin utilization: a review of lignin depolymerization from various aspects. Renew Sust Energ Rev 107:232–249
Chiranjeevi PV, Pandian MR, Sathish T (2014) Enhancement of laccase production from Pleurotus ostreatus PVCRSP-7 by altering the nutritional conditions using response surface methodology. Bioresources 9:4212–4225
Claus H (2003) Laccases and their occurrence in prokaryotes. Arch Microbiol 179(3):145–150
Claus H (2004) Laccases: structure, reactions, distribution. Micron 35:93–96
Coll PM, Fernandez-Abalos JM, Villanueva JR, Santamaria R, Perez P (1993) Purification and characterization of a phenoloxidase (laccase) from the lignin-degrading basidiomycete PM1 (CECT 2971). Appl Environ Microbiol 59(8):2607–2613
Collins PJ, Dobson ADW (1995) Extracellular lignin and manganese peroxidase production by the white-rot fungus Coriolus versicolor 290. Biotechnol Lett 17(9):989–992
Copete LS, Chanagá X, Barriuso J, López-Lucendo MF, Martínez MJ, Camarero S (2015) Identification and characterization of laccase-type multicopper oxidases involved in dye-decolorization by the fungus Leptosphaerulina sp. BMC Biotechnol 15. https://doi.org/10.1186/s12896-015-0192-2
Cordi L, Minussi RC, Freire RS, Duran N (2007) Fungal laccase: copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. Afr J Biotechnol 6(10):1255–1259
Costa S, Dedola DG, Pellizzari S, Blo R, Rugiero I, Pedrini P, Tamburini E (2017) Lignin biodegradation in pulp-and-paper mill wastewater by selected white rot fungi. Water 9(12):935
Couto SR, Toca-Herrera JL (2007) Laccase production at reactor scale by filamentous fungi. Biotechnol Adv 25(6):558–569
Couto SR, Moldes D, Liebanas A, Sanroman A (2003) Investigation of several bioreactor configurations for laccase production by Trametes versicolor operating in solid-state conditions. Biochem Eng J 15(1):21–26
Couto SR, Lopez E, Sanroman MA (2006) Utilisation of grape seeds for laccase production in solid-state fermentors. J Food Eng 74(2):263–267

Curir P, Thurston CF, Daquila F, Pasini C, Marchesini A (1997) Characterization of a laccase secreted by Armillaria mellea pathogenic for Genista. Plant Physiol Biochem 35:147–153

D'Souza T, Boominathan K, Reddy C (1996) Isolation of laccase gene-specific sequences from white rot and brown rot fungi by PCR. Appl Environ Microbiol 62(10):3739–3744. https://doi.org/10.1128/aem.62.10.3739-3744.1996

D'Souza-Ticlo D, Verma AK, Mathew M, Raghukumar C (2006a) Effect of nutrient nitrogen on laccase production, its isozyme pattern and effluent decolorization by the fungus NIOCC, isolated from mangrove wood. Indian J Mar Sci 35(4):364–372

D'Souza-Ticlo D, Verma AK, Mathew M, Raghukumar C (2006b) Effect of nutrient nitrogen on laccase production, its isozyme pattern and effluent decolorization by a fungus isolated from mangrove wood. Indian J Mar Sci 35:364–372

D'Souza-Ticlo D, Garg S, Raghukumar C (2009a) Effects and interactions of medium components on laccase from a marine-derived fungus using response surface methodology. Mar Drugs 7(4):672–688

D'Souza-Ticlo D, Sharma D, Raghukumar C (2009b) A thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus. Mar Biotechnol 11(6):725–737

Da Cunha MAA, Barbosa AM, Giese EC, Dekker RFH (2003) The effect of carbohydrate carbon sources on the production of constitutive and inducible laccases by Botryosphaeria sp. J Basic Microbiol 43(5):385–392

Daâssi D, Rodríguez-Couto S, Nasri M, Mechichi T (2014) Biodegradation of textile dyes by immobilized laccase from Coriolopsis gallica into Ca-alginate beads. Int Biodeterior Biodegradation 90:71–78

Dai Y, Niu J, Liu J, Yin L, Xu J (2010) In situ encapsulation of laccase in microfibers by emulsion electrospinning: preparation, characterization, and application. Bioresour Technol 101(23):8942–8947

Dalfard A, Khajeh K, Soudi M, Naderi-Manesh H, Ranjbar B, Sajedi R (2006) Isolation and biochemical characterization of laccase and tyrosinase activities in a novel melanogenic soil bacterium. Enzym Microb Technol 39(7):1409–1416. https://doi.org/10.1016/j.enzmictec.2006.03.029

Daroch M, Houghton CA, Moore JK, Wilkinson MC, Carnell AJ, Bates AD, Iwanejko LA (2014) Glycosylated yellow laccases of the basidiomycete Stropharia aeruginosa. Enzym Microb Technol 58:1–7

De Jesus M, Nicola A, Rodrigues M, Janbon G, Casadevall A (2008) Capsular localization of the Cryptococcus neoformans polysaccharide component galactoxylomannan. Eukaryot Cell 8(1):96–103. https://doi.org/10.1128/ec.00331-08

De la Mora E, Lovett JE, Blanford CF, Garman EF, Valderrama B, Rudino-Pinera E (2012) Structural changes caused by radiation-induced reduction and radiolysis: the effect of X-ray absorbed dose in a fungal multicopper oxidase. Acta Crystallogr D Biol Crystallogr 68(5):564–577

De Marco A, Roubelakis-Angelakis K (1997) Laccase activity could contribute to cell-wall reconstitution in regenerating protoplasts. Phytochemistry 46(3):421–425. https://doi.org/10.1016/s0031-9422(97)00301-4

De Salas F, Cañadas R, Santiago G, Virseda-Jerez A, Vind J, Gentili P et al (2019) Structural and biochemical insights into an engineered high-redox potential laccase overproduced in Aspergillus. Int J Biol Macromol 141:855–867

Dec J, Bollag JM (2000) Phenol oxidase-mediated interactions of phenols and anilines with humic materials. J Environ Qual 29(3):665–676

Dhakar K, Pandey A (2013) Laccase production from a temperature and pH tolerant fungal strain of Trametes hirsuta (MTCC 11397). Enzyme Res 2013:869062

Dhakar K, Jain R, Tamta S, Pandey A (2014) Prolonged laccase production by a cold and pH tolerant strain of Penicillium pinophilum (MCC 1049) isolated from a low temperature environment. Enzyme Res 2014:120708

Dhawan S, Kuhad RC (2002) Effect of amino acids and vitamins on laccase production by the bird's nest fungus Cyathus bulleri. Bioresour Technol 84(1):35–38

Ducros V, Brzozowski AM, Wilson KS, Brown SH, Østergaard P, Schneider P, Davies GJ (1998) Crystal structure of the type-2 Cu depleted laccase from Coprinus cinereus at 2.2 Å resolution. Nat Struct Biol 5(4):310–316

Dwivedi UN, Singh P, Pandey VP, Kumar A (2011) Structure–function relationship among bacterial, fungal and plant laccases. J Mol Catal B Enzym 68:117–128

Eaton D, Chang HM, Kirk TK (1980) Decolorization of kraft bleach effluent. TAPPI 63:103

Edens WA, Goins TQ, Dooley D, Henson JM (1999) Purification and characterization of a secreted laccase of Gaeumannomyces graminis var. tritici. Appl Environ Microbiol 65(7):3071–3074

Eggert C, Temp U, Eriksson KE (1996a) The ligninolytic system of the white rot fungus Pycnoporus cinnabarinus: purification and characterization of the laccase. Appl Environ Microbiol 62(4):1151–1158

Eggert C, Temp U, Dean JFD, Eriksson KEL (1996b) A fungal metabolite mediates degradation of non-phenolic lignin structures and synthetic lignin by laccase. FEBS Lett 391:144–148

Faraco V, Giardina P, Sannia G (2003) Metal-responsive elements in Pleurotus ostreatus laccase gene promoters. Microbiology 149:2155–2162

Faramarzi MA, Forootanfar H (2011) Biosynthesis and characterization of gold nanoparticles produced by laccase from Paraconiothyrium variabile. Colloids Surf B Biointerfaces 87:23–27

Farnet AM, Criquet S, Tagger S, Gil G, Le PJ (2000) Purification, partial characterization and reactivity with aromatic compounds of two laccase from Marasmius quercophillus strain 17. Can J Microbiol 46:189–194

Farver O, Pecht I (1984) The reactivity of copper sites in the "blue" copper proteins. In: Lontie R (ed) Copper proteins and copper enzymes, vol 1. CRC Press, Boca Raton, FL, pp 183–214

Faure D, Bouillant M, Bally R (1994) Isolation of Azospirillum lipoferum 4T Tn5 mutants affected in melanization and laccase activity. Appl Environ Microbiol 60(9):3413–3415. https://doi.org/10.1128/aem.60.9.3413-3415.1994

Feng B, Li P, Fu L, Yu X (2015) Exploring laccase genes from plant pathogen genomes: a bioinformatic approach. Genet Mol Res 14:14019–14036. https://doi.org/10.4238/2015.October.29.21

Fernández-Fernández M, Sanromán MÁ, Moldes D (2013) Recent developments and applications of immobilized laccase. Biotechnol Adv 31:1808–1825

Ferraroni M, Myasoedova NM, Schmatchenko V, Leontievsky AA, Golovleva LA, Scozzafava A, Briganti F (2007) Crystal structure of a blue laccase from Lentinus tigrinus: evidences for intermediates in the molecular oxygen reductive splitting by multicopper oxidases. BMC Struct Biol 7(1):60

Ferraroni M, Matera I, Chernykh A, Kolomytseva M, Golovleva LA, Scozzafava A, Briganti F (2012) Reaction intermediates and redox state changes in a blue laccase from Steccherinum ochraceum observed by crystallographic high/low X-ray dose experiments. J Inorg Biochem 111:203–209

Flores C, Vidal C, Trejo-Hernandez MR, Galindo E, Serrano-Carreon L (2009) Selection of Trichoderma strains capable of increasing laccase production by Pleurotus ostreatus and Agaricus bisporus in dual cultures. J Appl Microbiol 106:249–257

Fonseca MI, Molina MA, Winnik DL, Busi MV, Fariña JI, Villalba LL, Zapata PD (2018) Isolation of a laccase-coding gene from the lignin-degrading fungus Phlebia brevispora BAFC 633 and heterologous expression in Pichia pastoris. J Appl Microbiol 124(6):1454–1468

Freire RS, Durám N, Kubota LT (2001) Effects of fungal laccase immobilization procedures for the development of a biosensor for phenol compounds. Talanta 54(4):681–686

Fritz-Langhals E, Kunath B (1998) Synthesis of aromatic aldehydes by laccase-mediator assisted oxidation. Tetrahedron Lett 39(33):5955–5956

Gaitan IJ, Medina SC, González JC, Rodriguez A, Espejo AJ, Osma JF et al (2011) Evaluation of toxicity and degradation of a chlorophenol mixture by the laccase produced by Trametes pubescens. Bioresour Technol 102:3632–3635

Galhaup C, Wagner H, Hinterstoisser B, Haltrich D (2002) Increased production of laccase by the wood-degrading basidiomycete Trametes pubescens. Enzym Microb Technol 30(4):529–536

Galli C, Gentili P, Jolivalt C, Madzak C, Vadalà R (2011) How is the reactivity of laccase affected by single-point mutations? Engineering laccase for improved activity towards steri-

cally demanding substrates. Appl Microbiol Biotechnol 91:123–131. https://doi.org/10.1007/s00253-011-3240-4
Gavnholt B, Larsen K (2002) Molecular biology of plant laccases in relation to lignin formation. Physiol Plant 116(3):273–280. https://doi.org/10.1034/j.1399-3054.2002.1160301.x
Gayazov R, Rodakiewicz-Nowak J (1996) Semi-continuous production of laccase by P. radiata in different culture media. Folia Microbiol Lett 41:480–484
Ghindilis A (2000) Direct electron transfer catalysed by enzymes: application for biosensor development. Biochem Soc Trans 28(2):84–89
Gianfreda L, Xu F, Bollag J (1999) Laccases: a useful group of oxidoreductive enzymes. Biorem J 3(1):1–26. https://doi.org/10.1080/10889869991219163
Giardina P, Sannia G (2015) Laccases: old enzymes with a promising future. Cell Mol Life Sci 72(5):855–856
Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G (2010) Laccases: a never-ending story. Cell Mol Life Sci 67:369–385
Givaudan A, Effosse A, Faure D, Potier P, Bouillant M, Bally R (1993) Polyphenol oxidase in Azospirillum lipoferum isolated from rice rhizosphere: evidence for laccase activity in non-motile strains of Azospirillum lipoferum. FEMS Microbiol Lett 108(2):205–210. https://doi.org/10.1111/j.1574-6968.1993.tb06100.x
Gomez J, Pazos M, Rodrıguez Couto S, Sanroman MA (2005) Chestnut shell and barley bran as potential substrates for laccase production by Coriolopsis rigida under solid-state conditions. J Food Eng 68:315–319
Grimm D, Wösten HAB (2018) Mushroom cultivation in the circular economy. Appl Microbiol Biotechnol 102:7795–7803
Grotewold E, Taccioli GE, Aisemberg GO, Judewicz ND (1998) Purification of an extracellular fungal laccase. MIRCEN J Appl Microbiol Biotechnol 4:357–363
Hajdok S, Leutbecher H, Greiner G, Conrad J, Beifuss U (2007) Laccase initiated oxidative domino reactions for the efficient synthesis of 3,4-dihydro-7,8-dihydroxy-2H-dibenzofuran-1-ones. Tetrahedron Lett 48(29):5073–5076
Hakulinen N, Kiiskinen LL, Kruus K, Saloheimo M, Paananen A, Koivula A, Rouvinen J (2002) Crystal structure of a laccase from Melanocarpus albomyces with an intact trinuclear copper site. Nat Struct Biol 9:601–605
Hatvani N, Kredics L, Antal Z, Mecs I (2002) Changes in activity of extracellular enzymes in dual cultures of Lentinula edodes and mycoparasitic Trichoderma strains. J Appl Microbiol 92:415–423
Hautphenne C, Penninckx M, Debaste F (2016) Product formation from phenolic compounds removal by laccases: a review. Environ Technol Innov 5:250–266
Heinfling A, Bergbauer M, Szewzyk U (1997) Biodegradation of azo and phthalocyanine dyes by Trametes versicolor and Bjerkan-dera adusta. Appl Microbiol Biotechnol 48:261–266
Heinzkill M, Bech L, Halkier T, Schneider P, Anke T (1998) Characterization of laccases and peroxidases from wood-rotting fungi (Family Coprinaceae). Appl Environ Microbiol 64(5):1601–1606. https://doi.org/10.1128/aem.64.5.1601-1606.1998
Hess J, Leitner C, Galhaup C, Kulbe KD, Hinterstoisser B, Steinwender M, Haltrich D (2002) Enhanced formation of extracellular laccase activity by the white-rot fungus Trametes multicolor. In: Biotechnology for fuels and chemicals. Humana, Totowa, NJ, pp 229–241
Hilden K, Hakala TK, Lundell T (2009) Thermotolerant and thermostable laccases. Biotechnol Lett 31(8):1117–1128
Hofrichter M, Steinbüchel A (2001) Lignin, humic substances and coal. Wiley-VCH, Weinheim
Hoopes J, Dean J (2004) Ferroxidase activity in a laccase-like multicopper oxidase from Liriodendron tulipifera. Plant Physiol Biochem 42(1):27–33. https://doi.org/10.1016/j.plaphy.2003.10.011
Huber M, Lerch K (1987) The influence of copper on the induction of tyrosinase and laccase in Neurospora crassa. FEBS Lett 219(2):335–338
Hyde KD, Jones EBG (1988) Marine mangrove fungi. Mar Ecol 9(1):15–33

Ihssen J, Reiss R, Luchsinger R, Meyer LT, Richter M (2015) Biochemical properties and yields of diverse bacterial laccase like multicopper oxidases expressed in Escherichia coli. Sci Rep 5:10465

Ikeda R, Sugita T, Jacobson E, Shinoda T (2003) Effects of melanin upon susceptibility of Cryptococcus to antifungals. Microbiol Immunol 47(4):271–277. https://doi.org/10.1111/j.1348-0421.2003.tb03395.x

Imran M, Asad MJ, Hadri SH, Mehmood S (2012) Production and industrial applications of laccase enzyme. J Cell Mol Biol 10:1–11

Iwasaki H, Matsubara T, Mori T (1967) A fungal laccase, its properties and reconstitution from its protein and copper. J Biochem 61(6):814–816

Iyer G, Chattoo B (2003) Purification and characterization of laccase from the rice blast fungus, Magnaporthe grisea. FEMS Microbiol Lett 227(1):121–126. https://doi.org/10.1016/s0378-1097(03)00658-x

Jain KK, Kumar A, Shankar A, Pandey D, Chaudhary B, Sharma KK (2020) De novo transcriptome assembly and protein profiling of copper-induced lignocellulolytic fungus Ganoderma lucidum MDU-7 reveals genes involved in lignocellulose degradation and terpenoid biosynthetic pathways. Genomics 112:184–198. https://doi.org/10.1016/j.ygeno.2019.01.012

Jaiswal N, Pandey VP, Dwivedi UN (2015) Purification of a thermostable alkaline laccase from papaya (Carica papaya) using affinity chromatography. Int J Biol Macromol 72:326–332

Jang MY, Ryu WR, Cho MH (2002) Laccase production from repeated batch cultures using free mycelia of Trametes sp. Enzyme Microb Technol 30:741–746

Janusz G, Pawlik A, Świderska-Burek U, Polak J, Sulej J, Jarosz-Wilkołazka A, Paszczyński A (2020) Laccase properties, physiological functions, and evolution. Int J Mol Sci 21(3):966

Jaouani A, Tabka MG, Penninckx MJ (2006) Lignin modifying enzymes of Coriolopsis polyzona and their role in olive oil mill wastewaters decolourisation. Chemosphere 62(9):1421–1430

Jaouani A, Neifar M, Prigione V, Ayari A, Sbissi I, Ben Amor S et al (2014) Diversity and enzymatic profiling of halotolerant micromycetes from Sebkha El Melah, a Saharan salt flat in southern Tunisia. Biomed Res Int 2014:439197

Jensen PR, Fenical W (2002) Secondary metabolites from marine fungi. In: Hyde KD (ed) Fungi in marine environments. Fungal Diversity Press, Hong Kong, China, pp 293–315

Johnson D, Thompson J, Brinkmann S, Schuller K, Martin L (2003) Electrochemical characterization of purified Rhus vernicifera laccase: voltammetric evidence for a sequential four-electron transfer. Biochemistry 42(34):10229–10237. https://doi.org/10.1021/bi034268p

Junghanns C, Moeder M, Krauss G, Martin C, Schlosser D (2005) Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. Microbiology 151(1):45–57. https://doi.org/10.1099/mic.0.27431-0

Junghanns C, Pecyna MJ, Böhm D, Jehmlich N, Martin C, Von Bergen M, Schlosser D (2009) Biochemical and molecular genetic characterisation of a novel laccase produced by the aquatic ascomycete Phoma sp. UHH 5-1-03. Appl Microbiol Biotechnol 84(6):1095–1105

Kahraman SS, Gurdal IH (2002) Effect of synthetic and natural culture media on laccase production by white rot fungi. Bioresour Technol 82(3):215–217

Kallio JP, Auer S, Jänis J, Andberg M, Kruus K, Rouvinen J, Hakulinen N (2009) Structure–function studies of a Melanocarpus albomyces laccase suggest a pathway for oxidation of phenolic compounds. J Mol Biol 392(4):895–909

Kallio JP, Gasparetti C, Andberg M, Boer H, Koivula A, Kruus K, Hakulinen N (2011a) Crystal structure of an ascomycete fungal laccase from Thielavia arenaria–common structural features of asco-laccases. FEBS J 278(13):2283–2295

Kallio JP, Rouvinen J, Kruus K, Hakulinen N (2011b) Probing the dioxygen route in Melanocarpus albomyces laccase with pressurized xenon gas. Biochemistry 50(21):4396–4398

Kanunfre CC, Zancan GT (1998) Physiology of exolaccase production by Thelephora terrestris. FEMS Microbiol Lett 161(1):151–156

Karimi S, Abdulkhani A, Karimi A, Ghazali AHB, Ahmadun FLR (2010) The effect of combination enzymatic and advanced oxidation process treatments on the colour of pulp and paper mill effluent. Environ Technol 31(4):347–356

Kertesz MA, Thai M (2018) Compost bacteria and fungi that influence growth and development of Agaricus bisporus and other commercial mushrooms. Appl Microbiol Biotechnol 102:1639–1650

Khammuang S, Sarnthima R (2009) Laccase activity from fresh fruiting bodies of Ganoderma sp. MK05: purification and Remazol Brilliant Blue R decolorization. J Biol Sci 9(1):83–87

Kiiskinen LL, Viikari L, Kruus K (2002) Purification and characterisation of a novel laccase from the ascomycete Melanocarpus albomyces. Appl Microbiol Biotechnol 59(2–3):198–204

Klonowska A, Le Petit J, Tron T (2001) Enhancement of minor laccases production in the basidiomycete Marasmius quercophilus C30. FEMS Microbiol Lett 200(1):25–30

Koschorreck K, Richter S, Ene A, Roduner E, Schmid R, Urlacher V (2008) Cloning and characterization of a new laccase from Bacillus licheniformis catalyzing dimerization of phenolic acids. Appl Microbiol Biotechnol 79(2):217–224. https://doi.org/10.1007/s00253-008-1417-2

Kour D, Rana KL, Kaur T, Singh B, Chauhan VS, Kumar A et al (2019a) Extremophiles for hydrolytic enzymes productions: biodiversity and potential biotechnological applications. In: Molina G, Gupta VK, Singh B, Gathergood N (eds) Bioprocessing for biomolecules production. Wiley, Hoboken, NJ, pp 321–372. https://doi.org/10.1002/9781119434436.ch16

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019b) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kour D, Kaur T, Devi R, Yadav A, Singh M, Joshi D et al (2021) Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: present status and future challenges. Environ Sci Pollut Res 28:24917–24939. https://doi.org/10.1007/s11356-021-13252-7

Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA, Druzhinina IS, Thon M, Mukherjee M (2011) Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of Trichoderma. Genome Biol 12(4):R40

Kumar S, Mishra A (2011) Optimization of laccase production from WRF-1 on groundnut shell and cyanobacterial biomass: by application of Box-Behnken experimental design. J Microbiol Biotechnol Res 1:33–53

Kumar S, Nussinov R (2001) How do thermophilic proteins deal with heat? Cell Mol Life Sci 58(9):1216–1233

Kumar SV, Phale PS, Durani S, Wangikar PP (2003) Combined sequence and structure analysis of the fungal laccase family. Biotechnol Bioeng 83:386–394

Kumar VV, Sivanesan S, Cabana H (2014) Magnetic crosslinked laccase aggregates—bioremediation tool for decolorization of distinct classes of recalcitrant dyes. Sci Total Environ 487:830–839

Kumar A, Chaturvedi AK, Yadav K, Arunkumar KP, Malyan SK, Raja P et al (2019) Fungal phytoremediation of heavy metal-contaminated resources: current scenario and future prospects. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for sustainable environments, vol 3. Springer, Cham, pp 437–461. https://doi.org/10.1007/978-3-030-25506-0_18

Kumar A, Yadav AN, Mondal R, Kour D, Subrahmanyam G, Shabnam AA et al (2021) Myco-remediation: a mechanistic understanding of contaminants alleviation from natural environment and future prospect. Chemosphere 284:131325. https://doi.org/10.1016/j.chemosphere.2021.131325

Kunamneni A, Camarero S, García-Burgos C, Plou FJ, Ballesteros A, Alcalde M (2008) Engineering and applications of fungal laccases for organic synthesis. Microb Cell Factories 7(1):32

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, López R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Larsson DJ (2014) Antibiotics in the environment. Ups J Med Sci 119(2):108–112
Lawal AT (2017) Polycyclic aromatic hydrocarbons. A review. Cogent Environ Sci 3(1):1339841
Leatham GF, Kent Kirk T (1983) Regulation of ligninolytic activity by nutrient nitrogen in white-rot basidiomycetes. FEMS Microbiol Lett 16(1):65–67
Lee IY, Jung KH, Lee CH, Park YH (1999) Enhanced production of laccase in Trametes vesicolor by the addition of ethanol. Biotechnol Lett 21(11):965–968
Lee K, Wi S, Singh A, Kim Y (2004) Micromorphological characteristics of decayed wood and laccase produced by the brown-rot fungus Coniophora puteana. J Wood Sci 50(3):281–284. https://doi.org/10.1007/s10086-003-0558-2
Lee KM, Kalyani D, Tiwari MK, Kim TS, Dhiman SS, Lee JK, Kima IW (2012) Enhanced enzymatic hydrolysis of rice straw by removal of Phenolic compounds using a novel laccase from yeast Yarrowia lipolytica. Bioresour Technol 123:636–645
Leite OD, Fatibello-Filho O, Barbosa A d M (2003) Determination of catecholamines in pharmaceutical formulations using a biosensor modified with a crude extract of fungi laccase (Pleurotus ostreatus). J Braz Chem Soc 14(2):297–303
Leonowicz A, Sarkar JM, Bollag J-M (1988) Improvement in stability of an immobilized fungal laccase. Appl Microbiol Biotechnol 29:129–135
Levasseur A, Saloheimo M, Navarro D, Andberg M, Pontarotti P, Kruus K, Record E (2010) Exploring laccase-like multicopper oxidase genes from the ascomycete Trichoderma reesei: a functional, phylogenetic and evolutionary study. BMC Biochem 11(1):32. https://doi.org/10.1186/1471-2091-11-32
Levin L, Forchiassin F, Ramos AM (2002) Copper induction of lignin-modifying enzymes in the white-rot fungus Trametes trogii. Mycologia 94(3):377–383
Li K, Xu F, Eriksson KEL (1999) Comparison of fungal laccases and redox mediators in oxidation of a nonphenolic lignin model compound. Appl Environ Microbiol 65(6):2654–2660
Li WX, Sun HY, Zhang RF (2015) Immobilization of laccase on a novel ZnO/SiO_2 nanocomposited support for dye decolorization. In: IOP conference series: materials science and engineering, vol 87
Liu Y, Zeng Z, Zeng G, Tang L, Pang Y, Li Z, Liu C, Lei X, Wu M, Ren P, Liu Z, Chen M, Xie G (2012) Immobilization of laccase on magnetic bimodal mesoporous carbon and the application in the removal of phenolic compounds. Bioresour Technol 115:21–26
Livernoche D, Jurasek L, Desrochers M, Dorica J (1983) Removal of color from kraft mill wastewaters with cultures of white-rot fungi and with immobilized mycelium of Coriolus versicolor. Biotechnol Bioeng 25:2055–2065
Lo SC, Ho YS, Buswell JA (2002) Effect of phenolic monomers on the production of laccases by the edible mushroom Pleurotus sajor-caju, and partial characterization of a major laccase component. Mycologia 39(3):413–421
Lorenzo M, Moldes D, Couto SR, Sanroman A (2002) Improving laccase production by employing different lignocellulosic wastes in submerged cultures of Trametes versicolor. Bioresour Technol 82(2):109–113
Lorenzo M, Moldes D, Sanroman MA (2006) Effect of heavy metals on the production of several laccase isoenzymes by Trametes versicolor and on their ability to decolourise dyes. Chemosphere 63:912–917
Lu SXF, Jones CL, Lonergan GT (1996) Correlation between fungal morphology and laccase expression under the influence of cellobiose induction. In: Proceedings of the 10th international biotechnology symposium and the 9th international symposium on yeasts, Sydney, Australia, 1996, Poster session 1
Luis P, Walther G, Kellner H, Martin F, Buscot F (2004) Diversity of laccase genes from basidiomycetes in a forest soil. Soil Biol Biochem 36(7):1025–1036
Luke AK, Burton SG (2001) A novel application for Neurospora crassa: progress from batch culture to a membrane bioreactor for the bioremediation of phenols. Enzym Microb Technol 29(6–7):348–356
Luo H, Jin S, Fallgren PH, Park HJ, Johnson PA (2010) A novel laccase-catalyzed cathode for microbial fuel cells. Chem Eng J 165:524–528

Madhavi V, Lele SS (2009) Laccase: properties and applications. Bioresour Technol 4(4):1694–1717
Madzak C, Mimmi MC, Caminade E, Brault A, Baumberger S, Briozzo P et al (2006) Shifting the optimal pH of activity for a laccase from the fungus Trametes versicolor by structure-based mutagenesis. Protein Eng Des Sel 19(2):77–84
Majeau JA, Brar SK, Tyagi RD (2010) Laccases for removal of recalcitrant and emerging pollutants. Bioresour Technol 101(7):2331–2350
Mansur M, Suarez T, Gonzalez AE (1998) Differential gene expression in the laccase gene family from basidiomycete I-62 (CECT 20197). Appl Environ Microbiol 64:771–774
Marqués de Souza CG, Tychanowicz GK, Farani De Souza D, Peralta RM (2004) Production of laccase isoforms by Pleurotus pulmonarius in response to presence of phenolic and aromatic compounds. J Basic Microbiol 44:129–136
Martin C, Corvini PF, Vinken R, Junghanns C, Krauss G, Schlosser D (2009) Quantification of the influence of extracellular laccase and intracellular reactions on the isomer-specific biotransformation of the xenoestrogen technical nonylphenol by the aquatic hyphomycete Clavariopsis aquatica. Appl Environ Microbiol 75:4398–4409
Martins L, Soares C, Pereira M, Teixeira M, Costa T, Jones G, Henriques A (2002) Molecular and biochemical characterization of a highly stable bacterial laccase that occurs as a structural component of the Bacillus subtilis endospore coat. J Biol Chem 277(21):18849–18859. https://doi.org/10.1074/jbc.m200827200
Mate DM, Alcalde M (2015) Laccase engineering: from rational design to directed evolution. Biotechnol Adv 33:25–40. https://doi.org/10.1016/j.biotechadv.2014.12.007
Mate DM, Alcalde M (2017) Laccase: a multi-purpose biocatalyst at the forefront of biotechnology. Microb Biotechnol 10:1457–1467. https://doi.org/10.1111/1751-7915.12422
Maté D, García-Ruiz E, Camarero S, Alcalde M (2011) Directed evolution of fungal laccases. Curr Genomics 12(2):113–122
Mate DM, Gonzalez-Perez D, Falk M, Kittl R, Pita M, De Lacey AL et al (2013) Blood tolerant laccase by directed evolution. Chem Biol 20(2):223–231
Mateljak I, Monza E, Lucas MF, Guallar V, Aleksejeva O, Ludwig R et al (2019) Increasing redox potential, redox mediator activity, and stability in a fungal laccase by computer-guided mutagenesis and directed evolution. ACS Catal 9(5):4561–4572
Matsubara T, Iwasaki H (1972) Occurrence of laccase and tyrosinase in fungi of Agaricales and comparative study of laccase from Russula delica and Russula pseudodelica. Bot Mag Tokyo 85:71
Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60(6):551–565
Mayolo-Deloisa K, Trejo-Hernández MR, Rito-Palomares M (2009) Recovery of laccase from the residual compost of Agaricus bisporus in aqueous two-phase systems. Process Biochem 44:435–439
Mayolo-Deloisa K, González-González M, Rito-Palomares M (2020) Laccases in food industry: bioprocessing, potential industrial and biotechnological applications. Front Bioeng Biotechnol 8:222
Min K-L, Kim Y-H, Kim YW, Jung HS, Hah YC (2001) Characterization of a novel laccase produced by the wood-rotting fungus Phellinus ribis. Arch Biochem Biophys 392:279–286
Minussi RC, Pastore GM, Durán N (2002) Potential applications of laccase in the food industry. Trends Food Sci Technol 13(6–7):205–216
Minussi RC, Rossi M, Bologna L, Rotilio D, Pastore GM, Durán N (2007a) Phenols removal in musts: strategy for wine stabilization by laccase. J Mol Catal B Enzym 45:102–107
Minussi RC, Miranda MA, Silva JA, Ferreira CV, Aoyama H, Marangoni S et al (2007b) Purification, characterization and application of laccase from Trametes versicolor for colour and phenolic removal of olive mill wastewater in the presence of 1-hydroxybenzotriazole. Afr J Biotechnol 6(10)
Mirzadeh SS, Khezri SM, Rezaei S, Forootanfar H, Mahvi AH, Faramarzi MA (2014) Decolorization of two synthetic dyes using the purified laccase of Paraconiothyrium variabile immobilized on porous silica beads. J Environ Health Sci Eng 12(1):6

Missall TA, Moran JM, Corbett JA, Lodge JK (2005) Distinct stress responses of two functional laccases in Cryptococcus neoformans are revealed in the absence of the thiolspecific antioxidant Tsa1. Eukaryot Cell 4:202–208
Mohorcic M, Friedrich J, Pavko A (2004) Decoloration of the diazo dye reactive black 5 by immobilised Bjerkundera adusta in a stirred tank bioreactor. Acta Chim Slov 51(4):619–628
Moin S, Omar M (2013) Laccase enzymes: purification, structure to catalysis and tailoring. Protein Pept Lett 21(8):707–713. https://doi.org/10.2174/09298665113209990058
Moldes D, Gallego PP, Rodrıguez-Couto S, Sanroman A (2003) Grape seeds: the best lignocellulosic waste to produce laccase by solid state cultures of Trametes hirsuta. Biotechnol Lett 25:491–495
Morozova OV, Shumakovich GP, Gorbacheva MA, Shleev SV, Yaropolov AI (2007) "Blue" laccases. Biochemistry (Mosc) 72:1136–1150
Moya R, Hernandez M, Garcia-Martin AB, Ball AS, Arias ME (2010) Contributions to a better comprehension of redox-mediated decolouration and detoxification of azo dyes by a laccase produced by Streptomyces cyaneus CECT 3335. Bioresour Technol 3(7):2224–2229
Mugdha A, Usha M (2012) Enzymatic treatment of wastewater containing dyestuffs using different delivery systems. Sci Rev Chem Commun 2(1):31–40
Munoz C, Guillen F, Martınez AT, Martınez MJ (1997) Induction and characterization of laccase in the ligninolytic fungus Pleurotus eryngii. Curr Microbiol 34(1):1–5
Naclerio G, Falasca A, Petrella E, Nerone V, Cocco F, Celico F (2010) Potential role of Bacillus endospores in soil amended by olive mill wastewater. Water Sci Technol 61(11):2873–2879. https://doi.org/10.2166/wst.2010.082
Ng TB, Wang HX (2004) A homodimeric laccase with unique characteristics from the yellow mushroom Cantharellus cibarius. Biochem Biophys Res Commun 313(1):37–41
Nyanhongo GS, Gomesa BJ, Gubitzc GM, Zvauyab GR, Readd J, Steiner W (2002) Decolorization of textile dyes by laccases from a newly isolated strain of Trametes modesta. Water Resour 36:1449–1456
Nyman K, Hakala T (2011) Decolorization of inkjet ink and deinking of inkjet-printed paper with laccase-mediator system. Bioresources 6(2):1336–1350
O'Malley MD, Ross W, Wuli B, Chen-Loung C, Ronald S (1993) The role of laccase in lignification. Plant J 4:751–757
Oktem HA, Senyurt O, Eyidogan FI, Bayrac C, Yilmaz R (2012) Development of a laccase based paper biosensor for the detection of phenolic compounds. J Food Agric Environ 10(2):1030–1034
Omar HH (2008) Algal decolorization and degradation of monoazo and diazo dyes. Pak J Biol Sci 11(10):1310–1316
Osma JF, Toca-Herrera JL, Rodríguez-Couto S (2010) Uses of laccases in the food industry. Enzyme Res 2010:918761
Palmieri G, Giardina P, Bianco C, Scaloni A, Capasso A, Sannia G (1997) A novel white laccase from Pleurotus ostreatus. J Biol Chem 272(50):31301–31307
Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia G (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus Pleurotus ostreatus. Appl Environ Microbiol 66(3):920–924
Palvannan T, Sathishkumar P (2010) Production of laccase from Pleurotus florida NCIM 1243 using Plackett-Burman design and response surface methodology. J Basic Microbiol 50(4):325–335
Pandey A, Selvakumar P, Soccol CR, Nigam P (1999) Solid state fermentation for the production of industrial enzymes. Curr Sci 77(1):149–162
Pannu JS, Kapoor RK (2014) Microbial laccases: a mini-review on their production, purification and applications. Int J Pharm Arch 3(12):528–536
Papinutti VL, Diorio LA, Forchiassin F (2003) Production of laccase and manganese peroxidase by Fomes sclerodermeus grown on wheat bran. J Ind Microbiol Biotechnol 30(3):157–160
Park C, Lee B, Han EJ, Lee J, Kim S (2006) Decolorization of acid black 52 by fungal immobilization. Enzym Microb Technol 39(3):371–374
Perumal K (1997) Biochemical studies of lignolytic enzymes of Gonoderma lucidum, a white rot fungus and its application in treatment of paper mill effluent. Ph.D. thesis, University of Madras, Madras, India

Pickard MA, Roman R, Tinoco R, Vazquez-Duhalt R (1999) Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by Coriolopsis gallica UAMH 8260 laccase. Appl Environ Microbiol 65(9):3805–3809

Piontek K, Antorini M, Choinowski T (2002) Crystal structure of a laccase from the fungus Trametes versicolor at 1.90°A resolution containing a full complement of coppers. J Biol Chem 277:37663–37669

Piscitelli A, Pezzella C, Lettera V, Giardina P, Faraco V, Sannia G (2016) Fungal laccases: structure, function and application. In: Fungal enzymes. CRC Press, Boca Raton, FL, pp 113–151

Pointing SB (1999) Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. Fungal Divers 2:17–33

Pointing S (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57(1–2):20–33

Pointing SB, Jones EBG, Vrumed LLP (2000) Optimization of laccase production by P. sanguineus in submerged liquid culture. Mycologia 92:139–144

Polak J, Jarosz-Wilkolazka A (2012) Fungal laccases as green catalysts for dye synthesis. Process Biochem 47(9):1295–1307

Poli A, Finore I, Romano I, Gioiello A, Lama L, Nicolaus B (2017) Microbial diversity in extreme marine habitats and their biomolecules. Microorganisms 5(2):25

Polyakov KM, Fedorova TV, Stepanova EV, Cherkashin EA, Kurzeev SA, Strokopytov BV et al (2009) Structure of native laccase from Trametes hirsuta at 1.8 Å resolution. Acta Crystallogr D Biol Crystallogr 65(6):611–617

Pozdnyakova NN, Turkovskaya OV, Yudina EN, Rodakiewicz-Nowak Y (2006) Yellow laccase from the fungus Pleurotus ostreatus D1: purification and characterization. Appl Biochem Microbiol 42:56–61

Prakash O, Mahabare KU, Yadav KK, Sharma R (2019) Fungi from extreme environments: a potential source of laccases group of extremozymes. In: Tiquia-Arashiro S, Grube M (eds) Fungi in extreme environments: ecological role and biotechnological significance. Springer, Cham

Raghukumar C, Raghukumar S, Chinnaraj A, Chandramohan D, D'souza TM, Reddy CA (1994) Laccase and other lignocellulose modifying enzymes of marine fungi isolated from the coast of India. Bot Marina 37(6):515–524

Ralph J, Brunow G, Boerjan W (2007) Lignins. In: Ltd JWS (ed) eLS: Encyclopedia of life sciences. http://www.els.net. Chichester

Ranocha P, McDougall G, Hawkins S, Sterjiades R, Borderies G, Stewart D et al (1999) Biochemical characterization, molecular cloning and expression of laccases–a divergent gene family–in poplar. Eur J Biochem 259(1–2):485–495

Rehman AU, Thurston CF (1992a) Purification of laccase I from Armillaria mellea. J Gen Microbiol 138:1251–1257

Rehman AU, Thurston CF (1992b) Purification of laccase I from Armillaria mellea. Microbiology 138(6):1251–1257

Renzetti S, Courtin CM, Delcour JA, Arendt EK (2010) Oxidative and proteolytic enzyme preparations as promising improvers for oat bread formulations: rheological, biochemical and microstructural background. Food Chem 119(4):1465–1473

Revankar MS, Lele SS (2006) Increased production of extracellular laccase by the white rot fungus Coriolus versicolor MTCC 138. World J Microbiol Biotechnol 22(9):921–926

Riva S (2006) Laccases: blue enzymes for green chemistry. Trends Biotechnol 24(5):219–226

Rivera-Hoyos CM, Morales-Álvarez ED, Poutou-Piñales RA, Pedroza-Rodríguez AM, RodrÍguez-Vázquez R, Delgado-Boada JM (2013) Fungal laccases. Fungal Biol Rev 27(3–4):67–82

Rocasalbas G, Francesko A, Tourino S, Fernandez-Francos X, Guebitzc GM, Tzanov T (2013) Laccase-assisted formation of bioactive chitosan/gelatin hydrogel stabilized with plant polyphenols. Carbohydr Polym 92:989–996

Rodgers CJ, Blanford CF, Giddens SR, Skamnioti P, Armstrong FA, Gurr SJ (2010) Designer laccases: a vogue for high-potential fungal enzymes? Trends Biotechnol 28(2):63–72

Rodríguez Couto S, Toca Herrera J (2006) Industrial and biotechnological applications of laccases: a review. Biotechnol Adv 24(5):500–513. https://doi.org/10.1016/j.biotechadv.2006.04.003

Rodrıguez-Couto S, Gundın M, Lorenzo M, Sanroman MA (2002) Screening of supports and inducers for laccase production by Trametes versicolor in semi-solid-state conditions. Process Biochem 38:249–255

Rodríguez-Delgado M, Ornelas-Soto N (2017) Laccases: a blue enzyme for greener alternative technologies in the detection and treatment of emerging pollutants. In: Singh R, Kumar S (eds) Green technologies and environmental sustainability. Springer, Cham, pp 45–65. https://doi.org/10.1007/978-3-319-50654-8_2

Rosales E, Couto SR, Sanroman MA (2007) Increased laccase production by Trametes hirsuta grown on ground orange peelings. Enzym Microb Technol 40(5):1286–1290

Saito KO, Ikeda R, Endo K, Tsujino Y, Takagi M, Tamiya E (2012) Isolation of a novel alkaline-induced laccase from Flammulina velutipes and its application for hair coloring. J Biosci Bioeng 113(5):575–579

Sanchez-Amat A, Lucas-Elío P, Fernández E, García-Borrón J, Solano F (2001) Molecular cloning and functional characterization of a unique multipotent polyphenol oxidase from Marinomonas mediterranea. Biochim Biophys Acta Protein Struct Mol Enzymol 1547(1):104–116. https://doi.org/10.1016/s0167-4838(01)00174-1

Sanghi R, Dixit A, Verma P et al (2009) Design of reaction conditions for the enhancement of microbial degradation of dyes in sequential cycles. J Environ Sci (China) 21(12):1646–1651

Santhiago M, Vieira IC (2007) Determinación de l-cisteína en formulaciones farmacéuticas utilizando un biosensor a base de lacasa de Aspergillus oryzae. Sens Actuators B Chem 128:279–285

Saraiva JA, Tavares AP, Xavier AM (2012) Effect of the inducers veratryl alcohol, xylidine, and ligninosulphonates on activity and thermal stability and inactivation kinetics of laccase from Trametes versicolor. Appl Biochem Biotechnol 167:685–693

Savoie JM, Mata G (1998) The antagonistic action of Trichoderma sp. hyphae to Lentinula edodes hyphae changes lignocellulolytic activities during cultivation in wheat straw. World J Microbiol Biotechnol 15:369–373

Schäfer A, Specht M, Hetzheim A, Francke W, Schauer F (2001) Synthesis of substituted imidazoles and dimerization products using cells and laccase from Trametes versicolor. Tetrahedron 57(36):7693–7699

Scherer M, Fischer R (1998) Purification and characterization of laccase II of Aspergillus nidulans. Arch Microbiol 170(2):78–84. https://doi.org/10.1007/s002030050617

Schliephake K, Lonergan GT, Jones CL, Mainwaring DE (1993) Decolorization of a pigment plant effluent by Pycnoporus cinnabarinus in a packed-bed bioreactor. Biotechnol Lett 15:1185–1188

Schliephake K, Mainwaring DE, Lonergan GT, Jones IK, Baker WL (2000) Transformation and degradation of the disazo dye Chicago Sky Blue by a purified laccase from Pycnoporus cinnabarinus. Enzym Microb Technol 27(1–2):100–107

Schlosser D, Höfer C (2002) Laccase-catalyzed oxidation of Mn^{2+} in the presence of natural Mn^{3+} chelators as a novel source of extracellular H_2O_2 production and its impact on manganese peroxidase. Appl Environ Microbiol 68(7):3514–3521. https://doi.org/10.1128/aem.68.7.3514-3521.2002

Sedarati MR, Keshavarz T, Leontievsky AA, Evans CS (2003) Transformation of high concentrations of chlorophenols by the white-rot basidiomycete Trametes versicolor immobilized on nylon mesh. Electron J Biotechnol 6(2):27–37

Selinheimo E, Kruus K, Buchert J, Hopia A, Autio K (2006) Effects of laccase, xylanase and their combination on the rheological properties of wheat doughs. J Cereal Sci 43(2):152–159

Senthivelan T, Kanagaraj J, Panda RC (2016) Recent trends in fungal laccase for various industrial applications: an eco-friendly approach-a review. Biotechnol Bioprocess Eng 21(1):19–38

Shanmugapriya S, Manivannan G, Selvakumar G, Sivakumar N (2019) Extracellular fungal peroxidases and laccases for waste treatment: recent improvement. In: Recent advancement in white biotechnology through fungi. Springer, Cham, pp 153–187

Sharma RK, Arora DS (2010) Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by Phlebia floridensis. Bioresour Technol 101(23):9248–9253

Sharma KK, Kuhad RC (2008) Laccase: enzyme revisited and function refined. Ind J Microb 48:309–316

Sharma R, Prakash O, Sonawane MS, Nimonkar Y, Golellu PB, Sharma R (2016) Diversity and distribution of phenol oxidase producing fungi from soda lake and description of Curvularia lonarensis sp. nov. Front Microbiol 7:1847

Shuttleworth KL, Bollag JM (1986) Soluble and immobilized laccase as catalysts for the transformation of substituted phenols. Enzym Microb Technol 8(3):171–177

Singh G, Capalash N, Goel R, Sharma P (2007) A pH-stable laccase from alkali-tolerant γ-proteobacterium JB: purification, characterization and indigo carmine degradation. Enzym Microb Technol 41(6–7):794–799

Singh G, Bhalla A, Kaur P, Capalash N, Sharma P (2011) Laccase from prokaryotes: a new source for an old enzyme. Rev Environ Sci Biotechnol 10(4):309–326. https://doi.org/10.1007/s11157-011-9257-4

Soden DM, Dobson ADW (2001) Differential regulation of laccase gene expression in Pleurotus sajor-caju. Microbiology 147(7):1755–1763

Solomon EI, Augustine AJ, Yoon J (2008) O_2 Reduction to H_2O by the multicopper oxidases. Dalton Trans 30:3921–3932

Souza C, Zilly A, Peralta RM (2002) Production of laccase as the sole phenoloxidase by a Brazilian strain of Pleurotus pulmonarius in solid state fermentation. J Basic Microbiol 42(2):83–90

Strong PJ (2011) Improved laccase production by Trametes pubescens MB89 in distillery wastewaters. Enzyme Res 379176:1–8

Strong PJ, Claus H (2011) Laccase: a review of its past and its future in bioremediation. Crit Rev Environ Sci Technol 41(4):373–434

Suzuki T, Endo K, Ito M, Tsujibo H, Miyamoto K, Inamori Y (2003) A thermostable laccase from Streptomyces lavendulae REN-7: purification, characterization, nucleotide sequence, and expression. Biosci Biotechnol Biochem 67(10):2167–2175. https://doi.org/10.1271/bbb.67.2167

Tavares APM, Coelho MAZ, Agapito MSM, Coutinho JAP, Xavier AMRB (2006) Optimization and modeling of laccase production by Trametes versicolor in a bioreactor using statistical experimental design. Appl Biochem Biotechnol 134(3):233–248

Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140:19–26

Tinoco R, Pickard MA, Vazquez-Duhalt R (2001) Kinetic differences of purified laccases from six Pleurotus ostreatus strains. Lett Appl Microbiol 32:331–336

Torres E, Bustos-Jaimes I, Le Borgne S (2003) Potential use of oxidative enzymes for the detoxification of organic pollutants. Appl Catal B Environ 46(1):1–15

Urzua U, Fernando LL, Lobos S, Larrain J, Vicuna R (1995) Oxidation reactions catalyzed by manganese peroxidase isoenzymes from Ceriporiopsis subvermispora. FEBS Lett 371:132–136

Vasconcelos A, Barbosa A, Dekker R, Scarminio I, Rezende M (2000) Optimization of laccase production by Botryosphaeria sp. in the presence of veratryl alcohol by the response-surface method. Process Biochem 35(10):1131–1138. https://doi.org/10.1016/s0032-9592(00)00149-7

Vats A, Mishra S (2018) Identification and evaluation of bioremediation potential of laccase isoforms produced by Cyathus bulleri on wheat bran. J Hazard Mater 344:466–479. https://doi.org/10.1016/j.jhazmat.2017.10.043

Vavourakis CD, Ghai R, Rodriguez-Valera F, Sorokin DY, Tringe SG, Hugenholtz P, Muyzer G (2016) Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline soda lake brines. Front Microbiol 7:211

Verma AK, Raghukumar C, Verma P, Shouche YS, Naik CG (2010) Four marine-derived fungi for bioremediation of raw textile mill effluents. Biodegradation 21(2):217

Vianello F, Cambria A, Ragusa S, Cambria MT, Zennaro L, Rigo A (2004) A high sensitivity amperometric biosensor using a monomolecular layer of laccase as biorecognition element. Biosens Bioelectron 20(2):315–321

Vicente AI, Viña-Gonzalez J, Mateljak I, Monza E, Lucas F, Guallar V et al (2020) Enhancing thermostability by modifying flexible surface loops in an evolved high-redox potential laccase. AIChE J 66. https://doi.org/10.1002/aic.16747

Vijaykumar MH, Veeranagouda Y, Neelakanteshwar K, Karegoudar TB (2006) Decolorization of 1:2 metal complex dye Acid blue 193 by a newly isolated fungus, Cladosporium cladosporioides. World J Microbiol Biotechnol 22(2):157–162

Viswanath B, Subhosh Chandra M, Pallavi H, Rajasekhar Reddy B (2008) Screening and assessment of laccase producing fungi isolated from different environmental samples. Afr J Biotechnol 7(8):1129–1133

Viswanath B, Rajesh B, Janardhan A, Kumar AP, Narasimha G (2014) Fungal laccases and their applications in bioremediation. Enzyme Res 163242:1–21. https://doi.org/10.1155/2014/163242

Wahleithner JA, Xu F, Brown KM, Brown SH, Golightly EJ, Halkier T et al (1996) The identification and characterization of four laccases from the plant pathogenic fungus Rhizoctonia solani. Curr Genet 29(4):395–403

Wang HX, Ng TB (2004) Purification of a novel low-molecular-mass laccase with HIV-1 reverse transcriptase inhibitory activity from the mushroom Tricholoma giganteum. Biochem Biophys Res Commun 315(2):450454

Wang HX, Ng TB (2006) Purification of a laccase from fruiting bodies of the mushroom Pleurotus eryngii. Appl Microbiol Biotechnol 69:521–525

Wang W, Liu F, Jiang Y, Wu G, Guo L, Chen R et al (2015) The multigene family of fungal laccases and their expression in the white rot basidiomycete Flammulina velutipes. Gene 563(2):142–149

Wesenberg D, Kyriakides I, Agathos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv 22:161–187

Wong DW (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157(2):174–209

Wong K-S, Huang Q, Au C-H, Wang J, Kwan H-S (2012) Biodegradation of dyes and polyaromatic hydrocarbons by two allelic forms of Lentinula edodes laccase expressed from Pichia pastoris. Bioresour Technol 104:157–164

Woolery G, Powers L, Peisach J, Spiro T (1984) X-ray absorption study of Rhus laccase: evidence for a copper-copper interaction, which disappears on type 2 copper removal. Biochemistry 23(15):3428–3434. https://doi.org/10.1021/bi00310a008

Xenakis A, Zoumpanioti M, Stamatis H (2016) Enzymatic reactions in structured surfactant-free microemulsions. Curr Opin Colloid Interface Sci 22:41–45

Xu F (1997) Effects of redox potential and hydroxide inhibition on the pH activity profile of fungal laccases. J Biol Chem 272(2):924–928

Xu Q, Fu Y, Gao Y, Qin M (2009) Performance and efficiency of old newspaper deinking by combining cellulase/hemicellulase with laccase-violuric acid system. Waste Manag 29(5):1486–1490

Yadav AN (2020) Recent trends in mycological research, Agricultural and medical perspective, vol 1. Springer, Switzerland

Yadav AN, Verma P, Sachan SG, Kaushik R, Saxena AK (2018) Psychrotrophic microbiomes: molecular diversity and beneficial role in plant growth promotion and soil health. In: Panpatte DG, Jhala YK, Shelat HN, Vyas RV (eds) Microorganisms for green revolution, Microbes for sustainable agro-ecosystem, vol 2. Springer, Singapore, pp 197–240. https://doi.org/10.1007/978-981-10-7146-1_11

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N (2020a) Microbiomes of extreme environments, Biotechnological applications in agriculture, environment and industry, vol 2. CRC Press, Taylor & Francis Group, Boca Raton, FL

Yadav AN, Rastegari AA, Yadav N (2020b) Microbiomes of extreme environments: biodiversity and biotechnological applications. CRC Press, Taylor & Francis, Boca Raton, FL

Yang J, Li W, Ng TB, Deng X, Lin J, Ye X (2017) Laccases: production, expression regulation, and applications in pharmaceutical biodegradation. Front Microbiol 8:832

Yang X, Gu C, Lin Y (2020) A novel fungal laccase from Sordaria macrospora k-hell: expression, characterization, and application for lignin degradation. Bioprocess Biosyst Eng 43:1133–1139. https://doi.org/10.1007/s00449-020-02309-5

Yaropolov AI, Skorobogat'ko OV, Vartanov SS, Varfolomeyev SD (1994) Laccase: properties, catalytic mechanism, and applicability. Appl Biochem Biotechnol 49:257–280

Yaver DS, Xu F, Golightly EJ, Brown KM, Brown SH, Rey MW et al (1996) Purification, characterization, molecular cloning, and expression of two laccase genes from the white rot basidiomycete Trametes villosa. Appl Environ Microbiol 62(3):834–841

Yaver DS, Overjero MDC, Xu F, Nelson BA, Brown KM, Halkier T et al (1999) Molecular characterization of laccase genes from the basidiomycete Coprinus cinereus and heterologous expression of the laccase Lcc1. Appl Environ Microbiol 65(11):4943–4948

Yoshida H (1883) Chemestry of Lacquer (Urushi) Part 1. J Chem Soc 43:472–486

Yoshitake A, Katayama Y, Nakamura M, Iimura Y, Kawai S, Morohoshi N (1993) N-linked carbohydrate chains protect laccase III from proteolysis in Coriolus versicolor. J Gen Microbiol 139:179–185

Zadrazil F, Gonser A, Lang E (1999) Influence of incubation temperature on the secretion of extracellulare lignolytic enzymes of Pleurotus and Dichomitus squalus into soil. In: Proceedings of the Conf. on enzymes in the environment, Granada, Spain

Zahmatkesh M, Spanjers H, van Lier JB (2017) Fungal treatment of humic-rich industrial wastewater: application of white rot fungi in remediation of food-processing wastewater. Environ Technol 38(21):2752–2762

Zerva A, Simić S, Topakas E, Nikodinovic-Runic J (2019) Applications of microbial laccases: patent review of the past decade (2009–2019). Catalysts 9:1023. https://doi.org/10.3390/catal9121023

Zhang Q, Zhao L, Li Y, Wang F, Li S, Shi G, Ding Z (2020) Comparative transcriptomics and transcriptional regulation analysis of enhanced laccase production induced by co-culture of Pleurotus eryngii var. ferulae with Rhodotorula mucilaginosa. Appl Microbiol Biotechnol 104(1):241–255

Zhukova YN, Lyashenko AV, Lashkov AA, Gur'yanov VA, Kobyl'skaya YV, Zhukhlistova NE, Mikhailov AM (2010) Atomic structure of unligated laccase from Cerrena maxima at 1.76 Å with molecular oxygen and hydrogen peroxide. Crystallogr Rep 55(3):436–447

Zille A, Ramalho P, Tzanov T, Millward R, Aires V, Cardoso MH, Ramalho MT, Gübitz GM, Cavaco-Paulo A (2004) Predicting dye biodegradation from redox potentials. Biotechnol Prog 20(5):1588–1592

Zumárraga M, Bulter T, Shleev S, Polaina J, Martínez-Arias A, Plou FJ et al (2007) In vitro evolution of a fungal laccase in high concentrations of organic cosolvents. Chem Biol 14(9):1052–1064

Chapter 7
Fungal Cellulases: Current Research and Future Challenges

Soumya Ghosh, Liliana Godoy, Kenneth Yongabi Anchang, Conrad C. Achilonu, and Marieka Gryzenhout

Contents

S. Ghosh (✉) · C. C. Achilonu · M. Gryzenhout
Department of Genetics, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa
e-mail: GhoshS@ufs.ac.za

L. Godoy
Department of Fruit and Oenology, Faculty of Agronomy and Forestry, Pontifical Catholic University of Chile, Santiago, Chile

K. Y. Anchang
Faculty of Health Sciences, Department of Public Health, Imo State University, Owerri, Nigeria

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_7

7.1 Introduction

Cellulose is the most abundant and renewable component of plant biomass (Srivastava and Jaiswal 2016; Srivastava et al. 2015a, b, c; Tomme et al. 1995). It is the key product of photosynthesis, and the most abundant renewable bioresource (Zhang and Lynd 2004). Cellulose commonly exists in pure form, or otherwise in association with hemicellulose and lignin, and is found in plants as microfibrils (2–20 nm diameter and 100–40,000 nm long). Structurally, they form a strong framework in the plant cell wall (Zhang and Lynd 2004). Approximately 100 billion dry tons/year of cellulosic biomass is produced in the biosphere (Wang et al. 2016). Considered as a low-cost energy source based on energy content (Lynd et al. 2008; Zhang 2009), cellulose can bring benefits to local economies, the environment, and national energy securities (Zhang 2008).

Cellulose is a linear polysaccharide of glucose monomeric units bonded by β-1,4-glycosidic linkages. These monomeric units, consisting predominantly of small chain/s of cellobiose and glucose molecules (Taherzadeh and Karimi 2007), are released from the insoluble cellulose polymer through enzymatic hydrolysis by cellulases. Cellulases are a combination of enzymes, namely, endoglucanases, cellobiohydrolases, and β-glucosidases (Thota et al. 2017), which are distinctly folded with variable structural and functional modules (Henrissat et al. 1998; Taherzadeh and Karimi 2007). The catalytic modules of cellulases have been classified into numerous families based on their amino acid sequences and crystal structures (Henrissat 1991).

Cellulases are produced by fungi, bacteria, protozoans, plants, and some animal species such as termites and crayfish, whose cellulases differ substantially from the former organisms (Watanabe and Tokuda 2001). Among the organisms producing cellulases, fungi are studied extensively because their elongated hyphae can additionally inflict mechanical pressure on the cellulose structures aiding results of cellulase activities. Concurrently, fungal strains also have the capability to produce higher quantities of cellulases when compared to bacteria, plants, and animals (Amouri and Gargouri 2006; Chang et al. 2016; Gaur and Tiwari 2015; Have et al. 2002; Lange et al. 2019).

Fungal cellulases have significant applications in industries that deal with textiles, paper and pulp (Esteghlalian et al. 2001), food (Fernandes 2010), detergents, wine and brewery (Kuhad et al. 2011), amino acids synthesis (Ahmed and Bibi 2018), and animal feed and agriculture (Abdel-Azeem et al. 2021; Sharada et al. 2014; Yadav et al. 2019a, b). Furthermore, research of cellulases has grown rapidly in recent years due to the huge production of glucose that can occur at large scale by cellulase, which can be fermented to ethanol that has a variety of global applications, especially as biofuel (Agarwal et al. 2018; Brar et al. 2019; Johnson 2016; Li et al. 2020; Lynd et al. 2017). This chapter discusses microbial cellulases and related enzymes, their classifications and their action mechanisms, the fungal cellulases with their relevant aspects of the potential utilization, and their applications in diverse fields and industries.

7.2 Lignocelluloses

Lignocelluloses are the key structural polysaccharides of plant biomass, constituting cellulose, hemicellulose, and lignin, and are commonly spread across vascular plants (Marriott et al. 2016; Zhang et al. 2012; Zoghlami and Paes 2019). Cellulose (≈50%) and hemicellulose (≈30%) are polysaccharides of simple sugar units linked by linear β-1,4 linkages along with lignin that account for the remaining 20% of the lignocellulose. Additionally, hemicellulose may contain polymers of sugars such as pentoses (arabinose and xylose) and hexoses (glucose, galactose, mannose), making it a branched-chain polymer (Howard et al. 2003; Scheller and Ulvskov 2010). The natural variability in the hemicellulose content depends on the kind of plant source. For example, the softwoods (gymnosperms) such as spruce and pine plants have hemicellulosic regions that contain mostly structural and storage polysaccharides such as mannan (Girio et al. 2010). On the other hand, hardwoods (angiosperms) such as oak plants are mainly composed of xylans that increase. Lignin is a complex three-dimensional network of aryl or aromatic alcohol groups constituting of three phenyl propane units, such as coumaryl, coniferyl, and sinapyl alcohol, that are linked by numerous C–O and C–C bonds (Boerjan et al. 2003). The cell wall recalcitrance to enzymatic digestion (Faik 2013). Studies have shown that softwoods have a higher proportion of conferyl alcohol (90%) (Novaes et al. 2010) in comparison to hardwood where conferyl and sinapyl alcohols occur at equal proportions (Santos et al. 2012).

Hemicellulose and lignin form the amorphous network where the crystalline cellulose structures are attached (Rubin 2008; Sajith et al. 2016). The cellulosic microfibrils are stabilized through various intra- and intermolecular hydrogen bonds and Van der Walls interactions. Hemicellulose polymers surround these microfibrils. The matrices of cellulose–hemicellulose are protected from microorganisms by a covering of amorphous insoluble polymers lignin on the internal cellulosic structures (Sajith et al. 2016).

Intriguingly, some agricultural residues contain nonedible lignocelluloses such as forestry waste and agriculture crop residues (Mishra and Mohanty 2018). These can be used as renewable sources for the production of several value-added products, such as biofuel, that could be a replacement for fossil fuel (Wi et al. 2015). Furthermore, chemical pretreatment processes of lignocelluloses had various setbacks such as the release of by-products that discontinue sugar fermentation (Den et al. 2018; Singhvi and Gokhale 2019). To circumvent this problem, the use of lignocellulolytic enzymes as biocatalysts has proven to be significant in improving the quality of the pretreatment process of lignocellulose (Brijwani and Vadlani 2011; Lynd et al. 2008; Oberoi et al. 2014).

7.3 Microbial Lignocellulolytic Enzymes

A large amount of lignocellulosic material, such as herbaceous crops (alfalfa, switch grass) agricultural residues (corn stover, corn fiber, sugarcane bagasse), forestry residues (chips, sander dust, bark, sawdust, planer shavings), waste paper, and other wastes (municipal and industrial) (Acharya and Chaudhary 2012), is produced annually on the earth surface. Rapid microbial degradation prevents bulk accumulation of those wastes (Irbe et al. 2014). These microbes successfully convert synthesized organic debris into humus to provide carbon and energy for their survival as well as to recycle carbon to the ecosystem (He et al. 2009). Although a large number of microbes are reported to possess degradation capabilities, only a few have been proven to own a set of enzyme transcriptional systems for lignocellulolytic enzymes essential for the breakdown of lignocelluloses into simpler molecules (Adrio and Demain 2014; Ravindran et al. 2018; Ravindran and Jaiswal 2016).

There is an increasing demand for additional, active, and specific lignocellulolytic enzymes for an ultimate way to manage lignocellulosic biomass and for efficient industrial production of value-added products. Biotechnology and bioprocessing tools industrially aid the successful exploration of lignocelluloses for the production and exploitation of lignocellulolytic enzymes (Yadav et al. 2020). For instance, previous studies have shown the use of fungi for the large-scale production of lignocellulolytic enzymes in industries for other applications, as fungi can adhere and grown to solid substrates with little moisture content (Kour et al. 2019c; Sette et al. 2008; Toushik et al. 2017). Notably, filamentous fungi are more prolific in extracellular lignocellulolytic enzyme production in comparison to bacteria and yeast (Cunha et al. 2018; Soliman et al. 2012). Furthermore, these enzymes have been utilized in managing lignocellulose-containing environmental pollutants, such as textile and pulp effluents, organochloride agrochemicals, and crude oil residues (Couto and Toca-Herrera 2007; Kiiskinen et al. 2004; Mehandia et al. 2020; Santhanam et al. 2011; Sitarz et al. 2016).

7.3.1 Classification of Microbial Lignocellulolytic Enzymes

Numerous microbes thrive on the lignocelluloses materials; however, a few could produce an array of enzymes to degrade them into simpler molecules. Conventionally, the enzymatic degradation of lignocelluloses is carried out by a collection of complex enzymes called hemicellulases, cellulases, and ligninases (Fig. 7.1).

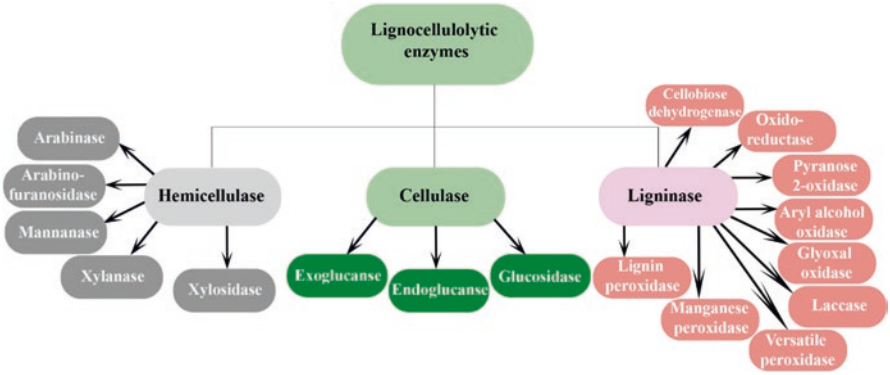


Fig. 7.1 Classification of lignocellulolytic enzymes involved in lignocellulose degradation

7.3.1.1 Hemicellulases

Hemicellulases are glycoside hydrolases that hydrolyze glycosidic bonds and ester linkages of acetate or ferulic acid side groups of hemicellulose substrates (Weiss et al. 2013; Zoghlami and Paes 2019). They have been primarily classified as arabinase (EC 3.2.1.99), arabinofuranosidases (EC 3.2.1.55), β-mannanases (EC 3.2.1.78), xylanases (EC 3.2.1.8), and β-xylosidases (EC 3.2.1.37) (Fig. 7.1). Mannanase and arabinose or arabinofuranosidases are essential for the complete degradation of hemicellulose (Sajith et al. 2016; Zoghlami and Paes 2019). Xylanase hydrolyzes xylan to oligomers that are further degraded by β-xylosidases to xylose (Sajith et al. 2016). For example, β-mannanase has been used in the poultry feed industry to digest antinutritional factors of soybeans and other leguminous seeds that are essential for the growth, digestion, health, and metabolism of nutrients in poultry birds (Saeed et al. 2019). Likewise, L-arabinose isomerases allow for the manufacture of D-tagatose from dairy products that has an antiglycemiant effect and possess potential in both pharmaceutical and agro-food industries (Boudebbouze et al. 2011). Another hemicellulases enzyme xylanase has been widely used for liquefying the mucilaginous hemicellulose matrix in the preparation of commercial coffee (Pedersen et al. 2009).

7.3.1.2 Ligninases

Ligninases or lignin-modifying enzymes (LMEs) are generated by fungi or bacteria that break down lignin into low-molecular-weight (MW) compounds to be absorbed by other microorganisms (Wong 2009). Ligninases are categorized into various types: (a) Lignin peroxidase, LiP (EC 1.11.1.14); (b) manganese peroxidases, MnP (EC 1.11.1.13); (c) versatile peroxidase, VP (EC 1.11.1.16); and (d) laccases (EC 1.10.3.2), glyoxal oxidase (EC 1.2.3.5), aryl alcohol oxidase (EC 1.1.3.7), pyranose 2-oxidase (EC 1.1.3.4), cellobiose/quinone oxidoreductase (EC 1.1.5.1), and

cellobiose dehydrogenase (EC 1.1.99.18) (Arora and Sharma 2010; Baldrian 2006; Kirk and Farrell 1987; Martinez et al. 2005) (Fig. 7.1).

Lignin peroxidase is the most crucial enzyme for lignin degradation. It is a hemiprotein (1,2-bis(3,4-dimethoxyphenyl) propane-1,3-diol: hydrogen-peroxide oxidoreductase) that catalyzes the H_2O_2-dependent oxidative depolymerization of lignin (Hammel et al. 1993; Vares et al. 1995). It was been first isolated from a white-rot fungus called *Phanerochaete chrysosporium* (de Paula et al. 2018; Edwards et al. 1993) and subsequently from other fungi, such as white-rot *Polyporales* species (Ayuso-Fernandez et al. 2019), *Trametes hirsuta* (Vasina et al. 2017), and *Schizophyllum commune* IBL-06 (Asgher et al. 2012).

Manganese peroxidase (MnP) catalyzes the Mn-dependent reaction $2Mn(II) + 2H^+ + H_2O_2 = 2Mn(III) + 2H_2O$. MnP occurs extracellularly and was first purified from *P. chrysosporium* by growing the fungus in a culture medium supplemented by Mn (II) (Bonnarme and Jeffries 1990). Studies in a solid-state fermentation process of rice straw with *P. chrysosporium* have shown the underlying mechanism involved in manganese amendment in the media that eventually controls the *mnp1* and *mnp2* gene transcription levels (Huang et al. 2017). Additionally, certain other stimulating factors such as Mn(II), glycolate, malonate, glucuronate, and 2-hydroxybutyrate, are identified that enhance the production of MnP by *P. chrysosporium* (Fujihara et al. 2008) and *Clitopiluss cyphoides* (Rao et al. 2019).

The molecular structure of the versatile peroxidase (VP) is comparable to lignin peroxidase and manganese peroxidase and has been isolated from fungi such as *Bjerkandera* species (Moreira et al. 2005) and *Pleurotus eryngii* (Chen et al. 2010; Palma et al. 2016). However, this VP group of enzymes is not only specific for Mn(II) as in the case of MnP but also oxidizes other aromatic substrates such as phenolic and non-phenolic compounds in the absence of manganese (Ruiz-Duenas et al. 2007; Wang et al. 2016).

Laccases are one of the earliest enzymes that belong to the multi-copper enzyme family and have been secreted from various bacteria, fungi, insects, and plants (Forootanfar and Faramarzi 2015). These enzymes catalyze the reaction of 4Benzenediol + $O_2 \leftrightarrow$ 4Benzosemiquinone + $2H_2O$ that involves the degradation of lignin via oxidation of phenolic compounds to yield phenoxy radicals and quinines (Brijwani and Vadlani 2011). Laccase activities have been identified in bacteria such as *Leptothrix discophora*, *Pseudomonas maltophilia*, *Pseudomonas syringae*, *Streptomyces* species (Chandra and Chowdhary 2015), *Azospirillum lipoferum* (Givaudan et al. 1993), *Geobacterial*, *Staphylococcus*, *Lysinibacillus*, *Aquisalibacillus*, *Proteobacterium, and Alteromonas* species (Guan et al. 2018). Fungi such as *Aspergillus nidulans*, *Phanerochaete chrysosporium*, *Lentinula edodes*, *Phellinus ribis*, and *Pleurotus pulmonarius* produce laccase that has extensive biotechnological applications, such as laccase-based bio-transfer, bio-oxidation, biosensor, and enzymatic synthesis of organic compounds (Arora and Sharma 2010). The plant laccases function in wound response and, along with peroxidases, catalyze the biosynthesis of lignin (Berthet et al. 2012; Tobimatsu and Schuetz 2019).

Glyoxal oxidases (GLOX) are copper-containing enzymes that exhibit similarity in active site structure and chemistry with galactose oxidases and are responsible for

the generation of H_2O_2 from plant biomass (Daou and Faulds 2017). GLOX was first identified in the fungus *Phanerochaete chrysosporium* and later isolated from *Utilago maydis*, the smut fungus where it was reported to cause filamentous growth and pathogenicity (Daou and Faulds 2017). Similar functionalities of H_2O_2 generation have been observed with aryl-alcohol oxidase (AAO) that was reported to be associated with lignolytic activities of white-rot fungus. AAO was first detected in *Polystictus versicolor* (current name *Trametes versicolor*) (Farmer et al. 1960) and later in a *Pleurotus* sp. (Muheim et al. 1990).

Pyranose 2-oxidase (P2O), the flavoenzyme, was first isolated from *Trametes multicolor* and found to be involved in catalyzing the oxidation of D-glucose and other aldopyranose sugars at the C2 position by supplying electron to the O_2 that results in 2-ketosugars and H_2O_2 (Prongjit et al. 2013). Cellobiose oxidoreductase (CBOR) and cellobiose dehydrogenase (CDH) are involved in lignocellulose degradation (Mason et al. 2002) and reduction of lytic polysaccharide monooxygenase (LPMO) by extracellular electron transfer, respectively (Scheiblbrandner and Ludwig 2020), and were detected in the white-rot fungus *P. chrysosporium* (Mason et al. 2002; Scheiblbrandner and Ludwig 2020).

7.3.1.3 Cellulases and Classification

Cellulases are glycosyl hydrolases produced by fungi and bacteria (Jayasekara and Ratnayake 2019; Juturu and Wu 2014; Yadav 2019) and are responsible for hydrolyzing the β-1,4-glycosidic bonds of intact cellulose and other related cello-oligosaccharide derivatives (Bajaj and Mahajan 2019; Barati and Amiri 2015; Kuhad et al. 2011; Mojsov 2016). Cellulases are classified based on their hydrolytic specificities toward the β-1,4 glycosidic linkages between two or more carbohydrates or one carbohydrate and a non-carbohydrate moiety (Lynd et al. 2002a; Mojsov 2016). There are three subcomponents, namely, exo-1,4-β-D-glucanase (exoglucanase, EC 3.2.1.91), endo-1,4-β-D glucanase (endoglucanases, EC 3.2.1.4), and β-D-glucosidases (β-D-glucoside glucanhydrolase, EC 3.2.1.21).

The enzymatic hydrolysis mechanism of cellulases involves the synergistic action of these three subcomponent key enzymes (Mojsov 2016; Soni et al. 2018; Yoon et al. 2014; Zhang and Lynd 2004). Exoglucanases attack the crystalline termini of cellulosic substrates to produce cellobiose. The endoglucanases hydrolyze the glycosidic bonds within the amorphous inner regions of the fibrils unsystematically, while the β-glucosidases complete the conversion process by hydrolyzing cellobiose and other cellodextrins to monomeric glucose units.

7.3.1.3.1 Endoglucanases (EG)

Endoglucanases (EG) are monomeric enzymes and the first cellulases to act in the hydrolysis of cellulose by hydrolyzing the internal β-1,4 glycosidic bonds at random in the low crystalline regions of the cellulose fibers (Fig. 7.2). These enzymes

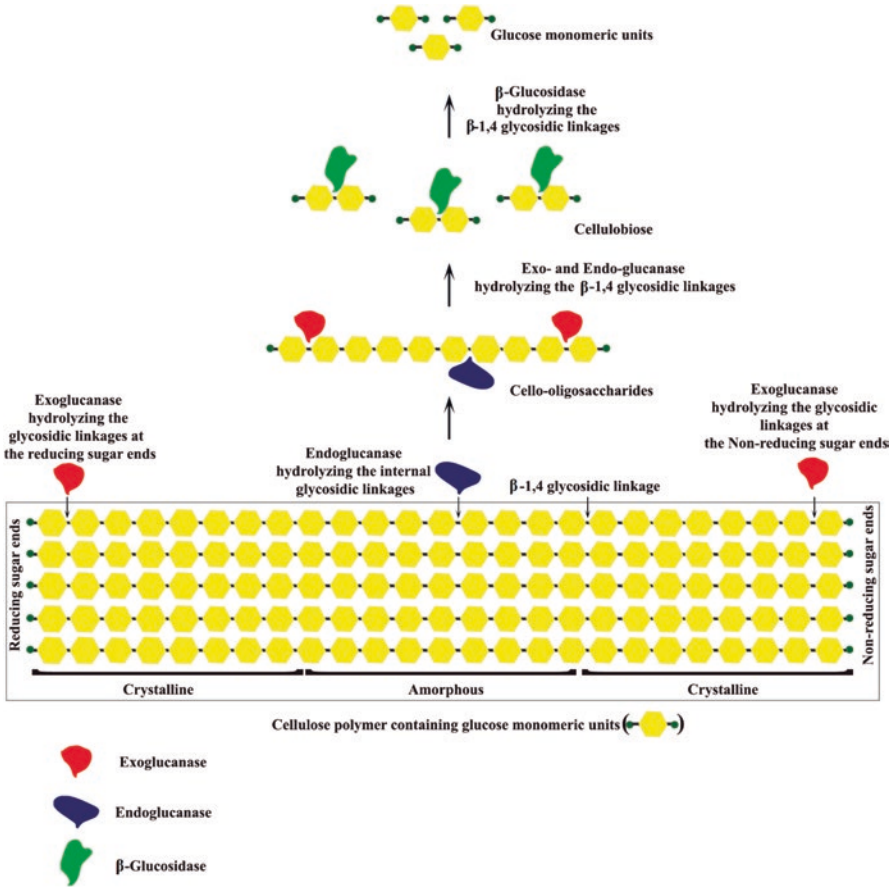


Fig. 7.2 Schematic representation of the synergistic hydrolytic action of cellulases on cellulose polymers

thus generate oligosaccharides of different lengths by inserting a water molecule into the β-1,4 junctions, generating reducing and non-reducing ends in the polymer chains that are susceptible to the subsequent action of exoglucanase (Gupta and Verma 2015). They may belong to various families of glycosyl hydrolases, described in the CAZy (www.CAZy.org) database of active carbohydrate enzymes, and their molecular weight varies from 22 to 45 kDa (Lombard et al. 2014).

Several studies have described fungi to possess multiple endoglucanases in their cellulase enzyme complexes. For example, the aerobic fungus *Trichoderma reesei* has been reported to produce five endoglucanases (EGI, EGII, EGIII, EGIV, and EGV) (Miettinen-Oinonen and Suominen 2002). Fungal endoglucanases commonly hold low or no glycosylation and have an open cleft that allows them to bind to the substrate (Baldrian and Valaskova 2008). It has been reported that endoglucanases

act at an optimal pH between 4 and 5 and temperature between 50 and 70 °C (Yennamalli et al. 2013).

7.3.1.3.2 Exoglucanases or Cellobiohydrolases (CBH)

Exoglucanases, also called Cellobiohydrolases (CBH), catalyze the successive hydrolysis of the cellulobiose residues produced by endoglucanase by progressively excising the β-1,4 glycosidic bonds from the reducing and non-reducing ends of cellulose (Fig. 7.2). CBHs have been shown to create a substrate-binding tunnel that surrounds cellulose. Cellobiose is released as the main product of the cellulose polymer, although larger oligomers are also released (Quiroz-Castañeda and Folch-Mallol 2013).

Biochemically, these enzymes are monomeric with molecular weight varying between 50 and 65 kDa. However, there are exemptions for some fungi such as the exoglucanase of *Sclerotium rolfsii* being 41.5 kDa (Sadana and Patil 1988). These enzymes exhibit low levels of glycosylation, up to 12%, and are functional at an optimal pH between 4 and 5, and a temperature range of 37–60 °C (Quiroz-Castañeda and Folch-Mallol 2013).

7.3.1.3.3 β-Glucosidase

β-Glucosidases (BGL) is the third and last major component of the cellulase complex system that degrades cellulose. They hydrolyze β-1,4-glycosidic bonds from soluble cellodextrins (cellulobiose) to fermentable monosaccharides. At the same time, they remove intermediates that act as inhibitors for EG and CBH. β-Glucosidases are competitively inhibited by their final product, glucose, by a feedback inhibition mechanism (Zhang and Lynd 2004) (Fig. 7.2).

BGL fungal enzymes have been well documented (Larue et al. 2016; Tiwari et al. 2013). Studies have shown that BGL's have been isolated from different fungal species, such as *T. reesei* and various white- and brown-rot fungi (Dashtban et al. 2009). β-Glucosidases belong to two families of glycosyl hydrolases (Glycosyl Hydrolase 1 and Glycosyl Hydrolase 3) and exhibit the most significant variability among cellulolytic enzymes (Henrissat 1991). For example, β-glucosidases from *Pleurotus ostreatus* have a monomeric structure and a molecular mass of about 35 kDa (Morais et al. 2002). However, in *Sporobolomyces singularis*, the BGL is dimeric (Ishikawa et al. 2005), and even in *Pisolithus tinctorius*, it is trimeric with a molecular mass of 450 kDa (Cao and Crawford 1993).

The β-glucosidases can occur intracellularly, where they are associated with the cell wall, as well as extracellularly. They lack cellulose-binding domains (CBD) and consist of a single catalytic module (Cao and Crawford 1993). Most of the β-glucosidases are glycosylated, being able to reach a degree of glycosylation of up to 90%, as is the case with β-glucosidase from *Trametes versicolor* (Du et al. 2015).

The optimum temperature and pH vary between 45–75 °C and 3.5–5.5, respectively (Dashtban et al. 2009).

7.4 Microbial Cellulases

Microbial cellulases are inducible enzymes and are predominantly synthesized by diverse bacterial and fungal species during their growth on cellulosic material (Kour et al. 2019a; Lee and Koo 2001). These cellulases have become important biocatalysts due to their complex nature and widely used industrial applications (Henrissat et al. 1998). The bacterial and fungal cellulases include microbes of both aerobic and anaerobic nature.

Among bacterial species, there are distinct differences between the aerobic and the anaerobic adopted cellulolytic strategies. Anaerobic bacterial species possess complex cellulase systems, called cellulosomes, that are localized on the cell-glycocalyx matrix (Demain et al. 2005; Lynd et al. 2002b; Rainey et al. 1994). For example, the thermophilic anaerobic bacterium *Clostridium thermocellum* possesses the well-defined polycellulosome organelles that contain nonenzymatic catalytic scaffolding proteins to which a number of enzymatic or catalytic subunits are attached (Bayer et al. 1998; Fontes and Gilbert 2010; Schwarz 2001). These anaerobic species cannot secrete measurable amounts of extracellular cellulase and, therefore, cannot effectively penetrate the cellulosic material.

In contrast, aerobic bacteria, fungi, and Actinomycetes produce secretary cellulases that hydrolyze the β-1,4-glycosidic linkages of cellulose. For example in the case of bacteria, species of *Cellulomonas* are coryneform bacteria and have cellulosome-like protuberant structures on their cell surfaces (Lynd et al. 2002a). These bacteria produce six endoglucanases and one exoglucanase (Cex) enzyme (Chaudhary et al. 1997). *Thermobifida fusca* is a thermophilic filamentous bacterium that degrades cellulosic material in soil and can produce six cellulase enzymes with three endoglucanases (E1, E2, E5), two exoglucanases (E3 and E6), and an uncommon cellulase with endoglucanase and exoglucanase activities (E4) (Lynd et al. 2002a).

Recurrent research on aerobic cellulolytic fungi has been prevalent (Kuhad et al. 2011; Sajith et al. 2016). Notably, *Trichoderma*, *Penicillium*, and *Aspergillus* species have been reported with the ability to produce high levels of extracellular cellulases (Barros et al. 2010). These fungal cellulases have significant roles by hydrolyzing diverse biomasses such as sugarcane bagasse, rice hulls, forest residues, corn stover, corn fiber, municipal solid waste, paper mill sludge, and industrial waste (Acharya and Chaudhary 2012). Therefore, these enzymes dominate industrial applications.

The *Trichoderma reesei* cellulase system has been studied well (Bischof et al. 2016; Druzhinina and Kubicek 2017). Two exoglucanases (CBHI and CBHII), five endoglucanases (EGI, EGII, EGIII, EGIV, and EGV), and two β-glucosidases (BGLI and BGLII) were characterized from *T. reesei* (de Paula et al. 2018; Zhang

et al. 2012). However, two *Penicillium* strains, *Penicillium* sp. GZ-2 and *Penicillium* sp. TG2, have been reported to be more efficient in cellulolytic enzyme production and enzymatic hydrolysis in comparison to *T. reesei* RUT-C30 during growth on agricultural residues (Liao et al. 2015). Studies have also identified and characterized an *Aspergillus* sp. that produces cellulolytic enzymes at a larger scale on lignocellulosic biomass (Kuhad et al. 2011). The protocol for a mixed substrate solid fermentation has been optimized for the production of cellulases and xylanases by *Aspergillus fumigatus* ABK9 (Das et al. 2013; Mehboob et al. 2014; Rana et al. 2014).

The cellulolytic activities of actinomycetes species are largely involved biomass modifications. Species of *Streptomyces*, *Micromonospora*, *Intrasporangium*, *Saccharopolyspora*, *Rhodococcus*, *Saccharomonospora*, *Nocardia*, *Cellulomonas*, *Microbispora,* and *Thermobifida* have been reported to possess cellulase activities (Dahm et al. 1987; Kathiresan et al. 2011; Sheng et al. 2015). Among them *Thermobifida fusca* (earlier an anaerobic bacterium), *Cellulomonas firmi*, and *Microbispora biospora* have been studied the most (Lynd et al. 2002b; Wilson 1992). *T. fusca* is a thermophile, and its genome encodes for 36 glycoside hydrolases distributed in 22 glycoside hydrolases families. The cellulase system in *T. fusca* comprises four extracellular endocellulases and two exocellulases and an intracellular β-glucosidase (Saini et al. 2015).

Intriguingly, although both *C. firmi* and *M. biospora* are facultative anaerobes, they also possess extracellular cellulases (Christopherson et al. 2013). The cellulase enzyme systems in *C. firmi* encompass three endocellulases (CenA, CenB, and CenD), two exocellulases (CenB and CenD), and a processive endocellulase (CenC) (Christopherson et al. 2013; Wilson 2004) while *M. biospora* synthesizes six distinct cellulases exhibiting exo-exo and endo-exo synergism (Wilson 1992).

7.5 Fungal Cellulases Structure and Production

Fungal cellulases are structurally less complicated in comparison to other cellulases of other organisms, in particular to those of the anaerobic bacteria (Sajith et al. 2016). These cellulases contain two separate domains, namely, the cellulose-binding module (CBM) and a catalytic domain (CD). The CD has a short polylinker region to its N-terminal to join the cellulose-binding module (CBM). The CBM contains 35 amino acid residues, while the polylinker region has numerous serine and threonine residues.

The major differentiating characteristics between cellulosomes and free cellulase are that cellulosomes cohesion has scaffolding and docker in containing enzymes. For fungi, cellulose-binding domains (CBMs) are replaced by dockerin in cellulosomal complexes in free cellulase and one scaffolding-born CBM directs the complete cellulosome complex to cellulosic biomass (Kuhad et al. 2011; Pandey 2003). In contrast, the fungal cellulase adheres to the lignocellulose biomass in the initial steps, followed by degradation of the biomass into components that are further absorbed by the fungi for nutrition (Gerwick and Fenner 2013).

The degradation of cellulose via fungal cellulase has been well studied. Several cellulose-degrading fungi such as *Aspergillus niger*, *Cladosporium cladosporioides*, *C. sphaerospermum*, *Penicillium chrysogenum*, *Scopulariopsis brevicaulis*, *Stachybotrys chartarum*, *Verticillium cyclosporum*, *T. reesei,* and *Chaetomium hamadae* have been identified for cellulase production based on their habitat (El-Morsy 2000; Luo et al. 2005; Maria et al. 2005). Among them, species of *Aspergillus* and *T. reesei* are prominent cellulase producers that possess a complete cellulase system (Kathiresan and Manivannan 2006; Maria et al. 2005; Rasmussen et al. 2010).

Aerobic fungal cellulases are available extracellularly, are adaptive in nature, and usually secreted in large quantities during growth. They are, therefore, preferred over cellulases of the anaerobic bacteria and fungi for the production of single products such as glucose, cellobiose, and cello-oligosaccharides (Jayasekara and Ratnayake 2019). Conversely, cellulases in anaerobic bacteria and fungi are organized into tight multi-enzyme complexes, often membrane-bound as cellosomes, and it is difficult to recover individual active enzyme species (Jayasekara and Ratnayake 2019; Mathew et al. 2008).

Fungal cellulase production can be carried out primarily by two methods: (1) solid-state fermentation (SSF) and (2) submerged fermentation (SmF) (Bansal et al. 2012; Cherian et al. 2016; Pandey 2003; Singhania et al. 2009; Subramaniyam and Vimala 2012; Xia and Cen 1999). In the case of SSF, solid substrates are used for the production of cellulases, for instance, agricultural waste such as rice straw, wheat bran, and sugarcane bagasse. The fermentation process can be carried out in the absence or nearly absence of free water using these solid substrates. The SmF method involves fermentation in the presence of water and uses primarily free molecules soluble in water as liquid substrates, such as molasses in broth (Subramaniyam and Vimala 2012).

The main advantages of the SSF technique are easy, cost-effectiveness, and recycling of cheap waste material. The SmF offers easy purification and product recovery (Couto and Sanroman 2006; Pandey 2003). Furthermore, SSF is mainly favorable for microorganisms that require less moisture content, while SmF is more suited to bacteria for cellulase production due to high water activity (Babu and Satyanarayana 1996).

SSF has been used for fermentation in Asian and Western countries since ancient times (Ryu and Mandels 1980; Swain and Ray 2007; Zhuang et al. 2007). SSF has gained importance in Western countries after the production of penicillin via the SmF technique in the 1940s. In the last two decades, SSF has been extensively used because of biotechnological advantages such as high fermentation ability, more stable end products, subordinate catabolic repression, as well as its use of cost-effective technology (Dhillon et al. 2013; Kasana et al. 2008; Liang et al. 2010; Sukumaran et al. 2009). Additionally, cellulase production via SSF is preferred over that with SmF because it yields two to three times higher enzyme production, has a high protein rate, and direct accessibility of dried fermentable solids as a source of enzyme that eliminates the costs involved in downstream processing (El-Bakry et al. 2015; Hendriks and Zeeman 2009; Sadhu and Maiti 2013).

The production of enzymes is dependent on various factors (Abusham et al. 2009). These include the substrate chosen, strain used cultivation strategy, medium supplementation, and cultivation parameters. A pretreatment step of the cellulosic material, to remove the lignin and to make the cellulosic component more accessible to the fungus, can also influence the final production of cellulase. Such modifications for the improvement in the cellulase production on sugarcane bagasse have been earlier (Jabasingh and Nachiyar 2011).

7.6 Industrial Applications

Six classes of lignocellulolytic enzymes have been nominated by the International Union of Biochemists in 1979 based on their functions and the overall reactions they catalyze. These classes include (a) Oxido-reductases, (b) Transferases, (c) Hydrolases, (d) Lyases, (e) Isomerases, and (f) Ligases. In industry, about 85% of the enzymes are hydrolases, with the remaining 15% divided among oxidoreductases and isomerases. Lignocellulolytic enzymes are widely used in industry including food, detergents analytical applications, medical sciences, production of chemicals, and waste treatment industries (Kuhad et al. 2011; Sharada et al. 2014).

Cellulases, as part of hydrolases, are progressively used for a large variety of industrial purposes, such as in the textile, pulp, paper, and food industries as well as an additive in detergents and improving the digestibility of animal feeds (Bhardwaj et al. 2020; Gübitz et al. 1996; Ishikawa et al. 2005). The world industrial enzyme market presently accounts for cellulase as a significant share. Growing concerns about the depletion of crude oil and the emissions of greenhouse gases have subsequently encouraged the production of bioethanol from lignocellulose, especially through enzymatic hydrolysis of lignocellulose materials-sugar platforms (Bayer et al. 1998; Himmel et al. 1999; Zaldivar et al. 2001). However, costs of cellulases for hydrolyzing pretreated lignocellulosic materials still need to be reduced, and their catalytic efficiency should be further increased to make the process more economically reasonable (Sheehan and Himmel 1999). The industrial production of cellulases from fungi is currently dominated by *Trichoderma* spp. and *Aspergillus* spp. (Pandey et al. 2015).

7.6.1 Wine and Brewery Industry

Cellulolytic enzymes such as hemicellulases, pectinases, and glucanases have been extensively used in the fermentation of alcoholic beverages, mainly to promote the quality and stability of wine and beer (Nuutila et al. 1999; Oksanen et al. 1985). These enzymes eventually reduce the viscosity of the must, clarify and improve the filtration process, as well as optimize color extraction and skin maceration (Bamforth 2009; Chakraborty et al. 2016; Kuhad et al. 2011; Sampathkumar et al. 2019). The

aromatic characteristics of the wine are chiefly attributed to β-glucosidases that act efficiently on glycosidic substrates, generate volatile compounds, and grant aromatic complexity (Nuutila et al. 1999; Oksanen et al. 1985). Currently, there are commercial preparations available in industries that aim to improve maceration and color extraction and facilitate clarification and filtration (Jayasekara and Ratnayake 2019). Cellulases are also used in beer production processes by improving the filtration process and preventing the formation of gels (Chakraborty et al. 2016).

7.6.2 Bioethanol Industry

Cellulose is a sustainable source of fermentable sugars that can be used for the generation of bioethanol (Sukumaran et al. 2009). Agricultural residues, such as rice, corn, and sugarcane bagasse, have been used as raw materials for the production of bioethanol, using cellulases produced by *Aspergillus*, *Trichoderma*, and *Penicillium* species (Binod et al. 2010; Jin et al. 2020; Swain et al. 2019; Talebnia et al. 2010; Xue et al. 2018). Bioethanol production involves four steps: (1) pretreatment of the lignocellulosic substrate (mechanical, chemical or enzymatic); (2) release of fermentable sugars from plant polysaccharides with cellulases (saccharification); (3) microbial fermentation; and (4) distillation (Chakraborty et al. 2012; Kaur et al. 2020; Kour et al. 2019b).

Mesophilic fungal enzymes functioning at the temperature range of 20–45 °C are usually used for the saccharification step. However, enzymes from psychrophilic fungi that are efficient at low temperatures (−20 to +10 °C) (Barroca et al. 2017) can also be used. Likewise, a thermostable and ethanol-resistant endoglucanase isolated from *Aspergillus niger* makes it an excellent candidate to be used simultaneously in saccharification and fermentation (Xue et al. 2018) (Table 7.1).

7.6.3 Food Processing Industry

Lignocellulolytic enzymes have been employed in the food industry to extract and clarify olive oil, fruit and vegetable juices, and fruit nectars (Galante et al. 1998; Pajunen 1986). Lignocellulolytic enzymes are important as part of macerating enzyme complexes (cellulases, xylanases, and pectinases). These macerating enzymes are also used to improve cloud stability and texture and to decrease the viscosity of the nectars and purees from tropical fruits such as mango, peach, papaya, plum, apricot, and pear. These complexes also improve the texture, flavor, and aromatic properties of fruits and vegetables, for instance, to reduce excessive bitterness of citrus fruits by infusing the nectar with enzymes such as pectinases and β-glucosidases. Due to their significant role in food biotechnology industries, the

Table 7.1 Industrial applications where cellulolytic activities of fungi are exploited

Industrial applications	Cellulolytic activities	Associated fungi	References
Wine and beer production	EG[a], CBH[b], β-glucanases	*A. niger*, *Penicillium emersonii*, *Trichoderma reesei*	Nuutila et al. (1999), Oksanen et al. (1985)
Bioethanol production and Fruit liquefaction in juice production	EG[a]	*A. niger*, *Penicillium*, *Trichoderma*	Bhardwaj et al. (2020), Jin et al. (2020), Thomas et al. (2016)
Pulp and paper industry	EG[a]	*Aspergillus aculeatus*, *Sporotrichum cellulophilum*, *S. rolfsii*, *T. reesei*	Araujo and Ward (1991), Christgau et al. (1994), Gübitz et al. (1996), Stalbrand et al. (1995)
Textile and laundry industry	EG[a], CBH[b], BGL[c]	*H. insolens*, *T. reesei*	Arja (2007), Cavaco-Paulo and Gübitz (2003)
Detergent industry	Cellulases[d]	*H. insolens*	Grethe et al. (1999)
Animal feed industry	Xylanase	*Trichoderma* sp.	Li et al. (2010)
Agricultural industry	β-Glucanases, CBH[b]	*Chaetomium* sp., *Geocladium* sp., *Penicillium* sp., *Trichoderma* sp.	Harman et al. (1991), Kuhad et al. (2011)
Olive oil extraction	Cellulases[d]	*A. aculeatus*	Petrakis (2006)
Carotenoid extraction	Cellulases[d]	*A. niger*, *A. terreus*	Cinar (2005)
Flavour improvement and dinking of wastepaper	BGL[c]	*P. ostreatus, P. tinctoriu, S. singularis, T. versicolor*	Cao and Crawford (1993), Ishikawa et al. (2005), Morais et al. (2002)
Wastewater management	Cellulases[d]	*A. niger*, *Penicillium decumbens*, *T. reesei*	Khan et al. (2019), Wilson (2011)
Biofuels production	EG[a], CBH[b], BGL[c]	*A. terreus*, *Chrysosporium*, *F. proliferatum*, *T. reesei*, *T. harzianum*, *T. viride*	Gusakov (2011)

[a]Endoglucanases
[b]Cellobiohydrolases
[c]β-Glucosidases
[d]Authors did not specify type of cellulases

demand for macerating enzymes will possibly increase for juice extractions for a wider range of fruits and vegetables (Raveendran et al. 2018; Sharada et al. 2014).

The extraction of olive oils is important for the international food market because of the huge health benefits of olive oil. However, extraction from the ripened fruits at high temperatures always results in oil with rancidity, high acidity, and poor aroma (Galante et al. 1998). The use of commercially available enzymes such as Olivex (a cocktail of cellulase, hemicellulases, and pectinase from *Aspergillus aculeatus*) and macerating food enzymes significantly increase the extraction process and antioxidants levels in extra virgin olive oil and reduce rancidity (Kuhad et al. 2011; Sharada et al. 2014). Under cold processing conditions, these enzymes increase extraction; improve centrifugal fractionation of the oily must; and decrease

olive paste viscosity during olive oil production and malaxation (Chiacchierini et al. 2007; Ranalli et al. 2003). The presence of the collateral activities of cellulases and hemicellulases ensure a quick and intense disintegration of the cell walls and membranes of the olive fruits, thereby favoring the passage of polyphenols and aromatic precursors into the final product (Kuhad et al. 2011; Ranalli et al. 2003; Sharada et al. 2014).

7.6.4 Pulp and Paper Industry

Cellulases in the pulp and paper manufacturing industry in combination with xylanases are used for biodeinking, generate an appearance of cleanliness, and shine in paper waste (Bajaj and Mahajan 2019; Jayasekara and Ratnayake 2019). A cocktail of enzymes of xylanases and cellulases from *Thermomyces lanuginosus and Aspergillus* sp., respectively, has been used to treat bleached kraft pine cellulosic pulp that has significantly improved susceptibility to refining, increased water retention value, and fines content (Przybysz Buzala et al. 2016). Another study (Kumar et al. 2018) has shown the production of a low-molecular-weight cellulase-xylanase complex (14 kDa) using the bacterium *Escherichia coli* SD5, demonstrating that the coexistence of these enzymes is advantageous in paper pulp modification and deinking applications. Furthermore, a study (Wang et al. 2017) has also shown the overexpression of a gene encoding for alkali-tolerant endoglucanase in *Bacillus subtilis* Y106 that could be used to improve the resistance properties against microbes of softwood pulp, hardwood, and non-wood materials.

7.6.5 Textile and Laundry Industry

Cellulases have been widely used for manufacturing in the textile and laundry industries. They have been primarily used for bio-polishing and softening of cellulosic fibers (Shin et al. 2016; Uddin 2015; Yu et al. 2015) besides improving the softness, color, shine, and appearance of textiles (Araujo et al. 2008; Arja 2007; de Souza et al. 2013; Juturu and Wu 2013; Shankar et al. 2017; Sharma 2015; Sreenath et al. 1996). Likewise, alkaline cellulases are also used, favoring the complete elimination of stains on the fabric and giving shine and softness to the clothes (Juturu and Wu 2014). The treatment of garments, using enzymes such as bio-polishing and bio-stonation of jeans, involves degradation of the fiber end of the fabric, the release of the dye from the textile, and facilitating the mechanical washing process (Cavaco-Paulo et al. 1998). The main advantage of cellulases is that they are easy to apply on the material and are biodegradable and thus do not pollute the environment (Jayasekara and Ratnayake 2019). Commercial cellulase used in the textile industry comes mainly from fungi such as *T. reesei* and *Humicola insolens* (Arja 2007; Cavaco-Paulo and Gübitz 2003).

7.6.6 Detergent Industry

Cellulases in combination with proteases and lipases in detergents are used in origination in industries (Singh et al. 2007). Cellulase preparations are capable of altering cellulose fibrils to improve brightness, color, feel, and dirt removal from cotton-blend garments (Niyonzima 2019; Sukumaran et al. 2005). Cellulases have also been added to detergents for the breakdown of hydrogen bonds under harsh environmental conditions such as alkaline or thermophile conditions (Menendez et al. 2015; Pajunen 1986). For instance, alkaline cellulases are used as a detergent additive to selectively attach to the cellulose within the interior of fibers so that soil can be removed from the internal fibril spaces in the presence of more conventional detergent ingredients. Currently, liquid laundry detergents contain anionic or nonanionic surfactants, citric acid, or a water-soluble salt (Karmakar and Ray 2011; Sharada et al. 2014).

7.6.7 Animal Feed Industry

Cellulases and hemicellulases applied in the animal feed industry have received considerable attention because of their potential to improve feed value and thus the performance and health of animals (Dhiman et al. 2002). The use of enzymes in animal nutrition became more important after the prohibition of using nutritive ionophore antibiotics, which were previously used in the European Union countries (Ali et al. 1995). Agricultural silage and grain feed pretreated by cellulases or xylanases can improve nutritional value by eliminating antinutritional factors with hydrolysis, degrading certain feed constituents to improve nutritional values, and providing supplementary digestive enzymes for plant-eating animals such as proteases, amylases, and glucanases (Ali et al. 1995; Godfrey and West 1996; Kuhad et al. 2011).

For instance, dietary fiber consists of many plant components such as inulin, chitins, waxes, dextrins, lignin, pectins, oligosaccharides, β-glucan, and nonstarch polysaccharides such as cellulose and arabinoxylans that act as antinutritional factors for animals such as swine (Ali et al. 1995). Cellulolytic actions hydrolyze these antinutritional factors that are mainly cellulose into easily absorbable and digestible components, such as simple sugars of cellulobiose and glucose monomeric units, thus improving animal health and performance (Kuhad et al. 2011). Moreover, cellulases and hemicellulases can cause partial hydrolysis of plant cell walls during silage and fodder preservation. Additionally, both these enzymes are further responsible for partial hydrolysis of lignocellulosic materials, dehulling of cereal grains, hydrolysis of β-glucans, and better emulsification and flexibility of feed materials, which results in the improvement in the nutritional quality of animal feed (Cowan 1996; Ibrahim et al. 2011; Mosier et al. 2005).

Animal feedstock production processes generally include heat treatments that inactivate potential viral and microbial contaminants (Kuhad et al. 2011). Applied, thermophilic cellulase during feedstock production to reduce pathogens by hydrolyzing their cell walls, while at the same time enhancing the digestibility and nutrition of the feed, thus providing a combination of heat treatment and feed transformation in a single step (Kuhad et al. 2011; Sharada et al. 2014). During caecal fermentation processes, cellulases have an additional positive effect by increasing the production of propionic acid, which acts as a bacteriostatic and thus can decrease the colonization of pathogenic bacteria (Kuhad et al. 2011; Pazarlioglu et al. 2005).

7.6.8 Agricultural Crop Industry

Numerous studies have been conducted to prove the uses of cellulases in combination with hemicellulases and pectinases for potential applications in agriculture and such resulted in enhancing the growth of crops and flowering, improved soil quality, and the controlling of plant diseases (Bhat 2000; Kuhad et al. 2011; Sharada et al. 2014). Cellulases and related enzymes from certain fungi are capable of degrading the cell wall of plant pathogens, thus controlling the plant disease (Kuhad et al. 2011; Singh and Yadav 2020). Various cellulolytic fungi, including species of *Trichoderma*, *Geocladium*, *Chaetomium*, and *Penicillium*, are known to play significant roles in agriculture by promoting rapid plant growth and flowering, seed germination, improved root system development, and thus increased crop yields (Bailey and Lumsden 1998; Harman and Björkman 1998; Harman and Kubicek 1998; Sharma et al. 2019; Yadav et al. 2018). Plant performance could be improved either by diffusing growth-promoting factors to the plant tissues by fungi or by controlling the plant disease and pathogens on plants (Bailey and Lumsden 1998; Harman and Björkman 1998; Kuhad et al. 2011). For soil quality improvement, incorporation of straw and subsequent microbial decomposition to soils has been adopted as an important strategy to improve soil quality and reduce dependence on chemical fertilizers (Escobar and Hue 2008; Tejada et al. 2008).

7.6.9 Carotenoid Extraction

Carotenoids have attracted much interest since they were first isolated from carrots. These are the main group of coloring agents in nature being responsible for many plant colors from red to yellow. The total production of carotenoids in nature has been estimated to be about 100 million tons/year (Bauernfeind 1981). There is a continuously growing market for carotenoids as food colorants and also due to their other desirable properties, such as their natural origin, null toxicity, high versatility, and providing both lipo- and hydro-soluble colorants. Additionally, it exhibits

provitamin A activity, lipid oxidation, and anti-carcinogenic properties that are crucial biological functions of these pigments (Cinar 2005).

Carotenoids can be released from the cell wall into the chloroplasts and in cell fluids of orange peels, sweet potatoes, and carrots by the combined action of cellulolytic and pectinolytic enzymes. Cellulases randomly split cellulose chains into glucose, whereas commercial pectinase preparations, mainly from *Aspergillus niger*, have pectinesterase (PE), polygalacturonase (PG), and pectin lyase (PL) activities (Dekker 1994a, b; Ory and Angelo 1977). These pigments remain in their natural state, bound with proteins that prevent the pigment from oxidation and ensures color stability (Dekker 1994a, b). Studies have shown that carotenoid extraction by solvents dissociates the pigments from the proteins and causes water insolubility and ease of oxidation (Bassi et al. 1993; Cinar 2005; Dekker 1994a, b; Nagodawithana and Reed 2013).

7.6.10 Wastewater and Waste Fill Management

Industries require large quantities of water. For instance, in the paper industry, it causes production and release of industrial wastewaters that contain a large amount of organic chemical contamination (Biological Oxygen Demand 5 and Chemical Oxygen Demand), hazardous substances such as sulfites, phenols and tannins, and lignin that significantly contaminate the environment (Hubbe et al. 2016; Kour et al. 2021; Kumar et al. 2021). Adequate measures are essential to purify the released water before it reaches the environment (Stanisavljevic et al. 2018).

Conversely, wastewater is also a very good source of carbon, nitrogen, phosphorus, and other nutrients. Several studies (Alma et al. 2003; Jamal et al. 2005) showed that sludge from domestic wastewater consists of 32% carbon, 3.8% nitrogen, 1.6% phosphorus, 0.05% magnesium, 0.15% potassium, and sufficient trace elements. These can be utilized for many microbial processes that could add value by producing certain valuable metabolic products such as ammonia (ammonification), nitrate (nitrification), nitrite (denitrification), and that can also be used as raw materials to produce various products such as organic acids, bio-solids, and bio-pesticides by liquid state bioconversion as an additional industrial process.

In waste landfills, the bio-decomposition (bioconversion) of organic matter principally occurs to methane and carbon dioxide by "anaerobic" digestion from microbes by using cellulases, which is a natural process in solid waste landfills (Gupta et al. 2011a, b; Karmakar and Ray 2011; Kuhad et al. 1997). In natural anaerobic digestion, some members of the microbial consortia collectively produce fermentable sugars from polysaccharides, with cellulases, while others specialize in converting these sugars to methane and carbon dioxide. Such mixed fermentations are extremely difficult to establish and maintain a large scale. Extra-cellular hydrolytic enzymes such as cellulase and lipases have been shown to be effective in the post hydrolysis of anaerobic digester effluent solids (Sharada et al. 2014).

7.7 Bioprospecting of Cellulases: An Essence of the Modern Time

Continuously growing industrial need for cellulases leads to an increasing demand to identify and isolate novel cellulases. The production and utilization of such cellulases are still continuously evaluated, aided with culture-dependent studies (Hussain et al. 2017; Liang et al. 2014; Yang et al. 2014). For example, six cellulase-producing fungi were isolated in *Trichoderma* and *Aspergillus* from decaying banana pseudostem and *Strelitzia alba* (Legodi et al. 2019). However, these approaches are restricted to cultivable microbial species (Ghosh 2015).

There has been incredible growth and development in the use of metagenomic, meta transcriptomic, and high-throughput sequencing approaches to tap into the holistic microbial diversity present within a given ecosystem in order to have a comprehensive and compelling view (Ghosh 2015; Handelsman 2004). Metagenomics deals with the direct extraction and amplification of the total amount of DNA in the environment, while meta transcriptomics is the isolation of their expressed version in the form of mRNA. Both the techniques are devoid of any prior cultivation for the microbial isolates (Ghosh 2015).

This technique has resulted in the isolation of novel cellulases from different matrices and ecosystems (Cui et al. 2019; Speda et al. 2017; Takasaki et al. 2013; Yang et al. 2016). For instance, a metagenomic sequence-based analysis of an anaerobically produced beer lees microbial consortium revealed three novel endo-β-1,4-glucanases genes that could be cloned and expressed in *E. coli* colonies (Yang et al. 2016). Another study (Nacke et al. 2012), has constructed a large insert grassland soil metagenomic library and detect/isolate a novel cellulase gene (cel01) and two xylanases genes (xyn01 and xyn02) based on function-based screening. However, constructing metagenomic libraries and subsequently, implementing next-generation sequencing techniques are not always feasible and affordable for most of the laboratories that have laboratory infrastructure constraints and strict budget restrictions (Martin et al. 2018; Wooley and Ye 2010).

Directed evolution involves manipulations of genes coding for certain enzymes as a step forward that came up in addition to bioprospecting. The approach involves random mutagenesis or DNA shuffling, followed by the selection for enhancements in desired traits, such as selected enzymes, thermostability, pH tolerance, and certain catalytic activities (Stephens et al. 2009; Wang and Xia 2008). This technique has led to the discovery of a number of enzymes, including cellulases and xylanases (Lin et al. 2011; Stephens et al. 2007, 2009). Methods accelerate and simplify the selection procedures (Liu et al. 2009) include structure-guided recombination process (SCHEMA) (Meyer et al. 2006), gene fusions (Hong et al. 2006), and protein module shuffling (Kittur et al. 2003). Although infrastructure-dependent, those techniques aid in increasing the thermostability and hydrolytic behavior of existing enzymes by incorporating beneficial mutations.

7.8 Conclusions

It was estimated that the world sale of industrial enzymes has already reached a market value of 10 billion US$ in 2019 and is expected to reach 14.7 billion US$ by 2025 of which the global cellulase (CAS 9012-54-8) market is valued at 1677.7 million US$ in 2020 and predicted to reach 2450.7 million US$ by the end of 2026. Microbial lignocellulolytic are preferred for their vast industrial applicability and relatively low cost of production. In fact, the trend for the search of potential cellulase enzymes is increasing and continuing worldwide in the interest of successful bioconversion of lignocellulosic biomass, especially for their use in waste management, food processing, pharmaceuticals, pulp and paper, and other industries. More and more research studies are improving scientific knowledge along with the success of meeting the growing demands of industry for cellulases and related enzymes to produce eco-friendly textiles, detergents, bio-pulping, and bio-alcohols.

A number of fungi produce cellulase and are considered with the most potential for use over other known microorganisms because they are more efficient in cellulolytic activities in comparison to the others. However, there still remain knowledge gaps about the different types of fungal cellulases that exist and their precise functionalities. Furthermore, efforts should be continued to develop more suitable, affordable, and optimized combinations processes and to isolate and improve the quality of the existing enzymes.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abusham RA, Rahman RNZRA, Salleh AB, Basri M (2009) Optimization of physical factors affecting the production of thermo-stable organic solvent-tolerant protease from a newly isolated halo tolerant Bacillus subtilis strain Rand. Microbial Cell Fact 8:20

Acharya S, Chaudhary A (2012) Bioprospecting thermophiles for cellulase production: a review. Braz J Microbiol 43:844–856. https://doi.org/10.1590/S1517-83822012000300001

Adrio JL, Demain AL (2014) Microbial enzymes: tools for biotechnological processes. Biomolecules 4:117–139. biom4010117 [pii]

Agarwal M, Goel A, Wati L (2018) Ethanol production from paddy straw using partially purified fungal cellulase. Int J Curr Microbiol Appl Sci 7:3709–3719

Ahmed A, Bibi A (2018) Fungal Cellulase; Production and Applications: Minireview. LIFE: International Journal of Health and Life Sciences 4:19–36

Ali S, Hall J, Soole KL, Fontes CMGA, Hazlewood GP, Hirst BH et al (1995) Targeted expression of microbial cellulases in transgenic animals in carbohydrate bioengineering. In: Petersen SB, Svensson B, Pedersen S (eds) Progress in biotechnology, vol 10. Elsevier, Amsterdam, The Netherlands, pp 279–293

Alma MZ, Fakhru'l-Razi A, Molla AH (2003) Biosolids accumulation and biodegradation of domestic wastewater treatment plant sludge by developed liquid state bioconversion process using a batch fermenter. Water Res 37:3569–3578. https://doi.org/10.1016/S0043-1354(03)00260-4

Amouri B, Gargouri A (2006) Characterization of a novel beta-glucosidase from a Stachybotrys strain. Biochem Eng J 32:191–197. https://doi.org/10.1016/j.bej.2006.09.022

Araujo A, Ward OP (1991) Studies on the galactomannan-degrading enzymes produced by *Sporotrichum cellulophilum*. J Ind Microbiol 8:229–236. https://doi.org/10.1007/Bf01576060

Araujo R, Casal M, Cavaco-Paulo A (2008) Application of enzymes for textile fibres processing. Biocatal Biotransformation 26:332–349. https://doi.org/10.1080/10242420802390457

Arja MO (2007) Cellulases in the textile industry. In: Polaina J, MacCabe AP (eds) Industrial enzymes. Springer, Dordrecht

Arora DS, Sharma RK (2010) Ligninolytic fungal laccases and their biotechnological applications. Appl Biochem Biotechnol 160:1760–1788. https://doi.org/10.1007/s12010-009-8676-y

Asgher M, Irshad M, Iqbal HMN (2012) Purification and characterization of LiP produced by *Schyzophyllum commune* IBL-06 using banana stalk in solid state cultures. Bioresources 7:4012–4021

Ayuso-Fernandez I, Rencoret J, Gutierrez A, Ruiz-Duenas FJ, Martinez AT (2019) Peroxidase evolution in white-rot fungi follows wood lignin evolution in plants. Proc Natl Acad Sci U S A 116:17900–17905. https://doi.org/10.1073/pnas.1905040116

Babu KR, Satyanarayana T (1996) Production of bacterial enzymes by solid state fermentation. J Sci Ind Res 55:464–467

Bailey BA, Lumsden RD (1998) Direct efects of Trichoderma and Gliocladium on plant growth and resistance to pathogens. In: Harman GF, Kubicek CP (eds) Trichoderma & Gliocladium-enzymes, Biological control and commercial applications, vol 2. Taylor & Francis, London, UK, pp 327–342

Bajaj P, Mahajan R (2019) Cellulase and xylanase synergism in industrial biotechnology. Appl Microbiol Biotechnol 103:8711–8724. https://doi.org/10.1007/s00253-019-10146-0

Baldrian P (2006) Fungal laccases - occurrence and properties. FEMS Microbiol Rev 30:215–242. https://doi.org/10.1111/j.1574-4976.2005.00010.x

Baldrian P, Valaskova V (2008) Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev 32:501–521. https://doi.org/10.1111/j.1574-6976.2008.00106.x

Bamforth CW (2009) Current perspectives on the role of enzymes in brewing. J Cereal Sci 50:353–357. https://doi.org/10.1016/j.jcs.2009.03.001

Bansal N, Tewari R, Soni R, Soni SK (2012) Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manag 32:1341–1346. https://doi.org/10.1016/j.wasman.2012.03.006

Barati B, Amiri IS (2015) Introduction of cellulase and its application. In: Barati B, Amiri IS (eds) In silico engineering of disulphide bonds to produce stable cellulase, SpringerBriefs in applied sciences and technology. Springer, New York, pp 1–5

Barroca M, Santos G, Gerday C, Collins T (2017) Biotechnological aspects of cold-adapted enzymes. In: Margesin R (ed) Psychrophiles: from biodiversity to biotechnology, 2nd edn. Springer, Berlin, pp 347–363

Barros RRO, Oliveira RA, Gottschalk LMF, Bon EPS (2010) Production of cellulolytic enzymes by fungi *Acrophialophora nainiana* and *Ceratocystis paradoxa* using different carbon sources. Appl Biochem Biotechnol 161:448–454. https://doi.org/10.1007/s12010-009-8894-3

Bassi R, Pineau B, Dainese P, Marquardt J (1993) Carotenoid-binding proteins of photosystem-II. Eur J Biochem 212:297–303. https://doi.org/10.1111/j.1432-1033.1993.tb17662.x

Bauernfeind J (1981) Carotenoids as food colorants and vitamin A precursors: technological and nutritional applications, 1st edn. Elsevier, New York

Bayer EA, Shimon LJW, Shoham Y, Lamed R (1998) Cellulosomes - structure and ultrastructure. J Struct Biol 124:221–234. https://doi.org/10.1006/jsbi.1998.4065

Berthet S, Thevenin J, Baratiny D, Demont-Caulet N, Debeaujon I, Bidzinski P et al (2012) Role of plant laccases in lignin polymerization. Lignins Biosynth Biodegrad Bioeng 61:145. https://doi.org/10.1016/B8978-0-12-416023-1.00005-7

Bhardwaj N, Kumar B, Agarwal K, Verma P (2020) Bioconversion of rice straw by synergistic effect of in-house produced ligno-hemicellulolytic enzymes for enhanced bioethanol production. Biresour Technol Rep 10:1–9. https://doi.org/10.1016/j.biteb.2019.100352

Bhat MK (2000) Cellulases and related enzymes in biotechnology. Biotechnol Adv 18:355–383. https://doi.org/10.1016/S0734-9750(00)00041-0

Binod P, Sindhu R, Singhania RR, Vikram S, Devi L, Nagalakshmi S et al (2010) Bioethanol production from rice straw: an overview. Bioresour Technol 101:4767–4774. https://doi.org/10.1016/j.biortech.2009.10.079

Bischof RH, Ramoni J, Seiboth B (2016) Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. Microb Cell Fact 15(1):106

Boerjan W, Ralph J, Baucher M (2003) Lignin biosynthesis. Annu Rev Plant Biol 54:519–546. https://doi.org/10.1146/annurev.arplant.54.031902.134938

Bonnarme P, Jeffries TW (1990) Mn(II) regulation of Lignin peroxidases and Manganese-dependent peroxidases from lignin-degrading White Rot Fungi. Appl Environ Microbiol 56:210–217. https://doi.org/10.1128/Aem.56.1.210-217.1990

Boudebbouze S, Maguin E, Rhimi M (2011) Bacterial L-arabinose isomerases: industrial application for D-tagatose production. Recent Pat DNA Gene Seq 5:194–201. https://doi.org/10.2174/187221511797636248

Brar KK, Agrawal D, Chadha BS, Lee H (2019) Evaluating novel fungal secretomes for efficient saccharification and fermentation of composite sugars derived from hydrolysate and molasses into ethanol. Bioresour Technol 273:114–121. https://doi.org/10.1016/j.biortech.2018.11.004

Brijwani K, Vadlani PV (2011) Cellulolytic enzymes production via solid-state fermentation: effect of pretreatment methods on physicochemical characteristics of substrate. Enzyme Res 2011:860134. https://doi.org/10.4061/2011/860134

Cao WG, Crawford DL (1993) Purification and some properties of Beta-glucosidase from the ectomycorrhizal fungus *Pisolithus tinctorius* strain Smf. Can J Microbiol 39:125–129. https://doi.org/10.1139/m93-018

Cavaco-Paulo A, Gübitz GM (2003) Textile processing with enzymes, 1st edn. Woodhead, Witney, UK

Cavaco-Paulo A, Almeida L, Bishop D (1998) Hydrolysis of cotton cellulose by engineered cellulases from *Trichoderma reesei*. Text Res J 68:273–280. https://doi.org/10.1177/004051759806800405

Chakraborty S, Aggarwal V, Mukherjee D, Andras K (2012) Biomass to biofuel: a review on production technology. Asia Pac J Chem Eng 7:S254–S262. https://doi.org/10.1002/apj.1642

Chakraborty S, Gupta R, Jain KK, Hemanshi, Gautam S, Kuhad RC (2016) Cellulases: application in wine and brewery industry. In: Gupta V (ed) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 193–200

Chandra R, Chowdhary P (2015) Properties of bacterial laccases and their application in bioremediation of industrial wastes. Environ Sci Process Impacts 17:326–342. https://doi.org/10.1039/c4em00627e

Chang HX, Yendrek CR, Caetano-Anolles G, Hartman GL (2016) Genomic characterization of plant cell wall degrading enzymes and in silico analysis of xylanses and polygalacturonases of *Fusarium virguliforme*. BMC Microbiol 16:147

Chaudhary P, Kumar NN, Deobagkar DN (1997) The glucanases of Cellulomonas. Biotechnol Adv 15:315–331. https://doi.org/10.1016/S0734-9750(97)00010-4

Chen M, Yao SJ, Zhang H, Liang XL (2010) Purification and characterization of a versatile peroxidase from edible mushroom *Pleurotus eryngii*. Chin J Chem Eng 18:824–829. https://doi.org/10.1016/S1004-9541(09)60134-8

Cherian E, Kumar MD, Baskar G (2016) Production and optimization of cellulase from agricultural waste and its application in bioethanol production by simultaneous saccharification and fermentation. Manag Environ Qual 27:22–35. https://doi.org/10.1108/Meq-07-2015-0128

Chiacchierini E, Mele G, Restuccia D, Vinci G (2007) Impact evaluation of innovative and sustainable extraction technologies on olive oil quality. Trends Food Sci Technol 18:299–305. https://doi.org/10.1016/j.tifs.2007.01.008

Christgau S, Kauppinen S, Vind J, Kofod LV, Dalboge H (1994) Expression cloning, purification and characterization of a beta-1,4-mannanase from *Aspergillus aculeatus*. Biochem Mol Biol Int 33:917–925

Christopherson MR, Suen G, Bramhacharya S, Jewell KA, Aylward FO, Mead D et al (2013) The genome sequences of *Cellulomonas fimi* and "Cellvibrio gilvus" reveal the cellulolytic strategies of two facultative anaerobes, transfer of "*Cellvibrio gilvus*" to the Genus Cellulomonas, and proposal of *Cellulomonas gilvus* sp nov. PLoS One 8:e53954

Cinar I (2005) Effects of cellulase and pectinase concentrations on the colour yield of enzyme extracted plant carotenoids. Process Biochem 40:945–949. https://doi.org/10.1016/j.procbio.2004.02.022

Couto SR, Sanroman MA (2006) Application of solid-state fermentation to food industry - a review. J Food Eng 76:291–302. https://doi.org/10.1016/j.jfoodeng.2005.05.022

Couto SR, Toca-Herrera JL (2007) Laccase production at reactor scale by filamentous fungi. Biotechnol Adv 25:558–569. https://doi.org/10.1016/j.biotechadv.2007.07.002

Cowan WD (1996) Animal feed. In: Industrial enzymology, 2nd edn. Macmillan Press, London, UK

Cui JM, Mai GQ, Wang ZW, Liu Q, Zhou V, Ma YF et al (2019) Metagenomic insights into a cellulose-rich niche reveal microbial cooperation in cellulose degradation. Front Microbiol 10:618

Cunha L, Martarello R, de Souza PM, de Freitas MM, Barros KVG, Ferreira Filho EX et al (2018) Optimization of xylanase production from *Aspergillus foetidus* in Soybean residue. Enzyme Res 2018:6597017. https://doi.org/10.1155/2018/6597017

Dahm H, Strzelczyk E, Majewska L (1987) Cellulolytic and pectolytic activity of mycorrhizal fungi, bacteria and actinomycetes associated with the roots of Pinus-Sylvestris. Pedobiologia 30:73–80

Daou M, Faulds CB (2017) Glyoxal oxidases: their nature and properties. World J Microbiol Biotechnol 33:87

Das A, Paul T, Halder SK, Jana A, Maity C, Das Mohapatra PK et al (2013) Production of cellulolytic enzymes by *Aspergillus fumigatus* ABK9 in wheat bran-rice straw mixed substrate and use of cocktail enzymes for deinking of waste office paper pulp. Bioresour Technol 128:290–296. https://doi.org/10.1016/j.biortech.2012.10.080

Dashtban M, Schraft H, Qin WS (2009) Fungal bioconversion of lignocellulosic residues; opportunities & perspectives. Int J Biol Sci 5:578–595. https://doi.org/10.7150/ijbs.5.578

de Paula RG, Antonieto ACC, Ribeiro LFC, Carraro CB, Nogueira KMV, Lopes DCB et al (2018) New genomic approaches to enhance biomass degradation by the industrial fungus *Trichoderma reesei*. Int J Genomics 2018:1974151

de Souza AAU, Ferreira FCS, Souza SMAGU (2013) Influence of pretreatment of cotton yarns prior to biopolishing. Carbohydr Polym 93:412–415. https://doi.org/10.1016/j.carbpol.2012.12.023

Dekker RFH (1994a) Enzymes in food and beverage processing. 1. Food Aust 46:136–139

Dekker RFH (1994b) Enzymes in food and beverage processing. 2. Food Aust 46:179–181

Demain AL, Newcomb M, Wu JHD (2005) Cellulase, clostridia, and ethanol. Microbiol Mol Biol Rev 69:124. https://doi.org/10.1128/Mmbr.69.1.124-154.2005

Den W, Sharma VK, Lee M, Nadadur G, Varma RS (2018) Lignocellulosic biomass transformations via greener oxidative pretreatment processes: access to energy and value-added chemicals. Front Chem 6:141

Dhillon GS, Kaur S, Brar SK (2013) Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: a review. Renew Sustain Energy Rev 27:789–805. https://doi.org/10.1016/j.rser.2013.06.046

Dhiman TR, Zaman MS, Gimenez RR, Walters JL, Treacher R (2002) Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. Anim Feed Sci Technol 101:115–125. Pii S0377-8401(02)00177-3

Druzhinina IS, Kubicek CP (2017) Genetic engineering of *Trichoderma reesei* cellulases and their production. Microb Biotechnol 10:1485–1499. https://doi.org/10.1111/1751-7915.12726

Du J, Pu G, Shao C, Cheng S, Cai J, Zhou L et al (2015) Potential of extracellular enzymes from Trametes versicolor F21a in Microcystis spp. degradation. Mater Sci Eng C Mater Biol Appl 48:138–144. https://doi.org/10.1016/j.msec.2014.11.004

Edwards SL, Raag R, Wariishi H, Gold MH, Poulos TL (1993) Crystal-structure of lignin peroxidase. Proc Natl Acad Sci U S A 90:750–754. https://doi.org/10.1073/pnas.90.2.750

El-Bakry M, Abraham J, Cerda A, Barrena R, Ponsa S, Gea T et al (2015) From wastes to high value added products: novel aspects of SSF in the production of enzymes. Crit Rev Environ Sci Technol 45:1999–2042. https://doi.org/10.1080/10643389.2015.1010423

El-Morsy E (2000) Fungi isolated from the endorhizosphere of halophytic plants from the red sea coast of Egypt. Fung Divers 5:43–54

Escobar MEO, Hue NV (2008) Temporal changes of selected chemical properties in three manure-amended soils of Hawaii. Bioresour Technol 99:8649–8654. https://doi.org/10.1016/j.biortech.2008.04.069

Esteghlalian AR, Bilodeau M, Mansfield SD, Saddler JN (2001) Do enzymatic hydrolyzability and Simons' stain reflect the changes in the accessibility of lignocellulosic substrates to cellulase enzymes? Biotechnol Prog 17:1049–1054. https://doi.org/10.1021/bp0101177

Faik A (2013) "Plant Cell Wall Structure-Pretreatment" the Critical Relationship in Biomass Conversion to Fermentable Sugars. In: Gu T. (eds) Green Biomass Pretreatment for Biofuels Production. SpringerBriefs in Molecular Science. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6052-3_1

Farmer VC, Henderson MEK, Russell JD (1960) Aromatic-Alcohol-Oxidase activity in the growth medium of *Polystictus versicolor*. Biochem J 74:257–262. https://doi.org/10.1042/bj0740257

Fernandes P (2010) Enzymes in food processing: a condensed overview on strategies for better biocatalysts. Enzyme Res 2010:862537. https://doi.org/10.4061/2010/862537

Fontes CMGA, Gilbert HJ (2010) Cellulosomes: highly efficient nanomachines designed to deconstruct plant cell wall complex carbohydrates. Annu Rev Biochem 79(79):655–681. https://doi.org/10.1146/annurev-biochem-091208-085603

Forootanfar H, Faramarzi MA (2015) Insights into laccase producing organisms, fermentation states, purification strategies, and biotechnological applications. Biotechnol Prog 31:1443–1463. https://doi.org/10.1002/btpr.2173

Fujihara S, Sakurai A, Sakakibara M (2008) Optimization of medium components for manganese peroxidase production by white rot fungus strain L-25. J Chem Eng Jpn 41:796–803. https://doi.org/10.1252/jcej.08we038

Galante YM, De Conti A, Monteverdi R (1998) Application of Trichoderma enzymes in textile industry. In: Harman GF, Kubicek CP (eds) Trichoderma and Gliocladium, Enzymes, biological control and commercial applications, vol 2. Taylor and Francis, London, pp 311–326

Gaur R, Tiwari S (2015) Isolation, production, purification and characterization of an organic-solvent-thermostable alkalophilic cellulase from Bacillus vallismortis RG-07. BMC Biotechnol 15:19

Gerwick WH, Fenner AM (2013) Drug discovery from marine microbes. Microb Ecol 65:800–806. https://doi.org/10.1007/s00248-012-0169-9

Ghosh S (2015) Metagenomic screening of cell wall hydrolases, their anti-fungal activities and potential role in wine fermentation. Ph.D. thesis, Stellenbosch University, South Africa

Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R (2010) Hemicelluloses for fuel ethanol: a review. Bioresour Technol 101:4775–4800. https://doi.org/10.1016/j.biortech.2010.01.088

Givaudan A, Effosse A, Faure D, Potier P, Bouillant ML, Bally R (1993) Polyphenol oxidase in *Azospirillum lipoferum* Isolated from rice rhizosphere - evidence for laccase activity in nonmotile strains of *Azospirillum lipoferum*. FEMS Microbiol Lett 108:205–210. https://doi.org/10.1111/j.1574-6968.1993.tb06100.x

Godfrey T, West S (1996) Textiles. In: Industrial enzymology, 2nd edn. Macmillan Press, London, UK

Grethe R, Moller MJ, Martin S, Anant PS, Fred H, Mailand HC et al. (1999) A cellulase preparation comprising an endoglucanase enzyme. Denmark Patent

Guan ZB, Luo Q, Wang HR, Chen Y, Liao XR (2018) Bacterial laccases: promising biological green tools for industrial applications. Cell Mol Life Sci 75:3569–3592. https://doi.org/10.1007/s00018-018-2883-z

Gübitz GM, Hayn M, Urbanz G, Steiner W (1996) Purification and properties of an acidic α-mannanase from *Sclerotium rolfsii*. J Biotechnol 45:165–172

Gupta A, Verma JP (2015) Sustainable bio-ethanol production from agro-residues: a review. Renew Sustain Energy Rev 41:550–567. https://doi.org/10.1016/j.rser.2014.08.032

Gupta R, Khasa YP, Kuhad RC (2011a) Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. Carbohydr Polym 84:1103–1109. https://doi.org/10.1016/j.carbpol.2010.12.074

Gupta R, Mehta G, Khasa YP, Kuhad RC (2011b) Fungal delignification of lignocellulosic biomass improves the saccharification of cellulosics. Biodegradation 22:797–804. https://doi.org/10.1007/s10532-010-9404-6

Gusakov AV (2011) Alternatives to *Trichoderma reesei* in biofuel production. Trends Biotechnol 29:419–425. https://doi.org/10.1016/j.tibtech.2011.04.004

Hammel KE, Jensen KA, Mozuch MD, Landucci LL, Tien M, Pease EA (1993) Ligninolysis by a purified lignin peroxidase. J Biol Chem 268:12274–12281

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669–685. 68/4/669 [pii]

Harman GE, Björkman T (1998) Potential and existing uses of Trichoderma and Gliocladium for plant disease control and plant growth enhancement. In: Trichoderma and Gliocladium, vol 2. Taylor and Francis, London, UK

Harman GE, Kubicek CP (1998) Trichoderma and Gliocladium: enzymes. In: Kubicek CP, Harman GE (eds) Biological control and commercial applications, vol 2. Taylor & Francis, London, UK

Harman GE, Jin X, Stasz TE, Peruzzotti G, Leopold AC, Taylor AG (1991) Production of conidial biomass of *Trichoderma harzianum* for biological control. Biol Control 1:23–28. https://doi.org/10.1016/1049-9644(91)90097-J

Have T, Tenberge KB, Benen JAE, Tudzynski P, Visser J, van Kan JAL (2002) The contribution of cell wall degrading enzymes to pathogenesis of fungal plant pathogens. In: Kempken F (ed) The mycota (a comprehensive treatise on fungi as experimental systems for basic and applied research), Agriculture application XI, vol 11. Springer, Berlin, pp 341–358

He J, Yu B, Zhang KY, Ding XM, Chen DW (2009) Strain improvement of *Trichoderma reesei* Rut C-30 for increased cellulase production. Indian J Microbiol 49:188–195. https://doi.org/10.1007/s12088-009-0030-0

Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18. https://doi.org/10.1016/j.biortech.2008.05.027

Henrissat B (1991) A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 280(Pt 2):309–316. https://doi.org/10.1042/bj2800309

Henrissat B, Teeri TT, Warren RAJ (1998) A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants. FEBS Lett 425:352–354. https://doi.org/10.1016/S0014-5793(98)00265-8

Himmel ME, Ruth MF, Wyman CE (1999) Cellulase for commodity products from cellulosic biomass. Curr Opin Biotechnol 10:358–364. https://doi.org/10.1016/S0958-1669(99)80065-2

Hong SY, Lee JS, Cho KM, Math RK, Kim YH, Hong SJ et al (2006) Assembling a novel bifunctional cellulase-xylanase from *Thermotoga maritima* by end-to-end fusion. Biotechnol Lett 28:1857–1862. https://doi.org/10.1007/s10529-006-9166-8

Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr J Biotechnol 2:602–619

Huang C, Lai C, Zeng GM, Huang DL, Xu PA, Zhang C et al (2017) Manganese-enhanced degradation of lignocellulosic waste by Phanerochaete chrysosporium: evidence of enzyme activity

and gene transcription. Appl Microbiol Biotechnol 101:6541–6549. https://doi.org/10.1007/s00253-017-8371-9

Hubbe MA, Metts JR, Hermosilla D, Blanco MA, Yerushalmi L, Haghighat F et al (2016) Wastewater treatment and reclamation: a review of pulp and paper industry practices and opportunities. Bioresources 11:7953–8091

Hussain AA, Abdel-Salam MS, Abo-Ghalia HH, Hegazy WK, Hafez SS (2017) Optimization and molecular identification of novel cellulose degrading bacteria isolated from Egyptian environment. J Genet Eng Biotechnol 15:77–85. https://doi.org/10.1016/j.jgeb.2017.02.007

Ibrahim NA, El-Badry K, Eid BM, Hassan TM (2011) A new approach for biofinishing of cellulose-containing fabrics using acid cellulases. Carbohydr Polym 83:116–121. https://doi.org/10.1016/j.carbpol.2010.07.025

Irbe I, Elisashvili V, Asatiani MD, Janberga A, Andersone I, Andersons B et al (2014) Lignocellulolytic activity of *Coniophora puteana* and *Trametes versicolor* in fermentation of wheat bran and decay of hydrothermally modified hardwoods. Int Biodeterior Biodegradation 86:71–78. https://doi.org/10.1016/j.ibiod.2013.06.027

Ishikawa E, Sakai T, Ikemura H, Matsumoto K, Abe H (2005) Identification, cloning, and characterization of a Sporobolomyces singularis beta-galactosidase-like enzyme involved in galacto-oligosaccharide production. J Biosci Bioeng 99:331–339. https://doi.org/10.1263/jbb.99.331

Jabasingh SA, Nachiyar CV (2011) Utilization of pretreated bagasse for the sustainable bioproduction of cellulase by Aspergillus nidulans MTCC344 using response surface methodology. Ind Crop Prod 34:1564–1571. https://doi.org/10.1016/j.indcrop.2011.05.017

Jamal P, Alam MZ, Ramlan M, Salleh M, Munirah MA (2005) Sewage treatment plant sludge: a source of potential microorganism for citric acid production. Am J Appl Sci 2:1236–1239

Jayasekara S, Ratnayake R (2019) Microbial cellulases: an overview and applications. Cellulose. IntechOpen, London, UK

Jin XC, Song JN, Liu GQ (2020) Bioethanol production from rice straw through an enzymatic route mediated by enzymes developed in-house from Aspergillus fumigatus. Energy 190:116395

Johnson E (2016) Integrated enzyme production lowers the cost of cellulosic ethanol. Biofuels Bioprod Biorefin 10:164–174. https://doi.org/10.1002/bbb.1634

Juturu V, Wu JC (2013) Insight into microbial hemi-cellulases other than xylanases: a review. J Chem Technol Biotechnol 88:353–363

Juturu V, Wu JC (2014) Microbial cellulases: engineering, production and applications. Renew Sustain Energy Rev 33:188–203. https://doi.org/10.1016/j.rser.2014.01.077

Karmakar M, Ray RR (2011) Current trends in research and application of microbial cellulases. Res J Microbiol 6:41–53

Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A (2008) A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's Iodine. Curr Microbiol 57:503–507. https://doi.org/10.1007/s00284-008-9276-8

Kathiresan K, Manivannan S (2006) α-Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. Afr J Biotechnol 5:829–832

Kathiresan K, Saravanakumar K, Anburaj R, Gomathi V, Abirami G, Sahu SK et al (2011) Microbial enzyme activity in decomposing leaves of Mangroves. Int J Adv Biotechnol Res 2:382–389

Kaur T, Devi R, Kour D, Yadav N, Prasad S, Singh A et al (2020) Advances in microbial bioresources for sustainable biofuels production: current research and future challenges. In: Yadav AN, Rastegari AA, Yadav N, Gaur R (eds) Biofuels production – sustainability and advances in microbial bioresources. Springer International Publishing, Cham, pp 371–387. https://doi.org/10.1007/978-3-030-53933-7_17

Khan MN, Luna IZ, Islam MM, Sharmeen S, Salem KS, Rashid TU et al (2019) Cellulase in waste management applications. In: Neha Srivastava N, Srivastava M, Mishra PK, Ramteke PW, Singh RL (eds) New and future developments in microbial biotechnology and bioengineering, 1st edn. Elsevier, Amsterdam, pp 237–256

Kiiskinen LL, Ratto M, Kruus K (2004) Screening for novel laccase-producing microbes. J Appl Microbiol 97:640–646. https://doi.org/10.1111/j.1365-2672.2004.02348.x

Kirk TK, Farrell RL (1987) Enzymatic combustion - the microbial-degradation of lignin. Annu Rev Microbiol 41:465–505. https://doi.org/10.1146/annurev.mi.41.100187.002341

Kittur FS, Mangala SL, Abu Rus'd A, Kitaoka M, Tsujibo H, Hayashi K (2003) Fusion of family 2b carbohydrate-binding module increases the catalytic activity of a xylanase from Thermotoga maritima to soluble xylan. FEBS Lett 549:147–151. https://doi.org/10.1016/S0014-5793(03)00803-2

Kour D, Rana KL, Kaur T, Singh B, Chauhan VS, Kumar A et al (2019a) Extremophiles for hydrolytic enzymes productions: biodiversity and potential biotechnological applications. In: Molina G, Gupta VK, Singh B, Gathergood N (eds) Bioprocessing for biomolecules production. Wiley, Hoboken, NJ, pp 321–372. https://doi.org/10.1002/9781119434436.ch16

Kour D, Rana KL, Yadav N, Yadav AN, Rastegari AA, Singh C et al (2019b) Technologies for biofuel production: current development, challenges, and future prospects. In: Rastegari AA, Yadav AN, Gupta A (eds) Prospects of renewable bioprocessing in future energy systems. Springer International Publishing, Cham, pp 1–50. https://doi.org/10.1007/978-3-030-14463-0_1

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019c) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kour D, Kaur T, Devi R, Yadav A, Singh M, Joshi D et al (2021) Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: present status and future challenges. Environ Sci Pollut Res 28:24917–24939. https://doi.org/10.1007/s11356-021-13252-7

Kuhad RC, Singh A, Eriksson KE (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57:45–125. https://doi.org/10.1007/BFb0102072

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzyme Res 2011:280696. https://doi.org/10.4061/2011/280696

Kumar NV, Rani ME, Gunaseeli R, Kannan ND (2018) Paper pulp modification and deinking efficiency of cellulase-xylanase complex from Escherichia coli SD5. Int J Biol Macromol 111:289–295. https://doi.org/10.1016/j.ibiomac.2017.12.126

Kumar A, Yadav AN, Mondal R, Kour D, Subrahmanyam G, Shabnam AA et al (2021) Myco-remediation: a mechanistic understanding of contaminants alleviation from natural environment and future prospect. Chemosphere 284:131325. https://doi.org/10.1016/j.chemosphere.2021.131325

Lange L, Pilgaard B, Herbst FA, Busk PK, Gleason F, Pedersen AG (2019) Origin of fungal biomass degrading enzymes: evolution, diversity and function of enzymes of early lineage fungi. Fungal Biol Rev 33:82–97. https://doi.org/10.1016/j.fbr.2018.09.001

Larue K, Melgar M, Martin VJ (2016) Directed evolution of a fungal beta-glucosidase in Saccharomyces cerevisiae. Biotechnol Biofuels 9:52. https://doi.org/10.1186/s13068-016-0470-9

Lee SM, Koo YM (2001) Pilot-scale production of cellulase using *Trichoderma reesei* rut C-30 in fed-batch mode. J Microbiol Biotechnol 11:229–233

Legodi LM, La Grange D, van Rensburg ELJ, Ncube I (2019) Isolation of Cellulose degrading fungi from decaying banana pseudostem and *Strelitzia alba*. Enzyme Res 2019:1390890. https://doi.org/10.1155/2019/1390890

Li XH, Yang HJ, Roy B, Park EY, Jiang LJ, Wang D et al (2010) Enhanced cellulase production of the *Trichoderma viride* mutated by microwave and ultraviolet. Microbiol Res 165:190–198. https://doi.org/10.1016/j.micres.2009.04.001

Li JY, Zhang YL, Li JG, Sun T, Tian CG (2020) Metabolic engineering of the cellulolytic thermophilic fungus Myceliophthora thermophila to produce ethanol from cellobiose. Biotechnol Biofuels 13:23

Liang YN, Feng ZS, Yesuf J, Blackburn JW (2010) Optimization of growth medium and enzyme assay conditions for crude cellulases produced by a novel thermophilic and cellulolytic bacterium, *Anoxybacillus* sp 527. Appl Biochem Biotechnol 160:1841–1852. https://doi.org/10.1007/s12010-009-8677-x

Liang YL, Zhang Z, Wu M, Wu Y, Feng JX (2014) Isolation, screening, and identification of cellulolytic bacteria from natural reserves in the subtropical region of China and optimization of cellulase production by *Paenibacillus terrae* ME27-1. Biomed Res Int 2014:512497

Liao HP, Fan XT, Mei XL, Wei Z, Raza W, Shen QR et al (2015) Production and characterization of cellulolytic enzyme from *Penicillium oxalicum* GZ-2 and its application in lignocellulose saccharification. Biomass Bioenergy 74:122–134. https://doi.org/10.1016/j.biombioe.2015.01.016

Lin HL, Li WG, Guo CH, Qu SH, Ren NQ (2011) Advances in the study of directed evolution for cellulases. Front Environ Sci Eng China 5:519–525. https://doi.org/10.1007/s11783-011-0326-2

Liu W, Hong J, Bevan DR, Zhang YH (2009) Fast identification of thermostable beta-glucosidase mutants on cellobiose by a novel combinatorial selection/screening approach. Biotechnol Bioeng 103:1087–1094. https://doi.org/10.1002/bit.22340

Lombard V, Ramulu HG, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495. https://doi.org/10.1093/nar/gkt1178

Luo W, Vrijmoed LLP, Jones EBG (2005) Screening of marine fungi for lignocellulose-degrading enzyme activities. Bot Mar 48:379–386. https://doi.org/10.1515/Bot.2005.051

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002a) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66:506–577. https://doi.org/10.1128/mmbr.66.3.506-577.2002

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002b) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66:739–739. https://doi.org/10.1128/Mmbr.66.4.739.2002

Lynd LR, Laser MS, Brandsby D, Dale BE, Davison B, Hamilton R et al (2008) How biotech can transform biofuels. Nat Biotechnol 26:169–172. https://doi.org/10.1038/nbt0208-169

Lynd LR, Liang XY, Biddy MJ, Allee A, Cai H, Foust T et al (2017) Cellulosic ethanol: status and innovation. Curr Opin Biotechnol 45:202–211. https://doi.org/10.1016/j.copbio.2017.03.008

Maria GL, Sridhar KR, Raviraja NS (2005) Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India. J Agric Technol 1:67–80

Marriott PE, Gomez LD, McQueen-Mason SJ (2016) Unlocking the potential of lignocellulosic biomass through plant science. New Phytol 209:1366–1381. https://doi.org/10.1111/nph.13684

Martin TC, Visconti A, Spector TD, Falchi M (2018) Conducting metagenomic studies in microbiology and clinical research. Appl Microbiol Biotechnol 102:8629–8646. https://doi.org/10.1007/s00253-018-9209-9

Martinez AT, Speranza M, Ruiz-Duenas FJ, Ferreira P, Camarero S, Guillen F et al (2005) Biodegradation of lignocellulosics: microbial chemical, and enzymatic aspects of the fungal attack of lignin. Int Microbiol 8:195–204

Mason MG, Wilson MT, Ball A, Nicholls P (2002) Oxygen reduction by cellobiose oxidoreductase: the role of the haem group. FEBS Lett 518:29–32. Pii S0014-5793(02)02633-9

Mathew GM, Sukumaran RK, Singhania RR, Pandey A (2008) Progress in research on fungal cellulases for lignocellulose degradation. J Sci Ind Res 67:898–907

Mehandia S, Sharma SC, Arya SK (2020) Isolation and characterization of an alkali and thermostable laccase from a novel Alcaligenes faecalis and its application in decolorization of synthetic dyes. Biotechnol Rep (Amst) 25:e00413. https://doi.org/10.1016/j.btre.2019.e00413

Mehboob N, Asad MJ, Asgher M, Gulfraz M, Mukhtar T, Mahmood RT (2014) Exploring thermophilic cellulolytic enzyme production potential of *Aspergillus fumigatus* by the solid-state fer-

mentation of wheat straw. Appl Biochem Biotechnol 172:3646–3655. https://doi.org/10.1007/s12010-014-0796-3

Menendez E, Garcia-Fraile P, Rivas R (2015) Biotechnological applications of bacterial cellulases. Aims Bioeng 2:163–182. https://doi.org/10.3934/bioeng.2015.3.163

Meyer MM, Hochrein L, Arnold FH (2006) Structure-guided SCHEMA recombination of distantly related beta-lactamases. Protein Eng Des Sel 19:563–570. https://doi.org/10.1093/protein/gzl045

Miettinen-Oinonen A, Suominen P (2002) Enhanced production of *Trichoderma reesei* endoglucanases and use of the new cellulase preparations in producing the stonewashed effect on denim fabric. Appl Environ Microbiol 68:3956–3964. https://doi.org/10.1128/Aem.68.8.3956-3964.2002

Mishra RK, Mohanty K (2018) Characterization of non-edible lignocellulosic biomass in terms of their candidacy towards alternative renewable fuels. Biomass Convers Bioref 8:799–812. https://doi.org/10.1007/s13399-018-0332-8

Mojsov KD (2016) Aspergillus enzymes for food industries. In: Gupta V (ed) New and future developments in microbial biotechnology and bioengineering: *Aspergillus* system properties and applications, vol 1. Elesevier, Amsterdam, pp 215–222

Morais H, Ramos C, Matos N, Forgacs E, Cserhati T, Almeida V et al (2002) Liquid chromatographic and electrophoretic characterisation of extracellular beta-glucosidase of *Pleurotus ostreatus* grown in organic waste. J Chromatogr B Anal Technol Biomed Life Sci 770:111–119. Pii S0378-4347(01)00561-8

Moreira PR, Duez C, Dehareng D, Antunes A, Almeida-Vara E, Frere JM et al (2005) Molecular characterisation of a versatile peroxidase from a *Bjerkandera* strain. J Biotechnol 118:339–352. https://doi.org/10.1016/j.jbiotec.2005.05.014

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M et al (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686. https://doi.org/10.1016/j.biortech.2004.06.025

Muheim A, Leisola MSA, Schoemaker HE (1990) Aryl-alcohol oxidase and lignin peroxidase from the white-rot fungus Bjerkandera-Adusta. J Biotechnol 13:159–167. https://doi.org/10.1016/0168-1656(90)90101-G

Nacke H, Engelhaupt M, Brady S, Fischer C, Tautzt J, Daniel R (2012) Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes. Biotechnol Lett 34:663–675. https://doi.org/10.1007/s10529-011-0830-2

Nagodawithana T, Reed G (2013) Enzymes in food processing, 3rd edn. Elsevier, Amsterdam

Niyonzima FN (2019) Detergent-compatible bacterial cellulases. J Basic Microbiol 59:134–147. https://doi.org/10.1002/jobm.201800436

Novaes E, Kirst M, Chiang V, Winter-Sederoff H, Sederoff R (2010) Lignin and biomass: a negative correlation for wood formation and lignin content in trees. Plant Physiol 154:555–561. https://doi.org/10.1104/pp.110.161281

Nuutila AM, Ritala A, Skadsen RW, Mannonen L, Kauppinen V (1999) Expression of fungal thermotolerant endo-1,4-beta-glucanase in transgenic barley seeds during germination. Plant Mol Biol 41:777–783. https://doi.org/10.1023/A:1006318206471

Oberoi HS, Rawat R, Chadha BS (2014) Response surface optimization for enhanced production of cellulases with improved functional characteristics by newly isolated *Aspergillus niger* HN-2. Anton Leeuw Int J Gen Mol Microbiol 105:119–134. https://doi.org/10.1007/s10482-013-0060-9

Oksanen J, Ahvenainen J, Home S (1985) Microbial cellulase for improving filterability of wort and beer. J Inst Brew 91:130–130

Ory R, Angelo AJS (1977) Enzymes in food and beverage processing. American Chemical Society, Washington, DC

Pajunen E (1986) Optimal use of β-glucanases in wort production. Paper presented at the EBC-symposium on wort production, Maffliers, France

Palma C, Lloret L, Sepulveda L, Contreras E (2016) Production of versatile peroxidase from *Pleurotus eryngii* by solid-state fermentation using agricultural residues and evaluation of its catalytic properties. Prep Biochem Biotechnol 46:200–207. https://doi.org/10.1080/10826068.2015.1084513

Pandey A (2003) Special issue - Solid-state fermentation - Preface. Biochem Eng J 13:79. Pii S1369-703x(02)00134-1

Pandey A, Höfer R, Taherzadeh M, Nampoothiri KM, Larroche C (2015) Industrial biorefineries and white biotechnology. Amsterdam, Elsevier

Pazarlioglu NK, Sariisik M, Telefoncu A (2005) Treating denim fabrics with immobilized commercial cellulases. Process Biochem 40:767–771. https://doi.org/10.1016/j.procbio.2004.02.003

Pedersen M, Hollensted M, Lange L, Andersen B (2009) Screening for cellulose and hemicellulose degrading enzymes from the fungal genus Ulocladium. Int Biodeterior Biodegradation 63:484–489. https://doi.org/10.1016/j.ibiod.2009.01.006

Petrakis C (2006) Olive oil extraction. Olive oil: chemistry and technology. AOCS Press, Champaign, IL

Prongjit M, Sucharitakul J, Palfey BA, Chaiyen P (2013) The oxidation mode of pyranose 2-oxidase is controlled by pH. Biochemistry 52:1437–1445. https://doi.org/10.1021/bi301442x

Przybysz Buzala K, Przybysz P, Kalinowska H, Derkowska M (2016) Effect of cellulases and xylanases on refining process and kraft pulp properties. PLoS One 11:e0161575

Quiroz-Castañeda RE, Folch-Mallol JL (2013) Hydrolysis of biomass mediated by cellulases for the production of sugars. In: Chandel AK, da Silva SS (eds) Degradation of lignocellulosic biomass - techniques, applications and commercialization. InTech, London

Rainey FA, Donnison AM, Janssen PH, Saul D, Rodrigo A, Bergquist PL et al (1994) Description of Caldicellulosiruptor-*Saccharolyticus* Gen-Nov, Sp-Nov - an obligately anaerobic, extremely thermophilic, cellulolytic bacterium. FEMS Microbiol Lett 120:263–266. https://doi.org/10.1111/j.1574-6968.1994.tb07043.x

Rana V, Eckard AD, Teller P, Ahring BK (2014) On-site enzymes produced from Trichoderma reesei RUT-C30 and *Aspergillus saccharolyticus* for hydrolysis of wet exploded corn stover and loblolly pine. Bioresour Technol 154:282–289. https://doi.org/10.1016/j.biortech.2013.12.059

Ranalli A, Pollastri L, Contento S, Lucera L, Del Re P (2003) Enhancing the quality of virgin olive oil by use of a new vegetable enzyme extract during processing. Eur Food Res Technol 216:109–115. https://doi.org/10.1007/s00217-002-0618-7

Rao RG, Ravichandran A, Kandalam G, Samanta AK, Swaraj S, Sridhar M (2019) Enhanced production of manganese peroxidase from *Clitopilus scyphoides* employing statistical optimization for application in improving crop residue digestibility by ruminants. J Dairy Vet Anim Res 8:190–203

Rasmussen ML, Shrestha P, Khanal SK, Pometto AL, van Leeuwen J (2010) Sequential saccharification of corn fiber and ethanol production by the brown rot fungus *Gloeophyllum trabeum*. Bioresour Technol 101:3526–3533. https://doi.org/10.1016/j.biortech.2009.12.115

Raveendran S, Parameswaran B, Ummalyma SB, Abraham A, Mathew AK, Madhavan A et al (2018) Applications of microbial enzymes in food industry. Food Technol Biotechnol 56:16–30. https://doi.org/10.17113/ftb.56.01.18.5491

Ravindran R, Jaiswal AK (2016) Microbial enzyme production using lignocellulosic food industry wastes as feedstock: a Review. Bioengineering (Basel) 3. https://doi.org/10.3390/bioengineering3040030

Ravindran R, Hassan SS, Williams GA, Jaiswal AK (2018) A review on bioconversion of agro-industrial wastes to industrially important enzymes. Bioengineering (Basel) 5. https://doi.org/10.3390/bioengineering5040093

Rubin EM (2008) Genomics of cellulosic biofuels. Nature 454:841–845. https://doi.org/10.1038/nature07190

Ruiz-Duenas FJ, Morales M, Perez-Boada M, Choinowski T, Martinez MJ, Piontek K et al (2007) Manganese oxidation site in *Pleurotus eryngii* versatile peroxidase: a site-directed mutagenesis, kinetic, and crystallographic study. Biochemistry 46:66–77. https://doi.org/10.1021/bi061542h

Ryu DDY, Mandels M (1980) Cellulases - biosynthesis and applications. Enzyme Microb Technol 2:91–102. https://doi.org/10.1016/0141-0229(80)90063-0
Sadana JC, Patil RV (1988) 1,4-Beta-D-glucan cellobiohydrolase from *Sclerotium rolfsii*. Methods Enzymol 160:307–314
Sadhu S, Maiti TK (2013) Cellulase production by bacteria: a review. Br Microbiol Res J 3:235–258
Saeed M, Ayasan T, Alagawany M, El-Hack MEA, Abdel-Latif MA, Patra AK (2019) The role of beta-Mannanase (Hemicell) in improving poultry productivity, health and environment. Braz J Poultry Sci 21:eRBCA-2019-1001
Saini A, Aggarwal NK, Sharma A, Yadav A (2015) Actinomycetes: a source of Lignocellulolytic enzymes. Enzyme Res 2015:279381. https://doi.org/10.1155/2015/279381
Sajith S, Priji P, Sreedevi S, Benjamin S (2016) An overview on fungal cellulases with an industrial perspective. J Nutr Food Sci 6:1–13
Sampathkumar K, Kumar V, Sivamani S, Sivakumar N (2019) An insight into fungal cellulases and their industrial applications. In: Srivastava M, Srivastava N, Ramteke P, Mishra P (eds) Approaches to enhance industrial production of fungal cellulases. Fungal biology. Springer, Cham, pp 19–35
Santhanam N, Vivanco JM, Decker SR, Reardon KF (2011) Expression of industrially relevant laccases: prokaryotic style. Trends Biotechnol 29:480–489. https://doi.org/10.1016/j.tibtech.2011.04.005
Santos RB, Capanema EA, Balakshin MY, Chang HM, Jameel H (2012) Lignin structural variation in hardwood species. J Agric Food Chem 60:4923–4930. https://doi.org/10.1021/jf301276a
Scheiblbrandner S, Ludwig R (2020) Cellobiose dehydrogenase: bioelectrochemical insights and applications. Bioelectrochemistry 131:107345
Scheller HV, Ulvskov P (2010) Hemicelluloses. Annu Rev Plant Biol 61(61):263–289. https://doi.org/10.1146/annurev-arplant-042809-112315
Schwarz WH (2001) The cellulosome and cellulose degradation by anaerobic bacteria. Appl Microbiol Biotechnol 56:634–649. https://doi.org/10.1007/s002530100710
Sette LD, de Oliveira VM, Rodrigues MFA (2008) Microbial lignocellulolytic enzymes: industrial applications and future perspectives. Microbiology Australia 29:18–20
Shankar SS, Gupta M, Shikha (2017) Microbial cellulases and their applications in the pulp and paper industry: an emerging paradigm. In: Bhargava RN (ed) Environmental pollutants and their bioremediation approaches. Taylor and Francis, United Kingdom, pp 273–304
Sharada R, Venkateswarlu G, Venkateswar S, AnandRao M (2014) Applications of cellulases – review. Int J Pharm Chem Biol Sci 4:424–437
Sharma SK (2015) Green chemistry for dyes removal from wastewater: research trends and applications. Wiley, Hoboken, NJ. https://doi.org/10.1002/9781118721001
Sharma S, Kour D, Rana KL, Dhiman A, Thakur S, Thakur P et al (2019) *Trichoderma*: biodiversity, ecological significances, and industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer, Cham, pp 85–120. https://doi.org/10.1007/978-3-030-10480-1_3
Sheehan J, Himmel M (1999) Enzymes, energy, and the environment: a strategic perspective on the US Department of Energy's Research and Development activities for bioethanol. Biotechnol Prog 15:817–827. https://doi.org/10.1021/bp990110d
Sheng P, Li YS, Marshall SDG, Zhang HY (2015) High genetic diversity of microbial cellulase and hemicellulase genes in the hindgut of Holotrichia parallela larvae. Int J Mol Sci 16:16545–16559. https://doi.org/10.3390/ijms160716545
Shin K, Yoon SM, Kim YK, Kim TJ, Kim YS (2016) Biopolishing of cotton fabric using crude cellulases from *Acanthophysium* sp. KMF001. J Kor Wood Sci Technol 44:381–388. https://doi.org/10.5658/WOOD.2016.44.3.381
Singh J, Yadav AN (2020) Natural bioactive products in sustainable agriculture. Springer, Singapore
Singh A, Kuhad RC, Ward OP (2007) Industrial application of microbial cellulases. In: Kuhad RC, Singh A (eds) Lignocellulose biotechnologgy: future prospects. I.K. International Publishing House, New Delhi, India

Singhania RR, Patel AK, Soccol CR, Pandey A (2009) Recent advances in solid-state fermentation. Biochem Eng J 44:13–18. https://doi.org/10.1016/j.bej.2008.10.019

Singhvi MS, Gokhale DV (2019) Lignocellulosic biomass: hurdles and challenges in its valorization. Appl Microbiol Biotechnol 103:9305–9320. https://doi.org/10.1007/s00253-019-10212-7

Sitarz AK, Mikkelsen JD, Meyer AS (2016) Structure, functionality and tuning up of laccases for lignocellulose and other industrial applications. Crit Rev Biotechnol 36:70–86. https://doi.org/10.3109/07388551.2014.949617

Soliman HM, Sherief A-DA, Tanash ABE (2012) Production of xylanase by *Aspergillus niger* and *Trichoderma viride* using some agriculture residues. Int J Agric Res 7:46–57

Soni SK, Sharma A, Soni R (2018) Cellulases: role in lignocellulosic biomass utilization. Methods Mol Biol 1796:3–23. https://doi.org/10.1007/978-1-4939-7877-9_1

Speda J, Jonsson BH, Carlsson U, Karlsson M (2017) Metaproteomics-guided selection of targeted enzymes for bioprospecting of mixed microbial communities. Biotechnol Biofuels 10:128. https://doi.org/10.1186/s13068-017-0815-z

Sreenath HK, Shah AB, Yang VW, Gharia MM, Jeffries TW (1996) Enzymatic polishing of jute/cotton blended fabrics. J Ferment Bioeng 81:18–20. https://doi.org/10.1016/0922-338x(96)83113-8

Srivastava N, Jaiswal P (2016) Production of cellulases using agriculture waste: application in biofuels production. Environmental biotechnology a new approach. Daya Publishing House® A Division of Astral International Pvt. Ltd., New Delhi

Srivastava N, Rawat R, Oberoi HS, Ramteke PW (2015a) A review on fuel ethanol production from lignocellulosic biomass. Int J Green Energy 12:949–960. https://doi.org/10.1080/15435075.2014.890104

Srivastava N, Singh J, Ramteke PW, Mishra PK, Srivastava M (2015b) Improved production of reducing sugars from rice straw using crude cellulase activated with Fe_3O_4/Alginate nanocomposite. Bioresour Technol 183:262–266. https://doi.org/10.1016/j.biortech.2015.02.059

Srivastava N, Srivastava M, Mishra PK, Singh P, Ramteke PW (2015c) Application of cellulases in biofuels industries: an overview. J Biofuels Bioenergy 1:55–63. https://doi.org/10.5958/2454-8618.2015.00007.3

Stalbrand H, Saloheimo A, Vehmaanpera J, Henrissat B, Penttila M (1995) Cloning and expression in *Saccharomyces cerevisiae* of a *Trichoderma reesei* beta-mannanase gene containing a cellulose binding domain. Appl Environ Microbiol 61:1090–1097

Stanisavljevic N, Levis JW, Barlaz MA (2018) Application of a life cycle model for European Union Policy-Driven waste management decision making in emerging economies. J Ind Ecol 22:341–355. https://doi.org/10.1111/jiec.12564

Stephens DE, Rumbold K, Permaul K, Prior BA, Singh S (2007) Directed evolution of the thermostable xylanase from Thermomyces lanuginosus. J Biotechnol 127:348–354. https://doi.org/10.1016/j.jbiotec.2006.06.015

Stephens DE, Singh S, Permaul K (2009) Error-prone PCR of a fungal xylanase for improvement of its alkaline and thermal stability. FEMS Microbiol Lett 293:42–47. https://doi.org/10.1111/j.1574-6968.2009.01519.x

Subramaniyam R, Vimala R (2012) Solid state and submerged fermentation for the production of bioactive substances: a comparative study. Int J Sci Nat 3:480–486

Sukumaran RK, Singhania RR, Pandey A (2005) Microbial cellulases - production, applications and challenges. J Sci Ind Res 64:832–844

Sukumaran RK, Singhania RR, Mathew GM, Pandey A (2009) Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. Renew Energy 34:421–424. https://doi.org/10.1016/j.renene.2008.05.008

Swain MR, Ray RC (2007) Alpha-amylase production by *Bacillus subtilis* CM3 in solid state fermentation using cassava fibrous residue. J Basic Microbiol 47:417–425. https://doi.org/10.1002/jobm.200710132

Swain MR, Singh A, Sharma AK, Tuli DK (2019) Bioethanol production from rice and wheat straw: an overview. In: Ray RC, Ramachandran S (eds) Bioethanol production from food crops sustainable sources, interventions, and challenges. Elsevier, Amsterdam, pp 213–231

Taherzadeh MJ, Karimi K (2007) Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bioresources 2:707–738

Takasaki K, Miura T, Kanno M, Tamaki H, Hanada S, Kamagata Y et al (2013) Discovery of glycoside hydrolase enzymes in an avicel-adapted forest soil fungal community by a metatranscriptomic approach. PLoS One 8:e55485. https://doi.org/10.1371/journal.pone.0055485

Talebnia F, Karakashev D, Angelidaki I (2010) Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation. Bioresour Technol 101:4744–4753. https://doi.org/10.1016/j.biortech.2009.11.080

Tejada M, Gonzalez JL, Garcia-Martinez AM, Parrado J (2008) Application of a green manure and green manure composted with beet vinasse on soil restoration: effects on soil properties. Bioresour Technol 99:4949–4957. https://doi.org/10.1016/j.biortech.2007.09.026

Thomas L, Parameswaran B, Pandey A (2016) Hydrolysis of pretreated rice straw by an enzyme cocktail comprising acidic xylanase from *Aspergillus* sp for bioethanol production. Renew Energy 98:9–15. https://doi.org/10.1016/j.renene.2016.05.011

Thota SP, Badiya PK, Yerram S, Vadlani PV, Pandey M, Golakoti NR et al (2017) Macro-micro fungal cultures synergy for innovative cellulase enzymes production and biomass structural analyses. Renew Energy 103:766–773. https://doi.org/10.1016/j.renene.2016.11.010

Tiwari P, Misra BN, Sangwan NS (2013) beta-Glucosidases from the fungus trichoderma: an efficient cellulase machinery in biotechnological applications. Biomed Res Int 2013:203735. https://doi.org/10.1155/2013/203735

Tobimatsu Y, Schuetz M (2019) Lignin polymerization: how do plants manage the chemistry so well? Curr Opin Biotechnol 56:75–81. https://doi.org/10.1016/j.copbio.2018.10.001

Tomme P, Driver DP, Amandoron EA, Miller RC Jr, Antony R, Warren J et al (1995) Comparison of a fungal (family I) and bacterial (family II) cellulose-binding domain. J Bacteriol 177:4356–4363. https://doi.org/10.1128/jb.177.15.4356-4363.1995

Toushik SH, Lee KT, Lee JS, Kim KS (2017) Functional applications of lignocellulolytic enzymes in the fruit and vegetable processing industries. J Food Sci 82:585–593. https://doi.org/10.1111/1750-3841.13636

Uddin MG (2015) Effects of biopolishing on the quality of cotton fabrics using acid and neutral cellulases. Textiles Clothing Sustain 1:9. https://doi.org/10.1186/s40689-015-0009-7

Vares T, Kalsi M, Hatakka A (1995) Lignin peroxidases, Manganese peroxidases, and other ligninolytic enzymes produced by *Phlebia radiata* during solid-state fermentation of wheat-straw. Appl Environ Microbiol 61:3515–3520. https://doi.org/10.1128/Aem.61.10.3515-3520.1995

Vasina DV, Moiseenko KV, Fedorova TV, Tyazhelova TV (2017) Lignin-degrading peroxidases in white-rot fungus *Trametes hirsuta* 072. Absolute expression quantification of full multigene family. PLoS One 12:e0173813

Wang Q, Xia T (2008) Enhancement of the activity and alkaline pH stability of Thermobifida fusca xylanase A by directed evolution. Biotechnol Lett 30:937–944. https://doi.org/10.1007/s10529-007-9508-1

Wang N, Ren K, Jia R, Chen WT, Sun RR (2016) Expression of a fungal manganese peroxidase in *Escherichia coli*: a comparison between the soluble and refolded enzymes. BMC Biotechnol 16:87

Wang MM, Du J, Zhang DL, Li XZ, Zhao J (2017) Modification of different pulps by homologous overexpression alkali-tolerant endoglucanase in *Bacillus subtilis* Y106. Sci Rep 7:3321

Watanabe H, Tokuda G (2001) Animal cellulases. Cell Mol Life Sci 58:1167–1178. https://doi.org/10.1007/Pl00000931

Weiss N, Borjesson J, Pedersen LS, Meyer AS (2013) Enzymatic lignocellulose hydrolysis: improved cellulase productivity by insoluble solids recycling. Biotechnol Biofuels 6:5

Wi SG, Cho EJ, Lee DS, Lee SJ, Lee YJ, Bae HJ (2015) Lignocellulose conversion for biofuel: a new pretreatment greatly improves downstream biocatalytic hydrolysis of various lignocellulosic materials. Biotechnol Biofuels 8:228

Wilson DB (1992) Biochemistry and genetics of actinomycete cellulases. Crit Rev Biotechnol 12:45–63. https://doi.org/10.3109/07388559209069187

Wilson DB (2004) Studies of *Thermobifida fusca* plant cell wall degrading enzymes. Chem Rec 4:72–82. https://doi.org/10.1002/tcr.20002

Wilson DB (2011) Microbial diversity of cellulose hydrolysis. Curr Opin Microbiol 14:259–263. https://doi.org/10.1016/j.mib.2011.04.004

Wong DWS (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotechnol 157:174–209. https://doi.org/10.1007/s12010-008-8279-z

Wooley JC, Ye YZ (2010) Metagenomics: facts and artifacts, and computational challenges. J Comput Sci Technol 25:71–81. https://doi.org/10.1007/s11390-010-9306-4

Xia LM, Cen PL (1999) Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. Process Biochem 34:909–912. https://doi.org/10.1016/S0032-9592(99)00015-1

Xue DS, Zeng XH, Lin DQ, Yao SJ (2018) Ethanol tolerant endoglucanase from *Aspergillus niger* isolated from wine fermentation cellar. Biocatal Agric Biotechnol 15:19–24. https://doi.org/10.1016/j.bcab.2018.04.016

Yadav AN (2019) Fungal white biotechnology: conclusion and future prospects. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for sustainable environments, vol 3. Springer International Publishing, Cham, pp 491–498. https://doi.org/10.1007/978-3-030-25506-0_20

Yadav AN, Verma P, Kumar V, Sangwan P, Mishra S, Panjiar N et al (2018) Biodiversity of the Genus *Penicillium* in different habitats. In: Gupta VK, Rodriguez-Couto S (eds) New and future developments in microbial biotechnology and bioengineering, *Penicillium* system properties and applications. Elsevier, Amsterdam, pp 3–18. https://doi.org/10.1016/B978-0-444-63501-3.00001-6

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N, Gaur R (2020) Biofuels production – sustainability and advances in microbial bioresources. Springer, Cham

Yang WP, Meng FX, Peng JY, Han P, Fang F, Ma L et al (2014) Isolation and identification of a cellulolytic bacterium from the Tibetan pig's intestine and investigation of its cellulase production. Electron J Biotechnol 17:262–267. https://doi.org/10.1016/j.ejbt.2014.08.002

Yang C, Xia Y, Qu H, Li AD, Liu RH, Wang YB et al (2016) Discovery of new cellulases from the metagenome by a metagenomics-guided strategy. Biotechnol Biofuels 9:138

Yennamalli RM, Rader AJ, Kenny AJ, Wolt JD, Sen TZ (2013) Endoglucanases: insights into thermostability for biofuel applications. Biotechnol Biofuels 6:136

Yoon LW, Ang TN, Ngoh GC, Chua ASM (2014) Fungal solid-state fermentation and various methods of enhancement in cellulase production. Biomass Bioenergy 67:319–338. https://doi.org/10.1016/j.biombioe.2014.05.013

Yu YY, Yuan JG, Wang Q, Fan XR, Wang P, Cui L (2015) Noncovalent immobilization of cellulases using the reversibly soluble polymers for biopolishing of cotton fabric. Biotechnol Appl Biochem 62:494–501. https://doi.org/10.1002/bab.1289

Zaldivar J, Nielsen J, Olsson L (2001) Fuel ethanol production from lignocellulose: a challenge for metabolic engineering and process integration. Appl Microbiol Biotechnol 56:17–34. https://doi.org/10.1007/s002530100624

Zhang YP (2008) Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. J Ind Microbiol Biotechnol 35:367–375. https://doi.org/10.1007/s10295-007-0293-6

Zhang YH (2009) A sweet out-of-the-box solution to the hydrogen economy: is the sugar-powered car science fiction? Energy Environ Sci 2:272–282

Zhang YH, Lynd LR (2004) Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnol Bioeng 88:797–824. https://doi.org/10.1002/bit.20282

Zhang ZS, Donaldson AA, Ma XX (2012) Advancements and future directions in enzyme technology for biomass conversion. Biotechnol Adv 30:913–919. https://doi.org/10.1016/j.biotechadv.2012.01.020

Zhuang J, Marchant MA, Nokes SE, Strobel HJ (2007) Economic analysis of cellulase production methods for bio-ethanol. Appl Eng Agric 23:679–687

Zoghlami A, Paes G (2019) Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. Front Chem 7:874

Chapter 8
Fungal Secondary Metabolites: Current Research, Commercial Aspects, and Applications

Marwa Tamim A. Abdel-Wareth

Contents

8.1 Introduction

Secondary metabolites are loosely defined as organic compounds that are not directly involved in primary metabolic processes such as cell growth, cell division, cell respiration, or photosynthesis. Furthermore, secondary metabolites are derived from a few common biosynthetic pathways which branch off the primary metabolic pathways and are often produced as families of related compounds, often specific

M. T. A. Abdel-Wareth (✉)
Environmental Research and Medical Malacology Department, Theodor Bilharz Research Institute, Giza, Egypt

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_8

for a group of organisms (Dewick 2009; Hartmann 2007; Hanson 2003; Devi et al. 2020). Fungi are a rich source of secondary metabolites and have been of interest to humans for thousands of years.

Secondary metabolites are biosynthesized of building blocks that are put together in various metabolic pathways. The pathways are usually named after enzymes or intermediates involved and are commonly used to classify secondary metabolites. The diversity and complexity of the structures that these relatively few building blocks can provide are fascinating (Dewick 2009). Generally, fungal secondary metabolites are classified into terpenoids, polyketides, alkaloids, carbohydrates, fatty acids, and peptides and proteins. Terpenoids are found essentially in all forms of life and they form a large group of secondary metabolites with more than 40,000 structures (Bohlmann and Keeling 2008).

They are built up from five-carbon segments, so-called isoprene units, which can combine to form different classes of terpenoids: hemi- (C5), mono- (C10), sesqui- (C15), di- (C20), sester- (C25), tri- (C30), and tetraterpenes (C40). The actual fundamental building block to all terpenes and terpenoids is isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). In fungi, IPP and DMAPP are biosynthesized via the mevalonic acid (MVA) pathway. Regarding polyketides, they are produced via the acetate pathway. They represent a large family of secondary metabolites found in bacteria, fungi, and plants (Rastegari et al. 2019a). They are particularly important for fungi because of being the most abundant fungal secondary metabolites; also many of them have important biological activities. For example, the cholesterol-lowering compound lovastatin, produced by the fungi *Monascus rubber* and *Aspergillus terreus* which was the first statin to be marketed (Dewick 2009; Keller et al. 2005; Rastegari et al. 2019b).

Alkaloids are cyclic organic compounds that contain one or more nitrogen atoms and are often basic. They have usually pronounced effects on the nervous system of humans and other animals. Alkaloids are commonly produced from amino acids such as ornithine, lysine, tryptophan, and tyrosine but other building blocks, for example, terpenes or acetate pathway derived moieties, are also often incorporated into structures of alkaloids. Ergolines are a group of alkaloids containing the indole ring system and have been found in several fungal genera, for example, *Claviceps*. These fungi are responsible for a fungal disease called ergot that affects cultivated grass such as wheat and rye and can be poisonous for humans and animals upon feeding. The fourth group is carbohydrates; their biosynthesis and degradation are essential for all organisms and are typical components of the primary metabolism. However, some of these carbohydrates, for example, glucose, are attached to secondary metabolites in glycosides. The noncarbohydrate moiety of such a compound is known as the aglycone and may originate from one or more biosynthetic pathways, for example, the shikimic acid pathway and the acetate pathway. Fusicoccin A, a fungal secondary metabolite from *Fusicoccum amygdali*, is an example of a glucopyranoside of a diterpene that has been shown to have growth-inhibiting effects on the deadly brain tumor; glioblastoma multiforme (Bury et al. 2013).

Concerning fatty acids, it was reported that they are biosynthesized via the acetate pathway in a similar way to that of the polyketides, although different enzymes

are working. Fatty acids may also, as carbohydrates, be attached to other metabolites. Finally, peptides and proteins consist of amino acids that are linked to form chains with different lengths; the length determines whether the product will be referred to as a peptide (up to 40–50 amino acids) or protein (generally more than 40–50 amino acids). Peptides and proteins, as a group, are difficult to classify as primary or secondary metabolites since many of them are large in size and found in materials that are widely distributed, and occur in many different organisms while others are small and restricted in occurrence. Peptides and proteins are produced by either ribosomal (produced on the ribosome) or non-ribosomal biosynthesis. Non-ribosomal peptides are synthesized by non-ribosomal peptide synthases (NRPSs) and are, in contrast to ribosomal synthesis, not dependent on mRNA (Dewick 2009; Keller et al. 2005).

Fungal secondary metabolites were shown to have multifaceted activities; on the one hand, some researchers found that some fungal metabolites are excellent antimicrobial candidates (Palanichamy et al. 2018; Orfali and Perveen 2019a, b), others proved their cytotoxic and antioxidant activities (Kiran and Mohan 2018; Abdel-Wareth et al. 2019a, b; Abdel-Azeem et al. 2021). On the other hand, some fungal metabolites like pigments have many industrial applications in textiles (Chadni et al. 2017; Morales-Oyervides et al. 2017), food (Caro et al. 2017; Kim and Ku 2018), and cosmetics (Rao et al. 2017; Sajid and Akber 2018), while some fungi and their metabolites have already been formulated and commercialized as biopesticides (Francardi et al. 2016). Industrial and pharmaceutical applications of fungal secondary metabolites, besides the potential biological control agents produced by fungi, will be discussed in this chapter.

8.2 Industrial Applications

8.2.1 Pigment Production

Since their discovery in the nineteenth century, pigments, especially synthetic ones, have occupied the entire market due to their wide range of applications in different industries. Different characteristics such as low production costs, ease of production, and superior coloring properties have largely contributed to the establishment of synthetic pigments in the market. However, the use of synthetic colors has been found to be detrimental to human health and the environment because of their disadvantages, such as poor degradation, longer persistence, the potential to cause cancers/allergies, and so on (Downham and Collins 2000; Osman et al. 2004; Babitha 2009; Samanta and Agarwal 2009; Ratna 2012). This has increased the demand for natural, organic, and eco-friendly pigments in the current era.

Synthetic pigments are used as colorants, color intensifiers, additives, antioxidants, and so on, in many aspects including the textile, pharmaceutical, cosmetic, painting, food, and beverage industries (Rao et al. 2017; Akilandeswari and Pradeep

2016; Yadav et al. 2019). In recent years, fungi have emerged among the prominent, eco-friendly sources of natural pigments. Fungi have immense advantages over plants as pigment producers; such as season-independent pigment production, easy and fast growth in a cheap culture medium, production of pigments with different color shades, and of more stable, soluble pigments, and easy processing (Joshi et al. 2003; Manikprabhu and Lingappa 2013).

Fungi belonging to the Monascaceae, Trichocomaceae, Nectriaceae, Hypocreaceae, Pleosporaceae, Cordycipitaceae, Xylariaceae, Chaetomiaceae, Sordariaceae, Chlorociboriaceae, Hyaloscyphaceae, Hymenochaetaceae, Polyporaceae, Ophiostomataceae, Tremellaceae, Herpotrichiellaceae, and Tuberaceae families have been described as potent pigment producers (Akilandeswari and Pradeep 2016; Dufossé 2006; Ramesh et al. 2019; Caro et al. 2017; Gmoser et al. 2017; Blanchette et al. 1992; Butler and Day 1998; Sakaki et al. 2002; Carvalho et al. 2003; Feng et al. 2012; Robinson et al. 2012; Tudor 2013; Tudor et al. 2013; Robinson et al. 2014; Hinsch et al. 2015; Tam et al. 2015; Hernandez et al. 2016; Robinson et al. 2016; Souza et al. 2016; Palomino et al. 2017; Avalos et al. 2017; Pombeiro-Sponchiado et al. 2017; Vega Gutierrez and Robinson 2017). These fungi are known to synthesize a variety of pigments as secondary metabolites. They are prolific producers of pigments belonging to several chemical classes, such as carotenoids, melanins, azaphilones, flavins, phenazines, quinones, monascin, violacein, indigo, and so on (Kirti et al. 2014; Caro et al. 2017; Gmoser et al. 2017; Mortensen 2006; Nagia and El-Mohamedy 2007; Mapari et al. 2010; Dufossé et al. 2014). The use of *Monascus* pigments for the production of red mold rice (ang-kak) is the oldest recorded use of fungal pigments by humans. Certain species of *Monascus*, viz., *Monascus ruber*, and *Monascus purpureus*, have been reported to be good potential producers of pigments worldwide. Many other fungal genera were recorded as pigment producers such as *Fusarium* spp. (Mapari et al. 2009; Zheng et al. 2017), *Trichoderma* spp. (Caro et al. 2017; Heo et al. 2018), *Hypoxylon* spp. (Caro et al. 2017), *Alternaria* spp. (Devi et al. 2014; Caro et al. 2017), *Penicillium* spp. (Chintapenta et al. 2014; Pandey et al. 2018), *Aspergillus* spp. (Mapari et al. 2009; Caro et al. 2017). and *Talaromyces* spp. (Frisvad et al. 2013; Caro et al. 2017).

8.2.1.1 Applications and Biological Activities of Fungal Pigments

Many fungal pigments have been reported to have a variety of biological applications because of their different properties such as antimicrobial, antioxidant, and anticancer activities, in addition to coloring properties (Rao et al. 2017; Ramesh et al. 2019; Caro et al. 2017; Sen et al. 2019), however, the degree of purity of pigments investigated in the various studies is not always known.

8.2.1.1.1 Food Colorants

The majority of work done on fungal pigments is related to their use as food colorants. The possibility of the use of fungal pigments in different industries, particularly in the food industry as food colorants or additives have been revealed long ago by many researchers (Fabre et al. 1993; Fink-Gremmels and Leistner 1989; Chattopadhyay et al. 2008; Caro et al. 2017; Mortensen 2006; Mapari et al. 2006, 2010; Sen et al. 2019; Dufossé et al. 2005; Simpson et al. 2012). Some of the fungal pigments that have already entered the market as food colorants are *Monascus* pigments, arpink red from *Penicillium oxalicum*, riboflavin from *Ashbya gossypii*, and β-carotene from *Blakeslea trispora* (Dufossé 2006; Caro et al. 2017; Kim and Ku 2018).

8.2.1.1.2 Cosmetic Industry

As the demand for natural products is increasing in the market, cosmetic industries are also in search of new types of natural pigments to replace synthetic ones. So, the use of fungal pigments is also rapidly expanding in cosmetics because of their advantages. Fungal pigments, especially melanin, carotenoids, and lycopene have been reported for their application in cosmetics, sunscreens, sun lotions, sunblocks, face creams, and anti-aging facials (Rao et al. 2017; Hill 1992; Sajid and Akber 2018). Excitingly, some of the fungal pigments (*Monascus* pigments and *Monascus*-like pigments) have already entered the market for their application in cosmetics such as lipsticks, skin conditioning, and skin care products, and so on (Caro et al. 2017).

8.2.1.1.3 Textile Industry

The textile industry is the largest industry after agriculture in terms of economic contribution and employment generation. It mainly depends on synthetic dyes for dyeing different types of fabrics (cotton, silk, and wool). Currently, natural pigments from fungi, with their many advantages over hazardous synthetic pigments, such as being eco-friendly, nontoxic, easily degradable, besides having high colorfastness and high staining capability, have proven their suitability to replace synthetic dyes. Many investigations have shown that organic pigments produced by fungi have extensive applications in the textile industry (Rao et al. 2017; Samanta and Agarwal 2009; Akilandeswari and Pradeep 2016; Kumar et al. 2015; Caro et al. 2017; Sajid and Akber 2018).

The literature reveals that only a handful of studies have investigated the application of fungal pigments in the textile industry, especially for dyeing different types of fabrics, such as cotton, silk, and wool. The dyeing potential of pigments of different species of fungal genera (*Monascus*, *Fusarium*, *Aspergillus*, *Penicillium*, *Talaromyces*, *Trichoderma*, *Alternaria*, *Curvularia*, *Chlorociboria*, *Scytalidium*,

Cordyceps, *Acrostalagmus*, *Bisporomyces*, *Cunninghamella*, *Thermomyces*, and *Phymatotrichum*) for different types of fabrics such as wool, cotton yarn, silk, polyester, and nylon have been reported (Hinsch et al. 2015; Palomino et al. 2017; Nagia and El-Mohamedy 2007; Chadni et al. 2017; Morales-Oyervides et al. 2017; Gupta et al. 2013; Poorniammal et al. 2013; Devi and Karuppan 2015; Velmurugan et al. 2010; Mabrouk et al. 2011; Sharma et al. 2012; Aishwarya 2014). Some studies investigated the dyeing potential of pigments from wood attacking fungi such as red pigment from *Scytalidium cuboideum*, a yellow pigment from *Scytalidium ganodermophthorum*, and green pigment from *Chlorociboria aeruginascens*.

This has shown the possible use of these pigments for dyeing bleached cotton, spun polyacrylic, spun polyamide (nylon 6.6), worsted wool, spun polyester (Dacron 54), and garment fabrics due to their high stability and good colorfastness to washing (Hinsch et al. 2015; Weber et al. 2014). Palomino et al. (2017) found that natural oils cannot be used in combination with these fungal pigments, as these fungal pigments are unstable in natural oils. All in all, most studies have shown that fungal pigments have good color stability and colorfastness properties. Moreover, these fungal pigments do not have any adverse effects on fabrics and are non-toxic to human skin. Therefore, the potential of application of fungal pigments has the opportunity to increase in the textile and clothing industry.

8.2.1.1.4 Dyeing Woods or as Color Modifiers

Pigments produced by wood-decaying fungi such as *Trichoderma versicolor*, *Xylaria polymorpha*, *Inonotus hispidus*, *Scytalidium cuboideum*, *Bjerkandera adusta*, *Chlorociboria aeruginascens*, and *Arthrographis cuboidea* have been used for dyeing different types of wood samples to increase their commercial importance (Robinson 2012, 2014; Robinson et al. 2012). Researchers have successfully used the red, green, and yellow pigments obtained from *Scytalidium cuboideum*, *Scytalidium ganodermophthorum*, and *Chlorociboria aeruginascens*, respectively, to attenuate the presence of blue stain on wood samples of *Pinus* spp. (Hernandez et al. 2016).

8.2.1.1.5 Optoelectronics

Recent studies of the optoelectronic properties of xylindein pigment extracted from *Chlorociboria aeruginascens* have demonstrated that this pigment has high photostability and electron mobility in amorphous films, which suggests its possible use for the development of sustainable organic semiconductor materials (Giesbers et al. 2018, 2019).

8.2.1.1.6 Antimicrobial Pigments

Numerous microbial pigments have been reported to possess many health benefits over synthetic pigments (Akilandeswari and Pradeep 2016; Nagpal et al. 2011). Several studies have proven that the pigments or pigment extracts of certain species of fungal genera (*Monascus*, *Fusarium*, *Talaromyces*, *Trichoderma*, *Penicillium*, and *Aspergillus*) and yeast *Rhodotorula glutinis* possess antimicrobial activity against different pathogenic bacteria as well as yeast and fungi.

All these studies suggest the potential use of bioactive pigments as food preservatives or as antibacterial ingredients in the food and pharmaceutical industries (Sarkar et al. 2017; Frandsen et al. 2016; Sibero et al. 2016; Wang et al. 2018; Patil et al. 2015; Seyedin et al. 2015; Kim and Ku 2018; Martinkova et al. 1995; Vendruscolo et al. 2014; Manon Mani et al. 2015; Saravanan and Radhakrishnan 2016; Yolmeh et al. 2016). Also, it was reported that pigments of *Alternaria alternata* and *Thermomyces* spp. had antimicrobial potential against specific pathogenic bacteria of different types of fabrics. The promising results of the studies on fungal pigments suggest their possible use in producing specific products for medical applications, such as bandages, suture threads, face masks, and so on (Poorniammal et al. 2013; Devi and Karuppan 2015; Prathiban et al. 2016).

8.2.1.1.7 Antioxidant Pigments

It has been reported that microbial pigments such as carotenoids, violacein, and naphthoquinones have antioxidant potential. Many studies have mentioned the antioxidant potential of pigments from certain fungi such as *Penicillium* (*P. miczynskii*, *P. purpureogenum*, and *P. purpuroscens*), *Fusarium* sp., *Thermomyces* sp., *Chaetomium* sp., *Sanghuangporus baumii*, *Stemphylium lycopersici*, and *Trichoderma afroharzianum* (Rao et al. 2017; Mata-Gómez et al. 2014; Ramesh et al. 2019; Sen et al. 2019; Tuli et al. 2015; Vendruscolo et al. 2016). Many researchers have demonstrated their possible application in the healthcare industry (Heo et al. 2018; Dhale and Vijay-Raj 2009; Manon Mani et al. 2015; Li et al. 2017; Poorniammal et al. 2019a).

8.2.1.1.8 Cytotoxic Pigments

The cytotoxic activity of pigments of certain fungal isolates (*Fusarium oxysporum*, *Talaromyces verruculosus*, and *Chaetomium* spp.) has been assessed by many researchers using different methods such as sour orange seeds toxicity assay or yeast toxicity test (YTT) using *Saccharomyces cerevisiae*, brine shrimp lethality bioassay, or cell counting kit-8 (CCK-8) assay. These studies found that these pigments could be applied in different industries, especially in health and pharmaceutical ones (Nagia and El-Mohamedy 2007; Wang et al. 2018; Chadni et al. 2017; Malik et al. 2016). Recently, Poorniammal et al. (2019b) evaluated the dermal

toxicity of pigments of *Thermomyces* spp. and *Penicillium purpurogenum* in Wistar rats and reported the nontoxic nature of such pigments suggesting their potential application in cosmetics and dyeing.

8.2.1.1.9 Anticancer Pigments

Several studies have shown that fungal pigments can be considered as potential anticancer drugs. It was found that pigments of *Monascus* species (*M. purpureus* and *M. pilosus*) such as monascin, ankaflavin, monaphilone A–B, monasphilone A–B, monapilol A–D, and monapurone A–C have been proven to possess anticancer potential against different types of cancers; such as mouse skin carcinoma, human laryngeal carcinoma, human colon adenocarcinoma, human hepatocellular carcinoma, and pulmonary adenocarcinoma (Fig. 8.1) (Feng et al. 2012; Hsu et al. 2010; Li et al. 2010; Hsu et al. 2011; Akihisa et al. 2005; Su et al. 2005).

Moreover, pigments from other fungi such as norsolorinic acid from *Aspergillus nidulans*, shiraiarin from *Shiraia bambusicola*, alterporriol K, alterporriol L, and alterporriol M from *Alternaria* spp., benzoquinone from *Fusarium* spp., and an uncharacterized red pigment from *Fusarium chlamydosporum* have also been reported to have anticancer or antiproliferative activity mainly against human breast cancer cell lines (MCF-7, MDA-MB-435, and MCF-7 b), whereas hypocrellin D from *Skeletocutis bambusicola* showed anticancer activity against other cancer cell lines such as hepatocellular carcinoma cell line (Bel-7721) and lung adenocarcinoma cell lines (A-549 and Anip-973) (Soumya et al. 2018; Zheng et al. 2017; Fang et al. 2006; Cai et al. 2008; Wang et al. 2008).

8.2.2 Organic Acids Production

Organic acids are produced abundantly in several genera of fungi (Magnuson and Lasure 2004; Kour et al. 2020). The reason for the production of organic acids by fungi is related to the local ecological niche as a competitive advantage over other microbes, especially low-pH-susceptible fungal strains. It has also been proposed that acidity increase in surrounding soil helps ectomycorrhizal fungi to solubilize soil minerals for better uptake by the host plants (Dutton and Evans 1996; Jones 1998; Plassard and Fransson 2009; Liaud et al. 2014). In the context of commercialization, the production of organic acids using fungi has evolved to be an efficient way to mass-produce organic acids for industries.

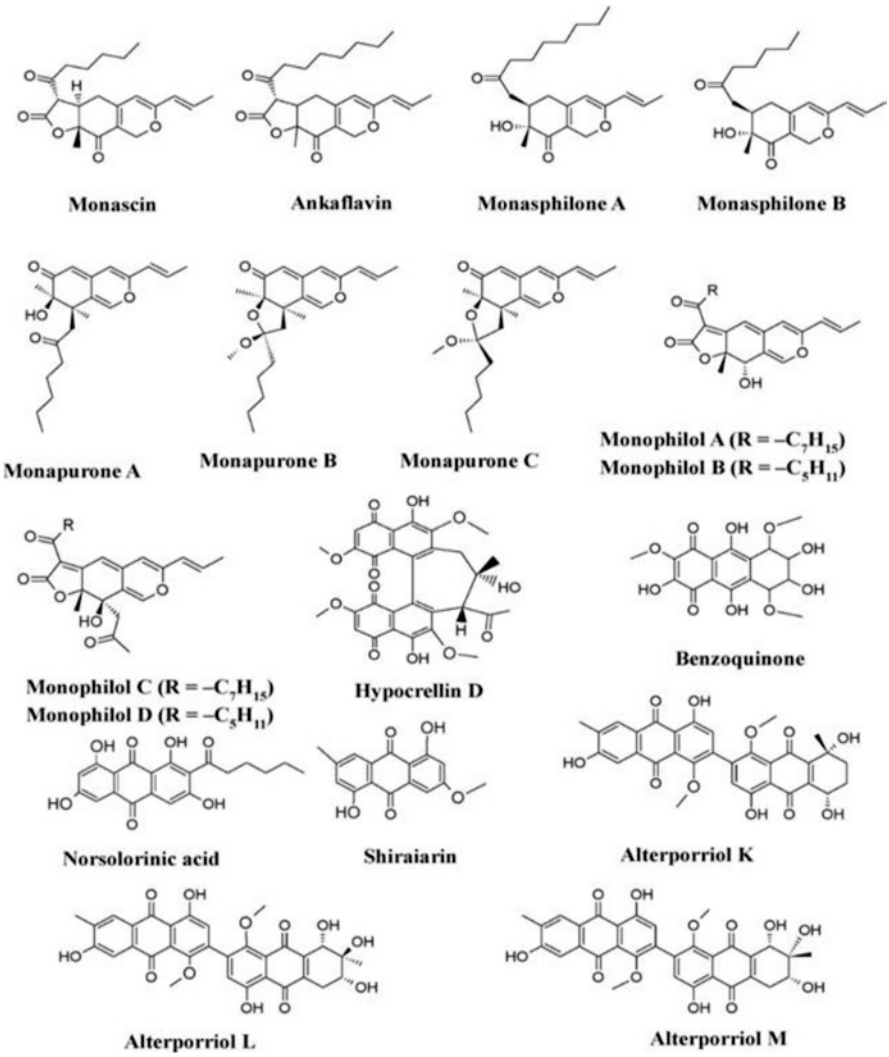


Fig. 8.1 Pigments from different taxonomic groups of fungi having promising anticancer or antitumor potential. (Re-drawn from Feng et al. (2012), Hsu et al. (2010), Li et al. (2010), Hsu et al. (2011), Soumya et al. (2018), Zheng et al. (2017), Fang et al. (2006), Cai et al. (2008), Wang et al. (2008), Akihisa et al. (2005), Su et al. (2005))

8.2.2.1 Citric Acid

Citric acid is the most important organic acid that could be harvested commercially via fungal biotechnology, and it carries the largest market demand compared to other organic acids (Lopez-Garcia 2002). One of the best known fungal producers of citric acid is *Aspergillus niger*, so that many biotechnology plants have

considered specific *A. niger* strains as standard bioprocessing agents for efficient citric acid production (Magnuson and Lasure 2004). The large market demand for citric acid can be attributed to its significance in food and beverage mass production due to its low toxicity and palatable taste. More than 70% of citric acid from global industrial output is used in food industries as acidifiers as well as food preservatives with the remaining 20–30% going into pharmaceutical pipelines (Max et al. 2010).

8.2.2.2 Succinic Acid

Succinic acid has been regarded as one of the top chemical building blocks that can be produced from sugars via biological processes (Werpy and Petersen 2004). Succinic acid is used as a precursor to produce chemicals ranging from plastic materials such as polybutylene succinate (PBS) to commodity chemicals such as 1,4-butanediol (1,4-BD) (Jansen and van Gulik 2014). Several fungi used in the industry as succinic acid producers include *Fusarium* spp., *Aspergillus* spp., *Saccharomyces cerevisiae*, *Candida krusei*, and *Penicillium simplicissimum*. Companies that employ fungal biotechnology to produce succinic acid are DSM/Roquette © (Joint Venture Reverdia), in which they developed a high succinic acid-producing *Saccharomyces cerevisiae* strain. In addition, in Bioamber/Mitsui © company, they used high succinic acid-producing strains of *Candida krusei* (Jansen and van Gulik 2014). Succinic acid is an intermediate in the tricarboxylic acid cycle (TCA) as well as in the glyoxylate cycle (Kornberg and Krebs 1957) making it feasible to be obtained from microorganisms (Beauprez et al. 2010). Furthermore, certain fungi such as *Penicillium simplicissimum* have been observed to overproduce succinic acid under certain anaerobic conditions (Beauprez et al. 2010).

8.2.2.3 Polyunsaturated Fatty Acids (PUFAs)

They are essential to humans; PUFAs such as docosahexaenoic acid (DHA) are needed for brain development and maintenance especially in infants, DHA also covers 60% of total fatty acids in the retina (Warude et al. 2006). Due to the increasing demands of PUFAs, various approaches have been applied for large quantity production including biotechnology. PUFA synthesis pathway in fungi involves two types of enzymes; desaturases and elongases (Warude et al. 2006). In fungi, *Mortierella* spp. have been found to produce PUFAs such as arachidonic acid and eicosapentaenoic acid (Lee et al. 2016). The arachidonic acid biosynthesis pathway in *Mortierella alpine* has been described, where two associated desaturase genes have been identified. Moreover, the mutant strain with a low activity of n3-desaturation produces a higher level of arachidonic acid (ARA) and a lower level of eicosapentaenoic acid (EPA) (Sakuradani et al. 2004). Arachidonic acid is a polyunsaturated omega-6 fatty acid, which is as equally important as DHA for brain development in infants (Bazinet and Laye 2014).

8.2.3 Other Useful Metabolites and Bioactive Molecules

The beneficial metabolites mentioned above are just the tip of the iceberg. Several other bioactive molecules that hold industrial significance and deserve to be briefly reviewed include carotenoids, terpenoides, and riboflavin.

8.2.3.1 Carotenoids

They are a class of natural lipid-soluble pigments that occur pervasively in photosynthetic systems, which include plant kingdom, algae, and photosynthetic microbes and fungi (Johnson and Schroeder 1995; Mata-Gómez et al. 2014). They act as vitamin A precursors (provitamin), as well as potent antioxidants. Some carotenoids, for instance, beta-carotene, are molecular precursors to vitamin A (Mata-Gómez et al. 2014; Britton et al. 1995) which renders them important for human health, and thus an industrially valuable nutritional product. One important carotenoid that is being optimized for production by fungal producers is lycopene, a carotenoid molecule that is well-known for its antioxidant activity, found generally in tomatoes. Yet tomatoes are an undesirable and inefficient source for industrial lycopene production due to their dependence on fruiting seasons and their low yield, so lycopene fungal producers, specifically of the genera *Phycomyces* and *Blakeslea*, are attractive fungal alternatives (Feofilova et al. 2006).

8.2.3.2 Terpenoids

It is known that some terpenoids made by fungi are mycotoxins, such as botrydial (from *Botrytis cinerea*), but some act as hormones, for example, gibberellic acid (GA3) (Avalos and Limón 2015). This phytohormone is one of the most industrially significant fungal terpenoids, as it acts as a plant growth hormone. It was first isolated from *Gibberella fujikuroi* (Kurosawa 1926). The biotechnologists became interested in the industrialization of GA3 from *Fusarium moniliforme* on a high-output solid-state fermentation (SSF) platform, due to its agro-industrial cost-effectiveness and better yield as compared to culturing on the submerged fermentation platform, besides the fact that the fungus generally produces a higher amount of GA3 as compared to plants (Silva et al. 2013). So, GA3 as a fungal terpenoid has become a commercially lucrative and economically demanding product in agricultural trade, specifically fruit harvest (Rodrigues et al. 2012).

8.2.3.3 Riboflavin

Another group of natural fungal metabolites that should not be ignored is the diverse heterogeneous group of compounds called vitamins, which remain one of the most important micronutrients in the human diet. Yet, most vitamins such as vitamin A (carotenoids), vitamin B1 (thiamine), and vitamin B3 (niacin) are still produced chemically as opposed to via biotechnology (Gavrilescu and Chisti 2005). However, several other vitamins, notably vitamin C, vitamin B2 (riboflavin), and vitamin B12 (cobalamin) have been tried to be produced through biotechnology and showed promising preliminary results (Zu Berstenhorst et al. 2009; Xia et al. 2015).

Riboflavin production is an interesting biosynthetic pathway that is currently being studied within several natural fungal overproducers, which include *Ashbya gossypii* and *Candida famata*. Riboflavin or vitamin B2 is needed as a micronutrient in human daily consumption due to its importance in skin and muscle maintenance, immune system boost, in addition to cell growth and division (Zu Berstenhorst et al. 2009). Leading companies using fungi to produce riboflavin are BASF ©, using *Ashbya gossypii* (Xia et al. 2015) and ADM Co., using *Candida famata*, although ADM Co. has terminated its use of *Candida famata* around 2006 due to low stability of the mutant strain (Dmytruk and Sibirny 2012).

Before 1996, riboflavin was conventionally synthesized using D-ribose reacting with 3,4-xylidine in methanol. It produced a lot of waste and the production was only 60% of maximum yield (Fischer and Bacher 2008; Stahmann et al. 2000). By using *Ashbya gossypii* to produce riboflavin on large scale, the eight steps applied in the conventional chemical synthetic process have been reduced to only one fermentation step. This contributed to more than 40% cost reduction. The observation on key environmental impact carried out by both BASF and Oeko Institutes showed that CO_2 emission has been lessened by 30%, besides a 60% reduction on consumable resources such as organic solvents, leading to a great reduction (95%) of wastes in vitamin B2 production (Sijbesma and Schepens 2003). Moreover, the market shares of riboflavin production through biotechnology had risen from 5% in 1990 to 75% in 2002 (Zu Berstenhorst et al. 2009).

8.2.4 Biosurfactants

Surfactants are among the most versatile materials in the chemical and process industry. Its amphiphilic nature, containing both hydrophilic and lipophilic functional groups in one molecule, plays an important role in numerous chemical applications (dispersion systems, as emulsions and colloids, personal hygiene products, detergents, fabric softeners, and food processing materials) (Raza et al. 2014; Banat et al. 2010). Researchers have demonstrated the advantages of biosurfactants (surfactants of biological origin) when compared with chemically produced surfactants. These advantages include lower toxicity, higher biodegradability, and possible biological activities (Banat et al. 2010; Pacwa-Płociniczak et al. 2011).

Biosurfactants properties are similar to those of synthetic surfactants; they have industrial applications in relation to detergency, emulsification, lubrication, and solubilization (Banat et al. 2010; Pacwa-Płociniczak et al. 2011; Das et al. 2008; Nitschke and Pastore 2002), and other interesting applications in catalysis, biosensing, and electronics using microstructures (Rehman et al. 2010).

Biosurfactants are mainly produced by bacteria and yeast, but, in recent years, studies have highlighted their production by filamentous fungi as well (Castiglioni et al. 2009; Velioglu and Urek 2015). The production of biosurfactants by microorganisms is influenced by several factors, including the nature of the carbon source and the concentrations of nutrients such as nitrogen, phosphorus, magnesium, iron, sulfur, and manganese, as well as the pH, temperature, agitation, and available oxygen (Banat et al. 2010; Fontes et al. 2008; Pirôllo 2006). These factors can make the production of biosurfactants more expensive than that of synthetic ones (Thavasi et al. 2007). So, several studies have been performed to make the price of the bioprocess more competitive (Pattanathu et al. 2008).

Although the field of production of biosurfactants by bacterial species is well explored, relatively fewer fungi are known to produce biosurfactants such as *Candida bombicola* (Pirôllo 2006; Thavasi et al. 2007; Pattanathu et al. 2008; Lacaz et al. 2001; Castiglioni et al. 2009; Velioglu and Urek 2015), *Candida lipolytica* (Barnett and Hunter 1998; Accorsini et al. 2012), *Candida ishiwadae* (Bodour and Miller-Maier 1998), *Candida batistae* (Cameron et al. 1988), *Ustilago maydis* (Luna-Velasco et al. 2007), and *Trichosporon ashii* (Camargo-de-Morais et al. 2003). Many of these are known to produce biosurfactants on low-cost raw materials.

The major type of biosurfactants produced by these strains is sophorolipids (glycolipids). Among soil-inhabiting fungi, the genera that showed a strong ability to emulsify toluene were *Penicillium*, *Trichoderma*, and *Fusarium*. The potential of *Penicillium* sp. to produce biosurfactants has already been demonstrated (Luna-Velasco et al. 2007; Camargo-de-Morais et al. 2003), but few studies have described the biosurfactant potentials of *Trichoderma* and *Fusarium* (Askolin et al. 2001). In addition, Lima et al. (2016) demonstrated that among eight analyzed fungi, only *Phoma* sp. (S31) was efficient as a biosurfactant producer. Moreover, Sena et al. (2018) reported that amongst 100 fungal cultures obtained from soil samples of the Amazon Forest, 61 strains produced biosurfactants, with *Penicillium* 8CC2 strain showing the highest emulsification index.

8.2.4.1 Applications of Biosurfactants

Biosurfactants have numerous applications in the bioremediation processes, food industries, cosmetic industries, and biomedical fields. The reported applications of the fungal biosurfactants are as follows:

8.2.4.1.1 Microbial Enhanced Oil Recovery and Cleaning of Oil Tanks

The sophorolipids from *Candida lipolytica* and *Candida bombicola* are very promising in the cleaning of oil tanks, decontamination of polluted areas, microbial enhanced oil recovery, industrial cleaning, low-end consumer products, and household applications (Rufino et al. 2007; Felse et al. 2007). Biosurfactants from *Torulopsis bombicola* and *Aspergillus ustus* MSF3 were used for the release of bitumen from the contaminated soil, and for the degradation of hydrocarbons (Cooper and Paddock 1984; Kiran et al. 2009). Mannosylerythritol lipids from *Candida antarctica* have potential applications in the removal and biodegradation of hydrocarbons in oil-contaminated soil (Kitamoto et al. 2001; Chaplin 1986).

8.2.4.1.2 Food and Oil Industry

Biosurfactants are able to stabilize various types of emulsions, so they are valuable in the food industry. Biosurfactants from *Candida lipolytica* and *Saccharomyces cerevisiae* are good candidates for food and oil industries applications (Sarubbo et al. 2007). For example, the bioemulsifier liposan from *Candida lipolytica* was able to stabilize the emulsions of vegetative oils and water. It was also able to stabilize the cottonseed oil, corn oil, soybean oil, and peanut oil emulsions (Cirigliano and Carman 1985; Cooper and Paddock 1983; Konishi et al. 2007; Adamczak and Bednarski 2000).

8.2.4.1.3 Biomedical Field

Biosurfactants are extensively useful in the biomedical fields, such as the biosurfactant from *Aspergillus ustus* MSF3 strain, which was reported to have significant antimicrobial activity against *Candida albicans* and gram-negative bacteria (Kiran et al. 2009).

8.2.4.1.4 Cosmetic Industry

Biosurfactants are used in the cosmetic industry due to their skin-friendly properties. For example, sophorolipids from the mutant strain *Candida bomobicola* ATCC 22214 have great uses in the cosmetic industries due to their anti-radical and hygroscopic properties, besides their ability to stimulate fibroblast metabolism to support healthy skin physiology (Williams 2009).

8.3 Pharmaceutical Applications

Natural products have been playing a vital role in the area of drug discovery. In the last 75 years, out of 175 new entities were labeled as anti-cancer, with 49% isolated from natural sources (Newman and Cragg 2016). From approximately 0.5 million secondary metabolites (natural products) that have been described to date, about 14% (70,000) were of microbial origin. Moreover, of about 33,500 bioactive microbial natural compounds, 47% were of fungal origin (Bills and Gloer 2016). Fungal secondary metabolites showed great potential as antiviral, antimicrobial, anticancer, and anti-inflammatory agents (Villa and Gerwick 2010).

Moreover, fungal secondary metabolites have contributed immensely to the drug discovery process by providing many novel drugs, including β-lactam antibiotics (penicillin G), cholesterol-lowering agents (lovastatin), and immunosuppressants (fingolimod) (Vagelos 1991; Strader et al. 2011; Kour et al. 2019). From an estimated 5.1 million species of fungi on earth (Blackwell 2011), only about 99,000 species have been described (Blackwell 2011), and a smaller fraction of these was explored for bioactive secondary metabolites (Kinghorn et al. 2016).

8.3.1 Antimicrobial Agents

Many researchers have investigated the antimicrobial effects of secondary metabolites produced by fungal species isolated from different ecological niches such as soil, freshwater, marine organisms, invertebrate tissues, and plant tissues.

8.3.1.1 From Endophytes

Endophytic fungi have a pronounced contribution as producers of bioactive compounds nearly identical to those produced by their harboring plants, among these bioactive compounds, antimicrobial metabolites have a great share. Sadrati et al. (2013) evaluated the antimicrobial activity of crude ethyl acetate of endophytic fungi isolated from wheat against 12 pathogenic bacteria, yeast, and 2 phytopathogenic fungi. All extracts showed inhibitory activity on at least one or more pathogenic microorganisms, where *Penicillium* sp. inhibited the growth of two phytopathogenic fungi; *Phytophthora infestans*, and *Fusarium oxysporum* f. sp. *albedinis*. Furthermore, *Aspergillus* sp., *Chaetomium* sp., *Penicillium* sp., and *Phoma* sp. were effective against *Escherichia coli*, while *Alternaria* sp. was the most effective on *Candida albicans*. Also, Shaaban et al. (2013) investigated the antimicrobial activity of the crude extract of endophytic fungus *Aspergillus fumigatus* against 11 microbial species. The strain extract showed high antibacterial activity against the Gram-positive *Bacillus subtilis* and *Streptomyces viridochromogenes*, besides *Staphylococcus aureus* and *Candida albicans*.

In another study, a total of 44 fungal endophytes were recovered from 400 leaflet segments of the *Prosopis juliflora* plant. Species of *Cladosporium*, *Colletotrichum*, and *Fusarium* were the most dominant. Seventeen endophytic fungi out of 44 showed significant antibacterial activity against test bacteria. Ethyl acetate extract of *Colletotrichum gloeosporioides* exhibited highly significant broad-spectrum antibacterial activity against each of *Xanthomonas campestris* pv. *vesicatoria*, *Xanthomonas oryzae*, *Pseudomonas syringae*, and *Ralstonia solanacearum* isolated from diseased plant material (Srivastava and Anandrao 2015).

The authors also demonstrated that this extract was effective on human pathogenic bacteria; *Bacillus subtilis*, *Bacillus cereus*, and *Escherichia coli*. On the other hand, 11 endophytes showed antifungal activity against all tested fungi (*Fusarium solani*, *Fusarium verticillioides*, *Aspergillus flavus*, and *Aspergillus ochraceus*). Moreover, they found that *Paecilomyces lilacinus* and *Trichoderma* sp. showed significant antifungal activity in dual culture, in addition, ethyl acetate extract of *Paecilomyces lilacinus* showed significant antifungal activity in the disc diffusion test. The preliminary chemical characterization of the active extract of *Colletotrichum gloeosporioides* and *Paecilomyces lilacinus* showed the presence of alkaloids, carbohydrates, sterols, and coumarins.

Sugijanto and Dorra (2016) found that ethyl acetate extracts of *Cladosporium oxysporum* isolated from *the Aglaia odorata* plant showed antimicrobial activity against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*. Moreover, Pinheiro et al. (2017) isolated 17 endophytic fungi from *Bauhinia guianensis*, a typical Amazonian plant used in combating infections, where the methanolic extract of the fungus *Exserohilum rostratum* showed good activity against *E. coli*, *Pseudomonas aureginosa*, and *Bacillus subtilis*. They reported that the polyketide monocerin was responsible for the broad antimicrobial spectrum.

Amongst 44 endophytes isolated from *Zingiber cassumunar*, Pansanit and Pripdeevech (2018) demonstrated that the ethyl acetate extract of the *Arthrinium* sp. showed activity against both Gram-positive and Gram-negative bacteria. Specifically *Staphylococcus aureus* and *E. coli*. Gas chromatography-mass spectrometry analysis revealed that the extract of the *Arthrinium* sp. contains various antibacterial compounds which are β-cyclocitral, 3*E*-cembrene A, laurenan-2-one, sclareol, 2*Z*,6*E*-farnesol, cembrene, β-isocomene, and γ-curcumene. In the same vein, a total of 42 endophytic fungi were isolated from 11 different medicinal plants collected from India, where the most frequently isolated fungi were *Alternaria* sp. and *Fusarium* sp. (Palanichamy et al. 2018). It was found that 15 ethyl acetate extracts showed antimicrobial activity towards potential human pathogens such as *E. coli*, *Salmonella typhi*, *Proteus mirabilis*, *Staphylococcus aureus*, and *Corynebacterium diphtheria*.

Furthermore, Handayani et al. (2018) declared that ethyl acetate extracts of endophytic fungi isolated from mangrove plant *Sonneratia alba* were effective against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*. Recently, the methanol extract produced by the endophytic fungus *Curvularia* sp. isolated from the medicinal plant *Rauwolfia macrophylla* was found to exhibit antimicrobial activities against *E. coli*, *Micrococcus luteus*, *Pseudomonas agarici*, and *Staphylococcus*

warner. The authors identified three bioactive compounds; 2′-deoxyribolactone, hexylitaconic acid, and ergosterol (Kaaniche et al. 2019). Also, Du et al. (2020) isolated a total of 420 endophytic fungal species from the *Securinega suffruticosa* plant grown in Shell Islands, from which 20 genera and 35 species were identified, where *Chaetomium*, *Fusarium*, *Cladosporium*, and *Ceratobasidium* were the dominant genera. *Chaetomium globosum*, *Fusarium* sp., and *Cladosporium ramotenellum* had a high antibacterial activity against *Enterococcus faecalis*, *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Table 8.1 shows some antimicrobial and antiviral compounds isolated from endophytic fungi.

8.3.1.2 From Entomopathogenic Fungi

It was reported that the cell-free culture filtrate of the entomopathogenic fungus *Paecilomyces fumosoroseus* exhibited high activity against *Escherichia coli* and good activity against *Bacillus subtilis* and *Salmonella typhi* (Gulwani et al. 2015). Dichloromethane extract of the indigenous entomopathogenic fungus *Metarhizium anisopliae* showed high antibacterial activity against each of *B. subtills*, *Poteus* sp., and *E. coli* (Ravindran et al. 2014). Fabelico (2015) attributed the antimicrobial properties of two entomopathogenic fungi to their phytochemical properties, as the significantly high antifungal activities of the *Pandora neoaphidis* against *Candida albicans* were due to the presence of sterols. Conversely, the significantly high antibacterial activity of *Beauveria alba* against *Bacillus subtilis* was suggested to be due to the presence of steroids, triterpenoids, glycosides, and fatty acids.

Moreover, Assadollahpour et al. (2011) tested the methanol extracts of the soilborne fungi *Paecilomyces variotii*, *Paecilomyces lilacinus* S1, and *Paecilomyces fumoroseus* isolated from soil samples (Iran), the nematophagous *Paecilomyces lilacinus* N1 isolated from the potato cyst nematode *Globodera rostochiensis*, and the entomopathogenic *Paecilomyes* sp.1 and *Paecilomyces* sp.2 isolated from the Colorado potato beetles *Leptinotarsa decemlineata*. All fungal spp. were capable of producing diffusible metabolites and volatile compounds with antifungal activities against *Pyricularia oryzae* and *Saccharomyces cerevisiae*. Besides, Mohammadi et al. (2016) indicated a good antibacterial activity of extracellular metabolites from *Paecilomyces* species against human pathogenic bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pyogenes* (G+) and *E. coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* (G−). Recently, Abdel-Wareth et al. (2019a) screened extracts of three entomopathogenic fungi on pathogenic bacteria and fungi, where acetone extracts of *Paecilomyces lilacinus*, and *Metarhizium anisopliae* showed the highest activities against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*.

Table 8.1 Biological activities of secondary metabolites produced by endophytic fungi

Endophytic fungus	Origin	Secondary metabolite	Importance	References
Phomopsis sp.	*Erythrina crista-galli*	Mevinic acid	Anti-inflammatory	Weber et al. (2005)
Pestalotiopsis microspora	*Terminalia morobensis*	Pestacin and isopestacin	Antioxidant	Strobel et al. (2002)
Aspergillus clavatonanicus	*Torreya mairei*	Clavatol	Antimicrobial	Leuchtman (2003)
Cytonaema sp.	*Quercus* sp.	Cytonic acid A and B	Inhibitor of human cytomegalovirus protease	Guo et al. (2000)
Aspergillus niger PN2	*Taxus baccata*	Lovastatin	Lowering blood cholesterol	Raghunath et al. (2012)
Xylaria sp. XC-16	*Toona sinensis*	Cytochalasins	Anticancer	Zhang et al. (2014a)
Fusarium subglutinans	*Tripterygium wilfordii*	Subglutinol A and B	Immunosuppressive activity	Lee et al. (1995)
Penicillium sp.	*Quercus variabilis*	Penicidones A, B and C	Cytotoxic	Ge et al. (2008)
Gliocladium roseum (NRRL 50072)	*Eucryphia cordifolia*	2,6-dimethyl, 3,3,5-trimethyl; cyclohexene, 4-methyl; decane, 3,3,6-trimethyl; and undecane, 4,4dimethyl (volatile hydrocarbons)	Biofuels	Strobel et al. (2008)
Alternaria alternata (RSF-6L)	*Solanum nigrum*	Indole acetic acid	Promote plant growth	Khan et al. (2015)
Penicillium chrysogenum	*Teucrium polium* L.	Indole acetic acid	Promote plant growth	Hassan (2017)
Trichoderma gamsii (YIM PH30019)	*Panax notoginseng*	VOCs such as dimethyl disulfide, dibenzofuran, methanethiol, ketones	Biocontrol agent	Chen et al. (2016)
Cochliobolus sp. (UFMGCB-555)	*Piptadenia adiantoides* (Fabaceae)	Cochlioquinone A and isocochlioquinone A	Leishmanicidal activity	Campos et al. (2008)
Penicillium brocae	Mangrove-derived	Spirobrocazines A–B (**57**–**58**)	Antibacterial, cytotoxicity	Meng et al. (2016)
Pestalotiopsis fici	Terrestrial	Chloropupukeananin (**72**)	Antiviral	Liu et al. (2008)

(continued)

Table 8.1 (continued)

Endophytic fungus	Origin	Secondary metabolite	Importance	References
Blennoria sp.	Terrestrial, *Carpobrotus edulis*	Blennolides A–G (**11–17**)	Antifungal, antialgal	Zhang et al. (2008a)
Cryptosporiopsis sp.	Terrestrial, *Viburnum tinus*	Viburspiran (**48**)	Antifungal	Saleem et al. (2011)
Cryptosporiopsis cf. *quercina*	Terrestrial, *Triptergyium wilfordii*	Cryptocin (**110**)	Antifungal	Li et al. (2000)
Cephalosporium acremonium	Terrestrial, *Trachelospermum jasminoides*	Cephalosol (**45**)	Antimicrobial	Zhang et al. (2008b)
Daldinia eschscholtzii	Terrestrial, *Paphiopedilum exul*	Daldionin (**47**)	Antimicrobial	Barnes et al. (2016)
Rhizoctonia solani	Terrestrial, *Cyperus rotundus*	Solanioic acid (**65**)	Antimicrobial	Ratnaweera et al. (2015)
Trichoderma spp.	Marine alga; *Codium fragile*	Harziandione (**85**) and harzianone (**86**)	Antimicrobial	Miao et al. (2012)
Paecilomyces variotii	Marine, algal-derived	Varioxepine A (**116**)	Antimicrobial	Zhang et al. (2014b)
Pestalotiopsis sp.	Marine, *Rhizophora mucronata*	Pestalotiopens A–B (**117–118**)	Antimicrobial	Hemberger et al. (2013)
Pestalotiopsis fici	Terrestrial	Chloropestolide A (**73**)	Anti-HIV, cytotoxicity	Liu et al. (2009)
Periconia sp.	Terrestrial, *Annona muricata*	Pericoannosin A (**96**)	Anti-HIV	Zhang et al. (2015)
Neosartorya udagawae	Marine, *Aricennia marina*	Neosartoryadins A–B (**59–60**)	Antiviral	Yu et al. (2016)
Periconia sp.	Terrestrial, *Annona muricata*	Periconiasins (**92–95**)	Antiviral, cytotoxicity	Zhang et al. (2016), Liu et al. (2016), Zhang et al. (2013)
Aspergillus versicolor	Terrestrial, *Polyphylla* var. *yunnanensis*	Aspergillines A–E (**125–129**)	Antiviral, cytotoxicity	Zhou et al. (2014)
Periconia sp.	Terrestrial, *Annonsa muricata*	Periconianone A (**79**)	Anti-inflammatory	Zhang et al. (2014c)
Phomopsis sp.	Terrestrial, *Isodon eriocalyx* var. *laxiflora*	Phomopchalasins A–B (**100–101**)	Antimigratory activity	Yan et al. (2016)

(continued)

Table 8.1 (continued)

Endophytic fungus	Origin	Secondary metabolite	Importance	References
Fusarium pallidoroseum	Terrestrial	Apicidins A–C (**62–64**)	Antiprotozoal, anticancer	Singh et al. (2001)
Actinoallomurus fulvus	Terrestrial, *Capsicum frutescens*	Actinoallolides A–E (**5–9**)	Anti-trypanosomal	Inahashi et al. (2015)
Aspergillus sp.	Marine, mangrove-derived	Asperterpenols A–B (**88–89**)	Acetylcholinesterase inhibition	Xiao et al. (2013)
Aspergillus sp.	Marine	Asperterpenoid A (**87**)	Antituberculosis	Huang et al. (2013)
Pestalotiopsis virgatula	Terrestrial, *Dracontomelon duperreanum*	Virgatolides A–C (**27–29**)	Cytotoxicity	Li et al. (2011)
Pestalotiopsis microspora	Terrestrial, *Torreya taxifolia*	Torreyanic acid (**36**)	Cytotoxicity	Lee et al. (1996)
Chaetomium globosum	Terrestrial, *Imperata cylindrical*	Chaetoglobins A–B (**38–39**)	Cytotoxicity	Ming Ge et al. (2008)
Alternaria sp.	Terrestrial, *Carex aridula*	(−)-Alternarlactam (**40**)	Cytotoxicity	Zhang et al. (2010)
Penicillium manginii	Terrestrial, *Panax notoginseng*	Duclauxamide A1 (**42**)	Cytotoxicity	Cao et al. (2015)
Mucor irregularis	Marine, *Rhizophora stylosa*	Rhizovarins A–C (**112–114**)	Cytotoxicity	Gao et al. (2016)
Peyronellaea coffeae arabicae	Terrestrial, *Pritchardia lowreyana*	Peyronellins A–C (**119–121**)	Cytotoxicity	Li et al. (2016)
Pestalotiopsis sp.	Terrestrial, *Taxus brevifolia*	Pestalotiopsin A (**80**)	Immunosuppressive	Pulici et al. (1996)

Sources: Gao et al. (2018) and Daba et al. (2018)

8.3.1.3 From Invertebrate-Associated Fungi

Fungi isolated from marine invertebrates are of considerable importance as new promising sources of unique secondary metabolites with significant biomedical potential. Yue et al. (2015) recovered symbiotic fungi from different tissues of jellyfish *Nemopilema nomurai*, where a total of seven morphotypes were isolated, which were assigned into four genera (*Aspergillus*, *Cladosporium*, *Purpureocillium*, and *Tilletiopsis*). Antibacterial and antifungal activities of their ethyl acetate extracts were tested against a panel of bacterial and fungal pathogens. Some extracts exhibited strong to significant antibacterial activity against the bacterial pathogens; *Staphylococcus aureus* and *Salmonella entrica*. Antifungal activity indicated that the extracts from a pure culture of *Aspergillus versicolor* and co-culture of *A.*

versicolor and *Tilletiopsis* sp. were promising, as the maximum mycelial growth inhibition was 82.32% for *Rhizoctonia solani* and 48.41% for *Botrytis cinerea* at 200μg/mL, respectively. In another study, Sibero et al. (2018) found that ethyl acetate extracts of sponge-associated fungi were effective on a number of vibrios; they demonstrated that from eight available strains, *Trichoderma asperellum* MT02 resulted in noticeable inhibition zones against *Vibrio harveyi* and *Vibrio alginolyticus*, while *Vibrio parahaemolyticus* was inhibited by *Trichoderma* sp. MT01.

Moreover, Abdel-Wareth et al. (2019b) isolated a number of fungal species from the tissues of *Biomphalaria alexandrina* snails exposed to environmental stresses, where the highest antimicrobial activities were reported from *Paecilomyces variotii* (ethyl acetate and acetone extracts), acetone extract of *Cladosporium cladosporioides* and ethyl acetate extract of *Mucor hiemalis*, as they inhibited the growth of *Staphylococcus aureus*, *Pseudomonas areuginosa*, *Candida albicans*, and *Aspergillus niger*. They identified two major compounds to be responsible for such activity; 1-(2-hydroxyphenyl)-1-(3-isopropyl-2-hydroxyphenyl) butane ($C_{19}H_{24}O_2$) which is an aromatic compound with an aliphatic side chain and 1-Acetyl-2,2,6-trimethyl-1,2,3,4-tetrahydroquinoline-3-one ($C_{14}H_{17}NO_2$) which is a quinoline derivative.

8.3.1.4 Fungi from Other Miscellaneous Sources

Aspergillus fumigatus and *A. flavus* strains isolated from the sediment of an Indian River highly contaminated with antibiotics were tested for antimicrobial activities (Svahn et al. 2012). The authors recorded a pronounced inhibitory effect on methicillin-resistant *Staphylococcus aureus*, extended-spectrum beta-lactamase-producing *Escherichia coli*, vancomycin-resistant *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Candida albicans*. On the other hand, Abdel-Hady et al. (2016) demonstrated that ethyl acetate extract of two freshwater-derived fungi; *Penicillium islandicum* and *Aspergillus tamarii* exhibited high antibacterial effect against *Escherichia coli*, they attributed that activity to two major compounds; namely 1, 2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, and 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester. Furthermore, Orfali and Perveen (2019a) isolated a thermophilic *Penicillium* species from Ghamiqa hot spring sediments in Saudi Arabia. They showed that the extract of *Penicillium* species cultured on solid rice medium yielded two new compounds 3-(furan 12-carboxylic acid)-6-(methoxycarbonyl)-4-hydroxy-4-methyl-4 and 5-dihydro-2*H*-pyran (**1**) and 3α-methyl-7-hydroxy-5-carboxylic acid methyl ester-1-indanone (**2**). In addition, three known compounds, austinol (**3**), emodin (**4**), and 2-methyl-penicinoline (**5**) were isolated. All isolated metabolites were studied for their antibiotic effect against several pathogenic bacteria, where Austinol (**3**) exhibited strong antibacterial activity against *Pseudomonas aeruginosa.*

In another study, ten soil fungal strains were selected and their ethyl acetate extracts were tested against pathogenic bacteria where the most efficient species was identified as *Chaetomium aureum* which showed remarkable inhibitory effect

against *Acinetobacter baumannii*; *Alcaligenes faecalis*, and *Pseudomonas aeruginosa* (Thamilvanan et al. 2018). In addition, Zhao et al. (2018a) carried out a fermentation study on 23 strains of Antarctic fungi to detect their bacteriostatic products on three aquatic pathogenic bacteria; subsequently, the active fungus was identified. It was indicated that the secondary metabolites of the 23 strains were distinct; of these, the extract of strain B-7 (belonging to *Bjerkandera* according to molecular identification) demonstrated a strong antibacterial activity on *Streptococcus agalactiae*, *Vibrio anguillarum*, and *Aeromonas hydrophila*.

Recently, Orfali and Perveen (2019b) carried out the first study on the metabolites produced by *Aspergillus* found in the rhizosphere of date palm trees in a temperate region. The soil-derived fungus *Aspergillus* sp. isolated from the rhizospheric soil of *Phoenix dactylifera* and cultured on the large scale solid rice medium yielded a novel compound; 1-(4-hydroxy-2,6-dimethoxy-3,5-dimethylphenyl)-2-methyl-1-butanone (**1**) and four known compounds; citricin (**2**), dihydrocitrinone (**3**), 2,3,4-trimethyl-5,7-dihydroxy-2, 3-dihydrobenzofuran (**4**), and oricinol (**5**). Compound (**1**) exhibited potent antimicrobial activities against *Staphylococcus aureus* and significant growth inhibitions of *Candida albicans* and *Candida parapsilosis*.

Other studies were carried out on identified fungal strains; Fawzy et al. (2011) tested the antimicrobial activity of the crude methanolic extracts of *Aspergillus niger* and *Aspergillus flavus* var. *columinaris* against ten species of microorganisms; *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Proteus vulgaris*, *Salmonella typhimurium*, *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*. The extracellular extracts of both fungi were effective against Gram-negative bacteria only.

They reported that the antibacterial activity of extracellular extract of *Aspergillus flavus* var. *columinaris* was higher than that of extracellular extract of *Aspergillus niger*, and *Candida albicans* was sensitive to the intracellular extract of *Aspergillus flavus* var. *columinaris*. Furthermore, Ali et al. (2011) investigated the efficacy of culture filtrates of five *Penicillium* species viz. *P. citrinum*, *P. digitatum*, *P. expansum*, *P. verrucosum*, and *P. viridicatum* against five different soil-borne bacteria namely *Salmonella gallinarum*, *Xenorhabdus luminescens*, *Xanthobacter autotrophicus*, *Acetobacter xylinum*, and *Carnobacterium mobile*. All *Penicillium* spp. showed marked antibacterial activity against all tested bacteria. They noticed that *Xenorhabdus luminescens* was the most sensitive to culture filtrates of all *Penicillium* species except *P. viridicatum.*

Swathi et al. (2013) declared that ethyl acetate extract of the marine fungus *Microascus* sp. showed an inhibitory effect on the bacterial pathogens; *Staphylococcus aureus*, *S. mutans*, *Lactobacillus casei*, *L. acidophilus*, *Enterococcus faecalis*, *Bacillus megaterium*, *Xanthomonas campestris*, and *E. coli*. Also, the extract was effective against *Candida albicans*, *Candida rugosa*, *Saccharomyces cervisciae*, and *Aspergillus niger*. By the same token, Oliveira Silva et al. (2009) evaluated the antimicrobial activity of ethyl acetate extract obtained from *Paecilomyces variotii* on eight clinical isolates of *Enterococcus faecalis*, where all clinical isolates were inhibited. Moreover, Dalinova et al. (2020) isolated a number

of compounds from the solid culture of *Alternaria sonchi*, where compounds **4**, **8**, and **9** showed antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, and *Candida tropicalis*. They added that chloromonilicin (**8**) was found to possess the highest antimicrobial activity against *B. subtilis* and *E. coli*, while 5-chloromoniliphenone (**2**) and α-, β-diversolonic esters (**13**) displayed antimicrobial activity against both *B. subtilis* and *C. tropicalis*. Finally, compounds **10**–**12** inhibited the growth of *B. subtilis.*

8.3.2 Cytotoxic Agents

Fawzy et al. (2011) found that the extracellular extracts of both *Aspergillus niger* and *Aspergillus flavus* var. *columinaris* showed more cytotoxic effect on hepatocellular carcinoma (HepG2) cell line than their intracellular extracts, as the extracellular extract of *Aspergillus niger* had the minimum IC_{50} (0.905μg/mL) followed by that of *Aspergillus flavus* var. *columinaris* (1.48μg/mL).

Kim et al. (2012) identified four new cytochalasin derivatives (**1**–**4**), together with cytochalasin B (**5**), from the fungus *Phoma* sp. obtained from the giant jellyfish *Nemopilema nomurai*. The four compounds showed significant cytotoxicity against a small panel of human tumor cell lines such as lung adenocarcinoma cell line (A549), ovarian cancer cell line (SK-OV-3), melanoma cell line (SK-MEL-2), central nervous system cancer cell line (XF 498), and colon cancer cell line (HCT15) with IC_{50} values in the range of 0.5–30μM. In addition, cytochalasin B (**5**) showed obvious cytotoxicity with IC_{50} of 7.9μM against HeLa human cervical carcinoma cells. Likewise, Yu et al. (2013) demonstrated that among 12 isolates from marine sponges, nine isolates belonging to *Aspergillus terreus* displayed strong in vitro cytotoxic activity (e.g., $IC_{50} < 50$μg/mL) against human lung carcinoma cell line (A-549), human liver carcinoma cell line (Bel-7402), human melanoma carcinoma cell line (A-375), and human normal embryo lung fibroblasts cell line (MRC-5).

In addition, cytotoxicity of selected fractions of *Penicillium verruculosum* culture filtrate containing different compounds revealed IC_{50} values ranging from 5 to 100μg/mL (Shah et al. 2014). It was significantly higher in the case of orevactaene and monascorubrine followed by pyripyropene against the KA3IT cancerous cell line. In another study, the ethyl acetate extract of an endophyte isolated from *Piper crocatum* inhibited the growth of human colon carcinoma (WiDr) and human ductal breast epithelial tumor (T47D) cell lines with IC_{50} of 120.38 and 37.43μg/mL, respectively (Astuti and Nababan 2014). Meanwhile, Saraiva et al. (2015) isolated a strain of *Aspergillus* sp. from the sediments of the northeast coast of Brazil, and the cytotoxic activity of its secondary metabolites was investigated against the human colon carcinoma (HCT-116) cell line. The cytotoxicity-guided fractionation of the extracts from this fungus yielded a number of compounds, among them, fumitremorgin C (**5**) and 12,13-dihydroxy fumitremorgin C (**6**) were the most active. Also, Ramos et al. (2015) demonstrated that ethyl acetate extracts of *Neosartorya paulistensis* and *Neosartorya siamensis* have selective cell death

activities in hepatocellular carcinoma (HepG2), colon carcinoma (HCT16), and malignant melanoma (A375) cell lines. In the same vein, Abdel-Hady et al. (2016) found that ethyl acetate extracts of both *Aspergillus tamarii* and *Penicillium islandicum* showed high cytotoxic effect on hepatocellular carcinoma (HepG2) cells.

Tenguria and Firodiya (2016) isolated a total of 496 endophytes from the leaves of Indian plants. Among them, extracts of *Asclepias curassavica* and *Alternaria alternata* showed good cytotoxic activity. They found that methanolic extracts were more cytotoxic to human breast cancer cell line (MCF-7) (IC_{50} = 31.53μg/mL) than human lung cancer cell line (A549) (IC_{50} = 55.26μg/mL). Whereas, chloroform extracts showed a significant potent cytotoxicity (IC_{50} = 15.63μg/mL) on MCF-7 cell line than on A549 cell line (IC_{50} = 19.56μg/mL). Table 8.1 shows some compounds produced by endophytes that were found to have cytotoxic activity.

Furthermore, the potential of fungi derived from the marine sponge *Neopetrosia chaliniformis* as producers of cytotoxic compounds was studied by Artasasta et al. (2017), they showed that ethyl acetate extracts of six fungal isolates were cytotoxically active against WiDr colon cancer cells with low percentages of viability. Additionally, Prabhu et al. (2018) studied the antitumor activity of greensporone C (GC), a new resorcylic acid lactone isolated from chloroform-methanol extract of a culture of a freshwater fungus; *Halenospora* sp., the compound was subjected to screening against a panel of leukemic cell lines (K562, U937, and AR320). In all three cell lines, cell proliferation was inhibited in a dose-dependent fashion. Also from the mangrove plant *Sonneratia alba*, Handayani et al. (2018) studied the cytotoxic activity of its fungal endophytes. Ethyl acetate extracts of three fungal strains were tested; *Trichoderma koningiopsis*, *Aspergillus sydowii*, and *Trichoderma lixii*, as the results revealed that 9 (69.2%) out of 13 extracts tested were cytotoxic to human ductal breast epithelial tumor (T47D) cell lines. Moreover, Kiran and Mohan (2018) reported that among the extracts of ten *Beauveria bassiana* isolates, ethyl acetate extract showed the highest cytotoxic activity on human lung carcinoma (A-549) cell lines. Moreover, the bioactive compounds of *Aspergillus aculeatus* strain have been studied (Yodsing et al. 2018). They identified ergosterol peroxide (**1**), secalonic acid D (**2**), secalonic acid F (**3**), variecolin (**4**), variecolactone (**5**), and ergosterol (**6**) claiming that compounds **1** and **4**–**6** were reported for the first time as fungal metabolites from this species. They also found that all compounds showed unprecedented anticancer activities against human epidermoid carcinoma in the mouth (KB) (compounds **1**–**6**), human breast cancer (MCF-7) (compounds **2, 4,** and **5**), and human lung cancer cells (NCI-H187) (compounds **1**–**4** and **6**).

Recently, Zhao et al. (2018b) evaluated 31 marine fungal ethyl acetate extracts against a panel of tumor cell lines including human lung carcinoma (A-549), human cervical carcinoma (HeLa), and human hepatoma (HepG2). A total of 12 fungal strains (38.7%) showed cytotoxicity with inhibition rates >50%. The fungi of the genera *Penicillium* and *Mucor* displayed the most potent cytotoxicity. By the same token, the cytotoxicity effect of crude metabolites of the endophyte *Alternaria* sp. was evaluated against cancer cell lines (Palanichamy et al. 2018), where all the tested malignant cell lines; hepatocellular carcinoma (HUH-7), lung adenocarcinoma (A549), and breast adenocarcinoma (MCF-7) cell lines showed significant

response towards the fungal extract. The authors added that the metabolites showed the strongest anti-proliferative activity against HUH-7 cell line, with a concentration of 75μg/mL. Also, Awad et al. (2018) found that the isolated proteins and carbohydrates from the soil-derived fungus *Trichoderma viride* exhibited 42.6% and 16.7% killing of the hepatocellular carcinoma (HepG2) cell line. While Orfali and Perveen (2019a) indicated that emodin 4 from the ethyl acetate extract of a thermophilic *Penicillium* species demonstrated significant cytotoxicity against the lymphoma human cancer (HTB-176) cell line with an IC_{50} value of 2μM.

Furthermore, Abdel-Wareth et al. (2019b) reported that acetone extracts of *Penicillium islandicum* and *Aspergillus niger* isolated from the tissues of *Biomphalaria alexandrina* snails were highly active against hepatocellular carcinoma (HepG2) cell lines, they found that the antitumor effect of these extracts was due to citric acid and organosulphur derivative compounds. In another study, Abdel-Wareth et al. (2019a) demonstrated that *Paecilomyces lilacinus* acetone extract exhibited high inhibitory activity against hepatocellular carcinoma (HepG2) cells as IC_{50} was 2.81μg/mL. Recently, Dalinova et al. (2020) isolated and identified a new chlorinated xanthone, methyl 8-hydroxy-3-methyl-4-chloro-9-oxo-9*H*-xanthene-1--carboxylate (**1**) and a new benzophenone derivative, 5-chloromoniliphenone (**2**), together with 11 structurally related compounds (**3**–**13**) from the solid culture of *Alternaria sonchi*. The mentioned compounds showed moderate cytotoxicity ($IC_{50} > 25$μg/mL) against human histiocytic lymphoma (U937) and human myelogenous leukemia (K562) cell lines. In the same year, Sahu et al. (2020) investigated the antiproliferative activity of the crude *Talaromyces purpureogenus* extracellular and intracellular extracts, where intracellular extracts demonstrated higher cytotoxicity. The extract was further purified by adsorption column chromatography, and the fractions were assessed for antiproliferative activity. Fraction A and C (toluene and ethyl acetate fractions) depicted more antiproliferative potential against breast cancer (MCF-7) and liver cancer (HepG2) cell lines with IC_{50} of 2.79 and 2.75μg/mL, respectively. They observed that breast cancer cells (MDA-MB-468) were highly sensitive to fraction B ($IC_{50} < 0.35$μg/mL) suggesting that these fractions might contain effective anticancer lead molecules. However, toluene and ethyl acetate fractions were also observed to be highly potent against liver cancer, but toluene fraction had similar toxicity in cancer as well as non-cancerous (control) cells with IC_{50} of 2.75 and 2.29μg/mL, respectively.

8.3.3 Other Pharmaceutically Promising Agents

Martínez-Luis et al. (2011) isolated endophytic fungi from different regions of Panama, and found that from 25 fungal isolates obtained, 10 had good anti-parasitic potential, showing selective activity against *Leishmania donovani*, such as *Edenia* sp., *Penicillium* sp., *Nectria* sp., *Mycosphaerella* sp. and *Hypocrea* sp., while *Stenocarpella* sp., *Nectria* sp., and *Mycosphaerella* sp. had significant anti-malarial activity on *Plasmodium falciparum*. In addition, *Mycosphaerella* sp. inhibited the

growth of *Trypanosoma cruzi*. For exploring the anti-leishmanial activity of fungal metabolites, some researches have been carried out, for instance, Santiago et al. (2012) tested the extracts of 12 fungi belonging to the genera *Alternaria*, *Antarctomyces*, *Cadophora*, *Davidiella*, *Helgardia*, *Herpotrichia*, *Microdochium*, *Oculimacula*, and *Phaeosphaeria*. All of them inhibited the proliferation of *Leishmania amazonesis*.

Similarly, Rosa et al. (2009, 2010) showed that 34 extracts of Basidiomycetous fungi and 11 extracts of *Alternaria*, *Arthrinium*, *Cochliobolus*, *Colletotrichum*, *Penicillium*, *Fusarium*, and *Gibberella* hindered the growth of *L. amazonensis* with IC_{50} values ranging from 4.60 to 24.40μg/mL. Moreover, Campos et al. (2008) found that the alcohol extract of the endophytic fungus *Cochliobolus* sp. killed 90% of the amastigote-like forms of *Leishmania amazonensis*. In another study, the anti-leishmanial activity of eight compounds isolated from *Edenia* sp., which were preussomerin EG1, palmarumycin CP2, palmarumycin CP17, palmarumycin CP18, CJ-12,371, palmarumycin CP19, preussomerin EG2, and 5-methylochracin was investigated. All these compounds inhibited the growth of amastigote forms of *Leishmania donovani* (Martínez-Luis et al. 2008). By the same token, Guimarães et al. (2008) reported that ethyl acetate extracts of *phaseolorum* sp., *Phyllosticta* sp., *Phomopsis* sp. and *Cercospora kikuchii* inhibited the growth of *Leishmania tarentolae*.

Also, Marinho et al. (2005) isolated citrinin, which is a phenolic compound, from *Penicillium janthinellum*, and recorded its activity against *Leishmania Mexicana*. Ebel (2010) found that certain *Drechslera* strains exhibited anti-plasmodial activity. He identified five new sesquiterpenoids named as isosativene-triol, drechslerines A and B, 9-hydroxyhelminthosporol, sativene epoxide, and drechslerines C–G, in addition to five known compounds; helminthosporol, *cis*-sativenediol, isocochlioquinone A, isocochlioquinone C and cochlioquinone B from *Drechslera dematioidea*. As Malaria is one of the most important health problems in the African region, researchers tried to combat it by fungal metabolites. Kanokmedhakul et al. (2011) isolated nine compounds from *Eurotium chevalieri* including chevalones A–D, aszonapyrone A and B (meroterpenoids), pyrrolobenzoxazine and eurochevalierine (sequiterpene alkaloid), and one sequiterpene. All these compounds showed anti-malarial activity.

Regarding antioxidant activities of fungal metabolites, a total of 20 endophytic fungi have been isolated from wheat (*Triticum durum*), where the antioxidant capacity of ethyl acetate extracts was evaluated by β-carotene/linoleic acid assay. The results showed that 60% of these extracts have antioxidant activity, exhibiting 50, 57% to 78 and 96% inhibitions (Sadrati et al. 2013). From *Aspergillus niger*, Suresha and Srinivasan (2013) identified the fungal metabolite nigerloxin and demonstrated that it was an effective antioxidant in different in vitro assays including the phosphomolybdenum, 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), 2,2′ azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) and ferric reducing antioxidant power (FRAP) methods.

Yadav et al. (2014) declared that alkaloides, phenols, flavonoids, saponins, and terpenes were the main phytochemicals present in 21 endophytes isolated from the

Eugenia jambolana plant. They found that ethyl acetate extracts of *Chaetomium* sp., *Aspergillus* sp., *Aspergillus peyronelii*, and *A. niger* showed high antioxidant activity ranging from 50% to 80%. Meanwhile, Jakovljević et al. (2014) showed that *Penicillium chrysogenum* ethanolic extract contained higher total phenolic content and better total antioxidant capacity as well as ferrous ion chelating ability. Also, *Penicillium fumiculosum* ethanolic extract showed higher DPPH free-radical scavenging activity, as well as reducing power. In the same vein, the endophytic fungi isolated from *Lannea coromandelica* plant were extracted with ethyl acetate, where *Aspergillus niger*, *Aspergillus flavus*, and *Alternaria alternata* were the most dominant, and their extracts showed high antioxidant potential (Premjanu and Jaynthy 2014).

Moreover, Kandasamy et al. (2015) declared that methanolic extracts of *Drechslera* sp. and *Nigrospora* sp. isolated from medicinal plants showed higher phenolic content and scavenging potentials, whereas *Phoma* sp. and *Chaetomium spiralis* showed increased phenolic content in aqueous extract. In addition, the endophytic fungus *Aspergillus* sp. from the Sudanese medicinal plant *Trigonella foenum-graecum* seeds demonstrated the highest total phenolic content in terms of gallic acid equivalent; 89.9 mg GAE/g and antioxidant activity for 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay with IC_{50} equal 18.0μg/mL (Khiralla et al. 2015). Furthermore, Wang et al. (2016) claimed that endophytic fungi can be a good source of radical scavengers.

They isolated 13 strains of endophytic fungi from *Astragalus monadelphus*, where the results showed that secondary metabolites and mycelium extracts from these strains had significant antioxidant activities. Also, Palanichamy et al. (2018) demonstrated that ethyl acetate extracts from the endophyte *Alternaria* sp. grown on five different fungal growth media showed radical scavenging properties (from 13.818% to 66.162%). They also found that the extract from Potato Dextrose Broth (PDB) exerted the highest radical scavenging property (66.162%). They identified the effective metabolite as alternariol methyl ether. Moreover, Gunasekaran et al. (2017) recorded a high scavenging activity (85.20%) of the ethyl acetate extract of the endophytic *Alternaria* sp. additionally, Hameed et al. (2017) investigated three important strains of *Mucor circinelloides* grown under submerged fermentation conditions for their potential antioxidants production. All mycelial extracts demonstrated effective antioxidant activities in terms of β-carotene/linoleic acid bleaching, radical scavenging, reduction of metal ions, and chelating abilities against ferrous ions. Simultaneously, Zohri et al. (2017) recorded the highest reducing power activities in 9, 20, and 14 strains of the three tested groups of fungi; endophytic, entomopathogenic, and saprophytic fungi, respectively.

They found that the more effective endophytic fungi were *Emericella* sp. followed by *Aspergillus Versicolor*, while *Beauveria bassiana* exhibited the highest antioxidant effect of the entomopathogenic group followed by *Aspergillus*. On the other hand, the best strain of saprophytic fungi was *Phoma herbarum* followed by *Aspergillus terreus* and *Botryotrichum piluliferum*. In another study, six fungal endophytes were isolated from the medicinal plants; *Garcinia kola* and *Cola nitida*, as ethyl acetate extracts of *Trichophyton* sp. and *Collectotrichum* sp. demonstrated

the most potent antioxidant activity (Nwobodo et al. 2017). Also, among all the mycelial extracts of *Beauveria bassiana* isolates, Kiran and Mohan (2018) declared that ethyl acetate extract exhibited the highest antioxidant activity with an IC_{50} of 202.45μg/mL. Furthermore, Awad et al. (2018) isolated volatile constituents, proteins, and carbohydrates from the fungus *Trichoderma viride*, and evaluated them as antioxidant agents. The isolated volatile constituents revealed high antioxidant effects; 29.62%, 63.12%, and 70.37% at concentrations of 10, 50, and 100μg, respectively. Moreover, proteins and carbohydrates recorded remarkable antioxidant effects (3.70%, 14.81%, and 33%) for proteins and (3%, 18%, and 23%) for carbohydrates at concentrations of 10, 50, and 100μg, respectively.

Recently, the endophytic fungus *Curvularia* sp. was isolated from *Rauwolfia macrophylla*, a medicinal plant from Cameroon, where three compounds were identified in the methanolic extract; 2′-Deoxyribolactone (**1**), Hexylitaconic acid (**2**), and Ergosterol (**3**). The three compounds showed good inhibitory potential towards acetylcholinesterase with IC_{50} values of 1.93, 1.54, and 1.52μM, respectively. Besides, they were active with EC_{50} values of 0.66, 0.56, and 1.09μM, respectively in antioxidant assay and 0.49, 0.88, and 0.83μM in radical scavenging capacity assay (Kaaniche et al. 2019). By the same token, Abdel-Wareth et al. (2019b) showed that acetone extract of *Trichoderma harzianum* and ethyl acetate extract of *Aspergillus flavus* isolated from tissues of *Biomphalaria alexandrina* snails exhibited the highest antioxidant capacity, followed by acetone extracts of *Penicillium variabile* and *Penicillium chrysogenum*. The authors attributed this activity to derivatives of heterocyclic compounds. Similarly, Abdel-Wareth et al. (2019a) reported that the highest antioxidant capacity amongst entomopathogenic fungi was that of the acetone extract of *Paecilomyces lilacinus* followed by *Beauveria bassiana*. Other activities of metabolites isolated from endophytic fungi are shown in Table 8.1.

Many researchers indicated other important activities of fungal secondary metabolites; for example, Abdel-Monem et al. (2013)reported that ethyl acetate extract of the marine-derived fungus *Trichurus spiralis* isolated from *Hippospongia communis* sponge showed hepatoprotective effect against heavy metal toxicity in rats. On the other hand, Patocka (2016) claimed that *Beauveria bassiana* and some of its metabolites represent a serious candidate for the prevention and treatment of neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease, as Tomoda and Doi (2008) have shown earlier that some cyclodepsipeptides, such as natural beauveriolides or their synthetically prepared derivatives, represent an interesting way in the fight against neurodegenerative diseases. Park et al. (2008) reported that the methanolic extract of *Beauveria bassiana* increased acetylcholinesterase (AChE) activity and reactive oxygen species (ROS) scavenging activity, which would be beneficial for the suppression of neurodegenerative disorders.

Moreover, Dominguete and Takahashiab (2018) selected about 50 filamentous fungi isolated from soil with different macroscopic characteristics. Then, they screened their broth culture media containing secondary metabolites for acetylcholinesterase (AChE) inhibitory activity, besides total antioxidant activity using the colorimetric phosphomolybdate method. The broth of *Hypocrea lixii* showed

promising results, even with bioactive compounds very diluted in the medium. It was found to have significant total antioxidant activity (18.22μg AAE/mL broths) and efficient inhibition of AChE (65.4%). Another activity of fungal metabolites was demonstrated by El-Neekety et al. (2016), they declared that *Aspergillus niger* and *Aspergillus fumigatus* isolated from soil samples in Egypt exhibited antiaflatoxigenic effect, as both fungal isolates inhibited the production of AFB1 by *Aspergillus flavus*. Moreover, *A. niger* was able to degrade AFB1 where 60.70% of the toxin was removed after 8 days. The authors also noticed that the crude ethyl acetate extract of *A. niger* showed high antioxidant capacity.

8.4 Applications in Biological Control Strategies

8.4.1 Molluscicidal Activity

Schistosomiasis is a parasitic disease caused by trematode worms (e.g., *Schistosoma mansoni* and *Schistosoma haematobium*) that affects approximately 260 million people worldwide. More than 90% of cases occur in the African region (WHO 2016). The life cycle of the parasites requires the presence of a freshwater snail as an intermediate host. The use of chemical molluscicides to control these snails could lead to toxic effects on non-target organisms and environmental pollution (Oliveria-Filho and Paumgartten 2000). So, researchers became interested in finding alternatives from biological origins (Moazami 2002). Consequently, many studies have focused on the control of *Biomphalaria alexandria* snails using biological agents like fungi and their metabolites. Two fungal species; *Aspergillus terreus* and *Penicillium janthinellum* were tested as filtrates against *Biomphalaria alexandrina* snails, where the LC_{50} values were 1.05% and 1.03%, respectively (Saad et al. 2014).

The authors also demonstrated that sublethal concentrations of the fungal filtrates reduced the survival rate and reproduction of the snails. Moreover, Abdel-Wareth and Ghareeb (2018) identified 22 phenolic compounds in the filtrate extracts of *Penicillium implicatum*, *Aspergillus niveus*, and *Aspergillus petrakii*, as methyl gallate and *p*-coumaric acid were amongst the major compounds. The authors found that both compounds were highly effective as larvicidal agents on each of miracidia and cercariae of *Schistosoma mansoni*. Recently, Abdel-Wareth et al. (2019a) found that acetone extracts of *Paecilomyces lilacinus* and *Beauveria bassiana* were effective against *Biomphalaria alexandrina* snails with LC_{50} values of 120 and 231 ppm, respectively. They reported that the sublethal concentrations of both extracts adversely affected survival rate, the digestive gland which is analogous to the liver in higher animals, besides causing deterioration of the hermaphrodite gland which is responsible for reproduction. Also, the authors reported a genotoxic effect of the metabolites of both fungi on the DNA of snails.

8.4.2 Anti-phytopathogenic Activity

The indolyl diketopiperazines (**1**–**6**) were isolated from the endophytic fungus *Aspergillus tamarii*, and tested for anti-phytopathogenic activity in vitro for the first time. Their structures were identified as fumitremorgin B (**1**), verruculogen (**2**), fumitremorgin C (**3**), cyclotryprostatins B (**4**), tryprostatin A (**5**), and tryprostatin B (**6**). The isolated metabolites showed significant anti-phytopathogenic activity on *Pyricularia oryzae*, *Penicillium chrysogenum*, *Fusarium graminearum*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Alternaria alternata*, and *Phytophthora capsici* (Zhang et al. 2012). In the same vein, Abd-El-Khair and El-Nagdi (2014) investigated four bio-control agents of fungal origin namely; Bio-Nematon® (*Paecilomyces lilacinus*; 108 unit/cm^3), *Trichoderma hamatum*, *Trichoderma album*, Stanes Symbion VAM plus® (*Glomus fasciculatum* and *Gigaspora* sp.). They were applied in commercial potato fields for controlling root-rot disease caused by *Fusarium solani* and *Rhizoctonia solani*, and nematode root-knot caused by *Meloidogyne arenaria*, compared to the chemical nematicide Nemacur.

The authors found that the biocontrol agents significantly reduced the frequency of *F. solani* and *R. solani*, as well as the percentages of disease incidence of root rot in the rhizospheres of treated potato plants, compared to Nemacur® as well as the untreated plants. After 2 months of treatment, reduction of the number of juveniles caused by these agents in soil was in the range of 33.1–76.9%, compared to 16.9% with Nemacur®. In addition, Zhao et al. (2018b) prepared crude ethyl acetate extracts from the fermentation broth of 31 selected marine-derived fungal isolates and evaluated them for their growth inhibitory activity against plant pathogenic bacteria including *Pseudomonas syringae* pv. *lachrymans*, *Acidovorax avenae*, *Erwinia carotovora*, *Xanthomonas oryzae* pv. *oryzae*, *Ralstonia solanacearum*, and *Clavibacter michiganensis*. They found that eight strains exhibited strong activity at 1 mg/mL, where the extracts of *Fusarium equiseti*, *Penicillium oxalicum*, and *Penicillium chrysogenum* at 0.1 mg/mL displayed stronger inhibitory activity against *P. syringae* pv. *lachrymans* and *A. avenae*, with *Fusarium equiseti* being the most efficient.

The marine-derived *Penicillium* sp. displayed the most potent anti-phytopathogenic bacterial activity. Also, the crude extract of *Alternaria* sp. showed the broadest antibacterial spectrum, since it inhibited the growth of all six tested plant pathogenic bacteria at 10 mg/mL. Moreover, these 31 fungal isolates were tested for their antifungal activity against two phytopathogenic fungal strains; *Alternaria alternata*, and *Phytophthora parasitica* var. *nicotianae*, as 14 strains were able to inhibit the mycelial growth of *P. parasitica* var. *nicotianae*, whereas only 4 strains restrained *A. alternata* growth. The authors isolated four compounds from *Fusarium equiseti* and *Alternaria* sp., and identified them where Alterperylenol (**4**) exhibited antibacterial activity against *Clavibacter michiganensis* with a minimum inhibitory concentration (MIC) of 1.95μg/mL, which was two fold stronger than that of the positive control; streptomycin sulfate. Besides, stemphyperylenol

(**3**) displayed potent antifungal activity against *Pestallozzia theae* and *Alternaria brassicicola* with MIC values equal to those of the positive control; carbendazim.

8.4.3 Insecticidal Activity

The pink hibiscus mealybug *Maconellicoccus hirsutus* is a serious pest on the ornamental plant *Hibiscus rosa sinensis.* Mohammad et al. (2010) evaluated the comparative effects of six commonly used insecticides including the biopesticides; Bio-fly and Biovar (which are formulations of *Beauveria bassiana*) besides Bioranza (which is a formulation of *Metarhizium anisopliae*). They recorded high percentages of reduction in mealybug populations ranging from 65% to 66.55%; these values were close to those resulting from the application of chemical pesticides. Also, El-Banna et al. (2013) indicated that application of Bioranza © (*Metarhizium anisopliae*) induced a reduction in the percentage of the larval population of lesser cotton leafworm *Spodoptera exigua* after spraying on cotton in Egypt.

The verroa mite, *Varroa destructor*, is known as the most serious ectoparasite mite on the honeybee, *Apis mellifera*. Based on the spores of entomopathogenic fungi, Ahmed and Abd-Elhady (2013) investigated two commercial preparations; Bioranza (*Metarhizium anisopliae*) and Biovar (*Beauveria bassiana*) through application into the hives against varroa mite. The results showed significant differences between treatments with Bioranza © and Biovar ©. The results were significant after 7 and 14 days of application, where Bioranza was more effective than biovar as it resulted in a significant increase of mites' daily mortality. In the same vein, Abdel-Raheem et al. (2015) evaluated the efficacy of the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on the insects affecting stored crops; *Rhyzopertha dominica*, *Sitophilus oryzae*, and *Oryzaephilus surinamensis*. They demonstrated that *M. anisopliae* was more effective against the tested insects than *B. bassiana* as LC_{50} of *B. bassiana* concentrations against *R. dominica*, *S. oryzae*, and *O. surinamensis* were 1.2×10^5 conidia/g, 1.6×10^5 conidia/g, and 1.4×10^5 conidia/g, respectively. While LC_{50} values of *M. anisopliae* concentrations against *R. dominica*, *S. oryzae*, and *O. surinamensis* were 2.7×10^5 conidia/g, 1.3×10^5 conidia/g, and 3.5×10^5conidia/g, respectively.

Moreover, Francardi et al. (2016) carried out a study to evaluate the efficacy of two commercial products, Met52 © and BioStorm ©, and of their fungal isolates, *M. anisopliae* (Man52) and (ManBS), respectively, against the adult red palm weevil; *Rhynchophorus ferrugineus*. The virulence of *M. anisopliae* strains (Man52) and (ManBS) was compared with that of an indigenous *M. anisopliae* (Man08/I05) strain obtained from *R. ferrugineus* specimens. The results indicated that the commercial formulations mixed directly into the soil were not effective against red palm weevil adults, and did not reduce female fecundity and fertility. On the contrary, the fungal strains; *M. anisopliae* (ManBS), (Man52), and (Man08/I05) inoculated on a rice substratum caused over 80% mortality of the phytopathogens. In particular, *M. anisopliae* (ManBS) and *M. anisopliae* (Man08/I05) resulted in the highest

mortality (100%), with LC_{50} and LC_{90} reached in 3 and 6 days, respectively. While, *M. anisopliae* (Met52) led to 85% mortality of red palm weevil specimens in 28 days, and took longer to reach LC_{50} (6 days) and LC_{90} (12 days). The reproductive potential of females infected with the fungal strains was also significantly reduced with respect to the control.

The greasy cutworm, *Agrotis ipsilon* (Order: Lepidoptera) is widely distributed all over the world, particularly in moderate and subtropical countries of the northern and southern hemispheres (Kononenko 2003). The greasy cutworm causes damage to vegetables, cucurbitaceous, and industrial crops. The greatest damage is caused to cotton, maize, tobacco, sunflower, tomatoes, sugar beet, and potato. The pest can strongly harm vegetables, and also cause damage to seedlings of tree species (pine, maple, and nut). This pest has a solitary habit, as it commonly feeds on seedlings at ground level, cutting off the stem and sometimes dragging the plants into their burrows. Gabarty et al. (2014) carried out scanning electron microscopy (SEM) of *Agrotis ipsilon* larvae treated with LC_{50} of *Beauveria bassiana*, and revealed adhesion, and penetration of the infected larvae. Growth of the fungus on the infected larvae and signs of hyphal penetration of insect cuticle as well as the proliferation of the cuticle was also observed.

On the other hand, *Metarhizium anisopliae* as declared by SEM showed a dense network, where green spores appeared on the insect cuticle. Also, SEM allowed observing the spores and hyphae of the fungus in the body cavity of infected larvae. Moreover, Elbanna et al. (2012) declared that infection of the desert locust; *Schistocerca gregaria* nymphs with Bioranza (*Metarhizium anisopliae* fungal spores) induced high mortality percentage, where the highest concentration resulted in faster mortality than the lowest one. In addition, Ibraheem et al. (2012) indicated that five tested biopesticides achieved a satisfactory reduction in *Saissetia oleae* insect population within an experimental period of 7 weeks. The total population showed that Stanes-biocatch (*Verticillium lecanii*) caused the highest reduction (91.68%) followed by Stanes-biomagic (*Metarhizium anisopliae*) (88.74%), Biovar (*Beauvaria bassiana*) (87.41%), and Bioranza (*Metarhizium anisopliae*) (82%). In addition, Biovar caused the highest reduction in the nymph population (97.28%), followed by Stanes-biocatch (95.1%), Bioranza (90.59%), and Stanes-biomagic (89.96%). Also, all biopesticides resulted in a 100% reduction of adult females.

8.4.4 Nematicidal Activity

Amongst a number of biopesticides tested against root-knot nematode under greenhouse conditions, *Paecilomyces lilacinus* was the most effective on both galls and egg masses achieving 88.23% and 76.94% reduction, respectively (Khalil et al. 2012). They added that *Paecilomyces lilacinus* product; Bio-Nematon was the best treatment in suppressing *Meloidogyne incognita* populations causing root-knot of tomato with 85.2% when compared with other microbial products. Furthermore, Sharma et al. (2014) showed that *Paecilomyces lilacinus* culture filtrate from

Karanja cake medium killed 100% of *Meloidogyne incognita* larvae, while only 78.28% mortality was recorded by Czapeck-Dox filtrate within 12 h of exposure. The filtrate, irrespective of culture medium, was found to be more nematotoxic when incubated for 15 days. Fourier Transform Infrared Spectroscopy (FT-IR) predicted the presence of phenolic and alcoholic compounds in the filtrate. The authors also reported that ethyl acetate and lyophilized aqueous extracts produced higher nematicidal activity than hexane extract, which indicated the polar nature of the active compounds produced by *P. lilacinus*. In addition, Sharma et al. (2016) carried out in vitro nematocidal bioassays, FT-IR, and HPLC analysis to demonstrate the involvement of toxins of *Purpureocillium lilacinum* in killing root-knot nematodes (*Meloidogyne incognita*). During the growth study, maximum mycelial biomass (10.52 g/l) in de-oiled Karanja cake medium was achieved on the eighth day, while complete mortality of nematodes was obtained by the sixth day. In addition, maximum production of the crude nematocidal toxin was recorded on the seventh day suggesting that the toxin production was paralleled with the growth of the fungus. The median lethal concentration (LC_{50}) determined for the crude toxin from the sixth to the tenth day ranged from 89.41 to 43.21 ppm.

8.5 Conclusion and Future Prospects

Fungal secondary metabolites are rich sources of bioactive compounds with multifaceted applications. As fungi inhabit various ecological niches, a subsequent variation of their secondary metabolites results. Also, varying the media, and/or the constituents of the known media, besides the conditions of fungal growth could result in the production of new metabolites which might be good candidates for combating diseases. More research should be carried out to explore fungal species from unusual or extreme environments, and molecular tools could be improved for identifying more fungal strains. In addition, genetic engineering can be used for modifying the genes responsible for the production of certain active compounds to increase the yield or to improve it. Also, advanced and rapid techniques have to be applied to detect, characterize, and separate promising effective fungal metabolites to pave the way for their formulation, and subsequent application in different relevant fields.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abdel-Hady H, Abdel-Wareth MTA, El-Wakil EA, Helmy EA (2016) Identification and evaluation of antimicrobial and cytotoxic activities of *Penicillium islandicum* and *Aspergillus tamarii* ethyl acetate extracts. World J Pharm Sci 5:2021–2039

Abd-El-Khair H, El-Nagdi WM (2014) Field application of bio-control agents for controlling fungal root rot and root-knot nematode in potato. Arch Phytopathol Plant Protect 47:1218–1230

Abdel-Monem NM, Abdel-Azeem AM, El-Ashry ESH, Ghareeb DA, Nabil-Adam A (2013) Pretreatment hepatoprotective effect of the marine fungus derived from sponge on hepatic toxicity induced by heavy metals in rats. Biomed Res Int 2013:1–15

Abdel-Raheem MA, Ismail IA, Abdel Rahman RS, Farag NA, Abdel Rhman IE (2015) Entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) as biological control agents on some stored product insects. J Entomol Zool Stud 3:316–320

Abdel-Wareth MTA, Ghareeb MA (2018) Bioprospecting certain freshwater-derived fungi for phenolic compounds with special emphasis on antimicrobial and larvicidal activity of methyl gallate and p-coumaric acid. Egypt J Chem 61:773–784

Abdel-Wareth MTA, Ghareeb MA, Abdel-Aziz MS, El-Hagrassi AM (2019a) Snailicidal, antimicrobial, antioxidant and anticancer activities of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces lilacinus* fungal extracts. Egypt J Aquat Biol Fish 23:195–212

Abdel-Wareth MTA, El-Hagrassi AM, Abdel-Aziz MS, Nasr SM, Ghareeb MA (2019b) Biological activities of endozoic fungi isolated from *Biomphalaria alexandrina* snails maintained in different environmental conditions. Int J Environ Stud 76:780–799

Accorsini FR, Mutton MJR, Lemos EGM, Benincasa M (2012) Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate. Braz J Microbiol 43:116–125

Adamczak M, Bednarski W (2000) Influence of medium composition and aeration on the synthesis of biosurfactants produced by *Candida antarctica*. Biotechnol Lett 22:313–316

Ahmed AA, Abd-Elhady HK (2013) Efficacy of two fungus-based biopesticides against the honeybee ectoparasitic mite, *Varroa destructor*. Pak J Biol Sci 16(16):819–825

Aishwarya AD (2014) Extraction of natural dyes from fungus—an alternate for textile dyeing. J Nat Sci Res 4:1–6

Akihisa T, Tokuda H, Ukiya M, Kiyota A, Yasukawa K, Sakamoto N, Kimura Y, Suzuki T, Takayasu J, Nishino H (2005) Anti-tumor-initiating effects of Monascin, an azaphilonoid pigment from the extract of *Monascus pilosus* fermented rice (red-mold rice). Chem Biodivers 2:1305–1309

Akilandeswari P, Pradeep BV (2016) Exploration of industrially important pigments from soil fungi. Appl Microbiol Biotechnol 100:1631–1643

Ali A, Haider MS, Khokhar I, Bashir U, Mushtaq S, Mukhtar I (2011) Antibacterial activity of culture extracts of *Penicillium* species against soil-borne bacteria. Mycopathologia 9:17–20

Artasasta MA, Yanwirasti AD, Handayani D (2017) Cytotoxic activity screening of ethyl acetate fungal extracts derived from the marine sponge *Neopetrosia chaliniformis* AR-01. J Appl Pharm Sci 7:174–178

Askolin S, Nakari-Setälä T, Tenkanen M (2001) Overproduction, purification, and characterization of the *Trichoderma reesei* hydrophobin HFBI. Appl Microbiol Biotechnol 57:124–130

Assadollahpour M, Zafari D, Zare R (2011) Insects *Hyphomycetous* fungi and their pathogenesis on Colorado potato beetle in Hamedan. J Plant Protect 24:465–470

Astuti P, Nababan OA (2014) Antimicrobial and cytotoxic activities of endophytic fungi isolated from *Piper crocatum* Ruiz & Pav. Asian Pac J Trop Biomed 4:S592–S596

Avalos J, Limón MC (2015) Biological roles of fungal carotenoids. Curr Genet 61(3):309–324

Avalos J, Pardo-Medina J, Parra-Rivero O, Ruger-Herreros M, Rodriguez-Ortiz R, Hornero-Mendez D et al (2017) Carotenoid biosynthesis in *Fusarium*. J Fungi 3:1–16

Awad NE, Kassem HA, Hamed MA, El-Feky AM, Elnaggar MAA, Mahmoud K, Ali MA (2018) Isolation and characterization of the bioactive metabolites from the soil derived fungus *Trichoderma viride*. Mycology 9:70–80

Babitha S (2009) Microbial pigments. In: Nigam PS, Pandey A (eds) Biotechnology for agro-industrial residues utilization. Springer, Dordrecht, the Netherlands, pp 147–162

Banat IM, Franzetti A, Gandolfi I et al (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol 87:427–444

Barnes EC, Jumpathong J, Lumyong S, Voigt K, Hertweck C (2016) Daldionin, an unprecedented binaphthyl derivative, and diverse polyketide congeners from a fungal orchid endophyte. Chem Eur J 22:4551–4555

Barnett HL, Hunter BB (1998) Illustrated genera of imperfect fungi, 4th edn. The American Phytopathological Society, St. Paul

Bazinet RP, Laye S (2014) Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 15:771–785

Beauprez JJ, De Mey M, Soetaert WK (2010) Microbial succinic acid production: natural versus metabolic engineered producers. Process Biochem 45:1103–1114

Bills GF, Gloer JB (2016) Biologically active secondary metabolites from the Fungi. Microbiol Spectr 4:VE-0001-2014

Blackwell M (2011) The fungi: 1, 2, 3 … 5.1 million species? Am J Bot 98:426–438

Britton G, Liaaen-Jensen S, Pfander H (1995) Carotenoids, Volume 1A: Isolation and analysis. Stuttgart, Germany: Birkhaüser

Blanchette RA, Wilmering AM, Baumeister M (1992) The use of green-stained wood caused by the fungus *Chlorociboria* in intarsia masterpieces from the 15th century. Holzforsch Int J Biol Chem Phys Technol Wood 46:225–232

Bodour AA, Miller-Maier RM (1998) Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. J Microbiol Methods 32:273–280

Bohlmann J, Keeling CI (2008) Terpenoid biomaterials. Plant J 54(4):656–669

Bury M, Andolfi A, Rogister B, Cimmino A, Megalizzi V, Mathieu V et al (2013) Fusicoccin A, a phytotoxic carbotricyclic diterpene glucoside of fungal origin, reduces proliferation and invasion of glioblastoma cells by targeting multiple tyrosine kinases. Trans Oncol 6(2):112–123

Butler MJ, Day AW (1998) Fungal melanins: a review. Can J Microbiol 44:1115–1136

Cai Y, Ding Y, Tao G, Liao X (2008) Production of 1,5-dihydroxy-3-methoxy-7-methylanthracene-9,10-dione by submerged culture of Shiraia bambusicola. J Microbiol Biotechnol 18:322–327

Camargo-de-Morais MM, Ramos SA, Pimentel MC, Morais J (2003) Production of an extracellular polysaccharide with emulsifier properties by *Penicillium citrinum*. World J Microbiol Biotechnol 19:191–194

Cameron DR, Cooper DG, Neufeld RJ (1988) The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifier. Appl Environ Microbiol 54:1420–1425

Campos FF, Rosa LH, Cota BB, Caligiorne RB, Rabello AL, Alves TM et al (2008) Leishmanicidal metabolites from *Cochliobolus* sp., an endophytic fungus isolated from *Piptadenia adiantoides* (Fabaceae). PLoS Negl Trop Dis 2:e348

Cao P, Yang J, Miao CP, Yan Y, Ma YT, Li XN, Zhao LX, Huang SX (2015) New duclauxamide from *Penicillium manginii* YIM PH30375 and structure revision of the duclauxin family. Org Lett 17:1146–1149

Caro Y, Venkatachalam M, Lebeau J, Fouillaud M, Dufossé L (2017) Pigments and colorants from filamentous fungi. In: Merillon JM, Ramawat KG (eds) Fungal metabolites. Springer International Publishing, Cham, Switzerland, pp 499–568

Carvalho JC, Pandey A, Babitha S, Soccol CR (2003) Production of Monascus biopigments: an overview. Agro Food Ind Hi-Tech 14:37–42

Castiglioni GL, Bertolin TE, Costa JAV (2009) Produção de biossurfactante por *Aspergillus fumigatus* utilizando resíduos agroindustriais como substrato. Quím Nova 32:292–295

Chadni Z, Rahaman MH, Jerin I, Hoque KMF, Reza MA (2017) Extraction and optimization of red pigment production as secondary metabolites from *Talaromyces verruculosus* and its potential use in textile industries. Mycology 8:48–57

Chaplin MF (1986) Carbohydrate analysis: a practical approach, The practical approach series. IRL Press, London

Chattopadhyay P, Chatterjee S, Sen SK (2008) Biotechnological potential of natural food grade biocolorants. Afr J Biotechnol 7:2972–2985

Chen JL, Sun SZ, Miao CP, Wu K, Chen YW, Xu LH et al (2016) Endophytic *Trichoderma gamsii* YIM PH30019: a promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of Panax notoginseng. J Ginseng Res 40:315–324

Chintapenta LK, Rath CC, Maringinti B, Ozbay G (2014) Pigment production from a mangrove *Penicillium*. Afr J Biotechnol 13:2668–2674

Cirigliano MC, Carman GM (1985) Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica*. Appl Environ Microbiol 50:846–850

Cooper DG, Paddock DA (1983) *Torulopsis petrophilum* and surface activity. Appl Environ Microbiol 46:1426–1429

Cooper DG, Paddock DA (1984) Production of a biosurfactant from *Torulopsis bombicola*. Appl Environ Microbiol 47:173–176

Daba GM, Elkhateeb WA, Thomas PW (2018) This era of biotechnological tools: an insight into endophytic mycobiota. Egypt Pharm J 17:121–128

Dalinova A, Chisty L, Kochura D, Garnyuk V, Petrova M, Prokofieva D et al (2020) Isolation and bioactivity of secondary metabolites from solid culture of the fungus, *Alternaria sonchi*. Biomolecules 10:2–18

Das P, Mukherjee S, Sen R (2008) Improved bioavailability and biodegradation of a model polyaromatic hydrocarbon by a biosurfactant producing bacterium of marine origin. Chemosphere 72(9):1229–1234

Devi S, Karuppan P (2015) Reddish brown pigments from *Alternaria alternata* for textile dyeing and printing. Indian J Fibre Text Res 40:315–319

Devi S, Kumar HAK, Ramachandran G, Subramanian C, Karuppan P (2014) Growth and mass spectrometry profile of *Alternaria alternata* pigment grown in maize grain extract. J Microbiol Biotechnol Food Sci 4:179–184

Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Dewick PM (2009) Medicinal natural products: a biosynthetic approach, 3rd edn. Wiley, Chichester, UK

Dhale MA, Vijay-Raj AS (2009) Pigment and amylase production in *Penicillium* sp. NIOM-02 and its radical scavenging activity. Int J Food Sci Technol 44:2424–2430

Dmytruk KV, Sibirny AA (2012) Candida famata (Candida flareri). Yeast 29:453–458

Dominguete LCB, Takahashiab JA (2018) Filamentous fungi as source of biotechnologically useful metabolites and natural supplements for neurodegenerative diseases treatment. Chem Eng Trans 64:295–300

Downham A, Collins P (2000) Coloring our foods in the last and next millennium. Int J Food Sci Technol 35:5–22

Du W, Yao Z, Li J, Sun C, Xia J, Wang B et al (2020) Diversity and antimicrobial activity of endophytic fungi isolated from *Securinega suffruticosa* in the Yellow River Delta. PLoS One 15(3):e0229589

Dufossé L (2006) Microbial production of food grade pigments. Food Technol Biotechnol 44:313–321

Dufossé L, Galaup P, Yaron A, Arad SM, Blanc P, Murthy KNC, Ravishankar GA (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci Technol 16:389–406

Dufossé L, Fouillaud M, Caro Y, Mapari SAS, Sutthiwong N (2014) Filamentous fungi are large-scale producers of pigments and colorants for the food industry. Curr Opin Biotechnol 26:56–61

Dutton MV, Evans CS (1996) Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Can J Microbiol 42(9):881–895

Ebel R (2010) Terpenes from marine-derived fungi. Mar Drugs 8:2340–2368

Elbanna SM, Elhadidy NM, Azazy AM, Elhallous EI, Semida FM (2012) Senergestic effects of Bioranza (*Metarhizium anisopliae*) and Nematode (*Steinernema feltiae*) on desert locust (*Schistocerca gregaria*) nymphs. Egypt J Nat Toxins 9:9–22

El-Banna AA, Abd El-Wahab IS, El-Akad AS, Amin NS (2013) Efficiency of some microbial control agents against the lesser cotton leafworm *Spodoptera exigua* Hbn. (*Lep*:*Noctuidae*) in Kaha, Egypt: a field study. Afr J Biol Sci 9:157–165

El-Neekety AA, Abdel-Aziz MS, Hathout AS, Hamed AA, Sabry BA, Ghareeb MA et al (2016) Molecular identification of newly isolated non-toxigenic fungal strains having antiaflatoxigenic, antimicrobial and antioxidant activities. Der Pharm Chem 8:121–134

Fabelico FL (2015) The phytochemical and antimicrobial properties of entomopathogenic fungi in Nueva Vizcaya, Philippines. Asia Pac J Multidisc Res 3:126–133

Fabre CE, Santerre AL, Loret MO, Baberian R, Pareilleux A, Goma G et al (1993) Production and food applications of the red pigments of *Monascus ruber*. J Food Sci 58:1099–1102

Fang LZ, Qing C, Shao HJ, Yang YD, Dong ZJ, Wang F, Zhao W, Yang WQ, Liu JK (2006) Hypocrellin D, a cytotoxic fungal pigment from fruiting bodies of the ascomycete *Shiraia bambusicola*. J Antibiot 59:351–354

Fawzy GA, Al-Taweel AM, Melake NA (2011) In vitro antimicrobial and anti-tumor activities of intracellular and extracellular extracts of *Aspergillus niger* and *Aspergillus flavus* var. *columinaris*. J Pharm Sci Res 3(1):980–987

Felse PA, Shah V, Chan J, Rao KJ, Gross RA (2007) Sophorolipid biosynthesis by *Candida bombicola* from industrial fatty acid residues. Enzyme Microb Technol 40:316–323

Feng Y, Shao Y, Chen F (2012) Monascus pigments. J Appl Microbiol Biotechnol 96:1421–1440

Feofilova EP, Tereshina VM, Memorskaya AS, Dul'kin LM, Goncharov NG (2006) Fungal lycopene: the biotechnology of its production and prospects for its application in medicine. Microbiologiia 75(6):629–633

Fink-Gremmels J, Leistner L (1989) Biologische Wirkungen von Monascus purpureus. Fleischwutsch 69:116–122

Fischer M, Bacher A (2008) Biosynthesis of vitamin B2: structure and mechanism of riboflavin synthase. Arch Biochem Biophys 474(2):252–265

Fontes GC, Amaral PFF, Coelho MAZ (2008) Produção de Biossurfactante por Levedura. Quim Nova 31:2091–2099

Francardi V, Benvenuti C, Barzanti GP, Roversi PF (2016) Metarhizium anisopliae biopesticides and fungus isolates: control efficacy against *Rhynchophorus ferrugineus* (Olivier) (*Coleoptera Dryophthoridae*) on different contamination substrata. Redia 98(1):25–29

Frandsen RJN, Rasmussen SA, Knudsen PB, Uhlig S, Petersen D, Lysoe E et al (2016) Black perithecial pigmentation in *Fusarium* species is due to the accumulation of 5-deoxybostrycoidin-based melanin. Sci Rep 6:1–13

Frisvad JC, Yilmaz N, Thrane U, Rasmussen KB, Houbraken J, Samson RA (2013) *Talaromyces atroroseus*, a new species efficiently producing industrially relevant red pigments. PLoS One 8:e84102

Gabarty A, Salem HM, Fouda MA, Abas AA, Ibrahim AA (2014) Pathogencity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotis ipsilon* (*Hufn*.). J Rad Res Appl Sci 7(1):95–100

Gao SS, Li XM, Williams K, Proksch P, Ji NY, Wang BG (2016) Rhizovarins A–F, indole-diterpenes from the mangrove-derived endophytic fungus *Mucor irregularis* QEN-189. J Nat Prod 79:2066–2074

Gao H, Li G, Lou HX (2018) Structural diversity and biological activities of novel secondary metabolites from endophytes. Molecules 23:2–31

Gavrilescu M, Chisti Y (2005) Biotechnology-a sustainable alternative for chemical industry. Biotechnol Adv 23(7):471–499

Ge HM, Shen Y, Zhu CH, Tan SH, Ding H, Song YC, Tan RX (2008) Penicidones A–C, three cytotoxic alkaloidal metabolites of an endophytic *Penicillium* sp. Phytochemistry 69:571–576

Giesbers G, Van Schenck J, Gutierrez SV, Robinson S, Ostroverkhova O (2018) Fungi-derived pigments for sustainable organic (opto) electronics. MRS Adv 3:3459–3464

Giesbers G, Van Schenck J, Quinn A, Van Court R, Vega Gutierrez SM, Robinson SC, Ostroverkhova O (2019) XylindeIn: naturally produced fungal compound for sustainable (opto) electronics. ACS Omega 4:13309–13318

Gmoser R, Ferreira JA, Lennartsson PR, Taherzadeh MJ (2017) Filamentous ascomycetes fungi as a source of natural pigments. Fungal Biol Biotechnol 4:1–4

Guimarães DO, Borges WS, Kawano CY, Ribeiro PH, Goldman GH, Nomizo A et al (2008) Biological activities from extracts of endophytic fungi isolated from *Viguiera arenaria* and *Tithonia diversifolia*. FEMS Immunol Med Microbiol 52:134–144

Gulwani N, Shukla H, Sandhu SS (2015) Screening the antibacterial potential of *Paecilomyces fumosoroseus* against some pathogenic bacteria. Open J Adv Drug Deliv 3:24–31

Gunasekaran S, Sathiavelu M, Arunachalam S (2017) In vitro antioxidant and antibacterial activity of endophytic fungi isolated from *Mussaenda luteola*. J Appl Pharm Sci 7(8):234–238

Guo B, Dai JR, Ng S, Huang Y, Leong C, Ong W, Carte BK (2000) Cytonic acids A and B: novel tridepside inhibitors of hCMV protease from the endophytic fungus *Cytonaema* species. J Nat Prod 63:602–604

Gupta C, Sharma D, Aggarwal S, Nagpal N (2013) Pigment production from *Trichoderma* spp. for dyeing of silk and wool. Int J Sci Nat 4:351–355

Hameed A, Hussain SA, Yang J, Ijaz MU, Liu Q, Suleria HAR, Song Y (2017) Antioxidants potential of the filamentous fungi (*Mucor circinelloides*). Nutrients 9:2–20

Handayani D, Rivai H, Mulyana R, Suharti N, Rasyid R, Hertiani T (2018) Antimicrobial and cytotoxic activities of endophytic fungi isolated from mangrove plant *Sonneratia alba* Sm. J Appl Pharm Sci 8:49–53

Hanson J (2003) Natural products: the secondary metabolites. Royal Society of Chemistry, London

Hartmann T (2007) From waste products to ecochemicals: fifty years research of plant secondary metabolism. Phytochemistry 68:2831–2846

Hassan SED (2017) Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. J Adv Res 8:687–695

Hemberger Y, Xu J, Wray V, Proksch P, Wu J, Bringmann G (2013) Pestalotiopens A and B: stereochemically challenging flexible sesquiterpene-cyclopaldic acid hybrids from *Pestalotiopsis* sp. Chem Eur J 19:15556–15564

Heo YM, Kim K, Kwon SL, Na J, Lee H, Jang S et al (2018) Investigation of filamentous fungi producing safe, functional water-soluble pigments. Mycobiology 46:269–277

Hernandez VA, Galleguillos F, Robinson S (2016) Fungal pigments from spalting fungi attenuating blue stain in *Pinus* spp. Int Biodeterior Biodegrad 107:154–157

Hill H (1992) The function of melanin or six blind people examine an elephant. BioEssays 14(1):49–56

Hinsch EM, Chen HL, Weber G, Robinson SC (2015) Colorfastness of extracted wood-staining fungal pigments on fabrics: a new potential for textile dyes. J Text Appar Technol Manag 9:1–11

Hsu Y-W, Hsu L-C, Liang Y-H, Kuo Y-H, Pan T-M (2010) Monaphilones A–C, three new antiproliferative azaphilone derivatives from *Monascus purpureus* NTU 568. J Agric Food Chem 58:8211–8216

Hsu Y-W, Hsu L-C, Liang Y-H, Kuo Y-H, Pan T-M (2011) New bioactive orange pigments with yellow fluorescence from *Monascus*-fermented Dioscorea. J Agric Food Chem 59:4512–4518

Huang X, Huang H, Li H, Sun X, Huang H, Lu Y, Lin Y, Long Y, She Z (2013) Asperterpenoid A, a new sesterterpenoid as an inhibitor of *Mycobacterium tuberculosis* protein tyrosine phosphatase B from the culture of *Aspergillus* sp. 16-5c. Org Lett 15:721–723

Ibraheem AAM, Al-Arnaouty SA, Moussa SF, Helmy SM (2012) Efficiency of certain biopesticides against the olive black scale insect, *Saissetia oleae* (Olivier) on olive trees at Giza Governorate, Egypt. Egypt Acad J Biol Sci A Entomol 5(2):87–93

Inahashi Y, Iwatsuki M, Ishiyama A, Matsumoto A, Hirose T, Oshita J et al (2015) Actinoallolides A–E, new anti-trypanosomal macrolides, produced by an endophytic actinomycete, *Actinoallomurus fulvus* MK10-036. Org Lett 17:864–867

Jakovljević VD, Milićević JM, Stojanović JD, Solujić SR, Vrvić MM (2014) Antioxidant activity of ethanolic extract of *Penicillium chrysogenum* and *Penicillium fumiculosum*. Hem Ind 68(1):43–49

Jansen MLA, van Gulik WM (2014) Towards large scale fermentative production of succinic acid. Curr Opin Biotechnol 30:190–197

Johnson EA, Schroeder WA (1995) Microbial carotenoids. In: Fiechter A (ed) Downstream processing biosurfactants carotenoids. Springer, Berlin, pp 119–178

Jones DL (1998) Organic acids in the rhizosphere–a critical review. Plant Soil 205(1):25–44

Joshi VK, Attri D, Bala A, Bhushan S (2003) Microbial pigments. Indian J Biotechnol 2:362–369

Kaaniche F, Hamed A, Abdel-Razek AS, Wibberg D, Abdissa N, El Euch IZ et al (2019) Bioactive secondary metabolites from new endophytic fungus *Curvularia* sp. isolated from *Rauwolfia macrophylla*. PLoS One 14(6):e0217627

Kandasamy P, Manogaran S, Dhakshinamoorthy M, Kannan KP (2015) Evaluation of antioxidant and antibacterial activities of endophytic fungi isolated from *Bauhinia racemosa* Lam and *Phyllanthus amarus Schum* and Thonn. J Chem Pharm Res 7(9):366–379

Kanokmedhakul K, Kanokmedhakul S, Suwannatrai R, Soytong K, Prabpai S, Kongsaeree P (2011) Bioactive meroterpenoids and alkaloids from the fungus *Eurotium chevalieri*. Tetrahedron 67:5461–5468

Keller NP, Turner G, Bennett JW (2005) Fungal secondary metabolism from biochemistry to genomics. Nat Rev Microbiol 3(12):937–947

Khalil MSEH, Allam AFG, Barakat AST (2012) Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematode on tomato plants under greenhouse conditions. J Plant Protect Res 52(1):47–52

Khan AR, Ullah I, Waqas M, Shahzad R, Hong SJ, Park GS et al (2015) Plant growth-promoting potential of endophytic fungi isolated from *Solanum nigrum* leaves. World J Microbiol Biotechnol 31:1461–1466

Khiralla A, Mohamed I, Thomas J, Mignard B, Spina R, Yagi S et al (2015) A pilot study of antioxidant potential of endophytic fungi from some Sudanese medicinal plants. Asian Pac J Trop Med 8(9):701–704

Kim D, Ku S (2018) Beneficial affects of *Monascus* sp. KCCM 10093 pigments and derivatives: a mini review. Molecules 23:2–15

Kim E, Li JL, Dang HT, Hong J, Lee CO, Kim DK et al (2012) Cytotoxic cytochalasins from the endozoic fungus *Phoma* sp. of the giant jellyfish *Nemopilema nomurai*. Bioorg Med Chem Lett 22(9):3126–3129

Kinghorn AD, De Blanco EJC, Lucas DM, Rakotondraibe HL, Orjala J, Soejarto DD et al (2016) Discovery of anticancer agents of diverse natural origin. Anticancer Res 36:5623–5637

Kiran KAP, Mohan CM (2018) Antioxidant and cytotoxic activity of *Beauveria bassiana* crude mycelia extracts against A-549 cell line. Indian J Pharm Sci 80(4):776–780

Kiran GS, Hema TA, Gandhimathi R, Selvin J, Thomas TA et al (2009) Optimization and production of a biosurfactant from the sponge-associated marine fungus *Aspergillus* ustus MSF3. Colloids Surf B Biointerfaces 73:250–256

Kirti K, Amita S, Priti S, Kumar AM, Jyoti S (2014) Colorful world of microbes: carotenoids and their applications. Adv Biol 1:1–13

Kitamoto D, Ikegami T, Suzuki GT, Sasaki A, Takeyama YI, Idemoto Y et al (2001) Microbial conversion of n-alkanes into glycolipid biosurfactants, mannosylerythritol lipids, by Pseudozyma (*Candida* antarctica). Biotechnol Lett 23:1709–1714

Konishi M, Morita T, Fukuoka T, Imura T, Kakugawa K et al (2007) Production of different types of mannosylerythritol lipids as biosurfactants by the newly isolated yeast strains belonging to the genus *Pseudozyma*. Appl Microbiol Biotechnol 75:521–531

Kononenko VS (2003) Noctuidae. In: Ler PA (ed) Trichoptera and Lepidoptera, Keys to the insects of the Russian Far East, vol 5(4). Dalnauka. (In Russian), Vladivostok, p 688

Kornberg HL, Krebs HA (1957) Synthesis of cell constituents from C2-units by a modified tricarboxylic acid cycle. Nature 179:988–991

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kour D, Rana KL, Yadav AN, Yadav N, Kumar M, Kumar V et al (2020) Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatal Agric Biotechnol 23:101487. https://doi.org/10.1016/j.bcab.2019.101487

Kumar A, Vishwakarma HS, Singh J, Dwivedi S, Kumar M (2015) Microbial pigments: production and their applications in various industries. Int J Phram Chem Biol Sci 5:203–212

Kurosawa E (1926) Experimental studies on the nature of the substance secreted by the "bakanae" fungus. Nat Hist Soc Formosa 16:213–227

Lacaz C, Porto E, Martins J (2001) Martins, Microbiologia Médica: Fungos, Actinomicetos e Algas de Interesse Médico, vol 33, 8th edn. Sarvier, Sao Paulo, Brazil

Lee JC, Lobkovsky E, Pliam NB, Strobel G, Clardy J (1995) Subglutinols A and B: immunosuppressive compounds from the endophytic fungus *Fusarium* subglutinans. J Org Chem 60:7076–7077

Lee JC, Strobel GA, Lobkovsky E, Clardy J (1996) Torreyanic acid: a selectively cytotoxic quinone dimer from the endophytic fungus *Pestalotiopsis microspora*. J Org Chem 61:3232–3233

Lee J, Lee H, Kang S, Park W (2016) Fatty acid desaturases, polyunsaturated fatty acid regulation, and biotechnological advances. Nutrients 8:2–13

Leuchtman A (2003) In: White J, Bacon CW, Hywel-Jones NL, Spatafora JW (eds) Natural products from plant associated endophytic fungi. Marcel-Dekker, New York, pp 175–341

Li JY, Strobel G, Harper J, Lobkovsky E, Clardy J (2000) Cryptocin, a potent tetramic acid antimycotic from the endophytic fungus *Cryptosporiopsis cf. quercina*. Org Lett 2:767–770

Li J-J, Shang X-Y, Li L-L, Liu M-T, Zheng JQ, Jin ZL (2010) New cytotoxic azaphilones from *Monascus purpureus*-fermented rice (red yeast rice). Molecules 15:1958–1966

Li J, Li L, Si YK, Jiang XJ, Guo LD, Che YS (2011) Virgatolides A–C, benzannulated spiroketals from the plant endophytic fungus *Pestalotiopsis virgatula*. Org Lett 13:2670–2673

Li CS, Ren G, Yang BJ, Miklossy G, Turkson J, Fei P et al (2016) Meroterpenoids with antiproliferative activity from a Hawaiian-plant associated fungus *Peyronellaea coffeae-arabicae* FT238. Org Lett 18:2335–2338

Li F, Xue F, Yu X (2017) GC-MS, FTIR and Raman analysis of antioxidant components of red pigments from *Stemphylium lycopersici*. Curr Microbiol 74:532–539

Liaud N, Giniés C, Navarro D, Fabre N, Crapart S, Herpoël-Gimbert I et al (2014) Exploring fungal biodiversity: organic acid production by 66 strains of filamentous fungi. Fungal Biol Biotechnol 1:1–10

Lima JMS, Pereira JO, Batista IH, Neto PDQC, dos Santos JC, de Araújo SP et al (2016) Potential biosurfactant producing endophytic and epiphytic fungi, isolated from macrophytes in the Negro River in Manaus, Amazonas, Brazil. Afr J Biotechnol 15:1217–1223

Liu L, Liu S, Jiang L, Chen X, Guo L, Che Y (2008) Chloropupukeananin, the first chlorinated pupukeanane derivative, and its precursors from *Pestalotiopsis fici*. Org Lett 10:1397–1400

Liu L, Li Y, Liu SC, Zheng ZH, Chen XL, Zhang H et al (2009) Chloropestolide A, an antitumor metabolite with an unprecedented spiroketal skeleton from *Pestalotiopsis fici*. Org Lett 11:2836–2839

Liu J, Zhang D, Zhang M, Liu X, Chen R, Zhao J et al (2016) Periconiasins I and J, two new cytochalasans from an endophytic fungus *Periconia* sp. Tetrahedron Lett 57:5794–5797

Lopez-Garcia R (2002) Citric acid. In: Kirk-Othmer (ed) Kirk-Othmer encyclopedia of chemical technology. Wiley, New York

Luna-Velasco MA, Esparza-García F, Cãnízares-Villanueva RO, Rodríguez-Vázquez R (2007) Production and properties of a bioemulsifier synthesized by phenanthrene-degrading *Penicillium* sp. Process Biochem 42:310–314

Mabrouk AM, El-Kkhrisy EAM, Youssef YA, Asem MA (2011) Production of textile reddish brown dyes by fungi. Malays J Microbiol 7:33–40

Magnuson JK, Lasure LL (2004) Organic acid production by filamentous fungi. In: Tkacz JS, Lange L (eds) Advances in fungal biotechnology for industry, agriculture, and medicine. Springer, New York, pp 307–340

Malik K, Tokas J, Anand RC (2016) Characterization and cytotoxicity assay of pigment-producing microbes. Int J Curr Microbiol Appl Sci 5:370–376

Manikprabhu D, Lingappa K (2013) Actinorhodin a natural and attorney source for the synthetic dye to detect acid production of fungi. Saudi J Biol Sci 20:163–168

Manon Mani V, Shanmuga Priya M, Dhaylini S, Preethi K (2015) Antioxidant and antimicrobial evaluation of bioactive pigment from *Fusarium* sp. isolated from the stressed environment. Int J Curr Microbiol Appl Sci 4:1147–1158

Mapari SAS, Meyer AS, Thrane U (2006) Colorimetric characterization for comparative analysis of fungal pigments and natural food colorants. J Agric Food Chem 54:7027–7035

Mapari SAS, Meyer AS, Thrane U, Frisvad JC (2009) Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. Microb Cell Factories 8:1–15

Mapari SAS, Thrane AU, Meyer AS (2010) Fungal polyketide azaphilone pigments as future natural food colorants? Trends Biotechnol 28:300–307

Marinho A, Rodrigues-Filho E, Moitinho M, Santos L (2005) Biologically active polyketides produced by *Penicillium janthinellum* isolated as an endophytic fungus from fruits of *Melia azedarach*. J Braz Chem Soc 16:280–283

Martínez-Luis S, Della-Togna G, Coley PD, Kursar TA, Gerwick WH, Cubilla-Rios L (2008) Antileishmanial constituents of the Panamanian endophytic fungus *Edenia* sp. J Nat Prod 71:2011–2014

Martínez-Luis S, Cherigo L, Higginbotham S, Arnold E, Spadafora C, Ibañez A et al (2011) Screening and evaluation of antiparasitic and in vitro anticancer activities of *Panamanian* endophytic fungi. Int Microbiol 14:95–102

Martinkova L, Juzlova P, Vesely D (1995) Biological activity of polyketide pigments produced by the fungus *Monascus*. J Appl Bacteriol 79:609–616

Mata-Gómez LC, Montañez JC, Méndez-Zavala A, Aguilar CN (2014) Biotechnological production of carotenoids by yeasts: an overview. Microb Cell Factories 13:1–11

Max B, Salgado JM, Rodríguez N, Cortés S, Converti A, Domínguez JM (2010) Biotechnological production of citric acid. Braz J Microbiol 41:862–875

Meng LH, Wang CY, Mandi A, Li XM, Hu XY, Kassack MU et al (2016) Three diketopiperazine alkaloids with spirocyclic skeletons and one bisthiodiketopiperazine derivative from the mangrove-derived endophytic fungus *Penicillium brocae* MA-231. Org Lett 18:5304–5307

Miao FP, Liang XR, Yin XL, Wang G, Ji NY (2012) Absolute configurations of unique harziane diterpenes from *Trichoderma* species. Org Lett 14:3815–3817

Ming Ge H, Yun Zhang W, Ding G, Saparpakorn P, Song YC, Hannongbua S et al (2008) Chaetoglobins A and B, two unusual alkaloids from endophytic *Chaetomium globosum* culture. Chem Commun 45:5978–5980

Moazami N (2002) Biopesticides production. In: Al Gobaisi D (ed) Encyclopedia of life support systems, 3-encyclopedias of biological physiological and health sciences, biotechnology, vol 6. EOLSS Publishers, Paris, France, pp 1–10

Mohammad AH, Moussa SF, Abo-Ghalia AH, Ahmed SA (2010) Efficiency of certain insecticides on the population(s) of the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) and their natural enemies under the field condition in Ismailia Governorate. Egypt Acad J Biol Sci F Toxicol Pest Control 2:11–17

Mohammadi S, Soltani J, Piri K (2016) Soilborne and invertebrate pathogenic *Paecilomyces* species show activity against pathogenic fungi and bacteria. J Crop Protect 5:377–387

Morales-Oyervides L, Oliveira J, Sousa-Gallagher M, Mendez-Zavala A, Montanez JC (2017) Assessment of the dyeing properties of the pigments produced by *Talaromyces* spp. J Fungi 3:2–9

Mortensen A (2006) Carotenoids and other pigments as natural colorants. Pure Appl Chem 78:1477–1491

Nagia FA, El-Mohamedy RSR (2007) Dyeing of wool with natural anthraquinone dyes from *Fusarium oxysporum*. Dyes Pigments 75:550–555

Nagpal N, Munjal N, Chatterjee S (2011) Microbial pigments with health benefits-a mini review. Trends Biosci 4:157–160

Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79:629–661

Nitschke M, Pastore GM (2002) Biossurfactantes: propriedades e aplicaçõoes. Química Nova 25(5):772–776

Nwobodo DC, Ugwu MC, Okoye FBC (2017) Screening of endophytic fungal secondary metabolites from *Garcinia kola* and *Cola nitida* for antioxidant properties. J Pharm Res 1:1–8

Oliveira Silva MR, Sena KX, Gusmão NB (2009) Secondary metabolites produced by endophytic fungus *Paecilomyces variotii* Bainier with antimicrobial activity against *Enterococcus faecalis*. In: Curr Res Top Appl Micro & Micro Biotech, Proceedings of the international conference on environmental, industrial and applied microbiology, University of Seville, Spain, pp 519–522

Oliveria-Filho EC, Paumgartten FJ (2000) Toxicity of Euphorbia milii latex and Niclosamide to snails and nontarget aquatic species. Ecotoxicol Environ Saf 46:342–350

Orfali R, Perveen S (2019a) New bioactive metabolites from the thermophilic fungus *Penicillium* sp. isolated from Ghamiqa hot spring in Saudi Arabia. J Chem 2019:1–7

Orfali R, Perveen S (2019b) Secondary metabolites from the *Aspergillus* sp. in the rhizosphere soil of *Phoenix dactylifera* (Palm tree). BMC Chem 19:2–9

Osman MY, Sharaf IA, Osman HMY, El-Khouly ZA, Ahmed EI (2004) Synthetic organic food coloring agents and their degraded products: effects on human and rat *cholinesterases*. Br J Biomed Sci 61:128–132

Pacwa-Płociniczak M, Płaza GA, Piotrowska-Seget Z, Cameotra SS (2011) Environmental applications of biosurfactants: recent advances. Int J Mol Sci 12(1):633–654

Palanichamy P, Krishnamoorthy G, Kannan S, Marudhamuthu M (2018) Bioactive potential of secondary metabolites derived from medicinal plant endophytes. Egypt J Basic Appl Sci 5(4):303–312

Palomino AME, Vega GSM, Chen HL, Robinson SC (2017) Wood-rotting fungal pigments as colorant coating on oil-based textile dyes. Coatings 7:1–9

Pandey N, Jain R, Pandey A, Tamta S (2018) Optimisation and characterization of the orange pigment produced by a cold-adapted strain of *Penicillium* sp. (GBPI_P155) isolated from mountain ecosystem. Mycology 9:81–92

Pansanit A, Pripdeevech P (2018) Antibacterial secondary metabolites from an endophytic fungus, *Arthrinium* sp. MFLUCC16-1053 isolated from *Zingiber cassumunar*. Mycology 9:264–272

Park SY, Song HH, Lee YG, Yoon CS, Lee C (2008) Biological activities and partial characterization of *Beauveria bassiana* mycelium. Food Sci Biotechnol 17(1):95–101

Patil SA, Sivanandhan G, Thakare DB (2015) Effect of physical and chemical parameters on the production of red exopigment from *Penicillium purpurogenum* isolated from spoilt onion and study of its antimicrobial activity. Int J Curr Microbiol Appl Sci 4:599–609

Patocka J (2016) Bioactive metabolites of entomopathogenic fungi *Beauveria bassiana*. Mil Med Sci Lett 85(2):80–88

Pattanathu K, Rahman M, Gapke M (2008) Production, characterisation and applications of biosurfactants – review. Biotechnology 2:360–370

Pinheiro EA, Pina JR, Feitosa AO, Carvalho JM, Borges FC, Marinho PS et al (2017) Bioprospecting of antimicrobial activity of extracts of endophytic fungi from *Bauhinia guianensis*. Rev Argent De Microbiol 49:3–6

Pirôllo MPS (2006) Estudo da produção de biossurfactantes utilizando hidrocarbonetos. Master's thesis, Instituto de Biociências-Universidade Estadual Paulista (Unesp), Sao Paulo, Brazil

Plassard C, Fransson P (2009) Regulation of low-molecular weight organic acid production in fungi. Fungal Biol Rev 23(1):30–39

Pombeiro-Sponchiado SR, Sousa GS, Andrade JC, Lisboa HF, Gonçalves RC (2017) Production of melanin pigment by fungi and its biotechnological applications. In: Blumenberg M (ed) Melanin. In Tech Open, London, UK, pp 45–75

Poorniammal R, Parthiban M, Gunasekaran S, Murugesan R, Thilagavathi G (2013) Natural dye production from Thermomyces sp. fungi for textile application. Indian J Fibre Text Res 38:276–279

Poorniammal R, Prabhu S, Sakthi AR (2019a) Evaluation of in vitro antioxidant activity of fungal pigments. Pharma Innov J 8:326–330

Poorniammal R, Prabhu S, Sakthi AR, Gunasekaran S (2019b) Subacute dermal toxicity of *Thermomyces* sp. and *Penicillium purpurogenum* pigments in wistar rats. Int J Chem Stud 7:630–634

Prabhu KS, Siveen KS, Kuttikrishnan S, Iskandarani AN, Khan AQ, Merhi M et al (2018) Greensporone C, a freshwater fungal secondary metabolite induces mitochondrial-mediated apoptotic cell death in leukemic cell lines. Front Pharmacol 720:1–10

Prathiban M, Thilagavathi G, Viju S (2016) Development of antibacterial silk sutures using the natural fungal extract for healthcare applications. J Text Sci Eng 6:1–4

Premjanu N, Jaynthy C (2014) Antioxidant activity of endophytic fungi isolated from *Lannea coromendalica*. Int J Res Pharm Sci 5(4):304–308

Pulici M, Sugawara F, Koshino H, Uzawa J, Yoshida S, Lobkovsky E, Clardy J (1996) Pestalotiopsins A and B: new caryophyllenes from an endophytic fungus of *Taxus brevifolia*. J Org Chem 61:2122–2124

Raghunath R, Radhakrishna A, Angayarkanni J, Palaniswamy M (2012) Production and cytotoxicity studies of lovastatin from *Aspergillus niger* PN2 an endophytic fungi isolated from *Taxus baccata*. Int J Appl Biol 3:342–351

Ramesh C, Vinithkumar NV, Kirubagaran R, Venil CK, Dufossé L (2019) Multifaceted applications of microbial pigments: current knowledge, challenges and future directions for public health implications. Microorganisms 7:2–46

Ramos AA, Prata-Sena M, Castro-Carvalho B, Dethoup T, Buttachon S, Kijjoa A, Rocha E (2015) Potential of four marine-derived fungi extracts as anti-proliferative and cell death-inducing agents in seven human cancer cell lines. Asian Pac J Trop Med 8(10):798–806

Rao MPN, Xiao M, Li WJ (2017) Fungal and bacterial pigments: secondary metabolites with wide application. Front Microbiol 8:1–13

Rastegari AA, Yadav AN, Yadav N (2019a) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5

Rastegari AA, Yadav AN, Yadav N, Tataei Sarshari N (2019b) Bioengineering of secondary metabolites. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 55–68. https://doi.org/10.1016/B978-0-444-63504-4.00004-9

Ratna PBS (2012) Pollution due to synthetic dyes toxicity and carcinogenicity studies and remediation. Int J Environ Sci 3:940–955

Ratnaweera PB, Williams DE, Patrick BO, de Silva ED, Andersen RJ (2015) Solanioic acid, an antibacterial degraded steroid produced in culture by the fungus *Rhizoctonia solani* isolated from tubers of the medicinal plant *Cyperus rotundus*. Org Lett 17:2074–2077

Ravindran K, Sathishkumar G, Rajkuberan C, Sivaramakrishnan S (2014) Antibacterial activity of indigenous entomopathogenic fungus *Metarhizium anisopliae* against clinically isolated human pathogens. Int J Pharm Sci 6:179–182

Raza ZA, Rehman A, Hussain MT et al (2014) Production of rhamnolipid surfactant and its application in bioscouring of cotton fabric. Carbohydr Res 391(1):97–105

Rehman A, Raza ZA, Saif-ur-Rehman et al (2010) Synthesis and use of self-assembled rhamnolipid microtubules as templates for gold nanoparticles assembly to form gold microstructures. J Colloid Interface Sci 347(2):332–335

Robinson SC (2012) Developing fungal pigments for "painting" vascular plants. Appl Microbiol Biotechnol 93:1389–1394

Robinson S (2014) The fine art of decay. Am Sci 102:206–213

Robinson SC, Tudor D, Cooper PA (2012) Utilizing pigment-producing fungi to add commercial value to American beech (*Fagus grandifolia*). Appl Microb Biotechnol 93:1041–1048

Robinson SC, Tudor D, Zhang WR, Ng S, Cooper PA (2014) Ability of three yellow pigment producing fungi to colour wood under controlled conditions. Int Wood Prod J 5:103–107

Robinson SC, Michaelsen H, Robinson JC (2016) Spalted wood, history, science and art of an unique material. Schiffer Publishing, Atglen, PA, pp 1–288

Rodrigues C, Vandenberghe LPDS, de Oliveira J, Soccol CR (2012) New perspectives of gibberellic acid production: a review. Crit Rev Biotechnol 32(3):263–273

Rosa LH, Machado KM, Rabello AL, Souza-Fagundes EM, Correa-Oliveira R, Rosa CA et al (2009) Cytotoxic, immunosuppressive, trypanocidal and antileishmanial activities of Basidiomycota fungi present in Atlantic Rainforest in Brazil. Antonie Van Leeuwenhoek 95:227–237

Rosa LH, Gonçalves VN, Caligiorne RB, Alves TM, Rabello A, Sales PA et al (2010) Leishmanicidal, trypanocidal, and cytotoxic activities of endophytic fungi associated with bioactive plants in Brazil. Braz J Microbiol 41:420–430

Rufino RD, Sarubbo LA, Campos-Takaki GM (2007) Enhancement of stability of biosurfactant produced by *Candida lipolytica* using industrial residue as substrate. World J Microbiol Biotechol 23:729–734

Saad AA, Khalil MT, Ragab FMA, Mekawey AAI, Abdel-Wareth MTA (2014) Efficacy of the fungi *Aspergillus terreus* and *Penicillium janthinellum* as biological control agents against *Biomphalaria alexandrina* snails. Int J Environ Sci Eng 5:27–39

Sadrati N, Daoud H, Zerroug A, Dahamna S, Bouharati S (2013) Screening of antimicrobial and antioxidant secondary metabolites from endophytic fungi isolated from wheat (*Triticum durum*). J Plant Protect Res 53(2):128–136

Sahu MK, Kaushik K, Das A, Jha H (2020) In vitro and in silico antioxidant and antiproliferative activity of rhizospheric fungus *Talaromyces purpureogenus* isolate-ABRF2. Bioresour Bioprocess 7:1–16

Sajid S, Akber N (2018) Applications of fungal pigments in biotechnology. Pure Appl Biol 7:922–930

Sakaki T, Shibata M, Mukai K, Sakai M, Wakamatsu K, Miyauchi S (2002) *Chlorociboria aeruginosa* pigment as algicide. Jpn Kokai Tokkyo Koho JP 2002:291493

Sakuradani E, Hirano Y, Kamada N, Nojiri M, Ogawa J, Shimizu S (2004) Improvement of arachidonic acid production by mutants with lower n-3 desaturation activity derived from *Mortierella alpina* 1S-4. Appl Microbiol Biotechnol 66:243–248

Saleem M, Hussain H, Ahmed I, Draeger S, Schulz B, Meier K, Steinert M, Pescitelli G, Kurtán T, Flörke U et al (2011) Viburspiran, an antifungal member of the octadride class of maleic anhydride natural products. Eur J Org Chem 2011:808–812

Samanta AK, Agarwal P (2009) Application of natural dyes on textiles. Indian J Fibre Text Res 34:384–399

Santiago IF, Alves TM, Rabello A, Sales Junior PA, Romanha AJ, Zani CL et al (2012) Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms Deschampsia antarctica Desv and *Colobanthus quitensis* (Kunth) Bartl. Extremophiles 16:95–103

Saraiva NN, Rodrigues BS, Jimenez PC, Guimarães LA, Torres MC, Rodrigues-Filho E et al (2015) Cytotoxic compounds from the marine-derived fungus *Aspergillus* sp. recovered from the sediments of the Brazilian coast. Nat Prod Res 29(16):1545–1550

Saravanan D, Radhakrishnan M (2016) Antimicrobial activity of pigments produced by fungi from the Western Ghats. J Chem Pharm Res 8:634–638

Sarkar SL, Saha P, Sultana N, Akter S (2017) Exploring textile dye from microorganisms, an eco-friendly alternative. Microbiol Res J Int 18:1–9

Sarubbo LA, Farias CB, Campos-Takaki GM (2007) Co-utilization of canola oil and glucose on the production of a surfactant by *Candida* lipolytica. Curr Microbiol 54:68–73

Sen T, Barrow CJ, Deshmukh SK (2019) Microbial pigments in the food industry-challenges and the way forward. Front Nutr 6:1–14

Sena HH, Sanches MA, Rocha DFS, Segundo Filho WOP, de Souza ÉS, de Souza JVB (2018) Production of biosurfactants by soil fungi isolated from the Amazon forest. Int J Microbiol 2018:1–8

Seyedin A, Yazdian F, Hatamian-Zarmi A, Rasekh B, Mir-derikvand M (2015) Natural pigment production by Monascus purpureus: bioreactor yield improvement through statistical analysis. Appl Food Biotechnol 2:23–30

Shaaban M, Nasr H, Hassan AZ, Asker MS (2013) Bioactive secondary metabolities from endophytic *Aspergillus fumigatus*: structural elucidation and bioactivity studies. Rev Latinoame Quími 41(1):50–60

Shah SG, Shier WT, Tahir N, Hameed A, Ahmad S, Ali N (2014) Penicillium verruculosum SG: a source of polyketide and bioactive compounds with varying cytotoxic activities against normal and cancer lines. Arch Microbiol 196:267–278

Sharma D, Gupta C, Aggarwal S, Nagpal N (2012) Pigment extraction from fungus for textile dyeing. Indian J Fibre Text Res 37:68–73

Sharma A, Sharma S, Dalela M (2014) Nematicidal activity of *Paecilomyces lilacinus* 6029 cultured on Karanja cake medium. Microb Pathog 75:16–20

Sharma A, Sharma S, Mittal A, Naik SN (2016) Evidence for the involvement of nematocidal toxins of *Purpureocillium lilacinum* 6029 cultured on Karanja deoiled cake liquid medium. World J Microbiol Biotechnol 32:1–7

Sibero MT, Triningsih DW, Radjasa OK, Sabdono A, Trianto A (2016) Evaluation of antimicrobial activity and identification of yellow pigmented marine sponge-associated fungi from Teluk Awur, Jepara, Central Java. Indones J Biotechnol 21:1–11

Sibero MT, Herdikiawan D, Radjasa OK, Sabdono A, Trianto A, Triningsih DW (2018) Antibacterial activity of sponge associated fungi against *vibriosis* agents in shrimp and its toxicity to *Litopenaeus vannamei*. AACL Bioflux 11(1):10–18

Sijbesma F, Schepens H (2003) White biotechnology: gateway to a more sustainable Future. EuropaBio, Brussels, Belgium

Silva ALL, Rodrigues C, Costa JL, Machado MP, Penha RO, Biasi LA (2013) Gibberellic acid fermented extract obtained by solid-state fermentation using citric pulp by *Fusarium moniliforme*: influence on Lavandula angustifolia Mill. cultivated in vitro. Pak J Bot 45:2057–2064

Simpson BK, Benjakul S, Klomklao S (2012) Natural food pigments. In: Simpson BK (ed) Food biochemistry and food processing, 2nd edn. Wiley-Blackwell, Hoboken, NJ, pp 704–722

Singh SB, Zink DL, Liesch JM, Dombrowski AW, Darkin-Rattray SJ, Schmatz DM, Goetz MA (2001) Structure, histone deacetylase, and antiprotozoal activities of apicidins B and C, congeners of apicidin with proline and valine substitutions. Org Lett 3:2815–2818

Soumya K, Narasimha Murthy K, Sreelatha GL, Tirumale S (2018) Characterization of a red pigment from *Fusarium chlamydosporum* exhibiting selective cytotoxicity against human breast cancer MCF-7 cell lines. J Appl Microbiol 125:148–158

Souza PNC, Grigoletto TLB, Moraes LAB, Abreu LM, Souza LH, Santos C, Galvao LR, Cardoso PG (2016) Production and chemical characterization of pigments in filamentous fungi. Microbiology 162:12–22

Srivastava A, Anandrao RK (2015) Antimicrobial potential of fungal endophytes isolated from leaves of *Prosopis juliflora* (SW.) DC. an important weed. Int J Pharm Sci 7:128–136

Stahmann KP, Revuelta JL, Seulberger H (2000) Three biotechnical processes using *Ashbya gossypii*, *Candida famata*, or *Bacillus subtilis* compete with chemical riboflavin production. Appl Microbiol Biotechnol 53(5):509–516

Strader CR, Pearce CJ, Oberlies NH (2011) Fingolimod (FTY720): a recently approved multiple sclerosis drugs based on a fungal secondary metabolite. J Nat Prod 74:900–907

Strobel GA, Ford E, Worapong J, Harper JK, Arif AM, Grant D et al (2002) Ispoestacin, an isobenzofuranone from *Pestalotiopsis microspora* possessing antifungal and antioxidant activities. Phytochemistry 60:179–184

Strobel GA, Knighton B, Kluck K, Ren Y, Livinghouse T, Griffin M et al (2008) The production of myco-diesel hydrocarbons and their derivatives by the endophytic fungus *Gliocladium roseum* (NRRL 50072). Microbiology 154:3319–3328

Su NW, Lin YL, Lee MH, Ho CY (2005) Ankaflavin from *Monascus*-fermented red rice exhibits selective cytotoxic effect and induces cell death on Hep G2 cells. J Agric Food Chem 53:1949–1954

Sugijanto NE, Dorra BL (2016) Antimicrobial activity of *Cladosporium oxysporum* endophytic fungus extract isolated from *Aglaia odorata Lour*. Indones J Med 1(2):108–115

Suresha BS, Srinivasan K (2013) Antioxidant properties of fungal metabolite nigerloxin in vitro. Appl Biochem Microbiol 49(6):587–591

Svahn KS, Göransson U, El-Seedi H, Bohlin L, Larsson DJ, Olsen B et al (2012) Antimicrobial activity of filamentous fungi isolated from highly antibiotic-contaminated river sediment. Inf Ecol Epidemiol 2:1–7

Swathi J, Narendra K, Sowjanya KM, Krishna SA (2013) Biological characterisation of secondary metabolites from marine fungi *Microascus* sp. Int J Res Pharm Biomed Sci 4:754–759

Tam WTE, Tsang CC, Lau KPS, Woo CYP (2015) Polyketides, toxins, and pigments in *Penicillium marneffei*. Toxins 7:4421–4436

Tenguria RK, Firodiya AD (2016) Cytotoxic activities of endophytic fungi isolated from central region of Madhya Pradesh. J Innov Pharm Biol Sci 3:29–38

Thamilvanan D, Ramkumar A, Ramesh R, Balakumar BS, Kumaresan S (2018) *In vitro* antibacterial activity of the soil fungal metabolites. Int Res J Pharm 9:175–181

Thavasi R, Jayalakshmi S, Balasubramanian T, Banat IM (2007) Biosurfactant production by *Corynebacterium kutscheri* from wastemotor lubricant oil and peanut oil cake. Lett Appl Microbiol 45(6):686–691

Tomoda H, Doi T (2008) Discovery and combinatorial synthesis of fungal metabolites beauveriolides, novel antiatherosclerotic agents. Acc Chem Res 41(1):32–39

Tudor D (2013) Fungal pigment formation in wood substrate. Ph.D. Thesis, Faculty of Forestry, University of Toronto, p 21

Tudor D, Robinson SC, Cooper PA (2013) The influence of pH on pigment formation by *lignicolous* fungi. Int Biodeterior Biodegrad 80:22–28

Tuli HS, Chaudhary P, Beniwal V, Sharma AK (2015) Microbial pigments as natural color sources: current trends and future perspectives. J Food Sci Technol 52:4669–4678

Vagelos P (1991) Are prescription drug prices high? Science 252:1080–1084

Vega Gutierrez P, Robinson S (2017) Determining the presence of spalted wood in Spanish marquetry woodworks of the 1500s through the 1800s. Coatings 7:188

Velioglu Z, Urek RO (2015) Biosurfactant production by *Pleurotus ostreatus* in submerged and solid-state fermentation systems. Turkish J Biol 39(1):160–166

Velmurugan P, Kim MJ, Park JS, Karthikeyan K, Lakshmanaperumalsamy P, Lee KJ et al (2010) Dyeing of cotton yarn with five water-soluble fungal pigments obtained from five fungi. Fibers Polym 11:598–605

Vendruscolo F, Tosin I, Giachini AJ, Schmidell W, Ninow JL (2014) Antimicrobial activity of *Monascus* pigments produced in submerged fermentation. J Food Process Preserv 38:1860–1865

Vendruscolo F, Buhler RMM, de Carvalho JC, de Oliveira D, Moritz DE, Schmidell W et al (2016) *Monascus*: a reality on the production and application of microbial pigments. J Appl Biochem Biotechnol 178:211–223

Villa FA, Gerwick L (2010) Marine natural product drug discovery: leads for treatment of inflammation, cancer, infections, and neurological disorders. Immunopharmacol Immunotoxicol 32:228–237

Wang CC, Chiang YM, Kuo PL, Chang JK, Hsu YL (2008) Norsolorinic acid from *Aspergillus nidulans* inhibits the proliferation of human breast adenocarcinoma MCF-7 cells via Fas-mediated pathway. Basic Clin Pharmacol Toxicol 102:491–497

Wang YG, Yang GR, Wang F, Yang MJ, Li J, Liu XF et al (2016) Antioxidant activity of secondary metabolites and mycelium extracts of endophytic fungi isolated from *Astragalus monadelphus*. Bulgarian Chem Commun 48(4):787–792

Wang W, Liao Y, Chen R, Hou Y, Ke W, Zhang B et al (2018) Chlorinated azaphilone pigments with antimicrobial and cytotoxic activities isolated from the deep sea-derived fungus *Chaetomium* sp. NA–S01–R1. Mar Drugs 16:1–11

Warude D, Joshi K, Harsulkar A (2006) Polyunsaturated fatty acids: biotechnology. Crit Rev Biotechnol 26:83–93

Weber D, Gorzalczany S, Martino V, Acevedo C, Sterner O, Anke T (2005) Metabolites from endophytes of the medicinal plant Erythrina crista-galli. Z Naturforsch C 60:467–477

Weber G, Chen HL, Hinsch E, Freitas S, Robinson S (2014) Pigments extracted from the wood-staining fungi *Chlorociboria aeruginosa*, *Scytalidium cuboideum*, and *S. ganodermophthorum* show potential for use as textile dyes. Color Technol 130:445–452

Werpy T, Petersen G (2004) Top value added chemicals from biomass. In: Werpy T, Petersen G (eds) Results of screening for potential candidates from sugars and synthesis gas, vol 1. U.S. Department of Energy; NREL/TP-510-35523, Washington, DC

WHO (2016) Schistosomiasis: number of people treated worldwide in 2014. Wkly Epidemiol Rec 91:53–60

Williams K (2009) Biosurfactants for cosmetic applications: overcoming production challenges. MMG 445 Basic Biotechnol 5:78–83

Xia W, Chen W, Peng WF, Li KT (2015) Industrial vitamin B12 production by *Pseudomonas denitrificans* using maltose syrup and corn steep liquor as the cost-effective fermentation substrates. Bioprocess Biosyst Eng 38(6):1065–1073

Xiao ZE, Huang HR, Shao CL, Xia XK, Ma L, Huang XS et al (2013) Asperterpenols A and B, new sesterterpenoids isolated from a mangrove endophytic fungus *Aspergillus* sp. 085242. Org Lett 15:2522–2525

Yadav M, Yadav A, Yadav JP (2014) In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana* Lam. Asian Pac J Trop Med 7:S256–S261

Yadav AN, Kour D, Rana KL, Yadav N, Singh B, Chauhan VS et al (2019) Metabolic engineering to synthetic biology of secondary metabolites production. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 279–320. https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Yan BC, Wang WG, Hu DB, Sun X, Kong LM, Li XN et al (2016) Phomopchalasins A and B, two cytochalasans with polycyclic-fused skeletons from the endophytic fungus *Phomopsis* sp. shj2. Org Lett 18:1108–1111

Yodsing N, Lekphrom R, Sangsopha W, Aimi T, Boonlue S (2018) Secondary metabolites and their biological activity from *Aspergillus aculeatus* KKU-CT2. Curr Microbiol 75(5):513–518

Yolmeh M, Hamedi H, Khomeiri M (2016) Antimicrobial activity of pigments extracted from Rhodotorula glutinis against some bacteria and fungi. Zahedan J Res Med Sci 18:1–7

Yu Z, Zhang B, Sun W, Zhang F, Li Z (2013) Phylogenetically diverse endozoic fungi in the South China Sea sponges and their potential in synthesizing bioactive natural products suggested by PKS gene and cytotoxic activity analysis. Fungal Divers 58(1):127–141

Yu G, Zhou G, Zhu M, Wang W, Zhu T, Gu Q et al (2016) Neosartoryadins A and B, fumiquinazoline alkaloids from a mangrove-derived fungus Neosartorya udagawae HDN13-313. Org Lett 18:244–247

Yue Y, Yu H, Li R, Xing R, Liu S, Li P (2015) Exploring the antibacterial and antifungal potential of Jellyfish-associated marine fungi by cultivation-dependent approaches. PLoS One 10(12):e0144394

Zhang W, Krohn K, Zia U, Florke U, Pescitelli G, Di Bari L et al (2008a) New mono- and dimeric members of the secalonic acid family: Blennolides A–G isolated from the fungus *Blennoria* sp. Chem Eur J 14:4913–4923

Zhang HW, Huang WY, Chen JR, Yan WZ, Xie DQ, Tan RX (2008b) Cephalosol: an antimicrobial metabolite with an unprecedented skeleton from endophytic *Cephalosporium acremonium* IFB-E007. Chem Eur J 14:10670–10674

Zhang AH, Jiang N, Gu W, Ma J, Wang YR, Song YC et al (2010) Characterization, synthesis and self-aggregation of (−)-alternarlactam: a new fungal cytotoxin with cyclopentenone and isoquinolinone scaffolds. Chem Eur J 16(48):14479–14485

Zhang HC, Ma YM, Liu R, Zhou F (2012) Endophytic fungus *Aspergillus tamarii* from Ficus carica L., a new source of indolyl diketopiperazines. Biochem Syst Ecol 45:31–33

Zhang DW, Ge HL, Xie D, Chen RD, Zou JH, Tao XY et al (2013) Periconiasins A–C, new cytotoxic cytochalasans with an unprecedented 9/6/5 tricyclic ring system from endophytic fungus *Periconia* sp. Org Lett 15:1674–1677

Zhang Q, Xiao J, Sun QQ, Qin JC, Pescitelli G, Gao JM (2014a) Characterization of cytochalasins from the endophytic *Xylaria* sp. and their biological functions. J Agric Food Chem 62:10962–10969

Zhang P, Mandi A, Li XM, Du FY, Wang JN, Li X et al (2014b) Varioxepine A, a 3H-oxepine-containing alkaloid with a new oxa-cage from the marine algal-derived endophytic fungus *Paecilomyces variotii*. Org Lett 16:4834–4837

Zhang D, Ge H, Zou JH, Tao X, Chen R, Dai J (2014c) Periconianone A, a new 6/6/6 carbocyclic sesquiterpenoid from endophytic fungus *Periconia* sp. with neural anti-inflammatory activity. Org Lett 16:1410–1413

Zhang D, Tao X, Chen R, Liu J, Li L, Fang X et al (2015) Pericoannosin A, a polyketide synthase-nonribosomal peptide synthetase hybrid metabolite with new carbon skeleton from the endophytic fungus *Periconia* sp. Org Lett 17:4304–4307

Zhang D, Tao X, Liu J, Chen R, Zhang M, Li L et al (2016) Periconiasin G, a new cytochalasan with unprecedented 7/6/5 tricyclic ring system from the endophytic fungus *Periconia* sp. Tetrahedron Lett 57:796–799

Zhao H, Cai C, Liu X, Jiao B, Chen B, Cai M et al (2018a) Secondary metabolites of Antarctic fungi antagonistic to aquatic pathogenic bacteria. Open Life Sci 13(1):11–21

Zhao DL, Wang D, Tian XY, Cao F, Li YQ, Zhang CS (2018b) Anti-phytopathogenic and cytotoxic activities of crude extracts and secondary metabolites of marine-derived fungi. Mar Drugs 16(1):36

Zheng L, Cai Y, Zhou L, Huang P, Ren X, Zuo A et al (2017) Benzoquinone from *Fusarium* pigment inhibits the proliferation of estrogen receptor-positive MCF-7 cells through the NF-kB pathway via estrogen receptor signalling. Int J Mol Med 39:39–46

Zhou M, Miao MM, Du G, Li XN, Shang SZ, Zhao W et al (2014) Aspergillines A–E, highly oxygenated hexacyclic indole-tetrahydrofuran-tetramic acid derivatives from *Aspergillus versicolor*. Org Lett 16:5016–5019

Zohri AA, Moharram AM, Abd El-Ghani OA (2017) Antioxidant potentialities of some strains belonging to endophytic, entomopathogenic and saprophytic fungi. Eur J Biol Res 7(1):76–85

Zu Berstenhorst SM, Hohmann HP, Stahmann KP (2009) Vitamins and vitamin-like compounds. In: Schaechter M (ed) Microbial production A2 - encyclopedia of microbiology, 3rd edn. Academic, Oxford, pp 549–561

Chapter 9
Bioprospecting of Thermophilic Fungal Enzymes and Potential Applications

Bassem A. Balbool, Fatma Ahmed Abo Nouh, Sara Atef Gezaf, Akram H. Mohamed, and Ahmed M. Abdel-Azeem

Contents

B. A. Balbool (✉)
Faculty of Biotechnology, October University for Modern Sciences and Arts, Giza, Egypt

F. A. Abo Nouh · A. M. Abdel-Azeem
Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

S. A. Gezaf
Botany and Microbiology Department, Faculty of Science, Arish University, North Sinai, Egypt

A. H. Mohamed
Microbial Genetic Resources Department, National Gene Bank, Agriculture Research Center, Giza, Egypt

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_9

9.1 Introduction

Enzymes are protein in nature they catalysis a specific reaction and act on specific substrates; they play various roles in medicine, industry, and agriculture. Enzymes are classified into many categories; they would be excreted extracellular or intracellular within eukaryotes, few fungal species can grow in temperatures ranging from 45 to 55 °C (Saxena et al. 2016; Suman et al. 2015). This species would be thermophilic or thermotolerant taxa which can be easily differentiated according to their minimum and maximum growing temperatures; thermophilic fungal species grow in between 20 to or above 50 °C, while thermotolerant species grow from or below 20 to 55 °C (Kumar et al. 2014a, b). Thermophilic fungi resemble chief components of the microflora that develops in heaped masses of plant material, piles of agricultural and forestry products, and other accumulations of organic matter wherein the warm, humid, and aerobic environment provides the basic conditions for their development. Although the thermophilicity of such fungal species didn't receive much attention as they don't inhabit toxic habitats and their degree of thermophily isn't very high as other archaebacteria and bacterial species which would reach 100 °C.

As an example of fungal producing enzymes; endophytes usually produce the enzymes necessary for the colonization of plant tissues. It has been demonstrated that most endophytes are able to utilize at least in vitro most plant nutrients and cell components. Most of the investigated endophytes utilize xylan and pectin, show lipolytic activity, and produce non-specific peroxidases and laccases, chitinase, and glucanase (Leuchtmann et al. 1992; Promputtha et al. 2011; Rana et al. 2019). Endophytes may be a novel and good producer of xylanase and the production of extracellular cellulase and hemicellulases other than xylanases are widespread but usually limited to organisms derived from selected hosts or even host tissues (Leuchtmann et al. 1992). Thermostable amylolytic enzymes are being investigated to improve industrial processes for starch degradation. *Streptosporangium* sp. an endophytic actinomycete isolated from leaves of maize (*Zea mays* L.) showed glucoamylase production. The isolated enzyme exhibited thermostable properties (Stamford et al. 2002). The ability of endophytes to produce various enzymes in vivo and in vitro means that the host supplies nutrients as well as habitats for endophyte colonization, and could be used for various biotechnological applications (Tomita 2003).

9.2 Biodiversity of Thermophilic Fungi

Thermophilic fungi are a small assemblage in eukaryote that have a unique mechanism of growing at elevated temperatures extending up to 60–62 °C. Cooney and Emerson's definition of thermophilic fungi is those that have a maximum temperature for growth at or above 50 °C and a minimum temperature for growth at or above

20 °C (Cooney and Emerson 1964; Yadav et al. 2020b). According to several reports, they have been found thermophilic fungi in a variety of environments. These include: composts, piles of hays, stored grains, wood chip piles, nesting material of birds and animals, snuff, and municipal refuse, and other accumulations of organic matter wherein the warm, humid, and aerobic environment provides the basic physiological conditions for their development (De Gannes et al. 2013). Tansey and Brock (1972) observed that thermophilic fungi are much more common in acid thermal habitats than those of neutral to alkaline pH. Thermophilic fungi have been recovered even from Antarctic soils (Satyanarayana et al. 1992). Also, thermophilic fungi are common in habitats wherever decomposition of organic matter takes place Hultman et al. (2010).

Thermophilic fungi are cosmopolitan and they may occur either as propagules or as active mycelia in both natural and human-made environments. Their growth and activity are mainly regulated by the temperature and availability of nutrients. Some authors suggest that the heat tolerance in fungi evolved from mesophilic ancestors associated with nests of birds able to thermoregulate their nests (Rajasekaran and Maheshwari 1993).

Fungi play an important role in this system; their exothermic metabolism raises the nest temperature to approximately 45 °C, similar to the heating process in natural composting (Tiquia et al. 1996, 2002; Abdel-Azeem et al. 2021; Yadav et al. 2019a). Thermophilic fungi are usually found in a self-heating environment. The composting system, where the temperature rises due to the exothermic metabolism of microorganisms, is by far the most suitable environment for their growth and dispersal. These fungi occur mainly from man-made environments, such as composting systems, due to the production of aerosols carrying mycelia or reproductive propagules when revolving the piles (Le Goff et al. 2010). Most species do not show any geographical restrictions. According to Salar and Aneja (2006) soils in tropical countries do not appear to have a higher population of thermophilic fungi than soils in temperate countries as believed earlier.

A large contribution to the taxonomy, biology, and economic importance of thermophilic fungi was given by Cooney and Emerson (1964) in their monograph. Eleven thermophilic species were documented with few being new to science (*Rhizomucor pusillus*, *R. miehei*, three varieties of *Chaetomium thermophilum* and *Chaetomium virginicum*, *Thermoascus aurantiacus*, *Melanocarpus albomyces*, *Malbranchea cinnamomea*, *Mycothermus thermophilus*, *Thermomyces lanuginosus*). Thermophily is found in taxa in different phylogenetic lineages in the fungal tree of life. A recent phylogenetic analysis showed the paraphyletic nature of heat tolerance in fungi (Morgenstern et al. 2012). It is clear that this ability had multiple origins in the kingdom of Fungi. However, in Chaetomiaceae (Sordariales), thermophily probably had a single origin and then multiple losses subsequently occurred within the family, whereas multiple independent gains seem to be more likely in Trichocomaceae (Eurotiales) (van Noort et al. 2013).

The thermophilic fungi comprise only 44 species belonging to 20 genera are represented in phylum Ascomycota, Mucoromycota, and Basidiomycota. In the Ascomycota, thermophiles are restricted to the orders Sordariales, Eurotiales, and

Onygenales. In the Sordariales, all known thermophilic species belong to the family Chaetomiaceae, which contains the greatest diversity of thermophilic fungi (Morgenstern et al. 2012). Among the Eurotiales, two families are considered to possess thermophilic members, the Trichocomaceae and the Thermoascaceae (Houbraken et al. 2014). A sole species of thermophilic fungus *Malbranchea cinnamomea* is found in the Onygenales (Morgenstern et al. 2012).

The thermophilic fungi in phylum Ascomycota particularly in Sordariales and Eurotiales orders, for example (*Acremonium*, *Arthrinium*, *Canariomyces*, *Chaetomidium*, *Chaetomium*, *Humicola*, *Malbranchea*, *Melanocarpus*, *Myceliophthora*, *Myriococcum*, *Rasamsonia*, *Remersonia*, *Scytalidium*, *Sordaria*, *Thermoascus*, *Thermomyces*, and *Thielavia*) that comprise the largest number of thermophilic species (Oliveira et al. 2015). Thermophiles in the Mucoromycota occur in the Mucorales and a recently created order, the Calcarisporiellales (Hirose et al. 2012). The order Mucorales contains two families with thermophiles, the Rhizopodaceae and the Lichtheimiaceae (Hoffmann et al. 2013), which is restricted to the two genera in Mucorales order (e.g., *Rhizomucor* and *Thermomucor*) (Oliveira et al. 2015). The Calcarisporiellales contains the thermophilic species *Calcarisporiella thermophile.* Thermophilic Basidiomycota have been described by Straatsma et al. (1994) genera in Basidiomycota belong to the order Polyporales (e.g., *Thermophymatospora*) (Oliveira et al. 2015). Currently, the total number of fungal species described is approximately 120,000 (Hawksworth and Lücking 2017). Among the species of thermophilic fungi are found *T. lanuginosus* and *Talaromyces thermophilus* which grow at 50 °C (Romdhane et al. 2010), and *Rhizomucor miehei* and *Myceliophthora* species which grow at 45 °C (Fawzi 2011).

9.3 Fungal Adaptations to Thermophily

Adaptations to tolerate adverse conditions such as extreme pH, high salt concentrations, and high temperatures are inherent in a few microorganisms (Gomes and Steiner 2004). Of all the factors affecting cell stability, temperature has the greatest influence on the function of biomolecules and the maintenance of biological structures, and most organisms can only grow within a narrow temperature range. However, the existence of geothermal stable environments allows for the selection and persistence of microorganisms that not only resist but also require high temperatures to survive (Van Noort et al. 2013).

Sustaining growth at high temperatures involves the adaptation of the cytoplasmic membrane, proteins, and DNA to temperatures above the mesophilic range. These adaptations provoked great interest from both biological and evolutionary perspectives, considering that the thermoresistance mechanisms of biomolecules of these microorganisms may be interesting models for bioengineering or to directly use in bioprocesses. In general, all the features observed in thermophilic fungi are similar to those of mesophiles. Thermophilic fungi do not appear to have any

specific organelles, structural modifications, or developmental patterns, which are not seen in their mesophilic counterparts (Singh et al. 2016).

Many organisms vary the fatty acid composition of their membrane phospholipids as a function of growth temperature so that their membrane fluidity is kept constant for the optimal functioning of membrane-localized transporters and enzymes. The adaptation of thermophilic microbial membranes corresponds with the process called homeoviscous adaptation, which consists of the replacement of unsaturated fatty acids with saturated fatty acids such that the membrane acquires the balance between density and fluidity necessary for the maintenance of physical and functional integrity at elevated temperatures. This adaptation occurs in the domains *Bacteria* and *Eukarya*, while the latter is found only in the Kingdom Fungi (Adams 1993). For example, with an increase in temperature, there is an increase in the proportion of saturated fatty acids incorporated into phospholipids, whereas, at a lower temperature, a higher proportion of unsaturated fatty acids is incorporated (Sinensky 1974). Crisan (1973) mentions four hypotheses that help to explain the ability of thermophiles to grow at high temperatures: (1) lipid solubilization, (2) rapid resynthesis of essential metabolites, (3) macromolecular thermostability, and (4) ultrastructural thermostability. Only the latter two still appear to be of major importance.

Two evolutionary strategies seem to define thermostability: (1) intrinsic factors or factors directly associated with the structure of the molecule, leading to stiffness and folding and (2) extrinsic factors that help to stabilize the proteins in a given environment, including solutes, binders, molecular chaperones, and the substrate it (Bruins et al. 2001). Extracellular proteins of filamentous fungi are mainly depolymerizing enzymes that in thermophilic environments must have dynamic and kinetic thermostability to be active. Some differences in the sequence, structure, function, dynamics, and thermodynamic properties can be observed between the psychrophilic, mesophilic, and thermophilic enzymes (Niehaus et al. 1999). The cytoplasmic membranes of thermophilic organisms consist of saturated fatty acids that confer increased stability and physical and functional integrity, unlike those of mesophilic organisms, which have unsaturated fatty acids.

The maintenance of DNA structure is an essential factor for all organisms, especially for hyperthermophilic which survive in high-temperature environments (Gomes and Steiner 2004). Wright et al. 1983 examined if an inability to regulate membrane fluidity may be a reason for the high minimum temperature of growth of thermophilic fungi. Firstly, *Talaromyces thermophilus* was shifted from a high (50 °C) to a low (33 °C) growth temperature, the degree of unsaturation of fatty acids at the two stated temperatures remained virtually unchanged. It is resulted because of a metabolic limitation, presumably due to a nonfunctional fatty acid desaturase, which restricted the ability of the fungus to convert oleate to linoleate at low temperature. In the second fungi, *T. lanuginosus* were different; the concentration of linoleic acid (18:2) was twofold higher at 30 than at 50 °C. The degree of unsaturation of phospholipid fatty acids was 0.88 in mycelia grown at 50 °C but 1.0 in the temperature-shifted cultures (from 50 to 30 °C) and 1.06 in cultures grown at constant 30 °C (Rajasekaran and Maheshwari 1990). Marguet and Forterre (1998)

reported that 2,3-DPG prevents depurination or depyrimidation of DNA, which causes mutations at high temperatures.

In addition, all hyperthermophilic organisms produce a different form of reverse DNA topoisomerase called DNA gyrase, which introduces positively supercoiled DNA. The positive supercoiling promotes greater DNA resistance to thermal denaturation (López-García 1999). According to Mehta and Satyanarayana (2013), some factors may combine to provide thermal stability to DNA in thermophiles, including high levels of K^+ and cyclic 2,3 diphosphoglycerate (2,3-DPG) that has been detected in the cytoplasm of thermophilic methanogens such as *Methanothermus fervidus*, *Methanothermus sociabilis*, and *Methanopyrus kandleri*. The molecular chaperones are also important for thermophilic adaptation of microorganisms.

They result in the folding and refolding of proteins, preventing possibly irreversible protein denaturation (Conway and Macario 2000). Oliveira et al. (2018) evaluating the peptidases from thermophilic fungi found that they contain a larger proportion of Ala, Glu, Gly, Pro, Arg, and Val residues and a lower number of Cys, His, Ile, Lys, Met, Asn, Gln, Ser, Thr, and Trp residues when compared to the mesophilic ones. Finally, to date, no fungi have been identified with growth above 62 °C. This may be related to the greater thermolability of their membrane systems than to the thermostability of enzymes or other cellular structures (van Noort et al. 2013).

9.4 Thermophilic Enzymes

The tolerance of the enzymes to high temperatures for long periods may be associated with their conformational structures, composition and/or amino acid sequences, and the origins of the enzymes (Gomes and Steiner 2004). The strategy for obtaining thermostable enzymes is to search in organisms that grow in high-temperature environments because their enzymes are more thermostable than those from mesophilic (Techapun et al. 2003), and also possible to obtain thermostable enzymes by the improvement of characteristics through small alterations in enzyme structure (Kour et al. 2019a; Yadav et al. 2020a).

These include site-directed mutagenesis (Xie et al. 2011), replacing the N-terminal region of a mesophilic enzyme by the N-terminal region of thermophilic organisms and the addition of disulfide bridges in the N-terminal region of the α-helix (Zhang et al. 2010). Higher thermal stability is one of the fundamental requirements for the application of an enzyme in industrial processes; it increases the efficiency of the enzyme. Therefore, searching for thermostable enzymes or improve the thermostability of enzymes has been the priority for researchers over the years (Nirmal and Laxman 2014), so the factors affecting thermostability are important for understanding the functions of proteins (Ruller et al. 2008). The advantages of employing enzymes with high optimum temperatures in biotechnological processes or biocatalytic conversions industrial include the lower risk of

microbial contamination by common mesophiles, the improvement of substrate solubility, increased reaction rates, and decreased viscosity (Joo et al. 2011).

9.4.1 Cellulases

The cellulase system in fungi is considered to comprise three hydrolytic enzymes: (1) the endo-(1,4)-b-D-glucanase (synonyms: endoglucanase, endocellulase, carboxymethyl cellulase) [EC 3.2.1.4], which cleaves b-linkages at random, commonly in the amorphous parts of cellulose; (2) the exo-(1,4)-b-D-glucanase (synonyms: cellobiohydrolase, exocellulase, microcrystalline cellulase, Avicelase) [EC 3.2.1.91], which releases cellobiose from either the nonreducing or the reducing end, generally from the crystalline parts of cellulose; and (3) the β-glucosidase (synonym: cellobiase) [EC 3.2.1.21], which releases glucose from cellobiose and short-chain cellooligosaccharides (Bhat and Bhat 1997).

Among the microorganisms, fungi, in particular, are dynamic cellulose decomposers, and possibly responsible for 80% of the cellulose breakdown on earth (Moore Landecker 1996; Devi et al. 2020; Yadav et al. 2019b). This is particularly true in the forest ecosystems, where fungi are the principal agents of decomposing cellulose and lignin (Alexopoulos et al. 1996). The cellulose decomposing fungi include members of the ascomycota, basidiomycota, and deuteromycota. Efficient cellulolytic fungi are represented by the species of *Aspergillus*, *Penicillium*, *Chaetomium*, *Trichoderma*, *Fusarium*, *Stachybotrys*, *Cladosporium*, *Alternaria*, *Acremonium*, *Ceratocystis*, *Myrothecium*, *Humicola*, and so on (Wood 1985). In the last few decades, thermophilic fungi have also been studied widely because of the fact that cellulose fibers bulge/swell up at higher temperatures so that they become easily accessible for hydrolytic enzymes (Li et al. 2011). *Talaromyces emersonii* is a typical thermophilic fungus capable of producing cellulase, which was active even at 70 °C and decomposes the intact cellulose (Murray et al. 2004). Two strains of *Penicillium* were identified from subtropical soils with potentials for the production of cellulase (Picart et al. 2007); *Chaetomium thermophilum*, *Sporotrichum thermophile*, *Talaromyces emersonii*, *and Thermoascus aurantiacus* grew well and decomposed cellulose very rapidly, producing thermostable cellulases (Mandels 1975).

Thermophilic fungi *Sporotrichum thermophile* (Coutts and Smith 1976) and *Talaromyces emersonii* (Folan and Coughlan 1978) produced cellulase activity nearly comparable to that of the mesophilic fungus *Trichoderma reesei*, regarded as the best source of fungal cellulase. Although cellulase productivity varies among strains (Oberson et al. 1992), using uniform procedures for the measurement of cellulase activity, Bhat and Maheshwari (1987) demonstrated that the endoglucanase and exoglucanase activities in the culture filtrate of their best strain of *S. thermophile* were about tenfold lower and the b-glucosidase activity was about 1.6-fold lower than in *T. reesei*. Despite these lower activities, *S. thermophile* degraded cellulose faster and grew at five times the rate of *T. reesei*.

The appearance of various cellulase components varied in different species: for example, *H. insolens* (Yoshioka and Hayashida 1980) and *T. aurantiacus* (Khandke 1986), all three cellulase components appeared simultaneously, while in *Chaetomium thermophile* var. *coprophile*, b-glucosidase activity preceded endo- and exoglucanase activities (Ganju 1986) and in *S. thermophile* it lagged behind endoglucanase and exoglucanase, which were typically formed during active growth. Kalogeris et al. (2003) investigated the production of extracellular cellulolytic enzymes in SSF by Thermoascus aurantiacus. For more details about thermophilic enzymes in Chaetomium please check Abdel-Azeem et al. (2020).

9.4.2 Xylanases

The xylanases belong to the family of glycosyl hydrolases (GH), which catalyze the hydrolysis of 1,4-linked β-D-xylosidic linkages in the main chain of xylan (Collins et al. 2005). Its complete degradation requires the cooperative action of a variety of hydrolytic enzymes: the endoxylanases (EC 3.2.1.8), which randomly cleave b-1,4-linked xylose (the xylan backbone); the b-xylosidases (EC 3.2.1.37), which hydrolyze xylooligomers; and the different side-branch splitting enzymes, for example, a-glucuronidase and a-arabinosidase, acetylxylan esterase, and acetyl esterase, which liberate other sugars (glucuronic acid arabinose) that are attached as branches to the backbone (Biely 1985). Based on the similarities between their amino acid sequences and the hydrophobic groups of their catalytic domains, fungal xylanases are classified into GH10 and GH11 families (Verma and Satyanarayana 2012). GH10 family groups xylanases with higher molecular weight (approximately 40 kDa) p*I* acid and three dimensional (β/α)8 structures, while the GH11 family has lower molecular weights (approximately 20 kDa) basic p*I*, and three-dimensional β barrel structures (Beaugrand et al. 2004).

Xylanases of both families (GH10 and GH11) have two glutamate residues conserved in their active sites and may possess carbohydrate-binding modules (CBMs) or amino- or carboxyl-terminal regions (Sydenham et al. 2014). Several authors have reported thermostable xylanases obtained from thermophilic fungi, such as *Humicola brevis* var. *thermoidea* (Masui et al. 2012), *Paecilomyces themophila* J18 (Yang et al. 2006), and *T. aurantiacus* RCKK (Jain et al. 2015). Most of these fungal xylanases thermostable belong to GH10 and GH11 families, and enzymes belonging to the same family, they present the same structure and amino acid sequence, and thus the thermal stability also did not differ (You et al. 2010).

The production of increased levels of xylanases from sugar beet pulp under SSF by *H. lanuginosa* and *T. aurantiacus*, and endoxylanase production from wheat straw under SSF was reported by Kalogeris et al. (1998). Dos Santos et al. (2003) reported xylanase production from *T. aurantiacus* on sugarcane bagasse in SSF. Pereira et al. (2015) isolated heat-tolerant *Myceliophthora thermophila* JCP 1–4 from the environment, which produced thermophilic endoglucanase, glucosidase, xylanase, and avicelase using lignocellulosic biomass as the substrate in

SSF. Xylanases of thermophilic fungi are receiving considerable attention because of their application in biobleaching of pulp in the paper industry, wherein the enzymatic removal of xylan from lignin-carbohydrate complexes facilitates the leaching of lignin from the fiber cell wall, obviating the need for chlorine for pulp bleaching in the brightening process. They also have applications in the pretreatment of animal feed to improve its digestibility (Kitpreechavanich et al. 1984).

The majority of xylan-degrading enzymes from thermophilic fungi are endoxylanases. *Malbranchea pulchella* var. *sulfurea* also produced an extracellular xylosidase (Matsuo and Yasui 1985), but in *H. grisea* var. *thermoidea* (Monti et al. 1991) and *Talaromyces emersonii* (Tuohy et al. 1993) the xylosidase was periplasmic. The majority of xylanases have pH optima ranging from 4.5 to 6.5. *T. emersonii* xylanases are unusual in having acidic pH optima. The temperature optima of most xylanases range from 55 to 65 °C. Xylanases of some strains of *T. aurantiacus* and *T. lanuginosus* are optimally active at 70–80 °C. The molecular masses of xylanases cover a wide range, from 21 to 78 kDa With the exception of the dimeric Xyl I and Xyl II of *T. emersonii*, most xylanases are single polypeptides. Xylanase I of *C. thermophile* var. *coprophile* and (Ganju et al. 1989) xylanase II of *H. insolens* were remarkable in their low molecular mass (7 kDa) (Dusterhoft et al. 1997). Outstanding yields of xylanases, requiring only three- to fourfold purification of culture filtrate protein, have been obtained from some wild isolates of thermophilic fungi (Tan et al. 1987). In *T. aurantiacus*, 258 mg of crystalline xylanase was obtained from 1622 mg of desalted crude culture filtrate protein, with 75% yield (Khandke et al. 1989). In *P. varioti*, the secreted protein was mostly xylanase with small amounts of b-glucosidase (Krishnamurthy 1989).

9.4.3 Laccases

Laccases (phenol oxidases: E.C. 1.10.3.2), also known as multicopper blue oxidases, belong to the oxidoreductase group of enzymes. Biochemically, they are glycoproteins carrying molecular mass between 50 and 130 kDa (Morozova et al. 2007). Fungi, belonging to ascomycetes, deuteromycetes, and basidiomycetes, are known to produce laccases of ecological as well as biotechnological importance, such as biodegradation and bioremediation (Mayer and Staples 2002). In addition, laccases are also responsible for various physiological functions in fungi (Claus 2004). Due to their broad specificity toward the substrate, they can oxidize a range of chemical compounds leading to various industrial applications (Kunamneni et al. 2007).

Laccase activity of thermophilic fungi could therefore be important in the polymerization of phenolic substances into humic substances. The gene encoding laccase of *Myceliophthora thermophila* was cloned and expressed in *A. oryzae*, and the recombinant enzyme (r-MtL) was purified from culture broth with a two- to fourfold-higher yield (11–19 mg/L) than that of native (MtL) laccase (Berka et al. 1997).

The optimal activity of the enzyme occurred at pH 6.5, and it retained full activity when incubated at 60 °C for 20 min. Laccase from culture filtrates of a thermophilic fungus reported as *Chaetomium thermophilium* was purified by ultrafiltration, anion-exchange chromatography, and affinity chromatography. The enzyme was a glycoprotein of 77 kDa. It was stable at a broad pH range from 5 to 10 and at 50 °C (Cheftez et al. 1998). *Chaetomium thermophilium* was isolated from composting municipal solid waste during the thermophilic stage of the process, exhibited laccase activity when it was grown at 45 °C both in solid media and in liquid media. Laccase activity reached a peak after 24 h in liquid shake culture. The purified enzyme was identified as a glycoprotein with a molecular mass of 77 kDa and an isoelectric point of 5.1. The laccase was stable for 1 h at 70 °C and had half-lives of 24 and 12 h at 40 and 50 °C, respectively (Cheftez et al. 1998).

Thermostable fungi laccases in the industrial application are mainly isolated from *Melanocarpus albomyces*, *Myceliophtora thermophila*, and *Chaetomium thermophilum*, which all belong to the *Ascomycota* (Hildén et al. 2009). Species belonging to genus *Corynascus* (*Myceliopthora*) have been of interest to mycologist as it produces thermostable enzymes. For example, *Corynascus thermophilus* (basionym: *Thielavia thermophila*) produced thermostable laccases with high activity and the ability to express in various hosts (Babot et al. 2011). Laccases produced by *C. thermophilus* ATCC 42464 are completely characterized, patented and genome sequenced (Beeson et al. 2011). Five thermophilic laccase enzyme isoforms were isolated, purified, and characterized from xerophytic plants *Cereus pterogonus* and *Opuntia vulgaris* (Gali and Kotteazeth 2012, 2013). Several fungi, viz., *Curvularia lonarensis*, *Penicillium* sp., and *Trametes* sp., have been reported from various extreme environments (thermophiles, alkaliphiles, psychrophiles, marine fungi, etc.) which have been studied for the laccase enzyme production potentials (Dhakar et al. 2014).

9.4.4 Amylase

α-Amylase (E.C. 3.2.1.1), the 1–4 alpha glucosidic links oligosaccharides and polysaccharides (Nielsen and Borchert 2000). Generally, amylases are classified into three categories. These are exoamylases (β-amylase), endoamylases (α-amylase), and the amylase enzymes (pullulanase, isoamylase) which do not show branching (Sahnoun Silva et al. 2016). The α-amylase enzyme allows for the formation of glucose, maltose, maltotriose, and α-limit dextrins by splitting off the α-1,4 links. Glucoamylase breaks the α-1,3, α-1,4, and α-1,6 links and converts them into glucose molecules (Haki and Raksit 2003).

All α-amylases of thermophilic and hyperthermophilic origin are monomeric enzymes and their molecular weights range from 42 to 68 kDa (Leveque et al. 2000). The fact that thermostable α-amylases do not require calcium ions for thermostability and activity makes thermophilic amylases different from the mesophilic α-amylases (Laderman et al. 1993). All species of thermophilic fungi studied so far

secrete amylase (Sadhukhan et al. 1992). However, only *T. lanuginosus* a-amylase has been characterized. The addition of Tween 80 to agitated submerged cultures increased a-amylase production 2.7-fold (Arnesen et al. 1998). Although a multiplicity of a-amylases is common in fungi, only one electrophoretically similar form of the enzyme was detected in culture filtrates of seven strains of *T. lanuginosus* (Mishra and Maheshwari 1996). Today, many enzymes of microbiological origin are used in the food industry. The field of the food industry in which the amylases are most commonly used is the bakery sector. It is recommended to use α-amylases which are thermostable and resistant to bread-baking temperature (Nguyen et al. 2002).

A thermophilic fungus, *Thermomyces lanuginosus* (syn. *Humicola lanuginosa*), was reported to produce glucoamylase (α-1,4-glucan glucohydrolase; EC 3.2.1.3) that was capable of quantitatively converting soluble starch into glucose. The enzyme was a glycoprotein with a molecular mass of ~57 kDa. It was active at 70 °C and was completely stable at 50 °C (Rao et al. 1981). Jensen et al. (1988) reported a glucoamylase in another strain of *T. lanuginosus* which had a molecular mass of 70–77 kDa. This strain also produced a thermostable α-amylase (α-1,4 glucan glucanohydrolase; EC 3.2.1.1) of molecular mass of 54–57 kDa that required Ca^{2+} for activity (Jensen and Olsen 1992). α-amylase producing *Fusarium* sp. was isolated from the soil at 50 °C. Growth and enzyme production occurred at 30, 45, and 55 °C. Soil samples were collected from a honey processing area in Eastern Nigeria (Nwagu and Okolo 2011).

A total of seven species of thermophilic fungi were isolated and identified from three locations, for example, two refuse-dump sites and a palm-kernel stack in Ibadan, Nigeria. These were *Absidia corymbifera*, *Gilmaniella humicola*, *Talaromyces helicus*, *Chaetomium elatum*, *Chaetomium* sp., *Humicola* sp., and *Rhizomucor pusillus*, respectively. Amylases were produced by all these thermophilic fungi (Olagoke 2014).

9.4.5 Lipase

Lipases are extracellular microbial are very important since they catalyze the hydrolysis of triacylglycerols and the synthesis of esters from glycerol and long-chain fatty acids. Microbial lipases are important in the industry due to their stability toward extremes of temperature and pH and because they have also broad substrate specificity (Dutra et al. 2008; Griebeler et al. 2011; Yadav 2015). Used in a wide range of applications in the field of food technology, clinical medicine, nutrition, and analytical and preparative chemistry. These enzymes are used to show the occurrence of interfacial activation, like an increase of catalytic activity on lipid aggregates (micelles) rather than on lipid monomers in an aqueous solution. When used as a component of laundry detergents, lipases that are stable at pH 10–11, at temperatures from 30 to 60 °C, and in the presence of surfactants are preferred.

Several thermophiles that have been reported to produce lipase include *Talaromyces thermophitus*, *Mucor pusillus*, *Humicola lanuginosa*, *H. grisea* var. *thermoidea*, and so on. *H. lanuginosa* produces an extracellular lipase at ~45 °C in a medium containing olive oil (Arima et al. 1972). *M. pusillus* and *T. thermophilus* produce extracellular lipase at 45 °C while those of *Thermoascus crustaceus* and *H. grisea* var. *thermoidea* produce extracellular lipase at 50 °C the pH optima for *H. grisea* var. *thermoidea* and *T. thermophilus* is 5.5 and for *M. pusiuus* and *T. crustaceus* at 6 (Ogundero 1980).

Arima et al. (1972) produced a purified extracellular lipase from *Humicola lanuginosa* strain Y 38. The enzyme was produced in a medium containing starch, corn steep liquor, soybean oil, and an antifoaming agent. It was purified to homogeneity from an 80-h-old culture medium by successive steps of ammonium sulfate precipitation, dialysis, ion exchange chromatography, and gel filtration chromatography, with 30% recovery. The protein, a single polypeptide (molecular weight, 27,500), was optimally active at pH 8.0 and was stable in the pH range of 4–11. Its temperature optimum for activity was at 60 °C. It exhibits activity at up to 65 °C but was inactivated on heating at 80 °C for 20 min (Liu et al. 1973).

Omar et al. (1987a, b) reported that the productivity and thermostability of lipase differed with different strains of *H. lanuginosa*. The enzyme was produced in a medium containing sorbitol, corn steep liquor, silicone oil as an antifoaming agent, and whale or castor oil as an enzyme inducer. With the pH maintained between 7 and 8 and the temperature set at 45 °C, maximum enzyme production by their strain occurred after 30 h. Following acetone precipitation and successive chromatographic steps, they obtained a more thermostable enzyme (stable at 60 °C for 20 h) than was obtained by (Arima et al. 1972).

9.4.6 Pectinase

Pectinases are enzymes that catalyzing break down pectin substances are of great industrial importance (Garg et al. 2016; Kour et al. 2019d), Pectinases is a polysaccharide found in plant primary cell walls of terrestrial plants, cereals, fibers, fruits, and vegetables (Anisa et al. 2013; Kohli and Gupta 2015). Pectinases comprise pectin methyl esterases, pectin acetyl esterases, polygalacturonases, polymethylgalacturonases, polygalacturonate lyases, polymethylgalacturonate lyases, rhamnogalacturonase, arabinases, and xylogalacturonases (Adapa et al. 2014). The most studied and widely used commercial pectinases are polygalacturonase. Many microorganisms have the ability to degrade pectin among these microorganisms are the filamentous fungi are efficient microorganisms.

They reported for their capability of secreting a wide range of pectin-degrading enzymes, and presently, most of the pectinolytic enzymes that used in industry are produced by filamentous fungi, particularly from genera *Aspergillus*, *Trichoderma*, and *Penicillium* (Benoit et al. 2012; Gupta and Kalpana 2011; Lara-Márquez et al. 2011). The most commonly used genera among them include *Aspergillus* sp.,

Rhizopus sp., *Trichoderma* sp., *Penicillium* sp., and *Fusarium* sp. (Ahmed et al. 2016). *Rhizopus* sp., *Gleosporium kake*, *Ciniothrium diplodella*, and *Aspergillus niger* have been reported to produce exo-Polygalacturonases as well as endo Polygalacturonase which release galacturonic acid from the terminal pectin chain (Chowdhury et al. 2017).

In a study by Martin et al. (2004) report the fungal strains isolated from decaying vegetable and soil are pectinase producing, *Moniliellla* sp. SB9, *Penicillium* sp. EGC5, *P. viridicatum* RFC3, *P. glabum* F1, *Penicillium* sp. RFC2, *P. itallicum*, *P. citrinum*, *Curvularia inaequalis* EM P11-1, *Curvularia* sp. 5.11.1, *Aspergillus* sp. EG66F, *Aspergillus* sp. EGC4, *A. niger*, *Thermoascus* sp., *Aureobasidium* sp. RE, *Phanerochaetes* sp. 291, *Cladosporium* sp. RFC1. In a study according to Nayebyazdi and Tajick (2012) report, several fungal isolated from agricultural soils producing pectinase; *Aspergillus foetidus*, *A. aculeatus*, *A. janthinellum*, *A. carbonarius*, *Rhizoctonia solani*, *Penicillium chrysogenum*, *P. flutanum*, *P. citreonigrum*, *P. citrinum*, *Trichoderma reesei*. In a study by Sandhya and Kurup (2013) a group of pectinolytic fungi was isolated from cheap sources like spoiled fruits and vegetables and *Penicillium citrinum* was found to be the potent source for pectinase.

In study by Priya and Sashi (2014) reports many fungal species isolated from soil producing pectinase, *Aspergillus niger*, *A. versicolor*, *A. flavus*, *Penicillium jenseni*, *P. citrinum*, *Fusarium oxysporum*, *Rhizopus stolonifera*, *Mucor racemous*, *M. hiemalis*, *Trichoderma viride*. On studying according to Abdullah et al. (2018) many fungal strains were isolated from different sources, that is, soil and fruits are producing pectinase, for example, *Aspergillus fumigatus*, *A. tamarrii*, *A. terraus*, *A. niger*, *A. oryzae*, *Penicillium* sp. *Rhizopus* sp., among all the strains the strain showing the highest pectinolytic potential were *Aspergillus niger* ABT-5. In a study by Okonji et al. (2019) report, *A. fumigatus* showed the maximum pectinase activity among all the isolates, isolated from the soil of decomposing plant materials.

On the contrary, very little is known about the pectinolytic activities from organisms from cold climates such as Antarctica. These mesophilic commercial pectinases possess optimal temperatures between 40 and 60 °C (Adapa et al. 2014). However, there are processes where pectin degradation is necessary at lower temperatures. Khatri et al. (2015) reported that pectinase enzyme was observed at 48 h of fermentation, the partial enzyme exhibited maximum activity of 60 U/mg, the enzyme was active at a broad range of temperature (30–70 °C) and pH (6.2–9.2). The optimum temperature and pH of the pectinase enzyme were 50 °C and 8.2, respectively.

The enzyme was stable up to 70 °C and about 82% of pectinase activity was still observed at 100 °C. Phutela et al. (2005) report the isolation and screening of pectinolytic thermophilic fungi using a semi-quantitative plate assay approach, a thermophilic fungal strain TF3 (*Aspergillus fumigatus* MTCC 4163) isolated from decomposing orange peel waste, was the best producer of pectinase and PG.

9.4.7 Phytase

The phytases of thermophilic fungi are histidine acid phosphatases that catalyze the hydrolysis of phytic acid (myoinositol hexakisphosphate) to inorganic phosphate and myo inositol through a series of myoinositol phosphate intermediates (Gontia-Mishra and Tiwari 2013; Kaur et al. 2017; Kumar et al. 2016, 2017a, b; Mitchell et al. 1997; Kour et al. 2020). Phytases can be produced in two different ways are solid-state and submerged fermentation by using thermophilic fungi.

The biochemical properties of the phytases from thermophilic fungi that are usually active at pH (4.5–7.0) and temperature (50–70 °C) (Singh and Satyanarayana 2011) when phytase add to seed-based poultry and pig feed increase the availability of phosphorus in the feed so it has commercial prospects. Phytases improve the nutritional value of foods by making minerals such as Ca^{2+}, K^{+}, Mg^{2+}, and Zn^{2+}, and proteins available for monogastric. Thermophilic fungal phytases have the ability to hydrolyze insoluble phytates, to improve the bread-making process, to dephytinized food ingredients, and to help in the encouragement of plant growth (Gontia-Mishra and Tiwari 2013; Reddy et al. 1989) animal waste that produced in high quantities rich with phytin phosphorus (calcium and magnesium salts of myoinositol hexakis-dihydrogen phosphate) (Greiner and Konietzny 2006) which phosphorous is known as an important pollutant. The entry of phosphorus in water bodies can lead to eutrophication resulting in algal blooms, hypoxia, and the death of aquatic animals (Naqvi et al. 2000).

This problem can be controlled by supplementing the animal feed with phytases, which improve the nutritional status of the feed and further, decrease environmental phosphorus pollution (Golovan et al. 2001). Phytic acid is the main storage form of phosphorus and involves 1–5% by weight in cereals, legumes, oilseeds, and nuts acting as an anti-nutritional factor (Harland and Morris 1995). Thus, for both environmental and economic reasons, phytases and phytase-producing microbes are attracting significant interest worldwide (Greiner and Konietzny 2006). Mesophilic fungi and bacteria are microbially known for their ability to produce phytase but now thermophilic molds have more attracted the attention of scientists all over the world because they produce enzymes with unique properties (Mitchell et al. 1997) and uses of thermophilic molds in industrial applications have been recognized recently (Satyanarayana and Singh 2004).

Chadha et al. (2004) studied the production of phytase from thermophilic fungus, Rhizomucor pusillus that isolated from composting soil by using solid-state fermentation and found the partially purified phytase was optimally active at 70 °C and pH 5.4, though the enzyme showed 80% activity over a wide pH range, 3.0–8.0. Chadha et al. (2004) isolated nine thermophilic fungal strains having the potential of phytase production like *Rhizomucor pusillus*, *Humicola grisea*, *Sporotrichum thermophile*, *Humicola insolens*, *Thermomyces lanuginosus*-I, *Thermomyces lanuginosus*-II, *Rhizomucor miehei*-I, *Rhizomucor miehei*-II, and *Aspergillus fumigatus*. Singh and Satyanarayana (2008) also investigated that *Sporotrichum thermophile* has the potential for enhanced production of phytase.

Javed et al. (2010) also isolated seven strains of five different thermophilic fungi such as *Aspergillus fumigatus*, *Humicola insolens*, *Rhizomucor miehei*-I & II, *Sporotrichum thermophile*, *Thermomyces lanuginosus*-I & II the potential for enhanced production of phytase. Between the seven thermophilic fungal strains screened for phytase production, *Sporotrichum thermophile* was found to produce higher extracellular phytase when grown on solid-state wheat bran.

9.4.8 Protease

Proteases are the enzymes that hydrolyze the peptide links of proteins or peptides into simpler proteins, peptides, and free amino acids. They are divided into endoenzymes promoting attack within the chain of macromolecules and exoenzymes hydrolyzing terminal amino acids (Neurath 1957; Kour et al. 2019b). Unlike other enzymes, they are regarded as a mix of enzymes (Lee et al. 2002) and contain proteinases, peptidases, and amidases, which hydrolyze intact proteins, peptides or peptones, and amino acids, respectively.

Proteases are classified in several ways basis on amino acid required for the catalytic function, for example (serine protease), the pH: acid proteases (pH from 2.0 to 6.0), neutral proteases (pH 7.0 or around 7.0), and alkaline proteases (pH from 8 to 11)., their site of cleavage, for example (aminopeptidases, which act at the free N terminus of the polypeptide chain, or carboxypeptidases, which act at the C terminus of the polypeptide chain), or their requirement of a free thiol group, for example (thiol proteinase) (Maheshwari et al. 2000). *Talaromyces thermophilus*, *Thermomyces ibadanensis*, *Thermoascus aurantiacus*, and *T. lanuginosus* can grow at optimum temperature 42–52 °C but can tolerate up to 61 °C for growth (Maheshwari et al. 2000). These thermophilic fungi have the ability to produce extracellular proteases and reduce microbial contamination from other organisms during protease production (Chen et al. 2004).

Restricted studies on proteases active within pH (3–6) and temperatures (45–55 °C) have been done on thermophilic fungi *Mucor pusillus*, *Penicillium duponti*, *Malbranchea pulchella* var. *sulfurea*, and *Humicola lanuginose*. Proteases from thermophilic fungi enter industrial applications due to stability and their high specific activity. Gene encoding protease from thermophilic *Chaetomium thermophilum* has been introduced in *P. pastoris* (Kim and Lei 2005; Li and Li 2009). Proteases have long been used in the food, dairy, detergent industries and for leather processing. To beat the limitation of obtaining chymosin, the milk-curdling enzyme from the stomach contents of milk feeding calves, which is used in the industrial preparation of cheese, led to a search for substitutes.

9.5 Potential Application

Fungal thermophilic enzymes such as xylanases, proteases, cellulases, amylases, and lipases were used extensively in the various biotechnological application as paper and pulp bleaching, sugar fermentation process, biofuel and bioethanol production, fruit juices extraction and refining, wastewater treatment and tobacco, coffee and cocoa curing (Singh et al. 2016). Thermophilic fungi produce a variety of enzymes with a high activity compared to mesophilic fungal strains and they have been used in different applications (Berka et al. 2011). During the self-heating of compost; thermophilic fungi such as *Trichoderma reesei* (40–50 °C) resemble the main component where it can degrade cellulose very quickly compared to mesophilic (Kaur et al. 2004).

9.6 Applications in Food, Bread and Drinks Production

Using enzymes in the food industry started with α-amylase, followed by the use of other enzymes as proteases, cellulases, xylanases, pullulanases, and lipases. Including the enzymes into food processing aims to improve the features of the final products by increasing the bread's softness and volume and modifying its color in order to attract customers (Pandey et al. 2000). Xylanases, proteases, cellulases increase the gluten network firmness leading to increasing the worthiness of the products (Gray and Bemiller 2003).

Xylanases also showed a significant role in rye baking, where it helps to convert the dough into more soft and sloppier state (Harbak and Thygesen 2002). Jiang et al. (2005) studied the effect of purified xylanase from *T. lanuginosus* CAU44 on bread quality and monitoring it during the state which showed a good result confirming that the thermostable xylanase may be used in bread baking industries. Xylanases and hemicellulases activate the depolymerization of hemicellulose present in the wheat flour, which leads to a uniform water circulation thereby the dough will be softer and easy to knead.

Laccases are also used in bakery industries, where applying laccases during the doughmaking process increases its strength and reduces the stickiness of the gluten due to its ability to cross-link the biopolymers. Labat et al. (2000) also reported that applying laccases improved the volume, structure, and softness of the bakery products even if low-quality flour were used. Also, phytases while being applied during the baking process improved the proteins levels and reducing sugars. Amylases and phytases combined when added to the dough improved the bread quality and properties compared to processed bread treated with other commercial enzymes (Singh and Satyanarayana 2008). Proteases extracted from *Aspergillus niger* showed an ability to degrade gluten peptides which can be stable under the stomach acidic environment (Heredia-Sandoval et al. 2016). Some enzymes such as cellulases, amylases, and pectinases were used in the beverage industries which improve the

flavors and reduce the turbidity and viscosity (Polizeli et al. 2005). *Penicillium pinophilum* produces a thermoacidophilic xylanase which can improve the filtration rate to 22% and the viscosity to 5.0% with 40 IU xyalanase (Cai et al. 2011).

Moreover, the addition of a higher dose of the enzyme (80 U) improved these parameters, resulting in the filtration rate to 26.7% and the viscosity of wort to 9.8%. Xylanases are used also in biscuits processing where it helps to make the crackers (cream) lighter and improves the texture and palpability of the wafers. Xylanase is used in beer industries where it activates the depolymerization of arabinoxylans to xylooligosaccharides leading to less viscous beer and improves its muddy appearance (Dervilly et al. 2002). Cellulases combined with other enzymes lead to the improvement of citrus aroma taste by reducing bitterness (Kuhad et al. 2011). Also, cellulases were used in the alcoholic industries such as wine and beer as it enhances the release of simple sugar (Karmakar and Ray 2010).

Starch processing steps involve applying glucoamylases extracted from *A. niger.* Where, isomerase activates the isomerization of glucose to fructose, β-amylase, and pullulanases could be used during the saccharification process to produce maltose syrup (Haki and Raksit 2003).

Fungal proteases got an interest in food applications especially cheese production, and some fungal species were approved by FDA for the use during cheese production such as *A. niger* var. *awamori*, *Endothia parasitica*, *Mucor miehei*, *Penicillium* spp., and *Rhizopus oryzae* (Adrio and Demain 2014). *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Mucor pusillus*, *Mucor miehei*, and *Rhizopus* species were used in oriental food preparations like the production of koji and tempeh cheese (Sternberg 1976).

9.7 Significant Role in Improving the Animal Feed

Improvement of animal foods received an international addition; xylanase was used as an additive to foods with low viscosity as maize and sorghum by enhancing the absorption of nutrients in the foremost part of the digestive tract (Van Paridon et al. 1992). Xylanase was used to enhance the digestion of ruminant feeds and speeding the composting process (Gilbert and Hazelwood 1993). Combined with xylanases; cellulases, amylases, galactosidases, glucanases, lipases, pectinases, proteases, and phytases were used in the utilization in animal feed. The cellulolytic thermophilic fungi have some advantages over mesophilic fungi such as higher growth rate and cellulose breakdown rate, active over a broad range of temperatures (20–55 °C), and good sources of protein. Phosphorus is an essential ingredient in animal and plant production. An inorganic phosphate was liberated from animal feed supplemented with phytases from *A. fumigatus* (Wyss et al. 1999).

Normally, inorganic phosphorus is released from phytic acid in ruminants with the help of microflora. While, monogastric as pigs, humans, chickens can't produce or little phytase in the intestine (Mullaney et al. 2000). The phytic acid present in the manure of these animals is enzymatically cleaved by soil and water-borne

microorganisms. This results in oxygen depletion due to excessive algal growth. Pretreatment of animal feed with phytases would increase the availability of inorganic phosphorus, thereby improving the nutritional value of food and also helping in combating phosphorus pollution (Vats et al. 2005). Phytases are well known to reduce pollution caused by the excess phosphorus accumulation in the soil and water (Nahm 2002).

9.8 Textile Industry

In textile industries, cellulases have been used for polishing cotton cloths biostoning of denim jeans to impart a stonewashed look for denims. Cellulase produced by *Humicola insolens* and *Trichoderma* is generally used for the biostoning of jeans (Cortez et al. 2001). Cellulases activate the hydrolyses of protrusion's small fibers and the release of indigo dyes, which result in the jean's dull look. It can replace the use of commercial pumice stones, and thus reduce the damage of the fibers (Arja 2007). Cellulase also helps in the absorbance of dyes through fibers and removes the excess (Kuhad et al. 2011). Also, cellulases were used in laundry household detergents to improve fabric softness and brightness. *Trichoderma viride*, *T. hurzianum*, *T. reesei*, *A. niger* and *Humicola insolens* were used as sources for thermotolerant mild alkaline cellulases producers (Kottwitz and Schambil 2004).

Laccases received attention through textile industries too where they can be applied in different phases during the industry (Rodríguez-Couto and Toca-Herrera 2006). Laccases play a role in dye changes like changing its color and strength of reactivity and bonding to textiles. Several patents targeted the coloration using laccase were reported (Kunamneni et al. 2008). Laccase biosensors were used to improve the cotton bleaching (Tian et al. 2012) and in biostoning (Paziarloglu et al. 2005). Laccase is also used to facilitate the attachment of functional molecules on textile fibers such as laccase-mediated grafting of lauryl gallate on wool resulted in a multifunctional textile material with antioxidant, antibacterial, and water repellent properties (Hossain et al. 2009).

9.9 Biofuel/Bioethanol

Biosynthesis of biofuels such as ethanol involved the treatment of lignocellulosic biomass with xylanases combined with hydrolases (Ahring et al. 1999). Biofuels especially bioethanol received global attention as a replacement for the massive exploitation of fossil fuels which have many environmental side effects (Msangi et al. 2007; Kaur et al. 2020; Kour et al. 2019c; Yadav et al. 2020c). Cellulose plays an important role in the conversion of cellulosic sources to glucose and fermentable sugars which would be used in bioethanol production steps. Bioethanol production is a multistep process starting with the pretreatment (mechanical, chemical, or

enzymatic) of the lignocellulosic biomass to remove the lignin and hemicellulose, followed by treatment with cellulase to release sugars which will be used in the fermentation process. Hydrolyzed cellulosic materials will be used for microbial fermentation to produce bioethanol (Sun and Cheng 2002). Different residues such as sugarcane bagasse, wheat straw, corn bran, and rice bran were used as raw materials to produce bioethanol after applying cellulase extracted from different fungi such as *Aspergillus*, *Trichoderma*, and *Penicillium* (Chen and Qiu 2010).

9.10 Pharmaceutical and Chemical Applications

Xylanases are used mixed with proteases, hemicellulases, and various enzymes as a dietary supplement and to cure weak digestion. One of xylanases byproducts "Xylitol" was reported to have many applications as it's highly suited for diabetics, used with lipid metabolism disorders and respiratory infections and for the prevention of osteoporosis as well as for persons suffering from kidney and parental lesions (Nigam and Singh 1995). Laccases byproducts were reported to have antimicrobial, detoxifying, or active personal-care agents.

Laccases are also used for the production of chemicals used in cosmetic industries such as isopropyl myristate, isopropyl palmitate, and 2-ethylhexyl palmitate which are used as emollients in personal care products such as skin and suntan creams, bath oils, and so on (Sharma et al. 2001). Laccase is used for the generation of iodine (Madhavi and Lele 2009). Laccases also showed antagonistic activity with HIV-1 reverse transcriptase (Wang and Ng 2004). Also, laccases are involved in other applications such as skin lightening, hair dyes, deodorants (Takase et al. 2011; Golz-Berner et al. 2004; Lang and Cotteret 1999). Proteases are involved in the production of household and industrial laundry and in household dishwashers. Proteases are also involved in the dehairing of animal skin which offers benefits over chemical treatment in soaking, dehairing, and bating for leather processing. Alkaline proteases display features such as time-saving, improved quality of leather, and also overcome pollution problems (Zambare et al. 2011). Proteases from *Aspergillus flavus* and *Conidiobolus coronatus* have been utilized in tanning during the industrial processing of leather (Laxman et al. 2005).

References

Abdel-Azeem A, El-Mansy M, Abo Nahas SA, Mousa HH, Gandal MK, Hamdy HE et al (2020) Thermophilic Chaetomium in biotechnology. In: Abdel-Azeem AM (ed) Recent developments on Genus Chaetomium, Fungal biology. Springer Nature, Switzerland AG, pp 421–440

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abdullah R, Jafer A, Nisar K, Kaleem A, Iqtedar M, Ifitkhar T, Saleem F, Naz S (2018) Process optimization for pectinase production by locally isolated fungal strain using submerged fermentation. Biosci J Uberlândia 34(4):1025–1032

Adams MW (1993) Enzymes and proteins from organisms that grow near and above 100 °C. Annu Rev Microbiol 47:627–658

Adapa V, Ramya L, Pulicherla K, Rao KS (2014) Cold active pectinases: advancing the food industry to the next generation. Appl Biochem Biotechnol 172:2324–2337

Adrio JL, Demain AL (2014) Microbial enzymes: tools for biotechnological processes. Biomol Ther 4:117–139

Ahmed I, Zia MA, Hussain MA, Akram Z, Naveed MT, Nowrouzi A (2016) Bioprocessing of citrus waste peel for induced pectinase production by *Aspergillus niger*; its purification and characterization. J Radiat Res Appl Sci 9(2):148–154. https://doi.org/10.1016/j.jrras.2015.11.003

Ahring BK, Licht D, Schimd AS et al (1999) Production of ethanol from wet oxidised wheat straw by *Thermoanaerobacter mathranii*. Bioresour Technol 68:3

Alexopoulos C, Mims C, Blackwell M (1996) Introductory mycology. Wiley, New York, p 869

Anisa SK, Ashwini S, Girish K (2013) Isolation and screening of *Aspergillus* spp. for pectinolytic activity. Electron J Biol 9(2):37–41

Arima K, Liu WH, Beppu T (1972) Studies on the lipase production of thermophilic fungus *Humicola lanuginosa*. Agric Biol Chem 36:893–895

Arja M-O (2007) Cellulases in the textile industry. In: Polaina J, MacCabe AP (eds) Industrial enzymes. Springer, Dordrecht, pp 51–63

Arnesen S, Eriksen SH, Olsen J, Jensen B (1998) Increased production of a-amylase from *Thermomyces lanuginosus* by the addition of Tween 80. Enzym Microb Technol 23:249–252

Babot ED, Rico A, Rencoret J, Kalum L, Lund H, Romero J, José C, Martínez ÁT, Gutiérrez A (2011) Towards industrially-feasible delignification and pitch removal by treating paper pulp with *Myceliophthora thermophila* laccase and a phenolic mediator. Bioresour Technol 102(12):6717–6722

Beaugrand J, Chambat G, Wong VW, Goubet F, Rémond C, Paës G, Benamrouche S, Debeire P, O'Donohue M, Chabbert B (2004) Impact and efficiency of GH10 and GH11 thermostable endoxylanases on wheat bran and alkali-extractable arabinoxylans. Carbohydr Res 339(15):2529–2540

Beeson WT, Iavarone AT, Hausmann CD, Cate JH, Marletta MA (2011) Extracellular aldonolactonase from *Myceliophthora thermophila*. Appl Environ Microbiol 77(2):650–656

Benoit I, Coutinho PM, Schols HA, Gerlach JP, Henrissat B, De Vries RP (2012) Degradation of different pectins by fungi: correlations and contrasts between the pectinolytic enzyme sets identified in genomes and the growth on pectins of different origin. BMC Genomics 13:321

Berka RM, Schneider P, Golightly EJ, Brown SH, Madden M, Brown KM, Halkier T, Mondorf K, Xu F (1997) Characterization of the gene encoding an extracellular laccase of *Myceliophthora thermophila* and analysis of the recombinant enzyme expressed in *Aspergillus oryzae*. Appl Environ Microbiol 63:3151–3157

Berka RM, Grigoriev IV, Otillar R et al (2011) Comparative genomic analysis of the thermophilic biomass degrading fungi Myceliophthora thermophila and Thielavia terrestris. Nat Biotechnol 29:922–929

Bhat MK, Bhat S (1997) Cellulose degrading enzymes and their potential industrial applications. Biotechnol Adv 15:583–620

Bhat KM, Maheshwari R (1987) *Sporotrichum thermophile*: growth, cellulose degradation, and cellulase activity. Appl Environ Microbiol 53:2175–2182

Biely P (1985) Microbial xylanolytic systems. Trends Biotechnol 3:286–290

Bruins ME, Janssen AE, Boom RM (2001) Thermozymes and their applications: a review of recent literature and patents. Appl Biochem Biotechnol 90(2):155–186

Cai H, Shi P, Bai Y, Huang H, Yuan T, Yang P, Luo H, Meng K, Yao B (2011) A novel thermoacidophilic family 10 xylanase from *Penicillium pinophilum* C1. Process Biochem 46(12):2341–2346

Chadha BS, Gulati H, Minhas M, Saini HS, Singh N (2004) Phytase production by the thermophilic fungus Rhizomucor pusillus. World J Microbiol Biotechnol 20:105–109
Cheftez B, Chen Y, Hadar Y (1998) Purification and characterization of laccase from *Chaetomium thermophilium* and its role in humification. Appl Environ Microbiol 9(64):3175–3179
Chen H, Qiu W (2010) Key technologies for bioethanol production from lignocellulose. Biotechnol Adv 28:556–562
Chen XG, Stabnikova O, Tay JH, Wang JY, Tay STL (2004) Thermoactive extracellular proteases of *Geobacillus caldoproteolyticus*, sp. nov., from sewage sludge. Extremophiles 8:489–498
Chowdhury TI, Jubayer F, Uddin B, Aziz G (2017) Production and characterization of pectinase enzyme from Rhrizopus oryzae. Potravinarstvo Slovak J Food Sci 11(1):641–651. https://doi.org/10.5219/656
Claus H (2004) Laccases: structure, reactions, distribution. Micron 35(1–2):93–96
Collins T, Gerday C, Feller G (2005) Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol Rev 29(1):3–23
Conway M, Macario AL (2000) Stressors, stress and survival: overview. Front Biosci 5:780–786
Cooney DG, Emerson R (1964) Thermophilic fungi – an account of their biology, activities and classification. W.H. Freeman and Co., San Francisco
Cortez JM, Ellis J, Bishop DP (2001) Cellulase finishing of woven, cotton fabrics in jet and winch machines. J Biotechnol 89:239–245
Coutts AD, Smith RE (1976) Factors influencing the production of cellulases by *Sporotrichum thermophile*. Appl Environ Microbiol 31:819–825
Crisan EV (1973) Current concepts of thermophilism and the thermophilic fungi. Mycologia 65:1171–1198
De Gannes V, Eudoxie G, Hickey WJ (2013) Insights into fungal communities in composts revealed by 454-pyrosequencing: implications for human health and safety. Front Microbiol 4:1–9
Dervilly G, Leclercq C, Zimmerman D et al (2002) Isolation and characterization of high molecular mass water-soluble arabinoxylans from barley malt. Carbohydr Polym 47:143
Devi R, Kaur T, Kour D, Rana KL, Yadav A, Yadav AN (2020) Beneficial fungal communities from different habitats and their roles in plant growth promotion and soil health. Microb Biosyst 5:21–47. https://doi.org/10.21608/mb.2020.32802.1016
Dhakar K, Jain R, Tamta S, Pandey A (2014) Prolonged laccase production by a cold and pH tolerant strain of *Penicillium pinophilum* (MCC 1049) isolated from a low temperature environment. Enzym Res 2014:120708
Dos Santos E, Piovan T, Roberto IC et al (2003) Kinetics of the solid state fermentation of sugarcane bagasse by Thermoascus aurantiacus for the production of xylanase. Biotechnol Lett 25:13–16
Dusterhoft E-M, Linssen VAJM, Voragen AGJ, Beldman G (1997) Purification, characterization, and properties of two xylanases from *Humicola insolens*. Enzym Microb Technol 20:437–445
Dutra JCV, Terzi SC, Bevilaqua JV, Damaso MCT, Couri S, Langone MAP (2008) Lipase production in solid state fermentation monitoring biomass growth of Aspergillus niger using digital image processing. Appl Biochem Biotechnol 147(63):75
Fawzi E (2011) Highly thermostable xylanase purified from *Rhizomucor miehei* NRL 3169. Acta Biol Hung 62(1):85–94
Folan MA, Coughlan MP (1978) The cellulase complex in the culture filtrate of the thermophyllic fungus, *Talaromyces emersonii*. Int J Biochem 9:717–722
Gali NK, Kotteazeth S (2012) Isolation, purification and characterization of thermophilic laccase from xerophyte *Cereus pterogonus*. Chem Nat Compd 48:451–456
Gali NK, Kotteazeth S (2013) Biophysical characterization of thermophilic laccase from the xerophytes: *Cereus pterogonus* and *Opuntia vulgaris*. Cellulose 20:115–125
Ganju RK (1986) Isolation and characterization of polysaccharide degrading enzymes of *Chaetomium thermophile* var. *coprophile*. Ph.D. thesis, Indian Institute of Science, Bangalore
Ganju RK, Vithayathil PJ, Murthy SK (1989) Purification and characterization of two xylanases from Chaetomium thermophile var. coprophile. Can J Microbiol 35:836–842

Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R (2016) Microbial pectinases: an ecofriendly tool of nature for industries. 3 Biotech 6:47. https://doi.org/10.1007/s13205-016-0371-4
Gilbert HJ, Hazelwood GP (1993) Bacterial cellulases and xylanases. J Gen Microbiol 139:187–194
Golovan SP, Hayes MA, Phillips JP, Forsberg CW (2001) Transgenic mice expressing bacterial phytase as a model for phosphorus pollution control. Nat Biotechnol 19:429–433
Golz-Berner K, Walze B, Zastrow L, Doucet O (2004) Cosmetic and dermatological preparation containing copperbinding proteins for skin lightening. WO Patent 2004017931
Gomes J, Steiner W (2004) The biocatalytic potential of extremophiles and extremozymes. Food Technol Biotechnol 42:223–235
Gontia-Mishra I, Tiwari S (2013) Molecular characterization and comparative phylogenetic analysis of phytases from fungi with their prospective applications. Food Technol Biotechnol 51:313–326
Gray JA, Bemiller (2003) Bread staling: molecular basis and control. Rev Food Sci Food Saf 2:1–21
Greiner R, Konietzny U (2006) Phytase for food application. Food Technol Biotechnol 44:125–140
Griebeler N, Polloni AE, Remonatto D, Arbter F, Vardanega R, Cechet JL (2011) Isolation and screening of lipase producing fungi with hydrolytic activity. Food Bioprocess Technol 4:578–586
Gupta R, Kalpana (2011) Optimization of production and reaction conditions of polygalacturonase from Byssochlamys fulva. Acta Microbiol Immunol Hung 58:339–349
Haki GD, Raksit SK (2003) Developments in industrially important thermostable enzymes: a review. Bioresour Technol 89:17–34
Harbak L, Thygesen HV (2002) Safety evaluation of a xylanase expressed in *Bacillus subtilis*. Food Chem Toxicol 40(1):1–8
Harland BF, Morris ER (1995) Phytate: a good or a bad food component. Nutr Res 15:733–754
Hawksworth D, Lücking R (2017) Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectr 5:1–17
Heredia-Sandoval NG, Valencia-Tapia MY, de la Barca AMC, Islas-Rubio AR (2016) Microbial proteases in baked goods: modification of gluten and effects on immunogenicity and product quality. Foods 30:E59
Hildén K, Hakala TK, Lundell T (2009) Thermotolerant and thermostable laccases. Biotechnol Lett 31:1117–1128
Hirose D, Degawa Y, Inaba S, Tokumasu S (2012) The anamorphic genus *Calcarisporiella* is a new member of the Mucoromycotina. Mycoscience 53:256–260
Hoffmann K, Pawłowska J, Walther G, Wrzosek M, De Hoog GS, Benny GL, Kirk PM, Voigt K (2013) The family structure of the Mucorales: a synoptic revision based on comprehensive multigene-genealogies. Persoonia 30:57–76
Hossain KHMG, González MD, Lozano GR, Tzanov T (2009) Multifunctional modification of wool using an enzymatic process in aqueous-organic media. J Biotechnol 141:58–63
Houbraken J, de Vries RP, Samson RA (2014) Modern taxonomy of biotechnologically important Aspergillus and Penicillium species. Adv Appl Microbiol 86:199–249
Hultman J, Vasara T, Partanen P, Kurola J, Kontro MH, Paulins L, Auvinen P, Romantschuk M (2010) Determination of fungal succession during municipal solid waste composting using a cloning-based analysis. J Appl Microbiol 108:472–487
Jain KK, Bhanja Dey T, Kumar S, Kuhad RC (2015) Production of thermostable hydrolases (cellulases and xylanase) from *Thermoascus Aurantiacus* RCKK: a potential fungus. Bioprocess Biosyst Eng 38(4):787–796
Javed M, Ahmed W, Zahoor S, Ikram-Ul HAQ (2010) Solid state culturing of thermophilic fungi for phytase production. Pak J Bot 42(5):3605–3611
Jensen B, Olsen J (1992) Physicochemical properties of a purified α-amylase from the thermophilic fungus *Thermomyces lanuginosus*. Enzym Microb Technol 14:112–116
Jensen B, Olsen J, Allerman K (1988) Purification of extracellular amylolytic enzymes from the thermophilic fungus *Thermomyces lanuginosus*. Can J Microbiol 34:218–223

Jiang ZQ, Yang SQ, Tan SS, Li LT, Li XT (2005) Characterization of a xylanase from the newly isolated thermophilic *Thermomyces lanuginosus* CAU44 and its application in bread making. Lett Appl Microbiol 41(1):69–76

Joo JC, Pack SP, Kim YH, Yoo YJ (2011) Thermostabilization of *Bacillus circulans* xylanase: computational optimization of unstable residues based on thermal fluctuation analysis. J Biotechnol 151(1):56–65

Kalogeris E, Christakopoulos P, Kekos D (1998) Studies on the solid state production of thermostable endoxylanases from Thermoascus aurantiacus, characterization of two isozymes. J Biotechnol 60:155–163

Kalogeris E, Christakopoulos P, Katapodis P et al (2003) Production and characterization of cellulolytic enzymes from the thermophilic fungus Thermoascus aurantiacus under solid state cultivation of agricultural wastes. Process Biochem 38:1099–1104

Karmakar M, Ray R (2010) Current trends in research and application of microbial cellulases. Res J Microbiol 6:41–53

Kaur G, Kumar S, Satyanarayana T (2004) Production, characterization and application of a thermostable polygalacturonase of a thermophilic mould Sporotrichum thermophile Apinis. Bioresour Technol 94:239–243

Kaur R, Saxena A, Sangwan P, Yadav AN, Kumar V, Dhaliwal HS (2017) Production and characterization of a neutral phytase of Penicillium oxalicum EUFR-3 isolated from Himalayan region. Nus Biosci 9:68–76

Kaur T, Devi R, Kour D, Yadav N, Prasad S, Singh A et al (2020) Advances in microbial bioresources for sustainable biofuels production: current research and future challenges. In: Yadav AN, Rastegari AA, Yadav N, Gaur R (eds) Biofuels production – sustainability and advances in microbial bioresources. Springer International Publishing, Cham, pp 371–387. https://doi.org/10.1007/978-3-030-53933-7_17

Khandke KM (1986) Isolation and functional characteristics of xylanases and cellulases of *Thermoascus aurantiacus*. Ph.D. thesis, Indian Institute of Science, Bangalore

Khandke KM, Vithayathil PJ, Murthy SK (1989) Purification of xylanase, b-glucosidase, endocellulase, and exocellulase from a thermophilic fungus, *Thermoascus aurantiacus*. Arch Biochem Biophys 274:491–500

Khatri BP, Bhattarai T, Shrestha S, Maharjan J (2015) Alkaline thermostable pectinase enzyme from Aspergillus niger strain MCAS2 isolated from Manaslu Conservation Area, Gorkha, Nepal. Springerplus 4:488

Kim T, Lei XG (2005) Expression and characterization of a thermostable serine protease (TfpA) from *Thermomonospora fusca* YX in *Pichia pastoris*. Appl Microbiol Biotechnol 68:355–359

Kitpreechavanich V, Hayashi M, Nagai S (1984) Purification and properties of endo-1,4-b-xylanase from *Humicola lanuginosa*. J Ferment Technol 5:415–420

Kohli P, Gupta R (2015) Alkaline pectinases: a review. Biocatal Agric Biotechnol 4:279–285

Kottwitz B, Schambil F (2004) Cellulase and cellulose containing detergent. US 20050020472 A1

Kour D, Rana KL, Kaur T, Singh B, Chauhan VS, Kumar A et al (2019a) Extremophiles for hydrolytic enzymes productions: biodiversity and potential biotechnological applications. In: Molina G, Gupta VK, Singh B, Gathergood N (eds) Bioprocessing for biomolecules production, pp 321–372. https://doi.org/10.1002/9781119434436.ch16

Kour D, Rana KL, Thakur S, Sharma S, Yadav N, Rastegari AA et al (2019b) Disruption of protease genes in microbes for production of heterologous proteins. In: Singh HB, Gupta VK, Jogaiah S (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 35–75. https://doi.org/10.1016/B978-0-444-63503-7.00003-6

Kour D, Rana KL, Yadav N, Yadav AN, Rastegari AA, Singh C et al (2019c) Technologies for biofuel production: current development, challenges, and future prospects. In: Rastegari AA, Yadav AN, Gupta A (eds) Prospects of renewable bioprocessing in future energy systems. Springer International Publishing, Cham, pp 1–50. https://doi.org/10.1007/978-3-030-14463-0_1

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019d) Agriculturally and industrially important fungi: current developments and potential biotechnological applications.

In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kour D, Kaur T, Yadav N, Rastegari AA, Singh B, Kumar V et al (2020) Phytases from microbes in phosphorus acquisition for plant growth promotion and soil health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, pp 157–176. https://doi.org/10.1016/B978-0-12-820526-6.00011-7

Krishnamurthy S (1989) Purification and properties of xylanases and b-glucosidases elaborated by the thermophilic fungus Paecilomyces varioti Bainier. Ph.D. thesis, Indian Institute of Science, Bangalore

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzym Res 2011:280696

Kumar M, Yadav AN, Tiwari R, Prasanna R, Saxena AK (2014a) Deciphering the diversity of culturable thermotolerant bacteria from Manikaran hot springs. Ann Microbiol 64:741–751

Kumar M, Yadav AN, Tiwari R, Prasanna R, Saxena AK (2014b) Evaluating the diversity of culturable thermotolerant bacteria from four hot springs of India. J Biodivers Bioprospect Dev 1:1–9

Kumar V, Yadav AN, Saxena A, Sangwan P, Dhaliwal HS (2016) Unravelling rhizospheric diversity and potential of phytase producing microbes. SM J Biol 2:1009

Kumar V, Yadav AN, Verema P, Sangwan P, Abhishake S, Singh B (2017a) β-Propeller phytases: diversity, catalytic attributes, current developments and potential biotechnological applications. Int J Biol Macromol 98:595–609

Kumar V, Yadav AN, Verma P, Sangwan P, Saxena A, Kumar K et al (2017b) β-Propeller phytases: diversity, catalytic attributes, current developments and potential biotechnological applications. Int J Biol Macromol 98:595–609. https://doi.org/10.1016/j.ijbiomac.2017.01.134

Kunamneni A, Ballesteros A, Plou FJ, Alcalde M (2007) Fungal laccase-a versatile enzyme for biotechnological applications. In: Mendez-Vilas A (ed) Communicating current research and educational topics and trends in applied microbiology. Formex, Badajoz, Spain, pp 233–245

Kunamneni A, Plou FJ, Ballesteros A, Alcalde M (2008) Laccases and their applications: a patent review. Recent Pat Biotechnol 2:10–24

Labat E, Morel MH, Rouau X (2000) Effects of laccase and ferulic acid on wheat flour doughs. Cereal Chem 77:823–828

Laderman KA, Davis BR, Krutzsch CH, Lewis MS, Griko YV, Privalov PL, Anfinsen BC (1993) The purification and characterization of an extremely thermostable α-amylase from the hyperthermophillic Archaebacterium Pyrococcus furiosus. J Biol Chem 32(15):24394–24401

Lang G, Cotteret J (1999) Hair dye composition containing a laccase. (L'Oreal, Fr.). WO Patent 9,936,036

Lara-Márquez A, Zavala-Páramo MG, López-Romero E, Cano Camacho H (2011) Biotechnological potential of pectinolytic complexes of fungi. Biotechnol Lett 33:859–868

Laxman RS, Sonawane AP, More SV et al (2005) Optimization and scale up of production of alkaline protease from *Conidiobolus coronatus*. Process Biochem 40:3152–3158

Le Goff O, Bru-Adan V, Bacheley H, Godon JJ, Wéry N (2010) The microbial signature of aerosols produced during the thermophilic phase of composting. J Appl Microbiol 108:325–340

Leveque E, Janecek S, Haye B, Belarbi A (2000) Thermophillic archaeal amylolytic enzyme. Enzym Microb Technol 26(1):3–14

Li AN, Li DC (2009) Cloning, expression and characterization of the serine protease gene from *Chaetomium thermophilum*. J Appl Microbiol 106(2):369–380

Li DC, Li AN, Papageorgiou AC (2011) Cellulases from thermophilic fungi: recent insights and biotechnological potential. Enzym Res 2011:308730

Liu WH, Beppu T, Arima K (1973) Physical and chemical properties of the lipase of thermophilic fungus *Humicola lanuginosa* S-38. Agric Biol Chem 37:2493–2499

López-García P (1999) DNA supercoiling and temperature adaptation: a clue to early diversif ication of life. J Mol Evol 49(4):439–452

Madhavi V, Lele SS (2009) Laccase properties, use. Bioresouces 4:1694–1717
Maheshwari R, Bharadwaj G, Bhat MK (2000) Thermophilic Fungi: their physiology and enzymes. Microbiol Mol Biol Rev 64(3):461–488
Mandels M (1975) Microbial sources of cellulase. Biotechnol Bioeng Symp (5):81–105
Marguet E, Forterre P (1998) Protection of DNA by salts against thermodegradation at temperatures typical for hyperthermophiles. Extremophiles 2(2):115–122
Martin N, de Souza SR, da Silva R, Gomes E (2004) Pectinase production by fungal strains in solid-state fermentation using agro-industrial bioproduct. Braz Arch Biol Technol 47(5):813–819
Masui DC, Zimbardi AL, Souza FH, Guimarães LH, Furriel RP, Jorge JA (2012) Production of a xylose-stimulated β-glucosidase and a cellulase-free thermostable xylanase by the thermophilic fungus *Humicola brevis* var. *thermoidea* under solid state fermentation. World J Microbiol Biotechnol 28(8):2689–2701
Matsuo M, Yasui T (1985) Properties of xylanase of *Malbranchea pulchella* var. *sulfurea* no. 48. Agric Biol Chem 49:839–841
Mayer AM, Staples RC (2002) Laccase: new functions for an old enzyme. Phytochemistry 60(6):551–565
Mehta D, Satyanarayana T (2013) Thermophilic microbes in environmental and industrial biotechnology: biotechnology of thermophiles. In: Satyanarayana T, Littlechild J, Kaw Arabayasi Y (eds) Diversity of hot environments and thermophilic microbes. Springer, Netherlands, pp 45–46
Mishra RS, Maheshwari R (1996) Amylases of the thermophilic fungus *Thermomyces lanuginosus*: their purification, properties, action on starch and response to heat. J Biosci 21:653–672
Mitchell DB, Vogel K, Weimann BJ, Pasamontes L, van Loon AP (1997) The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi Aspergillus terreus and Myceliophthora thermophila. Microbiology 143:245–252
Monti R, Terenzi HF, Jorge JA (1991) Purification and properties of an extracellular xylanase from the thermophilic fungus *Humicola grisea* var. *thermoidea*. Can J Microbiol 37:675–681
Moore Landecker E (1996) Fundamenttals of the fungi, 4th edn. Prentice Hall, Inc., Hoboken, NJ
Morgenstern I, Powlowski J, Ishmael N, Darmond C, Marqueteau S, Moisan M, Quenneville G, Tsang A (2012) A molecular phylogeny of thermophilic fungi. Fungal Biol 116:489–502
Morozova OV, Shumakovich GP, Gorbacheva MA, Shleev SV, Yaropolov AI (2007) Blue laccases. Biochemistry 72(10):1136–1150
Msangi S, Sulser T, Rosegrant M, Valmonte-Santos R (2007) Global scenarios for biofuels: impacts and implications for food security and water use. Paper presented at the 10th Annual conference on global economic analysis, Purdue University, Indiana
Mullaney EJ, Daly CB, Ullah AH (2000) Advances in phytase research. Adv Appl Microbiol 47:157–199
Murray P, Aro N, Collins C, Grassick A, Penttilä M et al (2004) Expression in Trichoderma reesei and characterisation of a thermostable family 3 β-glucosidase from the moderately thermophilic fungus Talaromyces emersonii. Protein Expr Purif 38:248–257
Nahm KH (2002) Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure. Crit Rev Environ Sci Technol 32:1–16
Naqvi SWA, Jayakumar DA, Narvekar PV, Naik H, Sharma VVSS, D'Souza W (2000) Increased marine production of N_2O due to intensifying anoxia on the Indian continental shelf. Nature 408:346–350
Nayebyazdi N, Tajick MAG (2012) Pectinase activity of some micromycetes isolated from agricultural soils. J Sci Islamic Repub Iran 23(4):305–311
Neurath H (1957) The multiple specificity of proteolytic enzymes. Ann N Y Acad Sci 68:1–24
Nguyen QD, Rezessy-Szabo JM, Claeyssens M, Stals I, Hoschke A (2002) Purification and characterization of amylolytic enzymes from thermophilic fungus *Thermomyces lanuginosus* strain ATCC 34626. Enzym Microb Technol 3:345–352
Niehaus F, Bertoldo C, Kahler M, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial applications. Appl Microbiol Biotechnol 51(6):711–729

Nielsen JE, Borchert TV (2000) Protein engineering of bacterial alpha amylase. Biochim Biophys Acta 1543:253–274
Nigam P, Singh D (1995) Processes for fermentative production of xylitol a sugar substitute. Process Biochem 30:117–124
Nirmal NP, Laxman RS (2014) Enhanced thermostability of a fungal alkaline protease by different additives. Enzym Res 2014:1–8
Nwagu TN, Okolo BN (2011) Extracellular amylase production of a Thermotolerant Fusarium sp. isolated from Eastern Nigerian soil. Braz Arch Biol Technol 54(4):649–658
Oberson J, Binz T, Fracheboud D, Canevascini G (1992) Comparative investigation of cellulose-degrading enzyme systems produced by different strains of Myceliophthora thermophila (Apinis) v. Oorschot. Enzyme Microb Technol 14:303–312
Ogundero VW (1980) Lipase activity of thermophilir fungi from mouldy groundnuts in Nigeria. Mycologia 72:118–126
Okonji RE, Itakorode BO, Ovumedia JO, Adedeji OS (2019) Purification and biochemical characterization of pectinase produced by Aspergillus fumigatus isolated from soil of decomposing plant materials. J Appl Biol Biotechnol 7(3):1–8. https://doi.org/10.7324/JABB.2019.70301
Olagoke OA (2014) Amylase activities of some thermophilic fungi isolated from municipal solid wastes and Palm-Kernel Stack. Am J Microbiol Biotechnol 1(2):64–70
Oliveira TB, Gomes E, Rodrigues A (2015) Thermophilic fungi in the new age of fungal taxonomy. Extremophiles 19:31–37
Oliveira TB, Gostinčar C, Gunde-Cimerman N, Rodrigues A (2018) Genome mining for peptidases in heat-tolerant and mesophilic fungi and putative adaptations for thermostability. BMC Genomics 19:152
Omar IC, Hayashi M, Nagai S (1987a) Purification and some properties of a thermostable lipase from *Humicola lanuginosa* no. 3. Agric Biol Chem 51:37–45
Omar IC, Nishio N, Nagai S (1987b) Production of a thermostable lipase by *Humicola lanuginosa* grown on sorbitol-corn steep liquor medium. Agric Biol Chem 51:2145–2151
Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000) Advances in microbial amylases. Rev Biotechnol Appl Biochem 31:135–152
Paziarloglu NK, Sariisik M, Telefoncu A (2005) Laccase production by Trametes versicolor and application to denim washing. Process Biochem 40:1673–1678
Pereira JC, Marques NP, Rodrigues A, Oliveira TB, Boscolo M, da Silva R, Gomes E, Martins DAB (2015) Thermophilic fungi as new sources for production of cellulases and xylanases with potential use in sugarcane bagasse saccharification. J Appl Microbiol 118:928–939
Phutela U, Dhuna V, Sandhu S, Chadha BS (2005) Pectinase and Polygalacturonase production by a thermophilic Aspergillus fumigatus isolated from decomposting orange peels. Braz J Microbiol 36:63–69
Picart P, Diaz P, Pastor FI (2007) Cellulases from two Penicillium sp. strains isolated from subtropical forest soil: production and characterization. Lett Appl Microbiol 45:108–113
Polizeli MLT, Rizzatti M, Monti R et al (2005) Xylanases from fungi: properties and industrial applications. Appl Microbiol Biotechnol 67:577–591
Priya V, Sashi (2014) Pectinase enzyme producing microorganisms. Int J Sci Res Publ 4(3):2250–3153
Rajasekaran AK, Maheshwari R (1990) Effect of growth temperature on lipid composition of a thermophilic fungus *Thermomyces lanuginosus*. Indian J Exp Biol 28:134–137
Rajasekaran AK, Maheshwari R (1993) Thermophilic fungi: an assessment of their potential for growth in soil. J Biosci 18:345–354
Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN et al (2019) Endophytic fungi: biodiversity, ecological significance and potential industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer, Switzerland, pp 1–62
Rao VB, Sastri NVS, Subba Rao PV (1981) Purification and characterization of a thermostable glucoamylase from the thermophilic fungus *Thermomyces lanuginosus*. Biochem J 193:379–387

Reddy NR, Pierson MD, Sathe SK, Salunkhe DK (1989) Phytates in cereals and legumes. CRC Press, Boca Raton, FL

Rodríguez-Couto S, Toca-Herrera JL (2006) Laccase in the textile industry. Biotechnol Mol Biol Rev 1:117–122

Romdhane IBB, Achouri IM, Belghith H (2010) Improvement of highly thermostable xylanases production by *Talaromyces thermophiles* for the agro-industrials residue hydrolysis. Appl Biochem Biotechnol 162:1635–1646

Ruller R, Deliberto L, Ferreira TL, Ward RJ (2008) Thermostable variants of the recombinant xylanase A from *Bacillus subtilis* produced by directed evolution show reduced heat capacity changes. Proteins 70(2):1280–1293

Sadhukhan R, Roy SK, Raha SK, Manna S, Chakrabarty SL (1992) Induction and regulation of a-amylase synthesis in a cellulolytic thermophilic fungus Myceliophthora thermophila D14 (ATCC 48104). Indian J Exp Biol 30:482–486

Sahnoun Silva NFS, Simões MR, Knob A, Moraes SS, Henn C, Conceiçã-Silva JL, Simão RCG, Maller A, Kadowaki MK (2016) Improvement in the bleaching of kraft pulp with xylanase from *Penicillium crustosum* FP11 isolated from the Atlantic forest. Biocatal Biotransform 34:1–9

Salar RK, Aneja KR (2006) Thermophilous fungi from temperate soils of northern India. J Agric Technol 2:49–58

Satyanarayana T, Singh B (2004) Thermophilic moulds: diversity and potential biotechnological applications. In: Gautam SP, Sharma A, Sandhu SS, Pandey AK (eds) Microbial diversity: opportunities and challenges. Shree Publishers and Distributors, New Delhi, pp 87–110

Satyanarayana T, Johri BN, Klein J (1992) Biotechnological potential of thermophilic fungi. In: Arora DK, Elander RP, Mukherji KG (eds) Handbook of applied mycology, vol 4. Marcel Dekker, New York, pp 729–761

Saxena AK, Yadav AN, Rajawat M, Kaushik R, Kumar R, Kumar M et al (2016) Microbial diversity of extreme regions: an unseen heritage and wealth. Indian J Plant Genet Resour 29:246–248

Sinensky M (1974) Homeoviscous adaptation—a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc Natl Acad Sci U S A 71:522–525

Singh B, Satyanarayana T (2008) Phytase production by a thermophilic mould *Sporotrichum thermophile* in solid-state fermentation and its potential applications. Bioresour Technol 99(8):2824–2830

Singh B, Satyanarayana T (2011) Phytases from thermophilic molds: their production, characteristics and multifarious applications. Process Biochem 46:1391–1398

Singh B, Pocas-Fonseca MJ, Johri BN, Satyanarayana T (2016) Thermophilic molds: biology and applications. Crit Rev Biotechnol 42:985–1006

Sternberg M (1976) Microbial rennets. Adv Appl Microbiol 20:135–157

Straatsma G, Samson RA, Olijnsma TW, Den Camp HJO, Gerrits JP, Van Griensven LJ (1994) Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. Appl Environ Microbiol 60:454–458

Suman A, Verma P, Yadav AN, Saxena AK (2015) Bioprospecting for extracellular hydrolytic enzymes from culturable thermotolerant bacteria isolated from Manikaran thermal springs. Res J Biotechnol 10:33–42

Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11

Sydenham R, Zheng Y, Riemens A, Tsang A, Powlowski J, Storms R (2014) Cloning and enzymatic characterization of four thermostable fungal endo-1,4-β-xylanases. Appl Microbiol Biotechnol 98(8):3613–3628

Takase T, Narise A, Sakurai K (2011) Deodorant composition. Int. Pat. Appl. WO Patent 2,011,105,042

Tan LUL, Mayers P, Saddler JN (1987) Purification and characterization of a thermostable xylanase from a thermophilic fungus *Thermoascus aurantiacus*. Can J Microbiol 33:689–692

Tansey MR, Brock TD (1972) The upper temperature limit for eukaryotic organisms. Proc Natl Acad Sci U S A 69:2426–2428

Techapun C, Poosaran N, Watanabe M, Sasaki K (2003) Thermostable and alkaline-tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocesses: a review. Process Biochem 38(9):1327–1340

Tian L, Branford-White C, Wang W, Nie H, Zhu L (2012) Laccase-mediated system pretreatment to enhance the effect of hydrogen peroxide bleaching of cotton fabric. Int J Biol Macromol 50:782–787

Tiquia SM, Tam NFY, Hodgkiss IJ (1996) Effects of composting on phytotoxicity of spent pig-manure sawdust litter. Environ Pollut 93:249–256

Tiquia SM, Wan JHC, Tam NFY (2002) Microbial population dynamics and enzyme activities during composting. Compost Sci Util 10:150–161

Tuohy MG, Puls J, Claeyssens M, Vrsanská M, Coughlan MP (1993) The xylan-degrading enzyme system of Talaromyces emersonii: novel enzymes with activity against aryl b-D-xylosides and unsubstituted xylans. Biochem J 290:515–523

van Noort V, Bradatsch B, Arumugam M, Amlacher S, Bange G, Creevey C, Falk S, Mende DR, Sinning I, Hurt E, Bork P (2013) Consistent mutational paths predict eukaryotic thermostability. BMC Evol Biol 13:7

Van Paridon PA, Booman JCP, Selten GCM et al (1992) Application of fungal endoxylanase in poultry diets. Elsevier, Amsterdam, pp 371–378

Vats P, Bhattacharyya MS, Banerjee UC (2005) Use of phytases (myoinositol hexakisphosphate phosphohydrolases) for combatting environmenta pollution: a biological approach. Crit Rev Environ Sci Technol 35:469–486

Verma D, Satyanarayana T (2012) Molecular approaches for ameliorating microbial xylanases. Bioresour Technol 117:360–367

Wang HX, Ng TB (2004) A novel laccase with fair thermostability from the edible wild mushroom (Albatrella dispansus). Biochem Biophys Res Commun 315:450–454

Wood TM (1985) Properties of cellulolytic enzyme systems. Biochem Soc Trans 13:407–410

Wyss M, Pasamontes L, Friedlein A, Remy R, Tessier M, Kronenberger A (1999) Biophysical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): molecular size, glycosylation pattern and engineering of proteolytic resistance. Appl Environ Microbiol 65:359–366

Xie J, Song L, Li X, Yi X, Xu H, Li J, Qiao D, Cao Y (2011) Site-directed mutagenesis and thermostability of xylanase XYNB from *Aspergillus niger* 400264. Curr Microbiol 62(1):242–248

Yadav AN (2015) Bacterial diversity of cold deserts and mining of genes for low temperature tolerance. Ph.D. Thesis, IARI, New Delhi/BIT, Ranchi, p 234, https://doi.org/10.13140/RG.2.1.2948.1283/2

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi, Diversity and enzymes perspectives, vol 1. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N (2020a) Microbiomes of extreme environments, Biotechnological applications in agriculture, environment and industry, vol 2. CRC Press, Taylor & Francis Group, Boca Raton, FL

Yadav AN, Rastegari AA, Yadav N (2020b) Microbiomes of extreme environments: biodiversity and biotechnological applications. CRC Press, Taylor & Francis, Boca Raton, FL

Yadav AN, Rastegari AA, Yadav N, Gaur R (2020c) Biofuels production – sustainability and advances in microbial bioresources. Springer, Cham

Yang SQ, Yan QJ, Jiang ZQ, Li LT, Tian HM, Wang YZ (2006) Highlevel of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid-state fermentation. Bioresour Technol 97(15):1794–1800

Yoshioka H, Hayashida S (1980) Purification and properties of b-glucosidase from *Humicola insolens* YH-8. Agric Biol Chem 44:1729–1735

You C, Huang Q, Xue H, Xu Y, Lu H (2010) Potential hydrophobic interaction between two cysteines in interior hydrophobic region improves thermostability of a family 11 xylanase from *Neocallimastix patriciarum*. Biotechnol Bioeng 105(5):861–870

Zambare V, Nilegaonkar S, Kanekar P (2011) A novel extracellular protease from *Pseudomonas aeruginosa* MCM B-327: enzyme production and its partial characterization. New Biotechnol 28:173–181

Zhang S, Zhang K, Chen X, Chu X, Sun F, Dong Z (2010) Five mutations in n-terminus confer thermostability on mesophilic xylanase. Biochem Biophys Res Commun 395(2):200–206

Chapter 10
Bioactive Secondary Metabolites from Psychrophilic Fungi and Their Industrial Importance

Chanda Vikrant Berde, Asha Giriyan, and Vikrant Balkrishna Berde

Contents

C. V. Berde (✉)
Marine Microbiology, School of Earth, Ocean & Atmospheric Sciences, Goa University, Taleigao Plateau, Goa, India
e-mail: chanda.berde@unigoa.ac.in

A. Giriyan
Coastal Ecology and Marine Resource Center, The Energy and Resource Institute, Panaji, Goa, India

V. B. Berde
Department of Zoology, Arts, Commerce and Science College, Lanja, Maharashtra, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_10

10.1 Introduction

Psychrophilic microorganisms are cold-adapted organisms that have an optimum growth temperature below 15 °C, and often below 5 °C. The cold biosphere includes aquatic and terrestrial environments, but many temperate habitats often have cold temperatures during autumn and winter (Margesin and Miteva 2011; Yadav et al. 2018). Cold-adapted fungi are ubiquitous in cold habitats such as the deep seas, Arctic and Antarctic areas, and glaciers. Psychrophilic fungi, including yeasts and filamentous fungi, are adapted to cold ecosystems like the Arctic and Antarctic zones. Despite the extreme conditions of glacial ice of Antarctica, such as temperatures below 0 °C, low nutrient availability (ultra-oligotrophic conditions), and low water activity, we detected a diverse fungal community, including species never before reported in the glacial ice of Arctic and Antarctica (Vincent 1988; Vishniac 1996; Del Frate and Caretta 1990; Robinson 2001; Deming 2002; Gocheva et al. 2005; Frisvad 2008; Yadav et al. 2020b).

Cold-adapted fungi have evolved special properties, for example, cold-adapted enzymes, change of membrane fluidity, and other cellular components, to enable them to grow at low temperatures at rates comparable to those of mesophiles at moderate temperatures (D'Amico et al. 2006; Ruisi et al. 2007). The terms stenopsychrophile and eurypsychrophile have therefore been proposed to modify the definitions of psychrophilic and psychrotolerant. The "steno-" and "eury-" are referred ecological terms derived from Shelford's law of tolerance that describe narrow or wide tolerance to an environmental determinant, respectively. The stenopsychrophile (equal to "psychrophile") refers to microorganisms with a restricted growth-temperature range that cannot tolerate higher temperatures. Eurypsychrophile (equal to "psychrotolerant microorganisms") describes microorganisms that "like" permanently cold environments, but can also tolerate a wide range of temperatures extending into the mesophilic range (Cavicchioli 2006).

In recent years, the diversity of filamentous fungi in cold niches has been increasingly investigated, and the number of known species has greatly expanded (Möller and Dreyfuss 1996; Robinson 2001; Blanchette et al. 2004; Arenz et al. 2006; Connell et al. 2006; Held et al. 2006; Malosso et al. 2006; Duncan et al. 2008; Onofri et al. 2008; Selbmann et al. 2008; Arenz and Blanchette 2009; Jurgens et al. 2009).

Most species in these studies, however, are psychrotolerant, and only a few were documented as psychrophiles such as *Thelebolus microsporus*, *Mucor strictus*, *Phoma herbarum*, *Humicola marvinii*, *Pseudogymnoascus destructans*, and some snow molds for example *Sclerotinia borealis*, *Microdochium nivale*, *Coprinus psychromorbidus* (Schipper 1967; Dejardin and Ward 1971; Traquair and Smith 1982; Richard et al. 1997; Hsiang et al. 1999; Tronsmo et al. 2001; Singh et al. 2006; Gargas et al. 2009; Hoshino et al. 2010; Anupama et al. 2011; Minnis, and Lindner 2013). Species in several yeast genera including *Mrakia*, *Mrakiella*, and *Rhodotorula* were usually described as psychrophilic. For example, *Mrakia frigida* grew well at 15 °C and 4 °C but poorly at 20 °C (Margaret 1966) thus proving its psychrophilic

nature; *Mrakia psychrophila* from Antarctic soil had an optimal growth temperature of 10 °C (Xin and Zhou 2007); *Mrakiella cryoconiti*, *M. aquatica*, and *M. niccombsii* from alpine and Arctic habitats also exhibited psychrophilic features and failed to grow at temperatures over 20 °C (Margesin and Fell 2008; Robin et al. 2010).

During the past two decades, research on cold-adapted fungi has increased, driven by their potential value for application in biotechnology (Margesin and Schinner 1994, 1999). Cold-adapted fungi have become important sources for the discovery of novel bioactive secondary metabolites and enzymes (Flam 1994; Pietra 1997; Biabini and Laatch 1998; Gudjarnnson 1999; Höller et al. 2000; Verbist et al. 2000; Bhadury et al. 2006; Ebel 2006; Blunt et al. 2007; Rateb and Ebel 2011). This chapter highlights the production of bioactive secondary metabolites by psychrophilic fungi.

10.2 Biodiversity and Distribution of Psychrophilic Fungi

Three-quarters of the earth's surface are dominated by the cold habitats spanning from the Arctic to the Antarctic and from high-mountain regions to the deep ocean (Deming and Eicken 2007; Rodrigues and Tiedje 2008). The major fraction of this extreme environment is represented by the deep sea (90% of the ocean volume), followed by snow (35% of land surface), permafrost (24% of land surface), sea ice (13% of the earth's surface), and finally glaciers (10% of land surface). Other cold environments are cold-water lakes, cold soils, cold deserts, and caves (Lauro and Bartlett 2008; Yadav et al. 2017, 2020a). These extreme environments are colonized by enormously diverse communities of prokaryotes and eukaryotes (Cavicchioli 2006; Kalanetra et al. 2009; Margesin and Miteva 2011; Buzzini et al. 2012; Lamilla et al. 2017) are able to survive and maintain metabolic activity at subzero temperatures.

As an attempt to understand the global climate change scenario, study of the glacial ice samples for their physicochemical composition (de Menezes et al. 2019) was undertaken. During this study, the presence of fungal spores or hyphal fragments trapped in the ice matrix was observed. The probable reasons for this may also be the growth of fungi due to the occasional melting and freezing of the ice in the Arctic regions as reported by Lutz et al. (2015), DuoSaito et al. (2018), and Perini et al. (2019a, b). Paleomycological and paleoecological investigations of the North and South Poles have indicated the presence of fungi in Antarctica since at least the Permian period because diverse fossil fungi have been found from the Triassic and Jurassic Periods (Stubblefield and Taylor 1983; Taylor and Osborne 1996; Harper et al. 2012).

Despite being an extreme and ultra-oligotrophic environment, the glacial ice of Antarctica seems to harbor rich fungal diversity. Latest reports indicate the presence of Basidiomycota and Ascomycota taxa among fungal assemblages to predominant the glacial ice from the Arctic (Perini et al. 2019a, b) and Patagonia (DuoSaito et al. 2018) as well as soil from Antarctica (Connell et al. 2008; Arenz and Blanchette

2011). Zygomycetes and Chytridiomycota species have also been isolated from Antarctic lakes and ponds (Lawley et al. 2004; Paterson 1973).

Bridge and Spooner (2012) listed over 400 fungal genera and more than 1000 species that had been reported from Antarctic regions and suggested that fungi may be the most diverse biota in Antarctica. Species such as *Thelebolus*, *Glaciozyma*, *Rhodotorula*, and *Penicillium* were also found in high densities and 11 taxa were found in low densities, which had not been recorded in Antarctic glacial ice. *Thelebolus* sp. are abundant in lakes and is associated with skuas, petrels, and other birds in Antarctica (de Hoog et al. 2005; Brunati et al. 2009; Gonçalves et al. 2012a, b) and are isolated from Arctic and Antarctic regions (Kobayasi et al. 1967; Montemartini et al. 1993; Sazanova et al. 2019; Alves et al. 2019). The psychrophilic fungal diversity and incidence of fungal species in different habitats of Antarctica are given in Table 10.1.

Bovio et al. (2018) reported *Thelebolus balaustiformis* a new psychrophilic fungal species isolated from the sponge, *Dyside fragilis*, in the South, Atlantic Ocean in the glacial ice habitats. Another psychrophile *Glaciozyma antarctica* (former *Leucosporidium antarcticum*) was isolated from various locations including the Antarctic marine waters (Fell et al. 1969); the soil around Lake Fletcher, Lichen, and Taylor Valleys, and on the dead sponge (Turchetti et al. 2011). Timling et al. (2014) sampled soils along the North American Arctic Transect and was successful in isolating more than 4350 fungal species. The most frequently isolated fungal isolates were *Leotiomycetes* sp., followed by *Thelebolus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Periconia*, *Geomyces*, *Cryptococcus*, and *Pueraria.* Ascomycota dominated the communities, followed by Basidiomycota. Five families in Chytridiomycota and one family in each of Zygomycota, Glomeromycota, Blastocladiomycota, and Neocallimastigomycota were detected, while Cryptomycota were only identified at the phylum level.

Compared to the polar regions, cold-adapted fungi in the Qinghai–Tibet Plateau are less documented except for a study from wherein more than 1400 fungal strains were isolated and 150 species including 6 new species were identified and described. Among those species, *Phoma sclerotioides* and *Pseudogymnoascus pannorum* were the most dominant species. Psychrotolerant species in *Helotiales* (*Leotiomycetes*, *Ascomycota*), the most commonly found group was studied in-depth and six new species, *Psychrophila antarctica*, *P. lutea*, *P. olivacea*, *Tetracladium ellipsoideum*, *T. globosum*, and *T. psychrophilum* were described (Wang et al. 2015a, b).

Hassan (2015) isolated 77 fungal strains representing 24 fungal genera from Batura Passu and Siachen Glaciers in the Hindu Kush and Karakoram mountains in Pakistan. Most of the fungal isolates showed antimicrobial activity and production of enzymes such as cellulase, lipase, protease, DNase, phosphatase (Hassan et al. 2017).

Psychrophilic endophytic fungi (PEF) were isolated from healthy foliar tissues of *Cupressus arizonica*, *Cupressus sempervirens*, and *Thuja orientalis* (*Cupressaceae*, *Coniferales*). Most of the 110 endophytic fungal isolates belonged to ascomycetous fungi, more specifically *Phoma herbarum*, *Phoma* sp., *and Dothideomycetes* sp., with the ability to produce secondary metabolites. *Phoma*

Table 10.1 Psychrophilic fungi isolated from different habitats of Antarctica, showing antagonistic activities

Habitat	Fungi	Metabolic activities	References
Benthic mats of Antarctic lakes	160 filamentous fungi belonging to 15 fungal genera	Antimicrobial and cytotoxic activity	Brunati et al. (2009)
Algae associates from the rocky coastline of Elephant, King George, and Deception Islands, in the Antarctic Peninsula	148 fungal strains consisting of *Penicillium* (35.8%), *Geomyces* (24.3%), and the yeast *Mestchnikowia australis* (4.7%)	Antioxidants, anti-algal, antifungal, and anti-insect metabolites	Godinho et al. (2013)
Associates of endemic macroalgae *M. harioti* and *Pyropia endiviifolia*	*Pseudogymnoascus* sp., *Guehomyces pullulans*, *M. australis*	Antifungal activities	Furbino et al. (2014)
	Penicillium steckii	Inhibition of yellow fever virus	
Associates of marine sponges of Fildes Bay, King George Island	101 fungal isolates including genera *Geomyces*, *Penicillium*, *Epicoccum*, *Pseudoeurotium*, *Thelebolus*, *Cladosporium*, *Aspergillus*, *Aureobasidium*, *Phoma*, and *Trichocladium*	Antimicrobial and antitumoral compounds	Henríquez et al. (2014)
Marine sediments of Admiralty Bay	23 of the 47 fungal strains belonging to the genera *Pseudogymnoascus*, *Penicillium*, *Cadophora*, *Paraconiothyrium*, and *Toxicocladosporium*	Antibacterial activity against *Xanthomonas* species	Purić et al. (2018)
Marine and lake sediments from Deception Island	*Penicillium* sp. *Pseudogymnoascus* sp. *Schizophyllum* sp.	Antimicrobial cytotoxic and antiprotozoal	Gonçalves et al. (2015)
Terrestrial soils of Admiralty Bay, King George Island, and Deception Island	8 strains from the genera *Bauveria*, *Penicillium*, *Phanerochaete*, *Pseudoeurotium*, *Pseudogymnoascus*, *Purpureocillium*, and *Trichoderma* sp.	Antimicrobial, cytotoxic, and antiprotozoal activities	Gonçalves et al. (2015)
Union Glacier, in the southern Heritage Range	17 fungi including *A. sydowii*, *P. allii-sativi*, *P. brevicompactum*, *P. chrysogenum*, and *P. rubens*	Antibacterial, antifungal, antitumoral, antiprotozoal, and herbicidal activities	Godinho et al. (2015)
Chinese Antarctic station at Fildes Bay, King George Island	14 fungal strains	Cytotoxic, antimicrobial	Ding et al. (2016)

(continued)

Table 10.1 (continued)

Habitat	Fungi	Metabolic activities	References
Robert, Nelson, King George, and Penguin Islands at South Shetland archipelago	Filamentous fungi *Pseudogymnoascus destructans*, *Mortierella parvispora*, and *P. chrysogenum*, *P. tardochrysogenm*	Antiviral activity against dengue and Zika virus; antiparasitic activity; herbicidal activity against *L. sativa* (lettuce) and *Allium schoenoprasum* (chive)	Gomes et al. (2018)
Deception Island	6 *Pseudogymnoascus* sp. out of 33 filamentous isolates	Anti-*Xhantomonas* activity	Purić et al. (2018)
Endophytes of *D. antarctica*	21 fungal strains	Antifungal activity	Gonçalves et al. (2015)
Endophytic to moss *Schistidum antarctic* found in Admiralty Bay, King George Island	*Mortierella alpine*	Antioxidant activity and antibacterial activity	Melo et al. (2014)
Endophytic association with *D. antarctica* and *C. quitensis*	313 fungal isolates from *D. antarctica* 251 isolates from *C. quitensis*	Antiparasitic to *L. amazonensis* and *T. cruzi* Antitumor	Santiago et al. (2012)

herbarum has been reported by the number of workers as soil psychrophilic fungi that is pathogenic to plants growing in cold regions (Domsch et al. 1980; Flanagan and Scarborough 1974; Selbmann et al. 2005; Singh et al. 2006).

10.3 Associations and Cold Adaptation Mechanisms of Psychrophilic Fungi

Fungi overcome cold tolerance through several physiological mechanisms and it is likely that they employ them in combinations. Numerous adaptations and mechanisms can be observed. One of the mechanisms is the production and accumulation of intracellular solutes, also called as cryopreservants or cryoprotectants, such as glycerol, trehalose, and so on. Cryoprotectants are exopolymeric substances (e.g., sugars, alcohols, and amino acids), generated in high amounts believed to be in response to cold. These prevent cold-induced aggregation of proteins as well as maintain optimum membrane fluidity under low temperatures (Krembs et al. 2002; Mancuso Nichols et al. 2005).

Ophiocordyceps sinensis, the Chinese caterpillar fungus, is adapted to cold temperature with putative antifreeze proteins and mechanisms for increasing lipid accumulation and fatty acid unsaturation (Xiao et al. 2013). *Pseudogymnoascus pannorum* (*Geomyces pannorum*) is a soil-inhibiting fungus, isolated from Arctic and Antarctic regions, as well as glacier bank soils in some Asian countries

(Deshmukh 2002; Arenz et al. 2006; Ozerskaya et al. 2004). *P. pannorum* grows slowly at temperatures below 0 °C to as low as −20 °C. This fungus maintains cell and membrane function at low temperatures by elevating levels of unsaturated fats and compounds with cryoprotectant properties such as trehalose and various polyols at low temperatures (Finotti et al. 1996; Hayes 2012). Some of the adaptation means are discussed below:

10.3.1 Trehalose Accumulation

Trehalose is an important storage sugar in fungal vegetative cells and spores (Lewis and Smith 1967) and the most widely distributed disaccharide in fungi (Thevelein 1984). In fungal vegetative structures, trehalose is commonly found with sugar alcohols and glycogen. As per Cooke and Whipps (1993), trehalose appears to function as a general stress protectant in the cytosol and also stabilizing membranes during dehydration (Goodrich et al. 1988).

More recently, authors have demonstrated the accumulation of trehalose in fungal hyphae as a response to low temperatures. An elevation in trehalose composition was observed after the exposure of the fungi to low temperature or during growth at low temperature. Trehalose concentration in Mycorrhizal roots as well as increased accumulation in *Hebeloma* sp. is reported (Niederer et al. 1992; Tibbett et al. 1998). A shift in growth temperature, that is, lowering growth temperature further, also resulted in higher production of trehalose as seen in *Humicola marvinii*, a psychrophile, isolated from fell-field soil at Jane Col, Signy Island in Antarctica as well as *Mortierella elongata*, a psychrotrophic fungus (Weinstein et al. 2000).

10.3.2 Polyol Production

Glycerol and mannitol both polyols may increase in concentration to maintain turgor pressure against heat-mediated decreases in external water potential (Cooke and Whipps 1993). Mannitol likewise is thought to be important in protection against water stress (Lewis and Smith 1967) and maybe a cryoprotectant (Weinstein et al. 1997). Initial evidence of their potential cryoprotectant role came from a study by Weinstein et al. (1997), using an Antarctic isolate of *Humicola marvinii*.

10.3.3 Antifreeze Proteins (AFPs)

AFPs either intra- or extracellular may allow fungi to function and survive under freezing conditions by preventing the formation of ice and also preventing the freezing of cell components (Snider et al. 2000). *Glaciozyma antarctica*, a psychrophilic

yeast, produces AFPs that help in its survival in glacial ice (de Menezes et al. 2019). AFP is found in the hyphae of three psychrophilic snow molds, the ascomycete *Sclerotinia Borealis*, and two basidiomycetes, *Coprinus psychromorbidus* and *Typhula incarnate* was reported back in 1994 by Newsted et al. (1994). Recently, antifreeze activity in snow-mold fungi *Typhula incarnata*, *T. ishikariensis*, and *T. phacorrhiza* has been reported by Snider et al. (2000).

10.3.4 Membrane Fluidity

Another adaptation strategy is the regulation of membrane fluidity as a response to freezing environments. The membrane is the first barrier, protecting the cells from external environments, thus acts as an interface for the fungi (Chintalapati et al. 2004). Shivaji and Prakash (2010) reported an increase in membranes rigidity at cold temperatures, which activates a membrane-associated sensor and subsequent upregulation of genes to mediate the exchange of metabolites to and from thus enhancing membrane fluidity of the cell. This process is aided by the modification of fatty acyl chains of the membrane fatty acids (Russell 2008) wherein saturated fatty acids are converted to unsaturated fatty acids by desaturase enzymes (Chintalapati et al. 2004).

It is evident from studies that membrane composition influences the survival and growth of fungi over the environmental range of temperature variations (Cooke and Whipps 1993). Such changes leading to increased fluidity of the cell membranes have been observed in *Candida*, *Leucosporidium*, *Mucor*, *Torulopsis* (Kerekes and Nagy 1980; Dexter and Cooke 1984a, b) where the degree of unsaturated fatty acids increased at low temperatures. Apart from fatty acids, changes in the membrane phospholipid saturation levels, membrane proteins, and sterols, also determine the membrane fluidity and thus help in survival in low freezing (Dexter and Cooke 1985; Hammonds and Smith 1986). Change in growth temperature of psychrophilic fungi *Geomyces pannorum*, *Mortierella elongata*, *Microdochium nivale*, showed the lipid composition to change towards unsaturation, thus modifying the membrane fluidity to adjust to the lowered temperatures and survive (Istokovics et al. 1998; Weinstein et al. 2000).

10.4 Bioactive Secondary Metabolites of Psychrophilic Fungi

Research and discovery of secondary metabolites from fungi from the tropics and temperate regions have been the focus for the last few decades. However, work on psychrophilic fungi started recently but has acquired a considerable pace, especially studies on isolation and secondary metabolite studies from different habitats of Antarctica (Table 10.2). Studies indicate dominance of *Penicillium* sp. in the glacial ice of Antarctica. Another well-studied niche for the psychrophilic fungi is the deep

Table 10.2 Secondary metabolites produced by psychrophilic fungi isolated from Antarctica

Fungi	Metabolite	Application	References
Tritirachium sp.	4-Carboxy-5,5′-dihydroxy-3,3′-dimethyl-diphenylether and macrosphelides A and J	Cell adhesion inhibitors and moderately cytotoxic agent	Ivanova et al. (2007), Hayashi et al. (1995)
Trichoderma asperellum	Asperelines A–F	Inhibitory activity against fungi and bacteria	Ren et al. (2009)
Oidiodendron truncatum	Chetracins B and C, and 5 new diketopiperazines, named chetracin D and oidioperazines A–D, melinacidin IV, T988 B, T988 C, T988 A, chetoseminudin C, and cyclo-L-Trp-L-Ser	Cytotoxic towards 5 human cancer cell lines	Li et al. (2012b)
Aspergillus sydowii SP-1	Acremolin C, and (*cyclo*-(L-Trp-L-Phe), 4-hydroxy-phenyl acetic acid, (7*S*)-(+)-hydroxyl-sydonic acid, and (7*S*,11*S*)-(+)-12-hydroxysydonic acid)	Antibacterial	Li et al. (2018), Nishanth Kumar et al. (2014), Li et al. (2015)
A. ochraceopetaliformis	Ochraceopones A–E, isoasteltoxin, asteltoxin and asteltoxin B; Ochracenes A–I, *trans* (3*R*,4*S*)-(−)-4-hydroxymellein, cis (3*R*,4*R*)-(−)-4-hydroxymellein, (3*R*,4*R*)-4,7-dihydroxymellein, 3,5-dimethylpyrone, stachyline B, and (*E*)-methyl-5-methylhexa-3,5-dienoate	Antiviral activities against the H1N1 and H3N2 influenza viruses Moderate inhibitory effects on lipopolysaccharide induced NO release in RAW 264.7 mouse macrophage cell lines	Wang et al. (2015a, 2016, 2017)
Cadophora luteo-olivacea	Spiciferone F, colomitides C and D, cadopheronenes A–D, similin C, and spicifernin B; polyketides spiciferone A, spiciferol A, dihydrospiciferone A, and dihydrospiciferol A	Phytotoxicity activity and plant growth-promoter activity	Nakajima et al. (1989), Nakajima et al. (1990), Rusman et al. (2018)
Geomyces sp.	Ethyl asterrate, n-butyl asterrate, and geomycins A–C. Asterric acid, methyl asterrate, and bisechlorogeodin	Antibacterial and antifungal activities	Li et al. (2008)
Pseudogymnoascus sp.	Pseudogymnoascins A–C and 3-nitroasterric acid, questin, pyriculamide	–	Figueroa et al. (2015)
Pseudogymnoascus pannorum	Pannomycin	Antibacterial activity	Parish et al. (2009)

(continued)

Table 10.2 (continued)

Fungi	Metabolite	Application	References
Penicillium nalgiovense	Amphotericin B	Antifungal	Svahn et al. (2015)
Penicillium sp. SCIO 05705	Penillines A and B and isopenilline A; (*E*)-3-(1*H*-imidazole-4-yimethylene)-6-(1*H*-indl-3-ylmethyl)-2,5-piperazinediol, penilloid, meleagrin, neoxaline, questiomycin A, *N*-(2-hydroxypehnyl)-acetamide, and 2-benzoxazolinone	Cytotoxicity; antituberculosis activity	Wang et al. (2015b)
Penicillium funiculosum GWT2-24	Chrodrimanins I and J Chrodrimanins A, B, E, F, and H	Inhibitory activity against influenza virus H1N1; lipid-lowering activity in HepG2 hepatocytes	Zhou et al. (2015, 2016)
Penicillium sp. S-1-18	Butanolide A, and guignarderemophilane F; penicyclone A, xylarenone A, callyspongidipeptide A, *cyclo*-(L-Phe-4*R*-hydroxyl-L--Pro), *cyclo*-(L-Pro-L-Phe), and *N*-(2-hydroxypropanoyl)-2-aminobenzoic acid amide	Tyrosine phosphatase 1B inhibition	Zhou et al. (2017)
Penicillium crustosum HDN153086	(8*E*,4*E*,6*E*,8*E*)-10-Hydroxyundeca-2,4,6,8-tetraenoic acid, fusaperazine F xylariolide D and two diketopiperazines	Cytotoxic activities	Liu et al. (2019)
Mortierella alpina	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)	Antibacterial activity	Melo et al. (2014)
Pseudogymnoascus strains	–	Antifungal activities	Furbino et al. (2014)
Purpureocillium lilacinum	–	Trypanocidal, antifungal, and antibacterial activities	Gonçalves et al. (2015)
218 fungal extracts including *P. destructans*, *Mortierella parvispora*, and *P. chrysogenum*	–	Antiviral activity against dengue and Zika viruses Antiparasitic activity against *Trypanosoma cruzi* and *Leishmania amazonensis*, and herbicidal activity	Gomes et al. (2018)

(continued)

Table 10.2 (continued)

Fungi	Metabolite	Application	References
Pseudogymnoasc, *Penicillium*, *Cadophora*, *Paraconiothyrium*, *Toxicocladosporium*, *Xanthomonas citri*	–	Antimicrobial inhibitory compounds against phytopathogen bacteria	Vieira et al. (2018)
Penicillium tardochrysogenum	Penicillin, secalonic acids D and F	–	Houbraken et al. (2012)
P. chrysogenum	–	Selective antimicrobial activities	Brunati et al. (2009)
P. chrysogenum	–	Antifungal and/or trypanocidal activities	Godinho et al. (2013)
Penicillium steckii	–	Antiviral activity against yellow fever virus	Furbino et al. (2014)
A. sydowii, *P. allii-sativi*, *P. brevicompactum*, *P. chrysogenum*, *P. rubens*	–	Antiviral, antimicrobial (antibacterial and antifungal), anticancer, antiprotozoal, and herbicidal activities	Godinho et al. (2015)

sea and a lot of research has been documented (Höller et al. 2000; Jensen and Fenical 2000; Verbist et al. 2000; Hentschel 2002; Bhadury et al. 2006; Ebel 2006; Konig et al. 2006; Newman and Hill 2006; Paul et al. 2006; Damare et al. 2006, 2008; Blunt et al. 2007).

Most of the species found in these cold regions have the ability to form extrolites in large amounts and have been reported to be species-specific (Larsen et al. 2005). The fungal communities found in the habitats of Antarctic regions have structures, which can be used for designing potential drugs and other products to treat tropical diseases and cancer. Rosa et al. (2019) described the extrolites produced by psychrophiles and have reported *Aspergillus*, *Cladosporium*, *Penicillium*, *Pseudogymnoascus*, *Phaeosphaeria*, *Microdochium*, *Mortierella*, and *Purpureocillium* sp. in their findings. Several such reports on the production of useful extrolites are available. Secondary metabolites produced by psychrophilic fungi are described below:

10.4.1 Antibiotics

Some unique components and products with potential bioactive properties have been isolated and characterized from psychrophilic fungi. *Penicillium* species are the best known fungal strains for their capability to produce diverse bioactive

compounds, including penicillin, produced by the strain *P. chrysogenum* (Houbraken et al. 2012; Devi et al. 2020; Rastegari et al. 2019a). *Penicillium* sp. was isolated from the Antarctic soil by Antipova et al. (2018) and has been reported to produce a number of unknown metabolites with numerous bioactivities (Rosa et al. 2019). Brunati et al. (2009) reported the production of rugulosin and skyrin (*bis*-anthraquinones) by strains of *P. chrysogenum* from Antarctica which had antibacterial activity against Gram-negative and Gram-positive organisms.

Another research group reported antifungal and anti-trypanocidal activities of *P. chrysogenum* extracts, associated with Antarctic algae *Palmaria decipiens* (Godinho et al. 2013). Strains of *P. chrysogenum* isolated from Antarctica soil samples demonstrated trypanocidal and herbicidal activities (Godinho et al. 2015). The most recent report on *P. palitans*, isolated from permafrost lying undisturbed for 30,000 years, produced two metabolites namely, festuclavine and fumigaclavines A and B (Kozlovsky et al. 2020). Psychrophile fungus *Penicillium rivulum*, producing new psychrophilins, and complex alkaloids communesins was characterized by Dalsgaard et al. (2004a, b, 2005a). Thus the *Penicillium* species incident in the cold habitats of Antarctica is "mycofactories" having the ability to produce a diverse range of biometabolites with several applications.

Santiago et al. (2012) studied the capabilities of Antarctic endophytic fungi recovered from *Deschampsia antarctica* to produce bioactive secondary compounds against neglected tropical diseases and tumor cells. Li et al. (2008) worked with *Geomyces* sp. strains isolated from Antarctica, that could produce asterric acid derivatives that are known for antibacterial, antifungal, and anti-angiogenic activities (Giddings and Newman 2014; Li et al. 2008; Mahmoodian and Stickings 1964; Lee et al. 2002). Further in 2012, Li and coworkers isolated a number of compounds from psychrophilic fungus *Oidiodendron truncatum* including two new epipolythiodioxopiperazines (ETPs), chetracins, and five new diketopiperaines, chetracin D, and oidioperaines A–D (Li et al. 2012a; Jiang and Guo 2011; Giddings and Newman 2014)

10.4.2 Cytotoxic Metabolites

Several cytotoxic compounds from psychrophilic fungi have been reported by the number of workers. The secondary metabolites, some of them, new records, have weak to moderately significant activity toward cancer cells. Table 10.3 gives a summary of some of the cytotoxic secondary metabolites isolated from psychrophilic and psychrotolerant fungi.

Table 10.3 Cytotoxic secondary metabolites isolated from psychrophilic and psychrotolerant fungi

Fungal species	Secondary metabolite	Nature of metabolite	Activity	References
Penicillium algidum	Psychrophilin D, Cycloaspeptide A and B	Nitropeptide and cyclopeptides	Cytotoxic to murine leukemia cells	Dalsgaard et al. (2005b)
Aspergillus sp.	Psychrophilin E and F	Nitropeptide	Cytotoxic to murine leukemia cells; lipid-lowering activities	Ebada et al. (2014), Peng et al. (2014)
Oidiodendron truncatum GW3-13	Epipolythiodioxopiperazines chetracins B and C; diketopiperazines chetracin D; melinacidin IV, T988, and T988 A	Piperazines	Cytotoxic activity	Li et al. (2012b)
Penicillium sp. PR19 N-1	Eremophilane-type compound	Sesquiterpene compounds	Cytotoxic to HL-60 cells and A-549 cell lines	Lin et al. (2014)
	Chloro-trinoreremophilane sesquiterpene	Chloroeremophilan sesquiterpene		Wu et al. (2013)
Trichoderma velutinum	Lipovelutibols B and D	Lipopeptaibols	HL-60, MDA-MB-231, A549, and LS180 cell lines	Singh et al. (2018)

10.4.3 Diterpenes

Diterpenes having antibacterial activity toward Gram-positive and Gram-negative bacteria namely *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*, were report to be produced by *Eutypella* sp. D-1. Among the diterpenes isolated from this psychrophilic fungus, libertellenone G and libertellenone H are new while two other known pimarane diterpenes were also detected (Liu et al. 2014).

10.4.4 Cyclic Peptides

Cyclic peptides have applications in different fields, such as the pharmaceutical industry for their anti-infective, antitumor, antimalarial activity, agricultural applications as fungicides, diagnostics, and vaccines (Demmer et al. 2009; Demain and Sanchez 2009; Claro et al. 2018). Studies on secondary metabolites from psychrophilic fungi producing cyclic peptides are sparse. A new cyclic nitropeptide, Psychrophilin Dan antitumor compound, was extracted from the fungus *Penicillium algidium* a psychrophilic fungus isolated from Greenland (Ivanova et al. 2001). From the same strain, two more cyclic peptides were obtained namely,

cycloaspeptide A and cycloaspeptide D. Later, Dalsgaard et al. (2004a) isolated cyclic peptides related to these, that is, psychrophilin A and cycoloaspeptide D from the extracts of the psychrophilic fungus *Penicillium reibeum*, both of which are new metabolites having antitumor and antagonistic activities (Demain and Sanchez 2009).

10.4.5 Polyketides

Polyketides (PKs) have antimicrobial activity and other clinically important applications. PKs help in nutrient assimilation resulting in lowering the capacities of competitors in the environments (Mukherjee et al. 2012). *Penicillium crustosum* PRB-2 from the deep sea of the Antarctic was found to produce Penilactones A and B, the oxygenated polyketides (Wu et al. 2012). While the same group also reported hybrid polyketides, like cladosins which were produced by *Cladosporium sphaerospermum* 2005-01-E3, a deep-sea isolates. One of the cladosins, Cladosin C showed antiviral activity influenza A H1N1 virus (Wu et al. 2014). The same workers further isolated polyketide Scequinadoline A from psychrophilic fungi *Dichotomomyces cejpii* F31-1(Wu et al. 2018) and polyketide, anthraquinone–xanthone from *Engyodontium album* LF069 (Wu et al. 2016). Scequinadoline A demonstrated antiviral activity against the dengue virus while the anthraquinone–xanthone could inhibit methicillin-resistant *Staphylococcus aureus*.

10.4.6 Exopolysaccharides (EPS)

Production of exopolysaccharide is a stress response observed in marine or aquatic organisms. Fungus EPS, apart from having good rheological properties, have numerous bioactive characteristic features such as antitumor, antioxidant, anti-inflammation, immune-stimulating, anti-anemics (Rastegari et al. 2019b). Thus it has a good potential in the health and drug industry (Li et al. 2013). It was observed that fungi with eps showed better growth under freeze-thaw conditions compared to fungi without eps (Selbmann et al. 2002; Yadav et al. 2019).

The fungal isolate from Antarctica, *Phoma herbarum* CCFEE 5080, showed the production of exopolysaccharide made up of glucan (Selbmann et al. 2002). Onofri (1999) and Selbmann et al. (2005) reported exopolysaccharide synthesis in meristematic black fungi isolated from Antarctica similar to *Friedmanniomyces endolithicus*. Exopolysaccharide has multiple applications such as in cryopreservation, for example, alginate beads containing EPS for sample preservation from freezing damage (Martínez et al. 1999). Psychrophilic fungi *Thelebolus* sp. IITKGP-BT12 isolated from Antarctica, produced EPS made up of glucan, having antiproliferative activity in cancer cells (Mukhopadhyay et al. 2014).

10.4.7 Pigment/Lipid Production

In the presence of low temperatures, fungi produce elevated concentrations of pigments and lipids. This rise in pigment or lipid content in the psychrophilic and psychrotolerant fungi is due to the synthesis of lipids like fatty acids and polyunsaturated triglycerides (Weinstein et al. 2000). These observations are supported by similar studies wherein increased amounts of carotenoid pigments and fatty acids (linoleic, stearic, linolenic, myristic, heptadecanoic, and palmitic acid) were seen in a cold-tolerant fungi *Thelebolus microspores* (Singh et al. 2014). Castrillo et al. (2018) similarly reported the synthesis of carotenoids by *Neurospora crassa*, when exposed to cold conditions. Carotenoids have application in the pharmaceutical industry as photoprotectors, incorporated in sunscreens and ointments used for protection from UV radiations. Likewise, mycosporin-derived molecules are of biotechnological interest due to their UV-absorbing properties (Volkmann et al. 2003).

Production of carotenoids and mycosporines by the number of psychrophilic yeast isolated from different sources from Antarctica, after exposure to UV radiations, has been observed by Libkind et al. (2009) and Vaz et al. (2011). The producers strain belonged to genera *Dioszegia*, *Cryptococcus*, *Exophiala*, *Microglossum*, and *Rhodotorula* genera.

10.4.8 Anti-allergic Compounds

There is a report of the production of the novel anti-allergic compounds from psychrophilic fungi. The marine isolates *Penicillium granulatum* MCCC 3A00475 from Prydz Bay, Antarctica was found to produce spirograterpene, a tetra-diterpene. This compound showed anti-IgE activity in rat mast cells (Niu et al. 2017).

10.5 Applications of Secondary Metabolites

Bioactive secondary metabolites of psychrophilic fungi have attracted a lot of attention due to their application in biotechnological and pharmaceutical fields. With new psychrophilic species being discovered and researched, bioprospecting of the fungal psychrophiles is in limelight. Some of the applications are discussed below:

10.5.1 Agriculture

The psychrophilic fungi having antibacterial, antifungal, anti-algal activities can be used against plant pathogens and pests. Some secondary metabolites having herbicidal activities as well as plant growth-promoting activities can be used to improve agriculture. *Pseudogymnoascus destructans* and *Penicillium tardochrysogenum* extracts showed strong and selective herbicidal activity against *Allium schoenoprasum* and *Lactuca sativa* (Gomes et al. 2018).

10.5.2 Medical and Pharmaceutical Applications

There is a sudden rise in the number of secondary metabolites reported from psychrophilic microorganisms in general and psychrophilic fungi in particular. Fungi are reported to produce pharmaceutical products (Schulz et al. 2002) but the recovery of such bioactive metabolites from fungi of cold regions is quite rare. The number of species of genera *Penicillium* itself is reported by the number of authors. Frisvad et al. (2006) reported the synthesis of cycloaspeptide A and griseofulvin by *Penicillium lanosum*, *P. soppii*, and *P. jamesonlandense* while *Penicillium ribium* was found to produce the metabolite, cyclic nitropeptide psychrophilin A (Dalsgaard et al. 2004a; Frisvad et al. 2006), whereas *Penicillium rivulorum* synthesized communesin G and H and psychrophilin B and C (Dalsgaard et al. 2004b, 2005a). Yet another species, *Penicillium algidum*, produced cycloaspeptide A and D and psychrophilin D (Dalsgaard et al. 2005b). All these cyclic peptides produced by the psychrophilic fungi showed bioactive properties, including antimalarial, insecticidal, and so on (Dalsgaard et al. 2005b; Lewer et al. 2006).

Some endophytic psychrophilic fungi namely were isolated *Phoma* sp., *P. herbarum*, and *Dothideomycetes* sp. having antifungal and antibacterial activity were isolated by Moghaddam and Soltani (2014). Psychrophilic fungi having antimicrobial potential were isolated from King George Island, Antarctic, and Svalbard as reported by Yogabaanu et al. (2017). Marinelli et al. (2004) and Rojas et al. (2009) have done documentation of the bioactive secondary metabolites of psychrophilic fungi of Antarctica. Table 10.4 gives a summary of the secondary metabolites produced by psychrophilic fungi along with their activity.

10.6 Conclusion and Future Prospects

With the research being focused nowadays on psychrophilic organisms, there is scope for the discovery of novel metabolites with biotechnological, medical, and industrial applications. The spectrum of bioactive compounds isolated and analyzed from psychrophilic fungi is fast broadening. Fungi from extreme environments,

Table 10.4 Secondary metabolites produced by psychrophilic fungi with their bioactivity

Psychrophilic fungi	Compound name	Application	References
Chaetomium sp.	Depsipeptide, chaetomiamide, diketopiperazines	Anticancer and cytotoxic activity	Wang et al. (2017)
Penicillium algidum	Cyclic nitropeptide, psychrophilin D; cyclic peptides, cycloaspeptide A, and cycloaspeptide D	Murine leukemia cell; anti-*Plasmodium falciparum*	Dalsgaard et al. (2005a, b)
Eutypella sp. D-1	Cytochalasins Z24, Z25, Z26; scoparasin B	Cytotoxicity toward human breast cancer MCF-7 cell line	Liu et al. (2014), Lu et al. (2014)
	Eutypenoids A–C	Immunosuppressive activities	Zhang et al. (2016)
Lindgomycetaceae strains	Lindgomycin; ascosetin	Antibiotic activities	Wu et al. (2015)
Trichoderma polysporum strain OPU1571	Novel compounds	Antifungal towards *Pythium iwayamai*	Kamo et al. (2016)
Geomyces sp.	Asterric acid; geomycins A–C	Antibacterial and antifungal	Li et al. (2008)
Trichoderma asperellum	Asperelines A–Z13	Antifungal and antibacterial	Ren et al. (2009, 2013)
Oidiodendron truncatum GW3-13	Epipolythiodioxopiperazines, diketopiperazines	Cytotoxicity to human cancer lines	Li et al. (2012b)
Penicillium sp. PR19N-1	Sesquiterpenes; eremofortine; eremophilane	Cytotoxic activity against HL-60 and A549 cancer cell lines	Wu et al. (2013), Lin et al. (2014)
Penicillium funiculosum GWT2-24	Chrodrimanins	Inhibitory activities against influenza virus A (H1N1)	Zhou et al. (2015)
Aspergillus ochraceopetaliformis SCSIO 05702	Ochraceopones A–E	Antiviral activities against the H1N1 and H3N2 influenza viruses	Wang et al. (2016)
Penicillium nalgiovense Laxa	Amphotericin B	Antifungal, antibacterial	Svahn et al. (2015)
Mrakia frigida	Toxin	Anti yeast	Hua et al. (2010), Liu et al. (2012)
Pseudogymnoascus sp.	Asterric acid derivatives	Antimicrobial activity	Henríquez et al. (2014), Figueroa et al. (2015)
Penicillium chrysogenum	*bis*-Anthraquinone (rugulosin and skyrin)	Insecticide and medicine	Parker et al. (2000), Sumarah et al. (2005)

(continued)

Table 10.4 (continued)

Psychrophilic fungi	Compound name	Application	References
Penicillium sp. PR19N-1	Chloro-trinoreremophilane sesquiterpene, eremophilane sesquiterpenes, eremofortine	Cytotoxic activity against cancer cell lines	Wu et al. (2013)
Penicillium tardochrysogenum	Penicillin, secalonic acids D and F	–	Houbraken et al. (2012)
Ophiocordyceps sinensis		Cancer, impotence, and fatigue	Chen et al. (2010), Lo et al. (2013), Stone (2008), Zhang et al. (2012)

including those living in Antarctica, may have developed specific metabolic pathways to produce singular natural products with bioactive properties. For this reason, these fungi represent potential sources of pharmaceutical molecules. Extracts obtained from fungi isolated in different Antarctic environments have shown promising antimicrobial, cytotoxic, antiparasitic, and antiviral activities. On the other hand, several pure compounds isolated from Antarctic fungal extracts show new carbon frameworks or unusual structural features, indicating that these fungi would be good sources of new chemical compounds.

This review provides a baseline or food for thought regarding the exploitation of cold-adapted fungi and their metabolites for biotechnology and industrial uses. Adaptive mechanisms of low-temperature fungi need to be investigated further, on a molecular and genetic basis. Two of the most important avenues are pharmaceuticals and replacing synthetic compounds with bio-based or biologically synthesized metabolites for use in industry and biotechnology. These fungi represent potential biological "factories" that can produce compounds with great potential for direct use in medicine and agriculture or as prototypical molecules that can be chemically modified for pharmaceutical and agrochemical applications.

Acknowledgment The authors are grateful to their respective institutions for continuous support.

References

Alves IM, Gonçalves VN, Oliveira FS, Schaefer CE, Rosa CA, Rosa LH (2019) The diversity, distribution, and pathogenic potential of cultivable fungi present in rocks from the South Shetlands archipelago, Maritime Antarctica. Extremophiles 23:327–336

Antipova TV, Zhelifonova VP, Baskunov BP, Kochkina GA, Ozerskaya SM, Kozlovskii AG (2018) Exometabolites of the *Penicillium* fungi isolated from various high-latitude ecosystems. Microbiology 87:642–651

Anupama PD, Praveen KD, Singh RK, Kumar S, Srivastava AK, Arora DK (2011) Psychrophilic and halotolerant strain of *Thelebolus microsporus* from Pangong Lake, Himalaya. Mycosphere 2:601–609

Arenz BE, Blanchette RA (2009) Investigations of fungal diversity in wooden structures and soils at historic sites on the Antarctic Peninsula. Can J Microbiol 55:46–56

Arenz BE, Blanchette RA (2011) Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo dry valleys. Soil Biol Biochem 43:308–315

Arenz BE, Held BW, Jurgens JA, Farrellb RL, Blanchettea RA (2006) Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. Soil Biol Biochem 38:3057–3064

Bhadury P, Mohammed BT, Wright PC (2006) The current status of natural products from marine fungi and their potential as anti-infective agents. J Ind Microbiol Biotechnol 33:325–337

Biabini MAF, Laatch H (1998) Advances in chemical studies of low-molecular weight metabolites of marine fungi. J Praktische Chemie 340:589–607

Blanchette RA, Held BW, Jurgens JA, McNew DL, Harrington TC, Duncan SM, Farrell RL (2004) Wood destroying soft-rot fungi in the historic expeditions huts of Antarctica. Appl Environ Microbiol 70:1328–1335

Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, Prinsep MR (2007) Marine natural products. Nat Prod Rep 24:31–86

Bovio E, Garzoli L, Poli A, Prigione V, Firsova D, McCormack GP, Varese GC (2018) The culturable mycobiota associated with three Atlantic sponges, including two new species: *Thelebolus balaustiformis* and *T. spongiae*. Fungal Syst Evol 1:141–167

Bridge PD, Spooner BM (2012) Non-lichenised Antarctic fungi: transient visitors or members of a cryptic ecosystem? Fungal Ecol 5:381–394

Brunati M, Rojas JL, Sponga F, Ciciliato I (2009) Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. Mar Genom 2:43–50

Buzzini P, Branda E, Goretti M, Turchetti B (2012) Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. FEMS Microbiol Ecol 82:217–241

Castrillo M, Luque EM, Carmen JPMM, Corrochano LM, Avalos J (2018) Transcriptional basis of enhanced photo-induction of carotenoid biosynthesis at low temperature in the fungus *Neurospora crassa*. Res Microbiol 169:278–289

Cavicchioli R (2006) Cold-adapted archaea. Nat Rev Microbiol 4:331–343

Chen JY, Sangwoo L, Cao YQ, Peng YQ, Winkler D, Yang DR (2010) Ethnomycological use of medicinal Chinese caterpillar fungus, *Ophiocordyceps sinensis* (Berk.) G. H. Sung et al. (Ascomycetes) in northern Yunnan province, SW China. Int J Med Mush 12:427–434

Chintalapati S, Kiran MD, Shivaji S (2004) Role of membrane lipid fatty acids in cold adaptation. Cell Mol Biol 50:631–642

Claro B, Bastos M, Garcia Fandino R (2018) Design and applications of cyclic peptides. In: Peptide applications in biomedicine, biotechnology and bioengineering. Woodhead Publishing, Cambridge, pp 87–129

Connell L, Redman R, Craig S, Rodrieudz RJ (2006) Distribution and abundance of fungi in the soils of Taylor Valley, Antarctica. Soil Biol Biochem 38:3083–3094

Connell LB, Redman R, Craig SD, Scorzetti G, Iszard M, Rodriguez R (2008) Diversity of soil yeasts isolated from South Victoria Land, Antarctica. Microb Ecol 56:448–459

Cooke RC, Whipps JM (1993) Ecophysiology of fungi. Blackwell Scientific, Oxford, UK

D'Amico S, Collins T, Marx JC, Feller G, Gerday C (2006) Psychrophilic microorganisms: challenges for life. EMBO Rep 7:385–389

Dalsgaard PW, Larsen TO, Frydenvang K, Christophersen C (2004a) Psychrophilin A and Cycloaspeptide D, novel cyclic peptides from the psychrotolerant fungus *Penicillium ribeum*. J Nat Prod 67:878–881

Dalsgaard PW, Blunt JW, Munro MH, Larsen TO, Christophersen C (2004b) Psychrophilin B and C: cyclic nitropeptides from the psychrotolerant fungus *Penicillium rivulum*. J Nat Prod 67:1950–1952

Dalsgaard PW, Blunt JW, Munro MHG, Frisvad JC, Christophersen C (2005a) Communesins G and H, new alkaloids from the psychrotolerant fungus *Penicillium rivulum*. J Nat Prod 68:258–261

Dalsgaard PW, Larsen TO, Christophersen C (2005b) Bioactive cyclic peptides from the psychrotolerant fungus *Penicillium algidum*. J Antibiot 58:141–144
Damare S, Raghukumar C, Raghukumar S (2006) Fungi in deep-sea sediments of the Central Indian Basin. Deep-Sea Res I 53:14–27
Damare S, Nagarajan M, Raghukumar C (2008) Spore germination of fungi belonging to *Aspergillus* species under deep-sea conditions. Deep-Sea Res I 55:670–678
de Hoog GS, Göttlich E, Platas G, Genilloud O, Leotta G, Van Brummelen J (2005) Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. Stud Mycol 51:33–76
de Menezes GCA, Porto BA, Simões JC, Rosa CA, Rosa LH (2019) Fungi in snow and glacial ice of Antarctica. In: Rosa LH (ed) Fungi of Antarctica, 1st edn. Springer, Basel, pp 127–146
Dejardin RA, Ward EWB (1971) Growth and respiration of *psychrophilic* species of the genus *Typhula*. Can J Bot 49:339–347
Del Frate G, Caretta G (1990) Fungi isolated from Antarctic material. Polar Biol 11:1–7
Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot 62:5–16
Deming JW (2002) Psychrophiles and polar regions. Curr Opin Microbiol 5:301–309
Deming JW, Eicken H (2007) Life in ice. In: Sullivan WT, Baross JA (eds) Planets and life: the emerging science of astrobiology. Cambridge University Press, Cambridge, pp 292–312
Demmer O, Frank AO, Kessler H (2009) Design of cyclic peptides. In: Peptide and protein design for biopharmaceutical applications. Wiley, Hoboken, NJ
Deshmukh SK (2002) Incidence of dermatophytes and other keratinophilic fungi in the glacier bank soils of the Kashmir valley, India. Mycologist 16:165–167
Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7
Dexter Y, Cooke RC (1984a) Fatty-acids, sterols and carotenoids of the psychrophile *Mucor strictus* and some mesophilic *Mucor* species. Trans Br Mycol Soc 83:455–461
Dexter Y, Cooke RC (1984b) Temperature-determined growth and sporulation in the psychrophile *Mucor strictus*. Trans Br Mycol Soc 83:696–700
Dexter Y, Cooke RC (1985) Effect of temperature on respiration, nutrient uptake and potassium leakage in the psychrophile *Mucor strictus*. Trans Br Mycol Soc 84:131–136
Ding Z, Li L, Che Q et al (2016) Richness and bioactivity of culturable soil fungi from the Fildes Peninsula, Antarctica. Extremophiles 20:425–435
Domsch KH, Gams W, Anderson TH (1980) Compendium of soil fungi. Academic, London
Duncan SM, Minasaki R, Farrell RL, Thwaites JM (2008) Screening fungi isolated from historic Discovery Hut on Ross Island, Antarctica for cellulose degradation. Antarct Sci 20:463–470
DuoSaito RA, Connell L, Rodriguez R, Redman R, Libkind D, de Garcia V (2018) Metabarcoding analysis of the fungal biodiversity associated with Castaño Overa Glacier—Mount Tronador, Patagonia, Argentina. Fungal Ecol 36:8–16
Ebada SS, Fischer T, Hamacher A, Du F-Y, Roth YO, Kassack MU, Wang B-G, Roth EH (2014) Psychrophilin E, a new cyclotripeptide, from co-fermentation of two marine alga-derived fungi of the genus Aspergillus. Nat Prod Res 28:776–781
Ebel R (2006) Secondary metabolites from marine-derived fungi. In: Proksch P, Muller WEG (eds) Frontiers in marine biotechnology. Horizon Bioscience, London, pp 73–143
Fell JW, Statzell AC, Hunter IL, Phaff L (1969) *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. Antonie Van Leeuwenhoek 35:433–462
Figueroa L, Jiménez C, Rodríguez J et al (2015) 3-Nitroasterric acid derivatives from an Antarctic sponge-derived *Pseudogymnoascus* sp. fungus. J Nat Prod 78:919–923
Finotti E, Paolino C, Lancia B, Mercantini R (1996) Metabolic differences between two Antarctic strains of *Geomyces pannorum*. Curr Microbiol 32:7–10
Flam F (1994) Chemical prospectors scour the seas for promising drugs. Science 226:1324–1325

Flanagan PW, Scarborough AM (1974) Physiological groups of decomposer fungi on tundra plant remains. In: Holding AJ, Heal OW, McLean SF Jr, Flannagan PE (eds) Soil organisms and decomposition in tundra. Tundra Biome Steering Committee, Stockholm, pp 159–181

Frisvad JC (2008) Fungi in cold ecosystems. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology, vol 9. Springer Nature, Switzerland, pp 137–156

Frisvad JC, Larsen TO, Dalsgaard PW, Seifert KA, Louis-Seize G, Lyhne EK, Jarvis BB, Fettinger JC, Overy DP (2006) Four psychrotolerant species with high chemical diversity consistently producing cycloaspeptide A, *Penicillium jamesonlandense* sp. nov., *Penicillium ribium* sp. nov., *Penicillium soppii* and *Penicillium lanosum*. Int J Syst Evol Microbiol 56:1427–1437

Furbino LE, Godinho VM, Santiago IF, Pellizari FM, Alves TMA, Zani CL, Junior AS, Romanha AJ, Carvalho AGO, Gil LHVG, Rosa CA, Minnis AM, Rosa LH (2014) Diversity patterns, ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic Peninsula. Microb Ecol 67:775–787

Gargas A, Trest MT, Christensen M, Volk TJ (2009) *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108:147–154

Giddings LA, Newman DJ (2014) Bioactive compounds from terrestrial extremophiles. Springer International Publishing, Cham

Gocheva YG, Krumova E, Slokoska L, Gesheva V, Angelova M (2005) Isolation of filamentous fungi from Antarctica. C R Acad Bulg Sci 58:403–408

Godinho VM, Furbino LE, Santiago IF, Pellizzari FM, Yokoya NS, Pupo D, Alves MA, Junior PAS, Romanha AJ, Zani CL, Cantrell CL, Rosa CA, Rosa LH (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. ISME J 7:1434–1451

Godinho VM, Gonçalves VN, Santiago IF, Figueredo HM, Vitoreli GA, Schaefer Carlos E-G-R, Barbosa EC, Oliveira JG, Alves MAT, Zani CL, Junior PAS, Murta SMF, Romanha AJ, Kroon EG, Cantrell CL, Wedge DE, Duke OS, Ali A, Rosa CA, Rosa LH (2015) Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. Extremophiles 19:585–596

Gomes ECQ, Godinho VM, Silva DAS, de Paula MTR, Vitoreli GA, Zani CL, Alves TMA, Junior PAS, Murta SMF, Barbosa EC, Oliveira JG, Oliveira FS, Carvalho CR, Ferreira MC, Rosa CA, Rosa LH (2018) Cultivable fungi present in Antarctic soils: taxonomy, phylogeny, diversity, and bioprospecting of antiparasitic and herbicidal metabolites. Extremophiles 22:381–393

Gonçalves VN, Vaz ABM, Rosa CA, Rosa LH (2012a) Diversity and distribution of fungal communities in lakes of Antarctica. FEMS Microbiol Ecol 82:459–471

Gonçalves DB, Teixeira JA, Bazzolli DMS, de Queiroz MV, de Araújo EF (2012b) Use of response surface methodology to optimize production of pectinases by recombinant *Penicillium griseoroseum* T20. Biocatal Agric Biotechnol 1:140–146

Gonçalves VN, Carvalho CR, Johann S, Mendes G, Alves TMA, Zani CL, Junior PAS, Murta SMF, Romanha AJ, Cantrell CL, Rosa CA, Rosa LH (2015) Antibacterial, antifungal and antiprotozoal activities of fungal communities present in different substrates from Antarctica. Polar Biol 38:1143–1152

Goodrich RP, Handel TM, Baldeschwieler JD (1988) Modification of lipid phase behaviour with membrane-bound cryoprotectants. Biochim Biophys Acta 938:143–154

Gudjarnnson S (1999) Bioactive marine natural products. Rit Fiskideildar 16:107–110

Hammonds P, Smith SN (1986) Lipid composition of a psychrophilic, a mesophilic and a thermophilic *Mucor* species. Trans Br Mycol Soc 86:551–560

Harper CJ, Bomfleur B, Decombeix AL, Taylor EL, Taylor TN, Krings M (2012) Tylosis formation and fungal interactions in an early Jurassic conifer from northern Victoria Land, Antarctica. Rev Palaeobot Palynol 175:25–31

Hassan H (2015) Isolation and characterization of psychrophilic fungi from Batura Passu and Siachen Glaciers, Pakistan. Dissertation, Department of Microbiology, Quaid-i-Azam University, Islamabad

Hassan N, Rafiq M, Hayat M, Nadeem S, Shah A, Hasan F (2017) Potential of psychrotrophic fungi isolated from Siachen glacier, Pakistan, to produce antimicrobial metabolites. Appl Ecol Environ Res 15:1157–1171

Hayashi M, Kim YP, Hiraoka H et al (1995) Macrosphelide, a novel inhibitor of cell-cell adhesion molecule. I. Taxonomy, fermentation, isolation and biological activities. J Antibiot 48:1435–1439

Hayes MA (2012) The Geomyces Fungi: ecology and distribution. Bioscience 62:819–823

Held BW, Jurgens JA, Duncan SM, Farrell RL (2006) Assessment of fungal diversity and deterioration in a wooden structure at New Harbor, Antarctica. Polar Biol 29:526–531

Henríquez M, Vergara K, Norambuena J, Beiza A, Maza F, Ubille P, Araya I, Chavez R, San-Martin A, Darias J, Darias MJ, Vaca I (2014) Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. World J Microbiol Biotechnol 30:65–76

Hentschel U (2002) Natural products from marine microorganisms. ChemBioChem 3:1151–1154

Höller U, Wright AD, Matthee GF, Konig GM, Draeger S, Aust H-J, Schulz B (2000) Fungi from marine sponges: diversity, biological activity and secondary metabolites. Mycol Res 104:1354–1365

Hoshino YT, Terami F, Tkachenko OB, Hoshino T (2010) Mycelial growth of the snow mold fungus, *Sclerotinia borealis*, improved at low water potentials: an adaptation to frozen environment. Mycoscience 51:98–103

Houbraken J, Frisvad JC, Seifert KA, Overy DP, Tuthill DM, Valdez JG, Samson RA (2012) New penicillin-producing *Penicillium* species and an overview of section Chrysogena. Persoonia 29:78–100

Hsiang T, Matsumoto N, Millett SM (1999) Biology and management of *Typhula* snow molds of turf grass. Plant Dis 83:788–798

Hua MX, Chi Z, Liu GL, Buzdar MA (2010) Production of a novel and cold-active killer toxin by *Mrakia frigida* 2E00797 isolated from sea sediment in Antarctica. Extremophiles 14:515–521

Istokovics A, Morita N, Izumi K, Hoshino T, Yumoto I, Sawada MT, Ishizaki K, Okuyama H (1998) Neutral lipids, phospholipids, and a betaine lipid of the snow mold fungus *Microdochium nivale*. Can J Microbiol 44:1051–1059

Ivanova V, Oriol M, Montes MJ, Garcia A, Guinea J (2001) Secondary metabolites from a *Streptomyces* strain isolated from Livingston Island, Antarctica, *Zeitschrift fur* Naturforschung C. J Biosci 56:1–5

Ivanova V, Kolarova M, Aleksieva K, Graefe U, Schlegel B (2007) Diphenylether and macrotriolides occurring in a fungal isolate from the Antarctic lichen *Neuropogon*. Prep Biochem Biotechnol 37:39–45

Jensen PR, Fenical W (2000) Marine drugs and drug discovery: current status and future potential. In: Fusetani N (ed) Drugs from the sea. Karger, Basel, pp 6–29

Jiang CS, Guo YW (2011) Epipolythiodioxopiperazines from fungi: chemistry and bioactivities. Mini Rev Med Chem 11:728–745

Jurgens JA, Blanchette RA, Filley TR (2009) Fungal diversity and deterioration in mummified woods from the ad Astra Ice Cap region in the Canadian High Arctic. Polar Biol 32:751–758

Kalanetra KM, Bano N, Hollibaugh T (2009) Ammonia-oxidizing Archaea in the Arctic ocean and Antarctic coastal waters. Environ Microbiol 11:2434–2445

Kamo M, Tojo M, Yamazaki Y, Itabashi T, Takeda H, Wakana D, Hosoe T (2016) Isolation of growth inhibitors of the snow rot pathogen *Pythium iwayamai* from an arctic strain of *Trichoderma polysporum*. J Antibiot 69:451–455

Kerekes R, Nagy G (1980) Membrane lipid composition of a mesophilic and psychrophilic yeast. Acta Aliment 9:93–98

Kobayasi Y, Hiratsuka N, Korf RP, Tubaki K, Aoshima K, Soneda M, Sugiyama J (1967) Mycological studies of the Alaskan Arctic. Annu Rep Inst Ferment Osaka 3:1–138

Konig GM, Kehraus S, Seibert SF, Abdel-Leteff A, Muller D (2006) Natural products from marine organisms and their associated microbes. ChemBioChem 7:229–238

Kozlovsky AG, Kochkina GA, Zhelifonova VP, Antipova TV, Ivanushkina NE, Ozerskaya SM (2020) Secondary metabolites of the genus *Penicillium* from undisturbed and anthropogenically altered Antarctic habitats. Folia Microbiol 65:95–102

Krembs C, Eicken H, Junge K, Deming JW (2002) High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. Deep Sea Res 49:2163–2181

Lamilla C, Pavez M, Santos A, Hermosilla A, Llanquinao V, Barrientos L (2017) Bioprospecting for extracellular enzymes from culturable Actinobacteria from the South Shetland Islands, Antarctica. Polar Biol 40:719–726

Larsen TO, Smedsgaard J, Nielsen KF, Hansen ME, Frisvad JC (2005) Phenotypic taxonomy and metabolite profiling in microbial drug discovery. Nat Prod Rep 22:672–695

Lauro FM, Bartlett DH (2008) Prokaryotic lifestyles in deep sea habitats. Extremophiles 12:15–25

Lawley B, Ripley S, Bridge P, Convey P (2004) Molecular analysis of geographic patterns of eukaryotic diversity in Antarctic soils. Appl Environ Microbiol 70:5963–5972

Lee HJ, Lee JH, Hwang BY, Kim HS, Lee JJ (2002) Fungal metabolites, asterric acid derivatives inhibit vascular endothelial growth factor (VEGF)-induced tube formation of HUVECs. J Antibiot 55:552–556

Lewer P, Graupner PR, Hahn DR, Karr LL, Duebelbeis DO, Lira JM, Anzeveno PB, Fields SC, Gilbert JR, Pearce C (2006) Discovery, synthesis, and insecticidal activity of cycloaspeptide E. J Nat Prod 69:1506–1510

Lewis DH, Smith DC (1967) Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytol 66:143–184

Li Y, Sun B, Liu S, Jiang L, Liu X, Zhang H, Che Y (2008) Bioactive asterric acid derivatives from the Antarctic ascomycete fungus *Geomyces* sp. J Nat Prod 71:1643–1646

Li HY, Shen M, Zhou ZP, Li T, Wei Y, Lin L (2012a) Diversity and cold adaptation of endophytic fungi from five dominant plant species collected from the Baima Snow Mountain, Southwest China. Fungal Divers 54:79–86

Li L, Li D, Luan Y, Gu Q, Zhu T (2012b) Cytotoxic metabolites from the Antarctic psychrophilic fungus *Oidiodendron truncatum*. J Nat Prod 75:920–927

Li JY, Jin MM, Meng J, Gao SM, Lu RR (2013) Exopolysaccharide from *Lactobacillus planterum* LP6: antioxidation and the effect on oxidative stress. Carbohydr Polym 98:1147–1152

Li XD, Li XM, Xu GM, Zhang P, Wang BG (2015) Antimicrobial phenolic bisabolanes and related derivatives from *Penicillium aculeatum* SD-321, a deep sea sediment-derived fungus. J Nat Prod 78:844–849

Li W, Luo D, Huang J, Wang L, Zhang F, Xi T, Liao J, Lu Y (2018) Antibacterial constituents from Antarctic fungus, *Aspergillus sydowii* SP-1. Nat Prod Res 32:662–667

Libkind D, Moliné M, Sampaio JP, Van Broock M (2009) Yeasts from high-altitude lakes: influence of UV radiation. FEMS Microbiol Ecol 69:353–362

Lin A, Wu G, Gu Q, Zhu T, Li D (2014) New eremophilane-type sesquiterpenes from an Antarctic deep-sea derived fungus, *Penicillium* sp. PR19 N-1. Arch Pharm Res 37:839–844

Liu GL, Wang K, Hua MX, Buzdar MA, Chia ZM (2012) Purification and characterization of the cold-active killer toxin from the psychrotolerant yeast *Mrakia frigida* isolated from sea sediments in Antarctica. Process Biochem 47:822–827

Liu JT, Hu B, Gao Y, Zhang JP, Jiao BH, Lu XL, Liu XY (2014) Bioactive tyrosine-derived cytochalasins from fungus *Eutypella* sp. D-1. Chem Biodivers 11:800–806

Liu YJ, Zhang J-L, Li C, Mu XG, Liu X-L, Wang L, Zhao Y-C, Zhang P, Li X-D, Zhang X-X (2019) Antimicrobial secondary metabolites from the seawater-derived fungus *Aspergillus sydowii* SW9. Molecules 24:4596

Lo HC, Hsieh C, Lin FY, Hsu TH (2013) A systematic review of the mysterious caterpillar fungus *Ophiocordyceps sinensis* in Dong-Chong Xia Cao (Dong Chong Xia Cão) and related bioactive ingredients. J Tradit Complement Med 3:16–32

Lu XL, Liu JT, Liu XY, Gao Y, Zhang J, Jiao BH, Zheng H (2014) Pimarane diterpenes from the Arctic fungus *Eutypella* sp. D-1. J Antibiot 67:171–174

Lutz S, Anesio AM, Edwards A, Benning LG (2015) Microbial diversity on Icelandic glaciers and ice caps. Front Microbiol 6:307

Mahmoodian A, Stickings CE (1964) Studies in the biochemistry of micro-organisms. 115. Metabolites of *Penicillium frequentans* Westling: isolation of sulochrin, asterric acid, (+)-bis-dechlorogeodin and two new substituted anthraquinones, questin and questinol. Biochem J 92:369–378

Malosso E, Waite IS, English L, Hopkins DW (2006) Fungal diversity in maritime Antarctic soils determined using a combination of culture isolation, molecular fingerprinting and cloning techniques. Polar Biol 29:552–561

Mancuso Nichols CA, Guezennec J, Bowman JP (2005) Bacterial exopolysaccharides from extreme marine environments with special consideration of the southern ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol 7:253–271

Margaret E (1966) Three new yeasts from Antarctic soils: *Candida nivalis*, *Candida gelida* and *Candida frigida* spp.n. Antonie Van Leeuwenhoek 32:25–28

Margesin R, Fell JW (2008) *Mrakiella cryoconiti* gen. nov., sp. nov., a psychrophilic, anamorphic, basidiomycetous yeast from alpine and arctic habitats. Int J Syst Evol Microbiol 58:2977–2982

Margesin R, Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. Res Microbiol 162:346–361

Margesin R, Schinner F (1994) Properties of cold-adapted microorganisms and their potential role in biotechnology. J Biotechnol 33:1–14

Margesin R, Schinner F (eds) (1999) Biotechnological applications of cold-adapted organisms. Springer, Berlin

Marinelli F, Brunati M, Sponga F, Ciciliato I, Losi D, Van Trappen S, Göttlich E, De Hoog S, Rojas JL, Genilloud O (2004) Biotechnological exploitation of heterotrophic bacteria and filamentous fungi isolated from benthic mats of Antarctic lakes. In: Kurtböke I, Swings J (eds) Microbial genetic resources and biodiscovery. Queensland Complete Printing Services, Queensland, pp 163–184

Martínez D, Rosa A-G, Revilla MA (1999) Cryopreservation of *in vitro* grown shoot-tips of *Olea europaea* L. var. *Arbequina*. Cryo-Letters 20:29–36

Melo IS, Santos SN, Rosa LH, Parma MM, Silva LJ, Queiroz SCN, Pellizari VH (2014) Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss *Schistidium antarctici*. Extremophiles 18:15–23

Minnis AM, Lindner DL (2013) Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in Bat hibernacula of eastern North America. Fungal Biol 117:638–649

Moghaddam MSH, Soltani J (2014) Psychrophilic endophytic fungi with biological activity inhabit Cupressaceae plant family. Symbiosis 63:79–86

Möller C, Dreyfuss MM (1996) Microfungi from Antarctic lichens, mosses and vascular plants. Mycologia 88:922–933

Montemartini A, Caretta G, Del Frate G (1993) Notes on *Thelebolus microsporus* isolated in Antarctica. Mycotaxon 48:343–358

Mukherjee M, Mukherjee PK, Horwitz B, Zachow C, Berg G, Zeilinger S (2012) *Trichoderma*—plant–pathogen interactions: advances in genetics of biological control. Indian J Microbiol 52:522–529

Mukhopadhyay SK, Chatterjee S, Gauri SS, Das SS, Mishra A, Patra M, Ghosh AK, Das AK, Singh SM, Dey S (2014) Isolation and characterization of extracellular polysaccharide Thelebolan produced by a newly isolated psychrophilic Antarctic fungus *Thelebolus*. Carbohydr Polym 104:204–212

Nakajima H, Hamasaki T, Kimura Y (1989) Structure of spiciferone A, a novel gamma-pyrone plant-growth inhibitor produced by the fungus *Cochliobolus spicifer*. Nelson Agric Biol Chem 53:2297–2299

Nakajima H, Hamasaki T, Maeta S et al (1990) A plant-growth regulator produced by the fungus *Cochliobolus spicifer*. Phytochemistry 29:1739–1743

Newman DJ, Hill RT (2006) New drugs from marine microbes: the tide is turning. J Ind Microbiol Biotechnol 33:539–544

Newsted WJ, Polvi S, Papish B, Kendall E, Saleem M, Koch M, Hussain A, Cutler AJ, Georges F (1994) A low molecular weight peptide from snow mold with epitopic homology to the winter flounder antifreeze protein. Biochem Cell Biol 72:152–156

Niederer M, Pankow W, Wiemken A (1992) Seasonal changes of soluble carbohydrates in mycorrhizas of Norway spruce and changes induced by exposure to frost desiccation. Eur J For Pathol 22:291–299

Nishanth Kumar SN, Mohandas C, Nambisan B (2014) Purification, structural elucidation and bioactivity of tryptophan containing diketopiperazines, from *Comamonas testosteroni* associated with a rhabditid entomopathogenic nematode against major human-pathogenic bacteria. Peptides 53:48–58

Niu S, Fan Z-W, Xie C-L, Liu Q, Luo Z-H, Liu G, Yang X-W (2017) Spirograterpene A, a tetracyclic spiro-diterpene with a fused 5/5/5/5 ring system from the deep-sea-derived fungus *Penicillium granulatum* MCCC 3A00475. J Nat Prod 80:2174–2177

Onofri S (1999) Antarctic microfungi. In: Seckbach J (ed) Enigmatic microorganisms and life in extreme environments. Kluwer Academic, Dordrecht, pp 323–336

Onofri S, Barreca D, Selbmann L, Isola D, Rabbow E, Horneck G, de Vera JPP, Hatton J, Zucconi L (2008) Resistance of Antarctic lack fungi and crypto-endolithic communities to simulated space and Martian conditions. Stud Mycol 61:99–109

Ozerskaya SM, Ivanushkina NE, Kochkina GA, Fattakhova RN, Gilichinsky DA (2004) Mycelial fungi in cryopegs. Int J Astrobiol 4:327–331

Parish CA, Cruz M, Smith SK, Zink D, Baxter J et al (2009) Antisense-guided isolation and structure elucidation of pannomycin, a substituted cis-decalin from *Geomyces pannorum*. J Nat Prod 72:59–62

Parker JC, McPherson RK, Andrews KM, Levy CB, Dubins JS, Chin JE, Perry PV, Hulin B, Perry DA, Inagaki T, Dekker KA (2000) Effects of skyrin, a receptor-selective glucagon antagonist, in rat and human hepatocytes. Diabetes 49:2079–2086

Paterson RA (1973) The occurrence and distribution of some aquatic phycomycetes on Ross Island and the dry valleys of Victoria Land, Antarctica. Mycologia 65:373–387

Paul VJ, Puglisi MP, Ritson-Williams R (2006) Marine chemical ecology. Nat Prod Rep 23:153–180

Peng J, Gao H, Zhang X, Wang S, Wu C, Gu Q, Guo P, Zhu T, Li D (2014) Psychrophilins E–H and versicotide C, cyclic peptides from the marine-derived fungus *Aspergillus versicolor* ZLN-60. J Nat Prod 77:2218–2223

Perini L, Gostinčar C, Gunde-Cimerman N (2019a) Fungal and bacterial diversity of Svalbard subglacial ice. Sci Rep 9:1–15

Perini L, Gostinčar C, Anesio AM, Williamson C, Tranter M, Gunde-Cimerman N (2019b) Darkening of the Greenland ice sheet: fungal abundance and diversity are associated with algal bloom. Front Microbiol 10:1–14

Pietra F (1997) Secondary metabolites from marine microorganisms – bacteria, protozoa, algae and fungi – achievements and prospectives. Nat Prod Rep 14:453–464

Purić J, Vieira G, Cavalca LB et al (2018) Activity of Antarctic fungi extracts against phytopathogenic bacteria. Lett Appl Microbiol 66:530–536

Rastegari AA, Yadav AN, Yadav N (2019a) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5

Rastegari AA, Yadav AN, Yadav N, Tataei Sarshari N (2019b) Bioengineering of secondary metabolites. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 55–68. https://doi.org/10.1016/B978-0-444-63504-4.00004-9

Rateb ME, Ebel R (2011) Secondary metabolites of fungi from marine habitats. Nat Prod Rep 28:290–344

Ren J, Xue C, Tian L, Xu M, Chen J, Deng Z, Proksch P, Lin W (2009) Asperelines A–F, peptaibols from the marine-derived fungus *Trichoderma asperellum*. J Nat Prod 72:1036–1044

Ren J, Yang Y, Liu D, Chen W, Proksch P, Shao B, Lin W (2013) Sequential determination of new peptaibols asperelines G-Z12 produced by marine-derived fungus *Trichoderma asperellum* using ultrahigh pressure liquid chromatography combined with electrospray-ionization tandem mass spectrometry. J Chromatogr A 1309:90–95

Richard WN, Palm ME, Johnstone K, Wynn-Williams DD (1997) Ecological and physiological characterization of *Humicola marvinii*, a new psychrophilic fungus from Fellfield soils in the Maritime Antarctic. Mycologia 89:705–711

Robin S, Hall T, Turchetti B, Buzzini P, Branda E, Boekhout T, Theelen B, Watson K (2010) Cold-adapted yeasts from Antarctica and the Italian Alps – description of three novel species*: Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. Extremophiles 14:47–59

Robinson CH (2001) Cold adaptation in Arctic and Antarctic fungi. New Phytol 151:341–353

Rodrigues DF, Tiedje JM (2008) Coping with our cold planet. Appl Environ Microbiol 74:1677–1686

Rojas JL, Martín J, Tormo JR, Vicente F, Brunati M, Ciciliato I, Losi D, Van Trappen S, Mergaert J, Swings J, Marinelli F (2009) Bacterial diversity from benthic mats of Antarctic lakes as a source of new bioactive metabolites. Mar Genomics 2:33–41

Rosa LH, Zani CL, Cantrell CL, Duke SO, Dijck PV, Desideri A, Rosa CA (2019) Fungi in Antarctica: diversity, ecology, effects of climate change, and bioprospection for bioactive compounds. In: Rosa LH (ed) Fungi of Antarctica, 1st edn. Springer, Cham, pp 1–17

Ruisi S, Barreca D, Selbmann L, Zucconi L (2007) Fungi in Antarctica. Rev Environ Sci Biotechnol 6:127–141

Rusman Y, Held BW, Blanchette RA et al (2018) Cadopherone and colomitide polyketides from *Cadophora* wood-rot fungi associated with historic expedition huts in Antarctica. Phytochemistry 148:1–10

Russell NJ (2008) Membrane components and cold sensing. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, pp 177–190

Santiago IF, Alves TMA, Rabello A, Sales-Júnior PA, Romanha AJ, Zani CL, Rosa CA, Rosa LH (2012) Leishmanicidal and antitumoral activities of endophytic fungi associated with the Antarctic angiosperms *Deschampsia antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. Extremophiles 16:95–103

Sazanova KV, Senik SV, Kirtsideli IY, Shavarda AL (2019) Metabolomic profiling and lipid composition of Arctic and Antarctic strains of *Micromycetes*, *Geomyces pannorum* and *Thelebolus microsporus* grown at different temperatures. Microbiology 88:282–291

Schipper MA (1967) *Mucor strictus hagem*, a psychrophilic fungus, and *Mucor falcatus* sp.n. Antonie Van Leeuwenhoek 33:189–195

Schulz B, Boyle C, Draeger S, Rommert AK, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106:996–1004

Selbmann L, Onofri S, Fenice M, Federici F, Petruccioli M (2002) Production and structural characterization of the exopolysaccharide of the Antarctic fungus *Phoma herbaru m* CCFEE 5080. Res Microbiol 153:585–592

Selbmann L, de Hoog GS, Mazzaglia A, Friedman EI, Onofri S (2005) Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. Stud Mycol 51:1–32

Selbmann L, de Hoog GS, Zucconi L, Isola D, Ruisi S, Gerrits van den Ende AHG, Ruibal C, De Leo F, Urzi C, Onofri S (2008) Drought meets acid: three new genera in a Dothidealean clade of extremotolerant fungi. Stud Mycol 61:1–20

Shivaji S, Prakash J (2010) How do bacteria sense and respond to low temperature? Arch Microbiol 192:85–95

Singh SM, Puja G, Bhat DJ (2006) Psychrophilic fungi from Schirmacher Oasis, East Antarctica. Curr Sci 90:1388–1392

Singh SM, Singh PN, Singh SK, Sharma PK (2014) Pigment, fatty acid and extracellular enzyme analysis of a fungal strain *Thelebolus microsporus* from Larsemann Hills, Antarctica. Polar Rec 50:31–36

Singh VP, Yedukondalu N, Sharma V, Kushwaha M, Sharma R, Chaubey A, Kumar A, Singh D, Vishwakarma RA (2018) Lipovelutibols A–D: cytotoxic lipopeptaibols from the Himalayan cold habitat fungus *Trichoderma velutinum*. J Nat Prod 81:219–226

Snider CS, Hsiang T, Zhao G, Griffith M (2000) Role of ice nucleation and antifreeze activities in pathogenesis and growth of snow molds. Phytopathology 90:354–361

Stone R (2008) Last stand for the body snatcher of the Himalayas? Science 322:1182–1182

Stubblefield SP, Taylor TN (1983) Studies of Paleozoic fungi. I. The structure and organization of Traquairia (Ascomycota). Am J Bot 70:387–399

Sumarah MW, Miller JD, Adams GW (2005) Measurement of a rugulosin-producing endophyte in white spruce seedlings. Mycologia 97:770–776

Svahn KS, Chryssanthou E, Olsen B, Bohlin L, Goransson U (2015) *Penicillium nalgiovense* Laxa isolated from Antarctica is a new source of the antifungal metabolite amphotericin B. Fungal Biol Biotechnol 2:1

Taylor TN, Osborne JM (1996) The importance of fungi in shaping the paleoecosystem. Rev Paleobot Palynol 90:249–262

Thevelein JM (1984) Regulation of trehalose mobilization in fungi. Microbiol Rev 48:42–59

Tibbett M, Sanders FE, Cairney JWG (1998) The effect of temperature and inorganic phosphorus supply on growth and acid phosphatase production in arctic and temperate strains of ectomycorrhizal *Hebeloma* spp. in axenic culture. Mycol Res 102:129–135

Timling I, Walker DA, Nusbaum C, Lennon NJ, Taylor DL (2014) Rich and cold: diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. Mol Ecol 23:3258–3272

Traquair JA, Smith DJ (1982) Sclerotial strains of *Coprinus psychromorbidus*, a snow mold basidiomycete. Can J Plant Pathol 4:27–36

Tronsmo AM, Hsiang T, Okuyama H, Matsumoto N (2001) Low temperature diseases caused by Microdochium nivale. In: Iriki N, Gaudet DA, Tronsmo AM et al (eds) Low temperature plant microbe interactions under snow. Hokkaido National Agricultural Experimental Station, Sapporo, pp 75–86

Turchetti B, Hall SRT, Connell LB, Branda E, Buzzini P, Theelen B, Müller WH, Boekhout T (2011) Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov. Extremophiles 15:573–586

Vaz ABM, Rosa LH, Vieira MLA, de Garcia V, Brandão LR, Lia Teixeira CRS, Moline M, Libkind D, van Broock M, Rosa CA (2011) The diversity, extracellular enzymatic activities and photoprotective compounds of yeasts isolated in Antarctica. Braz J Microbiol 42:937–947

Verbist JF, Sallenave C, Pruchus YF (2000) Marine fungal substances. In: Atta-Ur-Rahman (ed) Studies in natural products chemistry, vol 24. Elsevier, Amsterdam, pp 979–1092

Vieira G, Puric J, Morão LG, dos Santos JA, Inforsato FJ, Sette LD, Ferreira H, Sass DC (2018) Terrestrial and marine Antarctic fungi extracts active against *Xanthomonas citri* subsp. *citri*. Lett Appl Microbiol 67:64–71

Vincent WF (1988) Microbial ecosystems of Antarctica. Cambridge University Press, Cambridge

Vishniac HS (1996) Biodiversity of yeast and filamentous microfungi in terrestrial Antarctic ecosystems. Biodivers Conserv 5:1365–1378

Volkmann M, Whitehead K, Rütters H, Rullkötter J, Gorbushina AA (2003) Mycosporine-glutamicol-glucoside: a natural UV-absorbing secondary metabolite of rock-inhabiting microcolonial fungi. Rapid Commun Mass Spectrom 17:897–902

Wang M, Jiang X, Wu W, Hao Y, Su Y, Cai L, Xiang M, Liu X (2015a) Psychrophilic fungi from the world's roof. Persoonia 34:100–112

Wang J, He W, Qin X, Tian X, Liao L, Liao S, Yang B, Tu Z, Zhang T, Chen B, Wang F, Zhou X, Wei X (2015b) Three new indolyl diketopiperazine metabolites from the Antarctic soil derived fungus *Penicillium* sp. SCSIO 05705. RSC Adv 5:1039

Wang JF, Wei XY, Qin XC, Tian XP, Liao L, Li KM, Zhou XF, Yang XW, Wang FZ, Zhang TY et al (2016) Antiviral merosesquiterpenoids produced by the Antarctic fungus *Aspergillus ochraceopetaliformis* SCSIO 05702. J Nat Prod 79:59–65

Wang M, Tian J, Xiang M, Liu X (2017) Living strategy of cold-adapted fungi with the reference to several representative species. Mycology 8:178–188

Weinstein RN, Palm ME, Johnstone K, Wynn-Williams DD (1997) Ecological and physiological characterization of *Humicola marvinii*, a new psychrophilic fungus from fellfield soils in the maritime Antarctic. Mycologia 89:706–711

Weinstein RN, Montiel PO, Johnstone K (2000) Influence of growth temperature on lipid and soluble carbohydrate synthesis by fungi isolated from fellfield soil in the maritime Antarctic. Mycologia 92:222–229

Wu G, Ma H, Zhu T, Li J, Gu Q, Li D (2012) Penilactones A and B, two novel polyketides from Antarctic deep-sea derived fungus *Penicillium crustosum* PRB-2. Tetrahedron 68:9745–9749

Wu G, Lin A, Gu Q, Zhu T, Li D (2013) Four new chloro-eremophilane sesquiterpenes from an Antarctic deep-sea derived fungus, *Penicillium* sp. PR19N-1. Mar Drugs 11:1399–1408

Wu G, Sun X, Yu G, Wang W, Zhu T, Gu Q, Li D (2014) Cladosins A–E, hybrid polyketides from a deep-sea-derived fungus, *Cladosporium sphaerospermum*. J Nat Prod 77:270–275

Wu B, Weise J, Labes A, Kramer A, Schmaljohann R, Imhoff JF (2015) Lindgomycin, an unusual antibiotic polyketide from a marine fungus of the Lindgomycetaceae. Mar Drugs 13:4617–4632

Wu B, Wiese J, Wenzel-Storjohann A, Malien S, Schmaljohann R, Imhoff JF (2016) Engyodontochones, antibiotic polyketides from the marine fungus *Engyodontium album* strain LF069. Chem Eur J 22:7452–7462

Wu DL, Li HJ, Smith DR, Jaratsittisin J, Xia-Ke-Er XFKT, Ma WZ, Guo YW, Dong J, Shen J, Yang DP, Lan WJ (2018) Polyketides and alkaloids from the marine-derived fungus *Dichotomomyces cejpii* F31-1 and the antiviral activity of Scequinadoline A against Dengue Virus. Mar Drugs 16:229

Xiao HU, Zhang YJ, Xiao GH et al (2013) Genome survey uncovers the secrets of sex and lifestyle in caterpillar fungus. Chin Sci Bull 58:2846–2854

Xin MX, Zhou PJ (2007) *Mrakia psychrophila* sp. nov., a new species isolated from Antarctic soil. J Zhejiang Univ Sci B 4:260–265

Yadav AN, Verma P, Sachan SG, Saxena AK (2017) Biodiversity and biotechnological applications of psychrotrophic microbes isolated from Indian Himalayan regions. EC Microbiol ECO.01:48–54

Yadav AN, Verma P, Sachan SG, Kaushik R, Saxena AK (2018) Psychrotrophic microbiomes: molecular diversity and beneficial role in plant growth promotion and soil health. In: Panpatte DG, Jhala YK, Shelat HN, Vyas RV (eds) Microorganisms for green revolution, Microbes for sustainable agro-ecosystem, vol 2. Springer, Singapore, pp 197–240. https://doi.org/10.1007/978-981-10-7146-1_11

Yadav AN, Singh S, Mishra S, Gupta A (2019) Recent advancement in white biotechnology through fungi. In: Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N (2020a) Microbiomes of extreme environments, Biotechnological applications in agriculture, environment and industry, vol 2. CRC Press, Taylor & Francis Group, Boca Raton, FL

Yadav AN, Rastegari AA, Yadav N (2020b) Microbiomes of extreme environments: biodiversity and biotechnological applications. CRC Press, Taylor & Francis, Boca Raton, FL

Yogabaanu U, Weber JFF, Convey P, Rizman-Idid M, Alias SA (2017) Antimicrobial properties and the influence of temperature on secondary metabolite production in cold environment soil fungi. Pol Sci 14:60–67

Zhang Y, Li E, Wang C, Li Y, Liu X (2012) *Ophiocordyceps sinensis*, the flagship fungus of China: terminology, life strategy and ecology. Mycology 3:2–10

Zhang LQ, Chen XC, Chen ZQ, Wang GM, Zhu SG, Yan YF, Chen KX, Liu XY, Li YM (2016) Eutypenoids A–C: novel pimarane diterpenoids from the Arctic fungus *Eutypella* sp. D-1. Mar Drugs 14:44

Zhou H, Li L, Wang W, Ch Q, Li D, Gu Q, Zhu T (2015) Chrodrimanins I and J from the Antarctic moss-derived fungus *Penicillium funiculosum* GWT2-24. J Nat Prod 78:1442–1445

Zhou HB, Li LY, Wu CM, Kurtán T, Mándi A, Liu YK, Gu QQ, Zhu TJ, Guo P, Li DH (2016) Penipyridones A–F, pyridone alkaloids from *Penicillium funiculosum*. J Nat Prod 79:1783–1790

Zhou Y, Zhang W, Liu X, Yu H, Lu X, Jiao B (2017) Inhibitors of protein tyrosine phosphatase 1B from marine natural products. Chem Biodivers 14:e1600462

Chapter 11
Fungal Amylases and Their Industrial Applications

Anirudh G. Patil, Kounaina Khan, S. Aishwarya, Subrahmanya Padyana, Ravish Huchegowda, Kakarla Raghava Reddy, Roshan Pais, Haider Alrafas, Roshan Dsouza, J. Madhavi, Ajar Nath Yadav, Anjanapura V. Raghu, and Farhan Zameer

A. G. Patil · S. Aishwarya · R. Pais · F. Zameer (✉)
PathoGutOmic Laboratory, Department of Biochemistry, School of Basic and Applied Sciences, Department of Biological Sciences, Dayananda Sagar University, Shavige Malleshwara Hills, Bengaluru, Karnataka, India

K. Khan · S. Padyana
Post Graduate Department of Dravyaguna Vignana, Alva's Ayurveda Medical College, Dakshina Kannada, Karnataka, India

R. Huchegowda
Department of Neurochemistry, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, Karnataka, India

K. R. Reddy
School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, NSW, Australia

H. Alrafas
Department of Microbiology and Immunology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

R. Dsouza
J. Craig Venter Institute, Rockville, MD, USA

J. Madhavi
Department of Physics, Indian Institute of Science, Bengaluru, Karnataka, India

A. N. Yadav
Department of Biotechnology, Dr. KSG Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

A. V. Raghu (✉)
Department of Chemistry, School of Engineering and Technology, Jain Global Campus, Jain University, Bengaluru, Karnataka, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_11

Contents

11.1 Introduction

Enzymes are biological catalysts that are a vital component of biological reactions. The use of chemical catalysts has been followed for a very long time. Chemical catalysis though widely used was very cumbersome. The disadvantages that this method poses include the need for high temperature and pressure for catalysis and moderate specificity. These limitations were overcome by the use of the biocatalysts known as enzymes. Enzymes work at milder conditions when compared to those required by chemical catalysts for operation. Also, enzymes are highly specific and catalyze reactions faster than chemical catalysts (Zameer et al. 2010a, b). Many microbes such as bacteria, actinomycetes, fungi, and yeast extracellularly or intracellularly produce a group of versatile and attractive enzymes with a wide variety of structures and commercial applications (More et al. 2021). Many microbial enzymes, such as amylases, proteases, pectinases, lipases, xylanases, cellulases, and laccases, are extracellularly produced. Some enzymes such as catalase from *Saccharomyces cerevisiae* and *Aspergillus Niger* are intracellular (Fiedurek and Gromada 2000).

As biocatalytic molecules, microbial enzymes are ecologically effective and very specific, which results in the formation of stereo- and regiochemically defined reaction products with a rate acceleration of 10^5–10^8 (Gurung et al. 2013; Satapathy

et al. 2020). Among the industrial enzymes, 50% are made by fungi and yeast, 35% are from bacteria, while the remaining 15% are from plant origin (Saranraj and Naidu 2014). Among the types of enzymes, microbial enzymes have numerous advantages. First, microbial enzymes are more active and stable than plant and animal enzymes. Through the development of fermentation processes, particularly selected strains can produce purified, well-characterized enzymes on a large scale. Second, enzymes produced by microorganisms have a high yield and are easy for product modification and optimization owing to the biochemical diversity and susceptibility to gene manipulation. Engineering techniques have been applied to microorganisms to improve the production of enzymes and alter the properties of enzymes by protein engineering (Gopal et al. 2009; Zameer and Gopal 2010; Gurung et al. 2013).

Additionally, microbes represent a rich source for the discovery of microbial enzymes by many modern techniques such as metagenome screening, genome mining, and exploring the diversity of extremophiles (Adrio and Demain 2014). Currently, there are around 200 types of microbial enzymes of which 4000 enzymes are used commercially. However, only about 20 enzymes are produced on a truly industrial scale. The world enzyme demand is satisfied by about 12 major producers and 400 minor suppliers. Nearly 75% of the total enzymes are produced by three top enzyme companies, that is, Denmark-based Novozymes, US-based DuPont (through the May 2011 acquisition of Denmark-based Danisco), and Switzerland-based Roche. The market is highly competitive, has a small profit, and is technologically intensive (Li et al. 2012). With the improved understanding of microbial recombination, metagenome mining, fermentation processes, and recovery methods, an increasing number of industrial enzymes can be supplied. For example, recombinant DNA technology can be applied to microorganisms to produce enzymes commercially that could not be produced previously. Approximately 90% of industrial enzymes are recombinant versions (Adrio and Demain 2014; Ashwini et al. 2015; Kunnel et al. 2019; Satapathy et al. 2019).

The industrial applications for microbial enzymes have grown immensely in recent years. For example, the estimated value of worldwide sales of industrial enzymes for the years 2014, 2015, and 2016 are $1 million, $3 billion, and $3.74 billion, respectively (Deng et al. 2010). Amylases comprise about 30% of the world's enzyme production. Lipases represent the other major product segment in the market. Geographically, demand for industrial enzymes in matured economies, such as the United States, Western Europe, Japan, and Canada, was relatively stable during recent times, while the developing economies of Asia-Pacific, Eastern Europe, and Africa and Middle East regions emerged as the fastest growing markets for industrial enzymes (Mathew et al. 2020).

Based on the application, commercial applications of enzymes can be divided into nine broad categories including food and feed, detergents, and so on (Sharma et al. 2010; Zameer et al. 2010a, b; Beulah et al. 2015; Ashwini et al. 2019). Food and feed represent the largest segment for industrial enzymes (Zameer et al. 2016; Prasad et al. 2019). Detergents constitute the other major segment for industrial enzymes. This chapter covers the classification of amylase enzyme (Alpha, Beta,

and Gamma), different sources of amylase (Archaeal, Bacterial, and Fungal), production, thermostable amylases, and industrial applications of the enzyme in the domain of food, fuel, detergent, paper, and textile followed by challenges and future avenues associated with the enzyme (Shankara et al. 2016; Madhusudan et al. 2016; Sulthana et al. 2018).

11.2 Classification of Amylase

Amylases are the enzymes that break down starch or glycogen. These enzymes are produced by a variety of living organisms, ranging from bacteria to plants to humans (Pankaj et al. 2020). Although amylases are produced by several microorganisms, those produced by fungi and bacteria have dominated applications in the industrial sector (Pandey et al. 2000). Bacteria and fungi secrete amylases to the outside of their cells to carry out extracellular digestion, which breaks down the insoluble starch, and then the soluble end products (such as glucose or maltose) are absorbed into the cells. Amylases constitute a class of industrial enzymes occupying about 25% of the enzyme market. Because of the increasing demand for these enzymes in various industries, there is enormous interest in developing them with better properties, such as raw starch degrading amylases suitable for industrial applications and cost-effective production techniques. Although amylases can be derived from several sources, including plants, animals, and microorganisms, microbial enzymes generally meet industrial demands (Khan et al. 2020; Aishwarya et al. 2020).

A large number of microbial amylases are available commercially and they have almost completely replaced the chemical hydrolysis of starch in the starch processing industry (Gupta et al. 2003). One of the most important advantages of using microbes for the production of amylases is the bulk production capacity and the fact that microbes can be genetically modified to produce enzymes with desired characteristics (Lonsane and Ramesh 1990). These enzymes are of great significance in biotechnology, with various applications ranging from food, fermentation, and textiles to the paper industry. Each application of a-amylase requires unique properties concerning specificity, stability, and temperature, and pH dependence. Modern technologies such as computational packages and online servers are the current tools used in protein sequence analysis and characterization. The physicochemical and structural properties of these proteins are well understood with the use of computational tools (Pradeep et al. 2012).

11.2.1 α-Amylase

The α-amylase (E.C.3.2.1.1) is a hydrolase enzyme that catalyzes the hydrolysis of internal α-1,4-glycosidic linkages in starch to yield products such as glucose and maltose. It is a calcium metalloenzyme, that is, it depends on the presence of a metal

cofactor for its activity (Singh et al. 2011). There are two types of hydrolases: endo-hydrolase and exo-hydrolase. Endo-hydrolases act on the interior of the substrate molecule, whereas exo-hydrolases act on the terminal non-reducing ends. Hence, terminal glucose residues and α-1,6-linkages cannot be cleaved by α-amylase. The substrate that α-amylase acts upon is starch.

Starch is a polysaccharide composed of two types of polymers—amylose and amylopectin. Amylose constitutes 20–25% of the starch molecule. It is a linear chain consisting of repetitive glucose units linked by α-1,4-glycosidic linkage. Amylopectin constitutes 75–80% of starch and is characterized by branched chains of glucose units. The linear successive glucose units are linked by α-1,4-glycosidic linkage while branching occurs every 15–45 glucose units where α-1,6 glycosidic bonds are present. The hydrolysate composition obtained after hydrolysis of starch is highly dependent on the effect of temperature, the conditions of hydrolysis, and the origin of the enzyme (Gupta et al. 2003).

11.2.2 β-Amylase

Another form of amylase, β-amylase, is also synthesized by bacteria, fungi, and plants. Working from the nonreducing end, β-amylase catalyzes the hydrolysis of the second β-1,4 glycosidic bond, cleaving off two glucose units (maltose) at a time. During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. β-amylase enzyme breaks the glucose–glucose bonds by removing two glucose units at a time, thereby producing maltose. Amyloglucosidase is the enzyme that breaks successive bonds from the nonreducing end of the straight chain, producing glucose.

Many microbial amylases usually contain a mixture of these amylases. β-amylase (EC 3.2.1.2) is an exo-hydrolase enzyme that acts from the nonreducing end of a polysaccharide chain by hydrolysis of α-1,4-glucan linkages to yield successive maltose units. Since it is unable to cleave branch linkages in branched polysaccharides such as glycogen or amylopectin, the hydrolysis is incomplete and dextrin units remain uncleaved. Primary sources of β-Amylase are the seeds of higher plants and sweet potatoes. During the ripening of fruits, β-Amylase breaks down starch into maltose resulting in the sweetness of ripened fruit (Gupta et al. 2003).

11.2.3 γ-Amylase

The γ-Amylase (EC 3.2.1.3) cleaves α (1–6) glycosidic linkages, in addition to cleaving the last α(1–4) glycosidic linkages at the nonreducing end of amylose and amylopectin, unlike the other forms of amylase, yielding glucose. They are generally multi-domain enzymes structurally very distinct from both a-amylases and b-amylases and consist of a catalytic domain folded as a twisted (a/a) 6 barrel with

a central funnel-shaped active site connected to the starch binding domain. γ-amylase is also known as glucan 1,4-a-glucosidase, amyloglucosidase, exo-1,4-a-glucosidase, glucoamylase, lysosomal a-glucosidase, or 1,4-a-D-glucanglucohydrolase. Glucan1,4a-glucosidase acts on both α-(1–6) glycosidic linkages and terminal α (1–4) glycosidic linkages of starch molecules to yield glucose as the end product. Here also, catalysis is characterized by two Glu residues. Most of the γ-amylases are active at acidic pH; however, the search for newer and improved γ-amylases with the ability to act on higher pH is in demand (Kumar and Satyanarayana 2009).

Another classification of amylases is based on how the glycosidic bond is attacked. Accordingly, starch-hydrolyzing enzymes are grouped into four such as (1) endo-amylases; (2) exo-amylases; (3) de-branching enzymes; and (4) transferases which are used to hydrolyze starches particularly for the production of dextrin and glucose (Van der Maarel et al. 2002).

11.2.3.1 Endo-amylases

Endo-amylases can cleave α-(1–4) glycosidic bonds present in the inner part (endo) of the amylose or amylopectin chain. a-amylase is a well-known endo-amylase.

11.2.3.2 Exo-Amylases

The exo-amylases either exclusively cleave (1–4) glycosidic bonds such as b-amylase (EC 3.2.1.2) or cleave both α-(1–4) and α-(1–6) glycosidic bonds like amyloglucosidase or glucoamylase and a-glucosidase. These enzymes act upon the terminal glucose residues of starch molecules, thereby yielding maltose and limiting dextrin (e.g., β -amylase) or glucose (glucoamylase and α -glucosidase) (Pandey et al. 2000).

11.2.3.3 The De-branching Enzymes

De-branching enzymes act on branching points and hydrolyze a-(1–6) glycosidic bonds. A few of the examples are pullulanase enzymes and iso-amylase. Both act on amylopectin and hydrolyze to form linear polysaccharides. Apart from that, pullulanase could cleave a-(1–6) glycosidic bond in pullulan. These de-branching enzymes along with other amylolytic enzymes have been proved significant in the food industry, especially during the saccharification process (Hii et al. 2012).

11.2.3.4 Transferases

Transferases not only hydrolyze the glycosidic linkage but also transfer the glycoside bonds. They act by the cleavage of α-(1–4) glycosidic bond of donor molecule and transfer fragments of them to a glycosidic acceptor molecule. A few of the

members are amylo-maltase and cyclodextrin glycosyltransferase that forms a new α-(1–4) glycosidic bond. However, a new α-(1–6) glycosidic bond will be established by branching enzymes. They form cyclic molecules such as cyclodextrin (Horvathova et al. 2001).

11.3 Sources of Amylases

11.3.1 Archaeal Amylases

Bio-catalysis has emerged as an alternative process using enzymes or cells as biocatalysts, which are more selective, efficient, and environmentally friendly. Mesophilic enzymes have been used as biocatalysts but they have low stability at high temperatures or extreme pH. For this reason, there is a considerable demand for more stable enzymes. One approach to overcome this need is to search for new enzymes within extremophilic microorganisms. Extremophiles are organisms that can thrive in extreme environmental conditions (temperature, pressure, salinity, dryness, radiation, pH, or concentrations of heavy metals). Most of the extremophiles belong to the Archaea domain. These microorganisms and their enzymes have unique characteristics.

Extremophilic archaea that live under extreme conditions have developed enzymes with unique structure–function properties. These enzymes, known as extremozymes, have increased stability at high temperatures, extreme pH, in the presence of organic solvents and heavy metals, and against proteolytic attack (Cavicchioli 2011). Several of these enzymes come from hyper thermophilic archaea belonging to the genera *Pyrococcus* (Jung et al. 2014), *Thermococcus, Desulfurococcus, Staphylothermus, Methanococcus, and Sulfolobus.* Besides, there are also α-amylases from haloalkaliphilic archaea belonging to the genera *Haloarcula, Halorubrum, Haloferax, and Natronococcus* (Moshfegh et al. 2013). α-amylases from halo-alkaliphilic archaea are active at lower temperatures and higher pH than α-amylases from hyper/thermophilic archaea. For these reasons, they are not suitable for the starch industry, but they can be used in detergents for medium-temperature laundering because of their stability in detergents and organic solvents (Onodera et al. 2013).

11.3.2 Bacterial Amylases

Bacteria are one of the major sources of amylase enzymes. Bacterial enzymes are more preferred for their ease of production and genetic manipulation. Among bacterial amylolytic enzyme producers, *Bacillus* sp. is a predominant group, and most of the commercially applied enzymes are obtained from the genus. Few of the industrially significant producers are *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, and *Bacillus stearothermophilus* (Konsoula and

Liakopoulou-Kyriakides 2007). Among a-amylases from these strains, *B. licheniformis* has proved to be the most stable enzyme (Weemaes et al. 1996). The most widely used source among the bacterial species is the *Bacillus* spp. *B. amyloliquefaciens* and *B. licheniformis* are widely used for the commercial production of the enzyme. Other species that have been explored for the production of the enzyme include *B. cereus* and *B. subtilis* to name a few. α-Amylases produced from *Bacillus licheniformis*, *Bacillus stearothermophilus*, and *Bacillus amyloliquefaciens* show promising potential in several industrial applications in processes such as food, fermentation, textiles, and paper industries (Coronado et al. 2000). *Bacillus subtilis*, *Bacillus stearothermophilus*, *Bacillus licheniformis,* and *Bacillus amyloliquefaciens* are known to be good producers of thermostable α-Amylase. Thermostability is an important characteristic as enzymatic liquefaction and saccharification of starch are performed at high temperatures (100–110 °C). Thermostable amylolytic enzymes are being investigated to improve industrial processes of starch degradation and are useful for the production of valuable products like glucose, crystalline dextrose, dextrose syrup, maltose, and maltodextrins (Konsoula and Liakopoulou-Kyriakides 2007).

11.3.3 Fungal Amylases

11.3.3.1 Amylases-Producing Fungal Communities

Fungal enzymes have the advantage of being secreted extracellularly. Besides, the ability of fungi to penetrate hard substrates facilitates the hydrolysis process, and fungal species are highly suitable for solid-based fermentation. The first fungal-produced amylase for industrial applications was described several decades ago (Hussain et al. 2013; Yadav et al. 2021). Efficient amylase-producing species include those of genus *Aspergillus* (*A. oryzae, A. niger, A. awamori, A. fumigates, A. kawachii, and A. flavus*), as well as *Penicillium* species (*P. brunneum, P. fellutanum, P. expansum, P. chrysogenum, P. roqueforti, P. janthinellum, P. camemberti, and P. olsonii), Streptomyces rimosus, Thermomyces lanuginosus, Pycnoporussanguineus, Cryptococcus flavus, Thermomonospora curvata, and Mucor* sp. (Gupta et al. 2003; Kunamneni et al. 2005; Kathiresan and Manivannan 2006; Hussain et al. 2013). Among the fungal sources, the genus *Aspergillus* has been widely used for the production of a-amylases. *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus oryzae* are important sources used among the fungal sources. Other fungal strains producing a-amylase include *Thermomyces lanuginosus* (Singh et al. 2011).

11.3.3.2 Physio-Chemical Attributes of Fungal Amylases

11.3.3.2.1 Temperature, pH, and Ions

In general, many enzymes are denatured and lose their activity at temperatures above 50–60 °C. However, thermostable enzymes are of great industrial and biotechnological use because they are more suitable for harsh industrial processes that take place at high temperatures. The advantages of conducting biotechnological processes at elevated temperatures include high reaction rates and increased diffusion coefficient of the substrate due to decreased viscosity, reduced risk of contamination by mesophilic common features, and the facility of handling and transport (Haki and Rakshit 2003). The properties of bacterial amylase are mentioned in (Table 11.1).

Thermostable amylases have been isolated from many different sources, including plants, animals, and microorganisms. Despite the wide distribution of these enzymes, microbial sources such as fungi and bacteria are most commonly used for industrial production, due to their low cost, shorter length of production, and easiness in production and optimization. Amylases with high optimum temperatures are industrially desirable because they eliminate the chances of denaturing in bioreactors and the contamination of the solution. Many factors can affect the thermostability of enzymes, including the purity of the preparation, the presence of calcium, the type of substrate, and other stabilizers (Prakash and Jaiswal 2010). Therefore, some approaches such as chemical modifications in the presence of specific ions,

Table 11.1 Properties of bacterial amylase

Bacteria	pH optimal	Temperature optimal
Bacillus sp. B-10	7	50
Bacillus licheniformis SKB4	6.5	90
Bacillus sp. EF-TYK1-5	7	60
Pseudomonas sp. K6-28-040	7	50
Bacillus subtilis	6	45
Bacillus licheniformis	7	90
Bacillus subtilis DM03	7–10	50
Bacillus sphaericus JT3	7	50
Bacillus circulans GRS 313	4.9	48
Enterobacter cloacae IIT-BT 08	4	60
Trichoderma pseudokoningii	4.5–8.5	50
Aspergillus oryzae	7	45
Penicillium camemberti PL21	6	30
Penicillium citrinum HBF62	5.5	55
Aspergillus niger	4	30
Penicillium fellutanum	6.5	30
Aspergillus tamarii	4.5–6.5	50–55
Scytalidium thermophilum	6.5	60

immobilization, and genetic engineering have been used in an attempt to increase the stability against temperature.

The optimum pH for α-amylases varies in ranges of 2.0–10.5, indicating the evolutionary adaptation to different environments generally, the optimum pH for glucoamylases is between 2.0 and 7.0. The pH stability is also an important feature for continuous production, as pH changes are frequent in large-scale fermenters (Vihinen et al. 1994). Generally, the optimum pH for glucoamylases is between 2.0 and 7.0. The pH stability is also an important feature for continuous production, as pH changes are frequent in large-scale fermenters. The properties of fungal amylase are mentioned in (Table 11.2).

Many metal ions can activate fungal amylases. The ones that show the best effects are the divalent ions like Ca^{2+}, Ba^{2+}, Mn^{2+}, Mg^{2+}, and Fe^{2+}. The metal ions as Zn^{2+}, Cu^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Fe^{3+}, Ag^{+}, and Al^{3+} have been reported as inhibitors for glucoamylases, while others as Ba^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Na^{2+}, Mg^{2+}, Sn^{2+}, and Fe^{2+} have a positive effect on the enzyme, increasing its activity (Kumar and Satyanarayana 2009). Several cations, especially heavy metals, with sulfhydryl reagent groups, EDTA and EGTA, inhibit the activity of α-amylase. Ca^{2+}, in general, plays a role in the stabilization and activation of α-amylases. In general thiols, such as β-mercaptoethanol, dithiothreitol (DTT), and cysteine, increase the activity and stability of β-amylases. In some cases, enzymes partially inactivated by temperature or other agents may be partly reactivated by the addition of thiols (Ray and Nanda 1996).

11.3.3.2.2 Molecular Mass, Isoelectric Point, and Glycosylation

The molecular mass of α-amylases is in a range that varies from 10 to 139 kDa, and most of these enzymes are in the range of 50–60 kDa (Vihinen et al. 1994). For glucoamylases, molecular masses from 26.8 to 250 kDa can be found. The Isoelectric point, or pI, is the pH where the enzyme electric charge is zero. The p*I* values are important for understanding enzyme behavior against different purification

Table 11.2 Properties of fungal amylase

Fungi	pH optimal	Temperature optimal
Trichoderma pseudokoningii	4.5–8.5	50
Aspergillus oryzae	7	45
Penicillium camemberti PL21	6	30
Penicillium citrinum HBF62	5.5	55
Aspergillus niger	4	30
Penicillium fellutanum	6.5	30
Aspergillus tamarii	4.5–6.5	50–55
Scytalidium thermophilum	6.5	60

procedures at different pHs. The p*I* value of amylases varies widely, from 3.25 to 10.1. Oligosaccharides present in glycoproteins are responsible for the signaling of cell secretion, maintenance of protein structure, and protection against stress caused by temperature, pH, and pressure (Kumar and Satyanarayana 2009). The degree of glycosylation varies greatly in amylases, from 1.8 to 80%. For example, the *A. niger* glucoamylases have 19% neutral carbohydrates with an average size of two monosaccharides linked by α-1.2 and α-1.6 glycosidic linkages. Mannose is most often found in O-linked serine and threonine residues (Kumar and Satyanarayana 2009).

These days it is well known that protein glycosylation plays an extremely important role in cellular functioning. Glycosylation can contribute to protein secretion as well as to its function, stability, and immunogenicity. Concerning the membrane glycoproteins, glycans may mediate cell communication with the extracellular medium. In addition to this, recent studies have shown that N-glycosylation can also have important effects on enzyme activity, function, and substrate specificity.

In recent years, many studies have demonstrated the involvement of the systematic removal of putative sites from several glycosylated enzymes to identify the precise role glycan has on the regulation of enzyme secretion, activity, and substrate specificity. Studies on the removal of N-glycan from glucoamylases produced by *Aspergillus niger* resulted in the exposure of the protein hydrophobic regions, thereby leading to reduced thermal stability and a greater aggregation and flexibility of the enzyme; similar to the glucoamylase produced by *Aspergillus awamori*, it was observed a lower secretion and reduced thermostability, while the amylase produced by *Aspergillus oryzae*, the N-glycosylation, had no observed effect over the enzyme secretion, activity, or thermostability. Complementary studies showed an increase in thermostability when new glycosylation sites were artificially introduced in the glucoamylase produced by *Aspergillus awamori*.

The analysis of O-glucans makes it possible to verify some interesting data. A glucoamylase produced by *Aspergillus niveus* is highly O-glycosylated, and its catalytic activity is strongly dependent on this process (Kumar and Satyanarayana 2009; Prakash and Jaiswal 2010). The O-glycans structures of glucoamylases produced by *Aspergillus niveus are* highly variable, as well as their function on the structures, and this makes it necessary for a specific analysis of each glucoamylase.

The amount of glycosylation and its distribution in amylase varies among different fungi, a factor that can influence the function of other amylases studied. In recent decades, several studies have elucidated the role of glycosylation in enzyme secretion, stability, and intracellular trafficking and also its action in the regulation of enzyme activity and substrate specificity. However, many of the functions that glycosylation exerts on the enzyme activity remain unknown, and further studies are required. For the past few decades, the use of fungi in bioprocesses has gained importance due to the production of many enzymes with different physicochemical characteristics and excellent potential for industrial application.

11.3.3.3 Advantages of Fungal Amylases

Most reports about fungi that produce α-amylase have been limited to a few species of mesophilic fungi, and attempts have been made to specify the cultural conditions and to select superior strains of the fungus to produce on a commercial scale. Fungal sources are confined to terrestrial isolates, mostly to *Aspergillus* and *Penicillium* (Kathiresan and Manivannan 2006). The *Aspergillus* species produce a large variety of extracellular enzymes, and amylases are the ones with the most significant industrial importance.

Filamentous fungi, such as *Aspergillus oryzae* and *Aspergillus niger*, produce considerable quantities of enzymes that are used extensively in the industry. *A. oryzae* has received increased attention as a favorable host for the production of heterologous proteins because of its ability to secrete a vast amount of high-value proteins and industrial enzymes, for example, α-amylase (Jasti et al. 2008). *Aspergillus moryzae* has been largely used in the production of food such as soy sauce, an organic acid such as citric and acetic acids, and commercial enzymes including α-amylase. *Aspergillus niger* has important hydrolytic capacities in the α-amylase production and, due to its tolerance of acidity (pH <3), it allows the avoidance of bacterial contamination (Djekrif-Dakhmouche et al. 2006). Filamentous fungi are suitable microorganisms for solid-state fermentation (SSF), especially because their morphology allows them to colonize and penetrate the solid substrate. The fungal α-amylases are preferred over other microbial sources due to their more accepted GRAS (Generally Recognized as Safe) status (Manjunatha et al. 2013; Singh et al. 2016; Far et al. 2020).

11.4 Production of Fungal Amylases

11.4.1 Submerged Fermentation (SmF)

Industrially important enzymes have traditionally been obtained from submerged fermentation because of the ease of handling and greater control of environmental factors such as temperature and pH. However, solid-state fermentation constitutes an interesting alternative since the metabolites produced are less costly (Nigam 2013; Robinson et al. 2001). The effect of environmental conditions on the regulation of extracellular enzymes in batch cultures is well studied. A lot of work on the morphology and physiology of α-amylase production by *Aspergillus oryzae* during batch cultivation has been done. In a series of batch experiments, the authors observed that at pH 3.0–3.5, freely dispersed hyphal elements were formed and the pH range 4–5, both pellets and freely dispersed hyphal fragments were observed whereas at the highest pH 6 pellets were the only growth forms recorded.

The optimum growth temperature was found to be 35 °C. It is demonstrated that when glucose was exhausted, the biomass production stopped whereas the secretion

of α-amylase increased rapidly. A decline in enzyme production was also accompanied by morphological and metabolic variations during continuous cultivation. The industrial exploitation of SSF for enzyme production has been confined to processes involving fungi, and it is generally believed that these techniques are not suitable for bacterial cultivation (Mehta and Satyanarayana 2016).

The production is affected by a variety of physiological factors, which include pH, temperature, aeration, inoculum concentration, inoculums age, the composition of the growth medium, surfactants, carbon source, and nitrogen source. Interactions of these parameters have a significant influence on the production of the enzyme. Generally, SmF is carried out using synthetic media, incorporating medium constituents such as nutrient broth and soluble starch, as well as other components, which are very expensive. Replacement of such constituents by cheaper carbon and nitrogen sources as well as nutrients would benefit the process in cost reduction. Agricultural by-products offer potential benefits in this regard (Nigam 2013).

11.4.2 Solid-State Fermentation (SSF)

Solid-state fermentation compared to submerged fermentation is simpler; requires lower capital, has superior productivity, reduced energy requirement, simpler fermentation media, and absence of rigorous control of fermentation parameters; uses less water and produces lower wastewater; has easier control of bacterial contamination; and requires low cost of downstream processing (Santos et al. 2014). Agro-industrial substrates are considered the best substrates for SSF processes. It is of special interest in those processes in which a crude fermented product may be used directly as an enzyme source.

The common substrates used for SSF processes are wheat bran, rice bran, cassava waste, palm oil waste, banana waste, tea waste, coconut oil cake, coir pith, corn cobs, and so on. In SSF, it is important to provide optimized water content and to control the water activity of the fermenting substrate. At times, SSF is preferred to SmF because of its simple technique, low capital investment, lower levels of catabolite repression and end-product inhibition, low wastewater output, better product recovery, and high-quality production. The study conducted revealed that banana peel could be utilized as a potential substrate for a-amylase production by *A. niger*. Coconut oil cake (COC), a by-product of oil extraction from dried copra, is a substrate for the production of a-amylase from fungi.

COC supplemented with 0.5% starch and 1% peptone enhanced a-amylase production by *A. oryzae*. COC serves as a source of soluble proteins and lipids thus providing essential nutrients for the growth of enzyme synthesis by the organism. Production and optimization of α-amylase from *A. oryzae* using a by-product of wheat grinding (gruel) as the sole carbon source was done. Coffee by-products as a suitable substrate for the production of a-amylase under SSF. Coffee waste was converted into value-added products by fermentation using *Neurosporacrassa*

Table 11.3 Bacterial amylase production and the mode of fermentation used

Bacteria	Mode of fermentation
Bacillus amyloliquefaciens	SSF
Bacillus licheniformis	SSF
Bacillus coagulans	SSF
Bacillus polymyxa	SSF
Bacillus mesentericus	SSF
Bacillus vulgarus	SSF
Bacillus megaterium	SSF
Bacillus licheniformis GCB-U8	SmF
Bacillus licheniformis M27	SSF
Halomonas meridiana	SmF
Rhodothermus marinus	SmF
Bacillus cereus MTCC 1305	SSF

(Murthy et al. 2009). The bacterial and fungal strains used for submerged fermentation (Table 11.3) and solid-state fermentation (Table 11.4) are mentioned.

11.4.3 Purification and Characterization of α-Amylases

α-Amylases produced by fermentation are relatively crude preparations. Most of the commercial use of a-amylase does not require 100% purification of the enzyme. But high-purity enzymes are required when they are used in the clinical and pharmaceutical sectors. The first steps in the purification involve the isolation of crude enzymes after the fermentation (Fig. 11.1). In SmF, this is usually done by centrifuging the fermented medium and taking the supernatant as the source of crude enzyme; in the case of SSF, the fermented matter is usually mixed with water or buffers, and after suitable mixing, the contents are filtered, whereby the filtrate contains the crude enzyme. Then, the enzyme is concentrated (in the supernatant/filtrate), precipitated (using salts/solvents), and purified using various chromatographic techniques such as ion-exchange chromatography, gel filtration, and isoelectric focusing.

There are a large number of reports on the purification and characterization of α-amylases produced by bacterial or fungal sources in SmF and SSF. An enzyme produced in SSF was partially purified by ammonium sulfate fractionation. The enzyme was optimally active at pH 5.0 and 50 °C with a molecular mass of 66 kDa. The presence of $Mn^{2+}$and Fe^{2+} enhanced the enzyme activity, whereas in the presence of $Hg^{2+}$and Cu^{2+} the activity gets reduced (Ciloci et al. 2012).

Table 11.4 Fungal amylase production and the mode of fermentation used

Fungi	Mode of fermentation
Aspergillus oryzae	SSF
Penicillium fellutanum	SmF
Thermomyces lanuginosus	SSF
Aspergillus niger	SSF, SmF
Penicillium roquefortii	SSF
Streptomyces rimosus	SSF, SmF
Aspergillus kawachii	SSF, SmF
Penicillium chrysogenumm	SSF
Penicillium janthinellum (NCIM 4960)	SSF
Aspergillus awamori	SmF
Pycnoporus sanguineus	SSF

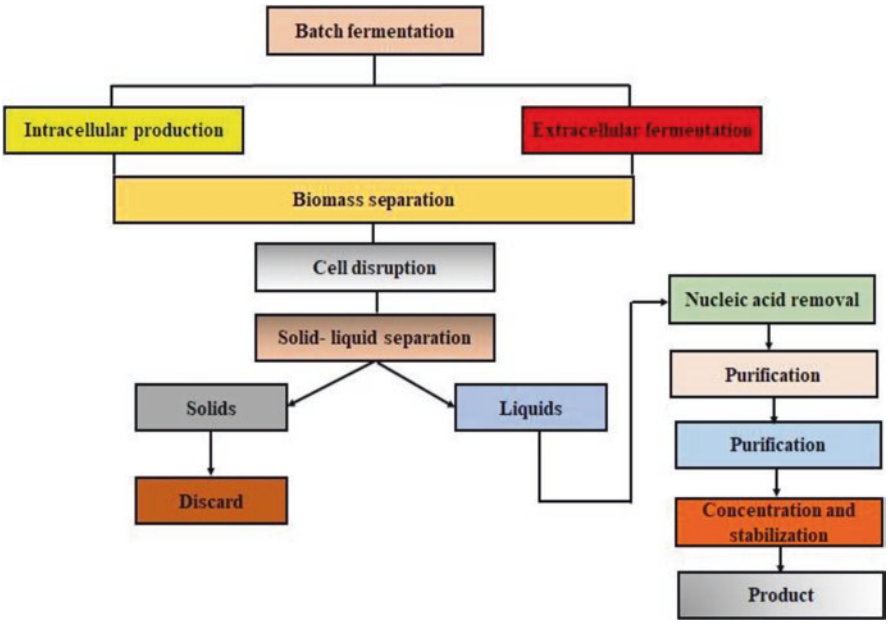


Fig. 11.1 Flow diagram of fungal enzyme production

11.4.4 Characterization of α-Amylase

Once the enzyme has been purified, the characterization of the enzyme is carried out. This can be done by determining the hydrolysates obtained after enzyme action on starch by PAGE. The purified enzyme sample along with molecular markers like BSA (67 kDa) and ovalbumin (43 kDa) is run on the gel. The resulting bands are then stained using staining agents like Coomassie Brilliant Blue and silver nitrate

and visualized. Various types of electrophoretic units are available for PAGE. The electrophoretic separation also indicates the purity and homogeneity of the enzyme obtained (Prasad et al. 2014).

11.5 Thermostable Fungal Amylases

Thermostable α-amylases are relatively stable at higher temperatures. Most studies focus on the purification and characterization of thermostable α-amylase secreted from bacteria but not from fungi and yeast. Thermophilic bacteria are the most commonly used as α-amylase producers as they can survive in high temperatures and produce enzymes having optimum temperatures higher than 50 °C. Thermostability is crucial in industrial applications, as most processes are optimally performed at elevated temperatures, where thermostable enzymes are not deactivated by heating the mixture to a certain temperature over a period due to their high denaturing temperature, unlike the mesophilic enzymes. The strategies adopted for strain improvement are enlisted (Table 11.5). Thermostable enzymes can be stored at room temperature, thus lowering the costs. There are three steps in starch hydrolysis, which are gelatinization, liquefaction, and saccharification. The gelatinization of starch is industrially carried out at 110 °C; thus, thermophilic and extremophilic α-amylases are preferred for their efficiency and economical value (Lim et al. 2020).

α-Amylase can be extracted from many sources such as animals, plants, and microorganisms. It is preferred to be industrially extracted and purified from microorganisms, especially bacteria and fungi. Microbial α-amylase can be easily isolated and selected using substrate specificity, serial dilution, and extreme conditions

Table 11.5 Strategies adopted for strain improvement/properties of alpha-amylase

Microorganism	Improved property
Bacillus subtilis BR151	Thermostability
Alternaria tenuissima FCBP 252	2.39-fold increased production
Thermobifida fusca NTU22	Increased production
Bacillus amyloliquefaciens	Increased production (1.4-fold)
Anoxybacillus spp.	High stability in the absence of Ca^{2+} ions at 60°C and high levels of maltose production
Aspergillus oryzae IIB 30	Increased production (2.1-fold)
Paenibacillus spp.	High rate of maltose production
Bacillus licheniformis MSG	Self-inducible, catabolite repression free, and glucose-activated expression system
Bacillus subtilis ASO1a	Increased production (sevenfold) and high stability in the absence of Ca^{2+}
Thermotoga maritima	Oxidative stability
Bacillus subtilis	Improved protein stability and catalytic efficiency
Bacillus sp. AAH-31	Increased production

such as temperature and extreme pH. The desired α-amylase properties for specific industrial applications can be designed and improved due to the advancement of genetic engineering and media optimization. This process involves the selection of mutants, recombinant organisms under the process of strain improvement for industrial enzymes for the selection of novel strains (Fig. 11.2) (Chen et al. 2015). Fungus is a preferred source compared to other microbial sources because fungal α-amylases have a more accepted GRAS status. *Aspergillus flavus* NSH9, *Aspergillus terreus* NCFT 4269.10, *Engyodontium* album TISTR 3645, *Komagataella phaffii* GS115, *Talaromyces pinophilus* 1–95, and *Trichoderma pseudokoningii* are the most widely used fungal strains for the production of fungal amylases.

Bacteria such as *Escherichia coli* forms inclusion bodies (IBs) containing infectious prion if it is used as an expression host for yeast proteins. As a eukaryotic expression host, yeast has its post-translational modifications (PTMs) more similar to higher level eukaryotes than bacteria. Although it is beneficial as a eukaryotic expression system, there has not been much research performed to purify and characterize α-amylase from yeast. In a study concerning marine yeast isolation and industrial applications, enzymes from marine yeast (*Aureobasidium* sp. and *Pichia* sp.) are expected to have high salt tolerance, thermostability, barophilicity, and cold adaptivity as the yeasts live in a high salinity environment. *M. guilliermondii* has been used as the research model organism named "flavinogenic yeasts," being capable of riboflavin over-synthesis during starvation for iron as well as the expression system of thermostable T1 amylase gene (Zaky et al. 2014).

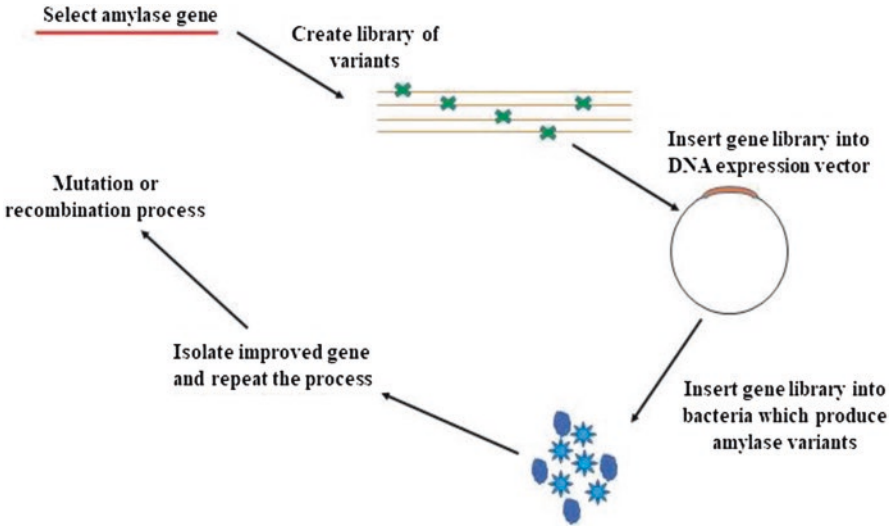


Fig. 11.2 Mutant library screening and selection of the best variant for recombinant amylase production

11.6 Industrial Applications

Amylase is one of the most important hydrolytic enzymes used in all starch-based industries and has been in practice since 1984 as a pharmaceutical aid for the treatment of digestive disorders. Amylases are applied in all the industrial processes such as food, detergents, textiles, and paper industries for the hydrolysis of starch. Nowadays, the chemical hydrolysis of starch is interchanged with enzymatic hydrolysis using microbial amylases in starch processing industries. One of the major applications of amylases is in the food industry, starch processing industry, and also been widely in bioethanol, soaps, and detergent industries (Fig. 11.3).

11.6.1 Food Industry

Starch is the major carbohydrate source and is mainly of plant origin. Starch derivatives also have a momentous role in the food, beverage, and feed industries, which include cyclodextrin, glucose syrup, hydrolysates, maltodextrin, and other modified starch. Production of starch derivatives is one of the growing industries, where starch-modifying enzymes find substantial roles. Amylases find their applications in

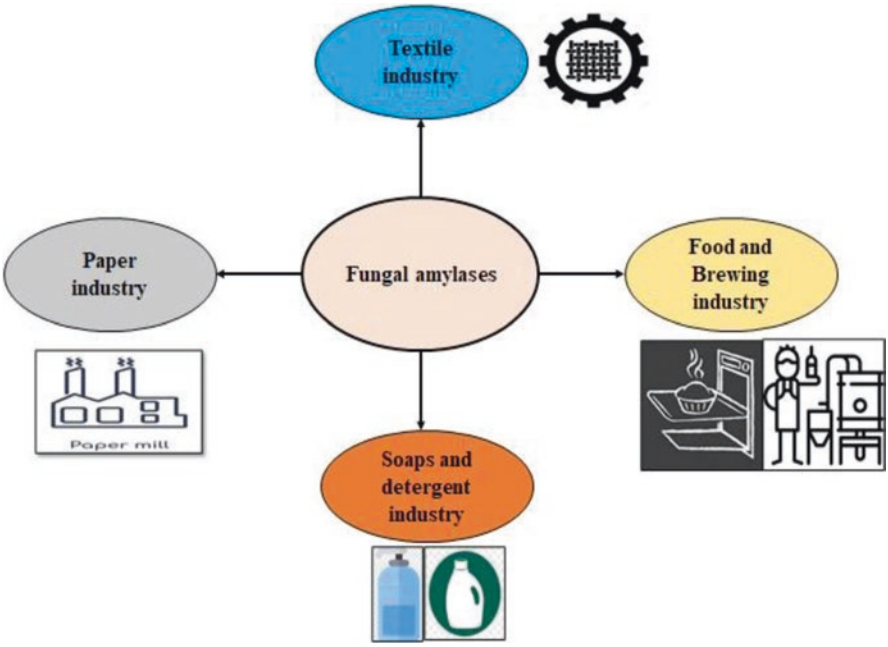


Fig. 11.3 Industrial application of amylases

many food processing industries, like brewing and baking sectors, preparation of fruit juices and starch syrups, and so on (Mobini-Dehkordi and Javan 2012).

Starch in the dough can be broken down to a-limit dextrins, the intermediate product starch hydrolysis; along with fermentable sugars in the bread baking process, further fermentation of these yields alcohol and CO_2 (Prakash and Jaiswal 2010). The presence of low-molecular-weight dextrins will reduce bread hardness. In wheat flour, the presence of beta-amylases is abundant and has little activity on undamaged native starch granules, while alpha-amylases are absent. Starch hydrolysis in the dough is by the combined action of heterogeneously supplied a-amylases and b-amylase. During the milling process, the starch granules in flour are sufficiently damaged and make more susceptible to amylases. During the baking process, gelatinization of the starch granules occurs, which together with the action of a-amylase cause liquefaction of the starch. Similarly, the b-amylase present in flour converts the dextrins to maltose, which is subsequently fermented by the baker's yeast. Only small amounts of fermentable sugars are available in the wheat flour.

The enzymatic hydrolysis made available enough fermentable sugars in the dough to sustain vigorous yeast fermentation required to produce lively doughs and large loaf volumes. Fungal a-amylases, mainly from *A. oryzae*, are usually used in bread baking to improve volume, color, and flavor, while bacterial alpha-amylases are used in the preparation of doughs for cakes, biscuits, and crackers, where it adds more sweetness. There may be staling effects during the storage of baked products, causing disagreeable changes affecting crumb firmness, crust crispness, moisture content of the crumb and loss of flavor, and so on. Upon storage, the short amylopectin side chains present in soft, fresh bread gradually gets crystallized to amylopectin network, which accounts for a major role in bread firming.

Following starch crystallization, moisture migration within the crumb structure occurs leading to increased crumb firmness and decreased crumb resilience. Bacterial alpha-amylases with intermediate thermostability are used in anti-staling agents. These limits recrystallization of amylopectin, its network formation, and consequent water immobilization and help to the retention of softness and improve the shelf life of baked food (Balakrishnan et al. 2019). The alpha-amylase from *B. stearothermophilus* has been employed in the baking industry as an anti-staling agent. However, overuse may result in gumminess of the bread, as it produces more branched dextrins. This can be reduced by the use of thermostable pullulanase along with amylase. The pullulanase help to hydrolyze the branched dextrins produced by the alpha-amylase.

Sweetening agents are the major and expensive elements of a large variety of confectionery products. Amylolytic enzymes enable starch from low-cost resources to be transformed into sugar syrup. Sucrose is used as a major sweetening agent, and starch syrups (glucose) and dextrose occupy a second position. Glucose, fructose, maltose, and higher oligosaccharides, mainly derived from uncooked starch, mostly from cereal and tuber starches. Starch enzymatic hydrolysis using amylases in the starch liquefaction process converts starch into fructose and glucose syrups (Regulapati et al. 2007). The process of liquefaction demands thermostable a-amylase that can act at high temperatures ranging from 70 to 100 °C. Due to

thermostability, enzymes from *Bacillus* sp. are most preferred for industrial applications. Alpha-amylase from *B. amyloliquefaciens* was used previously; however, it has been substituted by alpha-amylase of *B. licheniformis* or *B. Stearothermophilus* (Prakash and Jaiswal 2010).

Another major application of the amylase enzyme is in beer brewing. Beer is produced by yeast fermentation of sugars. Beer is traditionally based on barley, contains a large quantity of starch, and before the yeast fermentation to produce alcohol, starch must be converted to fermentable sugars. Mashing (malting) is the process in which enzymatic degradation of starch into fermentable sugars (maltose) occurs and is a complex process that involves many enzymes like a-amylase, b-amylase, a-glucosidase, and limit dextrinase (Bamforth 2000). The alpha-amylase acts on a-1,4linkages at random, while b-amylases are exo-enzymes that attack the liquefied starch chains forming maltose units from the nonreducing end. Modern brewers usually supplement these enzymes, and it is essential when grains other than barley are used. The thermostable a-amylase from *B. subtilis*, those from *A. oryzae* (with glucoamylase activity), and glucoamylases from *A. niger* are usually used in mashing for starch hydrolysis.

Enzymes are widely used in the maceration of fruit pulps and for clarification of fruit juices and have contributed to improving the quality and yield of different types of juices. The application of enzymes fruit juice clarification will depend on the kind of polysaccharides present in different fruit juices. Pectinase plays a major role in the clarification process (depectinization), whereas amylase is preferred in the presence of starch. These enzymes are used in combination with cellulose and hemicellulase to effectively reduce haziness in juices. Clarified apple juice is one of the most consumed fruit juices. Raw apple juice was obtained as turbid, viscous, and tends to settle during storage due to the presence of polysaccharides (pectin and starch), tannins, proteins, etc. Amylases are used to degrade starch present in apple juice (Suresh and Prathaban 2015).

11.6.2 Detergent and Soap Industry

Detergent industries are the primary consumers of enzymes, in terms of both volume and value. The use of enzymes in detergent formulations enhances the detergent stability to remove tough stains and making the detergent environmentally safe. Amylases are the second type of enzyme used in the formulation of enzymatic detergent, and 90% of all liquid detergents contain these enzymes (Hmidet et al. 2009).

These enzymes are used in detergents for laundry and automatic dishwashing to degrade the residues of starchy foods such as potatoes, gravies, custard, chocolate to dextrins, and other smaller oligosaccharides. Amylases have activity at lower temperatures and alkaline pH, maintaining the necessary stability under detergent

conditions and the oxidative stability of amylases is one of the most important criteria for their use in detergents where the washing environment is very oxidizing. Removal of starch from surfaces is also important in providing a whiteness benefit since starch can be an attractant for many types of particulate soils. Examples of amylases used in the detergent industry are derived from *Bacillus* and *Aspergillus* spp. (Mukherjee et al. 2009).

11.6.3 Fuel Bio-Ethanol

Ethanol is the most utilized liquid biofuel. For ethanol production, starch is the most used substrate due to its low price and easily available raw material in most regions of the world. In this production, starch has to be solubilized and then submitted to two enzymatic steps to obtain fermentable sugars. The bioconversion of starch into ethanol involves liquefaction and saccharification, where starch is converted into sugar using an amylolytic microorganism or enzymes such as alpha-amylase, followed by fermentation, where sugar is converted into ethanol using an ethanol fermenting microorganism such as yeast *Saccharomyces cerevisiae* (Fig. 11.4). Among bacteria, alpha-amylase obtained from thermo-resistant bacteria like *B. licheniformis* or engineered strains *of Escherichia coli* or *Bacillus subtilis* is used during the first step of hydrolysis of starch suspensions. Fungal alpha-amylases are produced using *Aspergillus, Penicillium,* and *Rhizopus* spp. (Sigoillot and Faulds 2016).

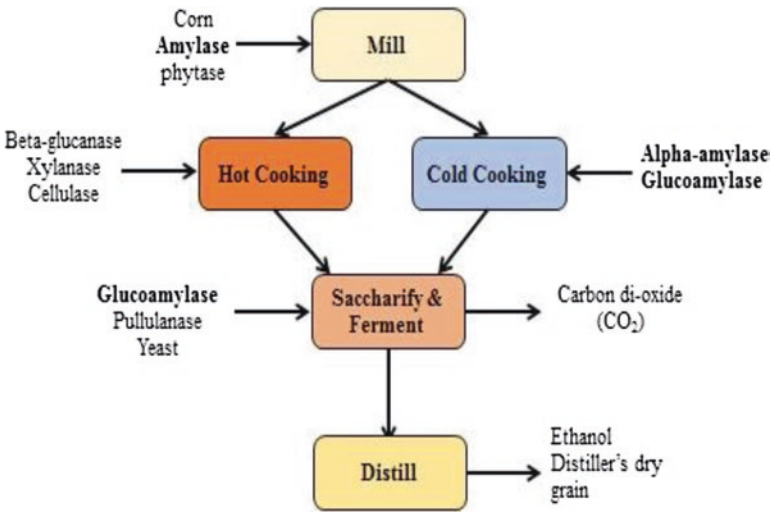


Fig. 11.4 Role of amylase in ethanol production

11.6.4 Textile Industry

Amylases are used in the textile industry for the desizing process. Sizing agents like starch are applied to yarn before fabric production to ensure a fast and secure weaving process. Starch is a very attractive size because it is cheap, easily available in most regions of the world, and it can be removed quite easily. Starch is later removed from the woven fabric in a wet-processing in the textile finishing industry. Desizing involves the removal of starch from the fabric that serves as the strengthening agent to prevent the breaking of the warp thread during the weaving process. The alpha-amylases remove selectively the size and do not attack the fibers (Feitkenhauer 2003).

11.6.5 Paper Industry

The use of alpha-amylases in the pulp and paper industry is for the modification of starch of coated paper, that is, for the production of low-viscosity, high-molecular-weight starch. The coating treatment serves to make the surface of paper sufficiently smooth and strong to improve the writing quality of the paper. In this application, the viscosity of the natural starch is too high for paper sizing, and this can be altered by partially degrading the polymer with alpha-amylases in a batch or continuous process. Starch is a good sizing agent for the finishing of paper and improving the quality and erasability, besides being a good coating for the paper. The size enhances the stiffness and strength of a paper (Saxena et al. 2004). Examples of amylases obtained from fungi used in the paper industry include Amizyme® (PMP Fermentation Products, Peoria, USA), Termamyl®, Fungamyl, BAN® (Novozymes, Denmark), and alpha-amylase G9995® (Enzyme Biosystems, USA).

11.7 Challenges and Future Prospects

Enzymes are some of the most important biomolecules, which have a wide range of applications in the industrial as well as the biomedical field. Today, enzymes are some of the most important molecules that are widely used in every sector, whether that may be dairy, industrial, agriculture, or pharmaceutical fields (Patil et al. 2021). The global market for industrial enzymes is estimated at $3.3 billion in 2010 and is expected to reach $5 billion by 2020. The market segmentation for various areas of the application shows that 34% of the market is for food and animal feed, followed by detergent and cleaners (29%). Paper and pulps share an 11% market while 17% of the market is captured by the textile and leather industries (Parameswaran et al. 2013).

The ongoing progress and interest in enzymes provide further success in areas of industrial bio-catalysis. There is a need for exciting developments in the area of biotransformation and molecular biology. Many factors are influencing the growing interest in biocatalysts, which include enzyme promiscuity, robust computational

methods combined with directed evolution, and screening technologies to improve enzyme properties to meet process prospects (Adrio and Demain 2014). Recent advances in genomics, proteomics, efficient expression systems, and emerging recombinant DNA techniques have facilitated the discovery of new microbial enzymes from nature or by creating enzymes with improved catalytic properties. A future trend is to develop more effective systems that use much smaller quantities of chemicals and less energy to attain maximum product yield. Modern biotechnology will lead to the development of enzyme products with improved effects with diverse physiological conditions.

Biotechnology offers an increasing potential for the production of goods to meet various human needs. Multidisciplinary research involving industry is required to develop application-oriented research on enzymes. Over the past 10 years, major advances in DNA technologies and bioinformatics have provided critical support to the field of bio-catalysis. These tools have promoted the discovery of novel enzymes in natural resources and have substantially accelerated the redesign of existing biocatalysts. Next-generation DNA sequencing technology has allowed parallel sequence analysis on a massive scale and at dramatically reduced cost (Bornscheuer et al. 2012).

New and exciting enzyme applications are likely to bring benefits in other areas like less harm to the environment, greater efficiency, lower cost, lower energy consumption, and the enhancement of product properties. New enzyme molecules capable of achieving this will be developed through protein engineering and recombinant DNA techniques. Industrial biotechnology has an important role to play in the way modern foods are processed. New ingredients and alternative solutions to current chemical processes will be a challenge for the enzyme industry. When compared with chemical reactions, the more specific and cleaner technologies made possible by enzyme-catalyzed processes will promote the continued trend toward natural processes in the production of food, energy, and textile (Table 11.6).

Table 11.6 Industrial applications of α-amylase

Industry	Application	Microorganism
Detergent (laundry and dish wash)	Starch stain removal	*Bacillus* spp./*Aspergillus* spp. (Amylases with activity at lower temperatures, alkaline pH, and oxidative conditions)
Starch liquefaction	Dispersion of insoluble starch granules in aqueous solution and decreasing viscosity followed by partial hydrolysis	*Bacillus* spp.(thermostable) *amyloliquefaciens*; *Bacillus stearothermophilus* or *Bacillus licheniformis*
Fuel alcohol production	Starch liquefaction and saccharification	As yeast *Saccharomyces cerevisiae*
Food industry	Bread softness and volume, flour adjustment; Juice treatment, low-calorie beer	Thermostablemaltogenicamylase of *Bacillus stearothermophilus*
Textile industry	De-sizing	*Bacillus* stains
Pulp and paper	De-sizing; Starchcoating, de-inking, drainage improvement	*Aspergillus* spp. and *Bacillus* spp.

11.8 Conclusion

The enzyme industry is one of the major industries of the world, and there exists a great market for enzymes. Enzymes are used in many different industrial products and processes, and new areas of applications are constantly being added because of advances in modern biotechnology. Microorganisms provide an impressive amount of catalysts with a wide range of applications across many industries, such as food, animal feed, technical industries, paper, fine chemicals, and pharmaceuticals. The unique properties of enzymes, such as high specificity, fast action, and biodegradability, allow enzyme-assisted processes in the industry to run under milder reaction conditions, with improved yields and a reduction in waste generation. Naturally occurring enzymes are often modified by molecular biology techniques to redesign the enzyme itself to fine-tune substrate specificity activity and thermostability. Enzyme technology offers great potential for many industries to meet challenges in the future with the help of recombinant technology.

Acknowledgments Mr. Anirudh Gururaj Patil (LIF-02-2019-20) would like to thank DST-KSTePS, GoK, for providing DST Ph.D. fellowship. Dr. Farhan Zameer (FZ) sincerely thanks Prof. Dr. Shubha Gopal, Department of Studies in Microbiology, University of Mysore, and Prof. Dr. Juergen Kreft, Department of Microbiology, University of Wurzburg, Germany, for their mentorship. FZ is also thankful to Dr. MN Nagendra Prasad, Department of Biotechnology, JSS Science and Technology University, Mysore, and Dr. Shaukath Ara Khanum, Department of Chemistry, Yuvaraja College, University of Mysore, Mysore, for their long-term collaboration in understanding the biology of chemical molecules. All authors thank Prof. Sunil S. More and Prof. Muthuchelian K, SBAS, Dayananda Sagar University (DSU), for continuous support. Further, we thank Mr. Vimal John Samuel, Mrs. K.B. Premakumari, Mr. Sunil, and Prof. V. Murgan, from the School of Pharmacy, DSU, for their technical assistance during the preparation of the manuscript. Further, we extend our gratitude toward the management and office bearers of Dayananda Sagar University, Bengaluru, Karnataka, India, for constant inspiration, motivation, and encouragement to pursue scientific research and for the DSU seed grant funding for the year 2020–2021.

References

Adrio JL, Demain AL (2014) Microbial enzymes: tools for biotechnological processes. Biomolecules 4(1):117–139

Aishwarya S, Khan K, Patil AG, Satapathy P, Devi AT, Avinash MG et al (2020) Nutraceutical attributes of *Tamarindus indica* L. - devils' tree with sour date. In: Choudhary MI, Yousuf S (eds) Science of spices and culinary herbs, vol 3. Bentham Science Publishers, Sharjah, pp 33–65

Ashwini P, Sumana MN, Shilpa P, Mamatha P, Manasa P, Dhananjaya BL et al (2015) A review on *Helicobacter pylori*: its biology, complications and management. Int J Pharm Pharm Sci 7:14–20

Ashwini P, Baker S, Prasad MN, Devi AT, Satish S, Zameer F et al (2019) Phytogenic synthesis of silver nanobactericides for anti-biofilm activity against human pathogen *H. pylori*. SN Appl Sci 1(4):1–7

Balakrishnan D, Kumar SS, Sugathan S (2019) Amylases for food applications-updated information. In: Green bio-processes. Springer, Singapore, pp 199–227

Bamforth CW (2000) Beer: ancient yet modern biotechnology. Chem Educator 5(3):102–112
Beulah KC, Aishwarya W, Meghashri H, Prasad N et al (2015) Phyto-antiquorumones: an herbal approach for blocking bacterial trafficking and pathogenesis. Int J Pharm Pharm Sci 7(1):29–34
Bornscheuer UT, Huisman GW, Kazlauskas RJ, Lutz S, Moore JC, Robins K (2012) Engineering the third wave of biocatalysis. Nature 485(7397):185–194
Cavicchioli R (2011) Archaea-timeline of the third domain. Nat Rev Microbiol 9(1):51–61
Chen J, Chen X, Dai J, Yan L, Lu L, Chen J, Xie G (2015) Cloning, enhanced expression and characterization of an α-amylase gene from a wild strain in *B. subtilis* WB800. Int J Biol Macromol 80:200–207
Ciloci A, Bivol C, Stratan M, Reva V, Clapco S, Tiurin S, Labliuc S (2012) Production and purification of α-amylase from *Aspergillus niger* 33-19 CNMN FD 02a mutant form. Analele Univ din Oradea Fasc Biol 19(1):74–79
Coronado MJ, Vargas C, Hofemeister J, Ventosa A, Nieto JJ (2000) Production and biochemical characterization of an α-amylase from the moderate halophile *Halomonas meridiana*. FEMS Microbiol Lett 183(1):67–71
Deng A, Wu J, Zhang Y, Zhang G, Wen T (2010) Purification and characterization of a surfactant-stable high-alkaline protease from *Bacillus sp*. B001. Bioresour Technol 101(18):7100–7106
Djekrif-Dakhmouche S, Gheribi-Aoulmi Z, Meraihi Z, Bennamoun L (2006) Application of a statistical design to the optimization of culture medium for α-amylase production by *Aspergillus niger* ATCC 16404 grown on orange waste powder. J Food Eng 73(2):190–197
Far BE, Ahmadi Y, Khosroshahi AY, Dilmaghani A (2020) Microbial alpha-amylase production: progress, challenges and perspectives. Adv Pharm Bull 10(3):350
Feitkenhauer H (2003) Anaerobic digestion of desizing wastewater: influence of pretreatment and anionic surfactant on degradation and intermediate accumulation. Enzyme Microb Technol 33(2–3):250–258
Fiedurek J, Gromada A (2000) Production of catalase and glucose oxidase by *Aspergillus niger* using unconventional oxygenation of culture. J Appl Microbiol 89:85–89
Gopal S, Srinivas V, Zameer F, Kreft J (2009) Prediction of proteins putatively involved in the thiol: disulfide redox metabolism of a bacterium (Listeria): the CXXC motif as query sequence. In Silico Biol 9(56):407–414
Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B (2003) Microbial α-amylases: a biotechnological perspective. Process Biochem 38(11):1599–1616
Gurung N, Ray S, Bose S, Rai V (2013) A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. Biomed Res Int 1:329121
Haki GD, Rakshit SK (2003) Developments in industrially important thermostable enzymes: a review. Bioresour Technol 89(1):17–34
Hii SL, Tan JS, Ling TC, Ariff AB (2012) Pullulanase: role in starch hydrolysis and potential industrial applications. Enzyme Res 2012:921362
Hmidet N, Ali NE, Haddar A, Kanoun S, Alya SK, Nasri M (2009) Alkaline proteases and thermostable α-amylase co-produced by *Bacillus licheniformis* NH1: characterization and potential application as detergent additive. Biochem Eng J 47(1–3):71–79
Horvathova V, Janecek S, Sturdik E (2001) Amylolytic enzymes: molecular aspects of their properties. Gen Physiol Biophys 20(1):7–32
Hussain I, Siddique F, Mahmood MS, Ahmed SI (2013) A review of the microbiological aspect of α-amylase production. Int J Agric Biol 15(5):1029–1034
Jasti N, Khanal SK, Pometto AL III, van Leeuwen J (2008) Converting corn wet-milling effluent into high-value fungal biomass in a biofilm reactor. Biotechnol Bioeng 101(6):1223–1233
Jung JH, Seo DH, Holden JF, Park CS (2014) Maltose-forming α-amylase from the hyperthermophilic archaeon *Pyrococcus sp.* ST04. Appl Microbiol Biotechnol 98(5):2121–2131
Kathiresan K, Manivannan S (2006) Amylase production by *Penicillium fellutanum* isolated from mangrove rhizosphere soil. Afr J Biotechnol 5(10):829–832
Khan K, Aishwarya S, Satapathy P, Veena SM, Melappa G, Zameer F et al (2020) Exploration of dill seeds (*Anethum graveolens*): an ayurpharmacomic approach. In: Science of spices

and culinary herbs-latest laboratory, pre-clinical, and clinical studies, vol 2. Bentham Books, Sharjah, pp 116–152
Konsoula Z, Liakopoulou-Kyriakides M (2007) Co-production of α-amylase and β-galactosidase by *Bacillus subtilis* in complex organic substrates. Bioresour Technol 98(1):150–157
Kumar P, Satyanarayana T (2009) Microbial glucoamylases: characteristics and applications. Crit Rev Biotechnol 29(3):225–255
Kunamneni A, Permaul K, Singh S (2005) Amylase production in solid-state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. J Biosci Bioeng 100(2):168–171
Kunnel SG, Subramanya S, Satapathy P, Sahoo I, Zameer F (2019) Acrylamide induced toxicity and the propensity of phytochemicals in amelioration: a review. Cent Ner Syst Agents Med Chem 19(2):100–113
Li S, Yang X, Yang S, Zhu M, Wang X (2012) Technology prospecting on enzymes: application, marketing and engineering. Comp Struct Biotechnol J 2(3):e201209017
Lim SJ, Oslan SN, Oslan SN (2020) Purification and characterization of thermostable α-amylases from microbial sources. BioResource 15(1):2005–2029
Lonsane BK, Ramesh MV (1990) Production of bacterial thermostable α-amylase by solid-state fermentation: a potential tool for achieving economy in enzyme production and starch hydrolysis, Adv Appl Microbiol, vol 35. Academic, New York, pp 1–56
Madhusudan M, Zameer F, Naidu A, Dhananjaya BL, Hegdekatte R (2016) Evaluating the inhibitory potential of *Withania somnifera* on platelet aggregation and inflammation enzymes: an *in vitro* and *in silico* study. Pharm Biol 54:1936–1941
Manjunatha HP, Singh H, Chauhan JB, Zameer F, Garampalli RH (2013) Induction of resistance against sorghum downy mildew by seed treatment with *Duranta repens* extracts. IOSR J Agric Vet Sci 3:37–44
Mathew T, Sree RA, Aishwarya S, Kounaina K, Patil AG, Satapathy P et al (2020) Graphene-based functional nanomaterials for biomedical and bioanalysis applications. Flat Chem 23:100184
Mehta D, Satyanarayana T (2016) Bacterial and archaeal α-amylases: diversity and amelioration of the desirable characteristics for industrial applications. Front Microbiol 7:1129
Mobini-Dehkordi M, Javan FA (2012) Application of alpha-amylase in biotechnology. J Biol Todays World 1:39–50
More VS, Ebinesar A, Prakruthi A, Praveen P, Fasim A, Archana R et al (2021) Isolation and purification of microbial exopolysaccharides and their industrial application, Microbial polymer. Springer, Singapore, pp 69–86
Moshfegh M, Shahverdi AR, Zarrini G, Faramarzi MA (2013) Biochemical characterization of an extracellular polyextremophilic α-amylase from the halophilic archaeon *Halorubrum xinjiangense*. Extremophiles 17(4):677–687
Mukherjee AK, Borah M, Rai SK (2009) To study the influence of different components of fermentable substrates on induction of extracellular α-amylase synthesis by *Bacillus subtilis* DM-03 in solid-state fermentation and exploration of feasibility for inclusion of α-amylase in laundry detergent formulations. Biochem Eng J 43(2):149–156
Murthy PS, Madhava Naidu M, Srinivas P (2009) Production of α-amylase under solid-state fermentation utilizing coffee waste. J Chem Technol Biotechnol 84(8):1246–1249
Nigam PS (2013) Microbial enzymes with special characteristics for biotechnological applications. Biomolecules 3(3):597–611
Onodera M, Yatsunami R, Tsukimura W, Fukui T, Nakasone K, Takashina T et al (2013) Gene analysis, expression, and characterization of an intracellular α-amylase from the extremely halophilic archaeon *Haloarcula japonica*. Biosci Biotechnol Biochem 77(2):281–288
Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000) Advances in microbial amylases. Biotech Appl Biochem 31(2):135–152
Pankaj S, Aishwarya S, Rashmi Shetty M, Akshaya Simha N, Dhanapal G, Aishwarya Shree R et al (2020) Phyto-nano-antimicrobials: synthesis, characterization, discovery, and advances, Chapter 7. In: Frontiers in anti-infective drug discovery, vol 8. Bentham Science International Publishers, Sharjah, pp 196–231

Parameswaran B, Piyush P, Raghavendra G, Nampoothiri KM, Duggal A, Dey K (2013) Industrial enzymes - present status and future perspectives for India. J Sci Ind Res 72:271–286
Patil AG, Kounaina K, Aishwarya S, Harshitha N, Satapathy P, Hudeda SP et al (2021) Myconanotechnology for sustainable agriculture: challenges and opportunities. In: Recent trends in mycological research. Springer, Cham, pp 457–479
Pradeep NV, Anupama AK, Pooja J (2012) Categorizing phenomenal features of α-amylase (Bacillus species) using bioinformatic tools. Adv Lif Sci Technol 4:27–31
Prakash O, Jaiswal N (2010) α-Amylase: an ideal representative of thermostable enzymes. Appl Biochem Biotechnol 160(8):2401–2414
Prasad MP, Sethi R, Anand M (2014) Evaluation of-amylase enzyme from *Bacillus sp.* isolated from various soil samples. Int J Curr Microbiol Appl Sci 3(10):783–789
Prasad A, Devi AT, Prasad MN, Zameer F, Shruthi G, Shivamallu C (2019) Phyto anti-biofilm elicitors as potential inhibitors of *Helicobacter pylori*. 3 Biotech 9(2):53
Ray RR, Nanda G (1996) Microbial β-amylases: biosynthesis, characteristics, and industrial applications. Crit Rev Microbiol 22(3):181–199
Regulapati R, Malav PN, Gummadi SN (2007) Production of thermostable α-amylases by solid-state fermentation - a review. Am J Food Technol 2(1):1–1
Robinson T, Singh D, Nigam P (2001) Solid-state fermentation: a promising microbial technology for secondary metabolite production. Appl Microbiol Biotechnol 55(3):284–289
Santos RD, Muruci LN, Santos LO, Antoniassi R, da Silva JP, Damaso MC (2014) Characterization of different oil soapstocks and their application in the lipase production by *Aspergillus niger* under solid-state fermentation. J Food Nutr Res 2(9):561–566
Saranraj P, Naidu MA (2014) Microbial pectinases: a review. Glob J Trad Med Syst 3(1):1–9
Satapathy P, Khan K, Devi AT, Patil AG, Govindaraju AM et al (2019) Synthetic gutomics: deciphering the microbial code for futuristic diagnosis and personalized medicine. Methods Microbiol 46:197–225
Satapathy P, Prakash JK, Gowda VC, More SS, Chandramohan V, Zameer F (2020) Targeting Imd pathway receptor in *Drosophila melanogaster* and repurposing of phyto-inhibitors: structural modulation and molecular dynamics. J Biomol Struct Dyn:1–12
Saxena RK, Malhotra B, Batra A (2004) The commercial importance of some fungal enzymes. In: Handbook of fungal biotechnology. CRC Press, Boca Raton, FL, pp 287–298
Shankara RBE, Ramachandra MS, Santosh MS, Sujan Ganapathy PS, Richard SA, Zameer F et al (2016) Evaluating the *In vitro* α-amylase inhibitory potential of gall extracts of *Terminalia chebula* (Gaertn.) Retz. (*Combretaceae*). Pharmacog J 8:4
Sharma VK, Kumar N, Prakash T, Taylor TD (2010) MetaBioME: a database to explore commercially useful enzymes in metagenomic datasets. Nucleic acids Res 38(Suppl 1):D468–D472
Sigoillot JC, Faulds C (2016) Second-generation bioethanol. In: Green fuels technology. Springer, Cham, pp 213–239
Singh SU, Sharma VI, Soni ML, Das S (2011) Biotechnological applications of industrially important amylase enzyme. Int J Pharma Bio Sci 2(1):486–496
Singh H, Zameer F, Khanum SA, Garampalli RH (2016) Durantol-a phytosterol antifungal contributor from *Duranta repens* Linn. For organic management of Sorghum Downy Mildew. Eur J Plant Pathol 146(3):671–682
Sulthana R, Taqui SN, Zameer F, Syed U, Syed AA (2018) Adsorption of ethidium bromide from aqueous solution onto nutraceutical industrial fennel seed spent: kinetics and thermodynamics modeling studies. Int J Phytoremediation 20(11):1075–1086
Suresh KC, Prathaban M (2015) Optimization of process parameters for carboxymethyl cellulase production under submerged fermentation by *Streptomyces lividians*. J Acad Ind Res 3(8):371
Van der Maarel MJ, Van der Veen B, Uitdehaag JC, Leemhuis H, Dijkhuizen L (2002) Properties and applications of starch-converting enzymes of the α-amylase family. J Biotechnol 94(2):137–155
Vihinen M, Peltonen T, Iitiä A, Suominen I, Mäntsälä P (1994) C-terminal truncations of a thermostable *Bacillus stearothermophilus* α-amylase. Prot Eng Des Select 7(10):1255–1259

Weemaes C, De Cordt S, Goossens K, Ludikhuyze L, Hendrickx M, Heremans K et al (1996) High pressure, thermal, and combined pressure-temperature stabilities of α-amylases from Bacillus species. Biotechnol Bioeng 50(1):49–56

Yadav AN, Kaur T, Devi R, Kour D, Yadav A, Yadav PK et al (2021) Environmental and industrial perspective of beneficial fungal communities: current research and future challenges, Chapter 18. In: Recent trends in mycological research, fungal biology, vol 2. Switzerland AG, Springer Nature, pp 497–517

Zaky AS, Tucker GA, Daw ZY, Du C (2014) Marine yeast isolation and industrial application. FEMS Yeast Res 14(6):813–825

Zameer F, Gopal S (2010) Transcriptome analysis of thiol disulfide redox metabolism genes in *Listeria monocytogenes* in biofilm and planktonic forms. Int J Pure Appl Sci 4(1):21–27

Zameer F, Meghashri S, Gopal S, Rao BR (2010a) Chemical and microbial dynamics during composting of herbal pharmaceutical industrial waste. J Chem 7(1):143–148

Zameer F, Kreft J, Gopal S (2010b) Interaction of *Listeria monocytogenes* and *Staphylococcus epidermidis* in dual species biofilms. J Food Saf 30(4):954–968

Zameer F, Rukmangada MS, Chauhan JB, Khanum SA, Kumar P, Devi AT et al (2016) Evaluation of adhesive and anti-adhesive properties of *Pseudomonas aeruginosa* biofilms and their inhibition by herbal plants. Iran J Microbial 8(2):108

Chapter 12
Fungal Phytases: Current Research and Applications in Food Industry

Parsa Mahmood Dar

Contents

12.1 Introduction

Phytases have been one of the most important enzymes in nutrition, environmental protection, and human health over the last two decades. These enzymes sequentially isolate orthophosphate groups from the core of phytate inositol, the principal chemical source of phosphorus in plants (Kumar et al. 2017). Various phytases have been

P. M. Dar (✉)
Institute of Chinese Medical Sciences, Avenida da Universidade, Taipa, Macau, China

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_12

isolated from plants or microbes and can be categorized according to their optimal pH and catalytic methods (histidine acid phosphatases, ß-propellate phytases, cysteine, or purple acid phosphatases). The urgent need to boost phytate phosphorus in diets for animals with simple stomachs to reduce the excretion of phosphorus fume into the environment has been motivated by recent phytase activities. However, future phytase applications may be extended to release dietary phytate-bound minerals for human nutrition and to produce specific inositol phosphates for human health (Konietzny and Greiner 2003; Kour et al. 2020).

There is also an excellent opportunity to use phytases in the production and manufacture of food for human consumption, where research focuses on the enhancement of the nutritional value of plant-based food as well as the technical development of food processing. A high diet of phytate contributes to a considerably reduced absorption of dietary mineral products (Sandberg et al. 1999). During food processing, phytate dephosphorylation results in the formation of only partly phosphorylated myo-inositol phosphate esters with a lower capacity to interfere with the dietary intake (Sandström and Sandberg 1992; Han et al. 1999; Shears 1998). Specific myo-inositol phosphate esters have proved to have many essential physiological functions in humans (Greiner et al. 2002). Phytases, therefore, that can be used for food processing to produce functional foods (Haros et al. 2001) should generate these biochemically active phosphate esters of myo-inositol and absorb them into the food tract. This chapter contains technical improvements to the implementation of phytases for bread making (Wang et al. 1999), plant protein isolation production (Fredrikson et al. 2001; Caransa et al. 1988), corn waters (Antrim et al. 1997; Kvist et al. 2005), and cereal bran fractionation (Andlid et al. 2004).

In the gastrointestinal monogastric tract, phytate (Inositol hexaphosphate, IP6), the dominant source of phosphorous and mineral food complexes (calcium, ferrous, magnesium, and zinc), is indigestible (Hellström et al. 2010; Brinch-Pedersen et al. 2007). Phytate is a key antinutritional factor for the bioavailability of dietary minerals that rely exclusively on cereals to dietary consumers (Cao et al. 2007). It involves the global burden of iron deficiency and the resulting complications, particularly among women and children, in low-income countries. Diät mineral bioavailability can be increased with the use of phytase, a catalytic enzyme for phytate sequence hydrolysis (Raghavendra and Halami 2009; Ogunremi et al. 2020; Mullaney and Ullah 2003).

As members of previously recognized phosphatases classes, the basic structural characteristics of several phytate-degrading enzymes were determined (Mullaney and Ullah 2003; Chu et al. 2004). X-ray crystallographic studies, among others, have confirmed their belonging to a class with a new catalytic mechanism (Ha et al. 2000). The description of the molecular 3D structure of the various phytate degrading enzymes strengthened our understanding of the relationship between the molecular structure and the catalytic functioning of the molecular structure. It is now clear that specific phytases have been established to satisfy the unique nutritional requirements of various forms of life in plants, animals, and microbials (Rastegari et al. 2020). A strong connection between the catalytic domain of an enzyme and unique molecular architectural elements also seems to exist. Although certain structural

Table 12.1 Phytase enzyme and their structural and adaptive features

Enzyme family	Unique structural feature	mechanism/adaptation to hydrolyzes phytate	Example	Reference
Histidine Acid Phosphatase	N-terminal RHGXRXP C-terminal HD consensus motif	N-terminal H forms a phosphohistidine intermediate, C-terminal acts as proton donor/ Substrate specificity site residues positively charged	*A. niger* *P. Lycii* *E. coli* Zea mays L.	Wodzinski and Ullah (1996)
Cysteine Phosphatase	P loop structure contains HCXXGXXR(T/S) consensus motif	Protein tyrosine phosphatase mechanism cleaves phosphate groups/ Deeper active site pocket accommodates phytate	*S. ruminatium*	Chu et al. (2004)
Purple Acid Phosphatase	Consensus motif: DXG/GDXXY/GNH (E,D) /VXXH/GHXH	Metalloenzymes, phylogenetically linked to large plant PAP/unknown	Glycine max *M. truncatula*	Oh et al. (2004)

components are important, certain nonessential parts of the molecule may therefore be altered to adapt the catalytic mechanism to the different substrates. In future research, the exact number of catalytic mechanisms formed to hydrolyze the phytate can be determined. It is now recognized that four phosphatase enzyme groups are representative of phytic acid that can be degraded as described in Table 12.1.

12.2 Phytases of Fungal Origin

The majority of phytases isolated from fungi and yeast, typically known as 3-phytases, are histidine acid phosphatases, glycosylated, and active for a wide variety of substrates (Wyss et al. 1999b). *Aspergillus niger* PhyA was the first phytase to be well characterized and marketable. This enzyme, encoded by a 1.4 kb DNA fragment, is a monomer with an estimated molecular weight of 80 kDa, a bi-hump pH profile with two optimal pH at 2.5 and 5.0–5.5, an optimal temperature at 55–60 °C, and a high phytic acid affinity (Han et al. 1999). *Aspergillus fumigatus* phytase has a sequence similarity of 66% to A. PhyA Phytase niger, however, exhibits greater thermo-tolerance (Pasamontes et al. 1997a; Wyss et al. 1998).

The thermo-tolerance was associated with high refolding efficiency after heat denaturation, and the specificity of the buffers used in heat treatment can be modulated (Rodriguez et al. 2000a). The enzyme has a wide range of pH and is highly active against low phosphorylation inositol phosphates (Wyss et al. 1999b; Rodriguez et al. 2000a). Yet its unique phytate activity is small (Tomschy et al. 2000). PhyA phytase *Peniophora lycii* was also sold out. It is a 6-phytase with optimum pH at 4.0–4.5 and optimum temperature at 50–55 °C and has dimeric conformation (Lassen et al. 2001). It seems vulnerable to thermal and protease treatments

(Simon and Igbasan 2002) or low pH (Quan et al. 2004) isolated a low-molecular-weight (32.6 kDa) phytase from *Cladosporium* sp., an airborne fungus. PS-1.

The enzyme is not glycosylated and has an average 3.5 pH and an average 40 °C temperature. It produces tri-phosphate inositol as the product. Phytases isolated from thermophilic fungi *Myceliophtora thermophila* and *Talaromyces thermophilus* (Mitchell et al. 1997; Pasamontes et al. 1997b) show a high degree of homology of sequence to other fungal phytases *A. niger*, *A. Terreus,* and *A. Fumigate*. Berka et al. (1998) isolated a phytase from the *Thermomyces lanuginosus* thermophilic fungus which showed better thermostability and catalytic performance and a higher transition temperature than the A. Phytase to the niger. Chadha et al. (2004) reported that phytase produced by the *Mucor pusillus* thermophilic fungus was active in a wide pH range of 3–7.8. Nakamura et al. (2000) found substantial levels of phytase activity in 35 species from a survey on 738 strains of yeast, with a wide range of optimal pH and temperature. *Arxula adeninivorans* developed well in media containing phytate as the sole source of phosphate and secreted phytase with an optimum pH of 4.5–5.0, and an optimum temperature of around 75 °C (Sano et al. 1999; Quan et al. 2002) also reported substantial phytase development from soil-isolated yeast *Candida Krusei* WZ-001. The isolated phytase produced two different subunits with 116- and 31-kDa molecular masses, had a glycosylation rate of about 35%, and had optimal pH and temperature at 4.6 and 40 °C, respectively. *Pichia anomala* (Vohra and Satyanarayana 2001), *Saccharomyces cerevisiae* (Türk et al. 2000), and *Schwanniomyces castellii* (Segueilha et al. 1992) also showed phytase activity (Loewus 2002). These enzymes were active in the range of acid pH, with the optimum temperature at 60–74 °C.

Phytats Dietary Effects Salts of phytic acid, designated as phytates, are regarded as the primary storage form of both phosphate and inositol in plant seeds and grains. Phytate is formed during the maturation of the plant seed, and in dormant seeds, it represents 60–90% of the total phosphate (Reddy 2002). Phytate is therefore a common constituent of plant-derived foods. Depending on the amount of plant-derived foods in the diet and the grade of food processing, the daily intake of phytate can be as high as 4500 mg (Grases et al. 2000). On average, daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries and 150–1400 mg for mixed diets. Potential health benefits of phytate-rich diets. Consumption of phytate, however, does not seem to have negative aspects on human health. Dietary phytate was reported to prevent kidney stone formation (Jariwalla et al. 1990) and to protect against atherosclerosis and coronary heart disease (Vucenik and Shamsuddin 2003) as well as against a variety of cancers (Grases et al. 2001).

The levels of phytate and its dephosphorylation products in the urine, plasma, and other biological fluids are fluctuating with ingestion or deprivation of phytate in the human diet. Therefore, the reduction in phytate intake in developed compared to developing countries might be a factor responsible for the increase in diseases typical for Western societies, such as diabetes mellitus, renal lithiasis, cancer, atherosclerosis, and coronary heart diseases. It was suggested that phytate exerts beneficial effects in the gastrointestinal tract and other target tissues through its chelating

ability, but other mechanisms have also been discussed. Because several myo-inositol phosphates, including phytate, are present as intracellular molecules, and because the second messenger D-myo-inositol(1,4,5)trisphosphate is bringing about a range of cellular functions including cell proliferation via mobilizing intracellular Ca^{2+} (16), phytate was proposed to exert its anticancer effect by affecting cell signaling mechanisms in mammalian cells (Grases et al. 2001). An effect of extracellular phytate on the concentration of several intracellular myo-inositol phosphate esters has already been demonstrated in human erythroleukemia cells (Ferry et al. 2002).

Furthermore, it has recently been reported that highly negatively charged myo-inositol polyphosphates can cross the plasma membrane and be internalized by cells. Myo-inositol hexakisphosphate was shown to enter HeLa cells followed by intracellular dephosphorylation to partially phosphorylated myo-inositol phosphates (Maffucci et al. 2005), whereas turnover of myo-inositol(1,3,4,5,6)pentakisphosphate was quite slow after internalization by SKOV-3 cells (Carrington et al. 1993). In addition, individual myo-inositol phosphate esters have been proposed to be metabolically active. D-myo-inositol (1,2,6), for example, has been studied with respect to prevention of diabetes complications and treatment of chronic inflammations as well as cardiovascular diseases (Claxson et al. 1990; Konietzny and Greiner 2003), and due to its antiangiogenic and antitumor effects, myo-inositol(1,3,4,5,6)pentakisphosphate was suggested as a promising compound for anticancer therapeutic strategies (Carrington et al. 1993).

12.3 Phytase Adverse Effects

Phytate is an antinutrient that functions as a strongly negatively charged ion in a wide pH range and thus has a considerable affinity to positive-charge food components, such as minerals, trace elements, and proteins (Lopez et al. 2002; Sandberg et al. 1999). This relationship not only has nutritional effects but also affects the yield and consistency of food ingredients such as starch, steep corn liquor, or isolates of plant protein (Sandström and Sandberg 1992; Han et al. 1999; Wang et al. 1999; Fredrikson et al. 2001; Caransa et al. 1988). The main issue about phytate's role in the human diet is its detrimental impact on mineral absorption. In this sense, minerals of concern include zinc, iron, calcium, magnesium, manganese, and copper (Antrim et al. 1997). Since such complexes are basically nonabsorbable from the human gastrointestinal tract, the development of insoluble mineral–phytate complexes at physiological pH values is known to be the main explanation for the poor bioavailability. In addition, owing to the lack of endogenous phytate-degrading enzymes and the restricted microbial community in the upper part of the digestive tract, the human small intestine has only a very limited capacity to hydrolyze phytate (Kvist et al. 2005). Myo-inositol phosphate–mineral complexes have been found to decrease in solubility and stability as the amount of phosphate residues on the myo-inositol ring decreases. The removal of phytate phosphate residues,

therefore, results in a decreased impairment of the intestinal absorption of essential dietary minerals (Cheryan 1980; Iqbal et al. 1994; Sandberg 1991).

Only myo-inositol pentakisphosphate in isolated form inhibited human absorption of iron, zinc, and calcium, while myo-inositol tetrakis and trisphosphates had no effect in the concentrations under investigation. Nonetheless, in the presence of higher phosphorylated myo-inositol phosphates, myo-inositol tetrakis- and trisphosphates have been shown to lead to the phytate's negative impact on iron absorption (Cheryan 1980). Since there has been a clear negative association between zinc absorption and the amount of myo-inositol tris- by hexakisphosphate from cereal and legume meals (Sebastian et al. 1998), such a contribution is also possibly true for zinc absorption. Phytate is well known to form protein complexes at both acidic and alkaline pH (Knuckles and Betschart 1987). This interaction can affect changes in protein structure that may reduce enzymatic activity, solubility of proteins, and digestibility of proteolytics. The importance of protein–phytate complexes in feeding is, however, still under scrutiny. There is clear evidence that phytate–protein interactions have an adverse effect on in vitro protein digestibility, and the degree of this effect depends on the source of the protein. Nonetheless, a negative effect of phytate on the protein's nutritional value was not explicitly established in monogastric animal studies (Desphande and Cheryan 1984).

Although some have indicated phytate does not influence the digestibility of proteins, others have found an increase in the supply of amino acids with declining phytate levels. This disparity may be attributed at least in part to the use of various sources of protein. The inhibition by phytate of digestive enzymes such as a-amylase (Knuckles 1988), lipase (Singh and Krikorian 1982), or proteinases (Desphande and Damodaran 1989; Inagawa et al. 1987; Jenab and Thompson 2002), such as pepsin, trypsin, and chymotrypsin, may also be of nutritional significance as shown in in vitro studies. With the amount of phosphate residues per myo-inositol molecule, and the concentration of myo-inositol phosphate, the inhibitory effect increases. This inhibition may be due to the nonspecific nature of phytate–protein interactions, the chelation of calcium ions necessary for the trypsin and a-amylase function, or the interaction with these enzyme substrates. Protease inhibition may be partially responsible for the decreased digestibility of the proteins. Phytate was also considered an in vivo a-amylase inhibitor as shown by the negative relationship between phytate intake and blood glucose response (D'Souza et al. 1987).

Food rich in phytate has therefore been regarded as having significant nutritional significance in the prevention and management of diabetes mellitus, one of Western society's most common nutrition-dependent diseases. The most serious phytate-attributable effects have occurred as a major dietary variable in populations with unrefined cereals and/or pulses. Deficiencies in zinc and iron have been reported due to high intakes of phytate (Lönnerdal 2000; Maberly et al. 1994). Various approaches have been developed to reduce the risk of mineral deficiency in vulnerable groups, such as child-bearing mothers, strictly vegetarians, inhabitants of developing countries, and fast-growing children. Supplementation with nutritional formulations, food fortification, dietary diversification, and disease prevention (Raboy 2002) is the most known methods for reducing micronutrient malnutrition.

None has been very good for different reasons. An alternative solution would be to increase the overall level of micronutrients in the edible parts of staple crops while at the same time increasing the concentration of compounds that encourage their uptake and/or decrease the amount of compounds that inhibit their uptake, either by plant breeding or by genetic engineering. Low phytate mutants have recently been isolated in maize, barley, rice, and soybeans (Mendoza 2002), and their capacity for enhancing the absorption of iron, zinc, and calcium has been shown (Lucca and Hurrell 2001). To boost rice as an iron source, three proteins were expressed in the rice seed's central endosperm: a *Phaseolus phytoferritin*, an endogenous metallothionine-like protein rich in cysteine, and an *Aspergillus fumigatus* phytase (Coello et al. 2001).

If properly managed, phytase overexpression during seed production may result in reduced levels of phytate in the mature seed (Greiner and Konietzny 2006). Increased levels of seed phytase can also lead to an increase in mineral absorption by decreasing phytate levels in plant-based food during human stomach processing and digestion after a meal is eaten. Furthermore, phytate degradation may be improved during food processing by adding exogenous phytases or modifying optimal conditions for the native plant or microbial phytases. In addition to enzymatic degradation, nonenzymatic phytate hydrolysis during food processing or physical separation of phytate-rich sections of plant seed may lead to reduced phytate levels in the final foods. In general, a loss of valuable nutrients that are either extracted along with the phytate-rich sections of the plant or destroyed by the strong acids or high temperatures needed for nonenzymatic phytate dephosphorylation must compensate for the lower phytate rates. However, enzymatic phytate degradation also occurs under moderate conditions and does not affect other components of food (Egli et al. 2002).

12.4 Enzymatic Phytate Dephosphorylation to Increase Mineral Bioavailability During Food Processing

Different methods of food processing and preparation contribute to a reduction of the phytate content of the raw products. As regards enzymatic phytate dephosphorylation during food processing and preparation, it is important to distinguish the modification of optimal conditions during food processing for the native plant or microbial phytases from the introduction of exogenous ones. For example, phytate hydrolysis during germination, soaking, cooking, and fermentation is a consequence of the naturally occurring phytate degrading behavior in plants and microorganisms. Due to variations in their intrinsic phytate-degrading activities (Viveros et al. 2000; Eeckhout and de Paepe 1994; Konietzny and Greiner 2002) and the properties of enzymes such as protein stability and pH, as well as optimum temperature for phytate degradation (Hurrell 2003), the ability to dephosphorylate phytate significantly differs between different plant and microbial organisms. To understand phytate

hydrolysis, it is important to recognize and account not only for phytase activity but also for other phosphatase activities present in the plant material.

All enzymes capable of dephosphorylated phytate are known as phytases per description. However, the products of phytase activity on phytate, myo-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates can be further dephosphorylated during food processing by phytases as well as phosphatases that do not accept phytate as a substratum. Phytate is generally not completely hydrolyzed during food processing or preparation by the phytases which occur naturally in plants and microorganisms. However, it has been found that phytate must be reduced to very low levels to greatly increase the bioavailability of minerals, particularly iron (Greiner and Konietzny 1999). Knowing the properties of the natural phytases is important to improve food processing and preparation for phytate degradation. Several cereal phytases (Greiner 2002; Greiner and Konietzny 1998; Hayakawa et al. 1989; Konietzny et al. 1995; Laboure et al. 1993; Nakano et al. 1999; Greiner et al. 2001; Greiner 2002;), legumes (Houde et al. 1990; Mandel et al. 1972; Gibson and Ullah 1988; Nayini and Markakis 1984; de Angelis et al. 2003), and microorganisms used for food fermentation (Greiner and Konietzny 1998, 1999) have been isolated in recent years, and their enzymatic properties have been established. However, the properties of a purified enzyme in a food matrix are not necessarily identical to the properties of the same enzyme. For example, the optimum temperature for phytate dephosphorylation was determined by a phytase of black beans (*Phaseolus vulgaris* var. Preto) as 50 °C for the isolated enzyme and 65 °C for the enzyme in the bean matrix (Fredlund et al. 1997) (Table 12.1).

12.4.1 Soaking

Soaking is also used as a pretreatment to promote the production of grains from the legumes and cereals. Soaking can last for a short time, about 15–20 min, or a very long time, about 12–16 h. Cereals and legumes are usually immersed in water overnight at room temperatures in household circumstances. Since phytate is water-soluble, by discarding the soak water, a significant reduction in phytate can be achieved. Additionally, endogenous phytase activity contributes to the reduction of phytate. Temperature and pH values during soaking (Fredlund et al. 1997; Greiner et al. 1997; Vidal-Valverde et al. 1998) have been shown to have a significant effect on enzymatic hydrolysis of the phytate. If the soaking stage is performed at temperatures between 45 and 65 °C and pH values between pH = 5.0 and 6.0, which are similar to the optimum conditions for phytate dephosphorylation by intrinsic plant phytases, a large percentage of phytate (26–100%) has been enzymatically hydrolyzed (Greiner et al. 1997; Vidal-Valverde et al. 1998; Egli et al. 2002).

12.4.2 Cooking

Since phytate is heat stable, phytate is not expected to cause significant heat loss during cooking. Thus, substantial phytate dephosphorylation during cooking occurs only by either discarding the cooking water or by enzymatic phytate hydrolysis due to the action of intrinsic plant phytases during the early part of the cooking process (Fredlund et al. 1997). Prolonged periods at high temperatures lead to a gradual inactivation of the endogenous enzymes. The supply of heat-stable phytases to plants or the introduction of exogenous heat-stable phytases is therefore possible.

12.4.3 Germination

Germination is a process widely used in legumes and cereals, especially through the breakdown of certain anti-nutrients, such as phytate and protease inhibitors, to increase their palatability and nutrient value. A little intrinsic phytate-degrading activity is found in nongerminated legume grains and cereal seeds, with the exception of rye and to some degree wheat, triticale, and barley (85–88), but a marked increase in phytate-degrading activity was observed during germination, with a concomitant decrease in phytate content (Mandel et al. 1972; Türk et al. 1996; Greiner et al. 2003). During germination, phytate is gradually hydrolyzed by phytases or concerted action of phytases and phosphatases that do not accept phytate as a substratum to supply the plant's nutritional needs without the accumulation of less phosphorylated intermediate myo-inositol. After 6–10 days of germination, phytate levels that resulted in a strong increase in mineral uptake could be achieved. Since there is a need for long periods of time to increase mineral bioavailability by germination, this approach is intended to be useful for household applications, but it does not appear to be an economical industrial food processing method.

12.4.4 Fermentation

Food fermentation covers a wide range of microbial and enzymatic processing of food and ingredients to achieve desirable features such as extended shelf-life, enhanced safety, attractive taste, nutritional enrichment, removal of anti-nutrients, and health promotion. Many cereals, legumes, and vegetables are used extensively to make a variety of fermented foods. Microorganisms used for the fermentation of food may be part of the normal microflora present in the fermented raw material or specially cultivated crops engineered to bring about changes in the fermented content. Established starter cultures and controlled conditions are widely used today in the fermentation of food. During the fermentation process, the form of microorganism, the fermentation conditions used, and the starting amount of phytate present in

the raw material greatly affect the extent of phytate removal. Major microorganisms for fermentation include lactic acid bacteria, molds, and yeast. For starters, in many countries, yeast and/or lactic acid bacteria are used to produce bread, a staple food. Phytate reduction takes place in the different stages of bread making and naturally depends on the type of bread that is being made. Phytase present in the cereal flour is primarily responsible for phytate dephosphorylation during bread fermentation, whereas the contribution of microbial phytate degrading activity from baker's yeast and lactic acid bacteria is very small or even nonexistent (Leenhardt et al. 2005; Lopez et al. 2000; Sutardi and Buckle 1988).

There is still some debate about the ability of the lactic acid bacteria to produce a phytate-degrading enzyme. Some studies appear to demonstrate the ability of lactic acid bacteria to hydrolyze phytate (Greiner and Konietzny 1999; Fujita et al. 2003), while others have failed to identify a phytate-degrading enzyme (Lopez et al. 2000). Lowering the pH value of the dough to a more suitable one for the operation of the endogenous cereal phytases is therefore very likely the contribution of the microorganisms during fermentation to phytate hydrolysis. However, there is convincing evidence in Oriental food fermentation that phytases of the micro-organisms used for fermentation contribute significantly to phytate degradation (Fujita et al. 2003; Konietzny et al. 1995). Food products such as tempeh, miso, koji, and soy sauce are produced by fermenting soybeans with, respectively, *Rhizopus oligosporus* and *Aspergillus oryzae*. Both molds have been shown to develop phytate-degrading activity intra- as well as extracellular (Kerovuo et al. 1998).

12.5 Isolated Phytases and Food Processing in Recent Times

It has been shown that adding a phytase preparation during food processing is an alternative to maximizing phytate dephosphorylation by enzymes already present in the raw material used in food processing. The effectiveness of supplementary phytase in reducing phytate content during food processing was demonstrated for cereals as well as for food products derived from legumes (Greiner et al. 1997; Shimizu 1992), and even full phytate degradation was demonstrated to be feasible. During food processing, the level of phytate hydrolysis is influenced by the raw material used, the manufacturing process, the source of phytase, and the amount of added enzyme activity. There is no suitable phytase for all diet applications. The added phytase must be highly active when processing or preparing food. Since temperature and pH value are the major factors deciding enzyme activity, high phytate degrading capability even at room temperature, appropriate heat resistance, and high activity over a broad pH range are beneficial properties for phytase that should be used in food processing (Kim et al. 1998; Jog et al. 2005; Yang et al. 1991; Wyss et al. 1999a; Greiner et al. 1993; Vohra and Satyanarayana 2002). The activity of the enzymes increases with temperature to the limit. A further rise in temperature results in enzyme denaturation which is heat-induced. The optimum temperature for phytate hydrolysis ranges from 35 to 80 °C, depending on the source of the enzyme.

Plant phytases generally exhibit maximum activity at lower temperatures as opposed to their microbial equivalent. The higher pH and thermal stability as well as the higher specific microbial activity relative to plant phytases make the former more suitable for food processing applications. Specific activity is a crucial factor in the commercial use of an enzyme, as it affects the expected use on the economy. Thus microbial phytases appear to exhibit higher specific activities compared to plant phytases. The stability of most plant phytases decreased significantly at pH values below pH = 4 and above pH = 7.5, while most of the corresponding microbial enzymes even at pH values above pH = 8.0 and below pH = 3.0 are very stable. For example, when exposed to 4 °C for 2 h (Segueilha et al. 1992), a phytase from *Escherichia coli* did not lose any activity at pH = 2.0 and pH = 10.0. Most plant phytases are irreversibly inactivated within minutes at temperatures above 70 °C while most of the corresponding microbial enzymes maintain significant activity even after extended incubation periods. *Pichia anomala* (Doekes et al. 1999), *Schwanniomyces castellii* (O'Conner et al. 2001), and *Lactobacillus sanfranciscensis* (Greiner and Konietzny 1999) have isolated the phytases most resistant to high temperatures reported so far. Incubation of these enzymes at 70 °C for 10 min did not result in significant loss of activity, and it was even confirmed that the phytase of *Pichia anomala* tolerated a 30-h treatment at 70 °C without any loss of activity (Doekes et al. 1999).

It is of practical interest for the technical application of phytases in food processing that a crude enzyme preparation as well as an enzyme present in a food matrix are more pH- and heat-resistant than the corresponding highly purified enzyme. Although microbial phytases are best suited for a food processing application, cereal and legume phytases are thought to be an alternative due to their higher consumer acceptance and their presumed low allergenic potential. Phytases that are found in cereals and legumes are already part of the human diet and none of them have been reported to be an allergen. Conversely, *Aspergillus niger* phytase was thought to be a high-risk factor for occupational asthma and rhinitis. The enzyme has been demonstrated to trigger unique immune responses to IgE among workers exposed to powered phytase preparation (Baur et al. 2002; Rodriguez et al. 2000; Garrett et al. 2004). The phytase preparations of *Aspergillus niger* are commercially available, making their use technically feasible in the food processing industry (Abdel-Azeem et al. 2021; Yadav et al. 2019a, b). Since phytases with the properties needed for food processing applications have so far not been found in nature, phytase engineering is seen as a promising strategy to optimize their catalytic features.

Improving thermal tolerance and rising specific activity are two important issues not only for animal feed but also for phytase applications for food processing. To obtain an enzyme capable of withstanding higher temperatures, various techniques were employed. After the introduction of three glycosylation sites into the amino acid sequence of the *Escherichia coli* phytase by site-directed mutagenesis (Lehmann et al. 2002), a shift in the optimum temperature of the *Escherichia coli* phytase from 55 to 65 °C and a significant increase in its thermal stability at 80 and 90 °C was achieved by expression of the enzyme in the yeast *Pichia pastoris*. A further technique used to improve the efficiency of the *Escherichia coli* phytase

(Tomschy et al. 2000) was the saturation mutagenesis technology of the gene site. A library of clones incorporating all 19 possible changes in amino acids in the 431 residues of the *Escherichia coli* phytase sequence was generated and screened for mutants showing increased thermal tolerance.

When exposed to 62 °C for 1 h and 27% of its initial activity after 10 min at 85 °C, the best mutant displayed no loss of activity, which is a major improvement over parental phytasis. Additionally, there has been a 3.5-fold enhancement in gastric stability. By using a consensus approach based on the comparison of amino acid sequences of homologous proteins and the subsequent calculation of a consensus amino acid sequence using one of the standard programs available, a fully synthetic phytase was generated, showing an increase in intrinsic thermal stability of 21–42 °C compared to the 19 parental fungal phytases used in its design (Tomschy et al. 2000) In addition, a threefold increase in specific activity was achieved by replacing one single amino acid in a fungal phytase sequence with site-directed mutagenesis (Miksch et al. 2002; Mayer et al. 1999). Finally, a phytase will not be efficient if an inexpensive device cannot produce it in high yield and purity. Due to the small amount of phytase obtained from wild-type organisms and their tedious and cost-intensive purification, wild-type organisms are not an appropriate source of enzymes for industrial applications. This has led to the development of highly efficient and cost-effective processes to produce phytase by recombinant microorganisms. The use of economically efficient expression/secretion systems for *Escherichia coli* (Yao et al. 1998) as well as for the yeasts has identified high rates of phytate-degrading activity accumulating in the fermentation media Hansenula (Kerovuo and Tynkkynen 2000) and *Pichia pastoris* (Haraldsson et al. 2005). There is no need for protein purification if phytate-degrading capability is introduced or increased in microorganisms used for food fermentation, such as *Saccharomyces cerevisiae*, *Lactobacillus sanfranciscensis*, or *Lactobacillus plantarum*. Improved use of microorganisms in the fermentation of raw material extracted from plants is expected to result in food products with substantially lower levels of phytate. A genetically modified phytase-secreting strain of *Lactobacillus plantarum* has recently been reported (Haros et al. 2001), but the levels of secretion were far too low for an industrial application. In addition, a *Saccharomyces cerevisiae* strain was constructed which produces high levels of extracellular phytase activity (Sandberg et al. 1996), but its ability to contribute significantly to phytate hydrolysis during fermentation needs to be studied first.

12.6 Isolated Phytase Applications in Food Production

In addition to improving the bioavailability of the mineral and trace elements, phytase addition during food processing has been documented to affect the economy of the production process as well as the yield and quality of the end products. Technical advances have been recorded by the introduction of phytase during food processing for bread making (Pasamontes et al. 1997), plant protein isolate production

(Sandström and Sandberg 1992; Han et al. 1999), maize wet milling (Wang et al. 1999; Fredrikson et al. 2001), and cereal bran fractionation (Caransa et al. 1988).

12.6.1 Bread Making

Phytase proved to be an outstanding improver in bread making (Pasamontes et al. 1997). In addition to reducing phytate content in doughs and fresh breads, phytase addition reduced fermentation time without affecting the pH of the dough. An increase in bread volume and an improvement in crumb texture were also observed. For both formulations, the breadcrumbs' hardness or firmness was popular so that softer crumbs were obtained with the addition of phytase. Certain criteria of texture such as gumminess and chewiness were also reduced. It has been proposed that these changes in bread consistency are consistent with an indirect effect of phytase on a-amylase activities. In the final breads, the introduction of phytase during bread making results in lower levels of phytate. Even so, a complete phytate removal was not attainable. This, in effect, releases calcium ions from calcium–phytate complexes that are important for a-amylase action. No phytase activity could be found in final breads. Therefore, both intrinsic cereal and augmented microbial phytases were inactivated during baking.

12.6.2 Generation of Isolated Plant Proteins

The application of plant protein isolates and concentrates has been finding increasingly important in food production because of their strong nutritional and functional properties. Nonetheless, the relatively high phytate content present in plant seeds and grains and their association with proteins under alkaline conditions, which are typically applied for protein extraction, adversely affect the yield and quality of the protein isolates obtained using standard production processes. The solubility of the proteins decreased by interacting with the phytate resulting in decreased protein content in the final concentrate. Therefore, a considerable amount of phytate ends up in the protein isolate which affects both its nutritional and its functional properties.

However, it was confirmed that the introduction of exogenous phytase into the production process resulted in significantly higher protein yields and almost complete removal of myo-inositolhexakis-, pentakis-, tetrakis-, and trisphosphates from the final plant protein isolate (Sandström and Sandberg 1992; Han et al. 1999). Such phytate-reduced plant protein isolates have been proposed as ideal protein sources for infant formulae due to an increase in mineral bioavailability, their amino acid composition, and their in vitro protein digestibility. In addition, certain phytate-reduced plant protein isolates are addressed in food products as functional additives, due to their strong properties of foaming, emulsifying, and gelling.

12.6.3 Wet Corn Milling

Steeping is a process needed to obtain the valuable corn steep liquor and soften the maize kernel as well as break the maize cell wall in wet maize milling. Starch yield, corn steep liquor consistency, and steeping time are the main issues of corn wet milling. Maize consists of phytate, which in large measure ends up in the steep liquor of corn and constitutes an undesirable portion. Phytate-free corn steep liquor is easier to absorb and is used in the fermentation industry to manufacture compounds such as enzymes, yeast, polysaccharides, antibiotics, and amino acids as well as a high-energy liquid feed product for animals. Through adding phytases to the steep liquor along with plant cell wall degrading enzymes, corn steep liquor was obtained which was completely free of phytate (Wang et al. 1999; Fredrikson et al. 2001). However, the steeping time was substantially decreased which was accomplished by promoting the separation of starch from fiber which gluten, higher starch and gluten yields, and lower energy consumption.

12.6.4 Cereal Branch Split

It is commonly recognized and accepted that the cereal bran, the by-product of flour production, is the most nutritious component of a grain of cereals. Recently, an industrial method was created to isolate economically the main branch fractions to produce high-value protein, soluble non-starch carbohydrates, oil fractions, and insoluble fibers (Antrim et al. 1997). The bran is first subjected to a combination of enzymatic treatment using starch- and phytate-hydrolyzing enzyme group proteins and wet milling, accompanied by concurrent centrifugation and ultrafiltration. The second step is to fractionate the insoluble phase of the above-mentioned first step by enzymatic treatment with xylanase and/or b-glucanase and wet milling, followed again by sequential centrifugation and ultrafiltration. All fractions obtained have much broader business applications and higher values than the branch initial.

12.7 Phytase Degradation in Human Body

Phytate hydrolysis in the human gastrointestinal tract can be achieved by the action of phytate-degrading enzymes from three sources: dietary phytases, small intestine mucosal phytases, and bacterial flora phytases in the colon. Except for calcium, phytate degradation in the colon is not expected to greatly affect mineral absorption, as minerals are mainly absorbed in the upper small intestine. In addition, it has been shown that only very low phytate-degrading activity exists in the small intestine of humans.

The human small intestine, therefore, has a very limited ability to hydrolyze phytate. In comparison, dietary phytases are an essential factor for phytate degradation during digestion because these enzymes are involved in the stomach of humans (Berka et al. 1998).

Generally speaking, the intrinsic phytate-degrading activity in plant-derived foods is not strong enough to hydrolyze the dietary phytate during the passage through the human stomach to such a degree that there is a substantial increase in iron absorption. The production of plants with higher phytase-degrading activities in the edible parts or the application of phytase preparations to the raw materials or the final foods may lead to more extensive phytate degradation in the human stomach. Such phytases should be effective in releasing phytate phosphate into the human stomach, stable to resist inactivation by storage, and may also be suitable to withstand food processing and preparation. Thermal stability is a concern of special significance as food processing and preparation typically require exposure to high temperatures. Until now, phytases with the necessary degree of thermal stability to withstand thermal treatments such as isolated cooking or within a certain food matrix have not been found in nature. It is therefore no surprise that isolation and characterization of thermostable enzymes, as well as engineering phytases, are hot spots of current phytase work (Phillippy 1999; Kim et al. 2003; Lehmann et al. 2002; Tomschy et al. 2000) to boost stability at elevated temperatures and the quest for determinants of thermal stability. Likewise, it is undisputedly desirable to have a phytase that can withstand long-term storage or transport at ambient temperature.

The enzymatic properties of a phytase are determined by its ability to hydrolyze phytate in the digestive tract. Since the stomach is the key functional site of dietary and/or supplementary phytase, it is beneficial to provide an enzyme with optimum acid pH, good stability under acidic pH conditions, and good pepsin resistance. Microbial phytases are thought to have advantages over their plant counterparts regarding their phytate-degrading ability in the human stomach. Microbial phytases demonstrate substantial enzymatic activity over a wide range of pH and are also active below pH = 3.5. Additionally, the stability of certain microbial phytases below pH = 3.0 is noteworthy. Additionally, plant phytases are more susceptible to gastrointestinal enzyme inactivation. It was confirmed that wheat phytase was less resistant to pepsin and pancreatin than *Aspergillus niger* phytase (Simon and Igbasan 2002) and that the phytases of *Escherichia coli* and *Citrobacter braakii* were even more resistant to pepsin and pancreatin than the *Aspergillus niger* phytase (Rodriguez et al. 1999).

Furthermore, *Citrobacter braakii* phytase was stable to trypsin (Rodriguez et al. 1999). The corresponding enzyme from *Bacillus subtilis* demonstrated a comparable vulnerability to pancreatin compared with the *Escherichia coli* phytase but a much higher resistance to pepsin (Haros et al. 2005). As recently recorded for the *Escherichia coli* and *Aspergillus niger* phytase produced in *Pichia pastoris* (Blanquet et al. 2004), it must also be noted that recombinant enzymes can differ in proteolytic resistance compared to their wild-type counterparts.

Quite recently (Sandberg et al. 1996), a radically different approach to enhancing phytate degradation in humans' stomach and upper small intestines was brought up. Healthy for human consumption microorganisms such as baker's yeast (Saccharomyces cerevisiae), lactobacilli, or bifidobacteria have been proposed as carriers of phytate reducing activity throughout the gastrointestinal tract. Tolerance to the conditions in the stomach and small intestine and the ability to produce extracellular phytate-degrading activity under gastrointestinal conditions are therefore characteristics that microorganisms require for such an application. *Saccharomyces cerevisiae* (Shears 1998), as well as some strains of Lactobacillus and Bifidobacterium (Vucenik and Shamsuddin 2003), has also demonstrated the ability to survive the passage across the human gastrointestinal tract.

Neither for *Saccharomyces cerevisiae* nor for any *Lactobacillus* or *Bifidobacterium* strains, however, was a sufficiently high extracellular phytate degrading activity demonstrated. Genetic engineering may be used to solve this restriction for the development of high-recombinant, phytase-producing strains. To be able to hydrolyze the dietary phytate, the microorganisms must secrete this phytase or attach it to its outer cell wall. Two separate strategies for improving the production and secretion of phytase in the target microorganisms were successfully applied. A *Bacillus subtilis* phytase-encoding gene was inserted into a *Lactobacillus plantarum* strain, but the secreted phytate degrading activity was far too small for any application (Haros et al. 2001).

Nevertheless, in yeast, the regulation of phytase synthesis has been changed by removing a gene encoding a negative regulator for the phytase-encoding genes to express themselves. Compared with the corresponding wild-type yeast, the recombinant yeast exhibited multiple-fold higher phytate hydrolysis capacity, both in the presence and in the absence of orthophosphate (Sandberg et al. 1996). However, recombinant yeast was shown to degrade up to 40% of the phytate present in wheat gruel under simulated gastric conditions, while with wild-type yeast no phytate hydrolysis was observed. Studies on in vivo are still lacking, however. Since yeast phytase at pH values above pH = 7 is practically inactive, this phytase will only be active in the human stomach, and no further phytate degradation is expected to occur in the small intestine. Thus, using one or a mixture of food-grade microorganisms with both characteristics, secretion of an optimally active phytase at acidic and another optimally active at alkaline conditions may increase phytate breakdown in the human stomach and upper small intestine during digestion with a concomitant improvement in the bioavailability of mineral products.

12.8 Production of Metabolically Active Phytate Breakdown Products

In recent years, a great deal of scientific evidence has been published connecting diet, foods, or individual food components with preserving human health and preventing chronic diseases such as coronary heart disease, cancer, or osteoporosis. Specific phosphate esters with myo-inositol have been shown to have essential physiological functions in humans (Brinch-Pedersen et al. 2007).

Several of these compounds, in particular D-myo-inositol(1,4,5)trisphosphate and D-myo-inositol(1,3,4,5)tetrakisphosphate, have been shown to play an important role as secondary intracellular messengers (Greiner and Konietzny 1996), and some isomers of myo-inositol phosphates have demonstrated major pharmacological effects, such as complications of diabetes and anti-inflammatory effects as well as an anti-inflammatory effect (Claxson et al. 1990; Konietzny and Greiner 2003). In addition, it has been proposed that dietary myo-inositol phosphates offer benefits for human health, such as improving heart disease conditions by regulating hypercholesterolemia and atherosclerosis (Vucenik and Shamsuddin 2003), preventing the development of renal stone (Jariwalla et al. 1990), and protecting against a variety of cancers, especially colon cancer (Grases et al. 2001).

Phytate can be partially dephosphorylated during food processing and digestion to produce many positional isomers of pentakis, tetrakis, tris, bis, and monophosphates in myo-inositol (Singh et al. 2020). The number and distribution of the residues of phosphates on the myo-inositol ring determine the metabolic effects caused by the individual phosphate isomer of myo-inositol. Different phytases exhibit varied pathways of phytate degradation, resulting in the generation and accumulation of various intermediate myo-inositol phosphate. Nonenzymatically, attempts to produce specified isomers of the various partially phosphorylated myo-inositol phosphates have resulted in mixtures of pentakis, tetrakis, tris, bis, and monophosphate isomers. The purification from the mixture of these isomers is arduous and uneconomical. An alternative approach to making phytate pure breakdown products available in appropriate amounts for physiological studies is the use of a bioreactor based on immobilized enzymes followed by the hydrolysis mixture anion-exchange chromatography. The quantity of the desired product for phytate degradation may be regulated by the number of phytate-containing solution passing through the bioreactor (Sandberg et al. 1987).

When individual phytate degradation products are known to be metabolically active, phytases can be used in food processing to produce foods with enhanced nutritional value, health benefits, and sensory (functional foods) properties. Through attaching phytase to the raw material, phytate is converted during food processing into metabolically active myo-inositol phosphates. To end up with foods with a reduced phytate content and a regulated content and composition of partially phosphorylated myo-inositol phosphate esters with health benefits, phytate dephosphorylation must be closely managed during food processing. An alternative may be

using pure phytate as the source material to produce metabolically active myo-inositol phosphates as food supplements.

Since partially phosphorylated myo-inositol phosphate esters are subject to degradation in the human gastrointestinal tract even if all dietary phosphatases like phytases are inactivated, the desired physiological effects may need to be stimulated by enriching foods with a precursor of the true active myo-inositol phosphate ester. It has already been demonstrated in ileostomy patients (Sandberg and Andersson 1988; Sandberg et al. 1987) that the human intestinal alkaline phosphatase exhibits activity against lower myo-inositol phosphate esters and that the microflora in the human colon is also considered capable of degrading phytate and phytate breakdown items. Myo-inositol phosphates must be consumed in the gastrointestinal tract to achieve their metabolic effects in tissues far away from the food tract. There is some evidence that the human digestive tract consumes myo-inositol phosphates since the levels of phytate and its dephosphorylation products in biological fluids fluctuate with ingestion or phytate deficiency in the human diet (Shamsuddin et al. 1992).

12.9 Conclusion

Phytases, the most significant enzyme in nutrition, human health, and environmental protection, are helpful in the elimination of phytate in the human stomach and upper small intestine during food processing. Surprisingly, in mankind phytase enzyme is reduced. In conclusion, research is required to classify metabolically active isomers and phytases of myo-inositol phosphate or a mixture of phytases and/or phosphatases without phytate-degrading activity able to produce such isomers. There isn't one phytase suitable for all food applications. Thus, scanning nature for phytases with more desirable properties for food applications and engineering phytases to improve their catalytic and stability characteristics are appropriate approaches to make a suitable phytase available for a particular food processing application. The phytases can be used in isolated form or generated at high levels in recombinant microorganisms used for the fermentation of food and/or in recombinant plant edible pieces. If the intrinsic phytases present in the material to be processed are to be used during food processing for phytate dephosphorylation, their catalytic properties must be elucidated to maximize phytate degradation with respect to the intended use of the product.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development. In: Biodiversity and ecological perspective, vol 1. Springer, Cham

Andlid TA, Veide J, Sandberg A-S (2004) Metabolism of extracellular inositol hexaphosphate (phytate) by *Saccharomyces cerevisiae*. Int J Food Microbiol 97:157–169. https://doi.org/10.1016/j.ijfoodmicro.2004.04.016

Antrim RL, Mitchinson C, Solheim LP (1997) Method for liquefying starch. US patent 5652127

Baur S, Melching-Kollmuss F, Koops K, Straßburger A (2002) Zober, IgE-mediated allergy to phytase—a new animal feed additive. Allergy 57:943–945

Berka RM, Rey MW, Brown KM, Byun T, Klotz AV (1998) Molecular characterization and expression of a phytase gene from the thermophilic fungus *Thermomyces lanuginosus*. Appl Environ Microbiol 64:4423–4427

Blanquet R, Antonelli L, Laforet S, Denis S, Marol-Bonin M (2004) Alric, living recombinant *Saccharomyces cerevisiae* secreting proteins or peptides as a new drug delivery system in the gut. J Biotechnol 110:37–49

Brinch-Pedersen H, Borg S, Tauris B, Holm PB (2007) Molecular genetic approaches to increasing mineral availability and vitamin content of cereals. J Cereal Sci 46:308–326. https://doi.org/10.1016/j.jcs.2007.02.004

Cao L, Wang W, Yang C, Yang Y, Diana J, Yakupitiyage A, Luo Z, li D (2007) Application of microbial phytase in fish feed. Enzyme Microbial Technol 40: 497–507. doi: https://doi.org/10.1016/j.enzmictec.2007.01.007

Caransa A, Simell M, Lehmussaari A, Vaara M, Vaara T (1988) A novel enzyme application for corn wet milling. Starch 40:409–411

Carrington AL, Calcutt NA, Ettlinger CB, Gustafsson T, Tomlinson DR (1993) Effects of treatment with myo-inositol or its 1,2,6-trisphosphate (PP56) on nerve conduction in streptozotocin-diabetes. Eur J Pharmacol 237:257–263

Chadha BS, Harmeet G, Mandeep M, Saini HS, Singh N (2004) Phytase production by the thermophilic fungus *Rhizomucos pusillus*. World J Microbiol Biotechnol 20:105–109

Cheryan (1980) Phytic acid interactions in food systems. Crit Rev Food Sci Nutr 13:297–355

Chu HM, Guo RT, Lin TW, Chou CC, Shr HL, Lai HL, Tang TY, Cheng KJ, Selinger BL, Wang AHJ (2004) Structures of Selenomonas ruminantium phytase in complex with persulfated phytate: DSP phytase fold and mechanism for sequential substrate hydrolysis. Structure 12:2015–2024

Claxson A, Morris C, Blake D, Siren M, Halliwell B, Gustafsson T et al (1990) The anti-inflammatory effects of D-myo-inositol-1.2. 6-trisphosphate (PP56) on animal models of inflammation. Agents Actions 29:68–70

Coello JP, Maughan A, Mendoza R, Philip DW, Bollinger TL, Veum LO, Vodkin JC (2001) Polacco, Generation of low phytic acid Arabidopsis seeds expressing an *E. coli* phytase during embryo development. Seed Sci Res 11:285–291

D'Souza SW, Lakhani P, Waters HM, Boardman KM, Cinkotai KI (1987) Iron deficiency in ethnic minorities: associations with dietary fibre and phytate. Early Hum Dev 15:103–111

de Angelis M, Gallo G, Corbo MR, McSweeney PLH, Faccia M, Giovine M, Gobbetti M (2003) Phytase activity in sourdough lactic acid bacteria: purification and characterization of a phytase from Lactobacillus sanfranciscensis CB1. Int J Food Microbiol 87:259–270

Desphande SS, Cheryan M (1984) Effects of phytic acid, divalent cations, and their interactions on alpha-amylase activity. J Food Sci 49:516–519

Desphande SS, Damodaran S (1989) Effect of phytate on solubility, activity and conformation of trypsin and chymotrypsin. J Food Sci 54:695–699

Doekes G, Kamminga N, Helwegen L, Heederik D (1999) Occupational IgE sensitisation to phytase, a phosphatase derived from *Aspergillus niger*. Occup Environ Med 56:454–459

Eeckhout W, de Paepe M (1994) Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim Feed Sci Technol 47:19–29

Egli L, Davidsson L, Juillerat MA, Barclay D, Hurrell RF (2002) The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. J Food Sci 67:3484–3488

Ferry S, Matsuda M, Yoshida H, Hirata M (2002) Inositol hexakisphosphate blocks tumor cell growth by activating apoptotic machinery as well as by inhibiting the Akt/NFcappaB-mediated cell survival pathway. Carcinogenesis 23:2031–2041

Fredrikson M, Biot P, Alminger ML, Carlsson N, Sandberg A-S (2001) Production process for high-quality pea-protein isolate with low content of oligosaccharides and phytate. J Agric Food Chem 49(3):1208–1212. https://doi.org/10.1021/jf000708x

Fredlund K, Asp NG, Larsson M, Marklinder I, Sandberg AS (1997) Phytate reduction in whole grains of wheat, rye, barley and oats after hydrothermal treatment. J Cereal Sci 25:83–91

Fujita S, Shigeta Y, Yamane H, Fukuda Y, Kizaki S, Wakabayashi K (2003) Ono, Production of two types of phytase from *Aspergillus oryzae* during industrial koji making. J Biosci Bioeng 95:460–465

Garrett JB, Kretz KA, O'Donoghue E, Kerovuo J, Kim W, Barton NR, Hazlewood GP, Short JM, Robertson DE, Gray KA (2004) Enhancing the thermal tolerance and gastric performance of a microbial phytase for use as a phosphate-mobilizing monogastric-feed supplement. Appl Environ Microbiol 70:3041–3046

Gibson DM, Ullah AHJ (1988) Purification and characterization of phytase from cotyledons of germinating soybean seeds. Arch Biochem Biophys 260:503–513

Grases F, March JG, Prieto RM, Simonet BM, Costa-Bauza A, García-Raja A, Conte A (2000) Urinary phytate in calcium oxalate stones formers and healthy people. Scand J Urol Nephrol 34:162–164

Grases F, Simonet BM, Prieto RM, March JG (2001) Variation of InsP4, InsP5 and InsP6 levels in tissues and biological fluids depending on dietary phytate. J Nutr Biochem 12:595–601

Greiner R (2002) Purification and characterization of three phytate-degrading enzymes from germinated lupin seeds (Lupinus albus var. Amiga). J Agric Food Chem 50:6858–6864

Greiner R, Konietzny U (1996) Construction of a bioreactor to produce special breakdown products of phytate. J Biotechnol 48:153–159

Greiner R, Konietzny U (1998) Endogenous phytate-degrading enzymes are responsible for phytate reduction while preparing beans (*Phaseolus vulgaris*). J Food Process Preserv 29:321–331

Greiner R, Konietzny U (1999) Improving enzymatic reduction of myo-inositol phosphates with inhibitory effects on mineral absorption in black beans (*vulgaris* var. Preto). J Food Process Preserv 23:249–261

Greiner R, Konietzny U (2006) Phytase for food application. Food Technol Biotechnol 44(2):125–140. (paper from which reference was taken)

Greiner R, Konietzny U, Jany KD (1993) Purification and characterization of two phytases from *Escherichia coli*. Arch Biochem Biophys 303:107–113

Greiner R, Konietzny U, Jany KD (1997) Properties of phytate-degrading enzymes and their biotechnological application in phytate reduction. In: Sandberg AS, Andersson H, Amadó R, Schlemmer U, Serra F (eds) Cost 916: bioactive inositol phosphates and phytosterols in foods. Office for the Official Publications of the European Communities, Luxembourg, pp 61–67

Greiner R, Muzquiz M, Burbano C, Cuadrado C, Pedrosa MM, Goyoaga C (2001) Purification and characterization of a phytate-degrading enzyme from germinated faba beans (Vicia faba var. Alameda). J Agric Food Chem 49:2234–2240

Greiner R, Larsson Alminger M, Carlsson NG, Muzquiz M, Burbano C, Cuadrado C, Pedrosa MM, Goyoaga C (2002) Enzymatic phytate degradation—a possibility to design functional foods? Pol J Food Nutr Sci 11:50–54

Greiner R, Konietzny U, Villavicencio ALCH (2003) Reduction in phytate content during lactic acid fermentation. In: Proceedings of the EURO Food Chem XII Congress: strategies for safe foods, Brugge, Belgium, vol 2, pp 670–673

Ha NC, Oh BC, Shin S, Kim HJ, Oh TK, Kim YO, Choi KY, Oh BH (2000) Crystal structures of a novel, thermostable phytase in partially and fully calcium-loaded states. Nat Struct Biol 7:147–153

Han YM, Wilson DB, Lei XG (1999) Expression of an *Aspergillus niger* phytase gene (phyA) in *Saccharomyces cerevisiae*. Appl Environ Microbiol 65:1915–1918

Haraldsson AK, Veide J, Andlid T, Larsson Alminger M, Sandberg AS (2005) Degradation of phytate by high-phytase *Saccharomyces cerevisiae* strains during simulated gastrointestinal digestion. J Agric Food Chem 54:5438–5444

Haros M, Rosell CM, Benedito C (2001) Fungal phytase as a potential breadmaking additive. Eur Food Res Technol 213:317–322

Haros M, Bielecka M, Sanz Y (2005) Phytase activity as a novel metabolic feature in bifidobacterium. FEMS Microbiol Lett 247:231–239

Hayakawa T, Toma Y, Igaue I (1989) Purification and characterization of acid phosphatases with or without phytase activity from rice bran. Agric Biol Chem 53:1475–1483

Hellström AM, Vázques-juárez R, Svanberg U, Andlid TA (2010) Biodiversity and phytase capacity of yeasts isolated from Tanzanian togwa. Int J Food Microbiol 136:352–358. https://doi.org/10.1016/j.ijfoodmicro.2009.10.011

Houde RL, Alli I, Kermasha S (1990) Purification and characterization of canola seed (Brassica sp.) phytase. J Food Biochem 114:331–351

Hurrell RF (2003) Influence of vegetable protein sources on trace element and mineral bioavailability. J Nutr 133(Suppl):2973–2977

Inagawa J, Kiyosawa I, Nagasawa T (1987) Effect of phytic acid on the digestion of casein and soyabean protein with trypsin, pancreatin and pepsin. Nippon Eiyo Shokuryo Gakkaishi 40:367–373

Iqbal TH, Lewis KO, Cooper BT (1994) Phytase activity in the human and rat small intestine. Gut 35:1233–1236

Jariwalla RJ, Sabin R, Lawson S, Herman ZS (1990) Lowering of serum cholesterol and triglycerides and modulation of divalent cations by dietary phytate. J Appl Nutr 42:18–28

Jenab M, Thompson LU (2002) Role of phytic acid in cancer and other diseases. In: Reddy NR, Sathe SK (eds) Food phytates. CRC Press, Boca Raton, FL, pp 225–248

Jog SP, Garchow BG, Mehta BD, Murthy PPN (2005) Alkaline phytase from lily pollen: investigation of biochemical properties. Arch Biochem Biophys 440:133–140

Kerovuo J, Tynkkynen S (2000) Expression of *Bacillus subtilis* phytase in Lactobacillus plantarum 755. Lett Appl Microbiol 30:325–329

Kerovuo J, Lauraeus M, Nurminen P, Kalkinnen N, Apajalahti J (1998) Isolation, characterization, molecular gene cloning and sequencing of a novel phytase from Bacillus subtilis. Appl Environ Microbiol 64:2079–2085

Kim YO, Kim HK, Bae KS, Yu JH, Oh TK (1998) Purification and properties of a thermostable phytase from Bacillus sp. DS11. Enzym Microb Technol 22:2–7

Kim HW, Kim YO, Lee JH, Kim KK, Kim YJ (2003) Isolation and characterization of a phytase with improved properties from *Citrobacter braakii*. Biotechnol Lett 25:1231–1234

Knuckles BE (1988) Effect of phytate and other myo-inositol phosphate esters on lipase activity. J Food Sci 53:250–252

Knuckles BE, Betschart AA (1987) Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J Food Sci 52:719–721

Konietzny U, Greiner R (2002) Molecular and catalytic properties of phytate-degrading enzymes (phytases). Int J Food Sci Technol 37:791–812

Konietzny U, Greiner R (2003) Phytic acid: nutritional impact. In: Caballero B, Trugo L, Finglas P (eds) Encyclopedia of food science and nutrition. Elsevier, London, UK, pp 4555–4563

Konietzny U, Greiner R, Jany KD (1995) Purification and characterization of a phytase from spelt. J Food Biochem 18:165–183

Kour D, Kaur T, Yadav N, Rastegari AA, Singh B, Kumar V et al (2020) Phytases from microbes in phosphorus acquisition for plant growth promotion and soil health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, pp 157–176. https://doi.org/10.1016/B978-0-12-820526-6.00011-7

Kumar V, Yadav AN, Verma P, Sangwan P, Saxena A, Kumar K et al (2017) β-Propeller phytases: diversity, catalytic attributes, current developments and potential biotechnological applications. Int J Biol Macromolec 98:595–609. https://doi.org/10.1016/j.ijbiomac.2017.01.134

Kvist S, Carlsson T, Lawther JM, DeCastro FB (2005) Process for the fractionation of cereal brans. US patent application US 20050089602

Laboure AM, Gagnon J, Lescure AM (1993) Purification and characterization of a phytase (myo–inositolhexakisphosphate phosphohydrolase) accumulated in maize (Zea mays) seedlings during germination. Biochem J 295:413–419

Lassen SF, Breinholt J, Østergaard PR, Brugger R, Bischoff A, Wyss M, Fuglsang CC (2001) Expression, gene cloning, and characterization of five novel phytases from four basidiomycete fungi: *Peniophora lycii*, *Agrocybe pediades*, a Ceriporia sp. and *Trametes pubescens*. Appl Environ Microbiol 67:4701–4707

Leenhardt F, Levrat-Verny MA, Chanliaud E, Rémésy C (2005) Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. J Agric Food Chem 53:98–102

Lehmann M, Loch C, Middendorf A, Studer D, Lassen SF, Pasamontes L, van Loon APGM, Wyss M (2002) The consensus concept for thermostability engineering of proteins: further proof of concept. Protein Eng 15:403–411

Loewus F (2002) Biosynthesis of phytate in food grains and seeds. In: Reddy NR, Sathe SK (eds) Food phytates. CRC Press, Boca Raton, FL, pp 53–61

Lönnerdal B (2000) Dietary factors influencing zinc absorption. J Nutr 130(Suppl):1378–1383

Lopez HW, Ouvry A, Bervas E, Guy C, Messager A, Demigne C, Rémésy C (2000) Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. J Agric Food Chem 48:2281–2285

Lopez HW, Leenhardt F, Coudray C, Rémésy C (2002) Minerals and phytic acid interactions: is it a real problem for human nutrition? Int J Food Sci Technol 37:727–739

Lucca R, Hurrell I (2001) Potrykus, approaches to improve the bioavailability and level of iron in rice seeds. J Sci Food Agric 81:828–834

Maberly GF, Trowbridge FL, Yip R, Sullivan KM, West CE (1994) Programs against micronutrient malnutrition: Ending hidden hunger. Ann Rev Public Health 15:277–301

Maffucci T, Piccolo E, Cumashi A, Iezzi M, Riley AM, Saiardi A, Godage HY, Rossi C, Broggini M, Iacobelli S, Potter BVL, Innocenti P, Falasca M (2005) Inhibition of the phosphatidylinositol-3-kinase/Akt pathway by inositol pentakisphosphate results in antiangiogenic and antitumor effects. Cancer Res 65:8339–8349

Mandel NC, Burman S, Biswas BB (1972) Isolation, purification and characterization of phytase from germinating mung beans. Phytochemistry 11:495–502

Mayer AF, Hellmuth K, Schlieker H, Lopez-Ulibarri R, Oertel S, Dahlems U, Strasser AWM, van Loon APGM (1999) An expression system matures: a highly efficient and cost-effective process for phytase production by recombinant strains of Hansenula polymorpha. Biotechnol Bioeng 63:373–381

Mendoza C (2002) Effect of genetically modified low phytic acid plants on mineral absorption. Int J Food Sci Technol 37:759–767

Miksch G, Kleist S, Friehs K, Flaschel E (2002) Overexpression of the phytase from *Escherichia coli* and its extracellular production in bioreactors. Appl Microbiol Biotechnol 59:685–694

Mitchell DB, Vogel K, Weimann BJ, Pasamontes L, van Loon APGM (1997) The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi Aspergillus terreus and Myceliophthora thermophila. Microbiol 143:245–252

Mullaney EJ, Ullah AHJ (2003) The term phytase comprises several different classes of enzymes. Biochem Biophys Res Commun 312:179–184

Nakamura Y, Fukuhara H, Sano K (2000) Secreted phytase activities of yeasts. Biosci Biotechnol Biochem 64:841–844

Nakano T, Joh T, Tokumoto E, Hayakawa T (1999) Purification and characterization of phytase from bran of *Triticum aestivum* L. cv. Nourin #61. Food Sci Technol Res 5:18–23

Nayini NR, Markakis P (1984) The phytase of yeast. Lebensm Wiss Technol 17:24–26
O'Conner TM, Bourke JF, Jones M, Brennan N (2001) Report of occupational asthma due to phytase and b-glucanase. Occup Environ Med 58:417–419
Ogunremi OR, Agrawal R, Sanni A (2020) Production and characterization of volatile compounds and phytase from potentially probiotic yeasts isolated from traditional fermented cereal foods in Nigeria. J Genet Eng Biotechnol 18:16. https://doi.org/10.1186/s43141-020-00031-z
Oh BC, Choi WC, Park S, Kim YO, Oh TK (2004) Biochemical properties and substrate specificities of alkaline and histidine acid phosphatase. Appl Microb Biotechnol 63:362–372
Pasamontes M, Haiker M, Wyss M, van Tessier APGM (1997) Loon, Gene cloning, purification, and characterization of a heat-stable phytase from the fungus *Aspergillus fumigatus*. Appl Environ Microbiol 63:1696–1700
Pasamontes L, Haiker M, Wyss M, Tessier M, van Loon APGM (1997a) Gene cloning and characterization of a heat-stable phytase from the fungus *Aspergillus fumigatus*. Appl Environ Microbiol 63:1696–1700
Pasamontes L, Haiker M, Henriquez-Huecas M, Mitchell DB, van Loon APGM (1997b) Cloning of the phytase from *Emericella nidulans* and the thermophilic fungus *Talaromyces thermophilus*. Biochim Biophys Acta 1353:217–223
Phillippy BQ (1999) Susceptibility of wheat and *Aspergillus niger* phytases to inactivation by gastrointestinal enzymes. J Agric Food Chem 47:1385–1388
Quan CS, Fan SD, Zhang LH, Wang YJ, Ohta Y (2002) Purification and properties of a phytase from Candida krusei WZ-001. J Biosci Bioeng 94:419–425
Quan CS, Tian WJ, Fan SD, Kikuchi Y (2004) Purification and properties of a low-molecularweight phytase from Cladosporium sp. FP-1. J Biosci Bioeng 94:260–266
Raboy (2002) Progress in breeding low phytate crops. J Nutr 132(Suppl):503–505
Raghavendra P, Halami PM (2009) Screening, selection and characterization of phytic acid degrading lactic acid bacteria from chicken intestine. Int J Food Microbiol 133:129–134. https://doi.org/10.1016/j.ijfoodmicro.2009.05.006
Rastegari AA, Yadav AN, Yadav N (2020) New and future developments in microbial biotechnology and bioengineering: trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam
Reddy NR (2002) Occurrence, distribution, content, and dietary intake of phytate. In: Reddy NR, Sathe SK (eds) Food phytates. CRC Press, Boca Raton, FL, pp 25–51
Rodriguez E, Porres JM, Han Y, Lei XG (1999) Different sensitivity of recombinant *Aspergillus niger* phytase (r-PhyA) and *Escherichia coli* pH 2.5 acid phosphatase (r-AppA) to trypsin and pepsin in vivo. Arch Biochem Biophys 365:262–267
Rodriguez E, Wood ZA, Karplus PA, Lei XG (2000) Site-directed mutagenesis improves catalytic efficiency and thermostability of *Escherichia coli* pH 2.5 acid phosphatase/ phytase expressed in Pichia pastoris. Arch Biochem Biophys 382:105–112
Rodriguez E, Mullaney EJ, Lei XG (2000a) Expression of the Aspergillus fumigatus phytase gene in Pichia pastoris and characterization of the recombinant enzyme. Biochem Biophys Res Commun 268:373–378
Sandberg AS (1991) The effect of food processing on phytate hydrolysis and availability of iron and zinc. Adv Exp Med Biol 289:499–508
Sandberg AS, Andersson H (1988) Effect of dietary phytase on the digestion of phytate in the stomach and small intestine of humans. J Nutr 118:469–473
Sandberg AS, Andersson H, Carlsson NG, Sandström B (1987) Degradation products of bran phytase formed during digestion in the human small intestine: effect of extrusion cooking on digestibility. J Nutr 117:2061–2065
Sandberg AS, Rossander Hulthén L, Türk M (1996) Dietary *Aspergillus niger* phytase increases iron absorption in humans. J Nutr 126:476–480
Sandberg AS, Brune M, Carlsson NG, Hallberg L, Skoglund E, Rossander-Hulthén L (1999) Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am J Clin Nutr 70:240–246

Sandström B, Sandberg AS (1992) Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. J Trace Elem Electrol Health Dis 6:99–103
Sano K, Fukuhara H, Nakamura Y (1999) Phytase of the yeast Arxula adenivorans. Biotechnol Lett 21:33–38
Sebastian S, Touchburn SP, Chavez ER (1998) Implications of phytic acid and supplemental microbial phytase in poultry nutrition: a review. World Poult Sci J 54:27–47
Segueilha L, Lambrechts C, Boze H, Moulin G, Galzy P (1992) Purification and properties of the phytase from *Schwanniomyces castellii*. J Ferment Bioeng 74:7–11
Shamsuddin AM, Baten A, Lalwani ND (1992) Effects of inositol hexaphosphate on growth and differentiation in K-562 erythroleukemia cell line. Cancer Lett 64:195–202
Shears SB (1998) The versatility of inositol phosphates as cellular signals. Biochim Biophys Acta 1436:49–67
Shimizu M (1992) Purification and characterization of a phytase from Bacillus subtilis (natto) N-77. Biosci Biotechnol Biochem 56:1266–1269
Simon O, Igbasan F (2002) In vitro properties of phytases from various microbial origins. Int J Food Sci Technol 37:813–822
Singh M, Krikorian AD (1982) Inhibition of trypsin activity in vitro by phytate. J Agric Food Chem 30:799–800
Singh B, Boukhris I, Pragya KV, Yadav AN, Farhat-Khemakhem A et al (2020) Contribution of microbial phytases to the improvement of plant growth and nutrition: a review. Pedosphere 30:295–313. https://doi.org/10.1016/S1002-0160(20)60010-8
Sutardi M, Buckle KA (1988) Characterization of extra- and intracellular phytases from *Rhizopus oligosporus* used in tempeh production. Int J Food Microbiol 6:67–79
Tomschy A, Tessier M, Wyss M, Brugger R, Broger C, Schnoebelen L et al (2000) Optimization of the catalytic properties of *Aspergillus fumigatus* phytase based on the three-dimensional structure. Protein Sci 9:1304–1311
Türk M, Carlsson NG, Sandberg AS (1996) Reduction in the levels of phytate during wholemeal bread making; Effect of yeast and wheat phytases. J Cereal Sci 23:257–264
Türk M, Sandberg AS, Carlsson N, Andlid T (2000) Inositol hexaphosphate hydrolysis from baker's yeast. Capacity, kinetics, and degradation products. J Agric Food Chem 48:100–104
Vidal-Valverde C, Frias J, Sotomayor C, Diaz-Pollan C, Fernandez M, Urbano G (1998) Nutrients and antinutritional factors in faba beans as affected by processing. Z Lebensm Unters Forsch A 207:140–145
Viveros C, Centeno A, Brenes R, Canales A, Lozano A (2000) Phytase and acid phosphatase activities in plant feedstuffs. J Agric Food Chem 48:4009–4013
Vohra A, Satyanarayana T (2001) Phytase production by the yeast, *Pichia anomala*. Biotechnol Lett 23:551–554
Vohra A, Satyanarayana T (2002) Purification and characterization of a thermostable and acid-stable phytase from Pichia anomala. World J Microbiol Biotechnol 18:687–691
Vucenik I, Shamsuddin AM (2003) Cancer inhibition by inositol hexaphosphate (IP6) and inositol: from laboratory to clinic. J Nutr 133(Suppl):3778–3784
Wang M, Hettiarachchy NS, Qi M, Burks W, Siebenmorgen T (1999) Preparation and functional properties of rice bran protein isolate. J Agric Food Chem 47:411–416
Wodzinski RJ, Ullah AHJ (1996) Phytase Adv Appl Microbiol 42:263–302
Wyss M, Pasamontes L, Rémy R, Kohler J, Kusznir E, Gadient M, Müller F, van Loon APGM (1998) Comparison of the thermostability properties of three acid phosphatases from molds: *Aspergillus fumigatus* phytase, *A. niger* phytase, and *A. niger* pH 2.5 acid phosphatase. Appl Environ Microbiol 64:4446–4451
Wyss M, Brugger R, Kronenberger A, Remy R, Fimbel R, Oesterhelt G, Lehmann M, van Loon APGM (1999a) Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolase): catalytic properties. Appl Environ Microbiol 65:367–373

Wyss M, Brugger R, Kronenberger A, Rémy R, Fimbel R, Oesterhelt G, Lehmann M, van Loon APGM (1999b) Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. Appl Environ Microbiol 65:367–373

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi. Volume 1: Diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 2: Perspective for value-added products and environments. Springer International Publishing, Cham

Yang WJ, Matsuda Y, Sano S, Masutani H, Nakagawa H (1991) Purification and characterization of phytase from rat intestinal mucosa. Biochim Biophys Acta 1075:75–82

Yao B, Thang C, Wang J, Fan Y (1998) Recombinant Pichia pastoris overexpressing bioactive phytase. Sci China 41:330–336

Chapter 13
Fungal Lipases: Insights into Molecular Structures and Biotechnological Applications in Medicine and Dairy Industry

Amira Mohamed Galal Darwish, Hebatallah H. Abo Nahas, Yasmin H. Korra, Alaa A. Osman, Wedad M. El-Kholy, Maria Reyes-Córdova, Essa M. Saied, and Ahmed M. Abdel-Azeem

Contents

A. M. G. Darwish (✉)
Department of Food Technology, Arid Lands Cultivation Research Institute (ALCRI), City of Scientific Research and Technological Applications (SRTACity), New Borg El-Arab, Alexandria, Egypt
e-mail: adarwish@srtacity.sci.eg

H. H. Abo Nahas
Zoology Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt

Y. H. Korra
Faculty of Pharmacy, Pharos University, Alexandria, Egypt

A. A. Osman
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, New Giza University, Cairo, Egypt

W. M. El-Kholy
Department of Dairy Research, Food Technology Research Institute (A.R.C.), Alexandria, Egypt

M. Reyes-Córdova
Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, National Agrarian University La Molina (UNALM), Lima, Perú

E. M. Saied
Chemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Institute for Chemistry, Humboldt Universität zu Berlin, Berlin, Germany

A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_13

13.1 Introduction

Recent consumers' interest in nature mimic for most industrial processes, especially bringing high product yield in short given time and mild conditions. Industrial waste by-products that may be very hazardous for human lead to a crucial need for production of less toxic, cost effective with low energy consumption. According to Global Industry Analysts report, the world market for industrial enzymes exceeded $2.9 billion by 2012, then reached $7 billion in 2013, with average annual increase of 6.3%, mostly in the biocatalyst enzymes. San Jose, California (PRWEB 2008) stated that USA was the fastest growing market for industrial enzymes with a CAGR of 5% in 2001–2010, while Europe was reported as the greatest regional market having an estimated share of 30.93% in 2008. Sales of industrial enzymes in Asia Pacific were estimated at US$327 million in 2008. Lipases, for their use in the detergent and cosmetics markets, reached a CAGR of 9.13% in 2001–2010,as estimated 1000 tons of lipases are added to the approximately 13 billion tons of detergents produced each year (PRWEB 2008). The global market size of lipase reached

$590.5 Million by 2020, at a CAGR of 6.5% between 2015 and 2020, with the increasing use of lipase in numerous feeds for livestock (Marketsandmarkets 2020).

Lipases occur widely in nature, but only fungal and bacterial lipases are commercially significant. The interest in fungal and bacterial lipase production has increased in the last decade, because of its large potentials in manufacturing applications as food additives (flavor modification),fine chemicals (synthesis of esters), waste water treatment (decomposition and removal of oil substances), cosmetics (removal of lipids), pharmaceutical (digestion of oils and fats in foods), leather (removal of lipids from animal skins), and medicine (blood triglyceride assay) (Mehta et al. 2017; Sahay et al. 2017; Yadav 2017). Lipase has been extensively used in detergent industry due to its potential capability to hydrolyze fats. Lipase-producing microbes have been found in diverse habitats such as industrial wastes, where they play major roles in waste biotransformation (Yadav et al. 2019c). In vegetable oil processing factories, lipases catalyze breaking down of lipids into glycerol and free fatty acids, dairies, soil contaminated with oil, etc. (Hasan et al. 2010).

Fungi are producing machinery lipase by excellence and considered as the best lipase sources. Lipase are of great importance in industry due to their activity, specificity, stability under physical and chemical conditions, and are regio- and esteroselective (Ken Ugo et al. 2017; Yadav et al. 2019b). Fungal lipases have benefits over bacterial ones due to the fact that present day technology favors the use of batch fermentation and low-cost extraction methods. Major genera of filamentous fungi include *Rhizopus, Aspergillus, Penicillium, Mucor, Ashbya, Geotrichum, Beauveria, Humicola, Rhizomucor, Fusarium, Acremonium, Alternaria, Eurotrium,* and *Ophiostoma* (Basheer 2007; Abdel-Azeem et al. 2021). Species of the mold *Aspergillus* are well known as lipase producers. Lipases from *Aspergillus niger* are produced both intracellularly and extracellularly. Fungal species which produce lipases are *Candida rugosa, Candida antarctica, T. lanuginosus, Rhizomucor miehei, Pseudomonas*, *Mucor,* and *Geotrichum* (Sharma et al. 2001). *Colletotrichum gloeosporioides* identified as the most productive lipase-producing strain among 59 fungal strains isolated from Brazilian savanna soil (Santhosh Kumar and Ray 2015).

Bioinformatics approaches of predicting the enzyme–substrate complex structure can be accomplished through two unified steps: first, by sampling conformations of the ligand (e.g., fatty acids, tripalmitin, etc.) in the active site of the protein, and second by classifying these conformations by a score that ranks most negative value among all the conformations generated which indicates greater stability. Homology modeling process checks stereochemical quality of protein structures, while molecular docking studies are performed to decipher the binding affinity and mode of interaction of selected compound(s) against fungal lipases (Moya-salazar et al. 2019).

This chapter provides an overview on the various sources of lipases, their properties, purification methods, classification, catalytic mechanism, and optimization. Additionally, it sheds the light on 3D molecular structure of fungal lipases, molecular docking techniques, homology modeling, and potential sustainable industrial

applications of fungal and bacterial lipases for green economy that makes lipases to be biocatalysts of choice for the present and future.

13.2 Historical Background of Fungal and Bacterial Lipase

Lipase was first discovered in pancreatic juice in the year 1856 by Claude Bernard (Parveen and Manikandaselvi 2011). Animal pancreatic extracts were traditionally used as the source of lipase for commercial applications. However, fungal and bacterial sources of lipase were explored when the industrial potential of lipases enhanced and when the demand for lipases could not be met by the supply from animal sources(Saxena et al. 1999).

In 1988, Novo Nordisk evolved a lipase produced by means of the fungus *Humicola*. Commercial detergent expressions with high temperature have been produced from *Humicolalanuginosa*(Lipolase) (Jaeger et al. 1994). As the volume of this enzyme was much less than required for commercial application; therefore, the yield of enzyme was elevated by cloning the gene coding for lipase produced by *Aspergillus oryzae* which can elevate amount of enzyme that can be used commercially in detergents. The first commercial lipase, Lipolase, was introduced in 1994 by Novo Nordisk. This enzyme was produced from *Trichoderma lanuginosus* and was expressed in *Aspergillus oryzae* (Hoq et al. 1985). They also produced a lipase containing detergent "LipoPrime®," laundering with lipase which contains detergents carried out in alkaline condition; for instance, lipase extracted from the *Aspergillus oryzae*(Umehara et al. 1990).

The first fungal lipase structure studied was that of the *Rhizomucor miehei* lipase by Brady et al. (1990) X-ray crystallographic analysis. It showed that this enzyme had an active site triad as that of the serine proteases. X-ray crystallography was still continued to be a powerful tool for structure determinations of most biological macromolecules. Recently, especially in this millennium, other approaches also have come into practice for structural analyses, including the use of bioinformatics tools for structure predictions up to the tertiary levels of protein organization(Mala and Takeuchi 2008).

13.3 Sources of Lipase

Lipase enzyme reported in many microbiota such as bacteria and fungi (filamentous yeast).The most commonly used bacterial genera for lipase applications are *Bacillus, Arthrobacter,* and *Pseudomonas*. Filamentous fungi are able to produce higher amounts of extracellular lipases and the most commonly used fungal genera in applications are *Mucor, Penicillium, Aspergillus, Geotrichum,* and *Rhizopus*. The most commonly used species from the genus *Penicillium are P. cyclopium, P. brevicompactum, P. chrysogenum, P. crustosum, P. roqueforti, and P. verrucosum*

(Ozturkoglu-Budak et al. 2016). Lipase-producing microbiota have been found in different habitats such as industrial wastes, dairy plants, and soil contaminated with oil vegetable oil processing factories, and oilseeds among others (Sharma et al. 2001; Yadav et al. 2020a).

Microbes being ubiquitous in distribution are incredibly successful at surviving in a broad range of environmental conditions owing to their great flexibility and physiological versatility due to their functional enzyme system. Microbes thrive properly in inhospitable habitats with mechanisms for adapting to harsh habitats and for the utilization of their trophic niche, the capability of microbiota to produce extracellular enzymes is great survival value (Hasan et al. 2006). Lipase extracted filamentous fungi are accepted as the potential source of extracellular lipase for mass production at industrial applications. Lipase production is costly and that could be a fundamental problem in industrial application. So, a variety of attempts have been made to reduce its production cost (Smaniotto et al. 2012).

Fungi are chosen as potential source for lipase that can be easily separated from the fermentation media. Basically, the most commercially significant lipase-producing fungi are recorded as related to the genera *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., *Mucor* sp., and *Rhizomucor* sp. Lipase production by filamentous fungi differs regarding to the strain, the composition of the growth medium, cultivation conditions, pH, temperature, and the kind of carbon and nitrogen sources (Mehta et al. 2017). Table 13.1 exhibits fungal strains for lipase production through different types of fermentation. The various species of *Aspergillus*, isolated from soil sources, have been reported to excrete lipase with noteworthy properties satisfactory for biotechnological applications (Basheer 2007; Kour et al. 2019).

According to Vakhlu and Kour (2006), the main terrestrial species of yeasts that were recorded to produce lipases are *Candida rugosa, Candida tropicalis, Candida antarctica, Candida cylindracea, Candida parapsilosis, Candida deformans, Candida curvata, Candida valida, Yarrowia lipolytica, Rhodotorula glutinis, Rhodotorula pilimornae, Pichia bispora, Pichia mexicana, Pichia silvicola, Pichia xylosa, Pichia burtonii, Saccharomycopsis crataegenesis, Torulaspora globosa, Trichosporon asteroids,* and *Sporidiobolus pararoseus* (Smaniotto et al. 2014). The genes that encode lipase i*n Candida* sp., *Geotrichum* sp., *Trichosporon* sp., and *Y. lipolytica* have been cloned and over-expressed (Thakur 2012).

Physicochemical properties of many extracellular fungal lipases have been determined. Lipases have been carefully characterized and purified in terms of their activity and stability profiles relative to PH, temperature, and impacts of metal ions, organic solvents, and chelating agents (Sharma et al. 2016). Fungal lipases have priority over bacterial ones due to the fact that present day technology favors the use of batch fermentation and low-cost extraction methods. Subsequently, fungal lipases considered incomparable biocatalysts which find applications in a quantity of catalytic strategies which are of industrial potential. Many fermentation techniques have been designed for easy cultivation with large scale. However, two most important methods are used for their production: submerged fermentation (SmF) and solid state fermentation (SSF) (Meghwanshi and Vashishtha 2018).

Table 13.1 Fungal strains for lipase production through fermentation (Mehta et al. 2017)

Microorganism	Time (h)	Lipase activity	Type of fermentation	Raw material
Penicillium aurantiogriseum	48	25	SmF	Soybean oil
Rhizopus rhizopodiformis	24	43	SSF	Olive oil cake-Bagasse
Rhizopus pusillus	25	10.8	SSF	Olive oil cake-Bagasse
Penicillium restrictum	24	30	SSF	Babassu oil cake
Penicillium simplicissimum	36	30	SSF	Babassu oil cake
Rhizopus oligosporus TUV-31	48	76.6	SSF	Egg yolk
Rhizopus oligosporus ISUUV-16	48	81.2	SSF	Almond meal
Aspergillus carneus	96	12.7	SSF	Sunflower oil
Candida cylindracea	179.5	23.7	SmF	Oleic acid
Candida rugosa	50	3.8	SmF	Olive oil
Penicillium verrucosum	48	40	SSF	Soybean bran
Geotrichum sp.	24	20	SmF	Olive oil
Rhizopus homothallicus	12	826	SSF	Olive oil
Penicillium chrysogenum	168	46	SSF	Wheat bran
Fusarium solani FS1	120	0.45	SmF	Sesame oil
Penicillium simplicissimum	48	21	SSF	Soycake
Aspergillus awamori	96	495	SmF	Ricebran oil
C. cylindracea NRRLY-17506	175	20.4	SmF	Olive mill wastewater

13.4 Classification of Lipases

Lipases are diverse, subsequently; they are grouped in different ways. They may be classified (i) according to their isoelectric point and their molecular mass, which ranges from 3.8 to 7.3 and 27 to 60 KDa, respectively, e.g., large molecular mass such as that from *Candida rugosa* (60 KDa), (ii) according to their substrate specification, for example, lipases from *Mucor miehei* and *Rhizopus delemar* attack the primary hydroxyl positions (1,3) of glycerol preferentially and are reported to be 1.3-specific. The lipases of *Candida rugosa* and *Chromobacterium viscosum* are not specific to their position; the lipase of *Geotrichum candidum* is selective towards cis-unsaturated (Δ9) fatty acids, such as oleic acid. The lipases of *M. miehei* can discriminate against polyunsaturated acids such as linoleic and docosahexaenoic acid (Ribeiro et al. 2011; Mehta et al. 2017). More classifications of lipases are exhibited in the following section according to their properties.

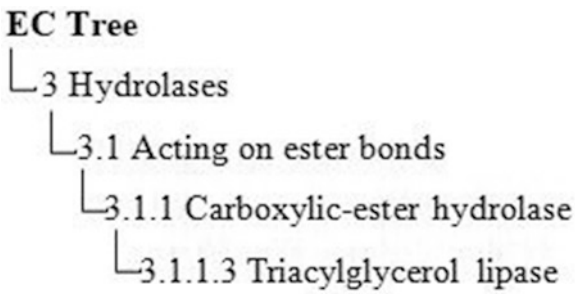


Fig. 13.1 EC Tree

13.4.1 Biocatalytic Reactions of Lipases

Lipases (E.C. 3.1.1.3) (Fig. 13.1) are enzymes that are primarily responsible for the hydrolysis of acylglycerides. What enlarges the variety of reactions is the fact that lipases are capable of catalyzing the reverse reaction of synthesis efficiently. Lipases can be classified into two categories based on their catalytic reaction: hydrolysis and synthesis (Gandhi 1997)

13.4.1.1 Hydrolysis

Hydrolysis of oils and fats is the applied term to the operation in which ethanolic KOH reacts with oil to form glycerol and fatty acids (FAs) in presence of water (Table 13.2) hydrolysis catalyzed by lipases with different substrates.

$$\mathrm{RCOOR' + H_2O \Leftrightarrow RCOOH + R'OH} \tag{13.1}$$

It is an unusual reaction, probably due to the opposite polarities of hydrophobic substrates, catalysts, and hydrophilic products. This characteristic is widely used in food, cosmetics, pharmaceutical applications, and the detergent industry such as soaps due to its lipolytic nature. The lipases from *Aspergillus oryzae* are used in the detergent industry, from *Candida rugosa*, used in leather manufacturing, from *Rhizopus nodosus* to obtain chamois (Muthukumaran and Dhar 1982; Christner et al. 1992).

13.4.1.2 Reaction Synthesis

Lipase-catalyzed esterification attracted interest due to the significance of derived products such as organic esters in biotechnology and the chemical industry. This category can be grouped into transesterification. The synthesis of esters *via* the reverse reaction in lipase-catalyzed esterification and transesterification processes

Table 13.2 Lipases catalyze hydrolysis of fat and oil

Lipase	Substrate	Remarks
Rhizopus delemar, Penicillium and Rhizopus niveus	Soybean oil	Combined lipase system
Candida rugosa, Aspergillus niger	Fish oil	Concentration of polyunsaturated fatty acids
C. rugosa	Soybean oil	Membrane reactor was used
C. rugosa	Cooking oil, Trex	Electrically enhanced dispersion
C. rugosa	*n*-Propylibuprofenate	Enantioselective hydrolysis in AOT micro-emulsions
C. rugosa	Olive oil	Lipase immobilized on Sephadex, Amberlite, etc. in isooctane
C. rugosa	Soybean oil	Hybrid membrane-emulsion reactor
C. rugosa	Butter oil	Spiral-wound membrane reactor
A. niger	Butter oil	Flat-sheet membrane reactor
C. rugosa	Tuna oil	Discrimination against docosahexaenoic acid
C. rugosa	Olive oil	Biphasic isooctane-aqueous system
Black cumin seed	Cumin seed oil	Kinetics determined
C. rugosa	Butter oil	Lecithin-isooctane reverse micelles
C. rugosa	Tributyrin	Kinetics determined
Mucor miehei	Soybean phosphatidylcholine	Solvents more polar than hexane better
M. miehei,	*Lesquerella*	Different reaction systems compared
Rhizopus arrhizus	*Fendleri* oil	
Pseudomonas putida	Olive oil	Cells as lipase source in organic-aqueous two-phase systems
C. rugosa	Fish oil	Lipase discrimination against docosahexaenoic acid
R. delemar	2-Naphthyl acetate,caprate, laurate, etc.	Interfacial kinetics determined
Pseudomonas	Hydrophobic diester	Stereospecific hydrolysis, a step in the synthesis of a selective leukotriene antagonist
C. rugosa, M. miehei,	Methyl-branchedoctanoic-	Effect of branching and stereobias studied
P. fluorescens, porcine pancreatic, lipoprotein lipases	acid thiol esters	
Rat hepatic lipase	Neutral glycerides and phospholipids	Higher hydrolytic rate for neutral lipids than phospholipids
Chromobacterium viscosum	Olive oil, triolein	AOT-isooctane reversed micelles
Thermomyces lanuginosus	Beef tallow	Kinetics studied in a flat-plate immobilized lipase reactor

(continued)

Table 13.2 (continued)

Lipase	Substrate	Remarks
C. rugosa, M. miehei	Tallow, cod liver oil, etc.	Effect of amines on hydrolysis investigated
C. rugosa		
R. niveus	Beef tallow, pork lard	Reactions in isooctane at temperatures lower than the melting points of substrates
C. rugosa	Fish oil	Docosahexaenoic acid concentration due to discrimination
M. miehei	Triacetin	Hollow fiber reactor
C. rugosa	Oleyl oleate	Thermodynamics and kinetics studied
P. fluorescens	Tributyrin	Various immobilization supports for lipase tested
C. rugosa	Acyl nucleosides	Regioselective deacylation
R. delemar	Olive oil	Hydrolysis enhanced by dimethyl ß-cyclodextrin
Porcine pancreatic	Palm oil	Monoglyceride preparation in microemulsions
C. rugosa	Glycidol esters	Enantiomers of glycidol-starting materials for manufacture of cardiovascular ß -blockers, etc.
	Methyl-2-chloropropionate	Carbon tetrachloride allows for stereospecific hydrolysis

(alcoholysis, acidolysis, interesterification) gives rise to alcohol, acid, or ester. Low and medium molecular weight esters such those produced by *R. miehei*, *R. delemar*, *P. cyclopium,* and *G. candidum* are used in the cosmetics industry in a process of preparing fatty acid esters of polyol with mixed groups (Kaufman and Garti 1982).

Reactions under this category can be further separated:

(a) Esterification

$$\mathrm{RCOOH} + \mathrm{R'OH} \Leftrightarrow \mathrm{RCOOR'} + \mathrm{H_2O} \tag{13.2}$$

(b) Interesterification

$$\mathrm{RCOOR'} + \mathrm{R''COOR*} \Leftrightarrow \mathrm{RCOOR*} + \mathrm{R''COOR'} \tag{13.3}$$

(c) Alcoholysis

$$\mathrm{RCOOR'} + \mathrm{R''OH} \Leftrightarrow \mathrm{RCOOR''} + \mathrm{R'OH} \tag{13.4}$$

(d) Acidolysis

$$\mathrm{RCOOR'} + \mathrm{R''COOH} \Rightarrow \mathrm{R''COOR'} + \mathrm{RCOOH} \tag{13.5}$$

13.4.2 Lipase Engineering Database (LED)

Lipases are very versatile, they include many protein families with no sequence similarity, however they share the same catalytic site, which is composed of the motif (serine-histidine-aspartate or glutamate) and the oxyanion hole. A classification was designed by Fischer and Pleiss (2003) in the Lipase Engineering Database (LED) (http://www.led.uni-stuttgart.de), in which includes bacterial, yeast, fungal, and mammalian lipases. This classification distributes the lipases based on the sequence, structure, function and the conformation of their oxyanion into three major classes: GX, GGGX, and Y. Based on this classification and of the amino acid sequence similarities, yeasts and fungal lipases have been grouped into five different subclasses, two in the GX class, two in the GGGX class, and one in the Y class (Fig. 13.2). In addition, they are grouped into 15 superfamilies based on the conserved peptide; it also has 32 homologous families (Martínez-Corona et al. 2020).

13.4.2.1 GX Class

G is conserved glycine and X is either hydrophobic or hydrophilic oxyanion hole residue. When the residue X is hydrophilic, this acts as an anchor residue. This class is the most diverse, with 27 superfamilies, in fungi we have abH23 – Filamentous fungi, abH15 – Burkholderia lipases, abH37 – *Candida antarctica* lipase like, abH36 – Cutinases, abH22 – Lysophospholipase, abH16 – *Streptomyces* lipases.

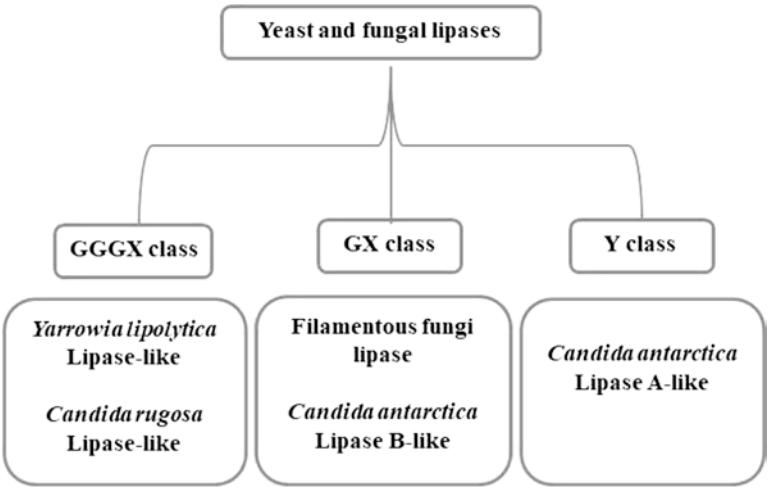


Fig. 13.2 Classification of lipases based on lipase engineering database

13.4.2.2 GGGX Class

Like the GX, G is a conserved glycine and is followed by a conserved hydrophobic residue. The oxyanion hole structure is highly preserved. Most of them have characteristics of activity and enantioselectivity. It has 6 superfamilies, in the case of fungi; we find homologous superfamilies abH02 – *Yarrowia lipolytica* lipase like, abH03 – *Candida rugosa* lipase like.

13.4.2.3 Y Class

Y class is formed by the hydroxyl group of tyrosine side chain. This type is found *C. antarctica* A lipase, some esterase, and a few bacterial lipases (Gupta et al. 2015). On the basis of the above classification, yeast and fungal lipases fall into five different subclasses: *Y. lipolytica* lipase like, *C. rugosa* lipase like, filamentous fungi lipases, *C. antarctica* lipase like, and *C. antarctica* lipase A like.

13.4.3 pH Stability

The fungal lipases have a stability pH at 6.0–7.5. There are lipases that work at acidic pH, for example, extracellular lipases from *A. Niger* and *Rhizopus* sp., that are stable at pH 4 (Saxena et al. 1999) and alkaline pH such as *Curvularia* sp. (El-Ghonemy et al. 2017).

13.4.3.1 Alkaline Lipase

As a result of growing global lipase market; the following criteria in formulations should be considered: (i) relative resistance to harsh conditions (30–60 °C and pH 10–11) requiring stable enzymes; (ii) the high variety triglycerides containing fatty acids, requiring less specific lipases to substrate with low substrate specificity; (iii) stability in the presence of different components in a detergent including metal ions, surfactants, oxidants, other enzymes particularly proteases and the effect of chemical denaturants caused by proteolytic detergent additives like surfactants linear alkyl benzene sulfonate (LAS). To get over these problems, the combination of developed lipases and attempting to improve the lipase properties on the basis of protein engineering could be a good alternative (Jaeger and Reetz 1998).

Since Novo Nordisks (1988) evolved lipase production as a detergent additive, the usage of alkaline lipase increased due to its affiliation with the nonphosphate detergents that able to decompose fatty material. Lipase is capable of removing fatty stains such as fats, butter, salad oil, sauces, and the tough stains on collars and cuffs. The factors that aid their increased application is the availability of wide enzyme

types, environmental friendly behavior, minimized energy consumption, easily controlled processes, possible modification of enzyme characteristics, environmentally friendly by-products, minimal greenhouse gas emissions, decreased use of non-renewable sources, and proliferation of chemicals in environment. The multi-faceted benefits availed through using enzymes are bound to convert into a greener world (Cherif et al. 2011). Laundering with lipase containing detergents is generally carried out in alkaline conditions; therefore, alkaliphilic lipases are preferred such as lipase obtained from the *A. oryzae* (Gerhartz 1990; Umehara et al. 1990). A presoak formulation was developed using an alkaline lipase produced by *Trichosporon asahii* MSR 54, used for removing oil stains at ambient temperature (Kumar et al. 2009).

Commercial detergent formulations with high-temperature optima have been produced from *P. mendocina* (Luma fast) and *Pseudomonas glumae* are used (Jaeger et al. 1994). In 1995, Luma fast and Lipoma bacterial lipases were introduced from *P. mendocina* and *Pseudomonas alcaligenes*, respectively, by Genencor International, AU-KBC Research Center, Life Sciences, Anna University, Chennai, India (http://www.au-kbc.org/beta/bioproj2/uses.htm) (Wang et al. 2012). Gerritse et al. (1998) reported an alkaline lipase from *P. alcaligenes* M-1, capable of removing fatty stains when used in a washing machine. A presoak procedure was advanced using an alkaline lipase produced by *Trichosporon asahii* MSR 54 used for eliminating oil stains at ambient temperature.

ABS fungal lipase is used in a many sanitary and waste treatment liquid formulations. This product exhibits activities in a wide range of pH. Enzymatic methods are preferred to chemical methods in many sanitary applications such as grease trap applications; other applications include detergents, pre-spotters, and industrial cleaning compounds (Hasan et al. 2010).

13.4.3.2 Acid Lipase

Lipases active at highly acidic pH are rarely reported from fungal or bacterial sources. Acid lipases have high potential application in the oleo chemical and food industries for hydrolysis and/or modification of triacylglycerols to improve the nutritional properties. *Aspergillus niger* is among the most well-known lipase producers and its enzyme is suitable for use in many industrial applications. Among the various fungal strains screened for acidic lipase production, *Rhizopus arrhizus* NCIM 877, 878, 879 and *Aspergillus niger* NCIM 1207 produced significant quantities of enzyme when grown in synthetic oil based (SOB) medium under submerged conditions. Most research concentrates on extracellular lipases that are produced by wide variety of organisms. Studies on conditions for the production of extracellular lipases by *A. niger* show variations among different strains but the requirement of lipid carbon source is essential for enzyme production.

The technique of solid state fermentation (SSF) involves the growth and metabolism of microorganisms on moist solids without any free-flowing water. This technique has many advantages over submerged fermentation (SmF) including economy

of space needed for fermentation, simplicity of fermentation media, no requirement of complex machinery, equipment and control systems, compactness of fermentation vessel owing to lower water volume, superior yields, less energy demand, lower capital, and recurring expenditure (Mahadik et al. 2002). Acid lipase produced from *Candida viswanathii* presented a broad specificity for triacylglycerols hydrolysis suggesting that this enzyme can be applied in lipid digestion and biotransformation (De Almeida et al. 2013).In addition, acidic lipase Lip I.3 was reported to be isolated from a *Pseudomonas fluorescens*-like strain although most reports on lipases from *Pseudomonas* have centered on enzymes belonging to Families I.1 and I.2 (Panizza et al. 2013).

13.4.4 Temperature Stability

Climate change is one of the most important issues the world currently facing, and as part of solution it calls for multi-pronged strategy. Application of cold-active enzymes is one of the important steps among them as it is expected to reduce energy consumption and consequently carbon dioxide emissions (Singh et al. 2016). Washing cloth at ambient temperature requires heating of water in the washing machine in colder areas. Cold washing with laundry detergent containing cold-active enzymes is regarded as an effective solution to this issue. Moreover, in poor countries where women do washing manually at ambient temperature have to put in much labor with currently available detergent. A detergent containing cold-active enzymes would thus save them from this drudgery. A switch-over to cold washing would thus require a huge amount of cold-active enzymes including lipase (Sahay and Chouhan 2018).

Generally, lipases are further divided into three divisions based on their degree of temperature stability, namely (i) psychrophilic, (ii) mesophilic, and (iii) thermophilic. Thermostable enzymes can be obtained from mesophilic and thermophilic organisms; even psychrophiles have some thermostable enzymes (Andualema and Gessesse 2012; Yadav et al. 2020b). Currently, lipases from thermophilic and psychrophilic organisms have been proved to be more useful for biotechnological applications (Imamura and Kitaura 2000).

Lipases were obtained from extremophiles, i.e., organisms adapted to life in high temperature, with maximum activity over 70 °C (*Bacillus thermocatenulatus*) or with high activity at low temperature as is the case for enzymes produced by Antarctic bacteria, such as *Pseudomonas* and *Moraxella* sp. Such extreme and unusual features open the possibility to apply these enzymes without further modification using molecular engineering approaches to adapt them for use in reactions carried out at high temperatures or, conversely low temperature processes such as that of detergents (low temperature washes) or in food processing (Demiorijan et al. 2001).

13.4.4.1 Thermophilic Lipases

The demand of thermostable lipases for different applications has been growing rapidly. Many lipases from mesophiles are stable at elevated temperatures. Proteins from thermophilic organisms have also been proved to be more useful for biotechnological applications than similar proteins from mesophiles due to their stability at high temperature (Imamura and Kitaura 2000). Enzymes with high thermostability are important to have higher reaction rate at higher operation temperature, increasing solubility of substrates and help to lower substrate viscosity and thereby avoid environmental contamination (Andualema and Gessesse 2012).

Enzymes produced from hyperthermophilic archaebacteria, such as *Pyrococcus furiosus* and *Thermotoga* sp., are highly advantageous for biotechnological applications with growing demand, since they can be produced at low cost and exhibit improved stability at high extreme temperature (de Miguel Bouzas et al. 2006). Thermostable lipases from various sources have been purified and characterized using appropriate procedures (Sugihara et al. 1991). Lipases operating chemical reaction at elevated temperatures have the following advantages. (1) A higher diffusion rates. (2) Increased solubility of lipids and other hydrophobic substrates in water. (3) Decreased substrate viscosities. (4) Increased reactant solubility (5) Higher temperature faster reaction rates. (6) Reduced risk of microbial contamination (Hasan et al. 2006). These enzymes have been applied to synthesis biopolymers, pharmaceutical chemicals, agrochemicals, cosmetics, flavors, and biodiesel (Haki and Rakshit 2003).

Thermostable lipases have been isolated from many sources, including *Pseudomonas fluorescens*; *Bacillus* sp., *B. coagulans* and *B. cereus*; *B. stearothermophilus*; *Geotrichum* sp. and *Aeromonas sobria* and *P. aeruginosa*. The enzyme from *P. aeruginosa* was significantly stabilized by Ca^{2+} and was inactivated by EDTA (Andualema and Gessesse 2012). Lipases from *Aspergillus niger* and *Rhizopus japonicus* are stable at 50 °C, and lipase of thermotolerant *Humicola lanuginosa* is stable at 60 °C(Mehta et al. 2017).

13.4.4.2 Psychrophilic Lipases

Cold adapted lipases are largely distributed in microorganisms existing at low temperatures nearly 5 °C. Although a number of lipase-producing organisms are available, only a few bacteria and yeast were exploited for the production of cold adapted lipases (Joseph et al. 2007; Yadav et al. 2017, 2019a). Attempts have been made to isolate cold adapted lipases from these microorganisms having high activity at low temperatures. Cold adapted bacterial strains were isolated mostly from Antarctic and Polar regions which represent a permanently cold (0 ± 2 °C). A marine bacterium *Aeromonas hydrophila* growing at a temperature range between 4 and 37 °C was found to produce cold-active lipolytic enzyme (Pemberton et al. 1997). Few bacterial genera have been isolated and characterized from deep-sea sediments where temperature is below 3°C. They include *Aeromonas* sp., *Pseudoalteromonas*

sp. and *Psychrobacter* sp. (Zeng et al. 2004) and *Photobacterium lipolyticum* (Ryu et al. 2006). Permanently cold regions such as glaciers and mountain regions are another habitat for psychrophilic lipase-producing microorganisms (Joseph et al. 2007). The soil and ice in Alpine region also harbor psychrophilic microorganisms which produces cold-active lipases. Psychrotrophic lipolytic moulds such as *Rhizopus* sp. and *Mucor* sp. were grown on milk and dairy products and soft fruits (Coenen et al. 1997).

An extensive research has been carried out in the cold-active lipase of *Candida antarctica* compared to the other psychrophilic fungi. *Candida lipolytica*, *Geotrichum candidum,* and *Penicillium roqueforti* have also been isolated from frozen food samples and reported to produce cold-active lipases (Alford and Pierce 1961). At the industrial level, the yeast *Candida antarctica* produces two lipases, A and B, the latter being sold for instance as Novozym435 by Novozymes (Bagsvaerd, Denmark). As a result of its substrate and stereospecificity, lipase B is involved in a very large number of organo synthesis applications related to food/feed processing, pharmaceuticals, or cosmetics, that is why *C. antarctica* dominated process- or product-based patents. This is a significant example of the potential for novel catalysts from genetic resources in cold environments (Feller 2013).

Cold-active lipases have lately attracted attention of communities as a result of their increasing use in the organic synthesis of chiral intermediates. Due to their low optimum temperature and high activity at very low temperatures which are favorable properties for the production of relatively frail compounds. Cold-active lipases are today the enzymes of choice for organic chemists, pharmacists, biophysicists, biochemical and process engineers, biotechnologists, microbiologists, and biochemists. Their current application includes additives in detergents (cold washing), additives in food industries (fermentation, cheese manufacture, bakery, meat tenderizing), environmental bioremediations (digesters, composting, oil degradation or xenobiotic biology applications, and molecular biology applications), biotransformation, and heterologous gene expression in psychrophilic hosts to prevent formation of inclusion bodies (Feller et al. 1996).

13.5 Lipase Catalytic Mechanism

The lipase-catalytic hydrolysis of an ester follows a two-step reaction mechanism and involves the catalytic triad amino acid in the lipase active site (serine-histidine-aspartate or sometimes glutamic acid). The first step in the hydrolysis is activated and stabilized by the histidine and aspartic acid residues in the active site, which enhance the nucleophilicity of the serine residue. The catalytic serine residue transfers a proton to the histidine residue to form an oxyanion which is then nucleophilically attack the carbonyl group of an ester to form a negatively charged tetrahedral acyl-lipase intermediate and an alcohol molecule (Fig. 13.3) (de Miranda et al. 2015). The charged acyl-lipase intermediate is stabilized by forming hydrogen bonds with the NH-groups of residues from the enzyme chain, which lead to the

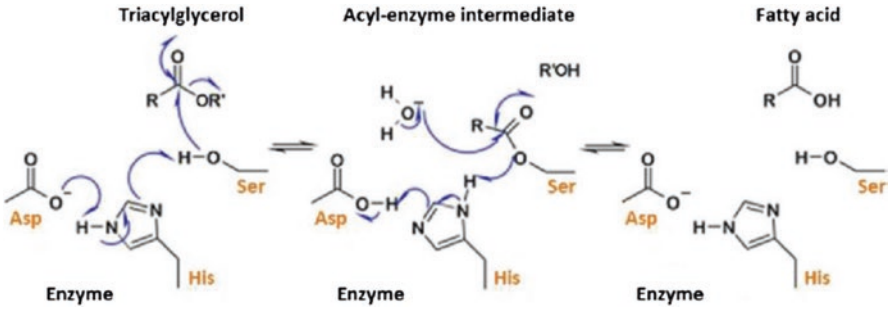


Fig. 13.3 Lipase-catalytic mechanism of ester hydrolysis. (i) A charged acyl-lipase intermediate is formed by nucleophilic attack a serine residue on the carbonyl carbon. (ii) The cleavage of acyl-enzyme intermediate by nucleophile, such as water, to afford the product and regenerating the catalytic residues (De Simone 2016)

formation of an oxyanion hole. In many of lipases this hole is correctly oriented, whereas in some lipases it requires the opening of the lid structure in order to be correctly oriented.

In the second step, a water molecule nucleophilically attacks the covalent acyl-enzyme intermediate to afford the carboxylic acid and the enzyme which repeats the same steps again. Several compounds can act as nucleophile and instead of water and deacylate the acyl-lipase intermediate. This indicates the broad reaction spectrum that can be catalyzed by lipases, such as transesterification, esterification, acidolysis (Adlercreutz 2013; Jung et al. 2013).

13.6 Optimization of Fermentation Condition for Production of Fungal and Bacterial Lipases

The number of available lipases has increased since the 1980s and used as industrial biocatalysts because of their properties like biodegradability (Mauti et al. 2016), high specificity (Das et al. 2016), high catalytic efficiency, temperature, pH dependency, activity in organic solvents (Kumar et al. 2016), and non-toxic nature. The most desired characteristics of the lipase are its ability to utilize all mono-, di-, and triglycerides as well as the free fatty acids in transesterification, low product inhibition, high activity/yield in non-aqueous media, low reaction time, resistance to altered temperature, pH, alcohol and reusability of immobilized enzyme. Additionally, lipases can carry out reactions under mild conditions of pH and temperature and this reduces energy required to direct reactions at unusual temperatures and pressures.

Fungal lipases are active over broad pH and temperature range (Barriuso et al. 2016). They possess stability over a wide range from pH 4.0 to 11.0 and temperature optima in the range from 10 to 96 °C. In addition they catalyze various reactions

since they have ability to act on wide range of substrates that may be artificial or natural (Patil et al. 2011). Fungal lipases are extracellular, and their production is influenced by nutritional and physicochemical factors such as temperature, pH, nitrogen, and carbon sources, and presence of lipids, inorganic salts, agitation rate, and dissolved oxygen concentration.

Production of lipase can be significantly influenced by carbon sources such as sugars, sugar alcohol, polysaccharides, whey, amino acids, and other complex sources (Rashid et al. 2001). Oleic acid (cis-9-Octadecenoic acid) has been reported as the most suitable inducer for the production of the main extracellular Lip2p lipase in *Yarrowia lipolytica* (Fickers et al. 2005). The major factor for the expression of lipase activity has always been carbon source, since lipases are inducible enzymes. Palm oil mill effluent has been used for lipase production by *Candida cylindracea* with an activity of 20.26 U/ml under the optimized conditions (Salihu and Alam 2012).

Various mineral and organic nitrogen sources were tested for their capacity to support cell growth and lipase production (Fickers et al. 2004). Corn steep liquor, yeast extract, and peptone have been reported as best nitrogen sources for lipase production from *Penicillium verrucosum*(Kempka et al. 2008). In order to improve the productivity of lipase from *Rhizopus chinensis*, the effect of oils and oil-related substrates were assessed by orthogonal test and Response Surface Methodology (RSM). The optimized medium for improved lipase activity consisted of peptone, olive oil, maltose, K_2HPO_4, and $MgSO_4{\cdot}7H_2O$ (Wang et al. 2008a). The Plackett–Burman statistical experimental design was used to evaluate the fermentation medium components (Rajendran et al. 2008). The effect of 12 medium components was studied in 16 experimental trials. Glucose, olive oil, and peptone were found to have more significant influence on lipase production by *Candida rugosa*. RSM approach was used to investigate the production of an extracellular lipase from *Aspergillus carneu*s. Interactions were evaluated for five different variables (sunflower oil, glucose, peptone, agitation rate, and incubation period) and 1.8-fold increase in production was reported under optimized conditions (Kaushik et al. 2006). Lipase production was observed in the range of 7.78–6.230 U/ml under various optimized conditions (Yadav et al. 1998; He and Tan 2006).

13.7 Purification Strategies

Many lipases have been extensively purified and characterized in terms of their activity and stability profiles at different pH, temperature, effects of metal ions, and chelating agents. Purification of enzymes allows determination of primary amino acid sequence and 3-D structure (Saxena et al. 2003), and X-ray studies of pure lipases enable the establishment of the structure–function relationships and contribute for a better understanding of the kinetic mechanisms of lipase action on hydrolysis, synthesis, and group exchange of esters (Ghosh et al. 1996). The purification of lipase from different microorganisms has been reported through several

techniques such as precipitation (Borkar et al. 2009), hydrophobic interaction chromatography, gel filtration, ion exchange chromatography (Patil et al. 2016), and affinity chromatography (Yang et al. 2016). Purification of lipase is needed in industries employing the enzymes for the biocatalytic production of fine chemicals, pharmaceuticals, and cosmetics (Metzger and Bornscheuer 2006). During the early stages of a purification method, precipitation was used as a crude separation step and was found to give a high average yield (Aires-Barros et al. 1994).

Lipase from *Penicillium cyclopium* MI was purified by using ammonium sulfate precipitation, Diethylaminoethyl (DEAE) cellulose, DEAE-Sepharose, hydroxyapatite chromatography, and gel filtration on Cellulofine GC-700. The purification of the preparation was 1380-fold and recovery yield 27%. The molecular weight of the enzyme was estimated to be 35,000 g/mol from Sephadex G-100 chromatography (Gombert et al. 1999). The lipase obtained from Trichoderma viride was purified 134-folds with 46% yield by ion exchange and gel permeation chromatography (Kashmiri et al. 2006). A novel thermostable lipase from *Aspergillus niger* was purified from a crude preparation by a procedure including precipitation followed by a series of chromatographic steps. The overall purification was 50-fold with a yield of 10% (Namboodiri and Chattopadhyaya 2000). The lipase from *Rhizopus japonicus* NR400 was purified to homogeneity by chromatography on hydroxyapatite, octyl Sepharose, and Sephacryl S-200 (Ghosh et al. 1996). Fungal and bacterial lipases showed different molecular weights ranging between 25 and 68 kDa (Mozaffar and Weete 1993; Laachari et al. 2015). The highest molecular weight of lipase, i.e., 70 kDa has been reported from thermophilic fungus *Neosartorya fischeri* P1 (Sun et al. 2016).

13.8 Molecular Structure and Functional Determinant of Fungal Lipases

Lipases, the well-known crucial enzymes, are produced by a wide range of organisms in the form of different isoenzymes. In spite of their primary structure diversity, lipases share the exact identical catalytic mechanisms and structural architecture, which is in turn similar to that of the hydrolases' α/β folds. The layout of the enzyme structure is based on the central β sheets, mostly eight, that are predominately parallel except for the antiparallel strand β2. It is also worth noticing, that the strand β3 is connected to strand β8 by packed α-helices on the two sides of the β-sheet. Variations in the enzyme structure can result from different β-sheet number or the presence of certain insertions (Murzin et al. 1995; Heikinheimo et al. 1999; Nardini and Dijkstra 1999). Resolving the3D structures of lipases paved the way for establishing the basic knowledge of the binding site structure of the enzyme.

The active site cavity is buried in the lipase enzyme and composed of a catalytic triad amino acid which includes a nucleophilic residue (serine), an acidic residue (aspartate or sometimes glutamate), and histidine residue, which are comparable to

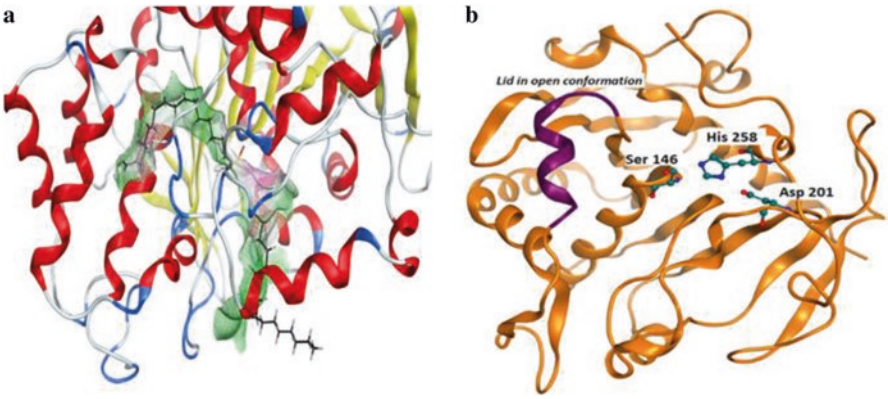


Fig. 13.4 The active site cavity buried, (**a**) in the *Candida rugosa* (PDB: 1LPP) lipase represented by green net, ligands are represented in black color and the protein of the enzyme is shown in ribbon representation, (**b**) Ribbon representation for the open form of *Thermomyces lanuginose*(PDB: 1EIN) which shows the active site and the position of catalytic triad

those found in serine proteases (Fig. 13.4) (Brady et al. 1990; Winkler et al. 1990). The substrate enters to the binding pocket of the enzyme which is located on the top of the central sheet. Although lipases share the exact structural architecture, they are considerably differing in the substrate specificity and the binding site located in the pocket. The structure, length, shape, and hydrophobicity of the binding pocket are the key factors that determine the enzyme activity, substrate specificity, and sterio- and regio-preference (Pleiss et al. 1998; Lotti and Alberghina 2007; Mehta et al. 2017). Even lipase isoenzymes of the same organism can differ in their activity and substrate selectivity. For example in *Candida rugosa* isoforms, the isoenzyme 1 hydrolyzes mainly the substrates with medium chain length (C8-C10), while isoenzymes 2 and 4 hydrolyze the substrates with longer chain length (C16-C18) (Lotti and Alberghina 2007; Mehta et al. 2017).

Most of lipases has a movable flap (Lid domain) located over the active site which protects the active site and the triad amino acids from being exposed to the solvent or being accessible by different substrate while it is in the inactive form (Fig. 13.5) (Schrag and Cygler 1997; Jaeger and Reetz 1998; Schmid and Verger 1998; Jaeger et al. 1999; Brzozowski et al. 2000). This fact does not assume that all lipases with a flap can carry out the activation conversion phenomenon or that all lipases share the existence of a movable flap (Chen et al. 1998; Nardini and Dijkstra 1999). In the area of the active site there are highly fixed water molecules which are conserved (Sussman et al. 1991; Schrag and Cygler 1993) and interacting directly by hydrogen bonds with the catalytic triad (Steitz and Shulman 1982). The lid domain together with the water molecules plays a crucial role in the formation of alternative conformational states. When the enzyme is in the closed state (inactive conformation), the flap is positioned to cover the catalytic tunnel of the enzyme from solvent. On the other hand, when it is in the active form, the flap moves up to afford an active enzyme conformation with the active binding site accessible to

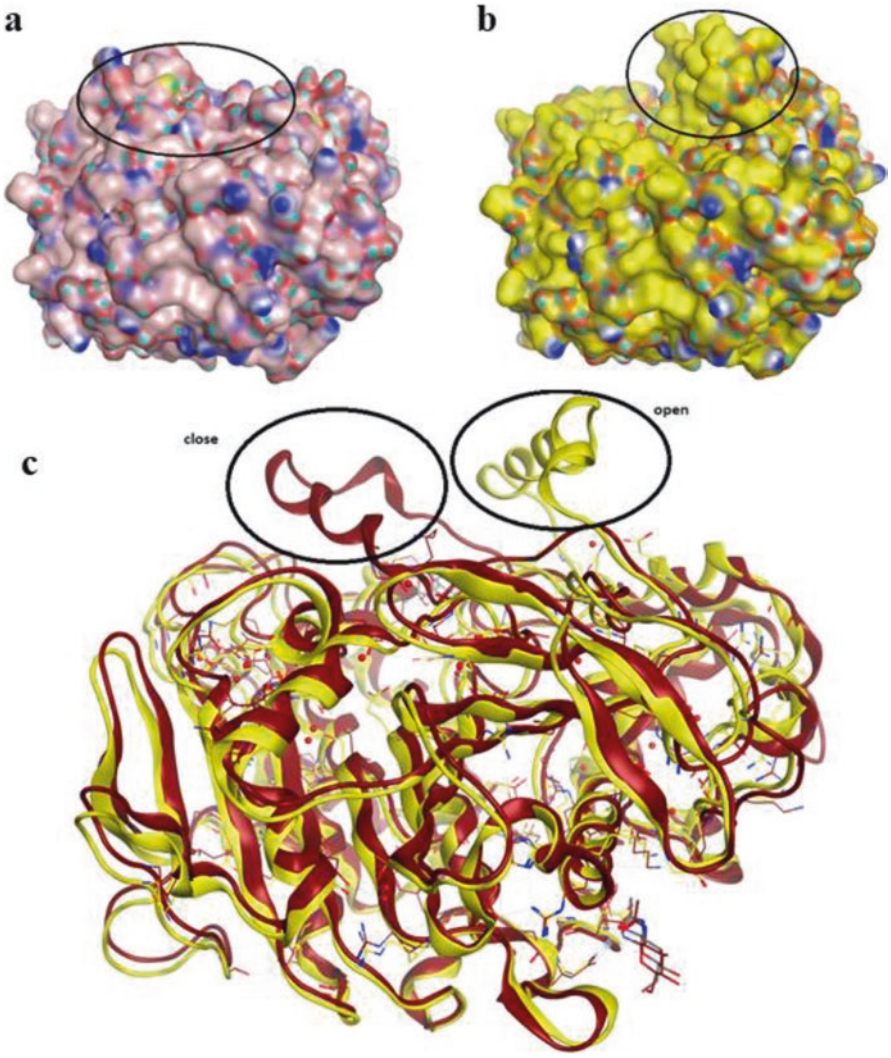


Fig. 13.5 (**a**) Closed conformation of Candida rugosa lipase (PDB: 1TRH), (**b**) open conformation of the same enzyme (PDB: 1CRL), (**c**) shows the position of the lid domain of the enzyme in its closed (dark red) and opened states (yellow)

substrates. Accordingly, the lid domain functions not only as a gate for the active site of the enzyme, but also plays a valuable role in determining the enzyme activity and substrate selectivity.

The opening mechanism of the flap domain differs between lipases, however, in all lipases the flap opening leads to the creation of a hole known as "oxyanaion hole." This anion hole is responsible for stabilization of the hydrolysis reaction intermediate (Brzozowski et al. 1991; Grochulski et al. 1994b).The rational for this

is that lid domain is amphipathic with two faces, one of them is hydrophobic and the other is hydrophilic. When the flap is in active state, the hydrophobic side faces the catalytic domain while the hydrophilic side faces the solvent phase. Changing from the inactive position to the active position will lead to exposure of the inner hydrophobic side more towards outside, leading to extension of the hydrophobic catalytic domain (Carrière et al. 1998).

Fungal lipases are one of seven families of lipases based on the elements of the basic fold that they contain. The other six families are acetyl cholinesterase-like, gastric lipase, lipase, fungal lipase, bacterial lipase, pancreatic lipase N-terminal domain, and cutinase-like (Lotti and Alberghina 2007). In this part, we will mainly focus on the known 3D structures of different fungal lipases available on protein data bank (PDB) (Table 13.3).

Table 13.3 The known 3D structures of different fungal lipases available on protein data bank (PDB)

Organism	PDB code	Title	Ligands	Reference
Candida rugosa	**1LPP**	*Analogues of reaction intermediates identify a unique substrate binding site in Candida rugosa lipase.*	1-Hexadecanesulfonic acid	Grochulski et al. (1994a)
	1LPO	*Analogues of reaction intermediates identify a unique substrate binding site in Candida rugosa lipase.*	1-Hexadecanesulfonic acid	
	1LPN	*Analogues of reaction intermediates identify a unique substrate binding site in Candida rugosa lipase.*	1-Dodecanesulfonate	
	1TRH	*Two conformational states of Candida rugosa lipases.*	–	Grochulski et al. (1994b)
	1GZ7	*Crystal structure of the closed state of lipase 2 from Candida rugosa.*	–	Mancheño et al. (2003)
	1CRL	*Insights into interfacial activation from an "open" structure of Candida rugosa lipase.*	–	Grochulski et al. (1993)
	3RAR	*X-ray structure of a bound phosphonate transition state analogue and enantioselectivity of Candida rugosa lipase toward chiral carboxylic acids.*	–	Colton et al. (2011)

(continued)

Table 13.3 (continued)

Organism	PDB code	Title	Ligands	Reference
	1LPM	*A structural basis for the chiral preferences of lipases*	(1*R*)-Menthyl hexyl phosphonate	Cygler et al. (1994)
	1LPS	*A structural basis for the chiral preferences of lipases.*	(1*S*)-Menthyl hexyl phosphonate	Cygler et al. (1994)
Candida antarctica	**5A6V**	*Open and closed conformations and protonation states of Candida antarctica lipase b: xenon complex.*	Xenon: complexed with the protein	Stauch et al. (2015)
	5A71	*Open and closed conformations and protonation states of Candida antarctica lipase b: atomic resolution native.*	–	
	1TCC	*The sequence, crystal structure determination, and refinement of two crystal forms of lipase b from Candida antarctica.*	–	Uppenberg et al. (1994)
	1TCB	*The sequence, crystal structure determination, and refinement of two crystal forms of lipase b from Candida antarctica.*	–	
	1TCA	*The sequence, crystal structure determination and refinement of two crystal forms of lipase b from Candida antarctica.*		
	2VEO	*X-ray structure of Candida antarctica lipase a in its closed state.*	–	Ericsson et al. (2008)
	6TP8	*Substrate protein interactions in the limbus region of the catalytic site of Candida antarctica lipase b.*	2,3-Di-(butanoyloxy)propyl butanoate	Silvestrini and Cianci (2020)
	3ICV	*Structural consequences of a circular permutation on lipase b from Candida antarctica.*	–	Qian et al. (2009)

(continued)

Table 13.3 (continued)

Organism	PDB code	Title	Ligands	Reference
	3ICW	*Structure of a circular permutation on lipase b from Candida antarctica with bound suicide inhibitor.*	–	
	3GUU	*X-ray structure of Candida antarctica lipase a*	–	–
	4ZV7	*Crystal structure of hexagonal form of lipase B from Candida antarctica.*	–	Strzelczyk et al. (2016)
	1LBT	*Lipase (e.c.3.1.1.3) (triacylglycerol hydrolase).*	Methylpenta(oxyethyl) heptadecanoate	Uppenberg et al. (1995)
	1LBS	*Lipase (e.c.3.1.1.3) (triacylglycerol hydrolase).*	*N*-Hexylphosphonate ethyl ester	
	4K5Q	*Crystal structure of CALB mutant DGLM from Candida antarctica.*	–	Xie et al. (2014)
	4K6G	*Crystal structure of CALB from Candida antarctica.*	–	
	4K6H	*Crystal structure of CALB mutant L278M from Candida antarctica.*		
	4K6K	*Crystal structure of CALB mutant D223G from Candida antarctica.*		
	3W9B	*Crystal structure of Candida antarctica lipase B with anion-tag.*	3,6,9,12,15,18,21-Heptaoxatricosane-1,23-diol	–
	5GV5	*Crystal structure of Candida antarctica lipase B with active ser105 modified with a phosphonate inhibitor*	(1*S*)-2-(methoxycarbonyl-amino)-1-phenyl-ethoxy-propyl-phosphinic acid	Park et al. (2016)
	6ISP	*Structure of Candida antarctica lipase B mutant.*	*N*,*N*-Bis(3-*D*-gluconanidopropyl)-deoxychloamide	Cen et al. (2019)
	6ISR	*Structure of lipase mutant with CYS-HIS-ASP catalytic triad.*	–	

(continued)

Table 13.3 (continued)

Organism	PDB code	Title	Ligands	Reference
	6ISQ	*Structure of lipase mutant with oxidized CYS-HIS-ASP catalytic triad.*	–	
	6J1P	*Crystal structure of Candida antarctica lipase B mutant—SR.*	–	Xu et al. (2019)
	6J1R	*Crystal structure of Candida antarctica lipase B mutant—RR.*		
	6J1Q	*Crystal structure of Candida antarctica lipase B mutant—RS.*		
	6J1T	*Crystal structure of Candida antarctica lipase B mutant SR with product analogue.*	(2*S*)-2-phenyl-N-[(1*R*)-1-phenylethyl] propanamide	
	6J1S	*Crystal structure of Candida antarctica lipase B mutant—SS.*	–	
Thermomyces lanuginosa	**1GT6**	*S146A mutant of Thermomyces (humicola) lanuginosa lipase complex with oleic acid.*	Oleic acid	Yapoudjian et al. (2002)
	1DU4	*The structural origins of interfacial activation in Thermomyces (humicola) lanuginosa lipase other structure details.*	–	Brzozowski et al. (2000)
	1DT3	*The structural origins of interfacial activation in Thermomyces (humicola) lanuginosa lipase.*	–	
	1DTE	*The structural origins of interfacial activation in Thermomyces (humicola) lanuginosa lipase.*	–	
	1DT5	*The structural origins of interfacial activation in Thermomyces (humicola) lanuginosa lipase.*	–	

(continued)

Table 13.3 (continued)

Organism	PDB code	Title	Ligands	Reference
	1EIN	*The structural origins of interfacial activation in Thermomyces (humicola) lanuginosa lipase.*	Di-undecylphosphatidyl choline	
	4ZGB	*Structure of untreated lipase from Thermomyces lanuginosa at 2.3 a resolution.*	–	Kumar et al. (2015)
	4FLF	*Structure of three phase partition treated lipase from Thermomyces lanuginosa at 2.15a resolution.*	–	–
	4GLB	*Structure of p-nitrobenzaldehyde inhibited lipase from Thermomyces lanuginosa at 2.69 A resolution.*	–	–
	4EA6	*Crystal structure of fungal lipase from Thermomyces(humicola) lanuginosa at 2.30 angstrom resolution.*	–	–
	4GI1	*Structure of the complex of three phase partition treated lipase from Thermomyces lanuginosa with 16-hydroxypalmitic acid at 2.4 A resolution.*	16-hydroxyhexadecanoic acid	–
	4GWL	*Structure of three phase partition treated lipase from Thermomyces lanuginosa at 2.55A resolution.*	–	–
	4GBG	*Crystal structure of ethyl acetoacetate treated lipase from Thermomyces lanuginosa at 2.9 A resolution.*	–	–

(continued)

Table 13.3 (continued)

Organism	PDB code	Title	Ligands	Reference
	4GHW	*Crystal structure of the complex of fungal lipase from Thermomyces lanuginosa with decanoic acid at 2.6 A resolution.*	–	–
	4KJX	*Crystal structure of the complex of three phase partition treated lipase from Thermomyces lanuginosa with lauric acid and p-nitrobenzaldehyde (pnb) at 2.1 resolution.*	–	–
	4N8S	*Crystal structure of the ternary complex of lipase from Thermomyces lanuginosa with ethyl acetoacetate and p-nitrobenzaldehyde at 2.3 A resolution.*	–	–
	4S0X	*Structure of three phase partition—treated lipase from Thermomyces lanuginosa in complex with lauric acid at 2.1 A resolution.*	–	–
	6OR3	*Structure of an acyl intermediate of Thermomyces lanuginosa lipase with palmitic acid in an orthorhombic crystal.*	Palmitic acid	–
	4DYH	*Crystal structure of glycosylated lipase from Humicola lanuginosa at 2 angstrom resolution.*	–	–
	1TIB	*Conformational lability of lipases observed in the absence of an oil–water interface: crystallographic studies of enzymes from the fungi Humicola lanuginosa and Rhizopus delemar.*	–	Derewenda et al. (1994b)

(continued)

Table 13.3 (continued)

Organism	PDB code	Title	Ligands	Reference
	6HW1	*Room temperature structure of lipase from T. Lanuginosa at 2.5 A resolution in chipx microfluidic device.*	–	De Wijn et al. (2019)
	5AP9	*Controlled lid-opening in Thermomyces lanuginosus lipase—a switch for activity and binding.*	–	Skjold-Jørgensen et al. (2017)
Rhizomucor miehei	**3TGL**	*Structure and molecular model refinement of Rhizomucor miehei triacylglyceride lipase: a case study of the use of simulated annealing in partial model refinement.*	–	Brzozowski et al. (1992)
	4TGL	*Catalysis at the interface: the anatomy of a conformational change in a triglyceride lipase.*	–	Derewenda et al. (1992)
	5TGL	*Catalysis at the interface: the anatomy of a conformational change in a triglyceride lipase.*	*N*-hexylphosphonate ethyl ester	
	6QPP	*Rhizomucor miehei lipase pro-peptide complex, native.*	–	Moroz et al. (2019)
	6QPR	*Rhizomucor miehei lipase pro-peptide complex, ser95/ile96 deletion mutant.*	–	
Rhizopus niveus	**1LGY**	*Lipase ii from Rhizopus niveus.*	–	Kohno et al. (1996)
Rhizopus microsporus var. chinensis	**6A0W**	*Crystal structure of lipase from Rhizopus microsporus var. chinensis.*	–	Zhang et al. (2019)

(continued)

Table 13.3 (continued)

Organism	PDB code	Title	Ligands	Reference
Rhizopus oryzae	**1TIC**	*Conformational lability of lipases observed in the absence of an oil–water interface: crystallographic studies of enzymes from the fungi Humicola lanuginosa and Rhizopus delemar.*	–	Derewenda et al. (1994b)
Geothricum candidum	**1THG**	*1.8 angstroms refined structure of the lipase from Geotrichum candidum.*	–	Schrag and Cygler (1993)
Penicillium camembertii	**1TIA**	*An unusual buried polar cluster in a family of fungal lipases.*	–	Derewenda et al. (1994a)
Penicillium expansum	**3G7N**	*Crystal structure of a triacylglycerol lipase from Penicillium expansum at 1.3.*	–	Bian et al. (2010)
Rasamsonia emersonii	**6UNV**	*Crystal structure of a methanol tolerant lipase/esterase from the fungus Rasamsonia emersonii.*	–	Rade et al. (2020)

The lipases have been recently classified into three main classes (GX, GGGX, and Y) based on the structure and orientation of oxyanion hole (Fischer and Pleiss 2003). According to this classification and based on similarities in the amino acid sequence, the fungal lipases have been assembled to five subclasses; one in Y class, two in GGGX class, and two in GX class (Gupta et al. 2015). Fungal lipases can have different enzyme isoforms ranging from one to five. Isoenzymes are related proteins that produced by the same organism and are closely related to biophysical properties and amino acid sequence, but greatly differ in substrate and reaction specificity, pH and temperature stability, and molecular mass.

An example is *Candida rugosa* which is known to produce the whole five isoenzymes. Although those five isoenzymes share very similar sequences, ranging from 77% to 88% for pairs of proteins, they show some specificity towards different substrates (Lotti et al. 1994). Lipase isoforms 2 and 3 can hydrolyze cholesterol esters, while isoform 1 is inactive to such substrates. The selectivity difference was considered to be a consequence of the differences found in the lid domains of the three isoforms of the enzyme (Brocca et al. 2000). The resolved structure of the lipase isoenzymes showed that the lid domain of lipase isoform 2 is more hydrophobic in comparison to isoforms 1 and 3 and differs in 11- and 9- positions (out of 30)

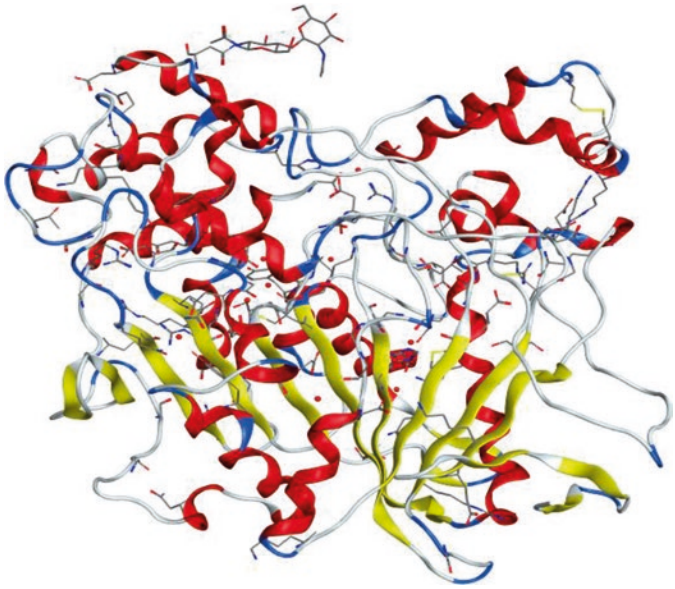


Fig. 13.6 Ribbon representation of Candida rugosa lipase 2 (PDB: 1GZ7) that shows the 11 β-sheets in yellow, α-helices in red and coils in white

(Grochulski et al. 1994b). The gene coding analysis showed a 534 amino acid polypeptide chain each, with molecular mass prediction of 60 kDa (Kawaguchi et al. 1989; Longhi et al. 1992; Lotti et al. 1993; Brocca et al. 1995).

The 3D structure of *Candida rugosa* lipase 2 (*CRL2*) consists of a core of 11-stranded mixed β-sheets, a small and nearly perpendicular N-terminal three-stranded β-sheets, and 16 α-helices with an outstanding twist in the central β-sheets (Fig. 13.6) (Mancheño et al. 2003).

The catalytic domain of the enzyme composed of a triad residue from Ser209, Glu341, and His449 with three water molecules. Two molecules of the water form hydrogen bonds with the main-chain nitrogen atoms of Glu341 and His449, while the last molecule forms hydrogen bonds with the side chain of Glu 208 and the main-chain nitrogen atom of Gly122. The lid domain composed of (65–94) residues with a conformationally restricted stem region due to the presence of a disulfide bridge (Cys60–Cys97) and an ionic interaction between Glu96 and Arg37. In addition, van der Waals interactions are formed between 9 residues of the internal side of the flap domain and the hydrophobic residues, which lead to the formation of a hinge point for the opening of the lid domain (Grochulski et al. 1994b).

To date, two lipases for filamentous *Candida antarctica* (*CalA* and *CalB*) have been distinguished (Høegh et al. 1995). Interestingly, *CaIA* lipase was found to be able to maintain its hydrolytic activity under extreme conditions and showed exceptional substrate selectivity by accepting tertiary alcohols unlike other lipases. Based on these unique properties, the sequence of *CaIA* lipase was intensively studied

which revealed that the *CaIA* primary structure is highly different than other lipases (Whitaker and Sonnet 1989; Domínguez De María et al. 2005). The active triad was found to be formed by Ser 184, Asp 334, and His 366 residues, while the lid domain is formed by the residues Gly426 to Gly436 (Schmid and Verger 1998; Ericsson et al. 2008).

The resolved structure of *Thermomyces lanuginosa* lipase showed that the catalytic site composed of a flap domain which contains (86–92) residues, and a catalytic triad from Ser146-His285-Asp201 residues. Importantly, the lid domain has a Trp89 residue which plays a significant role in the formation of a transition state with the substrate which helps the substrate to reach the catalytic tunnel beneath the flap (Lawson et al. 1994; Brzozowski et al. 2000; Yapoudjian et al. 2002).

The 3D structure of *Rhizomucor miehei* lipase showed a catalytic triad of Ser238, Asp297, and His351 residues and a flap domain formed by (178−186) residues. In a study by Moroz et al., the pro-peptide structure of the *Rhizomucor miehei* lipase enzyme was described which revealed the inactivity of the enzyme during its expression phase and the conversion of the pro-peptide to a mature active enzyme (Moroz et al. 2019).

For *Rhizopus niveus*, only one PDB code could be found for *Rhizopus niveus* fungal lipase-2. This lipase protein consists of a single 269 amino acid polypeptide chain, which lacks the A-chain and 28 N-terminal amino acids of the B-chain that found in the isoenzyme lipase-1. The catalytic domain of the fungal lipase-2 contains a catalytic triad residues of Serl45, Asp204, and His257, while the lid domain consists of (86–92) residues (Kohno et al. 1996). The crystal structure of *Rhizopus microsporus var. chinensis* lipase showed that the residues from Gly109 to Thr123 form the flag domain with its short α-helix (Phe113−Asp119) linked to the protein core. Additionally, the residues Ser172, His284, and Asp231form the catalytic triad in the active center of enzyme (Zhang et al. 2019). Noteworthy, the lipase structure contains a N-terminal polypeptide segment (NTPS) which showed to be crucial for the secretion, folding, and catalytic properties of *Rhizopus* lipase (Zhang et al. 2019).

Rhizopus oryzae fungal lipase, also named *Rhizopus delemar*, shares a 3D structure similar to that of *Rhizopus miehei*. The catalytic center of these lipases consists of a triad from Ser145, His258, and Asp204 residues. Additionally, the lid domain has two hinge regions, one N-terminal region (residues 83–843), and one C-terminal region (residues 91–95) with a six-residue-long region (85–90) (Derewenda et al. 1994b).

The 3D structure of *Geotricum candidum* lipase enzyme was one of the first three lipases that could be resolved by crystallography. The catalytic triad has glutamic acid at position 354 rather than aspartic acid, besides the other two residues (Serine 217 residue which locates approximately at 18 Å from the surface of the enzyme and Histidine 463 residue). Interestingly, Schrag*et al.* reported a second triad in the *Geotricum candidum* enzyme which consists of Ser 449, His 451, and Asn 465 residues buried away from surrounding solvent (Schrag et al. 1991). However, this triad does not have any lipolytic activity (Schrag and Cygler 1993).

In an interesting study, the sequence of *Penicillium camemberti* lipase was compared to that of *Humicola lanuginosa* lipase and it was found that they share 41%

identical amino acids (Derewenda et al. 1994a). Moreover, the active site of *Penicillium Expansum* lipase was found to have a catalytic triad of amino acid residues (Ser 132, Asp 188 and His 241)that is spatially arranged and superimposed with that of *Rhizomucor miehei* lipase. Bian et al. showed that the oxyanion hole of *Penicillium Expansum* is formed by the two amide groups found in residues 130 and 131 and the flap region contains (68–76) residues (Bian et al. 2010). Recently, the 3D structure of *Rasamsonia emersonii* fungal lipase was resolved which revealed a catalytic triad of Ser 177, His 290, and Asp 232 residues (Rade et al. 2020).

As described before, lipases have a wide diversity of substrate selectivity ranging from aliphatic esters and aromatic esters to thioesters and activated amines. Owning to their stability in most of organic solvents, lipases have been exploited in the organic synthesis such as aminolysis, esterification, and transesterification (Schmid and Verger 1998; Bornscheuer et al. 2002; Jaeger and Eggert 2002; Kirk et al. 2002; Gupta et al. 2004). Among different lipase sources, fungal lipases have been considered as the best lipase source due to the broad range of their enzymatic activities, substrate selectivity, and stability under different chemical and physical conditions (Facchini et al. 2016). Such unique properties make fungal lipases as key biocatalysis in the industrial applications (Hasan et al. 2006; Kapoor and Gupta 2012; Thakur 2012; Ray 2015)and for synthetic application (Azim et al. 2001). Based on this knowledge, the scientific efforts were dedicated to improve the lipase properties or to find novel lipases which meet the industrial requirements and laboratory use.

The molecular basis (amino acid sequence, 3D structure) of the catalytic site of lipases can have an effect on the efficiency, stability, and specificity of lipases. Accordingly, novel techniques were developed, such as molecular modeling and protein engineering, in order to modulate the catalytic behavior of lipases. The protein engineering technique based on creation of libraries of random mutants in the enzyme followed by selection of variants with improved properties (specificity, enantioselectivity, or catalytic efficiency). These variants are then used in more rounds of mutagenesis to finally obtain new lipases with the required activity for their applications (Klein et al. 1997; Rotticci et al. 2001).

More recently, the molecular modeling technique has been applied to improve the activity and property of lipases. This technique has been used to predict the 3D structure of novel lipases with unknown crystal structure by homology modeling and to predict the enzyme-substrate binding activity by *in silico* methods (Vasel et al. 1993). Compared to protein engineering technique, this technique has an advantage of being lower in costs, easy in handling, and provides the required information about the molecular structure of protein. Indeed, the molecular docking methods have been used to predict the lipase activity, specificity, and substrate selectivity. Based on this bioinformatics approach, several mutant enzymes can be created by introducing bulkier or more hydrophilic residues at different sites of lipases and the activity of created proteins is then screened by molecular docking for better interaction with a specific substrate (Tang et al. 2014).

This approach can be applied in industry for removing of pollutants and esters (Ericsson et al. 2008). Other approach for molecular modeling is based on resolved

3D structure of the enzyme, which can be used to screen a library of substrates to find a novel application of lipases.

Since the structure of many fungal lipases has not been resolved yet, a homology modeling approach has been applied to predict the 3D structure of the enzyme. The obtained predicted structure can be then used to investigate the interaction of the substrates with the modeled structure by molecular docking. This approach has intensively helped to find new applications for the lipases of unknown crystal structure and to predict the lipase activity on several substrates (Moya-salazar et al. 2019). Future work should be directed to use the molecular modeling techniques in order to improve the lipase properties and to find new substrates for the known lipases. This would definitely highlight the industrial and laboratory applications of lipases.

13.9 Medicinal Applications of Lipases

Lipases may be used as digestive aids and as the activators of Tumor Necrosis Factor, and therefore, can be used in the treatment of malignant tumors. Although human gastric lipase (HGL) is the most stable acid lipase and constitutes a good candidate tool for enzyme substitution therapy, lipases have earlier been used as therapeutics in the treatment of gastrointestinal disturbances, dyspepsias, cutaneous manifestations of digestive allergies, etc. (Ville et al. 2002).

13.9.1 Lipase in Fat Control (Obesity and Cholesterol)

Coronavirus disease 2019 (COVID-19), the worst pandemic in current century, has claimed >125,000 lives worldwide so far. There are many risk factors that would eventually put lives at risk with such virus, one of them is obesity. Being obese can increase risk of developing many potentially serious health conditions, including type 2 diabetes, hypertension, high cholesterol, and atherosclerosis, which might result in coronary heart condition and strokes. Additionally, studies proved that adipose tissue in individuals with obesity may act as a reservoir for more extensive viral spread, with increased shedding, immune activation, and cytokine amplification (Ryan and Caplice 2020).

Lipase is anticipated to attain a sales increase of roughly 6.8% by 2024. A serious area of the lipase market is alleged to the treatment of obesity, which is becoming a replacement challenging issue in developed countries. Due to its ability to break down fats into glycerol and free fatty acids under natural conditions, it is expected that there will be an increasing demand within the healthcare industry as a tool for weight management and management of overweight people (Ahuja and

Rawat 2019). Pancreatic enzymes like amylase, proteases, and lipase are developed as common digestive aids for people with weakened immune systems, for its effective role in reducing the danger of obesity in patients with HIV (Sarkissian et al. 1999; Carroccio et al. 2001). This enzyme is additionally useful within the treatment of pancreatic insufficiency in patients with chronic heart failure. A cocktail of pancreatic enzymes called "TheraCLECTotal™" is on the market (Meghwanshi et al. 2020).

Lipases from *Candida Rugosa* are accustomed to synthesize lovastatin, a drug that reduces serum cholesterol level. *S. marcescens* lipase was widely used for the asymmetric hydrolysis of 3-phenylglycidic acid ester which is essential intermediate within the synthesis of diltiazem hydrochloride (Matsumae et al. 1993). The lipase level in bodily fluid may be a diagnostic indicator for conditions like acute pancreatitis and pancreatic injury (Gutman et al. 1990). Lipase activity/level determination is additionally important within the diagnosis of heart ailments (Sharma and Kanwar 2014).

13.9.2 Lipases as Diagnostic Tool

A biosensor could be a combination of a biological component with a physicochemical detector and it helps in analysis of biomolecular interactions (Gopinath et al. 2013). Quantitative determination of triacylglycerol contains a great significance in clinical diagnosis and within the food industry. The utilization of lipid sensing device as a biosensor is a way cheaper and not as time consuming as compared to the chemical methods for the determination of triacylglycerol. Biosensors are three types: (a) chemical, (b) biochemical, or (c) electronic. Biochemical biosensor uses the enzymes or other proteins (antibodies), cells, or cell extract immobilized on an appropriate matrix linked to a transducer. An analytical biosensor was developed to work out of lipids for the clinical diagnosis (Masahiko et al. 1995). In quantitative determination lipases are accustomed to generate glycerol from triacylglycerol within the analytical sample and to quantify the released glycerol by enzymatic or chemical methods. This theory allowed diagnosing the patients with cardiac complaint. *Candida rugosa* lipase biosensor has been developed as a DNA probe (Salihu and Alam 2012).

Enantioselective interesterification and transesterification reactions by the assistance of lipases have great impact on pharmaceutical industry for selective acylation and deacylation reactions. The lipase concentration in humor will be used as a diagnostic tool to detect conditions like acute pancreatitis and pancreatic injury (Nagar et al. 2013). Lipases play a crucial role in improvement of monoglycerides to be used in emulsifiers in pharmaceutical applications (Sharma et al. 2001).

13.9.3 Lipases in Cosmetics

Lipases have potential applications in cosmetics and private care as perfumes because they exhibit activities in surfactants and in aroma production (Metzger and Bornscheuer 2006). Monoacylglycerols and diacylglycerols are produced by esterification of glycerols and are used as surfactants in cosmetics and perfume industries. Production of flavors by transesterification and therefore the resolution of racemic intermediates by lipases improved the cosmetic and perfume industry. Lipases produced by *Pseudomonas cepacia* are accustomed resolve the racemic rose oxides produced by the bromomethoxylation of citronellol (Taneja et al. 2005). Methyl butyrate (MB) or methyl ester of saturated fatty acid is an ester with a fruity odor of pineapple, apple, and strawberry (Garlapati and Banerjee 2013). Esters of aliphatic and aromatic acids and alcohols including terpene alcohols, aldehydes, and phenols are commonly present within the flavor materials employed in perfumes and other attention products (Franssen et al. 2005).

Retinoids (vitamin A and derivatives) are of great commercial potential in cosmetics like skin care products. Water-soluble retinol derivatives were prepared by catalytic reaction of immobilized lipase (Maugard et al. 2002). Esters of cinnamic acid, ellagic acid, ferulic acid, and organic compounds of biotechnological relevance would be properly modified as flavor/fragrance compounds, precursors of pharmaceuticals, and as additives in foods, cosmetics, and sunscreens (Chandel et al. 2011). Ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) the foremost abundant derivative of cinnamic acid is found in higher plants. Ester linked to cytomembrane constituents especially to arabinoxylans and lignins. Maximum UV absorption at 322 nm falls between UVB and UVA region and used as UV-absorbing substance for skin protection against sunlight (Kumar and Kanwar 2011).

13.9.4 Lipase CalB

13.9.4.1 Lipase CalB as Biocatalyst for Vitamin C Esters

Ascorbic acid esters L-ascorbic acid (vitamin C), is the major water-soluble natural antioxidant. Acting as an atom scavenger, L-ascorbic acid and its derivatives react with oxygen, thus removing it during a closed system. Esters of L-ascorbic acid with long-chain fatty acids (E-304) are employed as antioxidants in foods rich in lipids thanks to their solubility in fats compared to water-soluble vitamin (insoluble in oils) (Reyes-Duarte et al. 2011). *Candida antarctica* (CalB), the immobilized lipase B described by biocatalytic methods as biocatalyst and free fatty acids or activated esters as acyl donors (Burham et al. 2009). The biocatalytic conversion is able to do levels of approx. 95% depending on the operating temperature, the efficiency of the side product (water) removal, and therefore the length of the carboxylic acid.

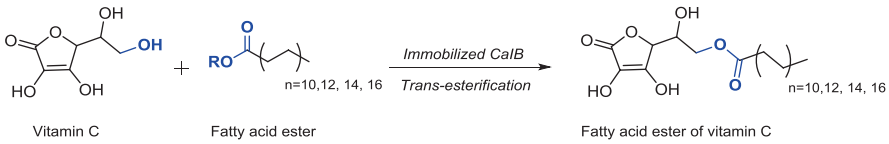


Fig. 13.7 Manufacture of vitamin C fatty acid ester by transesterification catalyzed by immobilized CalB (Basso and Serban 2019)

Although enzymatic synthesis offers some advantages compared with the present chemical processes, most of the assembly of ascorbyl esters is performed by chemical synthesis (Fig. 13.7).

13.9.4.2 Lipase CalB as a Biocatalyst for Omega-3 Esters

Polyunsaturated fatty acids (PUFA) like omega-3 fatty acids are essential for daily life and performance. For instance, the beneficial effects of omega-3 fatty acids like m-5,8,11,14,17-eicosapentaenoic acid (EPA) and c/s-4,7,10,13,16,19-docosahexaenoic acid (DHA) on lowering serum triglycerides are now well established. These compounds also are known for other cardioprotective benefits (Hooper et al. 2004).

Omega-3 fatty acids are often derived from marine fish, fungal, bacterial and/or algal oils. Such sources typically provide the PUFA during a triglyceride form where other undesired, fatty acids (e.g., saturated fatty acids) are present alongside a desired PUFA within the triglyceride molecule. The food industry is focusing efforts on modifying the composition of omega-3 fish oils to boost organoleptic and bioavailability properties. PronovaBioPharmaNorge AS reported on a process for separating ethyl ester EE fractions enriched in EPA (eicosapentaenoic acid, C20:5) from free carboxylic acid fraction (FFA) enriched in DHA (docosahexaenoic acid, C22:6) by direct esterification of animal oil free fatty acids with ethanol using immobilized CalB followed by distillation (Gudmundur et al. 2003).

To an answer containing 14% EPA and 15% DHA, three equivalents of ethanol were added and mixed at 40 °C within the presence of 50 w/w immobilized CalB on styrene/divinyl benzene (Novozym 435) as biocatalyst. Over 78% conversion of EE and 22% remaining FFA were reached after 4 h reaction, and therefore the residual free fatty acids were increased to contain 49% DHA and 6% EPA. Ocean Nutrition Canada Ltd. reported a technique of modification of omega-3 fish oils using immobilized lipase from TL (E.C. 3.1.1.3.) for hydrolysis of the glyceride, followed by a separation of the free saturated carboxylic acids FFA from the glycerides and a final enzymatic esterification of the hydrolyzed glyceride by immobilized CalB with a polyunsaturated fatty acid in water-free medium (Kralovec et al. 2009). The entire process is meant to extend the concentration of polyunsaturated carboxylic acid in an oil composition.

13.9.4.3 Lipase CalB in Sofosbuvir Synthesis

Hepatitis C is a communicable disease caused by hepatitis C virus (HCV), a member of the hepacivirus genera within the Flaviviridae family. Hepatitis C is the leading explanation for chronic disease worldwide (Boyer and Marcellin 2000). The infection is commonly asymptomatic, but its chronic infection can result in the scarring of the liver and eventually, to cirrhosis, which is usually apparent after a few years. In some cases, liver cirrhosis can change into liver failure, cancer, esophageal and gastric varices. HCV is transmitted primarily by direct contact with infected blood. Hence, the interest in developing improved methods of treating viral hepatitis will overcome the high mutagenicity of HCV and also the presence of various genotypes and subtypes (Domingo et al. 1995; Fukumoto et al. 1996).

In 2014, Gilead patented a compound that inhibits the hepatitis C virus NS3 protease, which has the good advantage of inhibiting multiple genotypes of the hepatitis C virus (Yang 2014; Cagulada et al. 2015; Bjornson et al. 2016). This compound, commercialized as Sofosbuvir, was reported with a replacement crystalline formulation, and employed for the treatment of HCV infection and therefore the related symptoms (Martin et al. 2016). Sofosbuvir could be a large molecule with a synthesis that needs many chemical steps, and immobilized CalB is employed within the patent from Gilead within the process for the enantioselective hydrolysis of an acetate ester into chiral alcohol (Fig. 13.8). The CalB immobilized on divinyl benzene/methacrylate polymer (Novozym 435) was utilized in MTBE saturated with aqueous 0.1M phosphate buffer pH 7 at a temperature of 10 °C; the conversion of the racemate to the specified enantiomer is sort of 40%.

Later in 2014, Chemelectiva-HC-Pharma reported another biocatalytic process to manufacture an intermediate of Sofosbuvir. The method involves the utilization of immobilized lipase CalB but the catalyzed reaction interestingly is on a unique position of the molecule. Immobilized CalB is employed for a regioselective monodeacetylation of Sofosbuvir intermediate in polar protic organic solvent to provide the corresponding alcohol at 60 °C (Fig. 13.9).

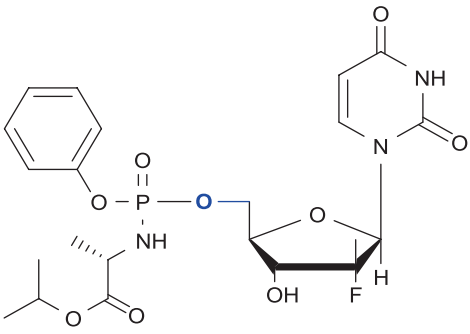


Fig. 13.8 Chemical structure of Sofosbuvir molecule, inhibitor of hepatitis C virus NS3 protease (Basso and Serban 2019)

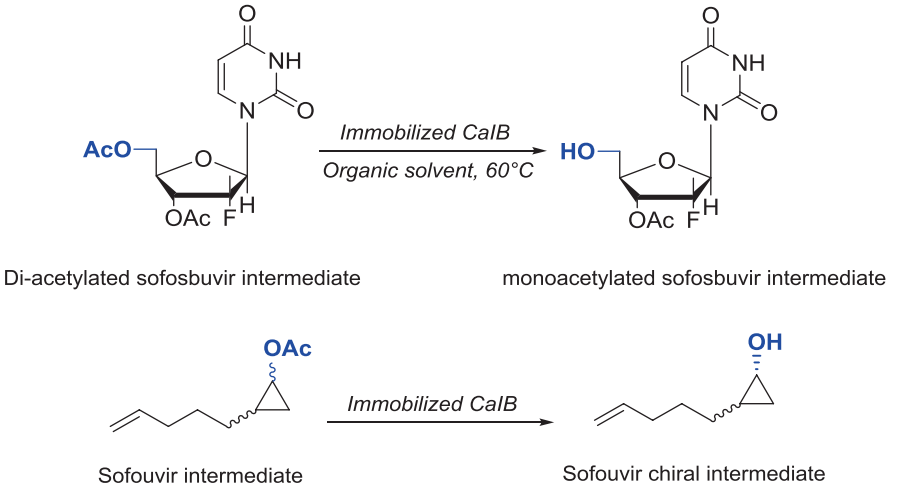


Fig. 13.9 Selective mono-deacetylation of a Sofosbuvir catalyzed by immobilized CalB in protic organic solvent to give the corresponding alcohol (Basso and Serban 2019)

13.9.5 Endophytic Fungi Pharmaceutical Lipases

Abdel-Azeem et al. (2015) recovered twenty one endophytic fungal species, isolated from medicinal plants (*Achillea fragrantissima*, *Artemisia herba-alba*, *Chiliadenusmontanus*, *Origanum syriacum*, *Teucrium polium*, *Tanacetumsinaicum*) collected from Saint Katherine Protectorate and screened for extracellular enzymes such as amylase, cellulase, chitinase, esterase, laccase, lipase, pectinase, protease, and tyrosinase on solid media. Sixty one percent of fungi (13 isolates) screened for enzymes showed positive for amylase, 92% for cellulase, 30% chitinase, 23% esterase, 53% laccase, 46% lipase, 53% pectinase, 76% protease, and only 30% showed positive for tyrosinase.

Their work investigated the powerful capability of the Egyptian endophytic fungi to produce a large number of pharmaceutical and industrial enzymes and more investigation and production on commercial scale are urgently needed. Attia et al. (2020) recovered eleven teleomorphic species of fungi from four medicinal plant species. *Chaetomium grande* and *Sordaria fimicola* were the most frequently isolated species and represented by 12 (Chg1-Chg12) and 7 (Sf1-Sf7) isolates, respectively, in their study. Enzymatically, Chg5 isolate considered as a resource of amylase, cellulase, protease, lipase, and chitinase. However, Sf3 isolates considered as a resource of amylase, laccase, and chitinase out of six screened enzymes during their study.

13.9.6 Leishmanicidal and Fungicidal Lipases

Leishmaniasis is a spectrum of disease caused by protozoan parasites belonging to the genus Leishmania. There are twenty pathogenic Leishmania species for human. They can be transmitted by the bit of an infected female sand fly bringing parasites into the host. There are thirty sand fly species proven vectors. They become infected when they take a blood meal from a reservoir host. Hosts are infected humans, wild and pet animals. The clinical manifestations of the disease depend on the infecting species (Handman 1999, 2001). Leishmaniasis presents in three main clinical forms which have devastating consequences. In visceral Leishmaniasis (VL or "kala-azar"), the parasites reside in the liver, spleen, and bone marrow causing a severe systemic disease which is fatal if not treated (Van Griensven et al. 2014).

In struggle against Leishmaniasis, natural products and biomolecules are important sources of narrative therapeutic agents. Idris (2012) studied secondary metabolites from endophytic fungi as a source of effective anti-leishmanial agents. Several crude extracts from endophytic fungi have been screened for anti-parasitic activity against and *Plasmodium falciparum* and *Leishmania donovani*. The active samples were further evaluated according to their toxicity versus mammalian cell lines. Eventually, DC401 was introduced as a promising source of new anti-parasitic compound. Another study described the manufacturing of lipases from endophytic fungi, *Vermisporium* such as *Emericella nidulan*s, *Dichotomophtora portulacae* and *D. boerhaaviae* and the biological activity against the dermatophyte fungi *Malassezia* sp*., Microsporum canis,* and the parasite *Leishmania amazonensis*.

Fungal enzymes extract exhibited lipolysis action in the media that contains long carbon chain lipids. The proteomic analysis of lipases exhibits many molecules mostly ranging in size from 220 to 20 kDa, with obvious differences in protein profile's yield. Fungal enzymes were efficient to eliminate promastigote forms of *Leishmania amazonensis*. The anti-leishmanial activity of lipases from *Vermisporium* such as *E. nidulans, D. portulacae* ,and *D. boerhaaviae* in amastigote forms induced the reduction in viability of 78.88, 39.65, 63.17, and 98.13%. In relation to antifungal activity, *Dichotomophtora* enzymes show best action with against *Microsporum canis* and *Malassezia* sp. *These* results promote us to deduce that lipases from entophytic fungi exhibit activity against dermatophyte fungi (*Malassezia* sp. and *Microsporum canis*) as well as Leishmania (Alves et al. 2018).

13.10 Lipases in Food Industry

In recent years, consumers have been increasingly confronted with functional foods and nutraceuticals, which are claimed to promote health and well-being beyond their nutritive properties. Large scale applications of lipases in industry can be found in production of trans-fatty acid free margarines. Fats and oils are important constituents of foods and their modification is one of the prime areas in food processing industry that demands novel economic and green technologies (Gupta et al.

2003). Besides the commercial utilization for flavor development in cheese products, they are used in processing of other foods, such as meat, vegetables, fruit, baked foods, milk products, and beer (Nagodawithana and Reed 1993).

Lipases from *A. niger, Rhizopus oryzae, Candida cylindracea* have been used in bakery products. Betapol was the first commercial product made by the 1,3-specific lipase treatment of tripalmitin with unsaturated fatty acids that resulted in 1,3-diunsaturated-2- saturated triglycerides intended for infant formula (Yang et al. 2003). Immobilized lipases from *Candida antarctica* (CALB), *Candida cylindracea* AY30, and *Geotrichum candidum* were used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants in sunflower oil (Buisman et al. 1998).

13.11 Applications of Fungal Lipases in Dairy Industry

Mold-ripened cheeses are manufactured and consumed in large quantities long before dairy microbiology acquired any importance. For many years, the role of fungi in cheese-ripening was not clearly understood, and yet during this time cheeses of excellent quality were manufactured. Recent studies on the growth and chemical activities of fungi in cheese-ripening have resulted in the adoption of scientific procedures for cheese manufacture which permit a large proportion of the products to be uniform in flavor.

13.11.1 Degree of Lipolysis in Cheese

Lipolysis in mold-ripened cheese is much more extensive than in other cheeses (Table 13.4). While the extent of lipolysis should not exceed 2% of the triglycerides in cheeses such as Gouda, Gruyere, or Cheddar, it is usually between 5 and 20% in mold-ripened cheeses. Data in the literature vary greatly, probably depending on the degree of ripening. Extensive lipolysis can be attained in mold-ripened cheeses without any rancid taste occurring, probably due to neutralization of fatty acids on elevation of the pH. Anderson and Day (1965) found high levels of free fatty acids in blue-veined cheese, about 65–100 meq/100 g of fat, i.e., 18–25% of the total fatty acids. Other workers reported lower lipolysis in Danish Blue (about 45 meq/I00 g of fat).

Table 13.4 Fat acidity of different cheese varieties (Fox 1994)

Cheese variety	meq acid/100 g of fat
Gouda	6.14 ± 0·50
Camembert	22.27 ± 13·73
6Danish Blue	45.34 ± 14·93
Roquefort	27.55 ± 12.6

Table 13.5 Relative proportions in camembert of free fatty acids and of fatty acids in glycerides (Kuzdzal-Savoie 1965)

Fatty acids	Glycerides	Free fatty acids
4:0 + 6:0 + 8:0	2.8	2.2
10:0 + i10:0 to 12:0	3.8	3.0
12:0 + i 12:0 to 14:0	4.5	4.7
14:0 + i 14:0 to 16:0	16.1	14.3
16:0 + i 16:0 to 18:0	28.6	23.3
8:0	10.2	7.3
18:1 + 18:2 + 18.3	34.0	45.2

The extent of lipolysis in Roquefort is 8–10% of the total fatty acids. Godinho and Fox (1981) noted a lower free fatty acid level in the outer part of blue cheese due to higher NaCl concentrations that limited the production of lipases and possibly their activity. Morris et al. (1963) noted a regular increase in free fatty acid levels during ripening, while others (Godinho and Fox 1981) observed that these levels decreased at the end of ripening. In Norman Camembert made from raw milk, lipolysis reaches 6–10% of total fatty acids, probably less typical, samples had lower values of 3–5% (Fox 1994). Lipolysis is always highest towards the surface (Kuzdza-Savoie 1968). Most of the free fatty acids arise from lipolysis: short-chain fatty acids, resulting from the breakdown of lactose or some amino acids, represent only 5% of the total free fatty acids. In Camembert, the relative proportions of free fatty acids are different from those of milk triglycerides since the former have a higher concentration of oleic acid (Kuzdzal-Savoie 1965) (Table 13.5).

13.11.2 Properties and Effect of Mold Lipases

The essential lipolytic agents in mold-ripened cheeses are *Penicillium* spp. The natural lipase of milk is not very active, even in raw milk cheeses; however, its effect has been shown in blue-veined cheeses made from homogenized milk (Morris et al. 1963). Except for *Geotrichum candidum*, microorganisms other than *P. roqueforti* or *P. camemberti* have very low lipolytic activity in mold-ripened cheeses. The particularly high proportion of free oleic acid in Camembert has been attributed to *G. candidum* lipase which preferentially releases this fatty acid (Godinho and Fox 1981).

Lamberet and Lenoir (1976) noted that *P. camemberti* produces only one extracellular lipase which has optimal activity on tributyrin at pH 9·0 and 35 °C. The production level of this enzyme varies from 1 to 10 (relative scale), depending on the strain. At pH 6.0, this lipase retains 50% of its maximal activity and remains very active between 0 and 20 °C. It is more active when calcium ions are present. The production of lipase has been studied during the ripening of raw milk Camembert; activity appears after 10 days of ripening, during or shortly after

mycelium growth, is maximal at 16 days and then decreases slightly until the 30th day when it increases again on the lysis of mycelia. In 10 cheeses of different origins, the lipase activity in the outer region of the cheeses varied from 1.2 to 4–45 units/g of cheese(Lamberet et al. 1982; Molimard and Spinnler 1996). *P. roqueforti* has been reported to produce two lipases, one with an optimum at acid pH values, the other with an optimum in the alkaline pH range (Imamura and Kataoka 1963).

Several authors (Morris and Jezeski 1953; Imamura and Kataoka 1963; Eitenmiller et al. 1970) reported that the alkaline lipase is optimally active at pH 7.5–8.0 but optimum pH values at 9.0–9.5 have also been reported. This enzyme still retains 20% of its activity at pH 6.0 (Menassa and Lamberet 1982). The activity of acid lipase is maximal at pH 6.0–6.5 (Lobyreva and Marchenkova 1981). The specificities of the two enzymes are different, the acid lipase being more active on tricaproin and the alkaline lipase on tributyrin. The activity of both enzymes has been measured in cheese (Lamberet and Lenoir 1976). More acid lipase was synthesized in six out of seven samples.

However, in spite of the favorable pH of the cheese, it may not always play the more important role since alkaline lipase has higher activity on milk fat(Menassa and Lamberet 1982). Samples in which alkaline lipase activity is high may have a slightly piquant taste or soapy aroma (Lamberet and Menassa 1983). This would reflect the relative activity levels of the two lipases and probably their different specificities (Lamberet and Lenoir 1976). A whole range of fungal lipase preparations such as *Mucor meihei* (Piccnate, Gist-Brocades; Palatase M, Novo Nordisk), *A. niger,* and *A. oryzae* (Palatase A, Novo Nordisk; Lipase AP, Amano; Flavour AGE, Chr. Hansen) have been developed for the cheese manufacturing industry.

13.12 Sustainable Applications of Fungal and Bacterial Lipases for Green Economy Bioremediation of Oil and Grease (O&G) Containing Wastewater

It is an avenue in lipase biotechnology where lipases have been used in wastewater treatment. Fungal species can be used to degrade oil spills in the coastal environment, which may enhance ecorestoration as well as help in the enzymatic oil processing in industries. Several lipase-producing bacterial, fungal, and yeast strains have been employed either individually or as a consortium to bring about O&G bioremediation. Among bacteria, *Pseudomonas* spp., *P. aeruginosa* have been especially useful (Kanmani et al. 2015).

Species belonging to the genera *Trichoderma*, *Fusarium*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Mortierella*, *Beauveria,* and *Engyodontium* are some examples of the fungi that have recently been described as tolerant to a variety of pollutants and indicated as potential bioremediation agents in soil (Islam and Datta 2015). Lipase from *Aspergillus niger* and *Aspergillus terreus* were used for the degradation of polyvinyl alcohol films and bioremediation of polluted soils, respectively (Mahmoud

et al. 2015). Lipase from *Aspergillus ibericus* and *Aspergillus uvarum* were also used in bioremediation processes (Salgado et al. 2016). Lipolytic enzyme obtained from *Aspergillus niger* isolated from oil polluted soil has been examined and found to degrade polyaromatic hydrocarbons found in petroleum contaminated soil (Mehta et al. 2017).

13.12.1 The Biodiesel Production

Production of biodiesel from waste and non-edible vegetable oil greatly reduces the cost of biodiesel production, and thus avoids the conflict between food and energy security, and is considered an important step in reducing pollution and recycling waste oil (Ken Ugo et al. 2017).

13.12.2 Textile Industry

Commercial preparations used for the desizing of denim and cotton fabrics contain lipase enzymes. Wang et al. (2008b) succeeded in preparation of a specific lipase catalyzing the hydrolysis of polyethylene terephthalate (PET) by *Aspergillus oryzae* CCUG 33812 as well as modification of PET fabrics by the enzyme.

13.12.3 Detergent Industries

Since lipases attack lipids, some fatty acyl soils can be removed through the lipolytic action of lipases. Fatty acyl soils could be found in form of sweat, lipstick, fry fats, butter, sauces (Kouker and Jaeger 1987). The use of lipases in detergents could reduce the washing period, agitation, and temperature, hence prolongs the life span of the textile materials and provides a habitable environment for living things. Lipases have been used in production of dishwashing detergents which brings down the quantity of surfactants used in the detergent production. An estimated 1000 tons of lipases are added to the approximately 13 billion tons of detergents produced each year (Ken Ugo et al. 2017).

13.12.4 Pulp and Paper Industry

They are used in paper industry is for “depitching” process, i.e., removal of “pitch” from the pulp during paper making (Kouker and Jaeger 1987). Pitch is generally described as the hydrophobic components of wood (triglycerides and waxes). These

lipid components appear as sticky deposits in the paper machines and cause holes and spots in the final paper.

13.12.5 Leather Industry

One of the major steps in leather production is soaking the hides and skins. This step is taken before the tanning step and the rational is to ensure smooth tanning process of the hides and skins. To degrade the emulsified fats, lipases are needed; also since they are specific in their action, lipases will leave the leather undamaged. For sheep skins, the use of solvent for degreasing is now being replaced by the use of lipases and surfactants (Berhanu and Amare 2012).

13.13 Conclusions and Future Prospects

Research and development in fungal lipases isolation and production are targeted to be compatible and efficient for the increasing industrial demands due to their multi-faceted benefits availed to convert into novel economic and greener world. Variable profiles of lipase characterization and activities have been extensively studied. Predicting the 3D structure of novel lipases and the enzyme-substrate binding activity by homology modeling in silico methods are recent approaches that can be used to screen a library of substrates to find the most appropriate application of lipases. More efforts are recommended in this area to boost fungal and bacterial lipases biotechnological applications to the choice for the present and future.

References

Abdel-Azeem MA, Abdel-Aziz DM, Salem FM, Abdel-Azeem AM (2015) Surveying of Egyptian Endophytic Fungi for Production of Some Pharmaceutical and Industrial Enzymes. In: 1st Scientific Forum for Student Research, Faculty of Science, Suez Canal University, Ismailia, Egypt, Abstract book. p 24

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Vol-1: Biodiversity and ecological perspective. Springer, Cham

Adlercreutz P (2013) Immobilisation and application of lipases in organic media. Chem Soc Rev 42:6406–6436. https://doi.org/10.1039/c3cs35446f

Ahuja K, Rawat A (2019) Enzymes market size by product (proteases, lipases, carbohydrases [amylases, xylanases, cellulases, pectinases, lactases], polymerases & nucleases, phytases, catalyses), by application (food & beverage, processed food, diary, bakery, confectionary), indus. In: Glob. Mark. Insights. https://www.gminsights.com/industry-analysis/enzymes-market

Aires-Barros M, Taipa M, Cabral J (1994) Isolation and purification of lipases. In: Wooley P, Petersen S (eds) In Lipases—their structure, biochemistry and application, vol 1, 1st edn. Cambridge University Press, Cambridge, pp 243–270

Alford JA, Pierce DA (1961) Lipolytic activity of microorganisms at low and intermediate temperatures. III. Activity of microbial lipases at temperatures below 0 °C. J Food Sci 26:518–524
Alves D, Morais SM, Tomiotto Pellissier F, Vasconcelos F et al (2018) Leishmanicidal and fungicidal activity of lipases obtained from endophytic fungi extracts. PLoS One 13:e0196796. https://doi.org/10.1371/journal.pone.0196796
Anderson D, Day E (1965) Quantitative analysis of the major free fatty acids in blue cheese. J Dairy Sci 48:248–249. https://doi.org/10.3168/jds.s0022-0302(65)88205-4
Andualema B, Gessesse A (2012) Microbial lipases and their industrial applications: review. Biotechnology 11:100–118. https://doi.org/10.3923/biotech.2012.100.118
Attia EA, Singh BP, Dashora K, Abdel-Azeem AM (2020) A potential antimicrobial, extracellular enzymes, and antioxidants resource: endophytic fungi associated with medicinal plants. Int J Biosci 17:119–132
Azim A, Sharma SK, Olsen CE, Parmar VS (2001) Lipase catalysed synthesis of optically enriched α-haloamides. Bioorganic Med Chem 9:1345–1348. https://doi.org/10.1016/S0968-0896(01)00006-2
Barriuso J, Vaquero ME, Prieto A, Martínez MJ (2016) Structural traits and catalytic versatility of the lipases from the *Candida rugosa*-like family: A review. Biotechnol Adv 34:874–885. https://doi.org/10.1016/j.biotechadv.2016.05.004
Basheer SM (2007) Lipase production by marine fungus Aspergillus awamori Nagazawa. Cochin University of Science and Technology, India
Basso A, Serban S (2019) Industrial applications of immobilized enzymes—a review. Mol Catal 479:110607. https://doi.org/10.1016/j.mcat.2019.110607
Berhanu A, Amare G (2012) Microbial lipases and their industrial applications. Rev Biotechnol 11:100–118
Bian C, Yuan C, Chen L et al (2010) Crystal structure of a triacylglycerol lipase from Penicillum expansum at 1.3 A determined by sulfur SAD. Proteins Struct Funct Bioinforma:78. https://doi.org/10.1002/prot.22676
Bjornson K, Canales E, Cottell J, Karki K (2016) Inhibitors of Hepatitis C virus, US 9296782 B2
Borkar P, Bodade R, Rao S, Khobragade C (2009) Purification and characterization of extracellular lipase from a new strain: Pseudomonas aeruginosa SRT 9. Brazilian J Microbiol 40:358–366
Bornscheuer UT, Bessler C, Srinivas R, Hari Krishna S (2002) Optimizing lipases and related enzymes for efficient application. Trends Biotechnol 20:433–437. https://doi.org/10.1016/S0167-7799(02)02046-2
Boyer N, Marcellin P (2000) Pathogenesis, diagnosis and management of hepatitis C. J Hepatol 32:98–112. https://doi.org/10.1016/s0168-8278(00)80419-5
Brady L, Brzozowski AM, Derewenda ZS et al (1990) A serine protease triad forms the catalytic centre of a triacylglycerol lipase. Nature 343:767–770. https://doi.org/10.1038/343767a0
Brocca S, Grandori R, Breviario D, Lotti M (1995) Localization of lipase genes on *Candida rugosa* chromosomes. Curr Genet 28:454–457. https://doi.org/10.1007/BF00310815
Brocca S, Alberghina L, Lotti M et al (2000) Mutants provide evidence of the importance of glycosidic chains in the activation of lipase 1 from *Candida rugosa*. Protein Sci 9:985–990. https://doi.org/10.1110/ps.9.5.985
Brzozowski AM, Derewenda U, Derewenda ZS et al (1991) A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex. Nature 351:491–494. https://doi.org/10.1038/351491a0
Brzozowski AM, Derewenda ZS, Dodson EJ et al (1992) Structure and molecular model refinement of *Rhizomucor miehei* triacyglyceride lipase: a case study of the use of simulated annealing in partial model refinement. Acta Crystallogr Sect B 48:307–319. https://doi.org/10.1107/S0108768191014647
Brzozowski AM, Savage H, Verma CS et al (2000) Structural origins of the interfacial activation in Thermomyces (Humicola) lanuginosa lipase. Biochemistry 39:15071–15082. https://doi.org/10.1021/bi0013905

Buisman G, Van-Helteren C, Kramer G et al (1998) Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. Biotechnol Lett 20:131–136

Burham H, Rasheed RAGA, Noor NM et al (2009) Enzymatic synthesis of palm-based ascorbyl esters. J Mol Catal B Enzym 58:153–157. https://doi.org/10.1016/j.molcatb.2008.12.012

Cagulada A, Chan J, Chan L, Colby D (2015) Synthesis of an antiviral compound, US 2015/0175626A1

Carrière F, Withers-Martinez C, Van Tilbeurgh H et al (1998) Structural basis for the substrate selectivity of pancreatic lipases and some related proteins. Biochim Biophys Acta: Rev Biomembr 1376:417–432. https://doi.org/10.1016/S0304-4157(98)00016-1

Carroccio A, Guarino A, Zuin G et al (2001) Efficacy of oral pancreatic enzyme therapy for the treatment of fat malabsorption in HIV-infected patients. Aliment Pharmacol Ther 15:1619–1625. https://doi.org/10.1046/j.1365-2036.2001.01070.x

Cen Y, Singh W, Arkin M et al (2019) Artificial cysteine-lipases with high activity and altered catalytic mechanism created by laboratory evolution. Nat Commun 10:1–10. https://doi.org/10.1038/s41467-019-11155-3

Chandel C, Kumar A, Kanwar SS (2011) Enzymatic synthesis of butyl ferulate by silica-immobilized lipase in a non-aqueous medium. J Biomater Nanobiotechnol 2:400–408

Chen JCH, Miercke LJW, Krucinski J et al (1998) Structure of bovine pancreatic cholesterol esterase at 1.6 Å: novel structural features involved in lipase activation. Biochemistry 37:5107–5117. https://doi.org/10.1021/bi972989g

Cherif S, Mnif S, Hadrich F et al (2011) A newly high alkaline lipase: an ideal choice for application in detergent formulations. Lipids Health Dis 10:221. https://doi.org/10.1186/1476-511X-10-221

Christner J, Pfleiderer E, Taeger T (1992) Enzymatic soaking method, United States Patent. 5

Coenen TMM, Aughton P, Verhagan H (1997) Safety evaluation of lipase derived from Rhizopus oryzae: summary of toxicological data. Food Chem Toxicol 35:315–322

Colton IJ, Yin DT, Grochulski P, Kazlauskas RJ (2011) Adv Synth Catal 353:2529–2544

Cygler M, Schrag JD, Bouthillier F et al (1994) A structural basis for the chiral preferences of lipases. J Am Chem Soc 116:3180–3186. https://doi.org/10.1021/ja00087a002

Das A, Shivakumar S, Bhattacharya S et al (2016) Purification and characterization of a surfactant-compatible lipase from *Aspergillus tamarii* JGIF06 exhibiting energy-efficient removal of oil stains from polycotton fabric. 3 Biotech 6:1–8. https://doi.org/10.1007/s13205-016-0449-z

De Almeida AF, Tauk-Tornisielo SM, Carmona EC (2013, 2013) Acid lipase from Candida viswanathii: production, biochemical properties, and potential application. Biomed Res Int. https://doi.org/10.1155/2013/435818

de Miguel BT, Barros-Velazquez J, Gonzalez Villa T (2006) Industrial applications of hyperthermophilic enzymes: a review. Protein Pept Lett 13:645–651. https://doi.org/10.2174/092986606777790548

de Miranda AS, Miranda LSM, de Souza ROMA (2015) Lipases: valuable catalysts for dynamic kinetic resolutions. Biotechnol Adv 33:372–393. https://doi.org/10.1016/j.biotechadv.2015.02.015

De Simone A (2016) Engineering the genetic code of Escherichia coli with methionine analogues and bioorthogonal amino acids for protein immobilization. Von der Fakultät II – Mathematik und Naturwissenschaften der Technischen Universität Berlin

De Wijn R, Hennig O, Roche J et al (2019) A simple and versatile microfluidic device for efficient biomacromolecule crystallization and structural analysis by serial crystallography. IUCrJ 6:454–464. https://doi.org/10.1107/S2052252519003622

Demiorijan DC, Moris-Varas F, Cassidy CS (2001) Enzymes from extremophiles. Curr Openion Biol 5:144–151

Derewenda U, Derewenda ZS, Brzozowski AM, Lawson DM (1992) Catalysis at the interface: the anatomy of a conformational change in a triglyceride lipase. Biochemistry 31:1532–1541. https://doi.org/10.1021/bi00120a034

Derewenda U, Swenson L, Green R et al (1994a) An unusual buried polar cluster in a family of fungal lipases. Nat Struct Biol 1:36–47. https://doi.org/10.1038/nsb0194-36

Derewenda U, Swenson L, Wei Y et al (1994b) Conformational lability of lipases observed in the absence of an oil- water interface: crystallographic studies of enzymes from the fungi Humicola lanuginosa and Rhizopus delemar. J Lipid Res 35:524–534. https://doi.org/10.2210/pdb1tib/pdb

Domingo E, Martinez-Salas E, Sobrino F et al (1995) The quasispecies (extremely heterogeneous) nature of viral RNA genome populations: Biological relevance-a review. Gene 40:1–8

Domínguez De María P, Carboni-Oerlemans C, Tuin B et al (2005) Biotechnological applications of *Candida antarctica* lipase A: State-of-the-art. J Mol Catal B Enzym 37:36–46. https://doi.org/10.1016/j.molcatb.2005.09.001

Eitenmiller RR, Vakil JR, Shahani KM (1970) Production and properties of *Penicillium roqueforti* lipase. J Food Sci 35:130–133

El-Ghonemy DH, El-Gamal MS, Tantawy AE, Ali TH (2017) Extracellular alkaline lipase from a novel fungus Curvularia sp. DHE 5: optimisation of physicochemical parameters, partial purification and characterisation. Food Technol Biotechnol 55:206–217. https://doi.org/10.17113/tb.55.02.17.4958

Ericsson DJ, Kasrayan A, Johansson P et al (2008) X-ray Structure of Candida antarctica Lipase A Shows a Novel Lid Structure and a Likely Mode of Interfacial Activation. J Mol Biol 376:109–119. https://doi.org/10.1016/j.jmb.2007.10.079

Facchini FDA, Vici AC, Pereira MG, Jorge JAPT (2016) Enhanced lipase production of Fusarium verticillioides by using response surface methodology and wastewater pretreatment application. J Biochem Technol 6:996–1002

Feller G (2013) Psychrophilic enzymes: From folding to function and biotechnology. Scientifica (Cairo) 2013:1–28. https://doi.org/10.1155/2013/512840

Feller G, Narinx E, Arpigny JL et al (1996) Enzymes from psychrophilic organisms. FEMS Microbiol Rev 18:189–202

Fickers P, Nicaud J, Gaillardin C et al (2004) Carbon and nitrogen sources modulated lipase production in the yeast Yarrowia lipolytica. J Appl Microbiol 96:742–749

Fickers P, Benetti P, Wache Y et al (2005) Hydrophobic substrate utilization by the yeast Yarrowia lipolytica, and its potential applications. FEMS Yeast Res 5:527–543

Fischer M, Pleiss J (2003) The lipase engineering database: a navigation and analysis tool for protein families. Nucleic Acids Res 31:319–321. https://doi.org/10.1093/nar/gkg015

Fox P (1994) Cheese: chemistry, physics and microbiology. Volume 2: Major cheese groups, 2nd edn. An Aspen Publication® Aspen Publishers, Inc., Gaithersburg, MD

Franssen MCR, Alessandrini L, Terraneo G (2005) Biocatalytic production of flavors and fragrances. Pure Appl Chem 77:273–279

Fukumoto T, Berg T, Ku Y et al (1996) Viral dynamics of hepatitis C early after orthotopic liver transplantation: evidence for rapid turnover of serum virions. Hepatology 24:1351–1354. https://doi.org/10.1002/hep.510240606

Gandhi NN (1997) Applications of lipase. J Am Oil Chem Soc 74:621–634. https://doi.org/10.1007/s11746-997-0194-x

Garlapati VK, Banerjee R (2013) Solvent-free synthesis of flavour esters through immobilized lipase mediated transesterification. Enzyme Res 2013:1–6

Gerhartz W (1990) Industrial uses of enzymes. In: Enzymes in industry production and application. VCH, Weinheim, Germany, pp 77–148

Gerritse G, Hommes R, Quax W (1998) Development of a lipase fermentation process that uses a recombinant Pseudomonas alcaligenes strain. Appl Environ Microbiol 64:2644–2651

Ghosh P, Saxena R, Gupta R et al (1996) Microbial lipases: production and applications. Sci Prog 79:119–157

Godinho M, Fox PF (1981) Ripening of blue cheese. Influence of salting rate on lipolysis and carbonyl formation. Milchwissenschaft 36(8):476–478

Gombert A, Pinto A, Castilho L, Freire D (1999) Lipase production by Penicillium restrictum in solid-state fermentation using babassu oil cake as substrate. Process Biochem 35:85–90
Gopinath SCB, Anbu P, Lakshmipriya T, Hilda A (2013) Strategies to characterize fungal lipases for applications in medicine and dairy industry. Biomed Res Int 2013:1–10
Grochulski P, Li Y, Schrag JD et al (1993) Insights into interfacial activation from an open structure of Candida rugosa lipase. J Biol Chem 268:12843–12847. https://doi.org/10.2210/pdb1crl/pdb
Grochulski P, Bouthillier F, Schrag JD et al (1994a) Analogs of reaction intermediates identify a unique substrate binding site in Candida rugosa lipase. Biochemistry 33:3494–3500. https://doi.org/10.1021/bi00178a005
Grochulski P, Li Y, Schrag JD, Cygler M (1994b) Two conformational states of Candida rugosa lipase. Protein Sci 3:82–91. https://doi.org/10.1002/pro.5560030111
Gudmundur G, Halldorsson A, Thorstad O (2003) Lipase-catalysed esterification of marine oil, US 7491522B2
Gupta R, Rathi P, Bradoo S (2003) Lipase mediated upgradation of dietary fats and oils. Crit Rev Food Sci Nutr 43:635–644
Gupta R, Gupta N, Rathi P (2004) Bacterial lipases: an overview of production, purification and biochemical properties. Appl Microbiol Biotechnol 64:763–781. https://doi.org/10.1007/s00253-004-1568-8
Gupta R, Kumari A, Syal P, Singh Y (2015) Molecular and functional diversity of yeast and fungal lipases: their role in biotechnology and cellular physiology. Prog Lipid Res 57:40–54. https://doi.org/10.1016/j.plipres.2014.12.001
Gutman AL, Zuobi K, Bravdo T (1990) Lipase-catalyzed preparation of optically active γ-butyrolactones in organic solvents. J Org Chem 55:546–3552
Haki GD, Rakshit SK (2003) Developments in industrially important thermostable enzymes: a review. Bioresour Technol 89:17–34
Handman E (1999) Cell biology of Leishmania. Adv Parasitol 44:1–39. https://doi.org/10.1016/S0065-308X(08)60229-8
Handman E (2001) Leishmaniasis: current status of vaccine development. Clin Microbiol Rev 14:229–243
Hasan F, Shah AA, Hameed A (2006) Industrial applications of microbial lipases. Enzyme Microb Technol 39:235–251. https://doi.org/10.1016/j.enzmictec.2005.10.016
Hasan F, Shah AA, Javed S, Hameed A (2010) Enzymes used in detergents: Lipases. African J Biotechnol 9:4836–4844. https://doi.org/10.5897/AJBx09.026
He Y, Tan T (2006) Use of response surface methodology to optimize culture medium for production of lipase with Candida sp. J Mol Catal B Enzym 43:99–125
Heikinheimo P, Goldman A, Jeffries C, Ollis DL (1999) Of barn owls and bankers: a lush variety of α/β hydrolases. Structure 7:R141–R146. https://doi.org/10.1016/S0969-2126(99)80079-3
Høegh I, Patkar S, Halkier T, Hansen MT (1995) Two lipases from *Candida antarctica*: cloning and expression in *Aspergillus oryzae*. Can J Bot 1:869–875. https://doi.org/10.1139/b95-333
Hooper L, Harrison RA, Summerbell CD et al (2004) Omega 3 fatty acids for prevention and treatment of cardiovascular disease. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD003177.pub2
Hoq M, Yamane T, Shimizu S et al (1985) Continuous hydrolysis of olive oil by lipase in microporous hydrophobic membrane bioreactor. J Am Oil Chem Soc 62:1016–1021
Idris A (2012) Antileishmania compounds from an unidentified endophytic fungus and biotransformation of 5,7,2',4',5'-pentahydroxyflavonol. University of Mississippi
Imamura T, Kataoka K (1963) Biochemical studies on the manufacturing of Roquefort type cheese. II. Characteristics of lipases produced by Penicillium roquetorti. Japanese J Zootech Sci 34:349–353
Imamura S, Kitaura S (2000) Purification and characterization of a monoacylglycerol lipase from the moderately thermophilic Bacillus sp. H-257. J Biochem 127:419–425. https://doi.org/10.1093/oxfordjournals.jbchem.a022623

Islam R, Datta B (2015) Fungal diversity and its potential in environmental cleanup. Int J Res 2:815–825

Jaeger KE, Eggert T (2002) Lipases for biotechnology. Curr Opin Biotechnol 13:390–397. https://doi.org/10.1016/S0958-1669(02)00341-5

Jaeger KE, Reetz MT (1998) Microbial lipases form versatile tools for biotechnology. Trends Biotechnol 16:396–403. https://doi.org/10.1016/S0167-7799(98)01195-0

Jaeger K, Ransac S, Dijkstra B et al (1994) Bacterial lipases. FEMS Microbiol Rev 15:29–63

Jaeger KE, Dijkstra BW, Reetz MT (1999) Bacterial biocatalysts: molecular biology, three-dimensional structures, and biotechnological applications of lipases. Annu Rev Microbiol 53:315–351. https://doi.org/10.1146/annurev.micro.53.1.315

Joseph B, Ramteke PW, Thomas G, Shrivastava N (2007) Standard review cold-active microbial Lipases: aversatile tool for industrial applications. Biotechnol Mol Biol Rev 2:39–48

Jung S, Kim J, Park S (2013) Rational design for enhancing promiscuous activity of *Candida antarctica* lipase B: a clue for the molecular basis of dissimilar activities between lipase and serine-protease. RSC Adv 3:2590–2594. https://doi.org/10.1039/c2ra23333a

Kanmani P, Aravind J, Kumaresan K (2015) An insight into microbial lipases and their environmental facet. Int J Environ Sci Technol 12:1147–1162. https://doi.org/10.1007/s13762-014-0605-0

Kapoor M, Gupta MN (2012) Lipase promiscuity and its biochemical applications. Process Biochem 47:555–569. https://doi.org/10.1016/j.procbio.2012.01.011

Kashmiri M, Adnan A, Butt B (2006) Production, purification and partial characterization of lipase from Trichoderma Viride. African J Biotechnol 5:878–882

Kaufman VR, Garti N (1982) Organic reactions in emulsions preparation of glycerol and polyglycerol esters of fatty acids by transesterification reaction. J Am Oil Chem Soc 59:471–474

Kaushik R, Saran S, Isar J, Saxena R (2006) Statistical optimization of medium components growth conditions by response surface methodology to enhance lipase production by *Aspergillus carneus*. J Mol Catal B Enzym 40:121126

Kawaguchi Y, Honda H, Taniguchi-Morimura J, Iwasaki S (1989) The codon CUG is read as serine in an asporogenic yeast Candida cylindracea. Nature 341:164–166. https://doi.org/10.1038/341164a0

Kempka A, Lipke N, Pinheiro T et al (2008) Response surface method to optimize the production and characterization of lipase from *Penicillium verrucosum* in solid state fermentation. Bioprocess Biosyst Eng 31:119–125

Ken Ugo A, Vivian Amara A, Igwe CN, Kenechuwku U (2017) Microbial lipases: a prospect for biotechnological industrial catalysis for green products: a review. Ferment Technol 6:144. https://doi.org/10.4172/2167-7972.1000144

Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13:345–351. https://doi.org/10.1016/S0958-1669(02)00328-2

Klein RR, King G, Moreau RA, Haas MJ (1997) Altered acyl chain length specificity of Rhizopus delemar lipase through mutagenesis and molecular modeling. Lipids 32:123–130. https://doi.org/10.1007/s11745-997-0016-1

Kohno M, Funatsu J, Mikami B et al (1996) The crystal structure of lipase II from Rhizopus niveus at 2.2 Å resolution. J Biochem 120:505–510. https://doi.org/10.1093/oxfordjournals.jbchem.a021442

Kouker G, Jaeger K (1987) Specific and Sensitive plate assay for bacterial lipase. Appl Environ Microbiol 53:211–213

Kour D, Rana KL, Kaur T, Singh B, Chauhan VS, Kumar A, Rastegari AA, Yadav N, Yadav AN, Gupta VK (2019) Extremophiles for hydrolytic enzymes productions: biodiversity and potential biotechnological applications. In: Molina G, Gupta VK, Singh B, Gathergood N (eds) Bioprocessing for biomolecules production, pp 321–372. https://doi.org/10.1002/9781119434436.ch16

Kralovec J, Wang W, Barrow J (2009) Enzymatic modification of oil, WO 2009/040676A2

Kumar A, Kanwar SS (2011) Synthesis of ethyl ferulate in organic medium using celite-immobilized lipase. Bioresour Technol 102:2162–2167

Kumar S, Kumar L, Sahai V, Gupta R (2009) A thiol-activated lipase from Trichosporon asahii MSR 54: detergent compatibility and presoak formulation for oil removal from soiled cloth at ambient temperature. J Ind Microbiol Biotechnol 36:427–432. https://doi.org/10.1007/s10295-008-0513-8

Kumar M, Mukherjee J, Sinha M, Kaur P, Sharma S, Gupta MN, Singh TP (2015) Enhancement of stability of a lipase by subjecting to three phase partitioning (TPP): structures of native and TPP-treated lipase from *Thermomyces lanuginosa*. Sustain Chem Process 3:14

Kumar A, Dhar K, Kanwar SS, Arora PK (2016) Lipase catalysis in organic solvents: advantages and applications. Biol Proceed:18. https://doi.org/10.1186/s12575-016-0033-2

Kuzdzal-Savoie S (1965) Étude comparée des acides gras ramifiés des aliments et du lait chez la vache. Le Lait, INRA Ed 45:627–636

Kuzdza-Savoie S (1968) Qualities Plantarum et Materiae Vegetabiles. 16:312

Laachari F, El Bergadi F, Ibnsouda SK (2015) Purification and characterization of a novel thermostable lipase from aspergillus flavus. Int J Res 2:342–352

Lamberet G, Lenoir J (1976) Les caractères du système lipolytique de l'espèce *Penicillium caseicolum*. Purification et proprietes de la lipase majeure. Le Lait 56:622–644

Lamberet G, Menassa A (1983) Détermination et niveau des activités lipolytiques dans les fromages à pâte persillée. Lait 63:333–344. https://doi.org/10.1051/lait:1983629-63020

Lamberet G, Auberger B, Canteri C, Lenoir J (1982) L'aptitude de Penicillium caseicolum it la degradation oxyda- tive des acides gras. Rev Lait Fr 406:13

Lawson DM, Brzozowski AM, Rety S et al (1994) Probing the nature of substrate binding in Humicola lanuginosa lipase through x-ray crystallography and intuitive modelling. Protein Eng Des Sel 7:543–550. https://doi.org/10.1093/protein/7.4.543

Lobyreva LB, Marchenkova AI (1981) Characteristics of lipases in the cultural broth of Penicillium roqueforti. Mikrobiologia 50:90–95

Longhi S, Fusetti F, Grandori R et al (1992) Cloning and nucleotide sequences of two lipase genes from *Candida cylindracea*. BBA – Gene Struct Expr 1131:227–232. https://doi.org/10.1016/0167-4781(92)90085-E

Lotti M, Alberghina L (2007) Lipases: molecular structure and function. In: Industrial enzymes. pp 263–281

Lotti M, Grandori R, Fusetti F et al (1993) Cloning and analysis of Candida cylindracea lipase sequences. Gene 124:45–55. https://doi.org/10.1016/0378-1119(93)90760-Z

Lotti M, Tramontano A, Longhi S et al (1994) Variability within the *Candida rugosa* upases family. Protein Eng Des Sel 7:531–535. https://doi.org/10.1093/protein/7.4.531

Mahadik ND, Puntambekar US, Bastawde KB et al (2002) Production of acidic lipase by *Aspergillus niger* in solid state fermentation. Process Biochem 38:715–721. https://doi.org/10.1016/S0032-9592(02)00194-2

Mahmoud G, Koutb M, Morsy F, Bagy M (2015) Characterization of lipase enzyme produced by hydrocarbons utilizing fungus *Aspergillus terreus*. Eur J Biol Res 5:70–77

Mala JGS, Takeuchi S (2008) Understanding structural features of microbial lipases: An overview. Anal Chem Insights 2008:9–19. https://doi.org/10.4137/ACI.S551

Mancheño JM, Pernas MA, Martínez MJ et al (2003) Structural insights into the lipase/esterase behavior in the Candida rugosa lipases family: crystal structure of the lipase 2 isoenzyme at 1.97 Å resolution. J Mol Biol 332:1059–1069. https://doi.org/10.1016/j.jmb.2003.08.005

Marketsandmarkets (2020) Lipase market by source (microbial lipases, animal lipases), application (animal feed, dairy, bakery, confectionery, others), & by geography (North America, Europe, Asia-Pacific, Latin America, RoW) - Global Forecast to 2020. https://www.marketsandmarkets.com/Market-Reports/lipase-market-205981206.html#:~:text=The global market size of,in numerous feeds for livestock

Martin N, Schone O, Spitzensteatter H, Benito-Garagorri D (2016) A process for preparing a crystalline form of sofosbuvir, WO 2016/156512 A

Martínez-Corona R, Vázquez Marrufo G, Cortés Penagos C et al (2020) Bioinformatic characterization of the extracellular lipases from *Kluyveromyces marxianus*. Yeast 37:149–162. https://doi.org/10.1002/yea.3449

Masahiko A, Masahiro K, Takasi K, et al (1995) Process for preparation of polyol fatty acid ester and glyceride mixture obtained. European Patent. EP-658629

Matsumae H, Furui M, Shibatani T (1993) Lipase-catalyzed asymmetric hydrolysis of 3-phenylglycidic acid ester, the key intermediate in the synthesis of diltiazem hydrochloride. J Ferment Bioeng 75:93–98

Maugard T, Rejasse B, Legoy MD (2002) Synthesis of water-soluble retinol derivatives by enzymatic method. Biotechnol Prog 18:424–428

Mauti GO, Onguso J, Kowanga DK et al (2016) Biodegradation activity of *Aspergillus niger* lipase isolates from a tropical country garage. J Sci Innov Res 5:15–18

Meghwanshi GK, Vashishtha A (2018) Biotechnology of fungal lipases. In: Fungi and their role in sustainable development: current perspective. pp. 383–411

Meghwanshi GK, Kaur N, Verma S et al (2020) Enzymes for pharmaceutical and therapeutic applications. Biotechnol Appl Biochem:1–16. https://doi.org/10.1002/bab.1919

Mehta A, Bodh U, Gupta R (2017) Fungal lipases: a review. J Biotech Res 8:58–77

Menassa A, Lamberet G (1982) Contribution à l'étude du système lipolytique de Penicillium roqueforti. Caractères comparés de deux activités exocellulaires. Lait 62:32–43. https://doi.org/10.1051/lait:1982611-6122

Metzger J, Bornscheuer U (2006) Lipids as renewable resources: current state of chemical and biotechnological conversion and diversification. Appl Microbiol Biotechnol 71:13–22

Molimard P, Spinnler HE (1996) Review: compounds involved in the flavor of surface mold-ripened cheeses: origins and properties. J Dairy Sci 79:169–184. https://doi.org/10.3168/jds.S0022-0302(96)76348-8

Moroz OV, Blagova E, Reiser V et al (2019) Novel inhibitory function of the rhizomucor miehei lipase propeptide and three-dimensional structures of its complexes with the enzyme. ACS Omega 4:9964–9975. https://doi.org/10.1021/acsomega.9b00612

Morris HA, Jezeski JJ (1953) The action of microorganisms on fats. II. Sorne characteristics of the lipase system of Penicillium roquejorti. J Dairy Sci 36:1285–1298

Morris HA, Jezeski JJ, Combs WB, Kuramoto S (1963) Free fatty acid, tyrosine, and pH changes during ripening of blue cheese made from variously treated milks. J Dairy Sci 46:1–6. https://doi.org/10.3168/jds.S0022-0302(63)88952-3

Moya-salazar J, Vértiz-osores J, Jibaja S et al (2019) Fungi lipases homology modeling and molecular docking with fatty acids and tripalmitin of palm oil effluent. An Arch Org Inorg Chem Sci:493–500. https://doi.org/10.32474/AOICS.2019.04.000182

Mozaffar Z, Weete J (1993) Purification and properties of an extracellular lipase from Pythium ultimum. Lipids 28:377382

Murzin AG, Brenner SE, Hubbard T, Chothia C (1995) SCOP: a structural classification of proteins database for the investigation of sequences and structures. J Mol Biol 247:536–540. https://doi.org/10.1016/S0022-2836(05)80134-2

Muthukumaran N, Dhar SC (1982) Comparative studies on the degreasing of skins using acid lipase and solvent with reference to the quality of finished leathers. Leather Sci 29:417–424

Nagar M, Dwivedi SK, Shrivastava D (2013) A review on industrial application in microbial lipases. Int J Pharm Res Sci 2:631–641

Nagodawithana T, Reed G (1993) Enzymes in food processing, 3rd edn. Academic Press

Namboodiri V, Chattopadhyaya R (2000) Purification and Biochemical characterization of a novel thermostable lipase from *Aspergillus niger*. Lipids 35:495–502

Nardini M, Dijkstra BW (1999) α/β hydrolase fold enzymes: the family keeps growing. Curr Opin Struct Biol 9:732–737. https://doi.org/10.1016/S0959-440X(99)00037-8

Ozturkoglu-Budak S, Wiebenga A, Bron PA, de Vries RP (2016) Protease and lipase activities of fungal and bacterial strains derived from an artisanal raw ewe's milk cheese. Int J Food Microbiol 237:17–27. https://doi.org/10.1016/j.ijfoodmicro.2016.08.007

Panizza P, Syfantou N, Pastor FIJ et al (2013) Acidic lipase Lip I.3 from a Pseudomonas fluorescens-like strain displays unusual properties and shows activity on secondary alcohols. J Appl Microbiol 114:722–732. https://doi.org/10.1111/jam.12089

Park A, Kim S, Park J et al (2016) Structural and experimental evidence for the enantiomeric recognition toward a bulky sec-alcohol by Candida antarctica lipase B. ACS Catal 6:7458–7465. https://doi.org/10.1021/acscatal.6b02192

Parveen BTN, Manikandaselvi S (2011) Production of Lipase enzyme by *Aspergillus flavus* using groundnut waste. Int J PharmTech Res 3:1299–1302

Patil KJ, Chopda MZ, Mahajan RT (2011) Lipase biodiversity. Indian J Sci Technol 4:971–982. https://doi.org/10.17485/ijst/2011/v4i8/30913

Patil U, Mokashe N, Chaudhari A (2016) Detergent compatible, organic solvent-tolerant alkaline protease from *Bacillus circulans* MTCC 7942: purification and characterization. Prep Biochem Biotechnol 46:56–64

Pemberton JM, Kidd SP, Schmidt R (1997) Secreted enzymes of aeromonas. FEMS Microbiol Lett 152:1–10

Pleiss J, Fischer M, Schmid RD (1998) Anatomy of lipase binding sites: the scissile fatty acid binding site. Chem Phys Lipids 93:67–80. https://doi.org/10.1016/S0009-3084(98)00030-9

PRWEB (2008) World Market for Industrial Enzymes. http://www.prweb.com/releases/industrial_enzymes/carbohydrases_proteases/prweb1569084.htm

Qian Z, Horton JR, Cheng X, Lutz S (2009) Structural redesign of Lipase B from *Candida antarctica* by circular permutation and incremental truncation. J Mol Biol 393:191–201. https://doi.org/10.1016/j.jmb.2009.08.008

Rade LL, da Silva MNP, Vieira PS et al (2020) A novel fungal lipase with methanol tolerance and preference for Macaw palm oil. Front Bioeng Biotechnol 8:304. https://doi.org/10.3389/fbioe.2020.00304

Rajendran A, Palanisamy A, Thangavelu V (2008) Evaluation of medium components by Plackett-Burman statistical design for lipase production by *Candida rugosa* and kinetic modeling. Clin J Biotechnol 24:436–444

Rashid N, Shimada Y, Ezaki S et al (2001) Low temperature lipase from psychrotrophic Pseudomonas sp strain KB700A. Appl Environ Microbiol 67:4064–4069

Ray S (2015) Applications of extracellular microbial lipase: a review. Int J Res Biotechnol Biochem 5:6–12

Reyes-Duarte D, Lopez-Cortes N, Torres P et al (2011) Synthesis and properties of ascorbyl esters catalyzed by lipozyme TL im using triglycerides as acyl donors. J Am Oil Chem Soc 88:57–64. https://doi.org/10.1007/s11746-010-1643-5

Ribeiro BD, De Castro AM, Coelho MAZ (2011) Freire DMG (2011) Production and use of lipases in bioenergy: a review from the feedstocks to biodiesel production. Enzyme Res. https://doi.org/10.4061/2011/615803

Rotticci D, Rotticci-Mulder JC, Denman S et al (2001) Improved enantioselectivity of a lipase by rational protein engineering. ChemBioChem 2:766770. https://doi.org/10.1002/1439-7633(20011001)2:10<766::AID-CBIC766>3.0.CO;2-K

Ryan PMD, Caplice NM (2020) Is adipose tissue a reservoir for viral spread, immune activation, and cytokine amplification in Coronavirus Disease 2019? Obesity 28:1191–1194. https://doi.org/10.1002/oby.22843

Ryu HS, Kim HK, Choi WC et al (2006) New cold-adapted lipase from Photobacterium lipolyticum sp. nov. that is closely related to filamentous fungal lipases. Appl Microbiol Biotechnol 70:321–326

Sahay S, Chouhan D (2018) Study on the potential of cold-active lipases from psychrotrophic fungi for detergent formulation. J Genet Eng Biotechnol 16:319–325. https://doi.org/10.1016/j.jgeb.2018.04.006

Sahay H, Yadav AN, Singh AK, Singh S, Kaushik R, Saxena AK (2017) Hot springs of Indian Himalayas: potential sources of microbial diversity and thermostable hydrolytic enzymes. 3 Biotech 7(2):1–11

Salgado J, Abrunhosa L, Venancio A et al (2016) Combined bioremediation and enzyme production by Aspergillus sp. in olive mill and winery wastewaters. Int Biodeterior Biodegrad 110:16–23

Salihu A, Alam M (2012) Production and applications of microbial lipases: a review. Sci Res Essays 7:2667–2677. https://doi.org/10.5897/SRE11.2023

Santhosh Kumar D, Ray S (2015) Fungal lipase production by solid state fermentation-an overview. J Anal Bioanal Tech 06. https://doi.org/10.4172/2155-9872.1000230

Sarkissian CN, Shao Z, Blain F et al (1999) A different approach to treatment of phenylketonuria: phenylalanine degradation with recombinant phenylalanine ammonia lyase. Proc Natl Acad Sci U S A 96:2339–2344. https://doi.org/10.1073/pnas.96.5.2339

Saxena R, Ghosh P, Gupta R et al (1999) Microbial lipases: potential biocatalysts for the future industry. Curr Sci 77:101–115

Saxena R, Sheoran A, Giri B, Davidson W (2003) Purification strategies for microbial lipases. J Microbiol Methods 52:1–18

Schmid RD, Verger R (1998) Lipases: interfacial enzymes with attractive applications. Angew Chemie Int Ed 37:1608–1633. https://doi.org/10.1002/(sici)1521-3773(19980703)37:12<1608::aid-anie1608>3.3.co;2-m

Schrag JD, Cygler M (1993) 1.8 Å refined structure of the lipase from Geotrichum candidum. J Mol Biol 230:575–591. https://doi.org/10.1006/jmbi.1993.1171

Schrag JD, Cygler M (1997) Lipases and α/β hydrolase fold. Methods Enzymol 284:85–107. https://doi.org/10.1016/S0076-6879(97)84006-2

Schrag JD, Li Y, Wu S, Cygler M (1991) Ser-His-Glu triad forms the catalytic site of the lipase from Geotrichum candidum. Nature 351:761–764. https://doi.org/10.1038/351761a0

Sharma S, Kanwar SS (2014) Organic solvent tolerant lipases and applications. Sci World J 2014:1–15. https://doi.org/10.1155/2014/625258

Sharma R, Chisti Y, Banerjee UC (2001) Production, purification, characterization, and applications of lipases. Biotechnology Adv 19:627–662. https://doi.org/10.1002/ejlt.200600201

Sharma AK, Sharma V, Saxena J (2016) A review paper on properties of fungal lipases. Int J Curr Microbiol Appl Sci 5:123–130. https://doi.org/10.20546/ijcmas.2016.512.014

Silvestrini L, Cianci M (2020) Principles of lipid–enzyme interactions in the limbus region of the catalytic site of *Candida antarctica* Lipase B. Int J Biol Macromol. https://doi.org/10.1016/j.ijbiomac.2020.04.061

Singh RN, Gaba S, Yadav AN, Gaur P, Gulati S, Kaushik R, Saxena AK (2016) First, high quality draft genome sequence of a plant growth promoting and cold active enzymes producing psychrotrophic arthrobacter agilis strain L77. Stand Genomic Sci 11(1):54. https://doi.org/10.1186/s40793-016-0176-4

Skjold-Jørgensen J, Vind J, Moroz OV et al (2017) Controlled lid-opening in *Thermomyces lanuginosus* lipase: an engineered switch for studying lipase function. Biochim Biophys Acta – Proteins Proteomics 1865:20–27. https://doi.org/10.1016/j.bbapap.2016.09.016

Smaniotto A, Skovronski A, Rigo E et al (2012) 'Synthetic lipase' production from a newly isolated Sporidiobolus pararoseus strain by submerged fermentation. Brazilian J Microbiol 40:1490–1498

Smaniotto A, Skovronski A, Rigo E et al (2014) Concentration, characterization and application of lipases from Sporidiobolus pararoseus strain. Brazilian J Microbiol 45:294–301. https://doi.org/10.1590/S1517-83822014000100043

Stauch B, Fisher SJ, Cianci M (2015) Open and closed states of *Candida antarctica* lipase B: protonation and the mechanism of interfacial activation. J Lipid Res 56:2348–2358. https://doi.org/10.1194/jlr.M063388

Steitz TA, Shulman RG (1982) Crystallographic and NMR studies of the serine proteases. Annu Rev Biophys Bioeng 11:419–444. https://doi.org/10.1146/annurev.bb.11.060182.002223

Strzelczyk P, Bujacz GD, Kiełbasiński P, Błaszczyk J (2016) Crystal and molecular structure of hexagonal form of lipase B from *Candida antarctica*. Acta Biochim Pol 63:103–109. https://doi.org/10.18388/abp.2015_1065

Sugihara A, Tani T, Tominaga Y (1991) Purification and characterization of a novel thermostable lipase from Bacillus sp. J Biochem 109:211–216

Sun Q, Wang H, Zhang H et al (2016) Heterologous production of an acidic thermostable lipase with broad-range pH activity from thermophilic fungus *Neosartorya fischeri* P1. J Biosci Bioeng 122:539–544. https://doi.org/10.1016/j.jbiosc.2016.05.003

Sussman JL, Harel M, Frolow F et al (1991) Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. Science (80) 253:872–879. https://doi.org/10.1126/science.1678899

Taneja SC, Sethi VK, Andotra SS et al (2005) Rose oxides: a facile chemo and chemo-enzymatic approach. Synth Commun 35:2297–2303

Tang L, Su M, Chi L et al (2014) Residue Val237 is critical for the enantioselectivity of Penicillium expansum lipase. Biotechnol Lett 36:633–639. https://doi.org/10.1007/s10529-013-1405-1

Thakur S (2012) Lipases, its sources, properties and applications: a review. Int J Sci Eng Res 3:1–29

Umehara K, Masago Y, Mukaiyama T, Okumura O (1990) Behaviour of alkaline lipase on detergency. Yukagaku 39:321–326

Uppenberg J, Hansen MT, Patkar S, Jones TA (1994) The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida antarctica*. Structure 2:293–308. https://doi.org/10.1016/S0969-2126(00)00031-9

Uppenberg J, Ohmer N, Norin M et al (1995) Crystallographic and molecular-modeling studies of lipase B from *Candida antarctica* reveal a stereospecificity pocket for secondary alcohols. Biochemistry 34:16838–16851. https://doi.org/10.1021/bi00051a035

Vakhlu J, Kour A (2006) Yeast lipases: enzyme purification, biochemical properties and gene cloning. Electron J Biotechnol 9:69–85

Van Griensven J, Carrillo E, López-Vélez R et al (2014) Leishmaniasis in immunosuppressed individuals. Clin Microbiol Infect 20:286–299. https://doi.org/10.1111/1469-0691.12556

Vasel B, Hecht HJ, Schmid RD, Schomburg D (1993) 3D-Structures of the lipase from Rhizomucor miehei at different temperatures and computer modelling of a complex of the lipase with trilaurylglycerol. J Biotechnol 28:99–115. https://doi.org/10.1016/0168-1656(93)90128-A

Ville E, Carrière F, Renou C, Laugier R (2002) Physiological study of pH stability and sensitivity to pepsin of human gastric lipase. Digestion 65:73–81. https://doi.org/10.1159/000057708

Wang D, Xu Y, Shan T (2008a) Effects of oils and oil-related substrates on the synthetic activity of membrane-bound lipase from Rhizopus chinensis and optimization of the lipase fermentation media. Biochem Eng J 41:30–37

Wang X, Lu D, Jönsson LJ, Hong F (2008b) Preparation of a PET-hydrolyzing lipase from Aspergillus oryzae by the addition of bis(2-hydroxyethyl) terephthalate to the culture medium and enzymatic modification of PET fabrics. Eng Life Sci 8:268–276. https://doi.org/10.1002/elsc.200700058

Wang H, Zhong S, Ma H et al (2012) Screening and characterization of a novel alkaline lipase from *Acinetobacter calcoaceticus* 1–7 isolated from Bohai Bay in China for detergent formulation. Brazilian J Microbiol 43:148–156. https://doi.org/10.1590/S1517-83822012000100016

Whitaker JR, Sonnet PE (1989) Biocatalysis in agricultural biotechnology. Am Chem Soc

Winkler FK, D'Arcy A, Hunziker W (1990) Structure of human pancreatic lipase. Nature 343:771–774. https://doi.org/10.1038/343771a0

Xie Y, An J, Yang G et al (2014) Enhanced enzyme kinetic stability by increasing rigidity within the active site. J Biol Chem 289:7994–8006. https://doi.org/10.1074/jbc.M113.536045

Xu J, Cen Y, Singh W et al (2019) Stereodivergent protein engineering of a lipase to access all possible stereoisomers of chiral esters with two stereocenters. J Am Chem Soc 141:7934–7945. https://doi.org/10.1021/jacs.9b02709

Yadav AN (2017) Beneficial role of extremophilic microbes for plant health and soil fertility. J Agric Sci 1(1):1–4

Yadav R, Saxena R, Gupta R, Davidson S (1998) Purification and characterization of a regiospecific lipase from *Aspergillus terreus*. Biotechnol Appl Biochem 28:243–249

Yadav AN, Verma P, Kumar V, Sachan SG, Saxena AK (2017) Extreme cold environments: a suitable niche for selection of novel psychrotrophic microbes for biotechnological applications. Adv Biotechnol Microbiol 2(2):1–4

Yadav AN, Kour D, Sharma S, Sachan SG, Singh B, Chauhan VS, Sayyed RZ, Kaushik R, Saxena AK (2019a) Psychrotrophic microbes: biodiversity, mechanisms of adaptation, and biotechnological implications in alleviation of cold stress in plants. In: Sayyed RZ, Arora NK, Reddy MS (eds) Plant growth promoting rhizobacteria for sustainable stress management. Volume 1: Rhizobacteria in abiotic stress management. Springer Singapore, Singapore, pp 219–253. https://doi.org/10.1007/978-981-13-6536-2_12

Yadav AN, Mishra S, Singh S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 1: Diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019c) Recent advancement in white biotechnology through fungi. Volume 2: Perspective for value-added products and environments. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N (2020a) Microbiomes of extreme environments, Volume 2: Biotechnological applications in agriculture environment and industry. CRC Press, Taylor & Francis Group, Boca Raton

Yadav AN, Rastegari AA, Yadav N (2020b) Microbiomes of extreme environments: biodiversity and biotechnological applications. CRC Press, Taylor & Francis, Boca Raton

Yang C (2014) Hepatitis C treatments with Sofosbuvir, WO 2014/185995 Al

Yang T, Xu X, He H, Li L (2003) Lipase-catalysed modification of lard to produce human milk fat substitutes. Food Chem 80:473–481

Yang W, He Y, Xu L et al (2016) A new extracellular thermo-solvent-stable lipase from Burkholderia ubonensis SL-4: identification, characterization and application for biodiesel production. J Mol Catal B Enzym 126:76–89

Yapoudjian S, Ivanova MG, Brzozowski AM, Patkar SA, Vind J, Svendsen A, Verger R (2002) Binding of thermomyces (Humicola) lanuginosa lipase to the mixed micelles of cis-parinaric acid/NaTDC: fluorescence resonance energy transfer and crystallographic study. Eur J Biochem 269:1613–1621

Zeng Z, Xiao X, Wang P, Wang F (2004) Screening and characterization of psychrotrophic, lipolytic bacteria from deep-sea sediments. J Microbiol Biotechnol 14:952–958

Zhang M, Yu XW, Xu Y et al (2019) Structural basis by which the N-Terminal polypeptide segment of rhizopus chinensis lipase regulates its substrate binding affinity. Biochemistry 58:3943–3954. https://doi.org/10.1021/acs.biochem.9b00462

Chapter 14
Fungal Xylanases for Different Industrial Applications

Farial Mehmood Dar and Parsa Mahmood Dar

Contents

F. M. Dar
District Headquarter Hospital, Jhelum, Punjab, Pakistan

P. M. Dar (✉)
Institute of Chinese Medical Sciences, Avenida da Universidade, Taipa, Macau, China

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_14

14.1 Introduction

The vast variety of life-including biochemical reactions are almost always regulated by biological catalysts, i.e., enzymes. They have a high degree of specificity for their substrates, and work under relatively mild conditions in aqueous solutions. The selection of the appropriate microorganism is very critical for the development of microbial enzymes (Singh et al. 2016; Yadav et al. 2016). The microorganisms are usually isolated from the soil. Demand for industrially essential enzymes of microbial origin is growing because of their low cost of production (Kour et al. 2019a, b). Since the versatility of microbial enzymes is fantastic, they offer a wide variety of features that make them ideal for different applications. Many of the microbial enzymes such as protease, amylase, xylanase, cellulase, tannase, lipase, etc. are used in the food, farming, medicinal, cosmetics, and other biotech industries (Yadav et al. 2019a).

Hemicelluloses are xylans (Fig. 14.1a) and the second most known polysaccharide (Collins et al. 2005). Both compounds are found in the cell wall of plant cells and in the middle lamella. Hemicellulose groups are called by the principal sugar classification. Therefore, when a polymer is hydrolyzed and yields xylose, it is a xylan; likewise, hemicelluloses include mannans, glucans, arabinans, and galactans (Whistler and Richards 1970; Viikari et al. 1994; Uffen 1997; Ebringerova and Heinze 2000). Hemicellulose from wood in nature hardly ever consists of only one form of sugar. These are typically complex structures composed of more than one polymer, the most common being glucuronoxylans, arabinoglucuronoxylans, glucomannans, arabinogalactans, and galactoglucomannans (Haltrich et al. 1996; Sunna and Antranikian 1997; Kulkarni et al. 1999; Subramaniyan and Prema 2002). The sum of each variable varies between species, and between tree and branch.

The plant cell wall consists of cellulose (35–50%), hemicellulose (20–30%, mostly xylan), and lignin (20–30%). Cellulose and hemicellulose bind through covalent and non-covalent interactions to lignin. Xylan is the second major hemicellulosic component with a linear backbone of β-1, 4-linked xylosis, and annual plant cell wall content accounts for 30, 15–30% of hard wood, and 7–10% of soft wood. Xylan is a heteropolysaccharide which contains substituents of O-acetyl,

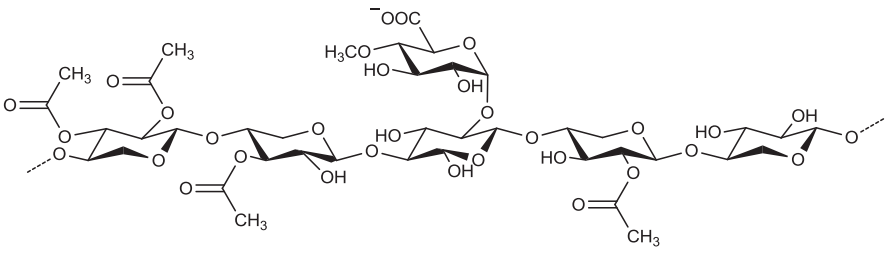


Fig. 14.1 (**a**) Structure of Xylan. (**b**) Structure plant xylan showing different substituent groups with sites of attack by microbial xylanases. (**c**) Glucuronoxylans a linear polymer chain showing linkages at different sites

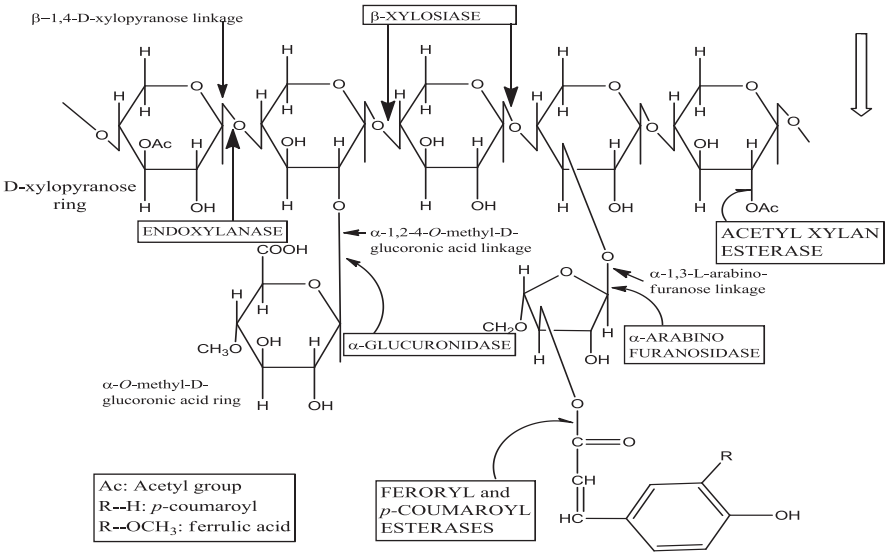


Fig. 14.1 (continued)

arabinosyl, and 4-O-methyl-d-glucuronic acid. It is substituted by ester bonds by glycosidic bonds with acetic acid and ferulic acid with l-arabinose, D-galactose, D-mannoses, and glucuronic acid (Collins et al. 2005; Ahmed et al. 2011). The depolymerization activity of endo-1,4-xylanases (1,4-β-xylan xylanohydrolase; EC 3.2.1.8) and β-d-xylosidase (1,4-β-xylan xylohydrolase; EC 3.2.1.37) results in the transformation of the polymer material into xylooligosaccharides and xylose (Gomez et al. 2008; Juturu and Wu 2014). Xylan skillfully forms a double extended ribbon-like structure by intrachain hydrogen bonding, which is said to be springier than the double helix of β-(1–4) cellulose.

β-(1, 2)-glycosidic connections with 4-group methyl glucuronic acid bind replacement brans to the backbone in hardwood xylan. Therefore, O-acetyl groups also replace OH groups in positions C-2 and C-3 (Fig. 14.1b), the presence of acetyl groups is responsible for the partial solubility of the water of xylan.

β-(1,2)-glycosidic connections with 4-methylglucuronic acid connect the substituent divisions with the backbone of hardwood xylan. In the backbone chain, the acetyl groups in softwood xylan are smaller. However, softwood xylan has other branches of β-(1, 3)-glycosidic bond composed of units of arabinofuranose bundled to the backbone (Puls and Schuseil, 1993). Softwood has more 4-O-methyl-αD-glucuronopyranosyl units than hardwood. These xylans are not acetylated and the furanoside structure of the arabinose side groups is readily hydrolyzed by acid (Ferreira-Filho Sunna and Antranikian, 1997). Both hardwood and softwood xylans are reduced to the rhamnosyl, galacturonosyl, and xylosyl residues in the end group (Andersson et al. 1983). Xylans in grass are usually arabinoxylan (Wilkie, 1979; Aspinall, 1980) and some xylans contain different combinations of arabinoxyl,

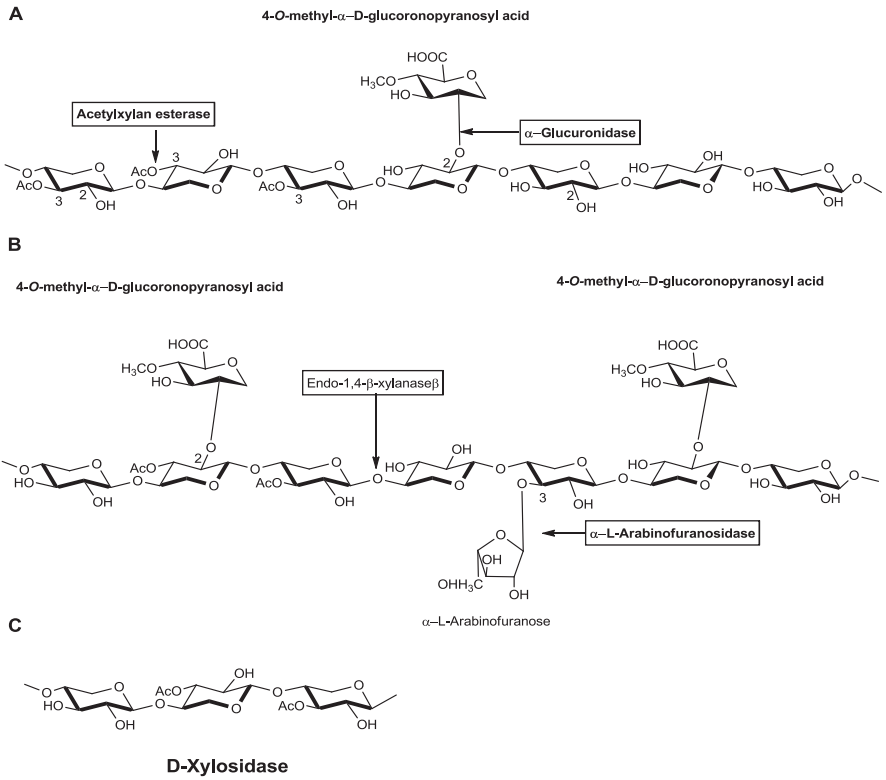


Fig. 14.1 (continued)

galactosyl, glucuronosyl, and xylosyl residues (Aspinall, 1980). Glucuronoxylans are linear polymers of units of (1–4) glycosidic (xylose) β-D-xylopyranosyl, as shown in (Fig. 14.1c). The polysaccharide base is packed with 4-O-methyl-α-D--glucuronosyl acid. Glucuronopyranosyl groups, groups of D-glucuronosyl methylated in position 4, and in position 2 or 3 joined to β-D-xylopyranosyl.

Angiosperm glucuronoxylans have a high substitution rate (70–80%) for acetyl groups in Position 2 and/or Position 3 of β-D-xylopyranosyl, giving xylan partial water solubility (Coughlan and Hazlewood 1993). Glucuronoarabinoxylans, usually found in softwood, have the same xylan backbone but in each of them ten β-D-xylopyranosyl units substitute α-L-arabinofuranosyl.

14.2 Occurrence of Xylan

Lignocellulosic biomass, one of the most plentiful materials in the world, is the largest repository of fixed carbon in nature. The primary sources of these materials are trees and crop residues. The lignocellulose key components are classified into four major classes, such as cellulose, hemicellulose, lignin, and extractives (Puls and Schuseil 1993; Yadav et al. 2020) (Table 14.1) and include a study on the distribution of lignocellulosic materials.

Fungi, yeast, bacteria, and marine algae contain xylanases. Microorganisms are the important xylanase creator (Yadav et al. 2019b). Endoxylanases usually have a peak activity between 40 and 80 °C and pH 4.0 and pH 6.5 although ideal conditions outside these ranges have also been identified. A list of microorganisms provided by xylan is given in (Table 14.2).

14.3 Xylanolytic Complex

Xylanases catalyze hydrolysis of xylans. Because of its complexity, xylan degradation requires not only an enzyme but also a complex enzyme. These are primarily produced by microorganisms and are used for the breakdown of the cell walls, the hydrolyzing of polysaccharides and xylanes during the sprouting of seeds (e.g., barley malt). The fish, protozoa, crustaceans, insects, snails, and land-plant grains can also be included in xylanases (Sunna and Antranikian 1997). Filamentous fungi are of special importance in microbial sources because they are secreted into the medium and their concentrations of xylanase are much higher than those present in yeasts and bacteria (Yadav 2020; Abdel-Azeem et al. 2021).

Endoxylanases and β-xylosidases are the most studied components of this system. Ferulic acid esterases, *p*-coumaric acid esterases, acetylxylan esterase, and α-glucuronidase were only discovered by the end of the 1980s, likely due to issues with the search for suitable substrates.

Table 14.1 Distribution of lignocellulosic components in softwoods, hardwoods, and wheat straw

	Weight, % of dry material		
	Softwoods	Hardwoods	Wheat straw
Cellulose	42 ± 2	45 ± 2	36 ± 5
Hemicellulose	27 ± 2	30 ± 5	27 ± 3
Lignin	28 ± 3	20 ± 4	11 ± 3
Extractives	3 ± 2	5 ± 3	26 ± 5

Table 14.2 Characteristics of some xylanases produced by different microorganisms. ND Not determined

Microorganisms	Molecular weight (kDa)	Optimal temperature (°C)	Optimal pH	Reference
Aspergillus aculeatus	18, 26, 52	50, 50, 70	4.0, 4.0, 5.0	Fujimoto et al. (1995)
Aspergillus awamori	39, 23, 26	45–55	4.0–5.5	Kormelink et al. (1993)
Aspergillus fischeri	31	60	6.0	Raj and Chandra (1996)
Aspergillus fumigatus	19, 8.5	55	5.5	Silva et al.(1999)
Aspergillus kawachii	35, 26, 29	60, 55, 50	5.5, 4.5, 2.0	Ito et al. (1992)
Aspergillus nidulans	22, 34	62, 56	5.5, 6.0	Fernández-Espinar (1994)
Aspergillus nidulans KK-99	ND	55	8.0	Taneja et al. (2002)
Aspergillus oryzae	35	60	5.0	Kitamoto et al. (1999)
Aspergillus sojae	33, 36	60, 50	5.0, 5.5	Kimura et al. (1995)
Aspergillus terreus	ND	50	7.0	Ghanen et al. (2000)
	ND	45	4.5	Ghareib and El Dein (1992)
Aspergillus versicolor	19	55	6.0	Carmona et al. (1998)
Acrophialophora nainiana	22	55	7.0	Salles et al. (2000)
Chaetomium cellulolyticum	25, 47, 57	50	5.0–7.0	Baraznenok et al. (1999)
Cryptococcus sp.	22	40	2.0	Iefuji et al. (1996)
Fusarium oxysporum F3	20.8, 23.5	60, 55	6.0	Christakopoulos et al. (1996)
H. grisea var. thermoidea	23	70	5.5	Monti et al. (1991)
Myceliophthora sp.	53	75	6.0	Chadha et al. (2004)
Penicillium brasilianum	31	ND	ND	Jørgensen et al. (2003)
Penicillium capsulatum	22	48	3.8	Ryan et al. (2003)
Penicillium sp.	25	50	2.0	Kimura et al. (2000)
Thermoascus aurantiacus	ND	70–75	4.0–5.0	Kalogeris et al. (1998)
Thermomyces lanuginosus	24.7	70	6.0–6.5	Singh et al. (2000)
Trichoderma harzianum	20	50	5.0	Tan et al. (1985)
	60	70	4.0–4.5	

14.3.1 Xylanolytic Enzymes

Xylanases catalyze hydrolysis of xylans. Because of its complexity, xylan degradation requires not only an enzyme but also a complex enzyme.

These are primarily produced by microorganisms and are used for the breakdown of the cell walls, the hydrolyzing of polysaccharides and xylanes during the sprouting of seeds (e.g., barley malt). The fish, protozoa, crustaceans, insects, snails, and land-plant grains can also be included in xylanases (Sunna and Antranikian 1997). Filamentous fungi are of special importance in microbial sources because they are secreted into the medium and their concentrations of xylanase are much higher than those present in yeasts and bacteria.

Endoxylanases and β-xylosidases are the most commonly studied components of this system. Ferulic acid esterases, p-coumaric acid esterases, acetylxylan esterase, and α-glucuronidase were only discovered by the end of the 1980s, likely due to issues with the search for suitable substrates.

14.3.2 Endo-1-4-β-Xylanases

The glycosidic bonds in the xylan backbone are cleaved by endo-1, 4-β-D-xylan xylanohydrolase (EC 3.2.1.8), which contribute to a reduction in the degree of substrate polymerization. Xylan is not randomly attacked but the selected bonds are dependent on the nature of the substratum molecule, i.e., chain length, branching degree, and substitution presence (Puls and Poutanen 1989; Li et al. 2000). The final products they release from xylan hydrolysis (i.e., xylose, xylobiose, xylotriose, and arabinose) differentiated endoxylanases. xylanases may also be categorized as non-debranching or debranching arabinose enzymes. Fungal endoxylanases are almost exclusively single unit proteins, usually glycosylated, with molecular weight values of 8.5–85 kDa and 4.0–10.3 isoelectric points (pI) (Coughlan and Hazlewood 1993), respectively. Endoxylanases typically exhibit a high activity of 40–80 °C and 4.0–6.5 pH, while conditions below these ranges are optimal.

Specific endoxylanase fungi which have three or more enzyme activity are isolated from one single culture (Rizzatti et al. 2004). A number of factors can be responsible for the various mechanisms often found for endoxylanases. These involve differential mRNA processing, post-translation shifts, such as glycosylation and self-aggregation, and proteolytics digestion. Various gene alleles, including distinct genes, can also express endoxylanases (Sung et al. 1995; Segura et al. 1998; Chavez et al. 2002). The xyn A gene, which encodes the endoxylanase in the yeast *Saccharomyces cerevisiae*, is known as heterologous (Nuyens et al. 2001). In S, the cloning and expression of *Trichoderma reesei* and *Aspergillus kawachii* endoxylanase genes among the fungi were achieved in *S. cerevisiae* (Dalboge, 1997).

14.3.3 β-Xylosidases

β-D-Xylosidases (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37) can be classified by their respective affinities for xylobiose and larger xylooligosaccharides. The molecular weight of the pathways may be monomeric, dimeric, or tetrameric between 26 and 360 kDa (Octavio and Francisco 2006). The most effective substratum for pure β-xylosidoses is xylobiose, and their resistance to xyloligosaccharides is inversely proportional to their degree of polymerization. It is possible to clear artificial substrates, such as p-nitrophenyl-β-d-xylopyranoside (Polizeli et al. 2005). When xylan has suffered from several consecutive hydrolyses, an integral feature attributed to β- xylosidases occurs.

14.3.4 α-Glucuronidases

α-Glucuronidase (EC 3.2.1.131) α-1, 2 is connections between residues of glucuronic acid and glucuronoxyl β-Dxylopyranosyl backbone (Kaneko et al. 1993).

14.3.5 α-Arabinofuranosidases

Arabinofuranosidases eliminate L-arabinose residues substituted at positions 2 and 3 of β-D-xylopyranosyl. There are two forms with distinct modes of action, exo-α-L-arabinofuranosidase (EC 3.2.1.55) which degrade p nitrophenyl-α-L-arabinofuranosides and branched arabinans and endo-1, 5-α-L-arabinase (EC 3.2.1.99) which hydrolyzes only linear arabinans (De Vries et al. 2000).

14.3.6 Acetylxylan Esterase

Acetylxylan esterase (EC 3.1.1.6) eliminates the O-acetyl substituents in acetylated xylans at 2 and 3 positions of xylose residues. In xylan hydrolysis, acetylxylan plays a key role, as the acetyl-side groups can interfere with the approach of enzymes that cleave the backbone through steric hindrance and their removal thus facilitates endoxylanase activity (Octavio and Francisco 2006).

14.3.7 *Ferulic Acid Esterase (EC 3.1.1.-) and* p-*Coumaric Acid Esterase*

The first is a clove between the arabinose and the ferulic acid side groups, while the second is a cleavage between arabinose and p-coumaric acid (Christov and Prior 1993; Williamson et al. 1998) (EC 3.1.1.) and *p*-coumaric acidivate esterase (EC 3.1.1.).

14.3.8 *Collaborative Effects of Enzymes in the Xylanolytic Complex*

Synergistic and cooperative effects among xylan-degrading enzymes increase the vulnerability of the heteropolymeric xylan to endoxylanase attacks (van Peij et al. 1997; de Vries et al. 2000). The addition of acetylxylan esterases to xylan thus leads to liberation of acetic acid and less acetylated xylan, which allows endoxylanase hydrolysis greater accessibility. By comparison, small acetylated polymers formed by endoxylanase are the preferred esterase substrates. Complex substrates such as wheat bran, which contains very large quantities of arabinoxylan, cannot be easily degraded by endoxylanases without α-arabinofuranosidase being tested beforehand. These enzymes, in conjunction with endoxylanases, enhance arabinoxylan saccharification.

As already described, β-xylosidases may be responsible for removing xylooligosaccharides, xylanase inhibition of the drug, allowing for more effective xylan hydrolysis. Therefore, the ideal microorganism for biotechnological purposes will be one that generates a sufficient amount of each of the enzymes of the xylanolytic complex. Such complexes have very high MW (500–600 kDa) and can consist of more than ten xylanolytic-activated proteins, some of which are endoxylanases (Beg et al. 2001).

Work on microorganisms using xylan, and on the enzyme systems involved, is becoming increasingly important in terms of ecology and economy. Mesophiles and thermophiles synthesize xylanases (Smith et al. 1991). The genera Aspergillus and Trichoderma are pre-eminent in the development of xylanase, among the mesophilic fungi. Much effort has been made in recent years to isolate thermophilic and even extremophilic microorganisms, as they generate enzymes of greater stability (Lasa and Berenguer 1993; Harris et al. 1997; Ishihara et al. 1997; Kalogeris et al. 1998; Andrade et al. 1999; Puchart et al. 1999; Maheshwari et al. 2000; Rizzatti et al. 2001; Bruins et al. 2001) which include *Chaetomium* thermophilic fungi, *Humicola insolens, Humicola lanuginosa, Humicola grisea*, *Melanocarpus albomyces, Paecilomyces variotii, Talaromyces byssochlamydoides*, *Talaromyces emersonii, Thermomyces lanuginosus,* and *Thermoascus aurantiacus*.

These fungi's xylanases have optimum temperatures between 60 and 80 °C, and are very stable in this range. Typically these enzymes are glycoproteins and mostly

show the highest activity at a pH level (4.5–6.5). They occur in a multiplicity of forms and in the range 6–38 kDa, the majority exhibit variable MWs. Most thermophile endoxylanases have some degree of structural homology with mesophilic homologues.

14.4 Xylanase Production: Submerged and Solid-State Fermentation

Xylanases are formed by a particular fermentation process, utilizing different microorganisms. Clear knowledge of the physiology of the microbial system and various metabolic processes enhanced the fermentation cycle. Nevertheless, enzyme efficiency can still be improved. Submerged (SmF) and solid-state (SSF) fermentation has produced xylanase (Motta et al. 2013). The range of the fermentation process, in general, depends on the type of microorganisms used (Table 14.3). SmF is

Table 14.3 Differences between SmF and SSF are given below

Factors	SmF	SSF
Substrate	Soluble substrate(sugars)	Insoluble substrate: starch, cellulose, pectin, lignin
Aseptic conditions	Heat sterilization and aseptic control	Vapor treatment Non-sterile conditions
Water	High volumes of water consumed and effluents discarded	Limited consumption of water, No effluent
Metabolic heating	Easy control of temperature	Low heat transfer capacity
Aeration	Limitation by soluble oxygen, high level of air required	Easy aeration and high surface exchange air/substrate
pH control	Easy pH control	Buffered solid substrate
Mechanical agitation	Good homogenization	Static conditions preferred
Scale up	Industrial equipment available	Need for engineering and new design equipment
Inoculation	Easy inoculation, continuous process	Spore inoculation, batch
Inoculation size	Small < 10%	Large > 10%
Contamination	Risk of contamination for single strain	Risk of contamination for low rate fungi growth
Energy consideration	High energy consumption	Low energy consumption
Volume equipment	High volumes	Low volumes
Cost	High cost technology	Low cost equipment
Effluent and pollution	High volumes of polluting effluents	High volumes, less pollution
Production rate	Low	High
Production yield	Low	High

favored because fungi need less moisture due to their mycelia presence and can be grown under SSF (Walia et al. 2017).

Some studies indicate that submerged fermentation with fungi is the most favored method for xylanase production. SmF produces about 90% of total xylanase worldwide. The synergistic effect of various enzymes can be observed during SmF and can also lead to a better use of biomass for increased production of xylanase (Polizeli et al. 2005; Bajpai 2014). Xylanase processing uses *Aspergillus oryzae* LC1 and *Aspergillus foetidus* as a residue of soybean and rice as a substratum under SmF (Bhardwaj et al. 2017). Similarly Irfan et al. (2016) proposed development of SmF xylanase with *B. Subtilis* BS04, and *B. Megaterium* BM07. Various SmF benefits over the medium were homogeneous; the mechanism is well defined and easily generalizable (Guleria et al. 2013). SmF has some drawbacks that also restrict its industrial use, such as high maintenance costs, intense energy and downstream volatility (Virupakshi et al. 2005; Walia et al. 2017).

Trichoderma koeningi SSF has shown improved production of xylanase using corn cob with pineapple powder (Bandikari et al. 2014). It has a number of benefits including low cost of production, service, and equipment, lower risk of contamination, rapid enzyme recovery, and high efficiency of reactors.

14.5 Strategies Employed for the Selection of the Method of Xylanase Production and Its Optimization

Initially a growing minimal medium is used to provide vital nutrients for the production of microorganisms. It will make it possible to test the strains to generate the appropriate enzymes / metabolite of the desired interest. The process is then further tailored for higher strain enzyme production (Walia et al. 2017). Different approaches are used to increase yields on the target product such as the optimization of media products, physical parameters of growth, and stress enhancement using different biotechnology methods (Sharma 2017). During SmF for the production of enzymes, various components that need to be optimized include selection of substrate and microorganisms, regulation of media concentrations of nutrients, i.e., carbon, nitrogen, trace elements, vitamins and amino acids, and physical parameters, i.e., temperature , pH, agitation, aeration, inoculum sizes, and incubation periods (Motta et al. 2013; Walia et al. 2015, 2017). The SSF optimization calls for particle sized, pre-treatment, humidity , water contents, and water activity (aw) regulating substrate, inoculum type and size, extra heat removal generated during microbial metabolism and mainly for the uniform environment (temperature) and evolution of CO_2 and O_2, i.e., gasses system (Murugan and others) (Table 14.4).

Table 14.4 Commercial xylanases produced by microorganisms. SbmF Submerged fermentation, SSF solid substrate fermentation, n.c. not cited

Commercial name	Distributors	Microorganism	Fermentation	Optimal pH	Optimal temperature (°C)	Application
Allzym PT	Alltech	*Aspergillus niger*	SbmF	5.3	65	Animal feed improvement
Amano 90	Amano Pharmaceutical		SSF wheat raw	4.5	50	Pharmaceutical analysis, food industry
Bio-feed Plus	Novo Nordisk	*Humicola insolens*	SbmF	n.c.	n.c.	Animal feed
Resinase	A/S	n.c.	n.c.	n.c.	n.c.	Cellulose and paper industry
Bleachzyme	Biocon, India	n.c.	n.c.	6.5–7.0	40–50	Cellulose pulp bleaching
Cartazyme	Clariant, UK	*Thermomonospora*	n.c.	5.0	45–55	
EcopulpX-200	Primalco	*Trichoderma*	SbmF	5.0–6.0	50–55	Cellulose pulp bleaching
Ecosane	Biotec	Reesei	SbmF	n.c.	n.c.	Animal feed
Ecozyme	Thomas Swan, UK	n.c	n.c.	7.0	50	Cellulose pulp industry
Grindazym GP e GV	Danisco ingredients	*A. niger*	SbmF	n.c.	n.c.	Bird and pig feed
Irgazyme 40	Nalco-Genencor, Ciba, Geigy	*Trichoderma Longibrachiatum*	SbmF	n.c.	n.c.	Paper industry and animal feed
Multifect XL	Genencor	*Trichoderma Longibrachiatum*	SbmF	5.0–5.5	55–60	Food industry
Solvay pentonase	Solvay Enzymes	*T. reesei*	SbmF	5.3–5.5	50	Cellulose and paper industry
Sternzym HC 46	Sternzym		SSF	n.c.	n.c.	Bread making
Sumizyme X	Shin Nihon	*Trichoderma Koningii*	SSF wheat Raw	5.0	55	Manufacture of mushrooms and vegetables extracts, bread making, enzymatic peeling of cereals, animal feed
Xylanases	Seikagaku	*Trichoderma* sp.	SbmF	n.c.	n.c.	Carbohydrate structural studies

Commercial name	Distributors	Microorganism	Fermentation	Optimal pH	Optimal temperature (°C)	Application
Xylanases	Granotec do Brazil	n.c.	n.c	n.c.		Weight decreasing in cream crackers, better texture and taste,Wafer's uniformity improvement
Xylanases GS35	Logen	*T. reesei*	SbmF	4.5	40	Cellulose pulp bleaching, animal feed
Biobrite		n.c.	n.c.	5.0–6.0	55	Cellulose and paper industry

14.6 Xylanases Applications

Commercial xylanases are produced in Japan, Finland, Germany, Ireland, Denmark, Canada, and the USA, for example, *Aspergillus niger*, *Trichoderma* sp, are the microorganisms used to produce these enzymes and Humicola insolens. The use of xylanase with these unusual thermo-alkaline-tolerant properties differs from one industry to the next, initially in the preparation of animal feed and subsequently in the fruit, fiber, paper and pulp processing, deinking, use of biomass to food industries, etc. Currently, together with pectinases, xylanase and cellulase represent 20% of the world market for enzymes. The use of xylans and xylanases in biotechnology has grown remarkably in recent years (Subramaniyan and Prema 2002; Beg et al. 2001; Techapun et al. 2003)

Two types of hydrolysis will convert xylan and its oligosaccharides into β-D-xylopyranosyl: acid or enzyme. Acid hydrolysis is also favored because it is faster but followed by toxic compounds that can interfere with subsequent microbial fermentation. It can also result in metallic machinery corrosion, which in the long term encounters acid. Several industrial companies have recently expressed an interest in developing active enzyme techniques used in the treatment of products containing hemicellulose rather than acid hydrolysis.

14.6.1 Bakery

The possible efficacy of xylanolytic enzymes in bread making has increased in the last few decades (Butt et al. 2008). Enzymatic hydrolysis of non-starch polysaccharides leads to improvement of the rheological characteristics of meal, basic bread volume, and crumb firmness (Martínez-Anaya and Jimenez 1997). The xylanases break down the hemicellulose in wheat flour, aid with water redistribution, and promote the kneading of the mass. The use of xylanases has increased bread length, increased water absorption, and increased fermentation power. (Bhardwaj et al. Bioresour. Bioprocess. (2019) 6:40 Page 22 of 36). The use of xylanases increases the longevity with lower intensity. A higher amount of arabinoxylan oligosaccharides would also improve safety (Polizeli et al. 2005). In the production of biscuits, xylanase is recommended to lighten cream crackers and increase wafer texture, smoothness, and consistency (Polizeli et al. 2005)

The bread made from xylanase showed low springiness and rubber content (Driss et al. 2013). Ghoshal et al. (2013) used microbial xylanase partially filtered to produce whole wheat bread with enhanced sensory properties (glossier color). Recombinant xylanase (r-XynBS27) from *Pichia pastoris* is used as an additive for the bread making. The recombinant xylanase increased specific volume and the sugar content, with lower friction, consistency, and stiffness.

14.6.2 Fruit and Vegetable Juices

A substantial part of the juice sector is in the enzyme business. The processing of fruit and vegetable juice includes methods of extraction, clearing, and stabilization. When the production of citrus fruit juices started in the 1930s, the yields were low and the turbidity of the juice filtration created problems. Awareness of fruit chemicals and the use of microbial enzymes have contributed to solving these problems. Nowadays, xylanase leads in conjunction with celluloses, amylases, and pectinases to an improved yield of juice by liquefaction of fruit and vegetables; stabilization of the fruit pulp; an improved recovery in aromas, essential oils, vitamins, mineral salts, comestible colors, pigments, etc. In combination with endoglucanase, xylanase participates in the hydrolysis of arabinoxylan and starch, which separates and isolates gluten from starch in wheat flour. This enzyme is also used in the preparations for coffee bean mucilage (Wong et al. 1988; Wong and Saddler, 1993)

Recently, recombinant wine yeast has been produced with the xlnA gene of Aspergillus nidulans, which has a more pronounced aroma than conventional wines (Ganga et al. 1999). The cell wall of the barley is hydrolyzed during its development and releases long chains of arabinoxylans which increase the viscosity of the beer, making it "muddy." So xylanases are used to hydrolyze arabinoxylan to the viscosity of beer oligosaccharides and thus to eliminate the muddy side of beer (Debyser et al. 1997; Dervilly et al. 2002).

14.6.3 Animal Feed

Xylanases are used as pre-treatment crops to improve the nutritional properties of silage and grain feed (Subramaniyan and Prema 2002; Kuhad and Singh 1993; Bedford and Classen 1992), thereby increasing ruminant foods' digestibility and promoting composting (Gilbert and Hazlewood 1993). But xylan removal is not required, as hemicelluloses are important dietary ingredients, which can increase bowel disease by removing xylan (Mandal, 2015).

In the presence of climate conditions enhancing the goat's ruminal activity, *Aspergillus japonicus* C03 showed applications for ruminant feed with good endoxylanase and cellulase development capability (Facchini et al. 2011). Xylanases were used for decades in animal feed, improving the digestible viscosity of poultry. The introduction of xylanase showed an increase in weight gain and an increased feed conversion ratio because of increased arabinoxylan digestibility in monogastric animal diets (Paloheimo et al. 2010; Van Dorn et al. 2018). Xylanase reported by Passos et al. (2015), used to complement the nutrient digestibility diet, digestive viscosity pigs feed soy meal-based morphology diets of maize. ECONASE XT is known to be a synthetic endo1.4-β-xylanase that was used for chicken fattening, piglet weaning, and pig fattening as feed additives (Rychen et al. 2018). The chicken

receives enough energy from less feed and leads to better weight and feed conversion efficiency. In a way, xylanase is applied to animal feed and the feed is predigested (Wu and Rabindran, 2004).

Xylanases are used in animal feed along with glucanases, pectinases, cellulases, proteases, amylases, phytases, galactosidases, and lipases. These enzymes break down arabinoxylans into feedstuffs, reducing the viscosity of the raw material (Twomey et al. 2003). Arabinoxylan in grain cell walls affects poultry as an antinutrient. When present in a soluble way, these components can increase the viscosity of the feed consumed, thereby interfering with mobility and other component absorption. Once xylanase is used in feeds containing maize and sorghum, both of which are feeds of low viscosity, nutrient digestion in the initial digestive tract may be increased, thus maximizing the use of energy. However, in the excreta (phosphore, phosphorus, copper, and zinc), this type of diet can reduce unwanted toxins, which can have a part to play in reducing emissions.

14.6.4 Paper and Pulp Industries

The use of xylanase eliminates organochlorine pollutants, including dioxins from the paper production process. Xylanase can minimize the chemical oxidation requirement by up to 20–40% (Garg et al. 1998). Xylanase does not affect cellulose; there is no adverse effect on paper product power.

The most popular pulping process is the Kraft or sulfate method, in which chips are cooked for lignin degradation and solubilization at approximately 170 °C for 2 h in a Na_2S/NaOH solution. The resulting pulp has a distinctive brown color primarily because of the presence of lignin and lignin residual derivatives. Pulp bleaching can be called the clearing process of lignin, organic colored content, and other undesirable remains, including deterioration, alteration, or solubilization (Madlala et al. 2001).

14.6.5 Bio-Pulp and Bleaching

Bio-pulping involves fungal preparation of wood chips and other lignocellulosic products with natural wood-decay fungi prior to mechanically or chemically pulping. The fungal therapy process is under way with the subsequent lignin removal. Wood is broken down, chipped and screened by frying. The bio-pulping approach is both technically feasible and economical. The key benefits of this approach are lower energy consumption and increased mechanical pulp efficiency. The method also increased paper strength and the environmental effect (Khonzue et al. 2011).

The use of xylanases helps to improve pulp fibrillation, decreases beating times in some pulp, and improves the free movement of reused fibers (Savitha et al. 2009). Some studies show that xylanase predicting is an environmentally sound and

economical invention and could minimize the amount of bleached chemicals needed in the subsequent chemical dehydration levels to achieve specified brightness. Pretreatment xylanase increases the efficacy of chemical pulp extraction of lignin and minimizes the need for chlorine dioxide (ClO_2) (Khonzue et al. 2011). The breakthrough further improves material strength, mass thickness, and breaking time, thus reducing the release of organic volatile compounds. Therefore xylanases used as part of the pulp and paper biobleaching may be a better option for the substitution of toxic chlorinated compounds without cellulase action (Golugiri et al. 2012).

14.6.6 Biorefinery

Production of biofuels has drastically gained importance as the energy resources are depleting as energy supplies are being the. Combining xylanase activity with other enzymes such as mannanase, lignase, xylosidase, glucanase, glucosidase, etc. can be implemented for the development of biofuels (ethanol and xylitol) from lignocellulosic biomass (Dominguez 1998). Ethanol from renewable resources has become an alternative fuel or an oxygen additive to current fossil fuels in recent decades (Sharma and Sharma 2016; Kaur et al. 2020; Prasad et al. 2020). The processing of bioethanol involves the dealignment of lignocellulose to release cellulose and hemicellulose. The next steps will include the depolymerization for free sugar processing carbohydrate polymers and fermentation for bioethanol development of mixed pentose and hexoses (Lee et al. 2009).

14.6.7 Chemical and Pharmaceutical Industries

Xylanase and xylanes in the pharmaceutical field are very little used. Xylanases are often used as a dietary supplement or as a combination of a complex of enzymes (hemicellulases, proteases, and others) to treat poor digestion, but few medicines are identified with these. Hydrolytic xylan products can be converted into fuel fluids (ethanol), solvents, or low-calorie artificial sweeteners, such as β-D-xylopyranosyl residue. The first steps are the dealignment of xylan-rich hemicellulose, followed by the hydrolysis of xylanases and hemicellulases for sugars like β-dxylopyranosyl. The products are then fermented in yeasts (Pichia stipitis and Candida shehatae) to produce xylitol or ethanol (Shapack et al. 1987; Screenath and Jeffries 2000).

Around 5% and 20% of sugars used in ethyl alcohol processing constitute β-D-xylopyranosyl residues. Xylitol is a sucrose relative sweetening polyalcohol (Parajó et al. 1998). Used as a non-cariogenic sweetener appropriate for diabetics and obesity to prevent osteoporosis and respiratory disorders, lipid metabolism diseases, kidney and parenteral lesions. A variety of consumer products, including chewing gum, contain xylitol. Although enzyme hydrolysis of xylan is a promising method

to obtain β-D-xylopyranosyl units, chemical catalysis currently produces large-scale commercial xylitol.

It is considered a cost-effective process, mainly because xylose must be purified in many stages initially. Furthermore, chemical reactions create fermentation-toxic by-products, as well as products derived from glucose (hydroxymethylfurfural) degradation (xylose (furfural) and lignin (aromatic and phenolic compounds and aldehyde) may be produced in the decomposition of lignocellulose material. Active microbial activity inhibitors can be substances released from lignocellulose structures, including acetic acid and related substances (e.g., terpenes and their derivatives, tropolones and phenolic compounds, flavonoids, stilbenes, quinones, lignans, and tannins) or system (iron, chromium, nickel, and copper). The invention of a better technology of xylitol processing has led to greater hope for its broader use in the food industry, pharmaceutical industry, and odontology.

14.6.8 Textile Industries

A combined xylanase-pectinase system is used in the debarking process, processed by the first stage of wood, which will increase the retting period. Certain uses of this integrated method are used to decompose fibers such as flax, cotton, jute, and fibers from plants instead of retting (Beg et al. 2001). The xylanolytic complex can be used in the textile industry to process plant fibers, such as hessian or linen. The enzyme preparation of textile fibers has done relatively little research, yet this appears to be a lucrative field which calls for the development of new techniques.

14.6.9 Solid Waste Treatment

Significant amounts of xylane can be found in agricultural and food waste products, where fungal xylanase can be used to transform xylane into xylose for the treatment of these waste materials. The development of an efficient enzymatic hydrolysis method offers new opportunities for hemicellulosic waste treatment (Rani and Nand 1996). Fungal xylanase involved in recycling waste paper contributing to increased paper resistance. High efficiency of decontamination leaves minimum ink load (Puneet Pathak, Nishi Bhardwaj volume 7, issue 3, 2018).

14.7 Conclusion

With the development of technologies, the production of microbial enzymes on the world market is making drastic progress. There is still room for new microorganisms that could possess better physiological characteristics in relation to

temperature, medium pH, and adaptability to low cost substrates on this great biodiversity. *Aspergillus* is the main xylanase producer. The apparent value of recombinant DNA technology is commendable as it expands the possibilities to further explore and have maximum advantages.

Soon, paper processing will lead to less harmful effects on nature, with a decrease in effluent release of toxins and thus better preservation of our fauna and flora. In the future there is great hope for emerging developments that will lead to advances in the production of xylanolytic systems or special xylanases. Various advantages of fungal xylanases have brought them to the fore. It was a major accomplishment to separate the mesophilic and thermophilic strains as their enzymes were more stable. To achieve high output of xylanase with desired industrial properties, the combination of current technology with emerging technologies such as synthetic biology (DNA oligo-synthesis), logical engineering, and directed growth can also be used.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Ahmed S, Jabeen A, Jamil A (2011) Xylanase from *Trichoderma harzianum*: enzyme characterization and gene isolation. J Chem Soc Pakistan 29:176

Andersson SI, Samuelson M, Ishihara O, Shimizu K (1983) Structure of the reducing end-groups in spruce xylan. Carbohydr Res 111:83–288

Andrade CMMC, Pereira N Jr, Antranikian G (1999) Extremely thermophilic microorganisms and their polymer-hydrolytic enzymes. Rev Microbiol 30:287–298

Aspinall GO (1980) Chemistry of cell wall polysaccharides. In: Preiss J (ed) The biochemistry of plants (a comprehensive treatise), Carbohydrates: structure and function, vol 3. Academic Press, Inc., New York, pp 473–500

Bajpai P (2014) Sources, production, and classification of xylanases. In: Xylanolytic enzyme. Academic Press (Imprint Elsevier), Tokyo, pp 43–52

Bandikari R, Poondla V, Obulam VSR (2014) Enhanced production of xylanase by solid state fermentation using *Trichoderma koeningi* isolate: effect of pretreated agro-residues. 3 Biotech 4:655–664. https://doi.org/10.1007/s13205-014-0239-4

Baraznenok VA, Becker EG, Ankudimova NV, Okunev NN (1999) Characterization of neutral xylanases from *Chaetomium cellulolyticum* and their biobleaching effect on eucalyptus pulp. Enzyme Microb Technol 25:651–659

Bedford, M. R. and Classen, H. L. (1992). The influence of dietary xylanase on intestinal viscosity and molecular weight distribution of carbohydrates in rye-fed broiler chick. In: Visser J, Beldman G, vanSomeren MAK, Voragen AGJ (Eds.), Xylans and xylanases,Elsevier, Amsterdam, pp. 362-370

Beg QK, Kapoor M, Mahajan L, Hoondal GS (2001) Microbial xylanases and their industrial applications: a review. Appl Microbiol Biotechnol 56:326–338

Bhardwaj N, Chanda K, Kumar B et al (2017) Statistical optimization of nutritional and physical parameters for xylanase production from newly isolated *Aspergillus oryzae* LC1 and its application in the hydrolysis of lignocellulosic agro-residues. BioResources 12:8519–8538

Bruins ME, Janssen AE, Boom RM (2001) Thermozymes and their applications: a review of recent literature and patents. Appl Biochem Biotechnol 90:155–186

Butt MS, Tahir-Nadeem M, Ahmad Z, Sultan MT (2008) Xylanases and their applications in baking industry. Food Technol Biotechnol 46:22–31

Carmona EC, Brochetto-Braga MR, Pizzirani-Kleiner AA, Jorge JA (1998) Purification and biochemical characterization of an endoxylanase from *Aspergillus versicolor*. FEMS Microbiol Lett 166:311–315

Chadha BS, Ajay BK, Mellon F, Bhat MK (2004) Two endoxylanases active and stable at alkaline pH from the newly isolated thermophilic fungus, Myceliophthora sp. IMI 387099. J Biotechnol 109:227–237

Chavez R, Schachter K, Navarro C, Peirano A, Aguirre C, Bull P, Eyzaguirre J (2002) Differences in expression of two endoxylanase genes (xynA and xynB) from *Penicillium purpurogenum*. Gene 293(1–2):161–168

Christakopoulos P, Nerinckx W, Kekos D, Macris B, Claeyssens M (1996) Purification and characterization of two low molecular mass alkaline xylanases from Fusarium oxysporum F3. J Biotechnol 51:181–189

Christov LP, Prior BA (1993) Esterases of xylan-degrading microorganisms, production, properties and significance. Enzyme Microb Technol 15:460–475

Collins T, Gerday C, Feller G (2005) Xylanases, xylanases families and extremophilic xylanases. FEMS Microbiol Rev 29:3–23

Coughlan MP, Hazlewood GP (1993) β-1,4-D-Xylan-degrading enzyme systems, biochemistry, molecular biology and applications. Biotechnol Appl Biochem 17:259–289

Dalboge H (1997) Expression cloning of fungal enzyme genes; a novel approach for efficient isolation of enzyme genes of industrial relevance. FEMS Microbiol Rev 21:29–42

De Vries RP, Kester HC, Poulsen CH, Benen JA, Visser J (2000) Synergy between enzymes from Aspergillus involved in the degradation of plant cell wall polysaccharides. Carbohydr Res 327(4):401–410

Debyser W, Derdelinckx G, Delcour JA (1997) Arabinoxylan solubilization and inhibition of the barley malt system by wheat during mashing with wheat whole meal adjunct, evidence for a new class of enzyme inhibitors in wheat. J Am Soc Brew Chem 55:153–157

Dervilly G, Leclercq C, Zimmerman D, Roue C, Thibault JF, Sauliner L (2002) Isolation and characterization of high molecular mass water-soluble arabinoxylans from barley malt. Carbohydr Polym 47:143–149

Dominguez JM (1998) Xylitol production by free and immobilized Debaryomyces hansenii. Biotechnol Lett 20:53–56

Driss D, Bhiri F, Siela M et al (2013) Improvement of breadmaking quality by xylanase GH11 from Penicillium occitanis Pol6. J Texture Stud 44:75–84. https://doi.org/10.1111/j.1745-4603.2012.00367.x

Ebringerova A, Heinze T (2000) Xylan and xylan derivatives— biopolymers with valuable properties. 1. Naturally occurring xylans structures, isolation procedures and properties. Macromol Rapid Commun 21:542–556

Facchini FDA, Vici AC, Reis VRA et al (2011) Production of fibrolytic enzymes by *Aspergillus japonicus* C03 using agro-industrial residues with potential application as additives in animal feed. Bioprocess Biosyst Eng 34:347–355

Fernández-Espinar M, Piñaga F, de Graaff L, Visser J, Ramón D, Vallés S (1994) Purification, characterization and regulation of the synthesis of an *Aspergillus nidulans* acidic xylanase. Appl Microbiol Biotechnol 42:555–562

Ferreira-Filho EX (1994) The xylan-degrading enzyme system. Braz J Med Biol Res 27:1093–1109

Fujimoto H, Ooi T, Wang S-L, Takizawa T, Hidaka H, Murao S, Arai M (1995) Purification and properties of three xylanases from *Aspergillus aculeatus*. Biosci Biotechnol Biochem 59:538–540

Ganga MA, Piñaga F, Vallés S, Ramón D, Querol A (1999) Aroma improving in microvinification processes by the use of a recombinant wine yeast strain expressing the *Aspergillus nidulans* xlnA gene. Int J Food Microbiol 47:171–178

Garg AP, Roberts JC, McCarthy AJ (1998) Bleach boosting effect of cellulase-free xylanase of Streptomyces thermoviolaceus and its comparison with two commercial enzyme preparations on birch wood Kraft pulp. Enzyme Microb Technol 22:594–598

Ghanen NB, Yusef HH, Mahrouse HK (2000) Production of Aspergillus terreus xylanase in solid-state cultures: application of the Plackett–Burman experimental design to evaluate nutritional requirements. Bioresour Technol 73:113–121

Ghareib M, El Dein MMN (1992) Purification and general properties of xylanase from Aspergillus terreus. Zentralbl Mikrobiol 147:569–576

Ghoshal G, Shivhare US, Banerjee UC (2013) Effect of xylanase on quality attributes of whole-wheat bread. J Food Qual 36:172–180. https://doi.org/10.1111/jfq.12034

Gilbert HJ, Hazlewood GP (1993) Bacterial cellulases and xylanases. J Gen Microbiol 139:187–194

Golugiri BR, Thulluri C, Cherupally M, Nidadavolu N, Achuthananda D, Mangamuri LN, Addepally U (2012) Potential of thermo and alkali stable xylanases from *Thielaviopsis basicola* (MTCC-1467) in biobleaching of wood Kraft pulp. Appl Biochem Biotechnol 167:2369–2380. https://doi.org/10.1007/s12010-012-9765-x

Gomez LD, Steele-King CG, McQueen-Mason SJ (2008) Sustainable liquid biofuels from biomass: the writing's on the walls. New Phytol 178:473–485

Guleria S, Walia A, Chauhan A, Shirkot CK (2013) Optimization of cultural conditions for cellulase-free xylanase production by mutant strain of alkalophilic Cellulosimicrobium sp. CKMX1 in submerged fermentation. Appl Biol Res 15:137–144

Haltrich D, Nidetzky B, Kulbe KD, Steiner W, Zupancic S (1996) Production of fungal xylanases. Bioresour Technol 58:137–161

Harris GW, Pickersgill RW, Connerton I, Debeire P, Touzel J-P, Breton C, Pérez S (1997) Structural basis of the properties of an industrially relevant thermophilic xylanase. Proteins 29:77–86

Iefuji H, Chino M, Kato M, Iimura Y (1996) Acid xylanase from yeast Cryptococcus sp. S-2: purification, characterization, cloning, and sequencing. Biosci Biotechnol Biochem 60:1331–1338

Irfan M, Asghar U, Nadeem M et al (2016) Optimization of process parameters for xylanase production by Bacillus sp. in submerged fermentation. J Radiat Res Appl Sci 9(2):139–147

Ishihara M, Tawata S, Toyama S (1997) Purification and some properties of a thermostable xylanase from thermophilic fungus strain HG-1. J Ferment Bioeng 835:478–480

Ito K, Iwashita K, Iwano K (1992) Cloning and sequencing of the xyn C gene encoding acid xylanase of *Aspergillus kawachii*. Biosci Biotechnol Biochem 56:1338–1340

Jørgensen H, Eriksson T, Börjesson J, Tjerneld F, Olsson L (2003) Purification and characterization of five cellulases and one xylanase from Penicillium brasilianum IBT 20888. Enzyme Microb Technol 32:851–861

Juturu V, Wu JC (2014) Microbial exo-xylanases: a mini review. Appl Biochem Biotechnol 174:81–92

Kalogeris E, Christakopoulos P, Kekos D, Macris BJ (1998) Studies on solid-state production of thermostable endoxylanases from *Thermoascus aurantiacus*. Characterization of two isozymes. J Biotechnol 60:155–163

Kaneko S, Shimasaki T, Kusakable I (1993) Purification and some properties of intracellular α-Larabinofuranosidase from *Aspergillus niger* 5–16. Biosci Biotechnol Biochem 57:1161–1165

Kaur T, Devi R, Kour D, Yadav N, Prasad S, Singh A, Negi P, Yadav AN (2020) Advances in microbial bioresources for sustainable biofuels production: current research and future challenges. In: Yadav AN, Rastegari AA, Yadav N, Gaur R (eds) Biofuels production – sustainability and advances in microbial bioresources. Springer International Publishing, Cham, pp 371–387. https://doi.org/10.1007/978-3-030-53933-7_17

Khonzue P, Laothanachareon T, Rattanaphan N, Tinnasulanon P, Apawasin S, Paemanee A, Ruanglek V, Tanapongpipat S, Champreda V, Eurwilaichitr L (2011) Optimization of xylanase production from *Aspergillus niger* for biobleaching of eucalyptus pulp. Biosci Biotechnol Biochem 75(6):1129–1134. https://doi.org/10.1271/bbb.110032

Kimura I, Sasahara H, Tajima S (1995) Purification and characterization of two xylanases and an arabinofuranosidase from *Aspergillus sojae*. J Ferment Bioeng 804:334–339

Kimura T, Ito J, Kawano A, Makino T, Kondo H, Karita S, Sakka K, Ohmiya K (2000) Purification, characterization, and molecular cloning of acidophilic xylanase from Penicillium sp. 40. Biosci Biotechnol Biochem 64:1230–1237

Kitamoto N, Yoshino S, Ohmiya K, Tsukagoshi N (1999) Purification and characterization of the overexpressed *Aspergillus oryzae* xylanase, XynF1. Biosci Biotechnol Biochem 6310:1791–1794

Kormelink FJM, Leeuwen MGFSL, Wood TM, Voragen AGJ (1993) Purification and characterization of three endo (1,4)-βD-xylanases and one β-xylosidase from Aspergillus awamori. J Biotechnol 27:249–253

Kour D, Rana KL, Kaur T, Singh B, Chauhan VS, Kumar A, Rastegari AA, Yadav N, Yadav AN, Gupta VK (2019a) Extremophiles for hydrolytic enzymes productions: biodiversity and potential biotechnological applications. In: Molina G, Gupta VK, Singh B, Gathergood N (eds) Bioprocessing for biomolecules production, pp 321–372. https://doi.org/10.1002/9781119434436.ch16

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA, Saxena AK (2019b) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kuhad RC, Singh A (1993) Lignocellulosic biotechnology: current and future prospects. Crit Rev Biotechnol 13:151–172

Kulkarni N, Shendye A, Rao M (1999) Molecular and biotechnological aspects of xylanases. FEMS Microbiol Rev 23:411–456

Lasa I, Berenguer J (1993) Thermophilic enzymes and their biotechnological potential. Microbiology 9:77–89

Lee JW, Park JY, Kwon M, Choi IG (2009) Purification and characterization of a thermostable xylanase from the brown rot fungus Laetiporus sulphurous. J Biosci Bioeng 107(1):33–37

Li K, Azadi P, Collins R, Tolan J, Kim JS, Eriksson Karl-Erik L (2000) Relationships between activities of xylanases and xylan structures. Enzyme Microb Technol 27:89–94

Madlala AM, Bissoon S, Singh S, Christove L (2001) Xylanase induced reduction of chlorine dioxide consumption during elemental chlorine-free bleaching of different pulp types. Biotechnol Lett 23(345):351

Maheshwari R, Bharadwaj G, Bhat MK (2000) Thermophilic fungi: their physiology and enzymes. Microbiol Mol Biol Rev 64:461–488

Mandal A (2015) Review on microbial xylanases and their applications. Int J Life Sci 4(3):178–187

Martínez-Anaya MA, Jimenez T (1997) Functionality of enzymes that hydrolyse starch and non-starch polysaccharide in bread making. Eur Food Res Technol 205(3):209–214

Monti R, Terenzi HF, Jorge JA (1991) Purification and properties of an extracellular xylanase from thermophilic fungus Humicola grisea var. thermoidea. Can J Microbiol 37:675–681

Motta FL, Andrade CCP, Santana MHA (2013) A review of xylanase production by the fermentation of xylan: classification, characterization and applications. In: Sustainable degradation of lignocellulosic biomass-techniques, applications and commercialization. InTechOpen, New York, pp 251–275

Nuyens F, van Zyl HW, Iserentant D, Verachtert H, Michiels C (2001) Heterologous expression of the Bacillus pumilus endo-β-xylanase (xynA) gene in the yeast Saccharomyces cerevisiae. Appl Microbiol Biotechnol 56:431–434

Octavio LC, Francisco VO (2006) Xylanases. Adv Agric Food Biotechnol:305–322

Paloheimo M, Piironen J, Vehmaanperä J et al (2010) Xylanases and cellulases as feed additives. In: Enzymes in farm animal nutrition. Nutr Finnfeeds Malborough, Wiltshire, pp 12–53

Parajó JC, Domíngues H, Domíngues JM (1998) Biotechnological production of xylitol. Part 1, interest of xylitol and fundamentals of its biosynthesis. Bioresour Technol 65:191–201

Passos AA, Park I, Ferket P et al (2015) Effect of dietary supplementation of xylanase on apparent ileal digestibility of nutrients, viscosity of digesta, and intestinal morphology of growing pigs fed corn and soybean meal based diet. Anim Nutr 1:19–23

Polizeli MLTM, Rizzatti ACS, Monti R, Terezni HF, Jorge JA, Amorim DS (2005) Xylanases from fungi: properties and industrial applications. Appl Microbiol Biotechnol 67:577–591

Prasad S, Yadav AN, Singh A (2020) Impact of climate change on sustainable biofuel production. In: Yadav AN, Rastegari AA, Yadav N, Gaur R (eds) Biofuels production – sustainability and advances in microbial bioresources. Springer International Publishing, Cham, pp 79–97. https://doi.org/10.1007/978-3-030-53933-7_5

Puchart V, Katapodis P, Biely P, Kremnický L, Christakopoulos P, Vršanská M, Kekos D, Macris BJ, Bhat MK (1999) Production of xylanases, mannanases and pectinases by the thermophilic fungus *Thermomyces lanuginosus*. Enzyme Microb Technol 24:355–361

Puls J, Poutanen K (1989) Mechanisms of enzymatic hydrolysis of hemicelluloses xylans and procedures for determination of the enzyme activities involved. In: Ericksson KEE, Ander P (eds) Proceedings of the 3rd International Conference on Biotechnology in the Pulp and Paper Industry. STFI, Stockholm, pp 93–95

Puls J, Schuseil J (1993) Chemistry of hemicelluloses: relationship between hemicellulose structure and enzyme required for hydrolysis. In: Coughlan MP, Hazlewood GP (eds) Hemicellulose and hemicellulases. Portland Press, London, pp 1–28

Raj KC, Chandra TS (1996) Purification and characterization of xylanase from alkali-tolerant *Aspergillus fischeri* Fxn1. FEMS Microbiol Lett 1453:457–461

Rani S, Nand K (1996) Development of cellulase free xylanase producing anaerobic consortia for the use of lignocellulosic wastes. Enzyme Microb Technol 18:23–28

Rizzatti ACS, Jorge JA, Terenzi HF, Rechia CGV, Polizeli MLTM (2001) Purification and properties of a thermostable extracellular β-xylosidase produced by a thermotolerant *Aspergillus phoenicis*. J Ind Microbiol Biotechnol 26:156–160

Rizzatti ACS, Sandrim VC, Jorge JA, Terenzi HF, Polizeli MLTM (2004) Influence of temperature on the properties of xylanolytic enzymes of the thermotolerant fungus *Aspergillus phoenicis*. J Ind Microbiol Biotechnol 31:88–93

Ryan SE, Nolan K, Thompson R, Gubitz GM, Savage AV, Tuohy MG (2003) Purification and characterization of a new low molecular weight endoxylanase from *Penicillium capsularium*. Enzyme Microb Technol 33:775–785

Rychen G, Aquilina G, Azimonti G et al (2018) Safety and efficacy of ECONASE®XT (endo-1, 4-$β$-xylanase) as a feed additive for pigs for fattening. EFSA J 16:e05217

Salles BC, Cunha RB, Fontes W, Sousa MV, Filho EXF (2000) Purification and characterization of a new xylanase from Acrophialophora nainiana. J Biotechnol 81:199–204

Savitha S, Sadhasivam S, Swaminathan K (2009) Modification of paper properties by the pretreatment of wastepaper pulp with *Graphium putredinis*, *Trichoderma harzianum* and fusant xylanases. Bioresour Technol 100:883–889. https://doi.org/10.1016/j.biortech.2008.07.014

Screenath HK, Jeffries TW (2000) Production of ethanol from wood hydrolysate by yeasts. Bioresour Technol 72:253–260

Segura BG, Durand R, Fèvre M (1998) Multiplicity and expression of xylanases in the rumen fungus Neocallimastix frontalis. FEMS Microbiol Lett 164(1):47–53

Shapack GE, Russel I, Stewart GG (1987) Thermophilic microbes in ethanol production. CRC

Sharma PK (2017) Xylanases current and future perspectives: a review Pawan Kumar Sharma. New Biol Rep 6:122

Sharma N, Sharma N (2016) Bioethanol production from alkaline hydrogen peroxide pretreated Populus deltoides wood using hydrolytic enzymes of Bacillus stratosphericus N12 (M) and Bacillus altitudinis Kd1 (M) under different modes of separate hydrolysis and fermentation by monoculture and co-culture culture combinations of ethanologens. Int J Bioassays 5(2):4810–4816

Silva CHC, Puls J, Sousa MV, Ferreira-Filho EX (1999) Purification and characterization of a low molecular weight xylanase from solid-state cultures of *Aspergillus fumigatus* Fresenius. Rev Microbiol 30:114–119

Singh S, Reddy P, Haarhoff J, Biely P, Janse B, Pillay B, Pillay D, Prior BA (2000) Relatedness of Thermomyces lanuginosus strains producing a thermostable xylanase. J Biotechnol 81:119–128

Singh RN, Gaba S, Yadav AN, Gaur P, Gulati S, Kaushik R, Saxena AK (2016) First, high quality draft genome sequence of a plant growth promoting and cold active enzymes producing psychrotrophic Arthrobacter agilis strain L77. Stand Genomic Sci 11(1):54. https://doi.org/10.1186/s40793-016-0176-4

Smith DC, Bhat KM, Wood TM (1991) Xylan-hydrolysing enzymes from thermophilic and mesophilic fungi. World J Microbiol Biotechnol 7:475–484

Subramaniyan S, Prema P (2002) Biotechnology of microbial xylanases, enzymology, molecular biology, and application. Crit Rev Biotechnol 22:33–64

Sung WL, Luk CK, Chan B, Wakarchuk W, Yaguchi M, Campbell R, Willick G, Ishikawa K, Zahab DM (1995) Expression of *Trichoderma reesei* and *Trichoderma viride* xylanases in *Escherichia coli*. Biochem Cell Biol 73(5–6):253–259

Sunna A, Antranikian G (1997) Xylanolytic enzymes from fungi and bacteria. Crit Rev Biotechnol 17(1):39–67

Tan LUL, Wong KKY, Yu EKC, Saddler JN (1985) Purification and characterization of two D-xylanases from *Trichoderma harzianum*. Enzyme Microb Technol 7:425–430

Taneja K, Gupta S, Kuhad RC (2002) Properties and application of a partially purified alkaline xylanase from an alkalophilic fungus Aspergillus nidulans KK-99. Bioresour Technol 85(1):39–42

Techapun C, Poosaran N, Watanabe M, Sasaki K (2003) Thermostable and alkaline-tolerant microbial cellulase free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocess: a review. Process Biochem 38:1327–1340

Twomey LN, Pluske JR, Rowe JB, Choct M, Brown W, McConnell MF, Pethick DW (2003) The effects of increasing levels of soluble non-starch polysaccharides and inclusion of feed enzymes in dog diets on faecal quality and digestibility. Anim Feed Sci Technol 108(1–4):71–82

Uffen RL (1997) Xylan degradation: a glimpse at microbial diversity. J Ind Microbiol Biotechnol 19:1–6

Van Dorn R, Shanahan D, Ciofalo V (2018) Safety evaluation of xylanase 50316 enzyme preparation (also known as VR007), expressed in *Pseudomonas fluorescens*, intended for use in animal feed. Regul Toxicol Pharmacol 97:48–56

van Peij NNME, Brinkmann J, Vrsanská M, Visser J, de Graaff LH (1997) β-xylosidase activity, encoded by xlnD, is essential for complete hydrolysis of xylan by *Aspergillus niger* but not for induction of the xylanolytic enzyme spectrum. Eur J Biochem 245:164–173

Viikari L, Kantelinen A, Sundquist J, Linko M (1994) Xylanases in bleaching, from an idea to the industry. FEMS Microbiol Rev 13:335–350

Virupakshi S, Babu KG, Gaikwad SR, Naik GR (2005) Production of a xylanolytic enzyme by a thermoalkaliphilic Bacillus sp. JB-99 in solid state fermentation. Process Biochem 40:431–435

Walia A, Mehta P, Guleria S, Shirkot CK (2015) Modification in the properties of paper by using cellulase-free xylanase produced from alkalophilic Cellulosimicrobium cellulans CKMX1 in biobleaching of wheat straw pulp. Can J Microbiol 61:671–681

Walia A, Guleria S, Mehta P et al (2017) Microbial xylanases and their industrial application in pulp and paper biobleaching: a review. 3 Biotech 7:1–12. https://doi.org/10.1007/s13205-016-0584-6

Whistler RL, Richards EL (1970) Hemicelluloses. In: Pigman W, Horton D (eds) The carbohydrates. Academic Press, New York, pp 447–469

Wilkie KCB (1979) The hemicelluloses of grasses and cereals. Adv Carbohydr Chem Biochem 36:215–226

Williamson G, Faulds CB, Kroon PA (1998) Specificity of ferulic acid (feruloyl) esterases. Biochem Soc Trans 26(2):205–209

Wong KKY, Saddler JN (1993) Applications of hemicellulases in the food, feed, and pulp and paper industries. In: Coughlan MP, Hazlewood GP (eds) Hemicelluloses and hemicellulases. Portland Press, London, pp 127–143

Wong KKY, Tan LUL, Saddler JN (1988) Multiplicity of β-1,4- xylanase in microorganisms, functions and applications. Microbiology 52:305–317

Wu YB, Rabindran V (2004) Influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurement of broiler chickens. Anim Feed Sci Technol 116:129–113

Yadav AN (2020) Recent trends in mycological research, Volume 1: Agricultural and medical perspective. Springer, Switzerland

Yadav AN, Sachan SG, Verma P, Kaushik R, Saxena AK (2016) Cold active hydrolytic enzymes production by psychrotrophic Bacilli isolated from three sub-glacial lakes of NW Indian Himalayas. J Basic Microbiol 56:294–307

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi. Volume 1: Diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 2: Perspective for value-added products and environments. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N, Gaur R (2020) Biofuels production – sustainability and advances in microbial bioresources. Springer, Cham

Chapter 15
Fungal Pigments for Food Industry

Tarek Nour Soliman, Marwa I. Wahba, and Ahmed Noah Badr

Contents

T. N. Soliman (✉)
Food Industries and Nutrition Research Division, Dairy Department, National Research Centre, Dokki, Cairo, Egypt

M. I. Wahba
Chemistry of Natural and Microbial Products Department, National Research Centre, Dokki, Giza, Egypt

Centre of Scientific Excellence-Group of Advanced Materials and Nanotechnology, National Research Centre, El-Behooth St., Dokki, Giza, Egypt

A. N. Badr
Toxicology and Food Contaminants Department, National Research Centre, Cairo, Egypt

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_15

15.1 Introduction

Recently, customer consciousness about the environment is increasing the use of natural products. Their biodegradable nature and sustainable production appear highly advantageous in the case of natural colors and pigments compared to synthetic pigments (Kant 2012; Shahid et al. 2013; Kour et al. 2021; Kumar et al. 2021). Furthermore, there is a set of consistent manufacturing staining methods tested for such molecules (Weber et al. 2014). To increase the use of fungal dyes to industrial level in manufacturing applications, testing of dyeing methods similar to industrial conditions and requirements is therefore a significant question. In coloration, there is growing interest in the revival of natural dyes. The importance of natural colors slackened because synthetic colors had some advantages over natural colors such as color speed, strong reproducibility of shades, the brightness of colors, and ease of use, as well as the ready availability of pure synthetic colors of various types/classes and their cost advantages, much of the fabric dyers switched to synthetic dyes.

Natural dye has been proposed as an environment and friendly substitutions to artificial pigments. A very diverse number of fungal species can be isolated from fungal dyes and pigments. Such chemicals are among the more natural products for use in the pharmaceutical, food, cosmetics, and textile manufacturing (Kulkarni and Gupta 2013; Robinson et al. 2014; Yadav 2021; Yadav et al. 2019c). Many fungi produce extracellular compounds that were tested as a colorant agent under the laboratory conditions and gave good results, among the various microorganisms that are capable of synthesizing natural dyes and colors. Yellow and red dyes producing by fungi, like *Penicillium murcianum* and *Talaromyces australis*, were isolated and utilized to dyestuff several materials. Satisfactory results were found when using dyes individually and mixed in different proportions.

Microorganisms could deliver excellent quantities of stable dyes such as anthraquinones, carotenoids, flavonoids, and quinines. Fungi are more environmentally friendly and important sources of dyes since they contain stable pigments. Fungi possess multiple molecules and colorants of anthraquinone, such as melanin, delphinidin, and volatile compounds that are being identified as a secondary metabolite. Fungal colors and pigments in nature have biological roles related to tolerance and defense against biotic and abiotic agents, such as ultraviolet radiation and antagonistic fungi and bacteria (Eisenman and Casadevall 2012; Yadav et al. 2019b). Furthermore, dyes were achieved by supplementing targeted material directly into the liquid cultures of extracellular pigment producing champignons (Chiba et al. 2006). No mordants were required in this process for the pigments fixation; and the dyed material showed acquired antimicrobial properties (Sharma et al. 2012).

Several fungi cells are implicated in produce for some types of pigment as their intermediate metabolites like *Alternaria alternata*, *Aspergillus niger*, *Curvularia lunata, Hapalopilus nidulans, Omphalotus olivaceous, Boletopsis grisea, Phaeolus schweinitzii, Hypomyces lactiflourum, Pisolithus tinctorius, Sarcodon fuscoindicus, Trichoderma virens, Monascus purpureus, Isaria farinosa, Emericella nidulans, Dermocybe sanguinea, Fusarium verticillioides,* and *Penicillium purpurogenum*.

Pigment development by the fungi regarded as a taxonomic feature due to their genetic history of the genus (Abdel-Azeem et al. 2021; Kour et al. 2019).

15.2 Challenges in Fungal Dyes Produces

A critical interest to be cautious of fungi for their ability to produce secondary metabolites may vary between useful and harmful molecules. Antibiotics, like penicillin and cephalosporin, cyclosporine, and statin are secondary metabolites type of fungal product (Asnaashari et al. 2012; Singh and Pandey 2013; Devi et al. 2020; Rana et al. 2020; Yadav 2019). Moreover, mycotoxins are another type of fungi secondary metabolites. It was registered as harmful metabolites, which affect public health if it was contaminated by human food or animal feed (Badr et al. 2017). Aflatoxins are a very dangerous group of fungi secondary metabolites that were classified as pre-carcinogenic molecules by the International Agency of Research for Cancer (Shahat et al. 2017; Shehata 2017). The health hazards to various tissues within living tissues have been confirmed (Shahat et al. 2017). The biologically active components, which are presented as minor components in plant extracts act a pivotal role in reducing the damage caused by mycotoxins and their biological impacts (Badr and Naeem 2019; Abdel-Razek et al. 2019; Rastegari et al. 2019a, b; Yadav et al. 2019a). Dyes are the third group of secondary metabolites that connected to the fungal lifecycle. It was reported to produce by fungi with different application benefits. It is well known that natural colorants are biodegradable and eco-friendly. Generally, it recognizes to be non-toxic and could be produced utilizing inexpensive materials.

15.3 Benefits of Fungal Pigments

15.3.1 Antimicrobial Activities of Fungal Pigments

In addition to giving or preserving desired colors in food, several of the dyes of food-grade provided by fungi could also be used as natural antimicrobial properties. For instance, *Monascus purpureus* dyes have been documented to prevent the growth from both species of fungi including such *Aspergillus, Trichoderma, Mucor, Penicillium*, and *Fusarium*, as well as, some bacterial species like *Bacillus, Pseudomonas, Escherichia,* and *Streptomyces* (Ungureanu and Ferdes 2010). The metabolic rate depending on the solution that used extract dyes and *Gram-positive* bacteria tends to be much less resistant than the *Gram-negative* one (Ungureanu and Ferdes 2010). The dyes from several fungi like *Monodictys castaneae; Sporobolomyces sp., Fusarium sp., Aspergillus sp., and Penicillium species* have both been reported to have antimicrobial activity against different fungal and

bacterial organisms (Manimala and Murugesan 2014). It also investigated the behavior of fungal pigments against other cells.

During production, the antimicrobial activities of dyes produced by the species *Monascus* can be enhanced by adding some L- and D-amino acids to the culture medium. The minimum inhibitory concentrations (MIC) of dye derivatives towards certain Grampositive and negative bacteria were determined. While; the quantity of pigment used to achieve the desired intensity of the color is adequate, relies on the quantity, it is significant to avoid the contamination occurrence insomuch as it has a toxicity impact. That is because the therapeutic potential differs with dye-form because specific nutrients need specific dye concentrations to achieve the desired intensity of suitable color. Cheng-yun and Wen-Ping (2008) reported the addition of Monascus dye to the sausage product, while El-Kholie et al. (2012) refer to its application in the beef burger.

15.3.2 Anticancer and Antitumor Activities of Fungal Pigments

The investigation of two fungal strains isolated from soil and had the ability to pigmentation Fungal pigments anticancer and antitumor activities and their anticancer activity was achieved. These two strains were recorded to produce antibiotics with biological efficacy, Anticancer, and antitumor activities with broad-spectrum application industries. The highest effectiveness of the fungal extract against pathogens and fungi was reported. Moreover, Turbyville et al. (2005) refer to the production of anticancer metabolites in the same media that contains the pigment produced by fungi. The antitumor activity of several components produced in media growth of *fusarium* strains had been reported (Ran et al. 2017). These strains were also capable to produce several pigments in a media of their growth. The application of the extracted pigment from these media will achieve the same effect by their implementation in several manufacturing processes.

15.3.3 Antioxidant Activities of Fungal Pigments

Increasing free radicals in the body enhances the chances of occurrence of chronic diseases such as cardiovascular, cancer, diabetes, and autoimmune disorders (Rankovic et al. 2011). To capture, this must be using antioxidants. Antioxidants are molecules that delay or inhibit cellular damage by donating electrons to a rampaging free radical and neutralizing them via their free radical scavenging properties (Lobo et al. 2010). Fungal pigments such as naphthoquinone, violacein, carotenoids, anthocyanins, and naphthoquinone demonstrated antioxidant activities (Sen et al. 2019). Violacein which is a purple pigment largely produced by *Pseudoalteromonas*

and *Chromobacterium violaceum* was reported as antioxidants (Duran et al. 2012). Many studies suggested that fungal pigments used as antioxidants may prevent the incidence of many chronic diseases such as cancer and heart disease.

15.4 The Extraction of Fungal Dyes

The growth media composition of fungi has a vital role in pigment production concentration. In previous studies, it was reported that the change of fungal growth media results in changes within the colorant concentration represented as a media darkness degree (Zhou et al. 2015; Da-Costa Souza et al. 2016). It is often present, in the fungal growth media, several substances linked to the secondary produced components by fungi. The yellow colorant, produced by *Penicillium aculeatum* ATCC 10409 in a growing media, was well-recorded in whey-enrichment growth media (Afshari et al. 2015). Moreover, the dark-reddish brown pigment was produced well if the strain of *Fusarium moniliforme* KUMBF 1201 implanted on media of potato dextrose (broth or agar), and the colorant concentration was more than other growth media (Pradeep et al. 2013). In most cases of pigment production by the filamentous fungi; suitable media for growth were both potato dextrose agar and Czapek Dox agar (Velmurugan et al. 2010; Aishwarya 2014; Da-Costa Souza et al. 2016).

Nevertheless, perhaps the harmful secondary metabolites, which also considered as a pigment in the fungal growth media, produced due to the growth-media construction and its components. *Averfin, Norsolorinic acid, Averantin, Versicolorin C, Nidurufin, Versicolorin A,* and *Versicolorin* A hemiacetal are classified as yellow pigments, which are produced by *Aspergillus sp.* strains. It classified to be fungal dyes existed through their growth on liquid media of potato dextrose agar. Moreover, these compounds considered as intermediates components of the aflatoxins synthesis steps by the fungi (Shier et al. 2005). The harmful metabolites of fungi, which expected to exist during the colorant production can be suppressed and controlled by the modification of the growth condition of fungal media (Zhou et al. 2015). For these reasons, more investigations are in demand for the determination and optimization of dyes-production condition by the fungal strains.

Fungal, which reportedly could produce pigments in growth media, extracted according to their solubility. Fungal dyes were diverse from polar to non-polar types with different solubility degrees. This considered as affecting their stability in the colorant media, while the polar degree of each dye will influence their fastness of the pigment after application (Velmurugan et al. 2010; Aishwarya 2014; Lagashetti et al. 2019). In most filamentous fungi types that possess the ability for pigment production, the resultant colorant was water-soluble while the fastness degree in light or pH levels may vary according to their polarity.

15.5 Fastness of the Colorant Sourced from Fungi

Several factors were reported affecting the fungal colorant production; these factors were including media contents, pH value, carbon source, nitrogen source, temperature, and media volume (Afshari et al. 2015). In order to the polarity degree, several solvent systems were applied for their extraction. For instance, water, methanol, ethanol, ethyl acetate, and dichloromethane were utilized for the fungal pigments extraction produced by different filamentous strains of fungi as described in Table 15.1. The fastness of fungal-colorants after their extraction mainly depends on the transformation to a fixed dye-fraction (Mabrouk et al. 2011). One more point that natural dyes specifically from fungal strains are pH-dependent, which mean changes of dye color by the pH of media or by the change of solvent of extraction (Zhou et al. 2015). The trend of natural pigment fastness was achieved by their application using encapsulation techniques. The encapsulation of natural dyes gives stability for the applicate material and avoids their natural degradation, particularly ones that sourced from fungi. Hence, the encapsulation of fungal pigments considered an effective solution for their broad spectrum in application food processing.

15.6 Food Applications of Fungal Pigments

Natural pigments could be considered as superior alternatives to synthetic pigments as they are non-toxic. Furthermore, they possess nutritional value and can exert pharmacological effects (Li et al. 2017).Noteworthy, enriching foods with such health-promoting entities is being focused on as it would promote health and well-being and would diminish chronic diseases' risk (Jain et al. 2020). Moreover, if these health-promoting entities were colorful, customers would be encouraged to consume their fortified food preparations. Thus, much research has focused on natural pigments and incorporating them in foods (Rocha et al. 2012; Jain et al. 2020; Zhao et al. 2020). Natural pigments are chiefly procured from plants, animals, and microorganisms. Nevertheless, the propagation of plants and animals is limited by season and origin; thus, it is expensive to procure pigments from these sources (Li et al. 2017). On the other hand, microorganisms, especially those propagated in-vitro, are less affected by seasonal variations. Moreover, the pigment extraction from microbial sources could also be greatly simplified, and this would help escalate the economic value of these pigments (De-Mejia et al. 2020). Additionally, the cost of producing fungal pigments could be further reduced if the costly fermentation media components were substituted with cheaper constituents. Thus, researchers devoted their efforts to exploit industrial wastes and side streams in the fermentation media. For instance, corn steep liquor was exploited as a nitrogen source and replaced yeast extract during pigments' production via *M. ruber. M. purpureus* pigments were also produced while utilizing grape waste (Rao et al. 2017). Moreover, red pigment extracted from *Penicillium purpurogenum* was applied in

Table 15.1 Common natural colorant and their producing fungi with secondary metabolites

Fungi producing dyes	Isolated from	Suitable media for growth	Mycotoxin (fungal metabolites)	Dye color	Solvent of extraction	Reference
Fusarium moniliforme KUMBF 1201	Paddy field soil	Potato Dextrose Agar (PDA) Potato Dextrose Broth (PDB)	Moniliformin, fusaric acid, fusarin C, fusariocin C,	Dark Reddish Brown	Methanol	Pradeep et al. (2013)
Aspergillus sydowii (CML2967), *Aspergillus aureolatus* (CML2964 and E.4.1), *Aspergillus keveii* (CML2968 and ONI75), *Penicillium flavigenum* (CML2965; E.2.7 and 3.1.a), *Penicillium chermesinum* (CML2966), one of *Epicoccum nigrum* (CML2971), *L. aphanocladii* (CML2970) *Fusarium sp.* (CML2969)	Brazilian caves	Potato Dextrose Agar (PDA)	Cyclopiazonic acid Sterigmatocystin Sterigmatocystin Penicillin, Penicillic acid Brevianamide A — — Fumonisin B	Yellow color Dark green Pale yellow Greenish-yellow Greenish-yellow Yellow–orange Reddish brown Pink- Yellow	Dichloromethane Dichloromethane Dichloromethane Ethyl acetate Dichloromethane Dichloromethane Ethyl acetate Ethyl acetate	Da-Costa Souza et al. (2016) Mapari et al. (2009) Meena et al. (2017)
Monascus Ruber	The Daqu (Shan Xi Vinegar starter)	Malt extract agar (MEA)	Citrinin	Red pigment	Water Ethanol	Zhou et al. (2015) Hajjaj et al. (2000)

(continued)

Table 15.1 (continued)

Fungi producing dyes	Isolated from	Suitable media for growth	Mycotoxin (fungal metabolites)	Dye color	Solvent of extraction	Reference
Acrostalagmus sp. *Alternaria alternata ,* *Aspergillus niger, Bisporomyces sp.* *Cunninghamella, Penicillium chrysogenum* *Penicillium italicum* *Penicillium oxalicum* *Penicillium regulosum* *Phymatotrichum sp.*	NRC culture collection	Czapek Dox agar	— Alternariol Ochratoxin — — Penicillin, Penicillic acid Citrinin Penicillin, Citrinin —	Brown to reddish brown	Water and Alcohols	Mabrouk et al. (2011) Meena et al. (2017)
Penicillium aculeatum	Textile	Whey media	Roquefortine Rugulovasine	Yellow pigment	Ethyl acetate	Afshari et al. (2015) Mapari et al. (2009)
Trichoderma harzianum *Trichoderma aureoviride,* *Trichoderma reesei* *Trichoderma hemantum*	Solid surfaces	Czapek Dox agar Potato Dextrose Agar (PDA)	Cyclosporine A	Yellow to pale yellow pigments	Ethyl acetate	Azam et al. (2012) Lagashetti et al. (2019)
Aspergillus flavus *Aspergillus parasiticus*	Food materials	Czapek Dox agar Potato Dextrose Agar (PDA)	Aflatoxins Cyclopiazonic Sterigmatocystin	Green pigment Olive pigment	Ethyl acetate Dichloromethane	Chang et al. (2020) Badr et al. (2019) Sabry et al. (2016)

Fungi producing dyes	Isolated from	Suitable media for growth	Mycotoxin (fungal metabolites)	Dye color	Solvent of extraction	Reference
Aspergillus nomius	Fruits & waste water	Potato Dextrose Agar (PDA)	Aflatoxins Sterigmatocystin	Yellow pigment	Methanol	Shier et al. (2005) Abbas et al. (2004)
Penicillium aculeatum ATCC 10409	Cereals	Potato Dextrose Agar (PDA)	Penicillin, Penicillic acid	Yellow pigments	Water	Afshari et al. (2015)
Penicillium purpurogenum DPUA 1275	Soil	Czapek Dox agar Potato Dextrose Agar (PDA)	Citrinin	Red colorant	Ethyl acetate	Santos-Ebinuma et al. (2013)
Monascus pilosus MS-1	Wood	Potato Dextrose Agar (PDA)	—	Red Colorant	Ethyl acetate	Feng et al. (2015)
Alternaria sp.	Horticulture	Czapek Dox agar	Alternariol	Yellow pigments	Dichloromethane	Mawthols et al. (2005)

meat product where it had the ability to increase the safety properties of the final product (Salama et al. 2021)

Nevertheless, concerns are raised regarding fungal pigments as some fungal secondary metabolites could be harmful to humans. Even the popular pigment producing, *Monascus* fungi could produce the mycotoxin, citrinin, and this hampered the approval of these fungi by the USA and the European Union although they have been exploited in Asia for hundreds of years (Rao et al. 2017). Such as an issue could be addressed after considering the fact that the metabolic pathway of citrinin production is independent from that of the pigments production (De-Mejia et al. 2020).Thus, manipulating the propagation of *Monascus* would impede critinin production. Another simple approach that could be adopted in order to impede the fungal toxins production is modulating the fermentation media where it was shown that adding or excluding certain metal ions, nitrogen sources, or carbon sources could influence the toxins' expression. On the other hand, fungal pigments can offer some advantages regarding their temperature and pH stabilities which would facilitate their industrial exploitation. For instance, various fungal pigments were reported to be stable at a wide pH range (Rao et al. 2017). Moreover, pigments secreted by *M. purpureus*, *Emericella* species, *Isaria* species, *Penicillium* species, and *Fusarium* species were shown to be stable at escalated temperatures (Velmurugan et al. 2010). Thus, we are going to discuss some of the fungal pigments while shedding the light on their safety and their beneficial effects that could improve foods' quality and shelf-life

15.6.1 Azaphilone Pigments

Azaphilone pigments could be considered as a class of pigments brought to food industries by fungal strains as such pigments are not concocted by plants, whereas various fungi are reported as azaphilone pigments' producers. For instance, six azaphilone pigments are procured from the fungal species *Monascus*. These are the yellow *ankaflavin* and *monascin*, the orange *rubropunctatin* and *monascorubrin*, and the red *rubropunctamine* and *monascorubramine* (Dufossé 2018). *Monascus* pigments are non-toxic and are already present in the market (Dufossé 2018; Vendruscolo et al. 2014). These pigments can be utilized as food colorants to augment the foods' colored morphology and sensory traits. *Monascus* pigments were also shown to exhibit antimicrobial traits. Antimicrobials and preservatives are critical in food industries as they guard against microbial spoilage, and this would extend the foods' shelf-life. Thus, fungal pigments with antimicrobial traits, such as the *Monascus* pigments, could be considered as safe and natural surrogates to the chemical preservatives. The antimicrobial traits of the *Monascus* pigments were evaluated against the foodborne bacteria, *Staphylococcus aureus* and *Escherichia coli*. *M. ruber* was incubated at various pHs in order to express two different pigments, the orange pigment and the red one. The red pigment, produced at pH 5, was more effective as it managed to inhibit the growth of both *S. aureus* (gram positive)

and *E. coli* (gram negative). On the other hand, the orange pigment, produced at pH 3, inhibited the growth of *S. aureus* only. Nevertheless, the efficiency of the orange pigment was escalated following its derivatization via the addition of glycine.

The glycine derivatization turned such an orange pigment into a red pigment capable of inhibiting both *S. aureus* and *E. coli* (Vendruscolo et al. 2014). Other amino acid derivatized *Monascus* pigments were also reported where Kim et al. (2006) concocted L-tyrosine, L-phenylalanine, L-glutamine, and L-asparagine derivatives of *Monascus* pigments. The hydrophobic amino-acids derivatives (L-tyrosine and L-phenylalanine) were more efficient as their minimum inhibitory concentrations (MIC) against *E. coli* were much lower than the MIC of the hydrophilic amino acids derivatives (L-glutamine and L-asparagine). Such increased antimicrobial efficiency was coupled with incremented adsorption of the hydrophobic derivatives onto the *E. coli's* surfaces. Noteworthy, the antimicrobial influence of *Monascus* pigments was debated to be ascribed to the induced disturbance in the permeability of the cell membrane (Zhao et al. 2015), which could diminish the transport of oxygen and of various nutrients and metabolites. Such antimicrobial traits could also be regarded to the interactions with cellular enzymes (Vendruscolo et al. 2014).

Penicillium marneffei is another fungus that can concoct azaphilone pigments. The red pigment of *P. marneffei* was shown to comprise over 16 amino acid conjugates of rubropunctatin and monascorubrin. Nevertheless, the pigment production via *P. marneffei* suffers from a serious drawback as the mycotoxin, citrinin is an early byproduct of its red pigment's biosynthetic pathway. Another more promising azaphilone pigments' producer is *Talaromyces atroroseus* as its pigments production are not coupled with mycotoxins concoction (Dufossé 2018).

15.6.2 Carotenoids

Food colorants also include carotenoids whose colors range from yellow to orange and red. Naturally procured carotenoids are diverse as they include over 700 carotenoids, such as β-cryptoxanthin, α-carotene, β-carotene, and lutein (De-Mejia et al. 2020). Carotenoids exhibit antioxidant traits where they dwindle the oxidative stress and scavenge free radicals which could trigger atherosclerosis, cancer, and various other diseases (Li et al. 2017; Özkan and Bilek 2014). Additionally, β-cryptoxanthin, α-carotene, and β-carotene (provitamin A carotenoids) can be transformed into vitamin A in human bodies (De-Mejia et al. 2020). Thus, it would be highly advantageous to blend such colored functional moieties with food products. Nonetheless, restrains are laid on the sources of the utilized carotenoids. For instance, the US regulations state that carotenes can only be procured from carrots (De-Mejia et al. 2020). Such restrictions are probably triggered by the concerns regarding the toxic fungal metabolites. However, the β-carotene procured by the fermentation of the fungus *Blakeslea trispora* was shown to be mycotoxin free, and it complies with the

specifications of the EC. The *B. trispora* β-carotene's production could reach up to 17 g/L (Chattopadhyay et al. 2008; Dufossé 2018).

Another *B. trispora* carotenoid is lycopene. The *B. trispora* lycopene was also shown to lack any evidence of toxicity up to a 1% (w/w suspension within sunflower oil) dietary level (Dufossé 2018), and this encouraged the exploitation of this *B. trispora* pigment which is now available in market (Rao et al. 2017). Noteworthy, lycopene could also be absorbed by humans following its dietary intake as was evidenced by it being detected in the human tissues and plasma following its ingestion (Dufossé 2018). This lycopene pigment could then impart several health benefits .Lycopene is among the most potent carotenoid antioxidant (Rocha et al. 2012), and it was shown that consuming lycopene rich products was linked to diminished cancer risk. Lycopene supplemented diet was also beneficial in the treatment of prostate cancer. Furthermore, lycopene consumption was shown to dwindle the total cholesterol concentration in humans and it was debated to have potential in lightening chronic sicknesses, such as coronary heart diseases (Herńandez-Almanza et al. 2016). It is worth mentioning that, within *B. trispora,* β-carotene is procured via lycopene cyclization. Thus, if such cyclization was inhibited more lycopene would be produced. Various inhibitors were investigated, such as imidazole which caused the carotenoid production of the *B. trispora* F-816 and F-744 to be a 100% lycopene production. A 96% lycopene production was also attained by the addition of 300 mg/L 2-isopropylimidazole to *B. trispora* (Herńandez-Almanza et al. 2016).

Owing to significance of carotenoids, some microbial strains were genetically modified in order to efficiently produce them. For instance, *Fusarium sporotrichioides* was genetically modified, and its isoprenoid pathway was redirected to the concoction of commercial carotenoids. *F. sporotrichioides'* strong promoter and terminator sequences were coupled to the *Erwinia uredovora*'s carotenoid biosynthetic genes, and the chimeric genes were inserted into the fungus causing it to attain 0.5mg/g lycopene and 5 mg/g β-carotene (Jones et al. 2004).

15.6.3 Anthraquinones

Anthraquinones are expressed by many fungal species including *Aspergillus, Fusarium, Drechslera, and Penicillium* (Dufossé 2018). For instance, red anthraquinone was procured from *Penicillium oxalicum*. This anthraquinone pigment was termed Arpink Red and it was considered as a safe colorant which was recommended to be blended with meat products, milk products, and confectionery at the concentrations of 100 mg/kg, 150 mg/kg, and 300 mg/kg, respectively (Chattopadhyay et al. 2008). Anthraquinones were also procured from the edible fungus *Pleurotus ostreatus* (Bindhu et al. 2020). Anthraquinones offer benefits beyond their colorful appearance as they exert different biological activities, such as bacteriostatic, antiviral, and insecticidal (Dufossé 2018). Thus, they could be exploited as food preservatives. Furthermore, the red antioxidant pigment expressed

by the endophytic fungus, *Stemphylium lycopersici was* shown to be constituted from anthraquinones and phenolics. This red pigment exhibited potent antioxidant traits which were comparable to that of ascorbic acid with respect to their DPPH radical scavenging ability (Li et al. 2017).

15.6.4 Riboflavin

Riboflavin is a yellow food colorant that is approved in most countries. Riboflavin is incorporated into beverages, dressings, sherbets, ice creams, and other food products. Some fungal strains, such as *Eremothecium ashbyii* and *Ashbya gossypii* are considered as strong over-producers of this riboflavin (Chattopadhyay et al. 2008). It is worth mentioning that *A. gossypii* riboflavin already exists in the market (Dufossé 2018). The effect of incorporating riboflavin in food preparations goes far beyond imparting color. Riboflavin is actually vitamin B2 which is a pivotal vitamin that should be incorporated into human diet at a 1.1–1.3 mg/day concentration as it is a structural constituent of the oxidation-reduction co-enzymes, flavin-mononucleotide and flavin-adenine-dinucleotide. These co-enzymes are involved in the concoction of ATP. Furthermore, riboflavin supplements were integrated in the neonatal jaundice's phototherapy treatments (Rao et al. 2017).

15.6.5 Melanin

Melanin is another pigment that could be procured from various fungi, such as *Cryptococcus neoformans, Sporothrixs chenckii, Aspergillus fumigatus*, and *Paracoccidioides brasiliensis*. Nevertheless, all of the aforementioned strains are human pathogens, and melanin is associated with their virulence. For instance, melanin guards *C. neoformans* against macrophage-mediated phagocytosis. Moreover, it was shown that the *A. fumigatus* conidia, which lacks pigmentation, is more susceptible to monocyte attack (in-vitro). These conidia also exhibited diminished virulence within animal models (Youngchim et al. 2004). Thus, special considerations should be adopted in order to ensure the biosafety of the fungal melanins if they were to be exploited in food industries. Melanin is known for its ability to confer light protection, and it also possesses antibacterial and antioxidant traits (Shankar et al. 2019). Thus, it would be advantageous to incorporate it in food packaging in order to provide light protection for light sensitive foods. Moreover, its antibacterial and antioxidant traits would help guard against microbial and chemical food spoilage. Shankar et al. (2019) concocted melanin nano-particles and incorporated them within gelatin-based nano-composite food packaging films. The incorporation of such nano-particles diminished the light transmittance of the films to the extent that UV-light was almost totally blocked when 1wt% of the melanin

nano-particles was incorporated into the gelatin films. Such nano-particles also significantly escalated the films' antioxidant traits.

15.7 Encapsulation

It should be noted that carotenoids suffer from stability issues when subjected to light, oxygen, or elevated temperatures. Moreover, they could impose solubility problems which would limit their incorporation within the various foods and beverages (Jain et al. 2020; Özkan and Bilek 2014). Similarly, riboflavin is extremely photosensitive and is sparingly soluble in water (0.05–0.33 g/L) (De-Fariasa et al. 2018). Such drawbacks could be surmounted via encapsulating these pigments as this process will lay barriers among such sensitive pigments and the external environment, and thus, would stabilize them. The water dispersibility of these pigments could also be improved if they were encapsulated within protein-carbohydrate matrices (Özkan and Bilek 2014).Various encapsulation techniques could be adopted including spray drying, emulsion, coacervation, inclusion within hydrogel beads, and inclusion within liposomes (De-Fariasa et al. 2018; Jain et al. 2020).

Spray drying is among the most commonly exploited industrial techniques as it is quick and cost effective. Spray drying comprises dispersing the moiety to be encapsulated within a matrix material. This dispersion is then atomized within heated air in order to promote the quick elimination of the solvent and the concurrent attainment of a fine powder. Spray drying was adopted during riboflavin's encapsulation within galactomannan (De-Fariasa et al. 2018). Lycopene was also encapsulated via spray drying within gum arabic and maltodextrin, gelatin and sucrose, gum arabic and sucrose, and also within the lipophilically modified starch Capsul®. The Capsul® encapsulated lycopene was significantly more stable relative to its free analogue when both samples were stored for 73days. The Capsul® encapsulated lycopene was also utilized during the preparation of a cake, and it managed to impart a homogenous pigmentation to the cake (Rocha et al. 2012).

Lycopene was also encapsulated within emulsions and nano-emulsions (Jain et al. 2020; Zhao et al. 2020). In emulsions, the lipophilic entities, such as lycopene are dispersed within an oil phase which is then emulsified with an aqueous phase. Thus, emulsions provide superior protection to the lipophilic bioactive entities against degradation. Moreover, the outer aqueous phase in oil-in-water emulsions would enhance the dispersion of the encapsulated lipophilic entities within food products. Additionally, the impact exerted by the lipophilic entities on the organoleptic properties of food could be reduced following their emulsification. Noteworthy, encapsulating lycopene within an oil-in-water emulsion consisting of a soybean oil phase and a modified resistant rice starch aqueous phase enhanced its stability as was evidenced from the increase in the retained antioxidant activity of the lycopene emulsion relative to the lycopene oily solution (Jain et al. 2020). As regard to nano-emulsions, they are characterized by the minute size of their droplets (diameter commonly <500 nm).

The Brownian motion of such minute droplets is robust enough to work against the kinetic instability triggered by gravity or viscosity. Thus, nano-emulsions are characterized by long-term stability and can avoid precipitation and coalescence (Zhao et al. 2020). It should be noted that the type of oil exploited to dissolve the lipophilic moieties could affect the stability of such moieties. For instance, when walnut oil, linseed oil, and sesame oil were compared as the oil phases for the lycopene nano-emulsions, it was shown that the nano-emulsion constituted from sesame oil was the most proficient in impeding lycopene's degradation. This was regarded to the sesame's oil escalated oxidative stability and reduced saturation (Zhao et al. 2020).

It should also be noted that emulsions could be spray dried into microcapsules. For instance, lycopene was integrated into a coarse emulsion, which was based on xylo-oligosaccharides glycosylated whey protein isolate (WPI). This emulsion was then spray dried in order to concoct lycopene loaded microcapsules. The glycosylated WPI was utilized instead of WPI as the base material for these microcapsules in order to surmount the shortcomings of protein based delivery vehicles which suffer from instability upon altering their pH. Moreover, they could be hydrolyzed via pepsin, and this would lead to the degradation of their loaded bioactive moieties if they were subjected to in-vitro digestion. On the other hand, glycosylation was shown to enhance proteins stability along extended pH range (Jia et al. 2020).

Glycosylated whey proteins were also reported to confer superior protection to their encapsulated bioactive entities under simulated gastrointestinal environment (Liu et al. 2017). Furthermore, glycosylated proteins could establish more compact interfacial layers which would exhibit superior antioxidant capability, and this would provide more protection to their encapsulated moieties. Thus, lycopene was encapsulated within glycosylated WPI microcapsules with encapsulation efficiency and encapsulation yield exceeding 80%. Such glycosylated WPI microcapsules offered lycopene more stability than did the neat WPI microcapsules as they retained 79% of lycopene after 36days storage at 4 °C. They were also more stable upon being subjected to simulated gastric digestion and escalated the bio-accessibility of their loaded lycopene (Jia et al. 2020).

Complex coacervation is another microencapsulation protocol. It is considered as a liquid/liquid phase separation which occurs secondary to the interaction among oppositely charged polyelectrolytes with the consequent concoction of polyelectrolyte rich coacervates. The opposite charges on gum tragacanth and casein enabled the construction of lycopene loaded microcapsules via complex coacervation. The lycopene loaded within such microcapsules retained around 80% of its initial titer after 60 days storage at 4 °C, whereas the oily lycopene solution retained only around 40% of its initial titer. The gum tragacanth-casein microcapsules also provided a sustained discharge of lycopene where 86.34% of the loaded lycopene was discharged during 24 h residence in simulated gastric fluid (pH 1.2). This sustained discharge was regarded to the structural integrity of the carbohydrate–protein complex which constituted a barrier against the lycopene discharge (Jain et al. 2016).

Hydrogel beads were also exploited to encapsulate lycopene. Simply, a lycopene rich extract was mixed with an alginate or a pectin solution, and the mixture was

kept at 50°C till complete dissolution. Afterwards, this solution was dropped onto a calcium chloride gelling solution. The lycopene loaded hydrogel beads were then either retained in their wet status or were dried at 60 °C in a vacuum oven. Nevertheless, the wet beads did not retain much of their lycopene content upon storage where only 29% and 21% of the initial lycopene contents of the wet alginate and pectinase beads were retained, respectively, after 8 weeks storage at 7 °C. This low percent of retained lycopene was partly regarded to the moisture content of the wet beads (~95%). Water is directly correlated to the occurrence of degrading chemical reactions (Sampaio et al. 2019), and it was previously shown that the stability of a chemical compound (betalain), which was encapsulated within alginate beads, diminished upon escalating the environment's moisture content (Otálora et al. 2016). On the other hand, good stability was conferred to lycopene by the dried beads where no significant alterations were recorded in the lycopene contents of the dried alginate beads which were stored at −10 °C and 7 °C for up to 8 weeks. As for the dried pectinate beads, they only lost ~10% of their lycopene contents within the first week of storage at the aforementioned temperatures and no other significant alterations were recorded for the rest of the 8 weeks storage period (Sampaio et al. 2019).

Inclusion within liposomes is another tool for encapsulation. Liposomes are regarded as colloidal vesicles that are established upon immersing amphiphilic lipids, such as phospholipids within aqueous solutions. The excess water then causes such amphiphilic lipids to self-assemble forming lipid bilayers that enclose aqueous compartments. Liposomes hold great potential for exploitation in food industries as they are biocompatible, safe, offer sustained release for their encapsulated entities, and can constitute vehicles for hydrophilic and hydrophobic entities (Michelon et al. 2016). β-carotene was successfully incorporated within various liposomes. For instance, the soy phosphatidylcholine based multilamellar liposomes, which were stabilized via the thickener, xanthan gum, protected β-carotene against degradation throughout a 90 days interval (Carvalho et al. 2015). The smaller versions of liposomes, the nano-liposomes, were also exploited to encapsulate β-carotene. The particles size of nano-liposomes ranges from 10 to 1000 nm. This minute size escalates the stability of the nano-liposomes against sedimentation and creaming. Moreover, it makes the nano-liposomes more efficient at protecting and delivery bioactive entities. The marine phospholipids based nano-liposomes managed to retain a β-carotene encapsulation efficiency of 92.95% after 70 days of storage (Hamadou et al. 2020).

15.8 Conclusion

Recently, the trend of using natural dyes instead of chemical dyes was increased, this could be related for the harmful impact of chemical dyes. Fungal dyes are considered the main source of natural colorant, for their easest and fruitful existence. Fungal dyes are also known to have several bioactivities and functions including antioxidant, anti-cancer, and antitumor. The challenge faced by pigment production

of fungi was connected to the presence of toxic components, particularly mycotoxins. Forward to this, it is significant to ensure that fungal pigment is free of mycotoxins before the application for food industries. The trend of natural pigment fastness was achieved by their application using encapsulation techniques. This could provide more stability and quality for their applications. Otherwise, the fungal pigments were reported to provide safety characteristics for food applications. These properties recommend their widespread insertion for food applications.

References

Abbas HK, Shier WT, Horn BW, Weaver MA (2004) Cultural methods for aflatoxin detection. J Toxicol: Toxin Rev 23(2-3):295–315

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Vol-1: Biodiversity and ecological perspective. Springer, Cham

Abdel-Razek AG, Shehata MG, Badr AN, Gromadzka K, Stępień L (2019) The effect of chemical composition of wild *Opuntia ficus indica* by-products on its nutritional quality, antioxidant and antifungal efficacy. Egypt J Chem 62(Special Issue (Part 1) Innovation in Chemistry):47–61

Afshari M, Shahidi F, Mortazavi SA, Tabatabai F, Eshaghi Z (2015) Investigating the influence of pH, temperature and agitation speed on yellow pigment production by *Penicillium aculeatum* ATCC 10409. Nat Prod Res 29(14):1300–1306

Aishwarya AD (2014) Extraction of natural dyes from fungus–An alternate for textile dyeing. J Nat Sci Res 4(7):1–7

Andayani DS, Sukandar U, Sukandar EY, Ketutadnyana I (2015) Antibacterial, antifungal and anticancer activity of five strains of soil microorganisms isolated from *Tangkuban Perahu* Mountain by fermentation. HAYATI J Biosci 22:186–190

Asnaashari M, Ghanbary MT, Tazick Z (2012) Optimization of penicillin G production by *Penicillium chrysogenum*. Ann Biol Res 3(12):5434–5440

Azam A, Anjum T, Irum W (2012). *Trichoderma harzianum*: a new fungal source for the production of cyclosporin. Bang J Pharmacol 7(1): 33–35

Badr AN, Naeem MA (2019) Protective efficacy using Cape-golden berry against pre-carcinogenic aflatoxins induced in rats. Toxicol Rep 6:607–615

Badr AN, Shehata MG, Abdel-Razek AG (2017) Antioxidant activities and potential impacts to reduce aflatoxins utilizing jojoba and jatropha oils and extracts. Int J Pharm, 2017, 13(8): pp. 1103–1114

Badr AN, El-Said M, Elmessery TM, Abdel-Razek AG (2019) Non-traditional Oils Encapsulation as Novel Food Additive Enhanced Yogurt Safety against Aflatoxins. Pak J Biol Sci: PJBS 22(2):51–58. https://doi.org/10.3923/pjbs.2019.51.58

Bindhu J, Das A, Sakthivel KM (2020) Anthraquinone from edible fungi *Pleurotus ostreatus* protects human SH-SY5Y neuroblastoma cells *a*gainst 6-hydroxydopamine-induced cell *d*eath—preclinical validation of gene knockout possibilities of PARK7, PINK1, and SNCA1 usingCRISPR SpCas9. Appl Biochem Biotechnol 191:555–566

Carvalho JP, Toniazzo T, Cavalcanti LP, Moraes IF, Oliveira CP, Pinho SC (2015) Physico-chemical stability and structural characterization of thickened multilamellar beta-carotene-loaded liposome dispersions produced using a proliposome method. Colloid Polym Sci 293:2171–2179

Chang PK, Cary JW, Lebar MD (2020) Biosynthesis of conidial and *sclerotial* pigments in *Aspergillus* species. Appl Microbiol Biotechnol 104(6):2277–2286

Chattopadhyay P, Chatterjee S, Sen SK (2008) Biotechnological potential of natural food grade biocolorants. Afr J Biotechnol 7:2972–2985

Cheng-yun L, Wen-Ping W (2008) Preservation and colouring of *Monascus* Color and Sorghum red. Meat Research. 2008, vol. 07

Chiba S, Tsuyoshi N, Fudou R, Ojika M, Murakami Y, Ogoma Y et al (2006) Magenta pigment produced by fungus. J Gen Appl Microbiol 52(4):201–207. https://doi.org/10.2323/jgam.52.201

Da-Costa Souza PN, Grigoletto TB, de Moraes LB, Abreu LM, Guimarães LS, Santos C, Cardoso PG (2016) Production and chemical characterization of pigments in filamentous fungi. Microbiology 162(1):12–22

De-Fariasa SS, Siqueira SC, Cunha AP, de-Souza CG, Fontenelle RS, de-Araújo TG et al (2018) Microencapsulation of riboflavin with galactomannan biopolymer and F127: physico-chemical characterization, antifungal activity and controlled release. Ind Crop Prod 118:271–281

De-Mejia EG, Zhang Q, Penta K, Eroğlu A, Lila MA (2020) The colors of health: chemistry, bioactivity, and market demand for colorful foods and natural food sources of colorants. Annu Rev Food Sci Technol 11:145–182

Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Dufossé L (2018) Red colorants from filamentous fungi: are they ready for the food industry? J Food Compos Anal 69:156–161

Duran M, Ponezi AN, Faljoni-Alario A, Teixeira MF, Justo GJ, Duran N (2012) Potential applications of violacein: a microbial pigment. Med Chem Res 21:1524–1532. https://doi.org/10.1007/s00044-011-9654-9

Eisenman H, Casadevall A (2012) Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol 93(3):931–940. https://doi.org/10.1007/s00253-011-3777-2

El-kholie EM, El shaver M, Mohammed AT, Abdelreheem GMA (2012) Detailed evaluation of a newly attained fungal pigment from *Monascus purpureus* in meat burgers. Int J Food Sci Nutr 63(7):860–865

Feng Y, Shao Y, Zhou Y, Chen F (2015) Effects of glycerol on pigments and monacolin K production by the high-monacolin K-producing but citrinin-free strain, *Monascus pilosus* MS-1. Eur Food Res Technol 240(3):635–643

Hajjaj H, Klaebe A, Goma G, Blanc PJ, Barbier E, François J (2000) Medium-chain fatty acids affect citrinin production in the filamentous fungus *Monascus ruber*. Appl Environ Microbiol 66(3):1120–1125

Hamadou AH, Huang WC, Xue C, Mao X (2020) Comparison of β-carotene loaded marine and egg phospholipids nanoliposomes. J Food Eng 283:110055

Herńandez-Almanza A, Montañez J, Martínez G, Aguilar-Jiménez A, Contreras-Esquivel JC, Aguilar CN (2016) Lycopene: progress in microbial production. Trends Food Sci Technol 56:142–148

Jain A, Thakur D, Ghoshal G, Katare OP, Singh B, Shivhare US (2016) Formation and functional attributes of electrostatic complexes involving casein and anionic polysaccharides: An approach to enhance oral adsorption of lycopene in rats in vivo. Int J Biol Macromol 93:746–756

Jain S, Winuprasith T, Suphantharika M (2020) Encapsulation of lycopene in emulsions and hydrogel beads using dual modified rice starch: Characterization, stability analysis and release behavior during *in-vitro* digestion. Food Hydrocoll 104:105730

Jia C, Cao D, Ji S, Lin W, Zhang X, Muhoza B (2020) Whey protein isolate conjugated with xylo-oligosaccharides via Millard reaction: characterization, antioxidant capacity, and application for lycopene microencapsulation. LWT- Food Sci Technol 118:108837

Jones JD, Hohn TM, Leathers TD (2004) Genetically modified strains of *Fusarium sporotrichioides* for production of lycopene and β-carotene. Society of Industrial Microbiology Annual Meeting, San Diego, USA, p 91

Kant R (2012) Textile dyeing industry and environmental hazard. Nat Sci 4(1):22–26

Kim C, Jung H, Kim JH, Shin CS (2006) Effect of *Monascus* pigments derivatives on the electrophoretic mobility of bacteria, and the cell adsorption and antibacterial activities of pigments. Colloids Surf B Biointer Faces 47:153–159

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kour D, Kaur T, Devi R, Yadav A, Singh M, Joshi D et al (2021) Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: present status and future challenges. Environ Sci Pollut Res 28:24917–24939. https://doi.org/10.1007/s11356-021-13252-7

Kulkarni P, Gupta N (2013) Screening and evaluation of soil fungal isolates for Xylanase production. Recent Res Sci Technol 5(2):33–36

Kumar A, Yadav AN, Mondal R, Kour D, Subrahmanyam G, Shabnam AA et al (2021) Myco-remediation: a mechanistic understanding of contaminants alleviation from natural environment and future prospect. Chemosphere 284:131325. https://doi.org/10.1016/j.chemosphere.2021.131325

Lagashetti AC, Dufossé L, Singh SK, Singh PN (2019) Fungal pigments and their prospects in different industries. Microorganisms 7(12):604

Li F, Xue F, Yu XH (2017) GC–MS, FTIR and Raman analysis of antioxidant components of red pigments from *Stemphylium lycopersici*. Curr Microbiol 74:532–539

Liu L, Chen P, Zhao W, Li X, Wang H, Qu X (2017) Effect of microencapsulation with the Millard reaction products of whey proteins and iso-maltooligosaccharide on the survival rate of *Lactobacillus rhamnosus* in white brined cheese. Food Control 79:44–49

Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4:118–126. https://doi.org/10.4103/0973-7847.70902

Mabrouk AM, Elkhrisy EM, Youssef YA, Mohamed AA (2011) Production of textile reddish brown dyes by fungi. Malaysian J Microbiol 7(1):33–40

Manimala MA, Murugesan R (2014) In vitro antioxidant and antimicrobial activity of carotenoid pigments extracted from *Sporobolomyces sp.* isolated from natural source. J Appl Nat Sci 6(2):649–653

Mapari SA, Meyer AS, Thrane U, Frisvad JC (2009) Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. Microb Cell Fact 8(1):24

Mawthols KR, Deshpande R, Ware D, Mahajan M (2005) Effect of pH on pigment production of fungi and their toxicity on seed germination. Ecol Environ Conserv 118(2):325–326

Meena M, Swapnil P, Upadhyay RS (2017) Isolation, characterization and toxicological potential of *Alternaria*-mycotoxins (TeA, AOH and AME) in different *Alternaria* species from various regions of India. Sci Rep 7(1):1–19

Michelon M, Mantovani RA, Sinigaglia-Coimbra R, Gaziola de la Torre L, Cunha RL (2016) Structural characterization of β-carotene-incorporated nanovesicles produced with non-purified phospholipids. Food Res Int 79:95–105

Otálora MC, Carriazo JG, Iturriaga L, Osorio C, Nazareno MA (2016) Encapsulating betalains from *Opuntiaficus-indica* fruits by ionic gelation: Pigment chemical stability during storage of beads. Food Chem 202:373–382

Özkan G, Bilek SE (2014) Microencapsulation of natural food colourants. Int J Nutr Food Sci 3:145–156

Pradeep FS, Begam MS, Palaniswamy M, Pradeep BV (2013) Influence of culture media on growth and pigment production by *Fusarium moniliforme* KUMBF1201 isolated from paddy field soil. World Appl Sci J 22(1):70–77

Ran X, Zhang G, Li S, Wang J (2017) Characterization and antitumor activity of camptothecin from endophytic fungus *Fusarium solani* isolated from *Camptotheca acuminate*. Afr Health Sci 17(2):566–574

Rana KL, Kour D, Kaur T, Devi R, Negi C, Yadav AN et al (2020) Endophytic fungi from medicinal plants: biodiversity and biotechnological applications. In: Kumar A, Radhakrishnan E (eds) Microbial endophytes. Woodhead Publishing, Cambridge, MA, pp 273–305. https://doi.org/10.1016/B978-0-12-819654-0.00011-9

Rankovic BR, Kosanic MM, Stanojkovic TP (2011) Antioxidant, antimicrobial, and anticancer activity of the lichens *Cladoniafurcata*, *Lecanoraatra* and *Lecanoramuralis*. BMC Complement Altern Med 11:97. https://doi.org/10.1186/1472-6882-11-97

Rao MN, Xiao M, Li WJ (2017) Fungal and bacterial pigments: secondary metabolites with wide applications. Front Microbiol 8:1113

Rastegari AA, Yadav AN, Yadav N (2019a) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5

Rastegari AA, Yadav AN, Yadav N, Tataei Sarshari N (2019b) Bioengineering of secondary metabolites. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 55–68. https://doi.org/10.1016/B978-0-444-63504-4.00004-9

Robinson S, Hinsch E, Weber G, Freitas S (2014) Method of extraction and resolubilisation of pigments from *Chlorociboria aeruginosa* and *Scytalidium cuboideum*, two prolific spalting fungi. Coloration Technol 130:221–225. https://doi.org/10.1111/cote.2014.130.issue-3

Rocha GA, Fávaro-Trindade CS, Grosso CF (2012) Microencapsulation of lycopene by spray drying: characterization, stability and application of microcapsules. Food Bioprod Process 90:37–42

Sabry BA, Hathout AS, Nooh A, Aly SE, Shehata MG (2016) The prevalence of aflatoxin and *Aspergillus parasiticus* in Egyptian sesame seeds. Int J Chem Tech Res 9(11):308–319

Salama HA, Badr AN, Elkhadragy MF, Hussein AS, Shaban IS, Yehia HM (2021) New antifungal microbial pigment applied to improve safety and quality of processed meat-products. Microorganisms 9(5):989. https://doi.org/10.3390/microorganisms9050989

Sampaio GA, Pacheco S, Ribeiro AO, Galdeano MC, Gomes FS, Tonon RV (2019) Encapsulation of a lycopene-rich watermelon concentrate in alginate and pectin beads: characterization and stability. LWT-Food Sci Technol 116:108589

Santos-Ebinuma VC, Teixeira MS, Pessoa A (2013) Submerged culture conditions for the production of alternative natural colorants by a new isolated *Penicillium purpurogenum* DPUA 1275. J Microbiol Biotechnol 23:802–810

Sen T, Barrow CJ, Deshmukh SK (2019) Microbial pigments in the food industry—challenges and the way forward. Front Nutr 6:1–15. https://doi.org/10.3389/fnut.2019.00007

Shahat MS, Badr AN, Hegaziy AI, Ramzy S, Samie MA (2017) Reducing the histopathological and biochemical toxicity of aflatoxins contaminated soybean using ozone treatment. Ann Res Rev Biol 15(3):1–14. https://doi.org/10.9734/ARRB/2017/35075

Shehata MG, Badr AN, Abdel-Razek AG, Hassanein MM, Amra HA (2017) Oil-bioactive films as an antifungal application to save post-harvest food crops. Annual Research & Review in Biology 16(4):1–16. https://doi.org/10.9734/ARRB/2017/36149

Shahid M, Ul-Islam S, Mohammad F (2013) Recent advancements in natural dye applications: a review. J Clean Prod 53:310–331. https://doi.org/10.1016/j.jclepro.2013.03.031

Shankar S, Wang LF, Rhim JW (2019) Effect of melanin nanoparticles on the mechanical, water vapor barrier, and antioxidant properties of gelatin-based films for food packaging application. Food Packag Shelf Life 21:100363

Sharma D, Gupta C, Aggarwal S, Naqpal N (2012) Pigment extraction from fungus for textile dyeing. Indian J Fibre Textile Res 37:68–73

Shier WT, Lao Y, Steele TW, Abbas HK (2005) Yellow pigments used in rapid identification of aflatoxin-producing *Aspergillus* strains are anthraquinones associated with the aflatoxin biosynthetic pathway. Bioorg Chem 33(6):426–438

Singh SK, Pandey A (2013) Emerging approaches in fermentative production of statins. Appl Biochem Biotechnol 171(4):927–938

Turbyville TJ, Kithsiri Wijeratne EM, Whitesell L, Leslie Gunatilaka AA (2005) The anticancer activity of the fungal metabolite terrecyclic acid A is associated with modulation of multiple cellular stress response pathways. Molecular Cancer Therapeutics 4(10):1569–1576. https://doi.org/10.1158/1535-7163.MCT-05-0050

Ungureanu C, Ferdes F (2010) Antibacterial and antifungal activities of red rice obtained from *Monascus purpureus*. Chem Eng Trans 20:223–228

Velmurugan P, Kim MJ, Park JS, Karthikeyan K, Lakshmana perumal P, Lee KJ et al (2010) Dyeing of cotton yarn with five water soluble fungal pigments obtained from five fungi. Fiber Polym 11:598–605

Vendruscolo F, Tosin I, Giachini AJ, Schmidell W, Ninow JL (2014) Antimicrobial activity of *Monascus* pigments produced in submerged fermentation. J Food Process Preserv 38:1860–1865

Weber G, Chen H, Hinsch E, Freitas S, Robinson S (2014) Pigments extracted from the wood-staining fungi *Chlorociboria aeruginosa*, *Scytalidium cuboideum*, and *S. ganodermophthorum* show potential for use as textile dyes. Coloration Technology 130(6):445–452. https://doi.org/10.1111/cote.12110

Yadav AN (2019) Fungal white biotechnology: conclusion and future prospects. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for sustainable environments, vol 3. Springer International Publishing, Cham, pp 491–498. https://doi.org/10.1007/978-3-030-25506-0_20

Yadav AN (2021) Microbial biotechnology for bio-prospecting of microbial bioactive compounds and secondary metabolites. J Appl Biol Biotechnol 9:1–6. https://doi.org/10.7324/JABB.2021.92ed

Yadav AN, Kour D, Rana KL, Yadav N, Singh B, Chauhan VS et al (2019a) Metabolic engineering to synthetic biology of secondary metabolites production. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 279–320. https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Yadav AN, Mishra S, Singh S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 1: Diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019c) Recent advancement in white biotechnology through fungi. Volume 2: Perspective for value-added products and environments. Springer International Publishing, Cham

Youngchim S, Morris-Jones R, Hay RJ, Hamilton (2004) Production of melanin by *Aspergillus fumigates*. J Med Microbiol 53:175–181

Zhao X, Huang X, Yao J, Zhou Y, Jia R (2015) Fungal growth and manganese peroxidase production in a deep tray solid-state bioreactor, and *invitro* decolorization of poly R 478 by MnP. J Microbiol Biotechnol 25:803–813

Zhao C, Yin LB, Liu F, Li J, Liu X, Wang J, Wang Y (2020) Encapsulation of lycopene within oil-in-water nanoemulsions using lactoferrin: impact of carrier oils on physicochemical stability and bio-accessibility. Int J Biol Macromol 153:912–920

Zhou ZG, Guo HZ, Xie CY (2015) Effects of culture conditions on production of red pigment and citrinin by fermentation of *Monascus ruber*. Chem EngTrans 46:1363–1368

Chapter 16
Fungal Production of Vitamins and Their Food Industrial Applications

Praveen Kumar Dikkala, Zeba Usmani, Shiv Kumar, Vijai Kumar Gupta, Atul Bhargava, and Minaxi Sharma

Contents

P. K. Dikkala
Department of Food Science and Technology, Punjab Agriculture University, Ludhiana, Punjab, India

Z. Usmani
Department of Applied Biology, University of Science and Technology, Meghalaya, India

S. Kumar
Department of Food Science and Technology (Hotel Management), Maharishi Markandeshwar University, Mullana, Ambala, Haryana, India

V. K. Gupta
Biorefining and Advanced Biomaterials Research Center, Scotland's Rural College (SRUC), Kings Buildings, Edinburgh, UK

A. Bhargava
Department of Botany, Mahatma Gandhi Central University, Motihari, India

M. Sharma (✉)
Department of Applied Biology, University of Science & Technology, Techno City, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_16

16.1 Introduction

Vitamins are essential organic compounds and mainly involved in the multi-metabolic pathways in living organisms, including human. The vitamins are acting as coenzymes which support the metabolism and help in the maintenance and proper functioning of the body. Vitamins are generally produced by plants and microorganisms, however, animals including human, need to consume them for the growth and maintenance of their health. Sometimes, the demand for vitamins is increased in the body due to pathogenesis, pregnancy, physical exercises, poor diet, malnutrition or in cases of stress and drug abuse (Acevedo-Rocha et al. 2019; Vandamme and Revuelta 2016). Furthermore, the techniques involved in food processing and preservation have an inverse effect on the quantity and quality of these vitamins (Vandamme and Revuelta 2016). Therefore, there is an increase in demand to produce the vitamins for applications in food, feed, cosmeceutical industry. Also, consumer awareness to have healthy food is a key factor to increase the demand for vitamins supplemented foods, and this demand promotes the food technologist to produce vitamins from other resources. In modern society, there is a constant increase in the demand for healthy foods, so to achieve the target, the food and feed industries are facing many challenges at industrial and consumers level.

From a recent report, it is anticipated that vitamin supplement market is expected to reach $74.61 billion in 2023 at a CAGR of 6.85%, globally (https://research.tdameritrade.com/grid/public/markets/news/story.asp?fromPage=results&docKey=100-203p8765). In this way, microbiological solutions have been tremendously explored for their ultimate bioactive potential to produce functional and nutraceutical food ingredients. Among all the microorganisms, filamentous fungi have excellent potential in the production of vitamins.

Vitamins are currently produced by chemical synthesis, and/or biotechnological applications by using microorganisms (Acevedo-Rocha et al. 2019; Abdel-Azeem et al. 2021; Yadav et al. 2019b). Through this, biotechnology has become an increasingly incredible solution for fashioning ways to feed the world. In the last decades, numerous microorganisms such as bacteria, fungi, yeast and algae have been comprehensively researched for their capability to synthesize various types of valuable bioactive compounds for food and animal feed applications (Bourdichon et al. 2012; Singh and Yadav 2020; Yadav et al. 2019c). In this context, filamentous fungi being no exception as they can produce a variety of useful food ingredients like enzymes, fatty acids, flavourings, organic acids, pigments & vitamins (Hüttner et al. 2020; Kour et al. 2019). Among the biotechnological applications, the use of fungal species for the production of vitamins is still a bottleneck and unexplored. From this book chapter, we reviewed the literature on various fungal species but a very few species involved in the production of vitamins, so there is a need to develop the fungal resources, and to explore the techniques to produce vital vitamins. Novel genetic engineering tools helped a lot to produce engineered high yielding vitamin-producing strains which improved the production of various bioactive nutrients, including vitamins (Nielsen and Keasling 2016; Rastegari et al. 2019a, b; Yadav

et al. 2019a). But still, there is a need to understand the biosynthetic pathway, its regulatory steps, technological advancements, and genetic makeup of fungal mycelia to enhance the production of vitamins.

16.2 Classification of Vitamins

Vitamins are micro-nutrients playing an essential role to maintain proper functioning of metabolism in human health. These organic molecules are essential micronutrients required in small quantities for the appropriate functioning of animal metabolism. These cannot be synthesized by the animals, either at all or scarcely, and so there is a need to obtain these vitamins to fulfill their needs, from other resources or diet (Yadav 2020).These are mainly classified into two on the basis of their solubility in water, i.e. water-soluble and fat-soluble vitamins. These are as follows:

16.2.1 Water-Soluble Vitamins and Their Fungal Sources

The water-soluble vitamins are further divided into two groups: B-vitamins and vitamin C (L-ascorbic acid). Figure 16.1 elaborates the structures for all the water-soluble vitamins with their active forms. Microbial production of all B-vitamins and vitamin C using various fungal and yeasts sources has shown in Table 16.1.

16.2.1.1 B-Vitamins

16.2.1.1.1 B_1 (Thiamin)

Chemically it is also known as thiamine hydrochloride and is a white crystalline powder, slightly hygroscopic in nature; odour resembles yeast and bitter in taste. It is completely soluble in water. Vitamin B_1 is widely found in the vegetable's kingdom. The richest sources of vitamin B_1 are yeast, grains, germs and rice bran, whole grain, potatoes. Animal tissues like liver, kidney, brain, heart are the sources of vitamin B_1 (Wu et al. 2020). The structure of vitamin B_1 consists of a thiazole moiety.

Vitamin B_1 is produced in the human gut, and is also synthesized by the aquatic microorganism, especially cyanobacteria (Putnam and Goodman 2020). The analyses of human gut flora microflora predict the bactericides *Fragilis, Prevotella copri*, *Clostridium difficile*, some *Lactobacillus* species and *Fusarium* species are vitamin B_1 producers that can be implying to intestine for complete vitamin B1 synthesis pathway (Yoshii et al. 2019). Thiamine is a cofactor for several enzymes which includes pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase, which play an important role in tricarboxylic acid (TCA) cycle (Abramova et al. 2020).

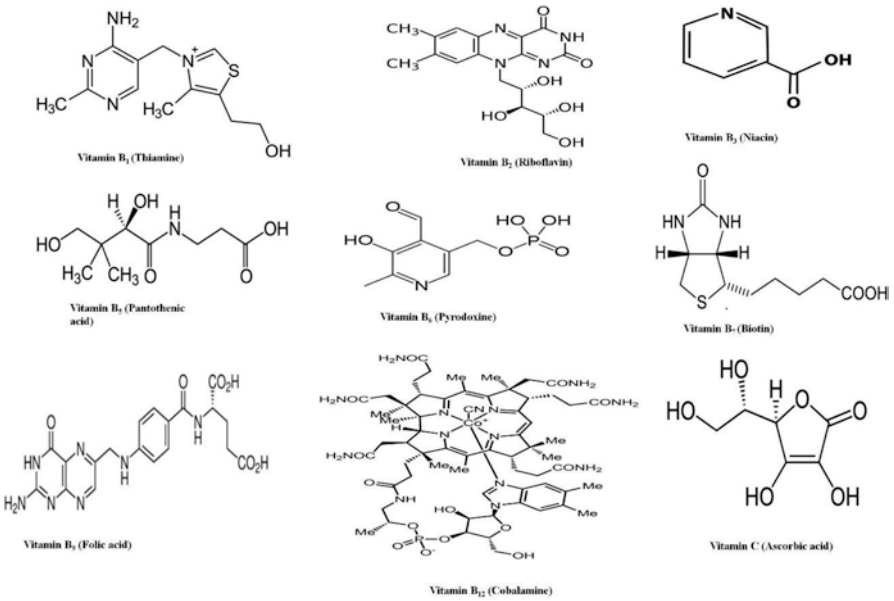


Fig. 16.1 Chemical structures of water-soluble vitamins

Vitamin B_1 deficiency causes lethargy and resulting in beriberi and also affects the nervous system and the cardiovascular system (Sharma et al. 2019).

Thiamin is an essential vitamin to accomplish the metabolic activity in all living organisms because it is a cofactor for many enzymes in metabolism. Unlike animals, fungal species such as filamentous fungi and yeasts can synthesize their thiamin including *Aspergillus oryzae*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe,* etc. Presence of thiamin primarily regulated the genes expression and thiamin transporters, during biosynthesis of thiamin. In the case of filamentous fungi, expression of thiamin biosynthesis genes is regulated by TPP riboswitches and RNA (located in messenger RNA). However, TPP riboswitches are rare in yeast cells, for the biosynthesis of thiamin (Donovan et al. 2018). In a study, to improve the productivity of thiamine pyrophosphate (TPP) in *Aspergillus oryzae*, Tokui et al. (2011) genetically improved the strain by overexpressing the genes thiA, nmtA and thiP, simultaneously. The resulting strain was responsible for accumulating 4-fold higher TPP than the control strain (Tokui et al. 2011). Shimizu et al. (2016) disordered thiA gene of the fungus *Aspergillus nidulans* and reported that the hypoxic stress modulates thiA expression through the thiamine riboswitch and alters cellular fermentation mechanisms by regulating the activity of the TPP enzymes. They demonstrated that under hypoxic stress, the fungus accumulated more thiamine (34 pmol mg^{-1} of thiamine) than under aerobic conditions. In a study, pyrithiamine (PT) resistance gene (ptrA) was cloned from a genomic DNA library prepared from a PT resistant mutant of *Aspergillus oryzae* (Kubodera et al. 2000). They reported that the introduction of the ptrA gene allowed an *A. oryzae* industrial

Table 16.1 Vitamins production from fungal and yeast sources

Vitamins	Coenzyme/ Cofactor	Fungal source	Genome modulation/conditions	Quantity produced	Reference
Water-soluble vitamins					
B1 (Thiamine Hydrochloride)	TPP (thiamine pyrophosphate)	*A. oryzae*	Overexpression of thiA, nmtA and thiP	4-fold > control	Tokui et al. (2011)
		Aspergillus nidulans	thiA gene expression under hypoxic stress, through the thiamine riboswitch	34 ± 5 pmol mg^{-1} of thiamine	Shimizu et al. (2016)
B2 (Riboflavin)	FMN (flavin mononucleotide), FAD (flavin adenine dinucleotide)	*A. gossypii*	Overexpression of riboflavin biosynthetic genes; Overexpression of threonine aldolase (GLY1); disruption of gene SHM2 encoding serine hydroxymethyltransferase	>20 g/L	Abbas et al. (2011)
		A. gossypii	RIB genes +	0.327 g/L 3.1-fold >control	Ledesma-Amaro et al. (2015)
		C. famata	SEF1 , RIB1 +, RIB7 +	16.4 g/L, 62-fold	Dmytruk et al. (2014)
		E. ashbyi	RIB1 +, RIB3 +	0.331 g/L, 1.44-fold	Sengupta et al. (2012)
		Ashbya gossypii	AgSHM2, Disruption abolished the conversion of glycine into serine	10.6-fold increase	Schlüpen et al. (2003)
		Candida famata	-	21 g/L	Abbas and Sibirny (2011)
		A. gossypii	AgURA3, increase the availability of riboflavin precursors	7.5-fold increase in riboflavin	Silva et al. (2015)
		A. gossypii	AgPRS2,4 and AgPRS3, Overexpression and elimination of feedback inhibition by site directed mutagenesis increased PRPP Availability	1.7- to 1.8-fold increase	Jiménez et al. (2008)
		A. gossypii	Overexpression of AgMLS1, increased riboflavin production from oils	By 1.7-fold	Sugimoto et al. (2009)

(continued)

Table 16.1 (continued)

Vitamins	Coenzyme/ Cofactor	Fungal source	Genome modulation/conditions	Quantity produced	Reference
B3 (Nicotinic acid (niacin))	NAD+ (nicotinamide adenine dinucleotide), NADP+ (nicotinamide	*S. cerevisiae*	KO of NR importer Nrt1 in salvage deficient nrk1 urh1 pnp1 strain	>8 mg/L nicotinamide riboside	Belenky et al. (2011)
B5 (calcium D-(+)-pantothenate)	Coenzyme A	*Fusarium oxysporum*	With pantolactone solution (700 g/L)	91.7% recovery	Shimizu et al. (2001)
B 6 (Pyridoxine Hydrochloride)	Pyridoxal phosphate	*Aspergillus flavus* and *Neurospora crassa*	PDX1 and PDX2 isolated from *Cercospora nicotianae*	4–7 ng/mg dry weight	Herrero and Daub (2007)
B7 (Biotin)	Biocytin	*Rhizopus nigricans*	–	10.4, 13.9, 5.7, 7.6 and 4.2-fold increases	El-Refai et al. (2010)
		Candida sp LEB 130	Sucrose 30 g/L, KH_2PO_4 2 g/L, $MgSO_4$ 1 g/L and $ZnSO_4$ 0.5 mL/L	2.9-fold higher than the control samples	Suzuki et al. (2011)
B9 (Folic Acid)	THF (tetrahydrofolic acid)	*A. gossypii*	Overexpression of *fol* genes and deletion of met7 and competing genes AgADE12 and AgRIB1	7 mg/L	Serrano-Amatriain et al. (2016)
B12 (Cyanocobalamine)	Adenosylcobalamin	*Lentinula edodes*	Cobalamin precursors (cobalt chloride) and methyl donors (betaine, methionine, and choline)	95 g/g of dry weight, 10,000-fold higher	Turło et al. (2008)
Vit C (L-ascorbic acid)	L-ascorbic acid	*Saccharomyces cerevisiae, Zygosaccharomyces bailii*	Overexpression of ALO1 and LGDH	13.2 and 2.8 mg L^{-1}, increased 3- and 15-fold for *S. cerevisiae* and *Z. bailii*, respectively	Sauer et al. (2004)
Fat-soluble vitamins					

Vitamins	Coenzyme/ Cofactor	Fungal source	Genome modulation/conditions	Quantity produced	Reference
Vitamin A	Beta-carotene (precursor)	*Candida utilis/ Saccharomyces cerevisiae*	GAL1 and GAL 10 are galactokinase genes that are induced in the galactose regulatory network	7.41 mg of dry cell weight	Xie et al. (2014)
	Beta-carotene	*S. cerevisiae*	overexpression of geranylgeranyl diphosphate (GGPP) synthase	5.9 mg/g (dry weight)	Verwaal et al. (2007)
	Beta-carotene	*Pichia pastoris* X-33	Overexpression of crtE, crtB, crtI genes from *Erwinia uredovora*	339 μg/g of cells, 1.5-fold increase	Araya-Garay et al. (2012)
	Beta-carotene	*Pichia pastoris* X-33	Overexpression of carotenogenic genes from *X. dendrorhous*	5.9 mg β-carotene/g (dw), 57-fold more production than control	Araya-Garay et al. (2012)
	Beta-carotene	*Saccharomyces cerevisiae*	Overexpression of HMG-CoA reductase	6.29 mg/g dry weight	Yan et al. (2012)
Vitamin D	Cholecalciferol	*Saccharomyces cerevisiae*	Removal of gene erg5, overexpression of upc2-1 and limiting enzymes (Erg1p, Hmg1p and Erg 11p)	Ergosterol production is increased by 86%	Nahlik et al. (2017)
Vitamin E	Tocopherol	*Lepista inversa*	–	1.18 μg/g	Heleno et al. (2010)
		Clitocybe alexandri		3.55 μg/g	
		Fistulina hepatica		2.26 μg/g	
		Hygrophoropsis aurantiaca		1.94 μg/g	
		Mycena rosea		4.89 μg/g	
		Laccaria laccata		8.04 μg/g	
Vitamin K	Dimethyl menaquinone	*Pichia pastoris*	The expression of Homo Sapiens UBIAD1	Increased by 0.24 mg/g	Sun et al. (2019)

strain to grow on the minimum medium containing PT (0.1 mg L^{-1}) on which an untransformed strain did not grow. This result indicates that the ptrA is applicable as a dominant selectable marker for transformation of *A. oryzae*.

16.2.1.1.2 B_2 (Riboflavin)

Vitamin B_2 is also chemically known as riboflavin dominantly found in nature as yellow pigments called flavin. The ribose (ribitol) is attached to the alcohol group, i.e. flavin ring. The role of riboflavin is an important compound of cellular disable because it is the precursor of flavoenzymes flavin mononucleotide and flavin adenine dinucleotide which are the electron carrier involved in the redox reactions (Thakur et al. 2016). It is orange-yellow in colour. This vitamin is widely distributed in plants as well as in animals in little quantity. Sources of vitamin B_2 are milk, liver, eggs, leafy vegetables and cheese (Liu et al. 2020). Dried yeast is the richest source of this vitamin. It is less water-soluble than thiamine but more stable to heat in acid or neutral media. As a prosthetic group of flavin enzymes, it is involved in the reaction of all nutrient's plants and animals (Shibata et al. 2017). It helps to regulate redox processes and thus catalyses the reactions of macromolecules like carbohydrates, fats, proteins and nucleic acids. Three groups of microorganisms are capable to produce large amount of vitamin B_2, such as bacteria (*Clostridium acetobutylicum*), yeast (*Candida)*, and ascomycetes (*Eremothecium ashbyii* and *A. gossypii)* (Sharma et al. 2019).

Riboflavin (vitamin B_2) vitamin is an essential nutritional component which is the precursor of coenzymes FMN and FAD that are involved in various reactions in metabolism. Commercial production of riboflavin is generally for feed, food, cosmetics and medicine. Nowadays, microbial production of riboflavin has increased significantly. Biosynthesis of riboflavin starts with guanosine triphosphate and ribulose-5-phosphate and is completed in six enzymatic steps (Fischer and Bacher 2005). Currently, industrial riboflavin production is achieved through the use of recombinant strains of microorganisms like bacteria and fungi. Among fungi, overproducing strains of *A. gossypii* and *Candida famata* are constructed using metabolic engineering approaches to produce riboflavin. In a study, Dmytruk et al. (2014) constructed *Candida famata* by overexpression of three genes SEF1, RIB1 and RIB7 coding for a putative transcription factor, GTP cyclohydrolase II and riboflavin synthase, respectively. They reported that under optimized conditions, the constructed strain accumulated up to 16.4 g/L of riboflavin in a 7 L laboratory bioreactor during fed-batch fermentation. They observed 4.1-fold increase in riboflavin production using overexpression of above-mentioned genes in overproducer AF-4 during shake-flask fermentation in their earlier study (Dmytruk et al. 2011). Riboflavin production has also been reported as 21 g/L and 15 g/L by other studies (Heefner et al. 1992; Bigelis 1989).

Similarly, *Ashbya gossypii* (syn. *Eremothecium gossypii*) is a highly flavinogenic mold of the Saccharomycetaceae family that has been extensively exploited for the production of riboflavin. Genetically improved *A. gossypii* strains have been used

since 1990 for the industrial production of this vitamin. In a study, riboflavin overproducing strain of *A. gossypii* was constructed by ultraviolet irradiation, to improve riboflavin production at industrial scale. After irradiated exposure of 10 min of ultraviolet light, a stable mutant of the wild strain was isolated. Riboflavin production of the mutant was two-fold higher than that of the wild strain in flask culture. The mutant strain was observed with improved riboflavin production upto 6.38 g/L and even more about 8.12 g/L under optimized pH (pH 6.0–7.0 using KH_2PO_4) in the later growth phase (Wei et al. 2012). Kato and Park (2012) worked on recombinant strains of *A. gossypii*, and reported the content of riboflavin up to 13.7, g/L, in fermenters. *Pichia pastoris* strain was reported to overexpress all RIB genes accumulating riboflavin (175 mg/L) in fed-batch fermentation (5L) at 25 °C, 5.0 pH, 25% ammonium hydroxide, and dissolved-oxygen kept above 20% by controlling stirrer speed between 600 and 1200 rpm (Marx et al. 2008). Mutated yeast strains of *Candida famata* were reported to accumulate up to 16.4 g/L riboflavin in optimized fed-batch fermentation using a stirred tank bioreactor for 126 h, 28 °C, 1200 rpm, 5.5 pH (Dmytruk et al. 2011). In *P. guilliermondii*, chromium ion treatment augmented riboflavin accumulation, and released exogenous riboflavin improved its resistance to metal ions (explaining tolerance for bioremediation) (Ksheminska et al. 2003). Metabolically engineered *Ashbya gossypii* MA2 produced 523 mg/L riboflavin after 120 h of fermentation (Ledesma-Amaro et al. 2015). Riboflavin fermentation using *Eremothecium ashbyii* was reported to be 3107.59 mg/L using a 15 L fed-batch stirred tank fermenter with a feed of glucose (0.5–0.8 g/100 mL), a feed flow of 60 g/L yeast extract for 48–96 h (Cheng et al. 2011).

16.2.1.1.3 B_3 (Niacin)

Vitamin B_3 is commonly called as niacin. There are three active forms of vitamin B_3 which are having role as essential cofactors in metabolism. Recently, the three forms of B_3, i.e. niacin (NA), niacinamide (NAM) and nicotinamide riboside (NR), gained attention in the treatments of age-related diseases (Acevedo-Rocha et al. 2019).The amides are very soluble in water so these are preferred therapeutically because they do not have any side chain (Yoshii et al. 2019). All are stable in air as well as heat acid or alkali. B_3 has important functions in our body as a component of two important enzymes and is also involved in respiration and breakdown of glucose to produce energy and necessary for growth and development (Lloyd-Price et al. 2019).

Deficiency of niacin change on skin, gastrointestinal tract and nervous system that changes into insomnia, dizziness, irritability, depression later may lead to dementia. The disease pellagra is occurred due to deficiency of niacin. Meat is rich source mainly organ meat such as liver. Groundnuts are also best source of niacin. Cereals are the major sources in the Indian diet. Whole cereal grains and parboiled cereals save more niacin than processed (Liu et al. 2020). The fungal sources (mushrooms) and brewer's yeast are the best for niacin.

S. cerevisiae (yeast) strain able to produce extracellular nicotinamide riboside (>8 mg/L nicotinamide riboside) by the native NAD^+ breakdown process using nicotinamide riboside importer Nrt1 (Belenky et al. 2011). Rather this is a high yielding process with the biocatalytic approach but a little motivation for the bioconversion of niacin or niacinamide. In contrast, fermentation of nicotinamide riboside is found favourable if market demand arises.

16.2.1.1.4 B_5(Pantothenic Acid)

Vitamin B_5 is also known as pantothenic acid. Pantothenic acid is widely distributed in foods and particularly abundant in animal tissues, pulses and whole cereal grains and in fewer amounts in milk (Gominak 2016). It is more stable in solution than dry form at pH range of 4–7 and decomposed by alkali or dry heat. Pantothenic acid as complex molecule as coenzyme-A containing sulphur-containing compounds (highly reactive), adenine, ribose, phosphoric acid etc. attributed to its functional role in the body (Watanabe and Bito 2018). Due to lack of these vitamins symptoms appear as loss of appetite, indigestion, and abdominal pain, mental depression, burning sensation in the feet, insomnia and respiratory infection. Important sources of pantothenic acids are animal tissues in the form of coenzyme-A and whole grains as well as legumes. Liver, yeast, egg yolk and meat particularly are the beast sources (Gonzalez-Lopez et al. 2016).

Pantothenic acid has a vital role in multiple metabolic reactions in all living cells and is a precursor of co-enzyme-A (CoA), and acyl carrier protein (ACP) which helps in lipid metabolism. The active form of vitamin B5 is pantothenate stabilized with calcium salt for commercial form (Leonardi and Jackowski 2007; Gonzalez-Lopez et al. 2016). Vitamin B_5 can be synthesized by bacteria, fungi and plants, but not by mammals, including human. Chemical synthesis of B_5 is diminishing and replaced by bio-catalytically microbial synthesis, at an industrial scale. Nowadays, commercial production of B_5 is carried out by using microbial biotechnological methods. Numerous filamentous fungi of genera *Gibberella* and *Fusarium,* which show higher activity of the enzyme (coenzyme-A) can synthesize pantothenic acid. Yeast cells, including *Saccharomyces cerevisiae* and *Debaryomyces castellii,* are pretty rich in CoA content and are used to produce the vitamin B_5. The yeast, *Debaryomyces castellii* in the nutrient growth media containing glucose, pantoic acid and β-alanine favours the production of D-pantothenic acid (Gonzalez-Lopez et al. 2016). In microbial cells, the biosynthesis of B_5 is starting from metabolic intermediates α-ketoisovalerate and aspartate. Expression of panB and panE genes is convoluted for the conversion of α-ketoisovalerate into pantothenate. Expression of ATP-dependent panC genes helps to combine two pantothenic precursors into the final product as vitamin B_5 (Stahmann 2019). The production yield of these vitamins can be enhanced by achieving genetic modifications in these microbes. Shimizu et al. (2001) isolated vitamin B_5 using *Fusarium oxysporum* mycelia incubated with pantolactone solution (700 g/L) for 24 h at 30 °C at controlled pH (6.8–7.2).

16.2.1.1.5 B_6(Pyridoxine)

Vitamin B_6 or pyridoxine is a water-soluble vitamin found naturally in plants and animals. Vitamin B_6 is an essential cofactor for numerous enzymatic reactions in its phosphate form. Pyridoxal 5" phosphate (PLP) is the effective form of coenzyme and most common measures of the bloodstream. Pyridoxine has pharmaceutical and numerous food functions. These vitamins can be synthesized by several potential stains of microorganisms like *Klebsiella sp.,Flavobacterium sp., Pichia guilliermondii, Bacillus subtilis,* and *Rhizobium meliloti,* etc. (Watanabe and Bito 2018; Yoshii et al. 2019).

In a study, Herrero and Daub (2007) demonstrated the effect of overexpression of two genes (PDX1 and PDX2) which involved in the biosynthesis of vitamin B_6 and the production efficiency of vitamin B_6. They isolated these genes from pathogenic fungi (*Cercospora nicotianae*) and overexpressed in two fungal strains, i.e. *Aspergillus flavus* and *Neurospora crassa*. They reported the values for B_6 varied from 4 to 7 ng/mg dry weight, in both the tested fungal species (*Aspergillus flavus* and *Neurospora crassa*). They concluded that no significant difference was observed in B_6 content of the two strains tested as compared to their corresponding wild type or vector-transformed controls.

Several yeasts strains have been shown to accumulate B_6 vitamin, extracellularly such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Pichia guilliermondii* (Chumnantana et al. 2001). Argoudelis (1999) investigated the production of vitamin B_6 by using a yeast mutant P-131 and reported that the yeast was able to produce higher vitamin B_6 content about 446 nmoles/g dried cells (PMP 30%, PM 22%, PLP 7%, PN 41%) as compared to control strain (23 nmoles/g dried cells (PMP 91%, PM 9%). Chumnantana et al. (2001) examined the production of vitamin B_6 especially pyridoxal 5′-phosphate (PLP) by using *Schizosaccharomyces pombe leu I* strain, extracellularly. They reported that L-leucine (30 mg/L), D-Glucose (1%, w/v) and supplementation of air and ammonium sulphate, significantly enhanced the B_6 (PLP) production. They observed the highest B_6 content (1203 nmol/g, wet weight) in the cells when menadione (0.09mM) was used in nutrient medium, extracellularly.

16.2.1.1.6 B_7(Biotin)

Vitamin B_7 is water-soluble and sulphur-containing vitamin widely distributed in the nature. It is stable at pH 5–8. Biotin helps in metabolization of carbohydrates and fats. D-(+)-Biotin (or biotin) is an essential cofactor for carboxylation reactions in metabolism of living organisms and has capacity to add or remove carbon dioxide. The symptoms of biotin deficiency appear as anorexia, nausea, mental depression and dry scaly dermatitis. Good sources of this vitamin include liver, kidney, egg yolk, groundnuts and some vegetables. The microbial sources of this vitamins are intestinal bacteria freely synthesized from malonyl CoA or pimeloyl-Co A (Satiaputra et al. 2016; Sharma et al. 2019).

Biotin is an essential component not only in growth but also help in metabolism of microorganisms including yeasts, fungi and bacteria. The fungi and *Streptomyces* yeast can generally accumulate huge amount of biotin in contrast bacteria. Addition of pimelic acid can significantly enhance the production of biotin in microbial cells. Pearson et al. (1986) screened 129 different yeasts species for the production of biotin vitamin and its vitamers. They reported that *Rhodotorula* and *Sporobolomyces* produced the highest content of biotin. The filamentous fungi, *Ashbya gossypii*, need many nutrients with biotin as well to produce other vitamin, i.e. riboflavin (Demain 1972). Özbas and Kutsal (1991) observed the highest riboflavin (0.6 kg/g) and biotin (0.4 µg/L) production using *Ashbya gossypii*. Similarly, Kojima et al. (1972) produced riboflavin using biotin in nutrient medium (glucose and salt) with *Eremothecium ashbyii* strain. Simultaneous production of biotin and riboflavin was reported by Suzuki et al. (2011) using *Candida* sp. LEB 130 strain. They reported the maximum production ratio of biotin and riboflavin was 8.3 µg/mL with the minimal nutrient medium containing sucrose 30 g/L, KH_2PO_4 2 g/L, $MgSO_4$ 1 g/L and $ZnSO_4$ 0.5mL/L. Additionally they reported that the yield of biotin was 2.9-fold higher than the control samples. Kalingan and Krishnan (1997) reported the highest vitamin production (30 kg/m^3) with increased production rate by 48% when used *Eremothecium ashbyii*, as compared to control strain. In a study, *Rhizopus nigricans* strain was utilized for the production of microbial biotin using multi-factorial experimental designs to analyse the effects on production efficiency of biotin. They reported about 10.4, 13.9, 5.7, 7.6 and 4.2-fold increases in production of biotin in nutrient medium enriched with sucrose and peptone (El-Refai et al. 2010).

16.2.1.1.7 B_9 (Folic Acid)

The common name of vitamin B_9 is folic acid (folates) generally synthesized by microorganism and plants with the help of precursors having mainly three chemical sub-compounds, i.e. guanosine 50-triphosphate (GTP), *p*-aminobenzoic acid and L-glutamic acid (Weimann et al. 2011). The folic acid name comes from the Latin word foliage or leaf (folium); these vitamins were first isolated from spinach leaves and distributed in green leaves. Biological forms of folate are tetrahydrofolate (THF), 5-methyl-THF, 5,10-methylene-THF, 5,10-methenyl-THF and 10-formyl-THF. Folic acid plays a key role as cofactor in one-carbon transfer reactions, involved in biosynthesis and metabolism of amino acids and nucleotides, and delivers methyl groups to various substrates such as DNA, hormones, proteins, lipids, etc. Animals, including human, cannot synthesize their own folic acid, so they need the folic acid in their diet, to maintain the biochemical reactions in their metabolism (Serrano-Amatriain et al. 2016). Diets deficient in folic acid in man result in poor growth, megaloblastic anaemia, macrocytic anaemia during pregnancy, gastrointestinal disturbance and other blood disorders. Consequently, the addition of folic acid in the diet to meet the need is increasing nowadays. Good sources of this vitamin are the kidney, green leafy vegetables, broccoli and dried whole wheat bread, etc. while the microbial sources include yeasts, *Bacteroides fragilis*, *Clostridium difficile*,

Lactobacillus plantarum, *L. reuteri species bulgaricus* and *Streptococcus thermophilus* (Maynard et al. 2018; Yoshii et al. 2019). A few studies have been reviewed here with fungal production of vitamin B_9.

Bioengineering tools in microbial biotechnology are recently being used to modulate the genetic makeup of microorganism to enhance the production of these vitamins. Yeasts (*Saccharomyces cerevisiae*) are major producers of folic acid in the form of substituted polyglutamates (77.4%) and unsubstituted polyglutamate (19.8%) which are quite bioavailable as well (Seyoum and Selhub 1998). Hjortmo et al. (2005) analysed the folate content of various strains of yeasts and observed that 65% of folate is available in 5 MTHF form. Serrano-Amatriain et al. (2016) produced folic acid by using metabolically engineered fungus *Ashbya gossypii* in order to enhance the production. They reported that the overexpression of *fol* genes accelerates the synthesis of folic acid about 146-fold higher (6595 μg/L) than the wild type strain and concluded that GTP cyclohydrolase I is the critical step in the process. Hjortmo et al. (2008) analysed the effect of growth medium on the production of folic acid by the yeast *Saccharomyces cerevisiae.* They allowed the aerobic fermentation for yeast in the synthetic, yeast peptone dextrose and molasses based medium, and they reported the highest yield of folic acid (120 μg/g).

16.2.1.1.8 B_{12}(Cyanocobalamin)

Vitamin B_{12} belongs to a group of cobalt-containing corrinoids known as cobalamins. It is also called as antipernicious-anaemia factor. It is composed of a corrinoid ring and an upper and lower legend. The upper ligand could be adenosine, methyl, hydroxyl or cyano groups which are metabolically active. In human metabolism, vitamin B12 is responsible to facilitate the enzymatic acitivity of two enzymes ((R)-methylmalonyl-A mutase and ado-coalmine catalse) (Shibata et al. 2017). (R)-methylmalonyl-A mutase assists in the metabolism of propionyl coenzyme-A, which make compounds like valine, thiamine, methionine and fatty acids of odd-chain produced after broken down (Sharma et al. 2019). The ado-coal mine catalyses the rearrangement of propionyl CoA, it follows the cycle of citric acid.

Methionine synthesis also needs vitamin B_{12} in the form of methylcobalamin. 5-methyltetrahydrofolate used as a methyl donor; this enzyme methylates homocysteine to form methionine. Human cannot synthesize vitamin B_{12}, thus it can be obtained with the help of microorganisms (biosynthesis of vitamins by probiotic bacteria). Vitamin B_{12} prevents the development of pernicious anaemia in animals which can be synthesized by prokaryotic organisms. The production of vitamin B_{12} occurs at large scale on industrial level which occurs by microbial fermentation, predominantly utilizing *Propionibacterium shermanii or Sinorhizobium meliloti, Pseudomonas denitrificans*. Recently engineers focus their attention to *Escherichia coli* for the production of vitamin B_{12} (Watanabe and Bito 2018; Liu et al. 2020). Fungi are incapable of producing B_{12} (cobalamin). For higher Basidiomycetes fungi, the process of biosynthesis of B_{12} is not well understood and there are a very few studies on fungal production of B_{12} vitamin. Recent advances in metabolic

engineering have been exploited to efficiently modulate many microbial chemical factories to produce vitamin B_{12} using bacteria but there are no studies using fungal mycelia. So, there is need to explore the genetic tools to confer the maximum production of B_{12}, using fungal cells.

Turło et al. (2008) optimized the submerged fermentation using mycelial cultures of *Lentinula edodes* for the production of vitamin B_{12}. By modifying the culture media with cobalamin precursors (cobalt chloride) and methyl donors (betaine, methionine and choline), they optimized the process for the growth of mycelia. Under optimum condition with Co^{2+} concentration (40 g/mL), vitamin B_{12} content of *L. edodes* mycelium was found as 95 g/g of dry weight. They also reported that the yield of vitamin B_{12} in submerged mycelia was 10,000-fold higher than that of un-submerged fruiting mycelia. The role of precursors such as betaine and choline has been investigated in methionine biosynthesis pathway using *Aspergillus nidulans* (Kacprzak et al. 2003) and *Saccharomyces cerevisiae* (Csaikl and Csaikl 1986). In a study, MET1 and MET8 mutants of *Saccharomyces cerevisiae* were overexpressed and complemented by *Salmonella typhimurium* cysG for the production of B_{12} synthesis (Raux et al. 1999). They reported that the mutant MET8 was capable to complement the cobalamin cobaltochelatase mutants.

16.2.1.2 Vitamin C

Vitamin C is the six-carbon lactone of alpha-keto-L-gluconic acid and is a derivative of carbohydrate, its structure resembles to monosaccharides. Vitamin C is found in green leafy vegetables and fruits like citrus fruits but the rich sources are amla and guava (Wheeler et al. 1998). Some microorganisms like *Cryptococcus dimennae* also produce the ascorbic acid and L-ascorbic acid which can be easily synthesized by microorganisms on industrial scale which is very cheap and have good demand in the market (Hancock et al. 2000). Vitamin C as ascorbic acid is also known as an anti-scurvy vitamin (Loewus 1999) and helps to improve the immune system as it is a natural antioxidant which impediments some kind of cancers and cardiac-related disease. Another important role of vitamin C is improving the absorption of non-haem iron (Ledesma-Amaro et al. 2013).

Industrial biotechnology has a prominent role in producing vitamin C, industrially. The significant production of vitamin C with a direct microbial fermentation step, without undergoing into the intermediate (2-keto-L-gulonic acid (2-KGA)) formation, is nowadays most promising approach. So, novel biotechnological processes to convert direct glucose to vitamin C in one step are taking into consideration which are desirable. *Saccharomyces cerevisiae* offers itself as a suitable candidate in this concern. Fungi cannot produce vitamin C in their biochemical pathway, endogenously, but can produce five-carbon erythroascorbic acid (analogue for vitamin C). Several fugal genera, i.e. Zygomycetes, Basidiomycetes and Ascomycetes can produce D-erythroascorbate, and other C5 analogues of ascorbate, in their biosynthetic pathway (inversion pathway) using D-arabinose (Laudert and Hohmann 2011). In yeasts cells, due to the presence of D-arabinono-1,4-lactone oxidase

(ALO) enzyme, they are capable of producing erythroascorbic acid, and help in synthesis of L-Asc (vitamin C) (Laudert and Hohmann 2011). Sauer et al. (2004) investigated the L-Asc production from *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* cells using overexpression of *S. cerevisiae* enzymes (D-arabinose dehydrogenase and D-arabinono-1,4-lactone oxidase) and *Arabidopsis thaliana* plant enzyme (L-galactose dehydrogenase). They concluded that the overexpressing of these enzymes produced about 100 mg of L-ascorbic acid L^{-1}, by utilizing 40% of the total glucose of nutrient medium. They observed that the overexpression of the enzymes increased the vitamin C content about 3- and 15- fold for *S. cerevisiae* and *Z. bailii*, respectively. Similarly, Branduardi et al. (2007) produced vitamin C (0.4 mg L^{-1}) from D-glucose using *S. cerevisiae* cells.

16.2.2 Fat-soluble Vitamins and Their Fungal Sources

Bioactive components (secondary metabolites from fungi) are positively influencing the cells or tissues in the biological system. Fat-soluble vitamins synthesized naturally from the different fungal strains are essential for the metabolic actions of different living organisms. Figure 16.2 showing the structures of all the fat-soluble vitamins. In the field of nutrition, the bioactive components are isolated from the essential nutrients which are required for sustainable health (Davies and Ryan 2012). Even though every vitamin is produced from the natural sources, all fat-soluble vitamins are commercially produced with the chemical process, which are used in the different food and pharmaceutical industries.

In this perspective, biotechnological processes play a major role. Vitamins that are synthesized from the fungal strains have overcome all the drawbacks in comparison with the synthetic processed vitamins (except the cost of production) With this perspective, the fermentation and biotechnological processes from the different fungal strains from liquid and solid substrate are producing a highly purified substance (such as vitamins). Microbial production of all fat-soluble vitamins using various fungal and yeasts sources is shown in Table 16.1. The fermentation process is playing an important role in increasing the production of the biomass and also the levels of bioactive components such as vitamin precursors (Gregori et al. 2007). The different steps involved to synthesize the vitamins include the identification of naturally vitamin-producing strains; developing the profitable cultural conditions; optimization of the downstream process (to obtain pure product); scaling up of the production (De baets et al. 2000; Eggersdorfer et al. 1996; Shimizu 2001; Rosenberg et al. 2017). In spite of the internal limitations associated with vitamin titers produced by chemical synthesis, the white biotechnology is gaining importance to modify the fungal strains with different biotechnological processes (genetic engineering) to produce fat-soluble vitamins (A, D, E and K) (De baets et al. 2000; Eggersdorfer et al. 1996). Different techniques that are involved include the genetic engineering, synthetic and systems biology, metabolomics, fluxomics and genetic

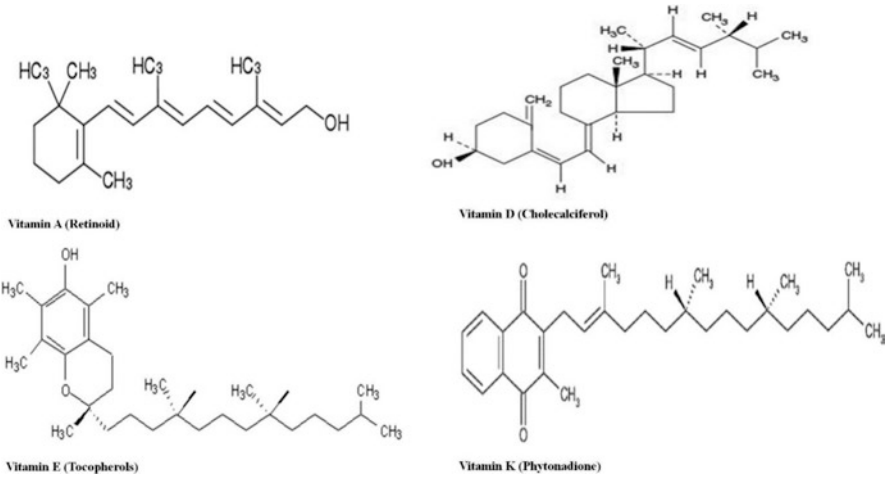


Fig. 16.2 Chemical structures of fat-soluble vitamins

engineering. The fungal strains that are used in the laboratory to produce large amounts of different kinds of vitamins are in a constructive way.

The basic notion about the vitamins from the fungal strains is that they help in biological functions, their dietary intake is discussed at the lab scale and the large scale production of vitamins is addressed. Moreover, the best results showed with the production of the vitamins with the different fungal strains (Rosenberg et al. 2017). The fat-soluble vitamins are the secondary metabolites which are produced from the enzymatic pathways than the ribosomal mechanisms. The enzymes involved in the enzymatic synthesis may be in the free form or in the complex form or these are the parts of large multifunctional polypeptides. These are involved in many of the enzymatic actions with the peptide synthetases and the polyketide synthases. Whether they are chromosomally borne, or the plasmid borne genes from the secondary metabolism are clustered usually, these are not necessarily single operons.

The clusters of different types of genes from fungus are used in the production of varieties of antibiotics such as penicillins, cephalosporins and trichothecenes (Rosenberg et al. 2017). It is used for biological activity of the compound, which performs the retinol activity, majorly as an antioxidant in the plants and animals (Zittermann et al. 2014). Vitamin D is available either in the ergocalciferol or cholecalciferol form in different types of fungal strains. Vitamin E is referred to group of chemical compounds containing four tocopherols and tocotrienols (DellaPenna and Last 2006). The fat-soluble vitamins such as vitamin K and vitamin E from the fungal strains are negligible, which is discussed in the further chapters. For the E-vitamin, alpha-tocopherol has the major activity, whereas the other toco-chromanols have the activity in the range of 0–50% with that of the alpha-tocopherol. Even, provitamin D is present in different fungal strains (shiitake mushrooms), which is converted into the vitamin D with the use of sunlight and ultraviolet radiation (Outila et al. 1999).

16.2.2.1 Vitamin A

The different forms of vitamin A are retinol, retinal and retinoic acid, and also their precursors (carotenoids) play a key role in the growth and development of human body, help in maintenance of immune system and good vision (O'Byrne and Blaner 2013). The microbial engineering process (particularly fungal strain) and their involvement improve the metabolic pathway and the cofactor generation. Usually, carotenoids (provitamin A) are the organic pigments present in the chloroplasts, chromoplasts and other fungal strains. The most commonly present carotenoids are lycopene and beta-carotene (precursor of vitamin A). The fungal carotenoids perform non-essential functions which are having stress tolerance and physiologically active by-products are synthesized (Avalos and Limon 2015).

In the process of fermentation, the different precursors such as beta-carotenes are produced from this biomass substrate. Different fungal strains contain vitamin A, some involved in the production of provitamins, but not all strains are involved in the production of the vitamin A. Many pathogenic fungal strains (*Candida* and *Cryptococcus*) are not producing the carotenoids. Carotenoid pathway is not a proper pathway in the development of the anti-fungal mechanisms to protect against the human beings (Avalos and Limon 2015; Meir and Osherov 2018). The types of fungal strains that are effectively used to produce the beta-carotene are *Blakeslea trispora* (filamentous fungi), *Pichia pastoris* and the yeasts such as *Phaffia rhodozyma*, *Torulopsis candida* and *Saccharomyces cerevisiae* (Verwaal et al. 2007; Araya-Garay et al. 2012). In the recent times, several metabolic engineering approaches are developed to produce different carotenoids from the fungal strains (Ye and Bhatia 2012).

The biosynthesis of the beta-carotene usually includes three relatively independent modules that include

- Glycolysis
- Precursor synthesis
- Beta-carotene synthesis

From several decades the researchers had been conducting an in-depth study on the synthesis of the beta-carotene (Christaki et al. 2013; Chen et al. 2017). Methylerythritol phosphate pathway is playing a key role in proving the precursor substances to synthesis of the beta-carotene in the different microbial strains, particularly in the bacterial strains. Along with this the galactose regulatory networking system is mostly characterized by transcriptional inducing systems. These systems are involved in repression and induction of the transcriptional induction systems which are regulated by the glucose and galactose. The GAL1 and also GAL 10 are galactokinase genes, can be induced in the galactose regulatory network at least of 1000-fold in the strains of yeast (*Saccharomyces cerevisiae*). The expression of the carotenogenic genes such as ERG19, ERG12 and ERG 20 (diphosphomevalonate decarboxylase, mevalonate kinase and FPP synthase) simultaneously supplies the palmitoleic acid, oleic acid in the recombinant yeast strains (particularly *Saccharomyces cerevisiae*).

By using bidirectional promoters GAL10-GAL1 in rate limiting step mevalonate pathway from farnesyl pyrophosphate to geranylgeranyl pyrophosphate, 7.41 mg of dry cell weight of beta-carotene was achieved in the strains of yeast (Xie et al. 2014). For this, there is a free dynamic control strategy over the repressor/inducer mechanisms. These are automatically switched in response to concentration of glucose in the media, which helped in production of beta-carotene in the growth environment. With the successive control over the competitive upstream and downstream pathways of farnesyl pyrophosphate in the yeast strain (*Saccharomyces cerevisiae*), the carotenoids production was about 20.79 mg with the process of fermentation (high cell density) (Xie et al. 2015). Furthermore, the productivity of the vitamin precursor always depends on the metabolic pathways including the activity, stability and specificity of the enzyme. This results in the low conversion or high conversion of the by-products. In this biotechnological process the process of protein engineering increases the specific properties such as specificity in the substrates. The directional degeneration and also the rational design are changed with the protein engineering (Damborsky and Brezovsky 2014; Zanghellini 2014; Jeschek et al. 2016) The energy supply regulation is playing a key role in the synthesis of beta-carotene (provitamin A). In the terpenoid compounds production, ATP and NADPH are two major factors, which are playing a key role in the synthesis of terpenoid compounds.

In the production of the terpenoid compounds, ATP and NADPH are the two significant cofactors. The central metabolic modulus of some strains of the microbes improves the production of beta-carotene (Zhao et al. 2013). In addition to this, the controlled nutrition and physiological stresses helped in the production of different types of carotenoids. Commercial demand for different carotenoids has attracted attention on the development of the appropriate biotechnological strategies, including the use of liquid waste substrates as nitrogen and carbon as sources. Yeast strains have important growth capabilities which were used to produce high levels of carotenoids with different agricultural and industrial wastes (Mata-Gómez et al. 2014). With the utilization of the waste from the poultries, pink yeast strains (*Rhodotorula glutinis*) effectively used as a novel substrate in the protection of several carotenoids, concentration of the carotenoids that are produced about 92 mg/L (Taskin et al. 2011). From the literature carotenoids particularly, beta-carotenes are produced from the fungus *Blakeslea Trispora* (Papaioannou and Liakopoulou-Kyriakides 2010). The fungus *Phycomyces Blakesleeanus* is responsible for the production of beta-carotene at industrial level (Almeida and Cerda-Olmedo 2008). Cerda-Olmedo (2001) reported that the production of carotenoid was more in the *Phycomyces species* (without agitation) in comparison with the *Blakeslea Trispora* strains. Some different authors are also described the production of the carotenoids by the *Rhodotorula* species. According to the researchers Tinoi et al. (2005) and Perrier and Dubreucq (1995) the yeast strains are distributed widely in nature and they are synthesizing some specific carotenoids such as beta-carotene, torulene and also torularhodin in various proportions. The recombinant *Saccharomyces Species* have potential to produce the beta-carotene at the rate of 6.29 mg/g (Yan et al. 2012).

16.2.2.2 Vitamin D

Plant kingdom produces wide range of sterols, but fungal strains produce sterols such as cholesterol (24-desmethylsterol) and also ergosterol (24-demethylsterol) (Hartmann 2004). The major sterol from the fungal strain is ergosterol which is synthesized through lanosterol. The types of enzymes involved in this process of ergosterol pathway in the *Saccharomyces cerevisiae* are identified. This yeast strain is used as a model in the ergosterols production. Ergosterol and cholesterol share the pathway until zymosterol formed in the pathway (Lees et al. 1995). Vitamin D is produced in fungi and yeast with the exposure of ultraviolet rays (UV-B) on the provitamin of the ergocalciferol. Small amounts of ergocalciferol are found in the plants contaminated with the fungal strains. Ergocalciferol is produced with the use of different fungal strains by the UV-B rays exposure producing ergosterol, whereas cholecalciferol is produced on the surface of the skin 7-dehydrocholestrol form.

Both the ergosterol and 7-dehydrocholestrol are the provitamins of vitamin D. With the UV-irradiation, ergocalciferol is formed into the ergosterol with different types of the fungal strains. Cholecalciferol is formed by the process of chemical conversion or irradiation of lanolin with the 7-dehydrocholesterol. The ergocalciferol utilization (in the edible fungal strains) increased the serum concentrations of 25-hydroxyvitamin D effectively. Different strains of yeast are used in producing ergosterol. Biosynthetic pathway of Vitamin D takes place by involvement of the methyl group at the C-24 position in sterols structure (Bach and Benveniste 1997). Lees et al. (1995) elucidated the production pathway of ergosterol and they identified that the cholesterol and ergosterol share the pathway till the zymosterol. The alkylation process of *S. cerevisiae* converted the zymosterol to fecosterol, in this process the side chain is catalysed by the S-adenosyl methionine sterol methyltransferase (ERG6) (Bach and Benveniste 1997).

The selection of the high yield ergosterol yeast species is the foremost important step in the production of ergocalciferol (Yuan et al. 2019). The main methods involved in preparation of the active cholecalciferol are divided into two kinds including the following:

- Thermalisation research method
- P_{450} enzymes in the microbial reforming process.

Ergosterol is mainly present in the plasma membranes of the different fungal strains. It is about 0.03–4.6 %. Particularly in the *Saccharomyces cerevisiae* it was about 7–10%. Pre-vitamin ergocalciferol is synthesized by the light singlet ring opening reactions of ergosterol. This is the photochemical reaction. HMG-CoA reductase is a speed limiting enzyme in the metabolic synthesis of ergosterols, which controls the synthesis of ergosterol. The interruption in feedback inhibition route improves the ergosterols production. The transcriptional profiling study of the *Penicillium digitatum* about the citrus green mold on the citrus fruits reveals some facts about vitamin D synthesis. The reactions with the citral usually inhibit the biosynthesis pathway of the ergosterols (Ouyang et al. 2011). There are no reports about the screening of ergosterols with high yields strains with metabolic

regulations (Yuan et al. 2019). The accumulated ergosterols are converted into the ergosta-5,7-dien3 β-ol with the metabolic engineering in the *Saccharomyces cerevisiae*. By removing the gene erg5, the over expression of the transcriptional factors such as upc2-1 and other rate limiting enzymes, i.e. Erg1p, Hmg1p and Erg 11p used in co-induction of post-squalene pathway (Ma et al. 2018). Ergosterol production by the different types of yeast strains, *Saccharomyces cerevisiae* fermentation is always dependent on the biomass of the yeast cells and the presence of the ergosterols in that cells.

Two preferred metabolic conditions such as oxidative fermentative growth on glucose and oxidative growth on ethanol are reported by a study. They reported the total ergosterol production was increased upto 86% with purity of 2.43%. The total ergosterols efficiency was about 103.84×10^{-6} g/L/h (Nahlik et al. 2017). Researchers are focused on the different aspects of restriction factors to solve the biological preparation of active cholecalciferol. For the production of cholecalciferol, the recombinant engineering technology was used to express the gene (for cholecalciferol) to solve the instability and low expression activity of vitamin D. In the vitamin D production, the different biotechnological processes (genetic engineering) are used in processing of the bacterial species rather than the fungal species.

By using different bacterial species (*Streptomyces Griseolus* and *Pseudonocardia Autotrophica*) and the successful application of P_{450} enzymatic reactions, the vitamin cholecalciferol is changed into 1α,25-dihydroxy cholecalciferol. With site directed mutagenesis of CYP105A1 (*Streptomyces griseolus*) and direct evolution of the CYP107 (*Pseudonocardia autotrophica*) the cholecalciferol hydroxylase activity was increased dramatically (Sakaki et al. 2011). *Pseudonocardia autotrophica* (actinomycete) is capable to convert the cholecalciferol vitamin into 1α,25-dihydroxy cholecalciferol (physiologically active vitamin D) (Fujii et al. 2009). It is important to select the optimum electron transport chains (set of electrons) to co-express with the hydroxylase simultaneously to solve the coenzyme NADH (NADPH) problem by the fusion protein construction (Imoto et al. 2011). Moreover, the cell catalytic system is used to co-express the coenzyme regeneration system which is very efficient in the bio-catalytic synthesis of 25-hydroxy cholecalciferol. This is key intermediate for the variety of hydroxylated vitamin D_3 derivatives that solves the problems in the coenzymes supply in reaction systems. The restriction factors of catalytic characters of the different enzymes and the existence of the fungal reforming process in the different recombinant strains improve the cholecalciferol production (Wang et al. 2019). The yeast species such as *Kluyveromyces*, *Saccharomyces cerevisiae*, *Torulaspora Delbrueckii* can produce significant amounts of vitamin D (Jäpelt and Jakobsen 2013).

16.2.2.3 Vitamin E

Tocopherols are present in some edible fungal strains. Some microalgal strains (green microalgae) are renewable natural sources of vitamin E (Sánchez 2017). Tocopherol content was increased with biotechnological processes in the higher

plants. A very little modification was done in the microalgae genes to increase the tocopherols accumulation (Yuan et al. 2019). Limited number of the tocopherols are present in the different fungal strains (Heleno et al. 2016). In different fungal hyphae mainly mushrooms (*Pleurotus ostreatus*, *Coprinus comatus*, *Agaricus bisporus* and *Mucor circinelloides*) have a little amount of tocopherols are present (Vamanu 2014). The presence of tocopherols in the different mushroom strains (Heleno et al. 2010) has shown in Table 16.1.

Tocopherols availability is lesser in the fungal strains as compared with the algal strains. In the dried edible mushrooms *Pleurotus ostreatus*, the large quantities of tocopherols were identified and extracted with fluidized bed extraction. Similar tocopherols are found in the mycelia of these species. The alpha tocopherols content in the plant oils is very low in comparison with algal species. In the algal tocopherols, 97% of the toco-chromanols are present which are having high bioactivity. The eukaryotic microalgae producing the vitamin E are *Dunaliella tertiolecta*, *Euglena gracilis*, *Diacronema vlkianum*, *Nannochloropsis oculate, Tetraselmis suecica* and *Isochrysis galbana*.

The production levels of tocopherols in the different algal strains depend upon the cultivation conditions, temperature and other factors which were discussed in the coming chapters (Mokrosnop and Zolotareva 2014). Major studies showed that the recognizable amounts of tocopherols are present, from the different types of fungal strains (edible and wild mushrooms), not at the industrial level, whereas in the algal species the amount of tocopherol content is more. *Euglena Gracilis* is the best producer of the tocopherols (7.35 mg/g of dry cell weight). A study clearly showed that the genetic and metabolic engineering from the *Synechocystis species* showed the fivefold increase in the tocopherol content (Qi et al. 2005). This species is very well purposefully used and organized in the biotechnological process to produce the tocopherols content.

16.2.2.4 Vitamin K

As we know that vitamin K is a fat-soluble vitamin and occurs in three forms such as vitamin K_1(phylloquinone), vitamin K_2 (menaquinone) and vitamin K_3 (menadione) (Shearer and Newman 2008).Different microbes have been cultured to generate this vitamin in food products, for example, *Propionibacterium* has been used for preparation of cheese with high content of this fat-soluble vitamin (Furuichi et al. 2007). Menoquinone is produced by a potent producer (*Flavobacterium* sp.) for commercial use.

After the comparison of the distribution of the isoprenoid units such as quinones amongst the bacterial and the fungal strains, it is clearly known that the bacteria are the major producers of the menaquinone (Tani et al. 1984). MK-7 strain is produced from the different bacterial strains, which is in contrast to the vitamin K with the fermentation process, which includes the solid state and liquid state fermentation. In the solid state fermentation, the water content is about 12–80% (Geleijnse et al. 2004; Ebrahiminezhad et al. 2016; Berenjian et al. 2013). The MK-7 production

process is a very costly process which is producing very low productivities (Berenjian et al. 2015). So that the enhancement of the MK-7 productivity has been a major issue.

Menaquinone is a prenylated product from the phylloquinone or menadione, it is produced through the *Homo sapiens* UBIAD1 gene expression (HsUBIAD1). This form of vitamin K produces many physiological and pharmacological functions. From the studies by Sun et al. (2019) on the methylotrophic yeast cells (*Pichia pastoris*) the attractive expression system has been successfully applied to the efficient expression of heterologous proteins (Sun et al. 2019). However, the biosynthetic pathway for the menaquinone has not discovered in the *Pichia Pastoris*. They constructed the innovative synthetic pathway in *Pichia Pastoris* for production of menaquinone-4 through the heterogenous expression of the HsUBIAD1. The constitutive promoter, glyceraldehyde 3-phosphate dehydrogenase is the most suitable for the expression of HsUBIAD1 for the various reasons. When compared with the initial conditions, the yield was increased by 4.37 times (Sun et al. 2019).

16.3 Factors Affecting Fungal Production of Vitamins

As we know that the vitamins are produced by the three ways of production by the chemical synthesis, fermentation process and by genetic engineering processes. Advantages of vitamins from different fungal strains include that the growth is very fast, does not depend on the seasons, these can be scaled up easily and these are not competitors for the human food requirements. It is very clear that the fat-soluble vitamins are required for both food and medicine (nutraceutical) so it requires an effective strategy for the process of the industrialization. To meet the medical requirements of the vitamins, they are synthesized from the different varieties of fungal strains artificially. With the organic synthesis of vitamins, non-renewable chemicals are used which produces hazardous waste materials. Besides sustainability concerns, from the last few years in the development of the bioprocess the economics is the majorly driving force. The factors which are influencing the production of provitamins or vitamins from the fungal strains are majorly:

- Environmental issues
- Regulatory issues
- Technical issues

16.3.1 Environmental Factors

Firstly, the most reliable factors such as physical, chemical and biotic factors with their interactions with each other are described in the changing of the environment. These changes lead to change in the environment (in both the internal and the

external factors). Different types of fungal strains accumulate carotenoids as a part of their response to different environmental stresses. Different types of cultural and environmental stimulants are used in achieving improvement in the carotenoid production. These cultural and environmental strains positively affect the content of carotenoids in the different microbial strains (Bhosale 2004).

16.3.1.1 Light and Temperature

In the development and growth of the yeast and the fungal strains light and temperature are playing an important role. The provitamins are produced in response with the white light irradiation in strains of fungus. It has a positive influence on the fungal strain. These two factors are the major factors changing the biosynthetic pathways, including vitamin biosynthesis (carotene biosynthesis). The concentration of the different enzymes changes with the temperature change during carotenoid synthesis (Hayman et al. 1974). The light intensity and temperature change in the different fungal strains, which changes the provitamin synthesis. In the production of the provitamin A, the improvement in the volumetric production of the provitamin A (carotenoids) is usually associated with the direct improvement of the growth of the fungal strain. In the carotenoids production, the white light illumination is playing an important role. The different enzymatic actions are always involved in the processing of the carotenoids in the different types of cells. This increases the carotenoid biosynthesis (Ausich 1997). Carotenoids production is significant in the aerobic mycelium, whereas they are almost undetectable in the yeast and anaerobic mycelium (Bhosale 2004). The provitamins content is always directly proportional to the amount of light reached to mycelium (Bhosale 2004; Bhosale and Gadre 2002).

16.3.1.2 Chemical Compounds (Metal Ions, Salts, Solvents)

Different types of chemical compounds including metal ions, salts, terpenes, ionones, amines, alkaloids and also antibiotics effects involve in the different provitamin production (Govind et al. 1982). Cations (potassium and magnesium) consist of bulk intracellular species. The other ions such as calcium, zinc and different transition elements are required in the production of carotenoids (Bhosale 2004). Chemical stimulators such as methylheptenone, pyridine and also imidazole are involved in stimulating the lycopene in the different fungal strains such as *Blakeslea trispora* and *Phycomyces blakesleeanus* (Bhosale 2004). The addition of different solvents such as ethanol, methanol and ethylene glycol in the growth media improved the growth of the provitamin synthesis. Supplementation of the ethanol improved the synthesis of beta-carotene in the yeast strains (*Rhodotorula glutinis*) (Margalith and Meydav 1968).

16.3.2 Regulatory Issues

It is one of the major issues which is associated with production of vitamins from different fungal strains. These laws and regulations vary with country to country. There are two types of European Union regulations involved in the production of vitamins from different fungal strains. Different food and the feed additives that are produced with the different natural hosts (genetically modified organisms) in the closed systems do not come in the regulations as discussed below (Paracchini et al. 2017).

1. The Food Safety Regulation (EC 178/2002) (excluding medicinal products)
2. The Novel Food Regulation (EC 258/97)

In 'The Food Safety Regulation', the commercialization of different types of foods and their ingredients are identified and their safety aspects are assured to make feasible to human consumption. In this perspective, the food and their ingredients should consider in the conventional form of food. The characteristics and the composition should come under the conventional food (Torregrosa-Crespo et al. 2018). In major cases, the vitamins that are synthesized from the fungal strains and from the chemical synthesis are identical. The presence of impurities affects the vitamin production. For example, aniline is an un-intentional impurity during chemical production of riboflavin that is not produced during fermentation process (Zu Berstenhorst et al. 2009).

In the chemically produced additives, it is very difficult to find out the presence of DNA, even it could be an issue in the different fermented food products. The EFSA (European Food Safety Authority) stated that it was mandatory for the food products or feed products which does not contain genetically modified materials, the final products should be placed in non-genetically modified foods or feed additives (Paracchini et al. 2017; EFSA 2011). Different regulatory steps are required to take on the replacement of chemical synthesis with microbial synthesis, the fungal strains used to produce different types of vitamins, stability of the product and the specific impurities presence in the product. The innovative product which is produced should be safe at a particular point, which is generally accepted through the toxicity studies and proves that this product is very closer to the previously approved product. For example, the European pharmacopoeia in the European Union is the principle organization to certify the safety of a food product before launching in the market. In the USA the standard practice is that the FDA should notify the ingredient is generally recognized as safe (GRAS). For this the example is that riboflavin biosynthesis by the fungal strain (*Eremothecium ashbyii*) is designated as GRAS (Belenky et al. 2011).

16.3.3 Technical Factors

The major challenges related to vitamin production are the technical applicability, high production cost, uneasy extraction & purification of the vitamins from the different fungal strains, selection of the fungal strains, and the lack of different types

of tools for the development of different hosts. The intermediates which are produced during the fermentation process are sometimes poisonous. In the niacin production, the fermentation process is the major technical issue, the production cost is very low for the niacin with the chemical synthesis. Similarly, in the folic acid production the cost of chemical synthesis is very low in comparison with the fermentation process (Chand and Savitri 2016).

The innovative possibilities in developing the fungal strains through the mutagenesis strategies are depending on the knowledge on the metabolic systems, enhancement of the biosynthetic pathways, by the inhibition of the pathways with the metabolic engineering or by the stain adaption to improve the cell tolerance to environment. The detailed process of biosynthesis pathways was used by the different fungal strains to produce different vitamins and other health compounds. The different fungal strains involved in the different health compounds production are yeasts (*Saccharomyces*, *Candida*, *Xanthophyllomyces*, *Yarrowia*), fungi (*Blakeslea*, *Ashbya*, *Mortierella*, *Mucor*, *Monascus*) (Laudert and Hohmann 2011; Borowitzka 2013; Ledesma-Amaro et al. 2013; Vandamme and Revuelta 2016). The different types of pathways that are involved in the metabolic regulations are complex in nature. It is difficult to deregulate the process and produce the desired components. Some of the vitamins, biological pigments (vitamins, minerals and other components) through the fungal strains to reach industrial level are still a challenge (Revuelta et al. 2016).

16.4 Industrial Food Applications of Fungal Vitamins

Today the modern society is facing a big issue with the avitaminoses, which was very well known in the western world. It occurs still in overpopulated, famine struck regions in the worlds and also large population countries in the world. Apart from this the essential growth factors, coenzymes for different plants and microorganisms, vitamins and also their relative components are improving the health related aids. Now in different varieties of processed foods, cosmetics and also pharmaceutical companies contain different types of vitamins and their related compounds (provitamins) (Revuelta et al. 2016; Devi et al. 2020). In the development of the efficient bioprocesses, the genetic tools must be involved (fungal/bacterial strains should be ready for that process) (Nielsen and Keasling 2016; Calero and Nikel 2018).

In the different food supply areas and in cosmetic areas, the consumers demand towards the natural pigments is very high. The advantages over the vitamin biosynthesis with the various fungal strains are that the properties remain unchanged and improved with the biosynthetic pathway. The advancements in the genetic engineering and biotechnological aspects are increasing every day. The rapidity in the process of genetic engineering and in biotechnological aspects of the microbes are increasing, this helps to produce innovative bioactive components. The use of the vitamins which are produced from the different fungal strains is having many benefits which includes the environmental friendly and there are no side effects during

the processing of the vitamins. Nutritional importance of different health related components are having more technical applications, for example, antioxidants (C5-epimer of citric acid, glutathione or GSH, tocopherols and carotenoids), acidulants such as (ascorbic acid or vitamin C) and biopigments (carotenoids in different colours, yellow coloured riboflavin and also the monascus pigments).

These are used in the different food, feed, different pharmaceutical and also in the nutraceutical industries. Some natural pigments are used in replacement of synthetic pigments and colourants. The fungal carotenoids used in the different colourants production are *Blakeslea Trispora* producing beta-carotene and *Xanthophyllomyces dendrorhous* produces astaxanthin. The algal carotenoids which are produced from the algal species are *Dunaliella salina* that produces xanthophylls and lycopenes. Presently, a few vitamins are produced with the chemical synthesis while the others are produced by the extraction process. This process is paying high cost for the disposal of waste. So, natural production with different fungal strains is playing a key role in the vitamins production. In the consumers also the consciousness about the food safety aspects on the different additives is increasing day-by-day. This improved the interest on the substitution of these processes (chemical synthesis and extraction process) with the different biotechnological processes. Consequently, some of these biotechnological processes for the different bioactive components production including vitamins and antioxidants are competing with the existing chemical processes.

16.5 Concluding Remarks and Future Aspects

The fat-soluble vitamins which are produced from the fungal strains are used in the different types of food, feed and also in pharmaceutical industries. For the chemical production of the bioactive substances such as vitamins, the cost of production is high and it has the problems with the waste disposal. This led to increase in the interest with the substitution towards the different biotechnological processes. Moreover, the vitamins extraction from the different fungal strains is helpful to extract the purest forms of the strains with the process of genetic engineering (biotechnological processes). In comparison with the chemical synthesis, the production of the vitamins from the fungal strains has these following advantages.

- Vitamins produced from the fungal strains are natural.
- Consumers recognize these vitamins very positively.
- The relevance in the bio-electron stereoisomers produced is very specific.
- The vitamins produced from the microbial strains are cheaper in cost.

However, the fungal strain production of the vitamin synthesis is not clear. Some of the plant systems are having the greatest potential in the biosynthesis of vitamins. The production of vitamins from the different fungal strains is increasing day-by-day due to its advantages and the progress made till today. Yet, some of the vitamins that are produced by this biosynthetic pathway have not been confirmed, for

example, phylloquinone. It is very clear that these are considered to be the weakest steroids in the terpenoid quinones. Some of vitamins that are produced from the different fungal strains are under the process of purification and commercialization. In the past decade with the protein and the metabolic engineering, the high yields of menaquinone and the vitamin A are obtained. The research in future is relied on the metabolic pathways (biosynthetic pathways) of fat-soluble vitamins by the industrial production. At present, the synthetic biological processes should focus on the comprehensive analysis of biological molecules, and also how these are generating the complex biological processes should be studied.

The synthetic biological process is involving more functions to construct different cell factories. With the use of knowledge of the plants it is easy to develop the different fungal strains to produce different vitamins. Several strategies had been developed to improve the secondary metabolites, which includes the selection of the high yield fungal strain, optimization of the environmental conditions and biotechnological processes and also genetic engineering. Vitamins are produced sustainably and economically with different combined modern engineering techniques. The use of advances in different laboratory techniques such as non-toxic fungal strains development, knowledge on biotechnological processes (advancements in the genetic engineering processes), biosynthesis regulation at the molecular level is allowed to achieve the safe food-grade materials at the higher levels. Besides, to this replacement of the chemical processes with the microbial process is encouraged to increase awareness about clean and labelled ingredients, increase in the costs for the waste disposal and also the shift towards the renewable substances to fulfill requirements of the sustainability.

References

Abbas CA, Sibirny AA (2011) Genetic control of biosynthesis and transport of riboflavin and flavin nucleotides and construction of robust biotechnological producers. Microbiol Mol Biol Rev 75:321–360

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Biodiversity and ecological perspective, vol 1. Springer, Cham

Abramova IM, Soloviev AO, Turshatov MV, Krivchenko VA, Kononenko VV (2020) Protein feedstuff production based on microbial biomass. In IOP conference series: earth and environmental science 548(8):082080. IOP Publishing

Acevedo-Rocha CG, Gronenberg LS, Mack M, Commichau FM, Genee HJ (2019) Microbial cell factories for the sustainable manufacturing of B vitamins. Curr Opin Biotech 56:18–29

Almeida ERA, Cerda-Olmedo E (2008) Gene expression in the regulation of carotene biosynthesis in phycomyces. Curr Genet 53:129–137

Araya-Garay JM, Feijoo-Siota L, Rosa-Dos-Santos F, Veiga-Crespo P, Villa TG (2012) Construction of new *Pichia pastoris* X-33 strains for production of lycopene and beta-carotene. Appl Microbiol Biotechnol 93:2483–2492

Argoudelis CJ (1999) Identification of the vitamers of vitamin B6 excreted by a yeast mutant growing in a glucose minimal culture medium. J Chromatogr B Biomed Appl 721(1):21–29

Ausich RL (1997) Commercial opportunities for carotenoid production by biotechnology. Pure Appl Chem 69:2169–2173

Avalos J, Limon MC (2015) Biological roles of fungal carotenoids. Curr Genet 61:309–324
Bach TJ, Benveniste P (1997) Cloning of cDNAs or genes encoding enzymes of sterol biosynthesis from plants and other eukaryotes: heterologous expression and complementation analysis of mutations for functional characterization. Prog Lipid Res 36:197–226
Belenky P, Stebbins R, Bogan KL, Evans CR, Brenner C (2011) Nrt1 and Tna1-independent export of NAD^+ precursor vitamins promotes NAD^+ homeostasis and allows engineering of vitamin production. PLoS One 6(5):e19710
Berenjian A, Chan NL, Mahanama R, Talbot A, Regtop H, Kavanagh J, Dehghani F (2013) Effect of biofilm formation by *Bacillus subtilis* natto on menaquinone-7 biosynthesis. Mol Biotechnol 54:371–378
Berenjian A, Mahanama R, Kavanagh J, Dehghani F (2015) Vitamin K series: current status and future prospects. Crit Rev Biotechnol 35:199–208
Bhosale P (2004) Environmental and cultural stimulants in the production of carotenoids from microorganisms. Appl Microbiol Biotechnol 63:351–361
Bhosale P, Gadre RV (2002) Manipulation of temperature and illumination conditions for enhanced β-carotene production by mutant 32 of *Rhodotorula glutinis*. Lett Appl Microbiol 34:349–353
Bigelis R (1989) Industrial products of biotechnology: application of gene technology. In: Rehm HJ, Reed G (eds) . Biotechnology, VCH, Weinheim, p 243
Borowitzka MA (2013) High value products from microalgae -their development and commercialization. J Appl Phycol 25:743–756
Bourdichon F, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP et al (2012) Food fermentations: microorganisms with technological beneficial use. Int J Food Microbiol 154(3):87–97
Branduardi P, Fossati T, Sauer M, Pagani R, Mattanovich D, Porro D (2007) Biosynthesis of vitamin C by yeast leads to increased stress resistance. PLoS One 2(10):1092
Calero P, Nikel PI (2018) Chasing bacterial chassis for metabolic engineering: a perspective review from classical to non-traditional microorganisms. Micro Biotechnol 12:1–27
Cerda-Olmedo E (2001) Phycomyces and the biology of light and color. FEMS Microbiol Rev 25:503–312
Chand T, Savitri B (2016) Vitamin B3, niacin. Industrial biotechnology of vitamins, biopigments, and antioxidants. Wiley-VCH Verlag GmbH & Co KGaA, pp 41–65
Chen CY, Kao AL, Tsai ZC, Shen YM, Kao PH, Ng IS, Chang JS (2017) Expression of synthetic phytoene synthase gene to enhance beta-carotene production in *Scenedesmus* sp CPC2. Biotechnol J 12:1–7
Cheng X, Zhou J, Huang L, Li K-T (2011) Improved riboflavin production by *Eremothecium ashbyii* using glucose and yeast extract. African J Biotech 10:15777–15782
Christaki E, Bonos E, Giannenas I, Florou-Paneri P (2013) Functional properties of carotenoids originating from algae. J Sci Food Agric 93:5–11
Chumnantana R, Hirose K, Baba H, Yagi T (2001) Production of pyridoxal phosphate by a mutant strain of *Schizosaccharomyces pombe*. Biosc Biotech Biochem 65:1789–1795
Csaikl U, Csaikl F (1986) Molecular cloning and characterization of the MET6 gene of *Saccharomyces cerevisiae*. Gene 46:207–214
Damborsky J, Brezovsky J (2014) Computational tools for designing and engineering enzymes. Curr Opin Chem Biol 19:8–16
Davies J, Ryan KS (2012) Introducing the parvome: bioactive compounds in the microbial world. ACS Chem Biol 7:252–259
De baets S, Vandedrinck S, Ej V (2000) Vitamins and related biofactors, microbial production. In: Encyclopedia of microbiology, vol 4. Academic Press, New York, pp 837–853
DellaPenna D, Last RL (2006) Progress in the dissection and manipulation of plant vitamin E biosynthesis. Physiol Plant 126:356–368
Demain A (1972) Riboflavin biosynthesis. Annual Rev Microbiol 26:369–388
Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N

(eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Dmytruk KV, Yatsyshyn VY, Sybirna NO, Fedorovych DV, Sibirny AA (2011) Metabolic engineering and classic selection of the yeast *Candida famata* (*Candida flareri*) for construction of strains with enhanced riboflavin production. Metabolic Eng 13:82–88

Dmytruk K, Lyzak O, Yatsyshyn V, Kluz M, Sibirny V, Puchalski C, Sibirny A (2014) Construction and fed-batch cultivation of Candida famata with enhanced riboflavin production. Journal of Biotechnology 172:11–17

Donovan PD, Holland LM, Lombardi L, Coughlan AY, Higgins DG, Wolfe KH, Butler G (2018) TPP riboswitch-dependent regulation of an ancient thiamin transporter in *Candida*. Plos Genet 14:1007429

Ebrahiminezhad A, Varma V, Yang S, Berenjian A (2016) Magnetic immobilization of *Bacillus subtilis* natto cells for menaquinone-7 fermentation. Appl Microbiol Biotechnol 100:173–180

EFSA (2011) Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA J 9(6):2193

Eggersdorfer M, Adam G, John MWH, Labler L (1996) Vitamins: biotechnology, vol 4. Weinheim, VCH, pp 114–158

El-Refai HA, El-Helow EL, Amin MA, Sallam LA, Salem H-AA (2010) Application of multifactorial experimental designs for optimization of biotin production by a *Rhizopus nigricans* strain. J Am Sci 6:179–187

Fischer M, Bacher A (2005) Biosynthesis of flavocoenzymes. Nat Prod Rep 22:324–350

Fujii Y, Kabumoto H, Nishimura K, Fujii T, Yanai S, Takeda K, Tamura N, Arisawa A, Tamura T (2009) Purification, characterization, and directed evolution study of a vitamin D3 hydroxylase from *Pseudonocardia autotrophica*. Biochem Biophys Res Commun 385:170–175

Furuichi K, Katakura Y, Ninomiya K, Shioya S (2007) Enhancement of 1,4-dihydroxy2-naphthoic acid production by *Propionibacterium freudenreichii* ET-3 fed-batch culture. Applied Env Microbiol 73:3137–3143

Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MHJ, Van Der Meer IM, Hof man A, JCM W (2004) Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam study. J Nutri 134:3100–3105

Gominak SC (2016) Vitamin D deficiency changes the intestinal microbiome reducing B vitamin production in the gut. The resulting lack of pantothenic acid adversely affects the immune system, producing a "pro-inflammatory" state associated with atherosclerosis and autoimmunity. Med Hypotheses 94:103–107

Gonzalez-Lopez J, Aliaga L, Gonzalez-Martinez A, Martinez-Toledo MV (2016) Pantothenic acid. In: Vandamme EJ, JLR (eds) Industrial biotechnology of vitamins, biopigments, and antioxidants. Wiley-VCH Verlag GmbH & Co., KGaA, Weinheim

Govind NS, Amin AR, Modi VV (1982) Stimulation of carotenogenesis in *Blakeslea trispora* by cupric ions. Phytochemistry 21:1043–1044

Gregori A, Švagelf M, Pohleven J (2007) Cultivation techniques and medicinal properties of *Pleurotus spp*. Food Tech Biotech 3:238–249

Hancock RD, Galpin JR, Viola R (2000) Biosynthesis of L-ascorbic acid (vitamin C) by *Saccharomyces cerevisiae*. FEMS Microbiol Lett 186:245–250

Hartmann MA (2004) Sterol metabolism and functions in higher plants. Lipid Metab Membr Biogenesis 6:57–81

Hayman EP, Yokoyama H, Chichester CO, Simpson KL (1974) Carotenoid biosynthesis in *Rhodotorula glutinis*. J Bacteriol 120:1339–1343

Heefner DL, Weaver CA, Yarus MJ, Burdzinski LA (1992) Method for producing riboflavin with *Candida famata* U.S. patent 5 164: 303

Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira ICFR (2010) Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. Food Chem 119:1443–1450

Heleno SA, Diz P, Prieto MA, Barros L, Rodrigues A, Barreiro MF, Ferreira ICFR (2016) Optimization of ultrasound-assisted extraction to obtain mycosterols from *Agaricus bisporus L.* by response surface methodology and comparison with conventional soxhlet extraction. Food Chem 197:1054–1063

Herrero S, Daub ME (2007) Genetic manipulation of Vitamin B-6 biosynthesis in tobacco and fungi uncovers limitations to up-regulation of the pathway. Plant Sci 172:609–620

Hjortmo S, Patring J, Jastrebova J, Andlid T (2005) Inherent biodiversity of folate content and composition in yeasts. Trends Food Sc Tech 16:311–316. https://research.tdameritrade.com/grid/public/markets/news/story.asp?fromPage=results&docKey=100-203p8765

Hjortmo S, Patring J, Andlid T (2008) Growth rate and medium composition strongly affect folate content in *Saccharomyces cerevisiae*. Int J Food Microbiol 123:93–100

Hüttner S, Nair R, Johansson A, Teixeira P, Achterberg P (2020) Recent advances in the intellectual property landscape of filamentous fungi. Fungal Biol Biotechnol 7:1–17

Imoto N, Nishioka T, Tamura T (2011) Permeabilization induced by lipid II-targeting lantibiotic nisin and its effect on the bioconversion of vitamin D3 to 25-hydroxyvitamin D3 by *Rhodococcus erythropolis*. Biochem Biophys Res Commun 405:393–398

Jäpelt RB, Jakobsen J (2013) Vitamin D in plants: a review of occurrence, analysis, and biosynthesis. Front in Plant Sci 4:1–20

Jeschek M, Gerngross D, Panke S (2016) Rationally reduced libraries for combinatorial pathway optimization minimizing experimental effort. Nat Commun 7:1–10

Jiménez A, Santos MA, Revuelta JL (2008) Phosphoribosyl pyrophosphate synthetase activity affects growth and riboflavin production in *Ashbya gossypii*. BMC Biotechnol 8:67–78

Kacprzak MM, Lewandowska I, Matthews RG, Paszewski A (2003) Transcriptional regulation of methionine synthase by homocysteine and choline in *Aspergillus nidulans*. Biochem J 376:517–524

Kalingan AE, Krishnan MRV (1997) Agro industrial by-products as flavinogenic stimulators for riboflavin production. Bioprocess Eng 17:87–91

Kato T, Park EY (2012) Riboflavin production by *Ashbya gossypii*. Biotechnol Lett 34:611–618

Kojima I, Yoshikawa H, Okazaki M, Terui G (1972) Studies on riboflavin production by *Eremothecium ashbyii* (I): on inhibiting factors of riboflavin production and their control. Ferment Technol 50:716–723

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Ksheminska H, Jaglarz A, Fedorovych D, Babyak L, Yanovych D, Kaszycki P et al (2003) Bioremediation of chromium by the yeast *Pichia guilliermondii*: toxicity and accumulation of Cr (III) and Cr (VI) and the influence of riboflavin on Cr tolerance. Microbiol Res 158(1):59–67

Kubodera T, Yamashita N, Nishimura A (2000) Pyrithiamine resistance gene (ptrA) of *Aspergillus oryzae*: cloning, characterization and application as a dominant selectable marker for transformation. Bioscience Biotechnol Biochem 64:1416–1421

Laudert D, Hohmann HP (2011) Application of enzymes and microbes for the industrial production of vitamins and vitamin-like compounds. In: Moo-Young M (Hrsg) Comprehensive biotechnology. 2. Aufl. Elsevier, Amsterdam, pp. 583–602

Ledesma-Amaro R, Santos MA, Jimenez A, Revuelta JL (2013) Microbial production of vitamins. In: McNeil B, Archer D, Giavasis I, Harvey L (eds) Microbial production of food ingredients, enzymes and nutraceuticals, vol 246. Woodhead Publishing, Oxford, pp 571–594

Ledesma-Amaro R, Serrano-Amatriain C, Jimenez A, Revuelta JL (2015) Metabolic engineering of riboflavin production in *Ashbya gossypii* through pathway optimization. Microb Cell Fact 14:163

Lees N, Skaggs B, Kirsch D, Bard M (1995) Cloning of the late genes in the ergosterol biosynthetic pathway of *Saccharomyces cerevisiae*: a review. Lipids 30:221–226

Leonardi R, Jackowski S (2007) Biosynthesis of pantothenic acid and coenzyme A. Eco Sal Plus 2:1–28
Liu S, Hu W, Wang Z, Chen T (2020) Production of riboflavin and related cofactors by biotechnological processes. Microb Cell Fact 19:1–16
Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Casero D (2019) Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature 569:655–662
Loewus FA (1999) Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. Phytochemistry 52:193–210
Ma BX, Ke X, Tang XL, Zheng RC, Zheng YG (2018) Rate-limiting steps in the *Saccharomyces cerevisiae* ergosterol pathway: towards improved ergosta-5,7-dien-3β-ol accumulation by metabolic engineering. World J Microbiol Biotechnol 34:1–12
Margalith P, Meydav S (1968) Carotenogenesis in the yeast *Rhodotorula mucilaginosa*. Phytochem 7:765–768
Marx H, Mattanovich D, Sauer M (2008) Overexpression of the riboflavin biosynthetic pathway in *Pichia pastoris*. Microbial Cell Fact 7:1–11
Mata-Gómez LC, Montañez JC, Méndez-Zavala A, Aguilar CN (2014) Biotechnological production of carotenoids by yeasts: an overview. Microb Cell Factories 13:1–11
Maynard C, Cummins I, Green J, Weinkove D (2018) A bacterial route for folic acid supplementation. BMC Biol 16(1):67
Meir Z, Osherov N (2018) Vitamin biosynthesis as an antifungal target. J Fungi 72:1–14
Mokrosnop VM, Zolotareva EK (2014) Microalgae as tocopherol producers. Biotechnol Acta 7:26–33
Nahlik J, Hrncirik P, Mares J, Rychtera M, Kent CA (2017) Towards the design of an optimal strategy for the production of ergosterol from *Saccharomyces cerevisiae* yeasts. Biotechnol Prog 33:838–848
Nielsen J, Keasling JD (2016) Engineering cellular metabolism. Cell 164:1185–1197
O'Byrne SM, Blaner WS (2013) Retinol and retinyl esters: biochemistry and physiology. J Lipid Res 54:1731–1743
Outila TA, Mattila PH, Piironen VI, Lamberg-Allardt CJE (1999) Bioavailability of vitamin D from wild edible mushrooms (*Cantharellus tubaeformis*) as measured with a human bioassay. Am J Clin Nutr 69:95–98
Ouyang SQ, He SJ, Liu P, Zhang WK, Zhang JS, Chen SY (2011) The role of tocopherol cyclase in salt stress tolerance of rice (*Oryza sativa*). Sci China Life Sci 54:181–188
Özbas T, Kutsal T (1991) Effects of growth factors on riboflavin production by *Ashbya gossypii*. Enzyme Microb Technol 13:594–596
Papaioannou EH, Liakopoulou-Kyriakides M (2010) Substrate contribution on carotenoids production in *Blakeslea trispora* cultivations. Food Bioprod Process 88:306–311
Paracchini V, Petrillo M, Reiting R, Angers-Loustau A, Wahler D, Stolz A, Schonig B, Matthies A, Bendiek J, Meinel DM, Pecoraro S, Busch U, Patak A, Kreysa J, Grohmann L (2017) Molecular characterization of an unauthorized genetically modified Bacillus subtilis production strain identified in a vitamin B2 feed additive. Food Chem 230:681–689
Pearson BM, Mackenzie DA, Keenan MHJ (1986) Production of biotin by yeasts. Lett Appl Microbiol 2:25–28
Perrier V, Dubreucq E, Galzy P (1995) Fatty acid and carotenoid composition of *Rhodotorula* strains. Arch Microbiol 164:173–179
Putnam EE, Goodman AL (2020) B vitamin acquisition by gut commensal bacteria. PLoS Pathog 16:e1008208
Qi Q, Hao M, Ng WO, Slater SC, Baszis SR, Weiss JD, Valentin HE (2005) Application of the *Synechococcus* nirA promoter to establish an inducible expression system for engineering the *Synechocystis* tocopherol pathway. Appl Environ Microbiol 71:5678–5684
Rastegari AA, Yadav AN, Yadav N (2019a) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial bio-

technology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5

Rastegari AA, Yadav AN, Yadav N, Tataei Sarshari N (2019b) Bioengineering of secondary metabolites. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 55–68. https://doi.org/10.1016/B978-0-444-63504-4.00004-9

Raux E, Mcveigh T, Peters SE, Leustek T, Warren MJ (1999) The role of *Saccharomyces cerevisiae* Met1p and Met8p in sirohaem and cobalamin biosynthesis. Biochem J 338:701

Revuelta JL, Buey RM, Ledesma-Amaro R, Vandamme EJ (2016) Microbial biotechnology for the synthesis of (pro) vitamins, biopigments and antioxidants: challenges and opportunities. Microb Biotechnol 9:564–567

Rosenberg J, Ischebeck T, Commichau FM (2017) Vitamin B6 metabolism in microbes and approaches for fermentative production. Biotechnol Adv 35:31–40

Sakaki T, Sugimoto H, Hayashi K, Yasuda K, Munetsuna E, Kamakura M, Ikushiro S, Shiro Y (2011) Bioconversion of vitamin D to its active form by bacterial or mammalian cytochrome P450. Biochim Biophys Acta 1814:249–256

Sánchez C (2017) Reactive oxygen species and antioxidant properties from mushrooms. Synth and Syst Biotechno 12:13–22

Satiaputra J, Shearwin KE, Booker GW, Polyak SW (2016) Mechanisms of biotin-regulated gene expression in microbes. Synth Syst Biotech 1:17–24

Sauer M, Branduardi P, Valli M, Porro D (2004) Production of L-ascorbic acid by metabolically engineered *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. Appl Environ Microbiol 70:6086–6091

Schlüpen C, Santos MA, Weber U, De Graaf A, Revuelta JL, Stahmann KP (2003) Disruption of the SHM2 gene, encoding one of two serine hydroxymethyltransferase isozymes, reduces the flux from glycine to serine in *Ashbya gossypii*. Biochem J 369:263–273

Sengupta S, Kaufmann A, Chandra TS (2012) Development of fluorescent reporter tagged RIB gene cassettes for replicative transformation, early expression, and enhanced riboflavin production in *Eremothecium ashbyii*. Fungal Biol 116:1042–1051

Serrano-Amatriain C, Ledesma-Amaro R, López-Nicolás R, Ros G, Jiménez A, Revuelta JL (2016) Folic acid production by engineered *Ashbya gossypii*. Metab Eng 38:473–482

Seyoum E, Selhub J (1998) Properties of food folates determined by stability and susceptibility to intestinal pteroylpolyglutamate hydrolase action. J Nutr 128:1956–1960

Sharma V, Rodionov DA, Leyn SA, Tran D, Iablokov SN, Ding H, Peterson SN (2019) B vitamin sharing promotes stability of gut microbial communities. Frontiers Microb 10:1485

Shearer MJ, Newman P (2008) Metabolism and cell biology of vitamin K. J Thromb Haemost 100:530–547

Shibata N, Kunisawa J, Kiyono H (2017) Dietary and microbial metabolites in the regulation of host immunity. Front Microbiol 8:2171

Shimizu S (2001) Vitamins and related compounds: microbial production. In: Rehm H-J, Reed G (eds) Biotechnology: special processes, vol 10, 2nd edn. Weinheim, Wiley-VCH Verlag GmbH

Shimizu S, Kataoka M, Honda K, Sakamoto K (2001) Lactone-ring-cleaving enzymes of microorganisms: their diversity and applications. J Biotechnol 92:187–194

Shimizu M, Masuo S, Itoh E, Zhou S, Kato M, Takaya N (2016) Thiamine synthesis regulates the fermentation mechanisms in the fungus *Aspergillus nidulans*. Biosci Biotechnol Biochem 80:1768–1775

Silva R, Aguiar TQ, Domingues L (2015) Blockage of the pyrimidine biosynthetic pathway affects riboflavin production in *Ashbya gossypii*. J Biotechnol 193:37–40

Singh J, Yadav AN (2020) Natural bioactive products in sustainable agriculture. Springer, Singapore

Stahmann KP (2019) Vitamins and vitamin-like compounds: microbial production. In: Schmidt TM (ed) Encyclopedia of microbiology, 4th edn. Academic, Oxford, pp 569–580

Sugimoto T, Kanamasa S, Kato T, Park EY (2009) Importance of malate synthase in the glyoxylate cycle of *Ashbya gossypii* for the efficient production of riboflavin. Appl Microbiol Biotechnol 83:529–539

Sun X, Liu H, Wang P, Wang L, Ni W, Yang Q et al (2019) Construction of a novel MK-4 biosynthetic pathway in *Pichia pastoris* through heterologous expression of HsUBIAD1. Microbial Cell Fact 18:169

Suzuki GT, Macedo JA, Macedo GA (2011) Medium composition influence on Biotin and Riboflavin production by newly isolated *Candida* sp. Braz J Microbiol 42:1093–1100

Tani Y, Asahi S, Yamada H (1984) Vitamin K2 (menaquinone): Screening of producing microorganisms and production by *Flavobacterium meningosepticum*. J Fermentation Technol 62:321–327

Taskin M, Sisman T, Erdal S, Kurbanoglu EB (2011) Use of waste chicken feathers as peptone for production of carotenoids in submerged culture of *Rhodotorula glutinis* MT-5. Eur Food Res Technol 233:657–665

Thakur K, Tomar SK, De S (2016) Lactic acid bacteria as a cell factory for riboflavin production. Microb Biotech 9:441–451

Tinoi J, Rakariyatham N, Deming RL (2005) Simplex optimization of carotenoid production by *Rhodotorula glutinis* using hydrolyzed mung bean waste flour as substrate. Proc Biochem 40:2551–2557

Tokui M, Kubodera T, Gomi K, Yamashita N, Nishimura A (2011) Construction of a thiamine pyrophosphate high-producing strain of *Aspergillus oryzae* by overexpression of three genes involved in thiamine biosynthesis. J Biosci Bioeng 111:388–390

Torregrosa-Crespo J, Montero Z, Fuentes JL, García-Galbis MR, Garbayo I, Vílchez C, Martínez-Espinosa RM (2018) Exploring the valuable carotenoids for the large-scale production by marine microorganisms. Marin Drug 16:1–25

Turło J, Gutkowska B, Herold F, Krzyczkowski W, Błażewicz A, Kocjan R (2008) Optimizing vitamin B12 biosynthesis by mycelial cultures of *Lentinula edodes* (Berk.) Pegl. Enzyme Microbial Tech 43:369–374

Vamanu E (2014) Antioxidant properties of mushroom mycelia obtained by batch cultivation and tocopherol content affected by extraction procedures. BioMed Res Int 2014:1–8

Vandamme EJ, Revuelta JL (2016) Vitamins, biopigments, antioxidants and related compounds: a historical, physiological and (bio) technological perspective. In: Vandamme EJ, Revuelta JL (eds) Industrial biotechnology of vitamins, biopigments, and antioxidants. Weinheim, Wiley-VCH Verlag GmbH KGaA, pp 1–14

Verwaal R, Wang J, Meijnen JP, Visser H, Sandmann G, Van Den Berg JA, Van Ooyen AJ (2007) High-level production of beta-carotene in *Saccharomyces cerevisiae* by successive transformation with carotenogenic genes from *Xanthophyllomyces dendrorhous*. Appl Environ Microbiol 73:4342–4350

Wang PP, Zeng WZ, Du GC, Zhou JW, Chen J (2019) Systematic characterization of sorbose/sorbosone dehydrogenases and sorbosone dehydrogenases from *Ketogulonicigenium vulgare* WSH-001. J Biotechnol 301:24–34

Watanabe F, Bito T (2018) Vitamin B12 sources and microbial interaction. Exper Biol Med 243:148–158

Wei S, Hurley J, Jiang Z, Wang S, Wang Y (2012) Isolation and characterization of an *Ashbya gossypii* mutant for improved riboflavin production. Brazilian J Microbiol 43(2):441–448

Weimann B-J, Hengartner U, de Saizieu A, Wehrli C (2011) Vitamins, 10. Folic acid. Ullmann's Encyclopedia Ind Chem

Wheeler GL, Jones MA, Smirnoff N (1998) The biosynthetic pathway of vitamin C in higher plants. Nature 393:365–369

Wu Y, Li S, Wang W, Zhang D (2020) Associations of dietary vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12 and folate equivalent intakes with metabolic syndrome. Int J Food Sci Nutr 71(6):738–749

Xie W, Liu M, Lv X, Lu W, Gu J, Yu H (2014) Construction of a controllable β-carotene biosynthetic pathway by decentralized assembly strategy in *Saccharomyces cerevisiae*. Biotechnol Bioeng 111:125–133

Xie W, Ye L, Lv X, Xu H, Yu H (2015) Sequential control of biosynthetic pathways for balanced utilization of metabolic intermediates in *Saccharomyces cerevisiae*. Metab Eng 28:8–18

Yadav AN (2020) Recent trends in mycological research, Volume 1: Agricultural and medical perspective. Springer, Switzerland

Yadav AN, Kour D, Rana KL, Yadav N, Singh B, Chauhan VS et al (2019a) Metabolic engineering to synthetic biology of secondary metabolites production. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 279–320. https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Yadav AN, Mishra S, Singh S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 1: Diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019c) Recent advancement in white biotechnology through fungi. Volume 2: Perspective for value-added products and environments. Springer International Publishing, Cham

Yan G, Wen K, Duan C (2012) Enhancement of β-carotene production by over-expression of HMG-CoA reductase coupled with addition of ergosterol biosynthesis inhibitors in recombinant *Saccharomyces cerevisiae*. Curr Microbiol 64:159–163

Ye VM, Bhatia SK (2012) Pathway engineering strategies for production of beneficial carotenoids in microbial hosts. Biotechnol Lett 34:1405–1414

Yoshii K, Hosomi K, Sawane K, Kunisawa J (2019) Metabolism of dietary and microbial vitamin B family in the regulation of host immunity. Front Nutr 6:48

Yuan P, Cui S, Liu Y, Li J, Du G, Liu L (2019) Metabolic engineering for the production of fat-soluble vitamins: Advances and perspectives. Appl Microb Biotechnol 104:935–951

Zanghellini A (2014) De novo computational enzyme design. Curr Opin Biotechnol 29:132–138

Zhao J, Li Q, Sun T, Zhu X, Xu H, Tang J, Zhang X, Ma Y (2013) Engineering central metabolic modules of *Escherichia coli* for improving beta-carotene production. Metab Eng 17:42–50

Zittermann A, Ernst JB, Gummert JF, Borgermann J (2014) Vitamin D supplementation, body weight and human serum 25-hydroxy vitamin D response: a systematic review. Eur J Nutr 53:367–374

Zu, Berstenhorst SM, Hohmann H-P, Stahmann K-P (2009) Vitamins and vitamin-like compounds: microbial production. Encyclo Microbiol:541–561

Chapter 17
Nutraceutical Potential of Wild Edible Mushroom *Hygrocybe alwisii*

Bijavara Ramakrishnappa Jagadish, Kandikere Ramaiah Sridhar, Hosamane Ramesh Dattaraj, Nagabhushana Chandramohana, and Shivannegowda Mahadevakumar

Contents

B. R. Jagadish · H. R. Dattaraj
Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

K. R. Sridhar (✉)
Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

Centre for Environmental Studies, Yenepoya (Deemed to be University), Mangalore, Karnataka, India

N. Chandramohana
Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore, Karnataka, India

S. Mahadevakumar
Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_17

17.1 Introduction

Mushrooms are ubiquitous as potential source of nutrition, medicinal values, and industrially important metabolites. Wild mushrooms are nutraceutically versatile compared to commercially cultivated mushrooms owing to nutritional composition, desired medicinal attributes, and value-added bioactive compounds (Hobbs 1995; Smith et al. 2002; Boa 2004; Karun and Sridhar 2016). They have desirable qualities like adequate protein, fiber, essential amino acids with a low quantity of lipids (Sanmee et al. 2003, Kavishree et al. 2008, Pavithra et al. 2018). Besides their nutritional value, they are composed of primary as well as secondary metabolites such as chitin, oxalic acids, peptides, steroids, terpenes, and quinones (Alves et al. 2012; Devi et al. 2020). Mushrooms have been considered as alternative source of various pharmaceuticals and medicinal principles against plant and animal-derived products. According to Maihara et al. (2012) about 2000 species of mushrooms are globally preferred for consumption.

The Western Ghats of India have been a treasure trove for a wide variety of wild mushrooms of traditional, agricultural, and industrial significance (Mohanan 2011; Karun and Sridhar 2016, 2017). Similarly, the scrub jungles of southwest India also support edible, medicinal, and ectomycorrhizal mushrooms (Sridhar 2018). The *Hygrocybe* (Order, Agaricales; Family, Hygrophoraceae) is a monophyletic genus characterized by brightly colored basidiocarps with waxy slimy cap, ring-less stipe, and white spores. This cosmopolitan genus has 721 records of about 350 species in the Index Fungorum. Assessment of 16 species of *Hygrocybe* in Europe revealed their adaptation to a diverse habitat but prefer grasslands rather than forests (Halbwachs et al. 2013). It is also confirmed that the *Hygrocybe* possesses the capability to grow in soils with low fertility. The Indian subcontinent comprises about 52 species of *Hygrocybe* (Lata and Manimohan 2018). Twenty five species of *Hygrocybe* (with 10 new species) have been described from the Kerala state (Leelavathy et al. 2006). A checklist by Farook et al. (2013) documents distribution up to 41 species of *Hygrocybe* in Kerala state. The updated list reveals occurrence of 48 species with 17 new species of *Hygrocybe* in the Western Ghats region (Maharashtra and Karnataka, Kerala states) (Table 17.1). Among these, *Hygrocybe alwisii* has been reported often in different parts of the Western Ghats followed by *H. astatogala* and *H. conica*.

During a diversity expedition in the scrub jungles of southwest Karnataka, the edible wild mushroom *H. alwisii* was found in large numbers on the litter strata (Dattaraj et al. 2020). A substantial quantity of this mushroom was found in the basins of the avenue trees of *Polyalthia longifolia*. This chapter aims to project the nutritional, bioactive, and antioxidant potential of *H. alwisii* in comparison with other wild edible mushrooms occurring in the scrub jungles of southwest India.

Table 17.1 Species of *Hygrocybe* reported from the Western Ghats of India (*, New species)

Hygrocybe acutoconica (Clem.) Singer	Karnataka	Senthilarasu and Kumaresan (2016)
	Kerala	Vrinda et al. (1995); Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe alwisii (Berk. & Broome) Pegler	Maharashtra	Senthilarasu (2014)
	Karnataka	Ghate and Sridhar (2016a); Karun and Sridhar (2016); Senthilarasu and Kumaresan (2016); Dattaraj et al. (2020)
	Kerala	Vrinda et al. (1996b); Leelavathy et al. (2006); Pradeep and Vrinda (2007); Mohanan (2011)
Hygrocybe anisa (Berk. & Broome) Pegler	Kerala	Mohanan (2011)
Hygrocybe apala (Berk. & Broome) Pegler & R.W. Rayner	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe astatogala (R. Heim) Heinem.	Maharashtra	Senthilarasu (2014); Borkar et al. (2015)
	Karnataka	Greeshma et al. (2015, 2016); Ghate and Sridhar (2016b); Pavithra et al. (2016a); Jagadish et al. (2019); Dattaraj et al. (2020)
	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe atrosquamosa Pegler	Kerala	Vrinda et al. (1997)
Hygrocybe aurantia Murrill	Kerala	Leelavathy et al. (2006)
**Hygrocybe aurantioalba* Leelav., Manim. & Arnolds	Karnataka	Greeshma et al. (2015, 2016)
	Kerala	Leelavathy et al. (2006); Mohanan (2011)
**Hygrocybe aurantiocephala* Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe atrosquamosa Pegler	Kerala	Vrinda et al. (1997)
Hygrocybe batistae Singer	Kerala	Vrinda et al. (2009)
Hygrocybe boriviliensis B.D. Sharma, S.D. Deshp. & S.G. Pradhan	Maharashtra	Sharma et al. (1986)
**Hygrocybe brunneosquamulosa* Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006); Mohanan (2011))
Hygrocybe caespitosa Murrill	Kerala	Vrinda et al. (1995)
Hygrocybe cantharellus (Schwein.) Murrill	Kerala	Vrinda et al. (1995); Leelavathy et al. (2006); Mohanan (2011)

(continued)

Table 17.1 (continued)

Hygrocybe cinerascens (Berk. & Broome) Pegler	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe coccinea (Schaeff.) P. Kumm.	Kerala	Mohanan (2003); Florence (2004)
Hygrocybe conica (Schaeff.) P. Kumm.	Karnataka	Karun and Sridhar (2016); Dattaraj et al. (2020)
	Kerala	Sankaran and Florence (1995); Vrinda et al. (1995); Florence (2004); Leelavathy et al. (2006)
**Hygrocybe corallina* Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006)
Hygrocybe cuspidata (Peck) Murrill	Kerala	Leelavathy et al. (2006); Mohanan (2011)
**Hygrocybe deceptiva* (A.H. Sm. & Hesler) Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe diversicolor (Petch) Pegler	Kerala	Vrinda et al. (2009); Mohanan (2011)
Hygrocybe erinacea (Pat.) Singer	Kerala	Vrinda et al. (1997)
Hygrocybe firma (Berk. & Broome) Singer	Kerala	Mohanan (2011)
**Hygrocybe globispora* Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006)
**Hygrocybe griseoalbida* Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006)
Hygrocybe hypohaemacta (Corner) Pegler	Kerala	Vrinda et al. (1996a)
**Hygrocybe indica* K.P.D. Latha & Manim.	Kerala	Latha and Manimohan 2018
Hygrocybe insipida (J.E. Lange) M.M. Moser	Kerala	Leelavathy et al. (2006)
**Hygrocybe keralensis* Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe konradii R. Haller Aar.	Kerala	Vrinda et al. (1995)
**Hygrocybe lobatospora* Leelav., Manim. & Arnolds	Kerala	Leelavathy et al. (2006)
**Hygrocybe manadukaensis* Senthil., Kumaresan & S.K. Singh	Karnataka	Senthilarasu et al. (2010a)
Hygrocybe martinicensis Pegler & Fiard	Kerala	Vrinda et al. (2009)
Hygrocybe mexicana Singer	Kerala	Leelavathy et al. (2006); Mohanan (2011)
**Hygrocybe natarajanii* Senthil. & Kumaresan	Karnataka	Senthilarasu et al. (2010b)
Hygrocybe nigrescens (Quél.) Kühner	Kerala	Vrinda et al. (1995)
**Hygrocybe nivosa* Berk. & Broome) Leelav., Manim. & Arnolds	Karnataka(	Ghate and Sridhar (2016a, 2016c)

(continued)

Table 17.1 (continued)

	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe ocutoconica (Clem.) Singer	Maharashtra	Senthilarasu and Kumaresan (2016)
Hygrocybe ortoniana Bon	Kerala	Leelavathy et al. (2006); Mohanan (2011)
**Hygrocybe parvispora* T.K. Abraham, K.B. Vrinda & C.K. Pradeep	Kerala	Abraham et al. (1996); Pradeep and Vrinda (2007)
Hygrocybe parvula (Peck) Murrill	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe pratensis (Fr.) Murrill	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe punicea (Fr.) P. Kumm.	Kerala	Pradeep et al. (1996); Pradeep and Vrinda (2007)
**Hygrocybe rubida* Vrinda & Pradeep	Kerala	Vrinda et al. (2013)
**Hygrocybe smaragdina* Leelav., Manim. & Arnolds	Karnataka	Ghate and Sridhar (2016a)
	Kerala	Leelavathy et al. (2006); Mohanan (2011)
Hygrocybe subminutula Murrill	Kerala	Vrinda et al. (1995)
Hygrocybe viridula Lodge & Pegler	Kerala	Vrinda et al. (1995)

17.2 Mushroom and Processing

The agaric mushroom *Hygrocybe alwisii* (Berk. and Broome) Pegler is one of the interesting edible mushrooms found in the scrub jungles of the southwest of Karnataka with a relative abundance of 0.5% (Dattaraj et al. 2020; Yadav et al. 2019b). The fruit body of the mushroom is a white to light-yellow waxy cap with prominent umbo and gills (Fig. 17.1a–c). Adequate quantity of fruit bodies was gathered in sterile polythene bags from three different locations as replicates. After rinsing, the fruit bodies eliminate the soil and dirt, they were blot-dried to remove excess water. After dividing each replicate into two parts, the first part designated as the uncooked sample, while the second part subjected to pressure-cooking at a very low amount of water (similar to leafy vegetables). The uncooked and cooked samples were oven-dried in a hot-air oven (52 ± 3 °C). Immediately on drying, the samples were milled using a hand grinder till getting fine to a coarse powder. They were transferred to sterile containers and preserved in a refrigerator until further analysis.

17.3 Qualitative Assessment

Uncooked and cooked samples of mushroom (5 g) were extracted with water (50 ml) on a rotary shaker (150 rpm) (24 h) (Banu and Catherine 2015). The extracts were centrifuged and supernatants were used for qualitative analysis of different

Fig. 17.1 Edible wild mushroom *Hygrocybe alwisii* occurring in the scrub jungles of southwest India: different stages of growth (**a**), a mature basidiomata (**b**) and longitudinal section showing characteristic gill pattern and hollow stipe (**c**) (Scale bar: 1 cm)

bioactive components (phenols, tannins, phlobatannins, cardiac glycosides, saponins, terpenoids, coumarins, flavonoids, and alkaloids) following standard methods (Trease and Evans 1989, 2002; Safowora 1993; Parekh and Chanda 2007; Soares et al. 2013; Rastegari et al. 2019; Yadav et al. 2019a).

17.3.1 Phenols

To the mushroom extract (1 ml), distilled water (2 ml) was added followed by the addition of a few drops of 10% $FeCl_3$. The appearance of a blue or green color indicated the presence of phenols.

17.3.2 Tannins

To the mushroom extract (2 ml), distilled water (2 ml) and 2–3 drops of aqueous $FeCl_3$ (1%) were added. The appearance of brownish-green, green, blue-black color indicated the presence of tannins.

17.3.3 Saponins

To carry out the foam test to the mushroom extract (5 ml), distilled water (5 ml) was added, heated, cooled, and shaken vigorously. The formation of froth indicated the presence of saponins. To carry out the emulsion test, to the mushroom extract (5 ml), distilled water (5 ml) was added, shaken vigorously, the froth formed was mixed with 3 drops of olive oil and shaken well for the formation of the emulsion as a characteristic feature of saponins.

17.3.4 Flavonoids

To the mushroom extract (2 ml), methanol (2 ml) was added and heated, followed by the addition of a few drops of concentrated hydrochloric acid. The development of red or orange color was considered as evidence for the presence of flavonoids.

17.3.5 Alkaloids

The mushroom extracts (2 ml) were treated with hydrochloric acid (1%) (5 ml), kept in a steam bath (20 min) and filtered. The filtrate (1 ml) was treated with 5–6 drops of Mayer's reagent (0.355 g mercuric chloride dissolved in 60 ml distilled water, 5.0 g of potassium iodide dissolved in 20 ml of distilled water; both the solutions were mixed and volume was made up to 100 using distilled water). The formation of cream-colored precipitate was an indication presence of alkaloids (Kour et al. 2019).

17.3.6 Terpenoids

The mushroom extracts (5 ml) were mixed with chloroform (2 ml) followed by addition of concentrated sulfuric acid. A reddish-brown color formation at the interface was considered a positive test for terpenoids.

17.3.7 Cardiac Glycosides

To the mushroom extract (5 ml), glacial acetic acid containing ferric chloride (a mixture of 1 volume of 5% $FeCl_3$ + 99 volume of glacial acetic acid) (2 ml) was added followed by addition of concentrated sulfuric acid (1 ml). The formation of the brown ring at the interface was considered a positive test for cardiac glycosides. A violet ring may appear beneath the brown ring and greenish coloration or ring can be seen in the acetic acid layer.

17.3.8 Coumarins

The mushroom extracts (2 ml) were treated with sodium hydroxide (10%) (3 ml), the formation of yellow color indicated the evidence for the presence of coumarins.

17.3.9 Phlobatannins

The mushroom extracts (2 ml) were boiled with hydrochloric acid (1%) (2 ml), the formation of red precipitate was considered a positive result for the presence of phlobatannins.

17.4 Quantitative Assessment

Uncooked and cooked mushroom samples were further subjected to quantitative assessment of total phenolics, vitamin C, and minerals by different methods.

17.4.1 Total Phenolics

The total phenolic content of mushroom samples was assessed according to the protocol outlined by Rosset et al. (1982). Fifty mg of the mushroom sample was extracted in methanol (50% in distilled water) (5 ml) in a water bath (95 ± 1 °C) (10 min), centrifuged (1500 rpm) and the supernatant was collected. The above extraction was repeated; the supernatants were pooled and made up to 10 ml of 50% methanol. An aliquot (0.1 ml) of the extract was made up to 1 ml with distilled water followed by the addition of 2% Na_2CO_3 (in 0.1 N NaOH) (5 ml). After a time-lapse of 10 min of incubation at room temperature, Folin–Ciocalteu's reagent (1:2 v/v) was added (0.5 ml) and absorbance of the reaction mixture was measured at 725 nm. Tannic acid served as standard and the results were expressed as milligram of tannic acid equivalents (TAEs) per gram of the mushroom sample (mg TAEs/g).

17.4.2 Vitamin C

The vitamin C content in uncooked and cooked mushroom samples was estimated according to the method by Roe (1954) with a slight modification. The mushroom samples (5 g) were extracted using TCA (trichloroacetic acid) (5%) (25 ml). Aliquots (0.5 ml) of the mushroom samples were made up to 1 ml using 5% TCA

followed by addition of chromogen (0.6% CuSO4, 5 ml + 5% thiourea, 5 ml + 2.2% of 2,4-dinitrophenyl hydrazine, 90 ml) (1 ml). The mixture was boiled (10 min), cooled to room temperature, sulfuric acid was added (65%, 4 ml) and incubated at room temperature (30 min). The absorbance was measured at 540 nm using ascorbic acid as standard to express vitamin C content in milligram of ascorbic acid equivalents per gram of mushroom sample (mg AAEs/g).

17.4.3 Functional Groups

Fourier transform infrared spectroscopy (FTIR) was carried out to follow the functional groups in uncooked and cooked mushrooms samples using Perkin Elmer Spectrum 1000 (Waltham, MA 02451 USA) for 4500–500 cm^{-1} with a resolution of 4 cm^{-1}.

17.4.4 Minerals

The energy dispersive spectroscopy (EDS) analysis was carried out (HITACHI Norn System 7, USA) for the detection of elemental composition in uncooked and cooked *Hygrocybe alwisii* samples.

17.5 Antioxidant Assay

Antioxidant potentials of mushroom sample were assessed for total antioxidant activity, ferrous ion chelation capacity, and DPPH radical scavenging activity by standard methods.

17.5.1 Total Antioxidant Activity

The total antioxidant activity (TAA) of the uncooked and cooked mushroom was measured according to the method by Prieto et al. (1999) with a few minor modifications. Aqueous extract of the uncooked and cooked mushroom sample (prepared for qualitative assessment of biochemical components) (0.2 ml) blended with a reagent mixture (Sulfuric acid, 0.6 M + sodium phosphate, 28 mM + ammonium molybdate, 4 mM) (2 ml). The mixtures were incubated at 95^0C in a water bath (90 min), after cooling to room temperature; the absorbance was measured at 695 nm against a blank (methanol). The total antioxidant activity was expressed as μM equivalent of ascorbic acid per gram of the mushroom (μM AAEs/g).

17.5.2 Ferrous Ion Chelation Capacity

Ferrous ion chelation capacity (FCC) was assessed based on the method by Hsu et al. (2003). Aqueous extract of the uncooked and cooked mushroom sample (prepared for qualitative assessment of biochemical components) (1 ml) was treated with ferrous chloride (2 mM) (0.1 ml) and ferrozine (5 mM) (0.2 ml). The final volume was made up to 5 ml using methanol. The mixture was incubated at room temperature (10 min) and absorbance was measured at 562 nm. The reagents without sample extract served as control.

$$\text{Ferrous ion chelation capacity}(\%) = \left[1 - (As_{562} \div Ac_{562})\right] \times 100 \tag{17.1}$$

(where *Ac* is the absorbance of the control and as is the absorbance of sample).

17.5.3 DPPH Radical Scavenging Activity

Aqueous extract of the uncooked and cooked mushroom sample (prepared for qualitative assessment of biochemical components) was assessed to follow the radical scavenging activity by the protocol by Singh et al. (2002). The sample extracts of different concentrations (0.2–1.0 mg in 0.2–1.0 ml) were made up to 1ml (using methanol). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.01 mM) (4 ml) was added and incubated at room temperature (20 min). The reagents without sample extract served as control and the absorbance was measured at 517 nm.

$$\text{Free radical scavenging activity}(\%) = [(Ac_{517} - As_{517})/(Ac_{517})] \times 100 \tag{17.2}$$

(where *Ac* is the absorbance of the control and as is the absorbance of the sample).

17.6 Data Analysis

The variations in bioactive components and antioxidant activities between uncooked and cooked mushroom samples were assessed by *t*-test with Statistica version #8.0 (StatSoft Inc., 2008).

17.7 Qualitative Components

The preliminary quantitative analysis provided a clue about the presence of various components in uncooked and cooked *H. alwisii*. Among the nine components tested, eight were present in uncooked samples, while six in cooked samples. The uncooked

and cooked samples were free from phlobatannins, while the cooked samples were also devoid of flavonoids and alkaloids. The presence of phenols, tannins, saponins, flavonoids, alkaloids, and terpenoids is indicative of the antibacterial activity of the uncooked and cooked *H. alwisii* (Machumi et al. 2010; Aboh et al. 2014). Besides, phenols, tannins, saponins, flavonoids, and coumarins are known for antioxidant activity (Poumale et al. 2013; Nithya et al. 2016). The cardiac glycosides (or aglycones) have a positive impact by increasing the capacity of the heart muscle to pump blood (Aldred 2008). Coumarins (1-benzopyran-2-one) are known for several bioactive properties (e.g., antimicrobial, antiviral, anti-inflammatory, antidiabetic, antioxidant and enzyme inhibition) (Poumale et al. 2013). Most of the bioactive compounds known from *H. alwisii* by the qualitative test are produced mainly by plant species. Further quantitative assessment of these bioactive components in *H. alwisii* provides support to obtain such components by *in vitro* cultivation.

17.8 Quantitative Components

The total phenolics content of uncooked samples is higher than the cooked samples of *H. alwisii* ($p < 0.01$) (Fig. 17.2a). Total phenolics is a major component to express antioxidant activity in mushrooms. On comparison with other edible wild mushrooms, the quantity of total phenolics is higher than *Amanita* sp., *Astraeus hygrometricus*, *Auricularia auricula,* and *Termitomyces umkowaan*, while comparable with *Lentinus squarrosulus* and *Termitomyces clypeatus* occurring in the southwest India (Karun et al. 2016; Pavithra et al. 2016b; Ghate and Sridhar 2017; Greeshma et al. 2018).

The content of vitamin C in uncooked samples of *H. alwisii* is higher than the cooked samples ($p < 0.05$) (Fig. 17.2b). Vitamin C in addition to providing nutritional benefits, it is also known for antioxidant activity in mushrooms. Its content is higher than *A. hygrometricus*, *A. auricula*, *L. squarrosulus*, *T. clypeatus,* and *T. umkowaan*, however lower compared to *Amanita* sp. occurring in the southwest India (Karun et al. 2016; Pavithra et al. 2016b; Ghate and Sridhar 2017; Greeshma et al. 2018).

In uncooked samples, the FTIR showed a broad peak with a medium intensity corresponding to 3302.27 cm^{-1} showed the presence belonging to amine group N–H stretching vibration and C–H stretching vibration (asym.) at 2918.56 cm^{-1} (Fig. 17.3a). The peaks observed at 1596.30 cm^{-1}; 1311.15 cm^{-1}; 1039.65 cm^{-1} corresponded to functional groups C=C stretching vibration; C–H deformation vibration and C–O stretching vibration, respectively. In cooked samples, the FTIR revealed broad O–H stretching vibration (associated) at 3271.93 cm^{-1} along with C–H stretching vibration-asym. at 2923.08 cm^{-1} (Fig. 17.3b). The sharp Peak observed at 1626.92 cm^{-1} corresponded to C=C stretching vibration. The CH_3 bending (sym.) was observed at 1398.23 cm^{-1}.

The peaks 1312.41 cm^{-1} 1037.10 cm^{-1} correspond to C–H deformation vibration and C–C skeleton vibration, respectively. Biogenic amines (BA) found in

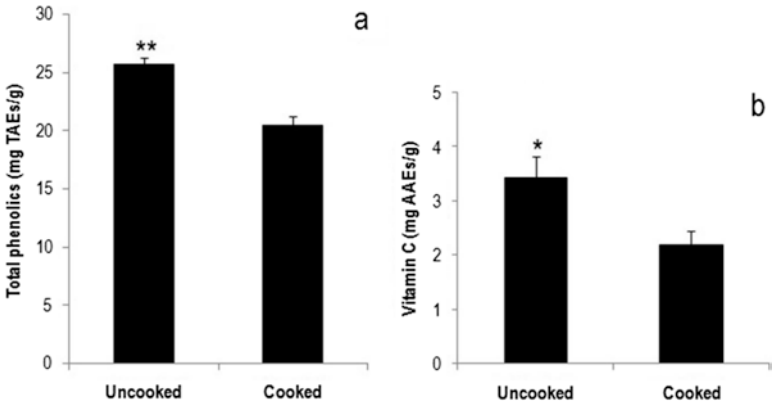


Fig. 17.2 Total phenolics (**a**) and vitamin C (**b**) in uncooked and cooked *Hygrocybe alwisii* ($n = 3 \pm$ SD; *t*-test: $^{*}p < 0.05$, $^{**}p < 0.01$)

mushrooms need to be stabilized as they may intimidate the food product safety. Some of the BAs present in food include: tyramine, cadaverine, 2-phenylethylamine, spermine, histamine, spermidine, putrescine, and tryptamine. The presence of BAs can elicit hypersensitivity reactions if consumed like itching, rash, vomiting, fever, and hypertension. In the present study, only the uncooked sample showed presence of BAs as confirmed by FTIR spectra. However, the cooked samples were devoid of BAs revealing its safety.

The energy dispersive spectroscopy (EDS) revealed difference in elemental composition of uncooked and cooked *H. alwisii* (Fig. 17.4). The uncooked sample showed two major elements (C and O), which account for 98.63% and 1.37% constituted by K. However, the element C composition reduced after cooking from 57.91 to 34.74, while the element O composition reduced from 40.72 to 30.44%. However, the element N was detected only in cooked samples. Further, presence of trace elements like P and K was also recorded, but the percent composition of K reduced from 1.37 in uncooked to 0.53 on cooking.

17.9 Antioxidant Potential

The total antioxidant activity (TAA) is significantly higher in uncooked than cooked samples of *H. alwisii* ($p < 0.01$) (Fig. 17.5a). The total antioxidant activity is comparable with *Amanita* sp., *A. hygrometricus*, *T. clypeatus, and T. umkowaan*, while lower than *A. auricula* and *L. squarrosulus* occurring in the southwest India (Karun et al. 2016; Pavithra et al. 2016b; Ghate and Sridhar 2017; Greeshma et al. 2018).

The ferrous ion chelation capacity (FCC) of *H. alwisii is* also significantly higher in uncooked samples ($p < 0.01$) (Fig. 17.5b). Its activity was also significantly lower in cooked compared to uncooked samples of other wild mushrooms like *Amanita*

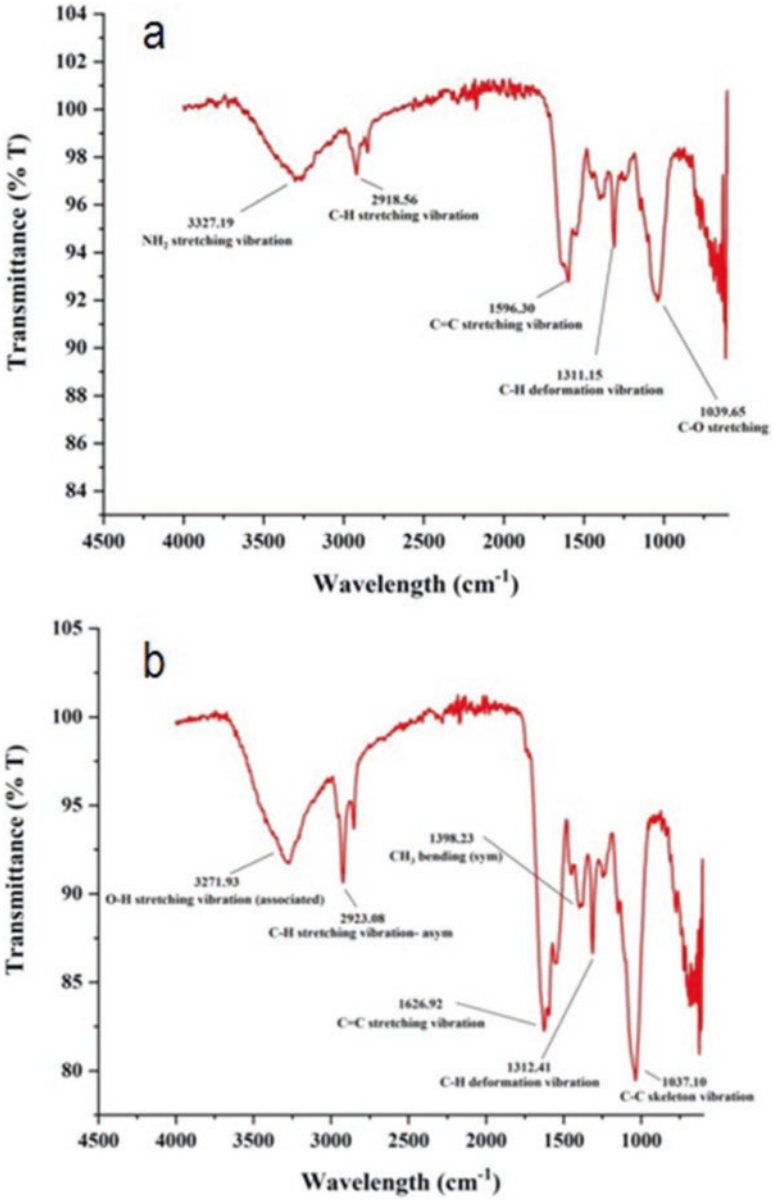


Fig. 17.3 The FTIR spectra of uncooked (**a**) and cooked (**b**) *Hygrocybe alwisii*

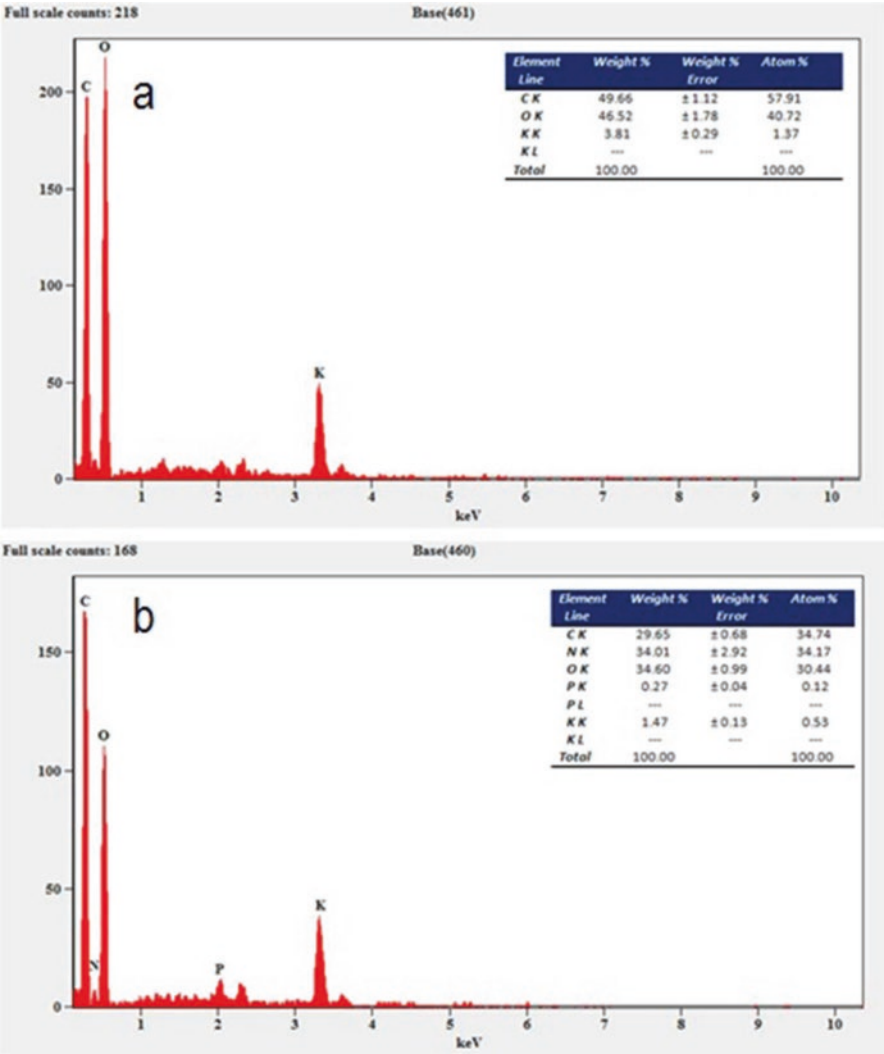


Fig. 17.4 The EDS spectra showing elemental composition of uncooked (**a**) and cooked (**b**) *Hygrocybe alwisii*

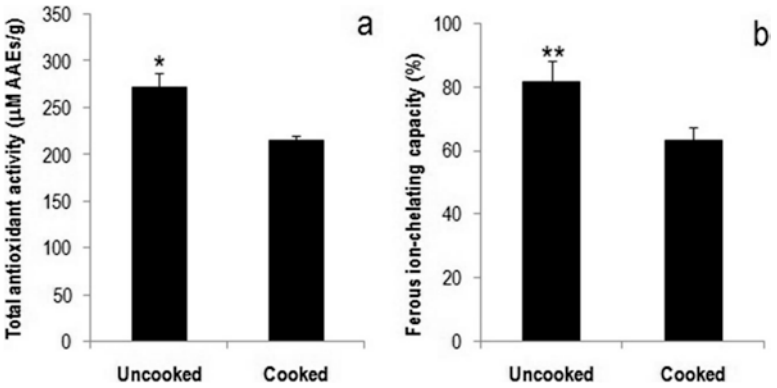


Fig. 17.5 Total antioxidant activity (a) and ferrous ion chelation activity (b) of uncooked and cooked *Hygrocybe alwisii* ($n = 3 \pm$ SD; *t*-test: $*p < 0.05$, $**p < 0.01$)

sp., *A. hygrometricus*, *A. auricula, L. squarrosulus, T. clypeatus,* and *T. umkowaan* occurring in the southwest India (Karun et al. 2016; Pavithra et al. 2016b; Ghate and Sridhar 2017; Greeshma et al. 2018). Its activity is higher than *Amanita* sp., *A. auricula,* while comparable to *A. hygrometricus*, *L. squarrosulus, T. clypeatus,* and *T. umkowaan*.

The DPPH radical scavenging activity was significantly higher in uncooked than cooked samples of *H. alwisii* in three concentrations (Fig. 17.6) ($p < 0.05$). Its activity is higher than other wild mushrooms like *A. auricula, L. squarrosulus, T. clypeatus, and T. umkowaan*, while lower than *Amanita* sp. and *A. hygrometricus* occurring in the southwest India (Karun et al. 2016; Pavithra et al. 2016b; Ghate and Sridhar 2017; Greeshma et al. 2018).

17.10 Conclusions and Outlook

The edible wild mushroom *Hygrocybe alwisii* occurring in the scrub jungles of southwest India consists of several bioactive components in uncooked and cooked samples. Qualitative tests revealed the occurrence of bioactive components usually occur in plant species. It possesses significant quantities of total phenolics as well as vitamin C. The FTIR spectra revealed the occurrence of amine residues (N–H) in uncooked samples and carboxylic acid (O–H) in cooked samples. In addition, presence of alkanes (C–H), conjugated alkenes (C=H) was also seen. The energy dispersive spectroscopy (EDS) revealed that the mushroom is rich in secondary metabolites and elements, which need to be substantiated further using techniques like GCMS and HPLC. The antioxidant capacity is higher than or comparable to many edible wild mushrooms occurring in the scrub jungles of southwestern India. This study provided further evidence that the wild edible agaric mushroom *H. alwisii* possess active nutritional as well as health-promoting components. These features are in

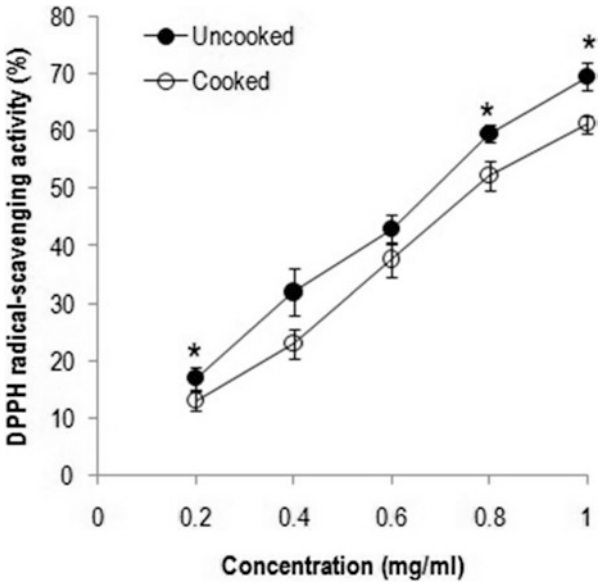


Fig. 17.6 The DPPH radical scavenging activity of uncooked and cooked *Hygrocybe alwisii* ($n = 3 \pm$ SD; *t*-test: $^*p < 0.05$)

line with the assessment of a wide range of structural and functional attributes of *Hygrocybe conica* in Malaysia (Chong et al. 2014). Compared to other mushrooms, the genus *Hygrocybe* has not been researched for nutritional and bioactive principles. Being cosmopolitan and wide varieties occurring in the Western Ghats of India, there is ample scope to tap this resource for nutraceutical industrial purposes.

Acknowledgements Authors are thankful to Dr. M. Pavithra, Department of Biosciences, Mangalore University and Dr. Sudeep Ghate, Yenepoya Research Centre, Yenepoya University for helpful suggestions. One of us (SM) is grateful to the Council of Scientific and Industrial Research, New Delhi for the award of Research Associate Fellowship.

References

Aboh MI, Olayinka BO, Adeshina GO, Oladosu P (2014) Antifungal activities of phyto compounds from *Mitracarpus villosus* (Sw.) DC from Abuja, Nigeria. J Microbiol Res 4:86–91

Abraham TK, Vrinda KB, Pradeep CK (1996) A new species of *Hygrocybe* from India. Mycotaxon 60:361–364

Aldred EM (2008) Pharmacology: a handbook for complementary healthcare professionals, 1st ed. Plant pharmacology, terpenes. Churchill Livingstone/Elsevier, Edinburgh, New York, pp 167–174

Alves MJ, Ferreira ICFR, Dias J, Teixeira V, Martins A, Pintado M (2012) A review on antimicrobial activity of mushroom (basidiomycetes) extracts and isolated compounds. Planta Med 78:1707–1718

Banu KS, Cathrine L (2015) General technique involved in phytochemical analysis. Int J Adv Res Chem Sci 2:25–32

Boa E (2004) Wild edible fungi. A global overview of their use and importance. Nonwood forest products 17. FAO, Rome, pp 1–147

Borkar P, Doshi A, Navathe S (2015) Mushroom diversity of Konkan region of Maharashtra, India. J Thr Taxa 7:7625–7640

Chong EL, Sia CM, Khoo HE, Chang SK, Yim HS (2014) Antioxidative properties of an extract of *Hygrocybe conica*, a wild edible mushroom. Malay J Nutr 20:101–111

Dattaraj HR, Sridhar KR, Jagadish BR (2020) Diversity and bioprospect significance of macrofungi in the scrub jungles of southwest India. In: Öztürk M, Egamberdieva D, Pešić M (eds) Biodiversity and biomedicine. Elsevier, New York. https://doi.org/10.1016/B978-0-12-819541-3.00012-8

Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Farook A, Khan S, Manimohan P (2013) A checklist of agarics (gilled mushrooms) of Kerala State, India. Mycosphere 4:97–131

Florence EJM (2004) Biodiversity documentation for Kerala, Part 2: Microorganisms (Fungi). KFRI Handbook No.17. Kerala Forest Research Institute, Peechi, Kerala, India

Ghate SD, Sridhar KR (2016a) Contribution to the knowledge on macrofungi in mangroves of the Southwest India. Plant Biosys 150:977–986

Ghate SD, Sridhar KR (2016b) Spatiotemporal diversity of macrofungi in the coastal sand dunes of Southwestern India. Mycosphere 7:458–472

Ghate SD, Sridhar KR (2016c) Macrofungi in coastal sand dunes and mangroves of Southwest India. In: Pullaiah T, Rani SS (eds) Biodiversity in India, vol 8. Regency Publications, New Delhi, pp 229–246

Ghate SD, Sridhar KR (2017) Bioactive potential of *Lentinus squarrosulus* and *Termitomyces clypeatus* from the southwestern region of India. Ind J Nat Prod Res 8:120–131

Greeshma AA, Sridhar KR, Pavithra M (2015) Macrofungi in the lateritic scrub jungles of southwestern India. J Thr Taxa 7:7812–7820

Greeshma AA, Sridhar KR, Pavithra M, Ghate SD (2016) Impact of fire on the macrofungal diversity of scrub jungles of Southwest India. Mycology 7:15–28

Greeshma AA, Sridhar KR, Pavithra M, Tomita-Yokotani K (2018) Bioactive potential of nonconventional edible wild mushroom *Amanita*. In: Gehlot P, Singh J (eds) Fungi and their role in sustainable development: Current perspectives. Springer Nature, Singapore, pp 719–738

Halbwachs H, Karasch P, Griffith GW (2013) The diverse habitats of *Hygrocybe:* peeking into an enigmatic lifestyle. Mycosphere 4:773–792

Hobbs C (1995) Medicinal mushrooms: an exploration of tradition, healing and culture. Botanica Press, Santa Cruz, CA

Hsu CL, Chen W, Weng YM, Tseng CY (2003) Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. Food Chem 83:85–92

Jagadish BR, Sridhar KR, Dattaraj HR (2019) Macrofungal assemblage with two tree species in scrub jungles of south-west India. Stud Fungi 4:72–82

Karun NC, Sridhar KR (2016) Spatial and temporal diversity of macrofungi in the Western Ghat forests of India. Appl Ecol Environ Res 14:1–21

Karun NC, Sridhar KR (2017) Edible wild mushrooms in the Western Ghats: Data on the ethnic knowledge. Data Brief 14:320–328

Karun NC, Sridhar KR, Niveditha VR, Ghate SD (2016) Bioactive potential of two wild edible mushrooms of the Western Ghats of India. In: Watson RR, Preedy VR (eds) Fruits, vegetables and herbs: bioactive foods in health promotion. Elsevier Inc, Oxford, pp 344–362

Kavishree S, Hemavathy J, Lokesh B, Shashirekha MN, Rajarathnam S (2008) Fat and fatty acids of Indian edible mushrooms. Food Chem 106:597–602

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, Perspective for value-added products and environments, vol 2. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Latha KPD, Manimohan P (2018) A new species of *Hygrocybe* from Kerala State, India. Phytotaxa 385:13–22

Leelavathy KM, Manimohan P, Arnolds EJM (2006) *Hygrocybe* in Kerala State, India. Personia 19:101–151

Machumi F, Samoylenko V, Yenesew A, Derese S, Midiwo JO, Wiggers FT et al (2010) Antimicrobial and antiparasitic abietane diterpenoids from the roots of *Clerodendrum eriophyllum*. Nat Prod Com 5:853–858

Maihara VA, Moura PLDC, Catharino MGM, Moreira EG, Castro LP, Figueira RCL (2012) Cadmium determination in *Lentinus edodes* mushroom species. Food Sci Technol 32:553–557

Mohanan C (2003) Mycorrhizae in forest plantations: association, diversity and exploitation in planting improvement. KFRI Research Report no. Peechi 252:1–133

Mohanan C (2011) Macrofungi of Kerala. KFRI Handbook no. 27, Kerala Forest Research Institute, Peechi, India, vol. 27, pp. 1-597

Nithya TG, Jayanthi J, Raghunathan MG (2016) Antioxidant activity, total phenol, flavonoid, alkaloid, tannin and saponin contents of leaf extracts of *Salvinia molesta* d.s. Mitchell (1972). Asian J Pharma Clin Res 9:200–203

Parekh J, Chanda S (2007) Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr J Biomed Res 10:175–181

Pavithra M, Sridhar KR, Greeshma AA, Karun NC (2016a) Spatial and temporal heterogeneity of macrofungi in the protected forests of Southwestern India. Int J Agric Technol 12:105–124

Pavithra M, Sridhar KR, Greeshma AA, Tomita-Yokotani K (2016b) Bioactive potential of the wild mushroom *Astraeus hygrometricus* in the southwest India. Mycology 7:191–202

Pavithra M, Sridhar KR, Greeshma AA (2018) Nutritional quality attributes of edible gasteroid wild mushroom *Astraeus hygrometricus*. In: Gehlot P, Singh J (eds) Fungi and their role in sustainable development: current perspectives. Springer Nature, Singapore, pp 367–382

Poumale HMP, Hamm R, Zang Y, Shiono Y, Kuete V (2013) Coumarins and related compounds from the medicinal plants of Africa. In: Kuete V (ed) Medicinal plant research in Africa, 1st edn. Elsevier, Oxford, pp 261–300

Pradeep CK, Vrinda KB (2007) Some noteworthy agarics from Western Ghats of Kerala. J Mycopathol Res 45:1–14

Pradeep CK, Joseph AV, Abraham TK, Vrinda KB (1996) *Hygrocybe punicea*: an edible mushroom new to India. Acta Bot Ind 24:103–104

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269:337–341

Rastegari AA, Yadav AN, Yadav N (2019) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5

Roe JH (1954) Chemical determination of ascorbic, dehydroascorbic and diketogluconic acids. In: Glick D (ed) Methods of biochemical analysis, vol 1. Inter Science Publishers, New York, pp 115–139

Rosset J, Bärlocher F, Oertli JJ (1982) Decomposition of conifer needles and deciduous leaves in two black Forest and two Swiss Jura streams. Int Rev Ges Hydrobiol 67:695–711

Safowora A (1993) Medicinal plants and traditional medicine in Africa. John Wiley and Sons Limited 2:96–106

Sankaran KV, Florence EJM (1995) Macrofungal flora and checklist of plant diseases of Malayattoor forests (Kerala). In: Mukherjee SK (ed) Advances in forestry research in India, vol 12. International Book Distributors, Dehra Dun, pp 147–168

Sanmee R, Dell B, Lumyong P, Izumori K, Lumyong S (2003) Nutritive value of popular wild edible mushrooms from northern Thailand. Food Chem 82:527–532

Senthilarasu G (2014) Diversity of agarics (gilled mushrooms) of Maharashtra, India. Cur Res Environ Appl Mycol 4:58–78

Senthilarasu G, Kumaresan V (2016) Diversity of agaric mycota of Western Ghats of Karnataka, India. Cur Res Environ Appl Mycol 6:75–101

Senthilarasu G, Kumaresan V, Singh SK (2010a) *Hygrocybe manadukaensis* sp. nov. in section *Firmae* from Western Ghats, India. Mycotaxon 114:343–349

Senthilarasu G, Kumaresan V, Singh SK (2010b) A new species of *Hygrocybe* in section *Firmae* from Western Ghats, India. Mycotaxon 111:301–307

Sharma BD, Deshpande S, Pradhan SG (1986) A new species of *Hygrocybe* Kummer from India. Bull Bot Sur India 28:171–173

Singh RP, Murthy CKN, Jayaprakasha GK (2002) Studies on antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro methods. J Agric Food Chem 50:81–86

Smith JE, Rowan N, Sullivan R (2002) Medicinal mushrooms: Their therapeutic properties and current medical usage with special emphasis on cancer treatments. Cancer Research, London

Soares MO, Alves RC, Pires PC, Oliveira MB, Vinha AF (2013) Angolan *Cymbopogon citratus* used for therapeutic benefits: Nutritional composition and influence of solvents in phytochemicals content and antioxidant activity of leaf extracts. Food Chem Toxicol 60:413–418

Sridhar KR (2018) Highlights on the macrofungi of south west coast of Karnataka, India. Int J Life Sci A 9:37–42

StatSoft (2008) Statistica version # 8. StatSoft Inc, Oklahoma, USA

Trease GE, Evans WC (1989) Pharmacognosy, 11th edn. Bailliere Tindall, London

Trease GE, Evans WC (2002) Pharmacognosy, 15th edn. Saunders Publishers, London

Vrinda KB, Pradeep CK, Pradeep NS, Abraham TK (1995) New records of *Hygrocybe* from India. J Econ Taxon Bot 19:576–584

Vrinda KB, Pradeep CK, Abraham TK (1996a) Agaricales from Western Ghats–III. Mush Res 5:47–50

Vrinda KB, Pradeep CK, Pratheep NS, Abraham TK (1996b) Agaricales from Western Ghats—I. J Mycopathol Res 34:119–123

Vrinda KB, Pradeep CK, Mathew S, Abraham TK (1997) Agaricales from Western Ghats—V. Mush Res 6:7–10

Vrinda KB, Pradeep CK, Varghese SP (2009) Noteworthy records of *Hygrocybe* section Firmae from Western Ghats of Kerala. Mush Res 18:1–4

Vrinda KB, Shibu P, Varghese P, Pradeep CK (2013) *Hygrocybe rubida*, a new species from Kerala State, India. Mycoscience 54:271–273

Yadav AN, Kour D, Rana KL, Yadav N, Singh B, Chauhan VS et al (2019a) Metabolic engineering to synthetic biology of secondary metabolites production. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 279–320. https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Yadav AN, Mishra S, Singh S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 1: Diversity and enzymes perspectives. Springer International Publishing, Cham

Chapter 18
Fungal Biopharmaceuticals: Current Research, Production, and Potential Applications

Amir Hosein Askari Pour, Fatemeh Rahmani, Masoomeh Shams-Ghahfarokhi, Mohammadhassan Gholami-Shabani, Fatemehsadat Jamzivar, and Mehdi Razzaghi-Abyaneh

Contents

A. Askari Pour
Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

Department of Basic Sciences, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

F. Rahmani · M. Gholami-Shabani · F. Jamzivar · M. Razzaghi-Abyaneh (✉)
Department of Mycology, Pasteur Institute of Iran, Tehran, Iran
e-mail: mrab442@pasteur.ac.ir

M. Shams-Ghahfarokhi
Department of Mycology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_18

18.1 Introduction

By defining biopharmaceuticals as therapeutic agents constructed by living organisms, the importance of fungal products is a remarkable point to expand study and researches upon them. Fungi can be described as eukaryotic organisms that produce spore to proliferate and wait for the desired condition to reproduce as fast as possible (Razzaghi-Abyaneh et al. 2015). Fungi live in different conditions from soil to deep water and could have a symbiotic relationship with variable organisms like plants that have made them very special and numerous among eukaryotic organisms. A metabolite can be classified into two groups of primary and secondary

where the 1st one is essential for the growth and development of the organism and the 2nd one is part of the survival defensive system (Razzaghi-Abyaneh and Rai 2013; Razzaghi-Abyaneh et al. 2014). If the therapeutic effects of these metabolites can prove with bioassays, they will be called bioactive metabolites as well. By the isolation and structural identification of these bioactive metabolites, they will be a fundamental base of further pharmaceutical procedures to novel drugs (Kensy et al. 2009). Even though there are differences between the extraction methods but the procedures are almost similar to each other. Once the fungi separate from their host, identification and separation of diverse strains will follow. To distinguish the metabolites several procedures may perform such as thin-layer chromatography, vacuum/high-performance liquid chromatography, and size exclusion chromatography (Zuckerkandl and Pauling 1965).

Fungal productions have gained ground as third biopharmaceuticals by 20% of total products (Kensy et al. 2009; Martínez et al. 2012; Gholami-Shabani et al. 2019; Seyedjavadi et al. 2019). The trace of fungi by humans is noticeable from pharaohs to Chinese traditional medicine but in last decades; by a progression of biotechnology vast valuable products such as anticancerous, antimicrobial agents, vitamin and amino acids, vaccines, immunoregulatory agents and anti-oxidants, probiotics and enzymes have been obtained from fungi as biopharmaceutical products. Identification of secondary metabolites of fungi is expanding and their eventual therapeutic role is under investigation and by considering the 0.3 hit ratio of being commercialized more than 2000 new pharmaceuticals by fungi is going to be discovered (Sneader 2005). Here we discuss about valuable fungal and fungal-like organisms from different genera and species especially those belong to *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Trichoderma,* and *Streptomyces* and their bioactive metabolites that have biopharmaceutical applications.

18.2 Antimicrobials from Fungi

Since 1928 by the discovery of penicillin, antibiotic production by fungi has been considered as a natural source of obtaining or a valuable inspiration for designing further pharmacologically active compounds against mankind's health by saving a large number of lives and increase in life expectancy. Even though many antibiotics have originated from Actinomycetes, *Aspergillus* and *Penicillium* species are the next two important sources of antibiotics. About 80% of antibiotics are produced by two genus *Streptomyces* and Actinomycetes. The spread of antibiotic resistance is raising danger upon human lives so that discovering and developing new antibiotics are fundamentally necessary. So that natural products are considered as a probable right therapeutic solution to this problem. The pharmaceuticals are produced from secondary metabolites or derived from secondary metabolites or by biotechnological procedures. Table 18.1 summarizes natural or derived antimicrobial drug and compounds from fungi distributed in cephalosporins (Fig. 18.1) and penicillins (Fig. 18.2).

Table 18.1 Natural antimicrobial drugs and compounds of fungal origin

Compound	Chemical group	Therapeutic index	Pharmacological effect	Commercial name	Producing organism
Cephalexin	Cephalosporins, 1st generation	Genitourinary tract, bone, skin, and respiratory tract infections, cellulitis and otitis media	Binds to one or more of the penicillin-binding proteins, which in turn inhibits synthesis of bacterial cell wall	Keflex, Panixine Disperdose	*Cephalosporium acremonium*
Cefazolin	Cephalosporins, 1st generation	Moderate to severe infections, mild infections with gram + Cocci, Uncomplicated UTI	A semisynthetic compound that binds to penicillin-binding sites to arrest cell wall synthesis and inhibition of bacterial replication	Kefzol, Ancef	*Cephalosporium acremonium*
Cephalothin	Cephalosporins, 1st generation	Parentally administrated during surgery	Binds to one or more of the penicillin-binding proteins that inhibits cell wall creation procedure		*Cephalosporium acremonium*
Cefaclor	Cephalosporins, 2nd generation	Lower respiratory tract infections, otitis media, skin and urinary tract infections, bronchitis, and pharyngitis	Binds to one or more of the penicillin-binding proteins, which in turn inhibits synthesis of bacterial cell wall Gram + and activity against *E. coli*, *H. influenza*, *Klebsiella pneumonia*	Ceclor, Raniclor	*Cephalosporium acremonium*

(continued)

Table 18.1 (continued)

Compound	Chemical group	Therapeutic index	Pharmacological effect	Commercial name	Producing organism
Cefuroxime axetil	Cephalosporins 2nd generation	Pharyngitis, sinusitis, bronchitis, skin infections, Uncomplicated UTI—Gonorrhea and severe to complicated infections	Binds to one or more of the penicillin-binding proteins, which in turn inhibits synthesis of bacterial cell wall Beta-lactamase inhibitor	Ceftin, Zinacef	*Cephalosporium acremonium*
Cefotaxime	Cephalosporins, 3rd generation	Gonococcal urethritis, preparation for surgery	Inhibiting the final transpeptidation step of peptidoglycan synthesis resulting in cell wall death Beta-lactamase resistance	Claforan	*Cephalosporium acremonium*
Ceftriaxone	Cephalosporins, 3rd Generation	Intra-abdominal, skin and soft necrotizing and prosthetic joint infections—meningitis	Inhibition of cell wall synthesis and interfering with the synthesis of peptidoglycan Beta-lactamase resistant Gram + Broad-spectrum while lower efficacy against gram – bacteria	Rocephin	*Cephalosporium acremonium*
Cefepime	Cephalosporins, 4th Generation	Pneumonia, febrile neutropenia, UTI, skin and intra-abdominal infections	A zwitterion that rapidly penetrates Gram – cells	Maxipime	*Cephalosporium acremonium*
Ceftaroline	Cephalosporins, 5th generation	Skin and skin structure infections, Community-acquired bacterial pneumonia	Beta-lactam cephalosporin with activity against aerobic & anaerobic Gram-positive and aerobic Gram-negative bacteria	Teflaro	*Cephalosporium acremonium*

(continued)

Table 18.1 (continued)

Compound	Chemical group	Therapeutic index	Pharmacological effect	Commercial name	Producing organism
Dicloxacillin	Penicillins	Infections	Binds to one or more of the penicillin-binding proteins, which in turn inhibits synthesis of bacterial cell wall		*Penicillium notatum*
Ampicillin	Penicillins	Genitourinary tract infections, gonorrhea, respiratory tract infections, bacterial meningitis	Interfering cell wall synthesis during replication	Ampi, Omnipen, Penglobe	*Penicillium notatum*
Amoxicillin	Penicillins	Ear, nose, & throat infections, genitourinary tract infections, *H. pylori* infection, anthrax		Amoxil – Moxatag - Trimox	*Penicillium notatum*
Ticarcillin	Penicillins		Inhibiting biosynthesis of cell wall mucopeptide anti-pseudomonal penicillin plus beta-lactamase inhibitor that provides coverage against most gram +/− and anaerobes	Ticar	*Penicillium notatum*
Piperacillin	Penicillins	UUTI, CAP, moderate infections, uncomplicated gonorrhoea, *Pseudomonas* infections	Inhibiting biosynthesis of cell wall mucopeptide Anti-pseudomonal activity	Piperacillin	*Penicillium notatum*
GT-1 (54) (LCB10 0200)	Cephalosporin siderophore (NP)		PBP (cell wall)		*Cephalosporium acremonium* Under clinical trial

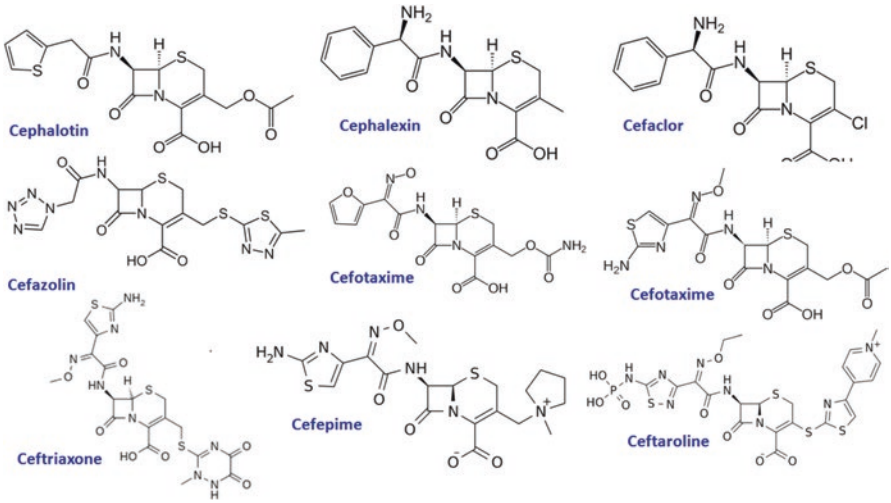


Fig. 18.1 Chemical structure of semisynthetic cephalosporins obtained from *Cephalosporium acremonium*

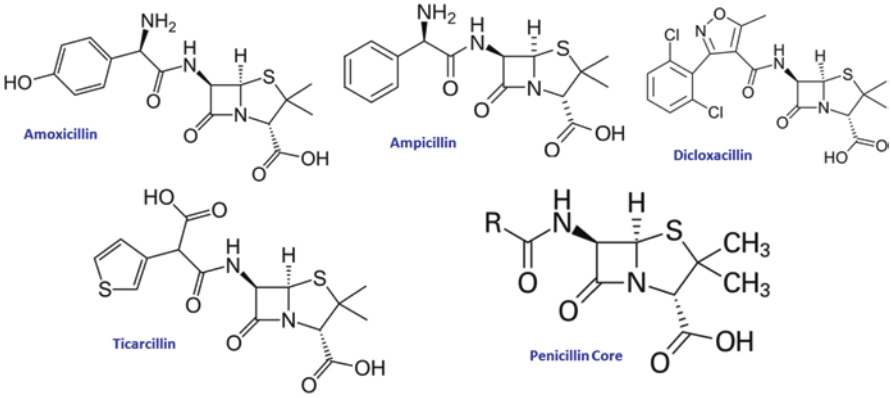


Fig. 18.2 Chemical structure of natural and semisynthetic penicillins obtained from *Penicillium notatum*

18.2.1 Cephalexin

As the 1st generation of cephalosporin classification, cephalexin ($C_{16}H_{17}N_3O_4S$) was approved by the Food and Drug Administration (FDA) in 1979. This drug was discovered from sewer examination of Giuseppe Brotzu from *Cephalosporium acremonium* (Crawford et al. 1952) and isolation by Abraham and Newton upon

N-acylation of the side chain and adding d-phenylglycyl to make a semisynthetic drug. By the famous commercial name of keflex, cephalexin is available in dosage forms of capsules (250, 500, and 750 mg), oral suspensions (125 mg/5 mL and 250 mg/5 mL), and tablets of 250 and 500 mg. This antibiotic has 90% of bioavailability which may take 1 h to reach its peak level in serum. Cephalexin's chemical structure has consisted of a beta-lactam and dihydrothiazine which binds to one or more penicillin-binding proteins, inhibiting the synthesis of the bacterial cell wall. Produced at the rate of 4000 tons per year worldwide, cephalexin is one of the most commonly prescribed medications in the world (Demain 2014).

18.2.2 Cefazolin

As the 1st generation of cephalosporins that interact with bacterial cell wall cefazolin ($C_{14}H_{14}N_8O_4S_3$) is one of the safest and high efficient antibiotics, reported by the World Health Organization (WHO 2019). Likewise other cephalosporins, its beta-lactam ring has been fused to six-membered ring containing sulfur and nitrogen making this semisynthetic drug bactericidal as well. Cefazolin is a parental antibiotic which powders are available from 500 mg to 300 g of active pharmaceutical ingredient. By having 86% of protein bonding it can reach its peak plasma level in 5 min if using intravenously. While having the poor capacity to cross the blood–brain barrier it is an active antibacterial against skin flora involving *S. aureus*.

18.2.3 Cephalothin

Cephalothin ($C_{16}H_{16}N_2O_6S_2$) is the earliest member of the cephalosporin family that is a parental, semisynthetic beta-lactam antibiotic. Like other members of the 1st generation, it is active against Gram-positive bacteria. Cephalothin provides its effectiveness by binding to penicillin-binding proteins (PBP) which is crucial in the final stages of assembling bacterial cell walls.

18.2.4 Cefaclor

As 2nd generation of the cephalosporin family this bactericidal antibiotic inhibits the cell wall synthesis by binding to PBP. While classified as a group, the 2nd generations have less Gram-positive effects while showing more Gram-negative effects against *Proteus mirabilis*, *Haemophilus influenza*, *Escherichia coli*, and *Klebsiella* sp. Cefaclor ($C_{15}H_{14}C_lN_3O_4S$) achieves its peak level concentration for about 1 h. It

is available in 250/500 mg capsules and 500 mg tablets as well. Cefaclor's structure presents chloro- and 2-amino-2-phenylacetamido groups at 3 and 7 positions of cephem structure.

18.2.5 Cefuroxime axetil

While providing oral and parental use, cefuroxime ($C_{20}H_{22}N_4O_{10}S$) is the only member of the 2nd generation of cephalosporins that can, respectively, pass the blood–brain barrier (BBB), and wildly spreads to cerebrospinal fluid (CSF) which could be used to treat meningitis. Cefuroxime reaches its peak plasma concentration from 3 min to 3 h upon the route of administration. Oral suspensions of 125 and 250 mg/5 mL plus injection powders of 750 mg to 225 g and 250 and 500 mg tablets provided desirable dosing forms and strengths to the drug.

18.2.6 Cefotaxime

It is a semisynthetic member of the 3rd generation of cephalosporins having bactericidal activity. By interfering with the final steps of transpeptidation needed for making cross-links among bacterial cell walls, cefotaxime ($C_{16}H_{17}N_5O_7S_2$) provides broad-spectrum activity against Gram-positive and Gram-negative bacteria. The specific distribution of drugs has made it proper in the therapy of bone, CSF, prostatic, and humor tissues. Parental solutions and injections are available from 20 mg/mL to 10 g powder of API.

18.2.7 Ceftriaxone

Having the chemical formula of ($C_{18}H_{18}N_8O_7S_3$), ceftriaxone is the 3rd generation of bactericidal cephalosporins that binds to PBP which may lead to bacterial lysis. Like other members of this group, the drug can penetrate the central nervous system bypassing the blood–brain barrier resulting in being active against pneumococci, meningococci, and penicillin-resistant *Neisseria gonorrhea*. Ceftriaxone will reach its peak plasma level 2–3 h while administrated as intramuscular (IM). Injectable drug solution and powder strengths provide 1 g/mL to 100 g of powder fighting against most Gram-negative bacteria.

18.2.8 Cefepime

4th generation is described as extended-spectrum cephalosporins that are active against *Enterobacter* spp., *Pseudomonas* spp., *Serratia marcescens*, and *Citrobacter* spp. Cefepime ($C_{19}H_{24}N_6O_5S_2$) was developed in 1987 that is the best beta-lactam against IM use by having complete absorption, on the other hand having poor penetration from blood–brain barrier (BBB) that makes it ineffective in meningitis. Cefepime has 1 and 2 g of powder and infusion solution of dosing forms and strengths.

18.2.9 Ceftaroline

While classically controversial, ceftaroline ($C_{22}H_{21}N_8O_8PS_4$) is considered as 5th generation of cephalosporins. Specific roles of ceftaroline have made it a powerful compound against antibiotic-resistant bacteria in vivo such as methicillin-resistant *Staphylococcus aureus* (MRSA) strains, while showing in vitro activity against linezolid-resistant *S. aureus* and vancomycin-resistant *S. aureus*. The drug has 400 and 600 mg vials which need 1 h to reach plasma levels.

18.2.10 Dicloxacillin and a Flashback to Penicillin History

After the discovery of penicillin by Fleming by *Penicillium notatum* of a plate mold, the antibiotic era started and, respectively, followed by Howard Flory and Ernst Chain. Moreover, penicillin was successfully tested on animals (1940) and the first adult patient (1942). Although natural penicillins cured diseases, by the emerging of bacterial resistance to natural penicillins (Penicillin G) researches of developing semisynthetic penicillins followed by isolation of 6-aminopenicillanic acid resulted in other penicillins that can be sub-classified by the spectrum of activity. The narrow-spectrum penicillin class involves penicillin V and G, procaine penicillin, and benzathine penicillin. The penicillinase-resistant penicillins or anti-staphylococcal penicillins are consisting of oxacillin, cloxacillin, methicillin, and dicloxacillin. In short, therapeutic index of this group is limited to methicillin-sensitive *S. aureus*. Subsequently, to other penicillins, dicloxacillin ($C_{19}H_{17}C_{l2}N_3O_5S$) makes its antibacterial effect by binding to PBP sites and representing as bactericidal by inhibition of peptidoglycan cross-linking of the cell wall that activates cell wall autolysis. Besides this 250/500 mg capsules have 96% of protein bonding and can reach their peak plasma level in 0.5–2 h.

18.2.11 Ampicillin

The development of broad-spectrum penicillin with desirable bioavailability and oral intake led to the synthesis of methicillin and ampicillin ($C_{16}H_{19}N_3O_4S•3H_2O$). On the contrary to amoxicillin, ampicillin can be used when the potential for oral intake is not available. The ampicillin has poor protein bonding (15–25%) and 30–40% of bioavailability by 1–2 h of reaching to peak plasma level. Additionally, it has 250/500 mg capsules, 125/250 mg/5 mL oral suspensions, and 125 mg to 10 g powders for injection.

18.2.12 Amoxicillin

Amoxicillin ($C_{16}H_{19}N_3O_5S$) is a derivative of ampicillin with the same antibacterial spectrum. On the other hand, it has a broader spectrum than penicillins with greater bioavailability while being well stable against digestive gastric acid. From a chemical view, it is penicillin in which there is a substituent of 2-amino-2-(4-hydroxyphenyl) acetamido group attached to position 6 of penam. While discovered in 1958, amoxicillin is one of the most common and universally antibiotics according to WHO. In the same way, it is one of the most prescribed drugs for children as well.

18.2.13 Ticarcillin

Alongside piperacillin, it is the next generation of penicillins naming extended-spectrum penicillins with the molecular formulation of ($C_{15}H_{16}N_2O_6S_2$).

18.3 Anticancer Compounds by Fungi

In addition to numerous drugs and compounds with anticancer effects, natural sources compounds are frontiers among chemotherapeutic agents. Fungal metabolites and natural products are under investigation to find novel medicines curing cancers. To elaborate survival and improving the quality of life of cancerous patients, developing biotechnological based drugs, and designing analogs of natural metabolites is an urgent necessity. In Table 18.2, a summary list of drugs and compounds by pharmacological effect and therapeutic index is sorted. Chemical structures of these compounds are shown in Fig. 18.3.

Table 18.2 Natural anticancer drugs and compounds of fungal origin

Compound	Chemical group	Therapeutic index	Pharmacological effect	Commercial name	Producing organism	Reference
Doxorubicin	Anthracycline	Breast, ovary, prostate, stomach, thyroid, small cell cancer of lung, liver, squamous cell cancer of head and neck, multiple myeloma, Hodgkin's disease, lymphoma, ALL, AML	Blocking an enzyme called topoisomerase 2	Rubex, Adriamycin	*Streptomyces peucetius*	Kornienko et al. (2015)
Daunorubicin	Anthracycline	Acute lymphocytic and non-acute lymphocytic leukemia	Inhibits topoisomerase II	Cerubidine	*Streptomyces peucetius*	Kornienko et al. (2015)
Mitomycin C		Stomach cancer, pancreas cancer, anal carcinoma	Inhibits the synthesis of deoxyribonucleic acid (DNA)	Mitosol, Mutamycin, Jelmyto	*Streptomyces caespitosus or Streptomyces lavendulae*	Kornienko et al. (2015)
Vincristine	Vinca alkaloid	Acute leukemia, Hodgkin's disease, non-Hodgkin's malignant lymphomas, rhabdomyosarcoma, neuroblastoma, Wilms tumor	Destroys microtubules in cells primarily by inhibition of microtubule polymerization	Oncovin, Vincasar	*Fusarium oxysporum* isolated from *Catharanthus roseus*	Kornienko et al. (2015)
Vinblastine	Vinca alkaloid	Testicular CA. squamous cell CA of head & neck, Hodgkinson Dz, kaposi's sarcoma, histiocytic lymphoma, mycosis fungoid, & Letterer–Siwe disease (histiocytosis X)	Destroys microtubules in cells primarily by inhibition of microtubule polymerization	Alkaban-AQ, Velban	*Fusarium oxysporum* isolated from *Catharanthus roseus*	Kornienko et al. (2015)
Paclitaxel	Natural taxane	Kaposi's sarcoma and cancer of the lung, ovarian, and breast. metastatic breast cancer and locally advanced or metastatic non-small cell lung cancer	Prevent depolymerization of cellular microtubules	Taxol	*Penicillium raistrickii*	Wani et al. (1971)
DothioreloneF	Polyketide	RAJI cell	IC_{50}: 2 μg/mL		*Dothiorella* sp.	Du and Su (2014)

(continued)

Epicocconigrone A	Polyketides	RAJI cell	50% inhibition of proliferation by 72 h at 5 μM and 30% cell death by 72 h at 25 μM		*Epicoccum nigrum*	Papich (2016)
PM181110	Peptides	40 human cancer cell lines 24 human tumor xenografts	IC_{50}:0.089 μM 0.245 μM		*Phomopsis glabrae*	Verekar et al. (2014)
Myrotheciumone A	Lactones	HepG2, SMMC-7721, A549, MCF-7, QSG-7701, HL-7702	IC_{50}: 5.36, 6.56, 5.88, 7.56, 16.30, 20.69 μM		*Myrothecium roridum*	Lin et al. (2014)
Phomopsidone A	Lactones	MDA-MB-435	IC_{50}:63 μM		*Phomopsis* sp. A123	Zhang et al. (2014)
3-epi-Waol A	Lactones	MCF-7, HCT116, H460	IC_{50}:22.46, 6.20, 1.0 mM		*Libertella blepharis*	Adames et al. (2015)

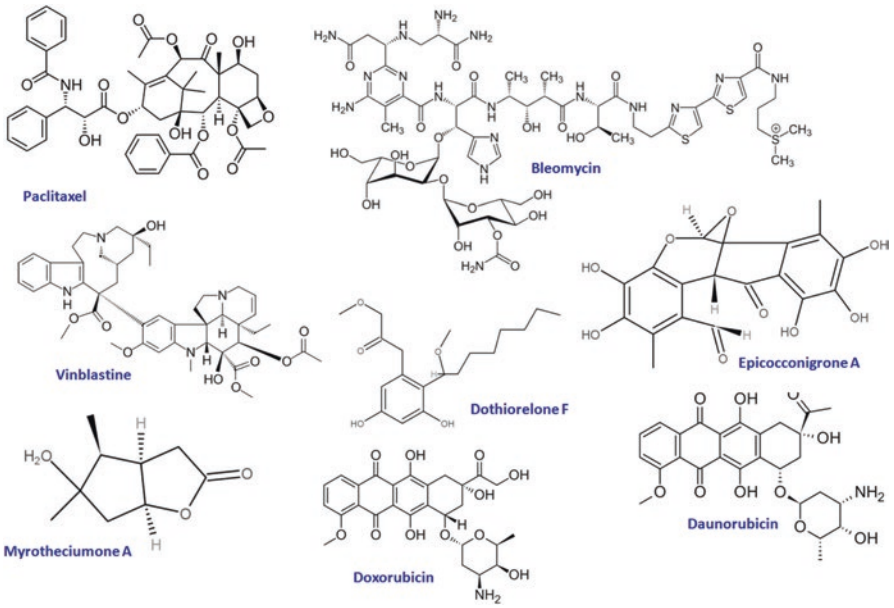


Fig. 18.3 Chemical structure of natural or semisynthetic anticancer drugs and compounds of fungal origin

18.3.1 Doxorubicin

Doxorubicin is an anthracycline antibiotic that is derived from the actinobacterium *Streptomyces peucetius*. Doxorubicin ($C_{27}H_{29}NO_{11}$) is used in the treatment of many human cancers with dosage form of injectable solution (2 mg/mL) and powder for injection (10 and 20 mg), that predominantly metabolize by liver with half-life of 1–3 h. This drug is a cell cycle-nonspecific agent that acts by blocking topoisomerase II activity (Thirumaran et al. 2007). This enzyme is responsible for DNA functions. When topoisomerase is inhibited, the DNA segments cannot perform transcription, leading to breaks in the DNA strands and cell death (Papich 2016).

18.3.2 Daunorubicin

Daunorubicin ($C_{27}H_{29}NO_{10}$) is an anthracycline antibiotic derived from the actinobacterium *Streptomyces peucetius* that is used in the treatment of acute leukemia, lymphoma, and breast cancer (Găman et al. 2020). The dosage forms are injectable solution (5 mg/mL) and powder for injection (20 mg) which metabolized by liver to

change to daunorubicinol. It has strong cytotoxicity against tumor cells by inhibition of topoisomerase II activity like doxorubicin.

18.3.3 Mitomycin

Mitomycin C is an alkylating agent derived from *Streptomyces caespitosus* that is used in stomach cancer, pancreas cancer, anal carcinoma (Crooke and Bradner 1976). Dosage form is just powder for injection (5, 20, and 40 mg) and metabolites by liver. The mechanism of action is inhibition of deoxyribonucleic acid (DNA) synthesis to prevent replication in transcription.

18.3.4 Bleomycin

Bleomycin ($C_{55}H_{84}N_{17}O_{21}S_3^+$) belongs to a subfamily of glycopeptide antibiotics which derived from *Streptomyces caespitosus*. It was first approved by the FDA for squamous cell carcinomas, malignant lymphomas, and testicular cancer treatment. Nowadays it has indications such as germinal cell tumors, gestational trophoblastic disease, Hodgkin lymphoma, and non-Hodgkin lymphoma treatments. Besides these dosage forms as 15 unit and 30 unit powder for injections have 1% of protein bonding and can reach their peak plasma level in 2 h. The mechanism of action is the induction of DNA strand breaks by making oxidative metallobleomycin complexes.

18.3.5 Vincristine

Vincristine ($C_{46}H_{56}N_4O_{10}$) **and vinblastine** ($C_{46}H_{58}N_4O_9$) are vinca alkaloid antimitotic drugs that are derived from *Fusarium oxysporum*. Their indications are treatment of many cancers which are available in table. Half-life of vincristine changes from 10.5 to 155 h which is triphasic in vinblastine as 4 min to 24.8 h. Inhibition of microtubule polymerization in M and S phases that destroys microtubules in cells primarily is the mechanism of these drugs (Beaver et al. 2018; Kong et al. 2018; Farrar and Jacobs 2020; Li et al. 2020).

18.3.6 Paclitaxel

Paclitaxel ($C_{47}H_{51}NO_{14}$) as a member of the taxane family is derived from *Penicillium raistrickii*. It is known as the most successful natural anticancer drug available. Although other antineoplastic agents prevent the association of tubulin

into microtubules, paclitaxel promotes that association. It blocks the progress of the cell cycle, prevents mitosis, and inhibits the growth of cancer cells (Weaver 2014). Indications of this drug are Kaposi's sarcoma and cancer of the lung, ovarian, and breast, metastatic breast cancer and locally advanced or metastatic non-small cell lung cancer (Farrar and Jacobs 2020).

18.3.7 Polyketides

Dothiorelone F ($C_{18}H_{28}O_5$) is a polyketide that has been isolated from endophytic fungus *Dothiorella* sp. obtained from the bark of *Aegiceras corniculatum*. This compound significantly inhibits RAJI cancer cell line with IC_{50} values 2 μg/mL (Du and Su 2014).

Epicocconigrone A ($C_{18}H_{14}O_9$) as another polyketide has been isolated from an endophytic fungus *Epicoccum nigrum* obtained from leaves of *Mentha suaveolens*. According to research, this compound inhibits 50% of proliferation by 72 h at 5 μM and 30% cell death at 25 μM (Amrani et al. 2014).

18.3.8 Peptides

PM181110 is a depsipeptide that has been isolated from an endophytic fungus *Phomopsis glabrate* that was in the leaves of the plant *Pongamia pinnata* (family Fabaceae). It was effective against different types of human cancer cell lines with an IC_{50} value of 0.089 μM and a mean IC_{50} value of 0.245 μM into 24 human tumor xenografts.

18.3.9 Lactones

Myrotheciumone A ($C_9H_{14}O_3$) belongs to the lactone class that is derived from the endophytic fungus *Myrothecium roridum* in *Ajuga decumbens* plant. It has a cytotoxicity effect against HepG2, SMMC-7721, A549, MCF-7, QSG-7701, HL-7702 with IC_{50} values of 5.36, 6.56, 5.88, 7.56, 16.30, 20.69 μM (Lin et al. 2014). Another lactone compound is phomopsidone A from endophytic fungus *Phomopsis* sp A123 in mangrove plant, *Kandelia candel*. It shows a cytotoxicity effect against the MDA-MB-435 cell line with an IC_{50} value of 63 μM. 3-epi-Waol A as a lactone compound has been derived from endophytic fungus *Libertella blepharis* in *Oryza latifolia* plant. 3-epi-Waol A demonstrated cytotoxicity MCF-7, H460 and HCT116, cancer cell lines with IC_{50} values of 22.46, 6.20, and 1.0 μM.

18.4 Lipid-Lowering Agents

Statins are a group of compounds that lower the plasma level of cholesterol by inhibition of 3-HMG-CoA reductase. This enzyme has a fundamental role in the production of cholesterol. To chemically summarize statins are a group of organic acids that interact by their acidic side to the enzyme. Views show lovastatin can be produced from two organisms, *Aspergillus terreus* and *Monascus ruber.* As an example of a commercial procedure, lovastatin is obtained from two fermentation procedures: Liquid fermentation (*Aspergillus terreus*) and solid-state fermentation (*Monascus ruber*). An equally important note in statins is their efficacy which is different from each other as affinity to enzyme active site varies from compound to compound (Table 18.3). Chemical structures of these compounds are shown in Fig. 18.4.

18.4.1 Lovastatin

Lovastatin ($C_{24}H_{36}O_5$), the first approved statin from FDA has been derived from *Aspergillus terreus*. Love protein in this fungus convinces the correct assembly of the nonaketide chain in lovastatin. The mechanism of hypocholesterolemic activity of statins is competitive inhibition of HMG-CoA reductase (Manzoni and Rollini 2002). Mevastatin as the first discovered statin is produced by *Penicillium citrinum*. The mechanism of this drug is lovastatin. Pravastatin is a carboxylic acid compound that can be obtained by the biotransformation of mevastatin by *Streptomyces carbophilus* (Manzoni and Rollini 2002).

18.5 Immunosuppressant Agents

Numerous immunosuppressive agents have been obtained from fungi. Cyclosporine and tacrolimus both affect as calcineurin inhibitor, a eukaryotic gene that regulates an organism's ability to react upon environmental changes and responding to environmental stress. Another essential point is the synergy effect of cyclosporine and tacrolimus with antifungal drugs which may end to enormous fungicidal action even with drug-resistant organisms (Table 18.4). Chemical structures of these compounds are shown in Fig. 18.5.

18.5.1 Cyclosporin

Cyclosporin ($C_{62}H_{11}N_{11}O_{12}$) is a natural cyclic polypeptide immunosuppressant that has been derived from *Tolypocladium inflatum* and *Beauveria nivea* (De Smet and Nussenblatt 1993).Dosage forms are capsule (25, 50, and 100 mg), oral solution

Table 18.3 Natural lipid-lowering drugs and compounds from fungi

Compound	Chemical group	Therapeutic index	Pharmacological effect	Commercial name	Producing organism	Reference
Lovastatin	Statins	Hypercholesterolemia	HMG-CoA reductase inhibitor	Mevacor, Altocor	*Aspergillus terreus*	Manzoni and Rollini (2002)
Mevastatin	Statins	Hypercholesterolemia	HMG-CoA reductase inhibitor	Mevastan, Mevasterol, Mevastin, Mevastina Mevatorte, Mevinacor, Compactin	*Penicillium citrinum*	Manzoni and Rollini (2002)
Pravastatin	Statins	Hypercholesterolemia	HMG-CoA reductase inhibitor	Pravachol, Selektine	*Streptomyces carbophilus*	Manzoni and Rollini (2002)

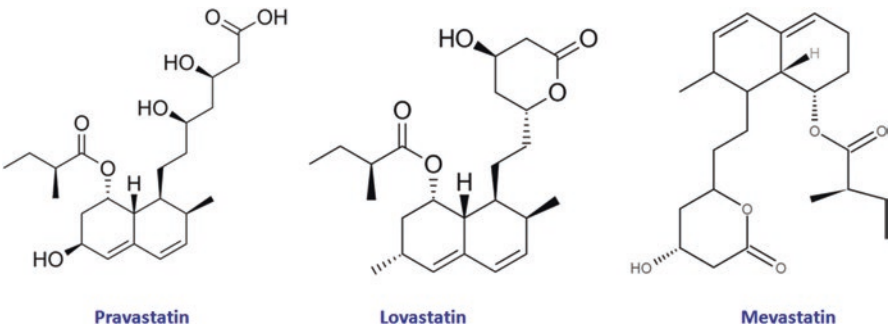


Fig. 18.4 Chemical structure of natural lipid-lowering drugs and compounds obtained from fungi

(100 mg/mL), and injectable solution (50 mg/mL) with protein bound of 90% that are metabolized to AM1AM9 and AM4N by liver. This drug suppresses cellular and humoral immunity (mainly T cells) via calcineurin inhibitor mechanism of action (Nakamura et al. 1993).

18.5.2 Tacrolimus

Another calcineurin inhibitor drug is tacrolimus ($C_{44}H_{69}NO_{12}$) that has been derived from *Streptomyces tsukubaensis*. Immediate release capsules, extended-release capsules and tablets, injectable solution and granules for oral suspension are the dosage forms of tacrolimus to prophylaxis of heart, liver, and kidney transplant rejection. Tacrolimus has reach protein bonding (99%) and different bioavailability in children (7–55%) and adults (7–33 %) by 0.5–6 h of reaching to peak plasma level.

18.5.3 Fingolimod

Fingolimod ($C_{19}H_{33}NO_2$) is as an analog of Sphingosine-1-phosphate approved by the FDA in 2010 for the treatment of relapsing forms of multiple sclerosis (MS) (Ward et al. 2016). It was derived from *Isaria sinclairii* (Chiba 2020). Sphingolipids have an important role in cell signaling. Fingolimod phosphorylates to fingolimod phosphate that binds to the S1P receptor and acts as an antagonist (Oo et al. 2007). This process causes the lymphoid tissue to maintain central memory T cells, preventing them from entering into the blood (Brinkmann 2009). Only one dosage form of this drug is available as (0.25 mg, 0.5 mg) capsule with reach bioavailability (93%) that can reach its peak plasma level in 12–16 h.

Table 18.4 Natural immunosuppressant drugs and compounds from fungi

Compound	Chemical group	Therapeutic index	Pharmacological effect	Commercial name	Producing organism	Reference
Cyclosporine	Natural cyclic polypeptide	Solid-organ transplant, rheumatoid arthritis, atopic dermatitis, chronic transplant rejection, psoriasis	Calcineurin inhibitor	Gengraf, Neoral, Sandimmune	*Tolypocladium inflatum, Beauveria nivea*	Döhren and Kleinkauf (1999)
Tacrolimus	Polyketide	Heart transplant rejection, kidney transplant rejection, liver transplant rejection, psoriasis	Inhibiting calcineurin phosphatase activity	Protopic, Prograf	*Streptomyces tsukubaensis*	Martínez-Castro et al. (2013)
Fingolimod		Relapsing multiple sclerosis (RMS)	Sphingosine 1-phosphate receptor modulator	Gilenya	*Isaria sinclairii*	Chiba (2020)
Mycophenolic acid	Benzofurans	Kidney transplant rejection, heart transplant rejection, liver transplant rejection, lupus nephritis	Inhibition of T- and B-cell proliferation, as well as antibody production	Cell Cept	*Penicillium brevicompactum*	Vinokurova et al. (2005)
(−) Mycousnine	Dibenzofuran	Novel effective immunosuppressant	Inhibition of T cell proliferation, suppressed expression of the surface activation antigens CD25 and CD69		*Mycospaerella nawae* ZJLQ129	Wang et al. (2017)
Curtachalasins 1&10	–	Selective inhibition on B-cell proliferation, selective inhibition on T cell proliferation	IC_{50}: 2.42 μM IC_{50}: 12.15 μM		*Xylaria* cf. *curta*	Wang et al. (2019)
Fusaperazine F	Diketopiperazine	Cytotoxicity against K562 cells	IC_{50}: 12.7 μM		*Penicillium crustosum* HDN153086	Liu et al. (2019)

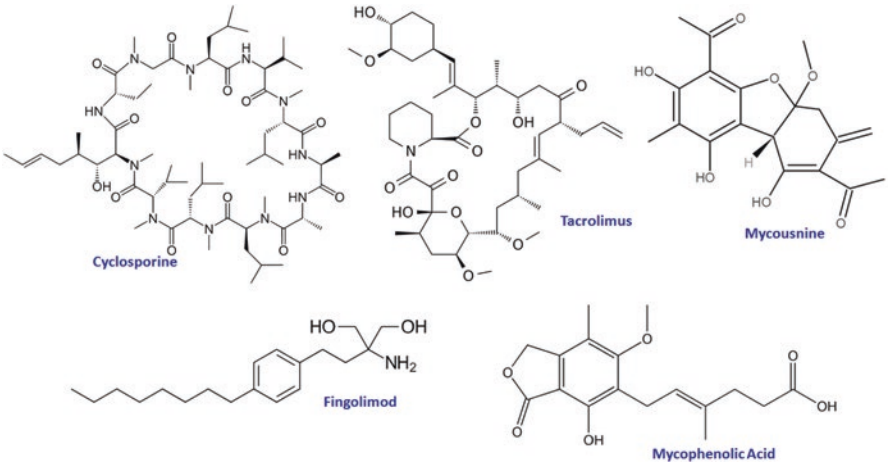


Fig. 18.5 Chemical structure of natural immunosuppressant drugs and compounds obtained from fungi

18.5.4 Mycophenolic Acid (MPA)

Mycophenolic acid (MPA) ($C_{17}H_{20}O_6$) is one of the benzofurans immunosuppressive drugs most used worldwide (Ferreira et al. 2020). Because of its low solubility the pro-drug mycophenolate mofetil (MMF) and an enteric-coated mycophenolate sodium salt (EC-MPS) are used in oral administration (Nowak and Shaw 1995; Shaw et al. 2001; Tönshoff et al. 2011). It is a selective, reversible inosine monophosphate dehydrogenase (IMPDH) inhibitor that inhibits guanine synthesis to limit lymphocyte proliferation to avoid allograft rejection of the organ (Korecka and Shaw 2009).

18.5.5 Mycousnine Enamine

Mycousnine enamine ($C_{19}H_{20}O_8$) is an amide derivative as a new candidate for developing immunosuppressive agent produced by an endophytic fungus, *Mycosphaerella nawae* (ZJLQ129). This selective T cell inhibitor compound suppresses the expression of the surface activation antigens CD25 and CD69 (Wang et al. 2017).

18.5.6 Curtachalasins 1 & 10

Curtachalasins 1 & 10 are other candidates for immunosuppressant drug development. According to research new curtachalasins F–P (1–11), from endophytic fungus *Xylaria* cf. *curta* were isolated. The result demonstrated significant selective inhibition on B-cell proliferation with an IC_{50} value of 2.42 μM for compound 1 and selective inhibition on T cell proliferation with an IC_{50} value of 12.15 μM for compound 10.

18.5.7 Fusaperazin F

Fusaperazin F is another new compound that obtained from Antarctic marine-derived fungus *Penicillium crustosum* HDN153086. This new diketopiperazine showed cytotoxicity against K562 cell, with an IC_{50} value of 12.7 μM (Zhang et al. 2014).

18.6 Antiviral Agents

Commonly, viral infections are among diseases that probably can affect most people worldwide. Despite these new viruses are found while antiviral drugs and vaccines acting against them are limited. By considering the vast number of fungal production and metabolites, investigating fungal bioactive molecules with antiviral effects is a trustable route in drug development researches. In this section, some important and new antiviral compounds derived from fungal sources with their class and chassis organism have been listed in Table 18.5. Chemical structures of these compounds are shown in Fig. 18.6.

18.6.1 Stachybogrisephenone B ($C_{16}H_{15}ClO_6$), Grisephenone A, and 3, 6, 8-Trihydroxy-1-Methylxanthone

Enterovirus 71 and coxsackievirus (EV71 & coxsackievirus) are responsible for hand, foot, and mouth (HFMD) acute viral disease(He et al. 2013). The fungus *Stachybotrys* sp produces 3 new sesquiterpenoid and xanthone compounds: stachybogrisephenone B ($C_{16}H_{15}C_lO_6$), grisephenone A, and 3, 6, 8-trihydroxy-1-methylxanthone. These compounds showed inhibitory activities against in vitro repetition of EV71 with IC_{50} values of 30.1, 50.0, and 40.3 μM (Qin et al. 2015).

Table 18.5 Natural antiviral drugs and compounds from fungi

Compound	Compound class	Therapeutic index	Pharmacological effect	Chassis organism (or fungal sp.)	References
Stachybogrisephenone B	Xanthone	EV-71, Coxsackie virus	IC_{50}: 30.1 μM	*Stachybotrys* sp.	Qin et al. (2015), Rabenau et al. (2010)
Grisephenone A	Xanthone	EV-71, Coxsackie virus	IC_{50}: 50.0 μM	*Stachybotrys* sp.	Qin et al. (2015); Rabenau et al. (2010)
3,6,8-Trihydroxy-1-methylxanthone	Xanthone	EV-71, Coxsackie virus	IC_{50}: 40.3 μM	*Stachybotrys* sp.	Qin et al. (2015); Rabenau et al. (2010)
Alternariol	Dibenzo-α-pyrones	HSV virus	IC_{50}:13.5	*Pleospora tarda*	Selim et al. (2018)
Alternariol-(9)-methyl ether	Dibenzo-α-pyrones	HSV virus	IC_{50}:21.3	*Pleospora tarda*	Selim et al. (2018)
11a-dehydroxyisoterreulactone A	Lactone	HSV-1 virus	IC_{50}: 33.38 μM	*Aspergillus terreus* SCSGAF0162	Nong et al. (2014)
Arisugacin A	Lactone	HSV-1 virus	IC_{50}: 12.76 μM	*Aspergillus terreus* SCSGAF0162	Nong et al. (2014)
Isobutyrolactone II	Lactone	HSV-1 virus	IC_{50}: 62.08 μM [	*Aspergillus terreus* SCSGAF0162	Nong et al. (2014)
Aspernolide A	Lactone	HSV-1 virus	IC_{50}: 68.16 μM	*Aspergillus terreus* SCSGAF0162	Nong et al. (2014)
Oxoglyantrypine	Indole alkaloid	H1N1 virus	IC_{50}: 85 μM	*Cladosporium* sp.	Peng et al. (2013)
Norquinadoline A	Indole alkaloid	H1N1 virus	IC_{50}: 82 μM	*Cladosporium* sp.	Peng et al. (2013)
Deoxynortryptoquivaline	Alkaloid	H1N1 virus	IC_{50}: 87 μM	*Cladosporium* sp.	Peng et al. (2013)
Deoxytryptoquivaline	Alkaloid	H1N1 virus	IC_{50}: 85 μM	*Cladosporium* sp.	Peng et al. (2013)
Tryptoquivaline	Alkaloid	H1N1 virus	IC_{50}: 89 μM	*Cladosporium* sp.	Peng et al. (2013)
Quinadoline B	Alkaloid	H1N1 virus	IC_{50}: 82 μM	*Cladosporium* sp.	Peng et al. (2013)

(continued)

Table 18.5 (continued)

Compound	Compound class	Therapeutic index	Pharmacological effect	Chassis organism (or fungal sp.)	References
Cladosin C	Hybrid polyketide	H1N1 virus	IC_{50}: 276 μM	*Cladosporium sphaerospermum* 2005-01-E3	Wu et al. (2014)
Isoaspulvinone E	Butenolide	H1N1 virus	IC_{50}: 32.3 μM	*Aspergillus terreus* Gwq-48	Gao et al. (2013)
Aspulvinone E	Butenolide	H1N1 virus	IC_{50}: 56.9 μM	*Aspergillus terreus* Gwq-48	Gao et al. (2013)
Pulvic acid	Butenolide	H1N1 virus	IC_{50}: 29.1 μM	*Aspergillus terreus* Gwq-48	Gao et al. (2013)
Sorbicatechols A & B	Sorbicillinoids	H1N1 virus	IC_{50}: 85 μM IC_{50}: 113 μM	*Penicillium chrysogenum* PJX-17	Peng et al. (2014)
Brefeldin A	Polyketide	Dengue virus (DENV)	IC_{50}: 54.6 ± 0.9 nM	*Penicillium* sp. FKI-7127	Raekiansyah et al. (2017)

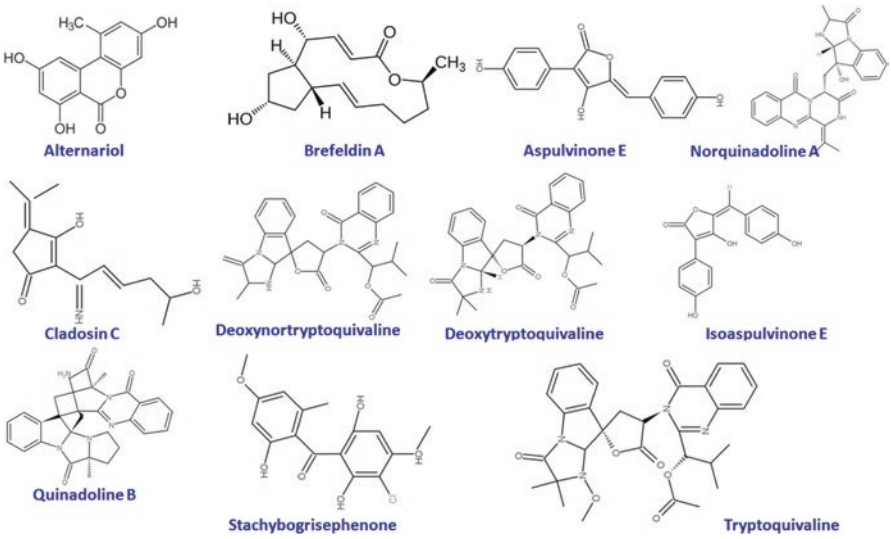


Fig. 18.6 Chemical structure of natural antiviral drugs and compounds obtained from fungi

18.6.2 Alternariol ($C_{14}H_{10}O_5$) and Alternariol-(9)-Methyl Ether

Herpes Simplex Viruses (HSVs)serotypes 1 and 2 (HSV-1 and HSV-2) are important pathogens in human orofacial infections (HSV-1) and sexually transmitted infections (HSV-2) (Whitley 2001). Alternariol ($C_{14}H_{10}O_5$) and Alternariol-(9)-methyl ether are novel compounds from the endophytic fungus *Pleospora tarda*. They have inhibition activity against HSVs with IC_{50} values of 13.5 and 21.3 μM with the selective index of 26.5 and 17.1 via polymerase or pre-integration steps inhibiting (Selim et al. 2018). Based on another research on *Aspergillus terreus* SCSGAF0162, produced compounds from this marine fungi: 11a-dehydroxy-isoterreulactone A, arisugacin A, isobutyrolactone II, and aspernolide, had antiviral activity against HSV-1 serotype with IC_{50} values of 33.38, 12.76, 62.08, and 68.16 μM (Nong et al. 2014).

18.6.3 Oxoglyantrypine, Norquinadoline A ($C_{26}H_{25}N_5O_4$), Deoxynortryptoquivaline ($C_{28}H_{28}N_4O_6$), Deoxytryptoquivaline ($C_{29}H_{30}N_4O_6$), Tryptoquivaline ($C_{29}H_{30}N_4O_7$), and Quinadoline B ($C_{25}H_{21}N_5O_3$)

H1N1 is a swine-origin influenza virus (S-OIV), A from the Orthomyxoviridae family. It is a negative (−) strand RNA virus that the names of subtypes are based on H (hemagglutinin) and N (neuraminidase) antigens (Scalera and Mossad 2009).

Oxoglyantrypine and norquinadoline A ($C_{26}H_{25}N_5O_4$) as indole alkaloids and deoxynortryptoquivaline ($C_{28}H_{28}N_4O_6$), deoxytryptoquivaline ($C_{29}H_{30}N_4O_6$), tryptoquivaline ($C_{29}H_{30}N_4O_7$), and quinadoline B ($C_{25}H_{21}N_5O_3$) as alkaloids from *Cladosporium* sp. inhibit viral envelope and the endosome fusion in H1N1 subtype. There inhibitory activity against influenza A (H1N1) revealed with IC_{50} values of 85, 82, 87, 85, 89, and 82 μM (Peng et al. 2013).

18.6.4 Cladosin C ($C_{13}H_{18}N_2O_3$)

Another compound isolated from a deep-sea-derived fungus *Cladosporium sphaerospermum* 2005-01-E3 is cladosin C ($C_{13}H_{18}N_2O_3$) that showed inhibitory activity against influenza A (H1N1) virus with an IC_{50} = 276 μM (Wu et al. 2014).

18.6.5 Isoaspulvinone E ($C_{17}H_{12}O_5$), Aspulvinone E ($C_{17}H_{12}O_5$), and Pulvic Acid

According to an analysis of aspulvinones from *Aspergillus terreus* Gwq-48, a mangrove rhizosphere soil-derived fungus; isoaspulvinone E ($C_{17}H_{12}O_5$), aspulvinone E ($C_{17}H_{12}O_5$), and pulvic acid demonstrated significant anti-influenza A H1N1 virus activities, with IC_{50} values of 32.3, 56.9, and 29.1 μg/mL (Gao et al. 2013). Isolation of a sorbicillin component in *Penicillium chrysogenum* PJX-17 fungi produced sorbicatechols A and B anti-H1N1 compounds with IC_{50} values of 85 and 113 μM (Peng et al. 2014).

18.6.6 Brefeldin A

Brefeldin A ($C_{16}H_{24}O_4$) is a novel compound from *Penicillium* sp. FKI-7127 showed some activities against the dengue virus (DENV), a pathogen for causing dengue fever (DF) and dengue hemorrhagic fever (DHF). Brefeldin A inhibited DENV-2 growth with an IC_{50} value of 54.6 ± 0.9 nM which makes it to be a candidate for new antiviral drug discovery (Raekiansyah et al. 2017).

18.7 Antifungal Agents

Among natural antifungal metabolite producers, fungal sources are an interesting source. In many cases, the antifungal metabolites are produced by endophytic fungi (i.e., within plants) which may be due to providing the right protection against

competitors within the host plant. These metabolites belong to different chemical and structural groups as benzofurans, isocoumarins, and xanthones as summarized in Table 18.6. Chemical structures of these compounds are shown in Fig. 18.7.

18.7.1 Griseofulvin ($C_{17}H_{17}C_lO_6$)

Griseofulvin is an antifungal drug that is produced by *Xylaria* sp. (Sica et al. 2016). It can cure tinea infection by depositing in keratin precursor cells and is tightly bound to new keratin. This process increases resistance to fungal invasion. Micronized oral suspension (125 mg/5 mL), micronized tablet (500 mg as Griseofulvin V) with variable absorption (25–70%), and ultra-micronized tablets (125 and 250 mg as Gris-PEG) with complete absorption are dosage forms of this drug.

18.7.2 5-(Undeca-3′, 5′, 7′-Trien-1′-yl) Furan-2-ol (A) and 5-(Undeca-3′, 5′, 7′-Trien-1′-yl)Furan-2-Carbonate (B)

Novel compounds 5-(undeca-3′, 5′, 7′-trien-1′-yl) furan-2-ol (A) and 5-(undeca-3′, 5′, 7′-trien-1′-yl) furan-2-carbonate (B) were isolated from the endophytic fungus *Emericella* sp. XL029. These alkylated furan derivatives showed antifungal activities against *R. solani*, *Verticillium dahliae*, *Harpophora maydis*, *Fusarium oxysporum*, *F. tricinctum*, *Botryosphaeria dothidea*, and *Aphelenchoides fragariae* (compound A) and *V. dahliae*, *H. maydis*, *F. tricinctum*, *B. dothidea*, and *A. fragariae* (compound B) (Wu et al. 2018).

18.7.3 Koninginins R and S

Trichoderma koningiopsis YIM PH30002 has been reported to produce 2 new polyketide compound: koninginin R and S with antifungal activity against *Fusarium oxysporum*, *F. flocciferum* (compound R), and *F. oxysporum* (compound S) (Hu et al. 2017).

Table 18.6 Natural antifungal drugs and compounds from fungi

Compound	Chemical group	Therapeutic index	Pharmacological effect	Commercial name	Producing organism	Reference
Griseofulvin	Benzofuran	Tinea infection	Fungistatic	Griseofulvin V and Gris-PEG	*Xylaria* sp.	Sica et al. (2016)
5-(undeca-30,50,70-trien-10-yl) furan-2-ol (A) 5-(undeca-30,50,70-trien-10-yl) furan-2-carbonate (B)	Furan	A: against *R. solani, V. dahliae, H. maydis, F. oxysporum, F. tricinctum, B. dothidea,* and *A. fragariae* B: displayed activity against *V. dahliae, H. maydis, F. tricinctum, B. dothidea,* and *A. fragariae*	A: MIC values from 25 to 3.1 g/mL B: (MIC values from 50 to 12.5 g/mL)	–	*Emericella* sp. XL029	Wu et al. (2018)
Koninginin R and S	Polyketide	R: active against *F. oxysporum* and *F. flocciferum* S: displayed activity against *F. oxysporum*	R: MICs at 128 g/Ml S: MIC at 128 g/mL	–	*Trichoderma koningiopsis* YIM PH30002	Hu et al. (2017)
5-hydroxy-8-methoxy-4--phenylisoquinolin-1(2H)-one (A), 3-O-methylviridicatin (B) and viridicatol (C)	Isoquinolone alkaloid (A) and quinolinone alkaloids (B,C)	A: active against *A. brassicae, A. alternata* and *V. mali* B: against *A. brassicae, B. cinerea* and *V. male* C: against *A. brassicae, A. alternata* and *B. cinerea*	A: MIC value of 31.2 g/mL B: MIC value of 31.2 g/mL C: MIC value of 31.2 g/mL	–	*Penicillium* sp. R22	Ma et al. (2017)
Fusaripeptide A	Cyclodepsipeptide	active against *C. albicans*, *C. glabrata*, *C. krusei*, and *A. fumigates*	IC_{50}: 0.11, 0.24, 0.19, and 0.14 M	–	*Fusarium* sp.	Ibrahim et al. (2018)

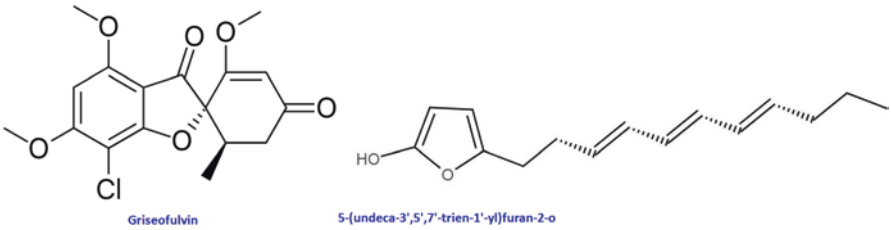


Fig. 18.7 Chemical structure of natural antifungal drugs and compounds obtained from fungi

18.7.4 5-Hydroxy-8-Methoxy-4-Phenylisoquinolin-1(2H)-One (A), 3-O-Methylviridicatin (B) and Viridicatol (C)

Another novel isoquinolone alkaloid compound, 5-hydroxy-8-methoxy-4--phenylisoquinolin-1(2H)-one (A), with antifungal activity against *Alternaria brassicae*, *A. alternata* has derived from *Penicillium* sp. R22. This fungus produces other quinolinone alkaloid compounds, 3-O-methylviridicatin (B), and viridicatol (C) that showed antifungal activities against *A. brassicae* and *Botrytis cinerea* (compound B) and against *A. brassicae*, *A. alternata,* and *B. cinerea* (compound C) (Ma et al. 2017).

18.7.5 Fusaripeptide A

Fusaripeptide A as a cyclodepsipeptide novel compound from *Fusarium* sp revealed antifungal activity against *Candida albicans*, *C. glabrata*, *C. krusei*, and *Aspergillus fumigatus* with IC_{50} values of 0.11, 0.24, 0.19, and 0.14 μM (Ibrahim et al. 2018).

18.8 Conclusion

Fungal biopharmaceuticals have been identified by mankind from centuries. Despite this traditional knowledge which was used as remedies, the exact mechanism of action was concealed. By isolating the bioactive compounds from natural sources and unraveling their mode of action, era of drug design and discovery was started. In the same way, scaling up bioactive compounds and expanding the diversion of them through semisynthetic chemical science resulted into introducing a vast number of drugs into health care system. It is apparent that discovery of new fungal

species and/or their pharmacologically active compounds are essential to reach new therapeutic agents. Nevertheless, a few metabolites will gain ground and become commercialize because several key points should be considered to develop a drug with least adverse effects and toxicity while being efficient. This chapter has focused on some valuable fungal and fungal-like organisms from different genera and species especially those belong to *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, *Trichoderma,* and *Streptomyces* and their bioactive metabolites that have been used as biopharmaceutical agents. Since a huge numbers of bioactive fungal metabolites remain to be discovered, further studies on finding of novel fungal strains and identifying their biopharmaceuticals are recommended.

Acknowledgments Research reported in this publication was supported by Elite Researcher Grant Committee under award numbers [958935 and 963366] from the National Institute for Medical Research Development (NIMAD), Tehran, Iran to MSG.

References

Adames I, Ortega HE, Asai Y, Kato M, Nagaoka K, TenDyke K et al (2015) 3-epi-Waol A and Waol C: polyketide-derived γ-lactones isolated from the endophytic fungus *Libertella blepharis* F2644. Tetrahedron Lett 56:252–255

Amrani E, Lai MD, Debbab A, Aly AH, Siems K, Seidel C et al (2014) Protein kinase and HDAC inhibitors from the endophytic fungus *Epicoccum nigrum*. J Nat Prod 77:49–56

Beaver A, Kluetz PG, Pazdur RJ (2018) Metastasis-free survival: a new end point in prostate cancer trials. New Eng J Med 378:2458

Brinkmann V (2009) FTY720 (fingolimod) in multiple sclerosis: therapeutic effects in the immune and the central nervous system. Br J Pharmacol 158:1173–1182

Chiba KJ (2020) Discovery of fingolimod based on the chemical modification of a natural product from the fungus *Isaria sinclairii*. J Antibiot 7(73):666–678

Crawford K, Heatley N, Boyd PF, Hale C, Kelly B, Miller G et al (1952) Antibiotic production by a species of *Cephalosporium*. Microbiology 6:47–59

Crooke ST, Bradner WT (1976) Mitomycin C: a review. Cancer Treat Rev 3:121–139

De Smet MD, Nussenblatt RB (1993) Clinical use of cyclosporine in ocular disease. Int Ophthalmol Clin 33:31

Demain AL (2014) Importance of microbial natural products and the need to revitalize their discovery. J Ind Microbiol Biotechnol 41:185–201

Döhren HV, Kleinkauf H (1999) 1.20 – Cyclosporin: the biosynthetic path to a lipopeptide. In: Barton SD, Nakanishi K, Meth-Cohn O (eds) Comprehensive natural products chemistry. Pergamon, Oxford, pp 533–555. https://doi.org/10.1016/B978-0-08-091283-7.00106-5

Du X-P, Su W-J (2014) Two new polyketides from mangrove endophytic fungus Dothiorella sp. Chem Nat Comp 50:214–216

Farrar MC, Jacobs TF (2020) Paclitaxel. StatPearls Publication

Ferreira PCL, Thiesen FV, Pereira AG, Zimmer AR, Fröehlich E (2020) A short overview on mycophenolic acid pharmacology and pharmacokinetics. J Clin Trans Res:e13997

Găman AM, Egbuna C, Găman M-A (2020) Natural bioactive lead compounds effective against haematological malignancies. In: Phytochemicals as lead compounds for new drug discovery. Elsevier, pp 95–115

Gao H, Guo W, Wang Q, Zhang L, Zhu M, Zhu T et al (2013) Aspulvinones from a mangrove rhizosphere soil-derived fungus *Aspergillus terreus* Gwq-48 with anti-influenza A viral (H1N1) activity. Bioorg Med Chem Lett 23:1776–1778. https://doi.org/10.1016/j.bmcl.2013.01.051

Gholami-Shabani M, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M (2019) Natural Product Synthesis by Fungi: Recent Trends and Future Prospects. In: Recent Advancement in White Biotechnology through Fungi. Springer-Verlag, pp. 195-228.

He S-J, Han J-F, Ding X-X, Wang Y-D, Qin C-F (2013) Characterization of enterovirus 71 and coxsackievirus A16 isolated in hand, foot, and mouth disease patients in Guangdong, 2010. Int J Infect Dis 17:e1025–e1030

Hu M, Li Q-L, Yang Y-B, Liu K, Miao C-P, Zhao L-X et al (2017) Koninginins RS from the endophytic fungus *Trichoderma koningiopsis*. Nat Prod Res 31:835–839

Ibrahim SR, Abdallah HM, Elkhayat ES, Al Musayeib NM, Asfour HZ, Zayed MF et al (2018) Fusaripeptide A: new antifungal and anti-malarial cyclodepsipeptide from the endophytic fungus *Fusarium* sp. J Asian Nat Prod Res 20:75–85

Kensy F, Zang E, Faulhammer C, Tan R-K, Büchs JJMCF (2009) Validation of a high-throughput fermentation system based on online monitoring of biomass and fluorescence in continuously shaken microtiter plates. Microb Cell Fact 8:31

Kong Y, Cheng L, Mao F, Zhang Z, Zhang Y, Ee F et al (2018) Inhibition of cholesterol biosynthesis overcomes enzalutamide resistance in castration-resistant prostate cancer (CRPC). J Biol Chem 293:14328–14341

Korecka M, Shaw L (2009) Review of the newest HPLC methods with mass spectrometry detection for determination of immunosuppressive drugs in clinical practice. Ann Transplant 14:61–72

Kornienko A, Evidente A, Vurro M, Mathieu V, Cimmino A, Evidente M et al (2015) Toward a cancer drug of fungal origin. Med Res Rev 35:937–967

Li W, Lu H, Zhang H, Sun X, Hu L, Wang D (2020) Squamous cell carcinoma associated with inverted papilloma: recurrence and prognostic factors. Oncol Lett 19:1082–1088

Lin T, Wang G, Shan W, Zeng D, Ding R, Jiang X et al (2014) Myrotheciumones: bicyclic cytotoxic lactones isolated from an endophytic fungus of *Ajuga decumbens*. Bioorg Med Chem Lett 24:2504–2507

Liu C-C, Zhang Z-Z, Feng Y-Y, Gu Q-Q, Li D-H, Zhu T-J (2019) Secondary metabolites from Antarctic marine-derived fungus *Penicillium crustosum* HDN153086. Nat Prod Res 33:414–419

Ma Y-M, Qiao K, Kong Y, Li M-Y, Guo L-X, Miao Z et al (2017) A new isoquinolone alkaloid from an endophytic fungus R22 of *Nerium indicum*. Nat Prod Res 31:951–958

Manzoni M, Rollini MJ (2002) Biosynthesis and biotechnological production of statins by filamentous fungi and application of these cholesterol-lowering drugs. Appl Microbiol Biotechnol 58:555–564

Martínez JL, Liu L, Petranovic D, Nielsen JJ (2012) Pharmaceutical protein production by yeast: towards production of human blood proteins by microbial fermentation. Curr Opin Biotechnol 23:965–971

Martínez-Castro M, Salehi-Najafabadi Z, Romero F, Pérez-Sanchiz R, Fernández-Chimeno RI, Martín JF et al (2013) Taxonomy and chemically semi-defined media for the analysis of the tacrolimus producer '*Streptomyces tsukubaensis*'. Appl Microbiol Biotechnol 97:2139–2152

Nakamura T, Liu Y, Hirata D, Namba H, Harada S-I, Hirokawa T et al (1993) Protein phosphatase type 2B (calcineurin)-mediated, FK506-sensitive regulation of intracellular ions in yeast is an important determinant for adaptation to high salt stress conditions. EMBO J 12:4063–4071

Nong X-H, Wang Y-F, Zhang X-Y, Zhou M-P, Xu X-Y, Qi S-H (2014) Territrem and butyrolactone derivatives from a marine-derived fungus *Aspergillus terreus*. Mar Drugs 12:6113–6124

Nowak I, Shaw L (1995) Mycophenolic acid binding to human serum albumin: characterization and relation to pharmacodynamics. Clin Chem 41:1011–1017

Oo ML, Thangada S, Wu M-T, Liu CH, Macdonald TL, Lynch KR et al (2007) Immunosuppressive and anti-angiogenic sphingosine 1-phosphate receptor-1 agonists induce ubiquitinylation and proteasomal degradation of the receptor. J Biol Chem 282:9082–9089

Papich M (2016) Ketoprofen. In: Saunders handbook of veterinary drugs. Elsevier, St. Louis

Peng J, Lin T, Wang W, Xin Z, Zhu T, Gu Q et al (2013) Antiviral alkaloids produced by the mangrove-derived fungus *Cladosporium* sp. PJX-41. J Nat Prod 76:1133–1140

Peng J, Zhang X, Du L, Wang W, Zhu T, Gu Q et al (2014) Sorbicatechols A and B, antiviral sorbicillinoids from the marine-derived fungus *Penicillium chrysogenum* PJX-17. J Nat Prod 77:424–428

Qin C, Lin X, Lu X, Wan J, Zhou X, Liao S et al (2015) Sesquiterpenoids and xanthones derivatives produced by sponge-derived fungus *Stachybotry* sp. HH1 ZSDS1F1-2. J Antibiot 68:121–125

Rabenau HF, Richter M, Doerr HW (2010) Hand, foot and mouth disease: seroprevalence of Coxsackie A16 and Enterovirus 71 in Germany. Med Microbiol Immunol 199:45–51

Raekiansyah M, Mori M, Nonaka K, Agoh M, Shiomi K, Matsumoto A et al (2017) Identification of novel antiviral of fungus-derived brefeldin A against dengue viruses. Trop Med Health 45:32

Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Rai M (2015) Medical Mycology: Current Trends and Future Prospects. CRC Press, Boca Raton, USA

Razzaghi-Abyaneh M, Rai M (2013) Antifungal Metabolites from Plants. Springer-Verlag, Heidelbergh, Germany.

Razzaghi-Abyaneh M, Chang P-K, Shams-Ghahfarokhi M and Rai M (2014) Global health issues of aflatoxins in food and agriculture: challenges and opportunities. Front. Microbiol. 5:420. doi: 10.3389/fmicb.2014.00420

Scalera NM, Mossad SB (2009) The first pandemic of the 21st century: review of the 2009 pandemic variant influenza A (H1N1) virus. Postgrad Med 121:43–47

Selim KA, Elkhateeb WA, Tawila AM, El-Beih AA, Abdel-Rahman TM, El-Diwany AI et al (2018) Antiviral and antioxidant potential of fungal endophytes of Egyptian medicinal plants. Fermentation 4:49

Shaw LM, Korecka M, DeNofrio D, Brayman KL (2001) Pharmacokinetic, pharmacodynamic, and outcome investigations as the basis for mycophenolic acid therapeutic drug monitoring in renal and heart transplant patients. Clin Biochem 34:17–22

Sica VP, Rees ER, Tchegnon E, Bardsley RH, Raja HA, Oberlies NH (2016) Spatial and temporal profiling of griseofulvin production in *Xylaria cubensis* using mass spectrometry mapping. Front Microbiol 7:544

Sneader W (2005) Drug discovery: a history. John Wiley & Sons

Seyedjavadi SS, Khani S, Zare-Zardini H, Halabian R, Goudarzi M, Khatami S et al (2019) Isolation, functional characterization, and biological properties of MCh-AMP1, a novel antifungal peptide from *Matricaria chamomilla* L. Chemical Biology & Drug Design 93:949-959.

Thirumaran R, Prendergast GC, Gilman PB (2007) Cytotoxic chemotherapy in clinical treatment of cancer. Cancer Immunother Elsevier:101–116

Tönshoff B, David-Neto E, Ettenger R, Filler G, van Gelder T, Goebel J et al (2011) Pediatric aspects of therapeutic drug monitoring of mycophenolic acid in renal transplantation. Transplant Rev 25:78–89

Verekar SA, Mishra PD, Sreekumar ES, Deshmukh SK, Fiebig H-H, Kelter G et al (2014) Anticancer activity of new depsipeptide compound isolated from an endophytic fungus. J Antibiot 67:697–701

Vinokurova N, Ivanushkina N, Kochkina G, Arinbasarov M, Ozerskaya SJ (2005) Production of mycophenolic acid by fungi of the genus *Penicillium* link. Appl Biochem Microbiol 41:83–86

Wang L-W, Wang J-L, Chen J, Chen J-J, Shen J-W, Feng X-X et al (2017) A Novel derivative of (−) mycousnine produced by the endophytic fungus *Mycosphaerella nawae*, exhibits high and selective immunosuppressive activity on T cells. Front Microbiol:8. https://doi.org/10.3389/fmicb.2017.01251

Wang W-X, Cheng G-G, Li Z-H, Ai H-L, He J, Li J et al (2019) Curtachalasins, immunosuppressive agents from the endophytic fungus *Xylaria* cf. *curta*. Org Biomol Chem 17:7985–7994

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc 93:2325–2327

Ward MD, Jones DE, Goldman MD, Disorders R (2016) Cryptococcal meningitis after fingolimod discontinuation in a patient with multiple sclerosis. Malt Scler Relat Disord 9:47–49

Weaver BA (2014) How taxol/paclitaxel kills cancer cells. Mol Biol Cell 25:2677–2681

Whitley RJ, Roizman BJTl (2001) Herpes simplex virus infections. Lancet 357:1513-1518.
WHO (2019) World Health Organization model list of essential medicines: 21st list 2019. World Health Organization
Wu G, Sun X, Yu G, Wang W, Zhu T, Gu Q et al (2014) Cladosins A–E, Hybrid polyketides from a deep-sea-derived fungus, *Cladosporium sphaerospermum*. J Nat Prod 77:270–275
Wu X, Pang X-J, Xu L-L, Zhao T, Long X-Y, Zhang Q-Y et al (2018) Two new alkylated furan derivatives with antifungal and antibacterial activities from the plant endophytic fungus *Emericella* sp. XL029. Nat Prod Res 32:2625–2631
Zhang W, Xu L, Yang L, Huang Y, Li S, Shen Y (2014) Phomopsidone A, a novel depsidone metabolite from the mangrove endophytic fungus *Phomopsis* sp. A123. Fitoterapia 96:146–151
Zuckerkandl E, Pauling L (1965) Evolutionary divergence and convergence in proteins. In: Evolving genes and proteins. Elsevier, pp 97–166

Chapter 19
Natural Pigments from Filamentous Fungi: Production and Applications

Ganapathy Ashok, Utpal Mohan, Meganathan Boominathan, Velayutham Ravichandiran, Chandran Viswanathan, and Venugopal Senthilkumar

Contents

G. Ashok (✉) · U. Mohan · V. Ravichandiran
National Institute of Pharmaceutical Education and Research, Kolkata, West Bengal, India

M. Boominathan
Department of Animal Behaviour and Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

C. Viswanathan
Department of Biotechnology, Sree Narayana Guru College, Coimbatore, Tamil Nadu, India

V. Senthilkumar
Pondicherry Centre for Biological Sciences, Puducherry, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_19

19.1 Introduction

Color, an appealing feature every individual is attracted to and nature has its own color, with which it pleases mankind and other living organisms. Beyond the limits of memory, colors have always played a pivotal role in all forms of life in the earth. Human life is always fascinated by the colors and it has been used in many aspects including foods, clothes, household things, etc. Prehistoric evidences suggest that naturally occurring colors are used by humans for esthetic purposes (Rao et al. 2017). In this world, usage of colors has been recorded in many ancient cultures including European, Chinese, Indian (Mohenjo-Daro and Harappa civilization), and central and North America (Aztec and Maya culture) (Aberoumand 2011; Gokhale et al. 2004),

Colors also have the power to attract living forms to foods which is associated with aroma and nutritional value. Food colors can be of natural or synthetic. Natural pigments are mainly derived from plants, animals, and microbes such as fungi, bacteria, and algae (Samanta and Agarwal 2009; Abdel-Azeem et al. 2021; Yadav et al. 2019b). Naturally occurring pigments were initially used for food colors but due to the less bio-availability and instability traditional colors were replaced by synthetic ones (Rohrig 2016). The discovery by Sir William Henry Perkin (1856), named "mauve" pigment led the stepping stone for the era of synthetic dyes (Garfield 2002). Over a greater extent, these synthetic dyes are used in textiles, cosmetics and pharmaceutical industries (Adam Burrows 2009). On the other hand, these synthetic colors have adverse effects on environment and human health (Carcinogenic and immune suppressive) (Osman et al. 2004; Ratna 2012; Arora 2014). Mainly due to less degradation and long-time persistence of artificial or synthetic colors in the environment led to the demand for natural pigments.

Over a period of time, natural pigments have been explored from different sources including plants, animals, and microorganisms. Pigments produced from microorganisms, especially fungi gain special attention, as they are able to produce huge amounts of pigments and are considered for safe use (Kirti et al. 2014). Microorganisms produce secondary metabolites which are not required for cellular function such as enzymes, pigments have therapeutic potential like anticancer, anti-inflammatory, antioxidant, immune modulation etc. (Velmurugan et al. 2010; Devi et al. 2020). Basidiomycetous fungi have been known to be used for dying wool and silk in ancient cultures (Hernández et al. 2019); however, large scale production for commercialization was not feasible. Pigments produced by filamentous fungi have inherent potential as they can be easily grown in laboratory and can be produced in large scale. These include wide range of fungi originated from different environmental origin such as marine, terrestrial, endophytic, and endolichenic (Dufossé et al. 2014). Natural pigments are gaining importance in the industrial sector over synthetic ones, lead to the investigation of filamentous fungi for their pigment production (biomass and toxin free) (Blumenthal 2004). Industries commercially produce pigments from filamentous fungi such as Arpink from *Penicillium oxalicum var. Armeniaca*, Ankaflavin from *Monascus.* sp. (Mapari et al. 2009).

Fungi belonging to the Monascaceae, Pleosporaceae, Chaetomiaceae, Tuberaceae, Nectriaceae, Sordariaceae, Chlorociboriaceae are known to be (Caro et al. 2017; Gmoser et al. 2017; Blanchette et al. 1992) the most promising and predominant producers of wide range of pigments of different chemical classes which includes carotenoids, quinines, indigo, melanin, phenazines, and violacein (Dufossé et al. 2014). More than 50 pigments have been discovered and characterized from the filamentous fungi *Monascus*, producing yellow (ankaflavin), purple (rubropunctatin), orange (monascorubrine) which have been used in food products in China, Japan and some Asian countries which possess antibacterial and anti-mutagenic properties (Dufosse et al. 2005; Feng et al. 2012). More than 200 fungal species belonging to the genera Zygomycetes and Basidiomycetes are reported for carotenoid production (Avalos and Carmen Limon 2015). Many species of Fusarium, for example, *Fusarium avenaceum, Fusarium tricinctum, Fusarium sporotrichioides,* etc. are reported for the production of a red pigment aurofusarin and *Fusarium verticillioides* for aurofusarin and bikaverin production (Caro et al. 2017). Anthraquinone is the most predominant class pigment used in dyeing industry and was commonly produced from fungi *Trichoderma, Fusarium,* and *Aspergillus* (Durán et al. 2002).

Pigments are bio-colorants that are produced by various plants and microorganisms. Colorants obtained from microbes are ideal than plants as they are more stable, have better solubility, high yield, and easy culturing of the producing microbes (Rao et al. 2017). Pigments consist of multitude of chemical compounds with varied structure having diverse bioactivities (Kim 2013). Microorganisms such as fungi, bacteria, and microalgae produce pigments (Joshi et al. 2003; Choi et al. 2015; Pandey et al. 2018; Ramesh et al. 2019). However, pigments from fungi have numerous advantages, not just as an alternate to synthetic pigments, but also as an value added product for bio-refineries (Dufossé et al. 2014; Caro et al. 2012; Mapari et al. 2010; Gusdinar et al. 2011; Sanchez et al. 2013). Fungi are known to produce wide range of pigments that include carotenoids, melanins, azaphilones, flavins, phenazines, quinones, monascins, violacein, and indigo (Dufosse 2006).

Microbes produce a variety of pigments that can be used as food colors such as carotenoids, flavins, melanins, quinines, monascins, and violacein were widely used as potential source for antioxidants, feed additives, major colour intensifiers and food ingredients. This chapter will give the detailed overview of pigment producing fungi from various sources and its application in industries with their therapeutic potential.

19.2 Biodiversity and Molecular Identification of Fungus

Fungi belonging to various classes were known to be a potential producer of pigments, which includes Zygomycetes, Basidiomycetes, and Ascomycetes. The highest amount of carotene production was reported in class Zygomycetes from order Mucorales, *Phycomyces,* and *Blakeslea* (Avalos and Carmen Limon 2015; Yadav et al. 2019a). Mold fungi such as *Monascus* sp. are known to produce six major azaphilone pigments, namely orange colored rubropunctatin and

monascorubramine, purple colored monascorubrin and rubropunctamine, yellow colored monascin and ankaflavin (Yang et al. 2015). Basidiomycetes fungi such as *Agaricus brunnescens* produce Tyrosinase (Malmstrom and Ryden 1968); *Ustilago, Sclerotinia*, *Sporidiobolus,* and *Rhodosporidium* sp. produce carotene (Avalos and Carmen Limon 2015).

The Ascomycetes genus such as *Aspergillus, Penicillium, Aschersonia,* and *Cercospora* are also reported to produce efficient amount of carotene (Avalos and Carmen Limon 2015). Melanin a dark brown pigment by *Aspergillus* sp. (Pal et al. 2014) and *Penicillium marneffei* (Liu et al. 2014) was reported. Bikaverin is a reddish pigment produced by *Fusarium fujikuroi* (Carmen et al. 2010). γ-carotene and neurosporaxanthin have been isolated from *Neurospora crassa* (Gusdinar et al. 2011; Pagano et al., 2015); *N. intermedia* produces yellow to orange carotenoids (Rebecca et al. 2018). *Penicillium marneffei* produced azaphilones (Woo et al. 2014); anthraquinones were isolated from *P. oxalicum* var. Americana (Dufosse 2006). Lycopene was isolated from *Fusarium sporotrichioides* (Jones et al. 2004). *Talaromyces purpurogenus* and *Talaromyces* atroroseus synthesized red pigments (Fig. 19.1); *Cordyceps unilateralis* has blood red pigment (Dufosse 2017); orange

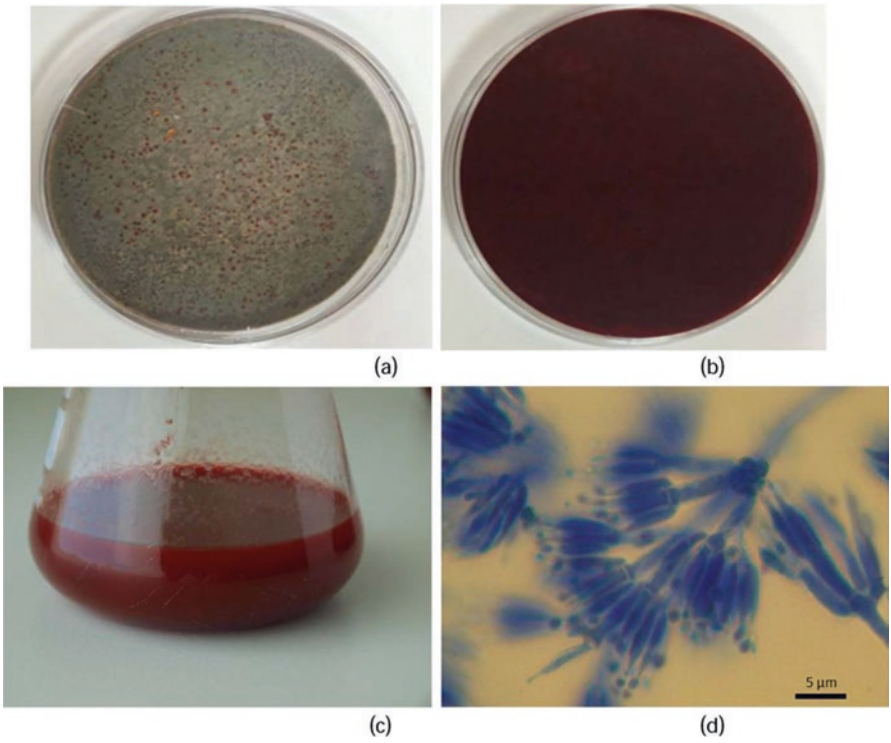


Fig. 19.1 Morphological features of *Talaromyces albobiverticillius* : (**a**) Obverse face of fungus grown on Potato Dextrose Agar (PDA) media; (**b**) Reverse face; (**c**) Red pigment production in Potato Dextrose Broth (PDB) medium incubated for 7 days at 24 °C; (**d**) Conidiophores produced on PDA, stained with lactophenol blue). (Venkatachalam et al. 2018)

pigments by *Herpotrichia rhodosticta*, *Fusarium oxysporum* produces red pigments (Torres et al. 2016); orange pigments from *Fusarium fujikuroi* (Avalos and Cerda-Olmedo 1987); yellow pigments in *Eurotium* sp. (Torres et al. 2016). Neilands (1952) reported organo-iron pigment from a Rust Fungus Ustilago sphaerogena. Endophytic fungi isolated from various host were known to produce pigments such as Lawsone an orange-red dye produced by *Gibberella moniliformis* (Sarang et al. 2017); melanin pigment from *Spissiomyces endophytica* (Suwannarach et al. 2019). The marine endophytic fungi such as *Halorosellinia* (Li et al. 2009), Eurotium rubrum (Xia et al. 2007), *Phaeotheca,* and *Trimmatostroma were identified and reported to produce huge quantity of pigments.* Endophytic fungal strains from the leaves of *Deschampsia antarctica* produced melanin pigment (Rosa et al. 2009).

In psychrophilic fungi pigments are produced to reduce photo-damage, tolerance against freeze-thaw cycles, and desiccation (Mueller et al. 2004). *Thelebolus microspores* isolated from Antarctic Peninsula produces astaxanthin, phoenicoxanthin, and β-carotene. Filamentous fungi *Penicillium* sp. isolated from Himalayan region produce carotenoid pigment (Pandey et al. 2018). *Friedmanniomyces endolithicus* isolated from Antarctic region produces melanin that protect cells from UV radiation (Onofri et al. 2004). Pigments produced by these fungi were gaining attention to be developed as food colorants, pharmaceutical drugs, cosmetics, and textile dyes (Caro et al. 2017) (Table 19.1 and Fig. 19.2).

Table 19.1 Some promising non-carotenoid fungal pigments as potential food colorants

Fungal source	Pigment	Color	Comments	References
Ascomycetes				
Monascus spp.	Monascorubrin	Orange	Well-known pigments of the orient, authorized in Japan, heat- and pH-stable, give rise to water-soluble red pigments on reacting with amino acids in the media	Juzlova et al. (1996), Jongrungruangchok et al. (2004), Jung et al. (2003)
	Rubropunctatin	Orange		
	Monascin	Yellow		
	Ankaflavin	Yellow		
	*Monascus*ones	Yellow		
Anamorphic Ascomycetes				
Paecilomyces sinclairii	Unknown	Red at pH 3–4, violet	Light-stable, high production by submerged cultivation, chemical characterization is needed	Cho et al. (2002a, 2002b)
		At pH 5–9 and pink		
		At pH 10–12		

(continued)

Table 19.1 (continued)

Fungal source	Pigment	Color	Comments	References
Penicillium herquei	Atrovenetin	Yellow	Atrovenetin is an antioxidant and might exert a dual functionality as a functional food additive, because it potentiates the antioxidant activity of tocopherol (likely mechanism: Regeneration of tocopherol by hydrogen donation)	Robinson et al. (1992), Bachmann et al. (1986), Ishikawa et al. (1991)
Roesleria hypogea	Herqueinone	Red		
Penicillium atrovenetum	Norherqueinone	Red		
	Several others	Bluish green		
Penicillium oxalicum var. armeniaca	Arpink red™	Dark red	Commercially produced, pH- and heat-stable, patented in more than 120 countries	Sardaryan (2002), Sardaryan (2004), Sardaryan et al. (2004)
Penicillium purpurogenum	Purpurogenone	Orange-yellow	Characteristic extracellular red-pink pigment depending on media	Francis (1996), Buchi et al. (1965)
	Mitorubrin	Yellow		
	Mitorubrinol	Orange to red		
Penicillium persicinum	Unknown	Reddish pink	High amount of exogenous pigment, not yet characterized	Wang et al. (2004)
Penicillium fagi	Unknown	Greenish blue	Mostly trapped in mycelium, uncharacterized	Martinez and Ramirez (1978)

19.3 Phylogenetic Analysis of 18S rRNA ITS Gene Sequence

Molecular identification of eukaryotic fungi isolates based on 18S rRNA marker were considered as due to conservation, universal, and relatively stable. The 18S rRNA sequence in the eukaryotes, 18S rRNA marker is considered as suitable approach for molecular classification of fungal isolates (Kaur et al. 2020). However, the 18S rRNA gene molecular marker also has some limitations due to conservation in some genera, sequence variations among multiple rRNA operons, and chance of horizontal gene transfer of these genes among taxa. Earlier, for delineation of new species, 18S rRNA gene similarity value was considered around 97%, whereas 98.7–99% cutoff value was recommended for different orders (Stackebrandt and Goebel 1994; Stackebrandt and Ebers 2006).

19.4 Primers, PCR Amplification, and Sequencing

Based on phenotypic characteristics of isolates specific primers were used for molecular identification. The calmodulin gene specific using primers like Cmd5/Cmd6 were used for PCR amplification and identification of Aspergillus species

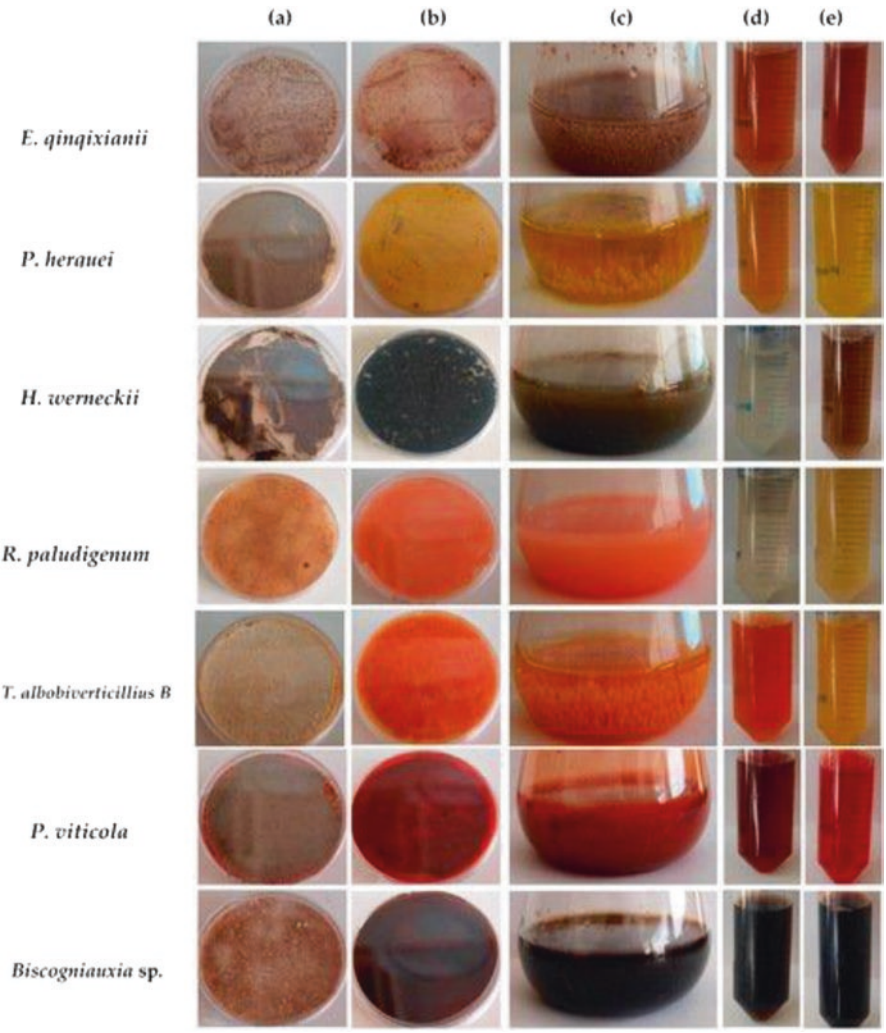


Fig. 19.2 Biodiversity of fungal isolates with pigmentation (Fouillaud et al. 2017)

and Penicillium species specific for β-tubulin using primers T10/Bt2b (Toju et al. 2012). EF-1H/EF-2T primer pair was used to amplify a fragment of the translation elongation factor 1 alpha gene (Tef1) for PCR amplification of translation elongation factor 1 alpha gene (Tef1) and identification of Trichoderma and Hypocreales species (Samson et al. 2014). ITS region were amplified using specific ITS1-F_KYO2/ITS2 or ITS3_KYO2/ITS4 for uncharacterised fungi, and, when necessary, the large subunit rDNA was also amplified using V9G/LR3 primer pair (Toju et al. 2012; Samson et al. 2014). The EzTaxon server was developed and used for analysis of sequenced isolates. EzTaxon provides calculation of multiple sequence alignment, pairwise similarity and for the phylogenetic tree construction (Table 19.2).

Table 19.2 PCR amplification and the sequencing primers used for the identification of fungal isolates

SN	Primer name	Primer sequence	References
1	ITS1-F_KYO2 Forward	TAGAGGAAGTAAAAGTCGTAA	Toju et al. (2012)
2	ITS2_KYO2 Reverse	TTYRCTRCGTTCTTCATC	Toju et al. (2012)
3	ITS3_KYO2 Forward	GATGAAGAACGYAGYRAA	Toju et al. (2012)
4	ITS1 Forward	TCCGTAGGTGAACCTGCGG	White et al. (1990)
5	ITS2 Reverse	GCTGCGTTCTTCATCGATGC	White et al. (1990)
6	ITS3 Forward	GCATCGATGAAGAACGCAGC	White et al. (1990)
7	ITS4-F Reverse	TCCTCCGCTTATTGATATGC	White et al. (1990)
8	Cmd-5 Forward	CCGAGTACAAGGARGCCTTC	Samson et al. (2011)
9	Cmd-6 Reverse	CCGATRGAGGTCATRACGTGG	Samson et al. (2011)
10	Tn10 Forward	CGATAGGTTCACCTCCAGAC	Samson et al. (2011)
11	Bt2b Reverse	ACCCTCAGTGTAGTGACCCTTGGC	Samson et al. (2011)
12	EF-728 Forward	CATCGAGAAGTTCGAGAAGG	Samson et al. (2011)
13	TEF1-LLE-Reverse	AACTTGCAGGCAATGTGG	Samson et al. (2011)

19.5 Genetic Fingerprinting of Fungi

Microbial diversity of exact microbial community can be investigated and studied using approaches either partial community analysis using 18S rRNA or single gene or whole community analysis through whole genome analysis (Fig. 19.3).

The partial community analysis can be investigated by using polymerase chain reaction (PCR) and PCR amplification of a specific gene product from the community, which reflects a mixture of gene signatures from a particular group of organisms.

The PCR amplification specific targeted genes can be used analyze differences between microbial communities through genetic fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE), ribosomal intergenic spacer analysis (RISA), single strand conformation polymorphism (SSCP), terminal restriction fragment length polymorphism (T-RFLP), temperature gradient gel electrophoresis (TTGE), length heterogeneity PCR (LH-PCR), amplified ribosomal DNA restriction analysis (ARDRA), and based on either sequence or fragment length polymorphism.

In addition, molecular markers such as random amplified polymorphic DNA (RAPD), D1/D2 regions of large subunit ribosomal RNA (LSU rRNA), internal transcribed spacer (ITS) and partial β tubulin genes, *Monascus* retrotransposon (MRT) as well as inter-simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) have been used for the *Monascus* classification and phylogenetic analysis, which are free from the effects of variation of cultural conditions on morphological and physiological characteristics, providing alternative tools for discrimination of *Monascus* spp. (Park et al. 2004).

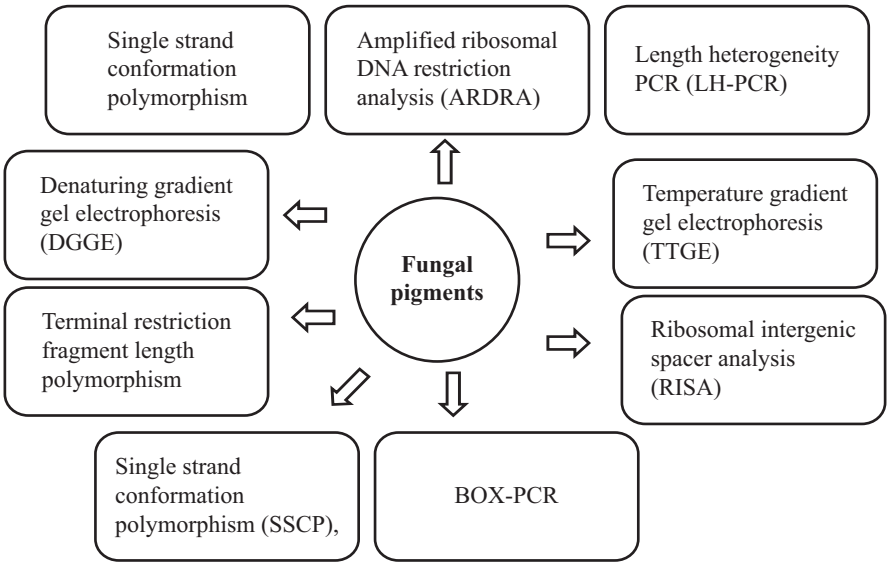


Fig. 19.3 Phylogenetic and genetic fingerprinting of fungi

19.6 Production and Purification of Fungal Pigments

Characterization of fungal pigments from natural sources involves several chromatographic and analytical techniques like thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), ultra performance liquid chromatography-tandem mass spectrometer (UPLC-MS), and UV-Vis (Ultraviolet-Visible) spectrophotometer. Bioactive pigments and compounds have been effectively separated in TLC with different solvents system (Feng et al. 2012). Dietary carotenoids in some food supplements were separated by TLC using solvent system methanol: acetone, 1:1(v/v) (Simonovska et al. 2012).

HPTLC is a chromatographic technique widely used for qualitative and quantitative analysis of the components extracted from bacterial, fungal pigments, and carotenoids like lutein, lycopene, and β-carotene were evaluated by HPTLC with methanol–dichloromethane (1:1, v/v) as developing solvent system with 0.5% triethylamine (TEA) (Visalakchi and Muthumary 2009). HPLC with reversed-phase C8, C18 or C30 columns is more suitable for isolating carotenoids in samples extracted from natural sources were reported earlier (Dugo et al. 2006). Hence, HPLC with reversed-phase C8, C18, or C30 columns is more suitable for isolating carotenoids in samples extracted from natural sources.

Spectroscopic methods such as ^{1}H NMR and ^{13}C NMR are suitable techniques to predict the structure of macromolecules or compounds. FTIR method is used to trace the functional group of macromolecules. Especially carotenoids like lycopene 3450, 2924, 2854, 1643, and 1510 cm^{-1} were attributed to OH, CH_2 asymmetrical, CH_2 symmetrical, C=C of olefin and C=C, respectively (Mohamed et al. 2011). Crocetin C=O (1664 cm^{-1}), C–O (1243 cm^{-1}), O–H (3400 cm^{-1}), C=C (1540 cm^{-1}), C–C (1166 cm^{-1}); for β-carotene C=C (1517 cm^{-1}), C–C (1160 cm^{-1}), C=O (1687), O–H (3372 cm^{-1}). Moreover, UPLC is a highly sensitive analytical technique for effective separation of pigments or compounds (Delpino-Rius et al. 2014).

Structurally correlated molecules and their epoxidized forms can be differentiated by using HPLC/MS-MS (Goupy et al. 2013). Several methods for ionization of carotenoids have been used which includes: electron impact (EI), matrix-assisted laser desorption/ionization (MALDI), fast atom bombardment (FAB), Electrospray ionization (ESI), atmospheric pressure chemical ionization, atmospheric solids analysis probe (ASAP) and atmospheric pressure photoionization (APPI) to analyse samples. Samples can be analyzed directly without preparation of sample or chromatographic separation via ASAP, Raman spectroscopy and MALDI-TOF-MS as well. Hence carotenoids have been efficiently determined by MALDI-TOF-MS (Manikandan et al. 2013).

FAB ionization reduces rearrangement and degradation of the structures of carotenoid scan. Carotenoid analysis are commonly performed using API coupled with MS and it has substituted FAB ionization method (Breemen et al. 2012). Carotenoids in spinach leaves have been detected by ASAP (Mcewen et al. 2005). LC combined with ESI and APCI has been found to be the most used and APCI has been found to be an excellent ionization technique for non-polar and lipophilic pigments(carotenoids), whereas polar compounds by ESI technology for ionization are mostly used (Allwood and Goodacre 2010). Thus, in the Raman scattering process, the energy is exchanged between the incident monochromatic light and the scattered molecules. Raman spectroscopy is a non-destructive technique for microbial pigments determination and carotenoids identification in various resources such as algae, bacteria, and coral which is effectively employed for halophilic research (Jelicka and Oren 2013).

Raman bands mainly correspond to respective vibrational modes,which involve motions of the atoms in the chromophore, which is that portion of the molecule where the electronic transition were exactly localized. Three intense Raman scattering modes of carotenoids are defined as υ1-conjugated C=C stretching vibrations, υ2-C-C vibrations coupled to C-CH_3 stretching or C–H in plane bending and υ3-CH stretching modes, of these three, υ band is the most diagnostic features of carotenoids (Kushwaha 2014). The chemical composition of the major pigments was discussed recently, and new methods for identifying natural dyes used frequently in foods were described (Molnar et al. 2010). Raman and NIR Raman spectroscopy were used recently for the characterization of natural dyes (Li-Chan 1996; Schrader et al. 1999). Structures and physico-chemical properties of 100 naphthoquinone metabolites produced by filamentous fungi were reviewed recently (Medentsev and Akimenko 1998).

19.7 Biotechnological Applications of Fungal Pigments

Fungal pigments are secondary metabolites, based on their chemical structure they can be grouped into distinct classes: polyketides, non-ribosomal peptides, terpenes, and aminoacid derived chemical compounds. Generally, polyketides are synthesized by polyketide synthases (PKS), are structurally complex which includes anthraquinones, naphthoquinone pigments, flavonoids, azaphilone pigments (polyketide derivatives), hydroxyanthraquinone pigments, etc. Different species of filamentous fungi are known to produce polyketide pigments with varying colors like orange, red, yellow, and brown (Dufossé et al. 2014; Gao et al. 2013).

The fungi *Fusarium* belonging to the *Nectriaceae* family have been known to produce a broad range of pigments with numerous biological activities (Mapari et al. 2009). Bostrycoidin, a red pigment (naphthoquinone) was isolated and characterized from *Fusarium bostrycoides* (Cajori et al. 1954). Aurofusarin (red dimeric naphthoquinone pigment) from *Fusarium graminearum* and bikaverin (red naphthoquinone pigment) from *Fusarium fujikuroi* are the most well studied polyketide pigments (Wiemann et al. 2009; Frandsen et al. 2006; Frandsen et al. 2011). Aurofusarin produced from *Fusarium culmorum* is pH dependent, which shows color shifts from yellow in acidic and red–purple in alkaline range (Ashley et al. 1937). A well-known producers of aurofusarin are *Fusarium graminearum, Fusarium tricinctum, Fusarium acuminatum, Fusarium culmorum*, etc. (Samson et al. 2002).The fungi *Fusarium lycopersici and Fusarium vasinfectum* were reported as the first ever producers of pigment bikaverin in cultures (Limón et al. 2010). The investigation of *Fusarium fujikuroi* for the production of bikaverin, a red naphthoquinone pigment has shown that the production is culture dependent and it is repressed when the culture media are alkaline and have high nitrogen content (Wiemann et al. 2009; Limón et al. 2010; Rodríguez-Ortiz et al. 2010). The fungal polyketide pigment, bikaverin has enormous biological properties including antibiotic activity and antitumor potential (Zhan et al. 2007).

Trichoderma is a group of fungi of the family *Hypocreaceae* are the long familiar producers of secondary metabolites with biological activity and are used as biocontrol agents and biopesticides (Vinale et al. 2008). Pachybasin, yellow hydroxyanthraquinone pigment and chrysophanol, orange pigment are known to be produced from several strains of *Trichoderma* which includes *Trichoderma aureoviride, Trichoderma viride, Trichoderma harzianum,* and *Trichoderma polysporum*. *Trichoderma viride* and *Trichoderma polysporum* also produce emodin (yellow pigment) (Caro et al. 2012) and *Trichoderma harzianum* is reported for the secretion of both emodin and pachybasin (Lin et al. 2012). Notably, the two commercial strains of *Trichoderma harzianum* T22 and T39 are widely used as biofertilizers and biopesticides (Vinale et al. 2009).

Hydroxyanthraquinone pigments are majorly produced by species of *Drechslera, Alternaria,* and *Curvularia* belonging to the family *Pleosporaceae*. Cyanodontin, catenarin, chrysophanol, and erythroglaucin are main pigments synthesized from *Curvularia lunata* (Caro et al. 2012). Disperse blue 7 and Acid green 28 are the anthraquinone pigments which are extracted from the fungus *Curvularia lunata* and are used as biodyes. Altersolanol A, a yellow-orange pigment is produced from

several species of *Alternaria* including *Alternaria solani, Alternaria tomatophila,* and *Alternaria porri* (Andersen et al. 2008) and is potentially safe without mycotoxins. Various species of fungus *Drechslera*, e.g., *Drechslera phlei, Drechslera avenae, Drechslera teres*, etc. are reported for the production of several hydroxyanthraquinone pigments such as helminthosporium, catenarin, etc. (Caro et al. 2012; Durán et al. 2002).

Fungal strains of the family *Cordycipitaceae,* which includes *Beauveria, lecanicillium, Isaria*, *Cordyceps* are the most promising producers of the orange to red bioactive pigments. Erythrostominone, red naphthoquinone pigments were known to be produced from the fungus *Cordyceps unilateralis* (Unagul et al. 2005). Red pigment (hydroxyanthraquinone type) extracted from the cultures of the fungus *Isaria farinosa* is resistant to pH and temperature provides new perspectives for the food coloring industry (Velmurugan et al. 2010). Several species of fungus *Beauveria* has been reported for the production of bassianin and tenellin (Watt et al. 1977). Tenellin is widely used for the control of agricultural pests such as white flies, mites, bugs, etc. Oosporein, a deep red mycotoxin produced by the fungal strains *Lecaniccilium aphanocladii* and *Beauveria bassiana* has many biological activities such as antimicrobial, phytotoxic effects on plant, antifungal, etc. (Vining et al. 1962; Souza et al. 2015). Skyrin, orange-red pigment discovered from *Hyperdermium bertonii* has the potential for use in agrochemical application which has selective toxicity against insects (Goldberg and Rokem 2009). Although pigments isolated from the fungal strains belonging to *Cordycipitaceae* have bioactive potential, these pigments are mostly mycotoxins and their harmlessness needs to be tested before using for industrial applications (Fig. 19.4 and 19.5).

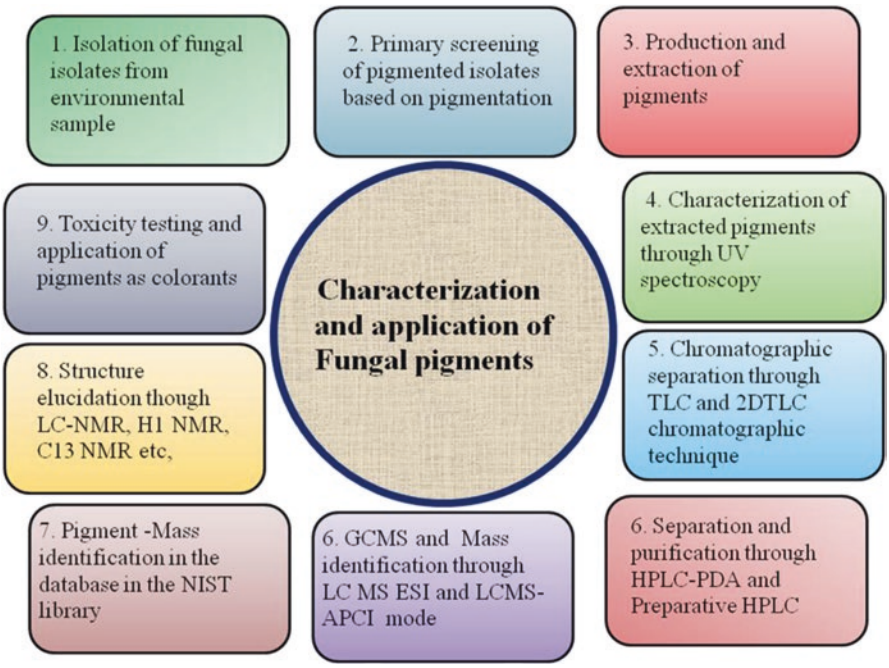


Fig. 19.4 Characterization and applications of pigmented fungal from environmental samples

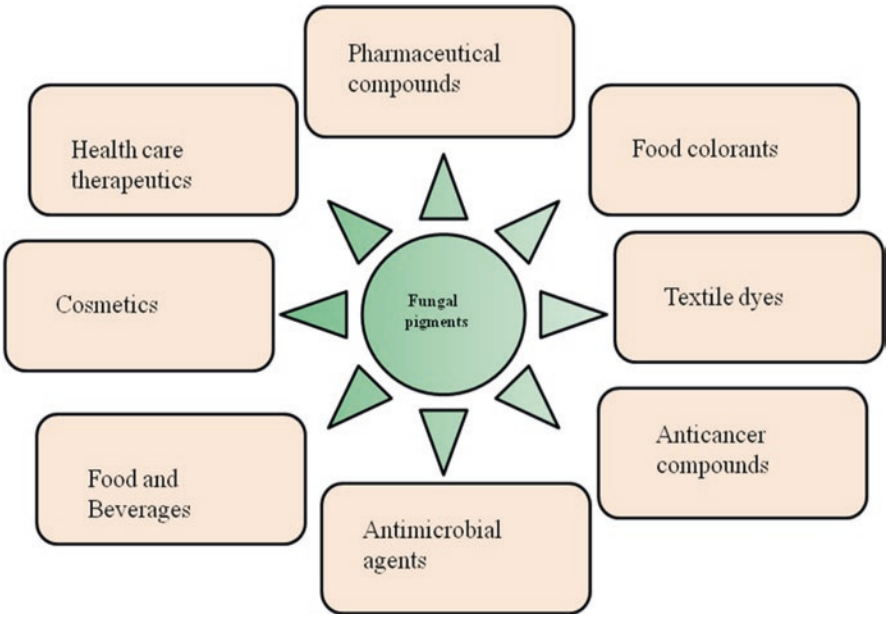


Fig. 19.5 Biotechnological applications of pigmented fungus

19.7.1 Edible Filamentous Fungi Monascus *and its Applications*

Monascus spp. have been used traditionally in fermented food products, nutraceutical and for medicinal purposes for curing dysentery, indigestion, and relieving pain (Lin et al. 2008). *Monascus* spp. belong to family Monascaceae, phylum ascomycota and nine species such as *Monascus ruber.*, *M. pilosus M. purpureus*, *M. floridanus*, *M. pallens*, *M. sanguineus*, *M. eremophilus*, *M. lunisporas*, *M. argentinensis* were accepted internationally Hawksworth D L, Pitt J I (1983); Barnard E L (1987).

The production of secondary metabolites from *Monascus* spp. and color constituents of *Monascus* pigments depend on fermentation condition. The secondary metabolites from *Monascus* spp. such as monacolins, γ-aminobutyric acid, dimerumic acid, etc. were reported earlier (Chen and Hu 2005).

In recent years, *Monascus* pigments showed multiple biological functions, such as anticancer properties, antimicrobial activities, anti-mutagenic and potential antiobesity characteristics, etc., *Monascus* pigments have potential applications in various fields (Feng et al. 2012). Japanese professor Akira Endo reported for first time Monacolin K or lovastatin, by separation from fermentation products of *M. ruber* fungal isolate (Endo and Monacolin 1979). Monacolin K plays keys role in inhibiting cholesterol synthesis by competitively inhibiting the activity 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), a rate limiting enzyme in cholesterol

biosynthesis and lowering cholesterol levels in animal and human blood. The study found at major 14 substances with hypolipidemic function in red yeast rice, including MK, monacolin J, monacolin L, monacolin M, monacolin X and their acidic structures, dehydroMK, dihydromonacolin L and compactin (Li et al. 2004).

Monascus pigments are produced through solid state or submerged fermentation method for enhanced production of pigments using substrates like rice and wheat meals (either as integral or broken residual cereal) and commercialized *Monascus* pigments as raw fermented powders after extraction with different suitable solvents. Stability of *Monascus* pigments stability depends on temperature the pigment resists processing for 30 min at 100 °C (Carvalho et al. 2005). Care must be taken for most strains producing citrinin, a yellow nephrotoxic mycotoxin; also, raw biomass may contain the anti-hypercholesteremic molecule lovastatin. *Pycnoporus sanguineus*, a ubiquitous wood-growing bioactive fungus which produces phenoxazine analogs with antimicrobial activity. *Monascus* pigments stability specifically depends on pH and temperature; for pH 7–8 in aqueous media, the pigment resists processing for 30 min at 100 °C but may lose up to 20% tinctorial strength at pH 4. More attention is needed for the production of *Monascus* pigments lovastatin, especially the presence of citrinin, a yellow nephrotoxic mycotoxin in the raw biomass (Carvalho et al. 2005) (Table 19.3 and Fig. 19.6).

Table 19.3 *Monascus* fermentation and optimized media for monacolin K production

SN	Microbial strains	Optimal nutrients	MK production (mg/kg)	References
1	*Monascus* mutant KU609	2.5% Soytone, 2.4% glucose and 0.26% MgSO4	977.70	Suh et al. (2007)
2	*M. purpureus* KCCM 60,168	1.32% glucose and 0.20% peptone	13,400	Suraiya et al. (2018)
3	*M. pilosus* KMU108	2.2% ganghwayakssuk and 3.8% glucose	3007	Lee and Lee (2012)
4	*M. purpureus* TISTR 3541	2% glycerol, 0.14% methionine and 0.01% sodium nitrate	5900	Jirasatid (2013)
5	*M. purpureus* 9901	5% soybean meal and 26%	12,900	Lu et al. (2013)
6	*M. purpureus*	4 mg/g NH_4Cl and 0.2 mg/g	6238.2	Kanpiengjai et al. (2018)
7	*M. pilosus* MS-1	35% water content, 0.6% (v/w) acetic acid and 0.004 Mol/kg MgSO4•7H2O	18,733	Feng et al. (2014)
8	*M. sanguineus*	20 g/L soybean, 2.5 g/L CaCl2 and 25 μL acetic acid	20,040	Dikshit and Tallapragada (2016)
9	*M. purpureus* MTCC 369	14.32 g/L NH4Cl, 0.76 g/L MgSO4, 14.65 g/L NaCl and 0.54 g/L CaCl2	3403	Kraboun et al. (2013)
10	*M. ruber*	20% glycerol and 3% soybean meal	19,8100	Zhang et al. (2018)

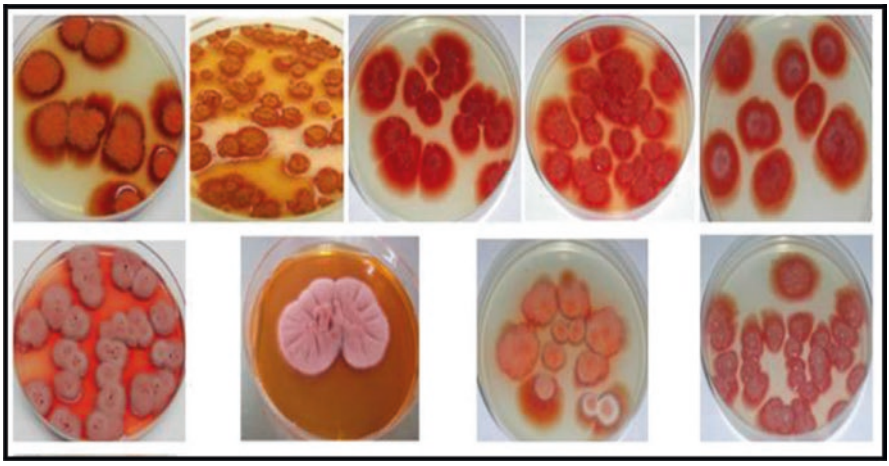


Fig. 19.6 Distinct morphology of several pigmented *Monascus* species. (Manan et al. 2017)

19.7.2 Carotenoids from Filamentous Fungi

Carotenoids are isoprenoid units with linear chain of alternate conjugate double bonds with different functional groups at terminal ends. Biosynthesis of carotenoids is initiated with five carbon structured isopentyl pyrophosphate as a precursor. Spectral characteristics of various carotenes mainly depend on the length of the conjugative bonds. Carotenoids are present in various vegetables like carrots, citrus peels, and tomatoes. In addition some microalgae, macroalgae, bacteria, and fungi species can also produce wide range of carotenoids with structural diversity (Avalos et al. 2015). More than seven hundred forms of different structural carotenoids have been identified so far, and it is widespread among both prokaryotes and eukaryotic organisms. These carotenoids appear in microbial cells as orange, yellow, and even red in colors. Carotenoids are naturally occurring pigments with widely used as food colorants, feed additives, antioxidants, dyes, and antitumor agents. Carotenoids like β-carotene, lycopene, astaxanthin, canthaxanthin, lutein, etc. are natural pigments have been used for nutraceutical and industrial applications (Dufoss 2006) (Fig. 19.7).

Carotenoids are lipid soluble polyisoprenoid compounds associated with the lipidic fractions sensitive to oxygen, heat, and light. Carotenoids can be divided into two main groups, they are (a) carotenes or hydrocarbon carotenoids (composed of carbon and hydrogen atoms), and (b) xanthophylls (oxygenated hydrocarbon derivatives contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy, or carboxylic acid groups) (Kirti et al. 2014). Specific names of the carotenes are preceded by the Greek letter prefixes that designate the two end groups out of seven (β, ζ, Ψ, ε, κ, ώ, γ, Φ, and χ). Some carotenoids have the structure consisting of more than 40 carbon atoms and derived formally by a loss of part of the C40 structure. When carbon atoms lost from terminal position of the molecule, they are commonly referred to as apocarotenoids or norcarotenoids (Liang et al. 2006). Carotenoids are linear chain of conjugated double bonds with different functional groups at the terminal ends. It is the conjugated double bond system in the

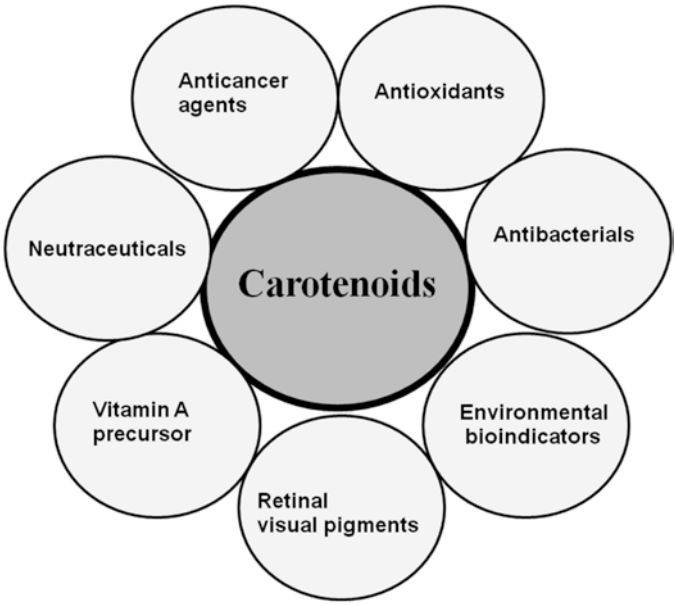


Fig. 19.7 Various applications of fungal carotenoids

carotenoid structure that acts as the chromophore for wavelength selective (light) absorption, giving these compounds an attractive bright yellow to red color.

Many biosynthetic enzymes involved in carotenoid biosynthesis which fall into few classes based on the type of reaction they catalyze like the transformation of geranylgeranyl pyrophosphate synthase, phytoene synthase, carotene desaturase, and lycopene cyclase. Modification of carotenes is further catalyzed by β-carotene ketolase and β-carotene hydrolase to generate various C-40 carotenoids.

Each desaturated reaction shifts the absorption maxima towards longer wavelengths resulting in different yellow to red colors of carotenoids (Joshi et al. 2003). The transformation from trans to cis can be done by acid, heat, oxygen and exposure to light (Molnar et al. 2010). Similar to other metabolites, carotenoids have ecological functions and protect from photooxidation. Sterols, dolichols, and ubiquinones fulfill the essential cell functions, while secondary carotenoids, such as astaxanthin and canthaxanthin, are mainly accumulated as a response to environmental stress and other abiotic factors (Dufoss 2006). Carotenogenic fungi Neurospora produce mixture of carotenoid like neurosporaxanthin and C35-apocarotenoid (Avalos and Corrochano 2013; Estrada et al. 2008). The filamentous fungus *F. fujikuroi* is well known to produce neurosporaxanthin and this fungal strain is very useful to explore the regulation and biosynthetic pathway of neurosporaxanthin production, accumulation, and storage in filamentous fungi (Díaz-Sánchez et al. 2011; Prado-Cabrero et al. 2007).

Genes involved in carotenoid and isoprenoid biosynthesis undergo both negative and positive negative feedback regulatory mechanism based on signaling molecules, substrates, and environmental conditions (Baranski and Cazzonelli 2016). Carotenoid biosynthesis gene remain silent in normal environment but its gene

expression is influenced by culture conditions and exploring its biosynthetic pathway or understanding molecular mechanism can help in large scale production (Torres et al. 2016). Filamentous fungus produces various secondary metabolites, especially carotenoids in response to external stimuli and signals proteins like Heterotrimeric G proteins (G proteins) which triggers upstream regulation of carotenoid biosynthesis (Yu and Keller 2005). The carotenoid pathway is initiated by the condensation of two geranylgeranyl pyrophosphate (GGPP) molecules by the bifunctional enzyme with phytoene synthase and lycopene cyclase activity, *N. crassa* synthesize carotene–phytoene, the main precursor to different carotenoids. Singgih et al. 2015 studied and reported carotenogenesis of *N. intermedia* N-1 in a liquid fermentation and identified five carotenoid such as lycopene, neurosporene, γ-carotene, β-carotene, and phytoene in the spores.

19.7.3 Melanin from Filamentous Fungi

Melanin is a highly complex, non-digestible, and insoluble dark brown to black pigment produced by some filamentous fungal species (in addition to other microbes and plants) under the genera *Auricularia*, *Eurotium, Wangiella, Penicillium, Sporothrix, Stachybotrys*, *Alternaria*, *Armillaria*, *Epicoccum*, *Ochroconis*, *Penicillium*, *Cladosporium*, *Magnaporthe*, *Phomopsis* and *Aspergillus*, *Spissiomyces* (Ellis and Griffiths 1974; Filip et al. 1974; Bell and Wheeler 1986; Hamada et al. 2014; Pal et al. 2014; Liu et al. 2014; De la Rosa et al. 2017; Martin and Haider 1969; Rajagopal et al. 2011; Zou and Hou 2017). Fungal melanin has a multifaceted molecular structure which is negatively charged, hydrophobic in nature with high molecular weight, produced by oxidative polymerization of phenolic and indolic compounds (Wheeler and Bell 1988; Huang et al. 2018; Plonka and Grabacka 2006).

Most fungal melanins were synthesized from tyrosine via 3,4-dihydroxyphenylalanine (DOPA) in media. However, in case of Basidiomycotina, they were isolated from their cell walls by glutaminyl-3,4-dihydroxybenzene (GDHB) or catechol. Similarly, in Ascomycotina and associated Deuteromycotina species, dark-brown to black melanins were produced via polyketide synthase pathway from the precursor 1,8-dihydroxynaphthalene (DHN). In addition, few types of extracellular melanins were also produced by fungi, mostly known as "heterogeneous" melanins.

The presence of melanin in fungi has helped them to survive under various abiotic stress conditions by showing resistance against heavy metal toxicity, temperature extremes, UV light damages, hydrolytic enzymes, and antimicrobial drugs (Butler and Day 1998; Fogarty and Tobin 1996; Garcia-Rivera and Casadevall 2001; Dadachova et al. 2007; Gomez and Nosanchuk 2003). Similarly regarding biotic stress, it acts as a barrier by protecting them against various enzymes (proteases and hydrolases) and other toxic proteins such as magainins, defensins, and protegrins (Nosanchuk et al. 2015) from different bacterial and animal sources. In addition, its presence has also been identified to enhance the virulence of certain parasitic fungi Exophiala (Wangiella) dermatitidis, Paracoccidioides brasiliensis, and *Sporothrix schenckii* (Gomez and Nosanchuk 2003; Schnitzler et al. 1999; Romero-Martinez et al. 2000).

A number of studies have been carried out to standardize the culture conditions for the production of melanin pigments from fungal species. Regarding temperature, fungal species such as *Monascus purpureus*, *Monascus sp*. J101, and melanin-overproducing mutant (MEL1) from *Aspergillus nidulans* showed highest pigment production at three different temperatures at 30, 25, and 28 °C, respectively. Generally, fungi produce high melanin pigment at alkaline pH condition, however, there are few fungi such as *Trametes versicolor* and *Xylaria polymorpha* produced maximum pigmentation at acidic pH range (4.5–5.0) (Lisboa 2003; Orozco and Kilikian 2008; Kang et al. 2014; Tudor et al. 2013). Reports also suggest that melanin production under dark light with proper aeration increased the intracellular and extracellular pigment production in addition to increased biomass (Velmurugan et al. 2010). Regarding carbon and nitrogen, the potential of fungus to produce melanin pigments differs based on the sources used. Whereas in *Gliocephalotrichum simplex*, the maximum pigment production was obtained with tyrosine (2.5%) and peptone (1%) in culture medium. In *Auricularia auricular*, a combination of tyrosine, soluble starch, CaCO3, peptone, and K2HPO4 showed high melanin pigment production. In another study with *A. auricular*, yeast extract, tyrosine, and lactose combination showed significant production of melanin (Sun et al. 2016). Similarly, under submerged culture condition, *A. auricular* obtained maximum melanin yield with L-tyrosine, wheat bran extract, and CuSO4 (Zou and Tian 2017). Fungal melanin has a wide range of application in the fields of dermocosmetics, nanotechnology, and biomedicine and material science.

Regarding bioelectronics application, melanin is used for the production of continuous thin films which has been used in chemi-sensors, next generation solar cells, and a range of other detectors including superconducting transition-edge bolometers (Morresi et al. 2010; Seppa 2001). Melanin is also used to prepare optical lenses or filters, which can be used in protective eyewear, windows, ophthalmic devices, canopies, and other such materials for protection against radiation (Gallas and Eisner 2006).

Melanin nanoparticles can be used as biocompatible drug nanocarriers. Metronidazole, a very interesting nanocarrier drug release device successfully used for the treatment of intestine and colon diseases (Araújo et al. 2014). Melanin has also been used to treat disorders of the immune system including malignant cancer tumors, diseases of blood origin, and disorders. It has also been used for treating genetic disorders such as Parkinson's disease and Alzheimer's disease (Berliner et al. 1998). In addition, melanin can also be used for the development of stem cells and in tissues repair engineering (Pezzella et al. 2015). In cosmetic industry, melanin is used as an ingredient in hand and face creams, body lotions, foundation make-ups, or anti-aging ointments, as it acts as an effective antioxidant by protecting the skin against the ultraviolet rays and photoinduced skin damages (Pawelek and Platt 1998). These melanin pigments are also been used for hair dyeing and improvement in hair recoloration without any side effects (Dlschia et al. 2013).

Melanin-mediated synthesis of silver nano particles shows broad-spectrum antimicrobial activity which can be used in effective paint additives and in food packaging industries (Apte et al. 2013). Melanin acts as an effective biosorbent due to its enhancing biomass-metal interaction leading to increased biosorption capacity for removal of rare earth metals from waste water. They are used for bioremediation process in the contaminated sites, to recover the heavy metals and radionuclides (Caporalin 2011).

19.7.4 Fungal Pigments as Ecofriendly Textile Dyes

Natural dyes from microbial sources gained more attention due to various applications, especially in the textile industry due to degradable in nature and prevent from environmental pollution when compared to synthetic dyes (Mansi and Gaurav 2016; Selvi 2014). Velmurugan et al. 2010 reported application of fungal *Monascus* pigments in dyeing cotton yarn effectively. The application of natural dyes in different fabrics with broad spectrum of antimicrobial activities against pathogens has been investigated and reported such as red colored prodigiosin from *Serratia marcescens*, purple pigmented bioactive compound violacein from *Chromobacterium violaceum* (Dura et al. 2012; Tobie 1934) (Fig. 19.8).

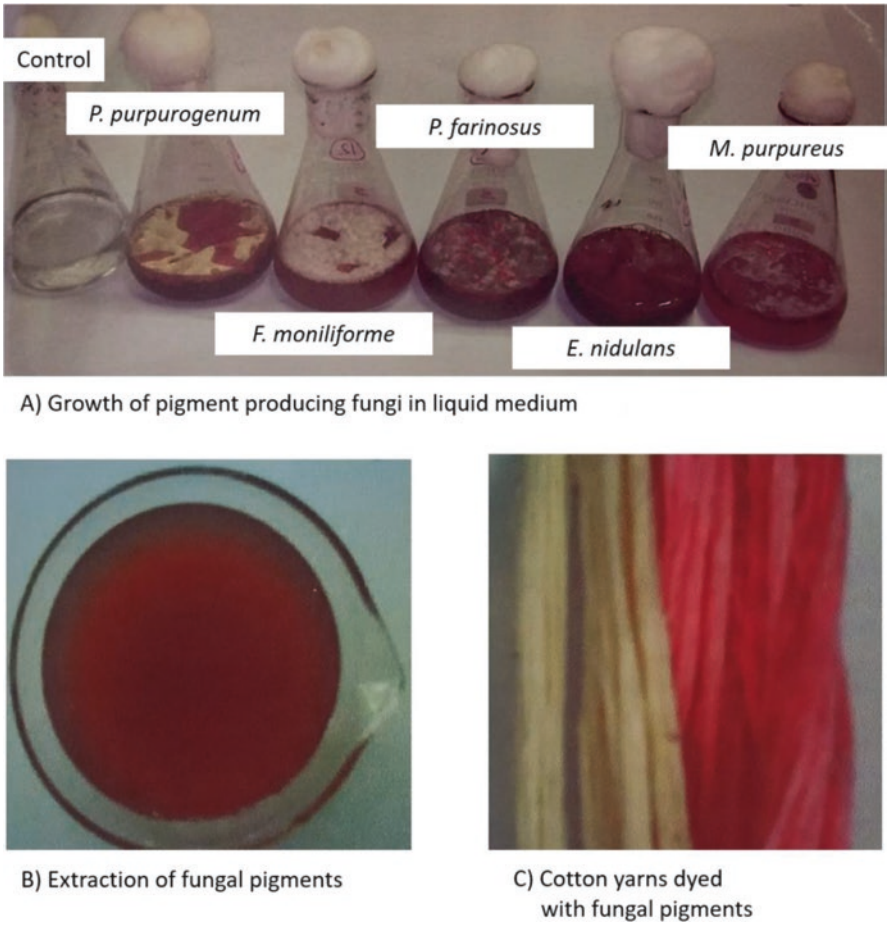


Fig. 19.8 Applications of fungal pigments in textile dyeing (Velmurugan et al. 2010). a) Growth of pigment producing fungi in liquid medium. b) Extraction of fungal pigments. c) Cotton yarns dyed with fungal pigments

19.8 Conclusion and Future Prospects

Fungal based pigments offer several advantages like solid state fermentation, higher yields, no seasonal variations and strain improvements for enhanced production of pigment production. Enhanced production of fungal pigments can be optimized using cheaper substrate or alternative media selection, pH, and temperature. Even though more careful attention is needed to avoid the presence of fungal mycotoxin like citrinin, which cause severe harmful effects to human being. Commercialization of edible fungal pigments can be further improved by improving shelf life of secondary metabolites through polymer encapsulation, green extraction, cheap organic substrate and designing novel fermentor designs to enhance production. In addition, fungi produce vast array of pigmented metabolites as compared to plants and other eukaryotic organisms. Fungal based pigments can be used widely for food colorants, nutraceuticals and pharmaceutical industry, which are sustainable and eco-friendly in nature. Genome mining and metabolic engineering of pathways of pigmented fungus can be used in the near future for industrial scale production of natural pigments.

Acknowledgements All the authors wish to thank National Institute of Pharmaceutical Education and Research (NIPER), Kolkata India for providing infra-structural facility.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Vol-1: biodiversity and ecological perspective. Springer, Cham

Aberoumand A (2011) A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. World J Dairy Food Sci 6:71–78

Allwood JW, Goodacre R (2010) An introduction to liquid chromatography-mass spectrometry instrumentation applied in plant metabolomic analyses. Phytochem Anal 21:33–47

Andersen B, Dongo A, Pryor BM (2008) Secondary metabolite profiling of *Alternaria dauci*, *A. porri*, *A. solani*, and *A. tomatophila*. Mycol Res 112:241–250

Arora S (2014) Textile dyes its impact on environment and treatment. J Biorem Biodegener 5(3):1

Ashley JN, Hobbs BC, Raistrick H (1937) Studies in the biochemistry of microorganisms: the crystalline colouring matters of *Fusarium culmorum* (W.G. Smith) Sacc. And related forms. Biochem J 31:385–397

Avalos J, Carmen Limon M (2015) Biological roles of fungal carotenoids. Curr Genet 61:309–324

Avalos J, Corrochano LM (2013) Carotenoid biosynthesis in *Neurospora*. In: Kasbekar DP, McCluskey K (eds) *Neurospora*: genomics and molecular biology. Horizon Scientific Press, Great Britain, pp 227–241

Apte M, Girme G, Bankar A, Ravikumar A, Zinjarde S (2013) 3,4-dihydroxy-L- phenylalanine derived melanin from Yarrowia lipolytica mediates the synthesis of silver and gold nanostructures. J Nanobiotechnol 11:3–11

Araújo M, Viveiros R, Correia TR, Correia IJ, Bonifácio VDB, Casimiro T (2014) Natural melanin: a potential pH-responsive drug release device. Int J Pharm 469(1):140–145

Avalos J, Carmen Limón M (2015) Biological roles of fungal carotenoids. Curr Genet 61(3):309–324

Avalos J, Cerdà-Olmedo E (1987) Carotenoid mutants of Gibberella fujikuroi. Curr Genet 11:505–511

Bachmann O, Kemper B, Musso H (1986) The green pigment from the fungus *Roesleria hypogea*. Liebigs Annalen Der Chemie 1986:305–309

Baranski R, Cazzonelli C (2016) Carotenoid biosynthesis and regulation in plants. In: Kaczor A, Baranska M (eds) Carotenoids: nutrition, analysis and technology. Wiley-Blackwell, London, pp 159–189

Blanchette RA, Wilmering AM, Baumeister M (1992) The use of green-stained wood caused by the fungus Chlorociboria in intarsia masterpieces from the 15th century. Holzforsch Int J Biol Chem Phys Technol Wood 46:225–232

Blumenthal CZ (2004) Production of toxic metabolites in *Aspergillus Niger*, *Aspergillus oryzae*, and *Trichoderma reesei*: justification of mycotoxin testing in food grade enzyme preparations derived from the three fungi. Regul Toxicol Pharmacol 39:214–228

Breemen RBV, Dong L, Pajkovic ND (2012) Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids Lycopene. Int J Mass Spectrom 312:163–172

Buchi G, White JD, Wogan GN (1965) The structures of mitorubrin and mitorubrinol. J Am Chem Soc 87:3484–3489

Burrows A (2009) Palette of our palates: a brief history of food coloring and its regulation. Compr Rev Food Sci Food Saf 8:394–408

Barnard EL (1987) A new species of *Monascus* from pine tissues in Florida. Mycologia 79(3):479–484

Bell AA, Wheeler MH (1986) Biosyntesis and functions of fungal melanins. Annu Rev Phytopathol 24:411–451

Berliner DL, Erwin RL, McGee DR (1998) Therapeutic uses of melanin. Patent No. 5,776,968

Butler MJ, Day AW (1998) Fungal melanins: a review. Can J Microbiol 44(12):1115–1136

Caporalin CB (2011) Comparison of biosorption of rare earth metals by melanised biomass of Aspergillus nidulans fungus in free and immobilized forms [thesis]. Araraquara: Sao Paulo State University

Carmen LM, Rodríguez-Ortiz R, Javier A (2010) Bikaverin production and applications. Appl Microbiol Biotechnol 87:21–29

Caro Y, Venkatachalam M, Lebeau J, Fouillaud M, Dufossé L (2017) Pigments and colorants from filamentous fungi. In: Fungal metabolites. Springer, Cham, pp 499–568

Chen F, Hu X (2005) Study on red fermented rice with high concentration of monacolin K and low concentration of citrinin. Int J Food Microbiol 103(3):331–337

Choi SY, Yoon KH, Lee JI, Mitchell RJ (2015) Violacein: properties and production of a versatile bacterial pigment. Biomed Res Int. https://doi.org/10.1155/2015/465056

Cajori FA, Otani TT, Hamilton MA (1954) The isolation and some properties of an antibiotic from Fusarium bostrycoides. J Biol Chem 208:107–114

Caro Y, Anamale L, Fouillaud M, Laurent P, Petit T, Dufossé L (2012) Natural hydroxyanthraquinoid pigments as potent food grade colorants: an overview. Nat Prod Bioprospect 2:174–193

Cho YJ, Hwang HJ, Kim SW, Song CH, Yun JW (2002b) Effect of carbon source and aeration rate on broth rheology and fungal morphology during red pigment production by *Paecilomyces sinclairii* in a batch bioreactor. J Biotechnol 95:13–23

Cho YJ, Park JP, Hwang HJ, Kim SW, Choi JW, Yun JW (2002a) Production of red pigment by submerged culture of *Paecilomyces sinclairii*. Lett Appl Microbiol 35:195–202

Delpino-Rius A et al (2014) Ultra performance liquid chromatography analysis to study the changes in the carotenoid profile of commercial monovarietal fruit juices. J Chromatogr A 1331:90–99

Devi R, Kaur T, Guleria G, Rana K, Kour D, Yadav N et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Díaz-Sánchez V, Estrada AF, Trautmann D, Al-Babili S, Avalos J (2011) The gene carD encodes the aldehyde dehydrogenase responsible for neurosporaxanthin biosynthesis in *fusarium fujikuroi*. FEBS J 278:3164–3176

Dufossé L, Fouillaud M, Caro Y, Mapari SAS, Sutthiwong N (2014) Filamentous fungi are large-scale producers of pigments and colorants for the food industry. Curr Opin Biotechnol 26:56–61

Dufosse L, Galaup P, Yaron A, Arad SM, Blanc P, Murthy KNC et al (2005) Microorganisms and microalgae as source of pigments for use: a scientific oddity or an industrial reality? Trends Food Sci Technol 16:389–406

Dugo P, Škeříková V, Trozzi A, Jandera P, Mondello L (2006) Elucidation of carotenoid patterns in citrus products by means of comprehensive normal-phase × reversed-phase liquid chromatography. Anal Chem 78:7743–7750

Dura M, Ponezi AN, Alario AF, Teixeira MFS, Justo GZ, DurN B (2012) Potential applications of violacein: a microbial pigment. Med Chem Res 21:1524–1532

Durán N, Teixeira MFS, De Conti R, Esposito E (2002) Ecological-friendly pigments from fungi. Crit Rev Food Sci Nutr 42:53–66

Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P (2007) Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS One 2(5):1–13

Dikshit R, Tallapragada P (2016) Statistical optimization of lovastatin and confirmation of nonexistence of citrinin under solid-state fermentation by Monascus sanguineus. J Food Drug Anal 24(2):433–440

DIschia M, Wakamatsu K, Napolitano A, Briganti S, Garcia-Borron JC, Kovacs D (2013) Melanins and melanogenesis: methods, standards, protocols. Pigment Cell Melanoma Res 26(5):616–633

Dufossé L (2006) Microbial production of food grade pigments. Food Technol Biotechnol 44:313–321

Ellis DH, Griffiths DA (1974) The location and analysis of melanins in the cell walls of some soil fungi. Can J Microbiol 20:1379–1386

Endo A, Monacolin K (1979) A new hypocholesterolemic agent produced by a *Monascus* species. J Antibiot 32:852–854

Estrada AF, Maier D, Scherzinger D, Avalos J, Al-Babili S (2008) Novel apocarotenoid intermediates in *Neurospora crassa* mutants imply a new biosynthetic reaction sequence leading to neurosporaxanthin formation. Fungal Genet Biol 45:1497–1505

Feng Y, Shao Y, Chen F (2012) Monascus pigments. Appl Microbiol Biotechnol 96:1421–1440

Fouillaud M, Venkatachalam M, Llorente M, Magalon H, Cuet P, Dufossé L (2017) Biodiversity of pigmented fungi isolated from marine environment in La Réunion Island, Indian Ocean: new resources for colored metabolites. J Fungi 3:36

Francis FJ (1996) Less common natural colorants. In: Hendry GAF, Houghton JD (eds) Natural food colorants. Springer, Boston, MA, pp 332–333. https://doi.org/10.1007/978-1-4615-2155-6_9

Frandsen RJ, Nielsen NJ, Maolanon N, Sørensen JC, Olsson S, Nielsen J, Giese H (2006) The biosynthetic pathway for aurofusarin in *Fusarium graminearum* reveals a close link between the naphthoquinones and naphthopyrones. Mol Microbiol 61:1069–1080

Frandsen RJN, Schutt C, Lund BW, Staerk D, Nielsen J, Olsson S, Giese H (2011) Two novel classes of enzymes are required for the biosynthesis of aurofusarin in *Fusarium graminear*um. J Biol Chem 286:10419–10428

Feng YL, Shao YC, Zhou YX, Chen FS (2014) Production and optimization of monacolin K by citrinin-free Monascus pilosus MS-1 in solid-state fermentation using non-glutinous rice and soybean flours as substrate. Eur Food Res Technol 239(4):629–636

Filip Z, Haider K, Beutelspacher H, Martin JP (1974) Comparison of IR spectra from melanins of microscopic soil fungi, humic acids and model phenol polymers. Geoderma 11:37–52

Fogarty RV, Tobin JM (1996) Fungal melanins and their interaction with metals. Enzym Microb Technol 19:317

Gallas J, Eisner M (2006) Melanin polyvinyl alcohol plastic laminates for optical applications. Patent No. 7,029,758

García-Rivera J, Casadevall A (2001) Melanization of Cryptococcus neoformans reduces its susceptibility to the antimicrobial effects of silver nitrate. Med Mycol 39:353–357
Gomez BL, Nosanchuk JD (2003) Melanin and fungi. Curr Opin Infect Dis 16(2):91–96
Gusdinar T, Singgih M, Priatni S, Sukmawati AE, Suciati T (2011) Neurospora intermedia N-1 (encapsulation and the stability of carotenoids from Neurospora intermedia N-1). J People Environ 18:206–211
Gao JM, Yang SX, Qin JC (2013) Azaphilones: chemistry and biology. Chem Rev 113:4755–4811
Garfield S (2002) Mauve: how one man invented a color that changed the world. W. W. Norton, New York
Gmoser R, Ferreira JA, Lennartsson PR, Taherzadeh MJ (2017) Filamentous ascomycetes fungi as a source of natural pigments. Fungal Biol Biotechnol 4(1):4
Gokhale SB, Tatiya AU, Bakliwal SR, Fursule RA (2004) Natural dye yielding plants in India. Nat Pro Radiance 3:228–234
Goldberg I, Rokem JS (2009) Organic and fatty acid production. Encyclopedia of microbiology. Academic Press, Oxford, pp 421–442
Goupy P, Abert M, Chemat F, Caris-veyrat C (2013) Identification and quantification of flavonols, anthocyanins and lutein diesters in tepals of *Crocus sativus* by ultra performance liquid chromatography coupled to diode array and ion trap mass spectrometry detections. Ind Crop Prod 44:496–510
Hamada T, Asnagi M, Satozawa T, Araki N, Banba S, Higashinura N, Akase T, Hirase K (2014) Action mechanism of the novel rice blast fungicide tolprocarb distinct from that of conventional melanin biosynthesis inhibitors. J Pestic Sci 39:152–158
Hawksworth DL, Pitt JI (1983) A new taxonomy for *Monascus* species based on cultural and microscopical characters. Aust J Bot 31(1):51–61
Hernández VA, Galleguillos F, Thibaut R, Müller A (2019) Fungal dyes for textile applications: testing of industrial conditions for wool fabrics dyeing. J Text Inst 110:61–66
Huang L, Liu M, Huang H, Wen Y, Zhang X, Wei Y (2018) Recent advances and progress on melanin-like materials and their biomedical applications. Biomacromolecules 19:1858–1868
Jirasatid S, Nopharatana M, Kitsubun P, Vichitsoonthonkul T, Tongta A (2013) Statistical optimization for Monacolin K and yellow pigment production and citrinin reduction by Monascus purpureus in solid-state fermentation. J Microbiol Biotechnol 23(3):364–374
Ishikawa Y, Morimoto K, Iseki S (1991) Atrovenetin as a potent antioxidant compound from *Penicillium* species. J Am Oil Chem Soc 68:666–668
Jelicka J, Oren A (2013) Raman spectroscopy in halophile research. Front Microbiol 4:1–7
Jones JD, Hohn TM, Leathers TD (2004) *Genetically modified strains of Fusarium sporotrichioides for production of lycopene and beta-carotene*. In: Society of Industrial Microbiology Annual Meeting, San Diego, pp 91
Joshi VK, Attri D, Bala A, Bhushan S (2003) Microbial pigments. Indian J Biotechnol 2:362–369
Jongrungruangchok S, Kittakoop P, Yongsmith B, Bavovada R, Tanasupawat S, Lartpornmatulee N et al (2004) Azaphilone pigments from a yellow mutant of the fungus *Monascus* kaoliang. Phytochemistry 65:2569–2575
Jung H, Kim C, Kim K, Shin CS (2003) Color characteristics of *Monascus* pigments derived by fermentation with various amino acids. J Agric Food Chem 51:1302–1306
Juzlova P, Martinkova L, Kren V (1996) Secondary metabolites of the fungus *Monascus*: a review. J Ind Microbiol 16:163–170
Kaur T, Yadav AN, Sharma S, Singh N (2020) Diversity of fungal isolates associated with early blight disease of tomato from mid Himalayan region of India. Arch Phytopathol Plant Protect:1–13. https://doi.org/10.1080/03235408.2020.1785098
Kirti K, Amita S, Priti S, Kumar AM, Jyoti S (2014) Colorful world of microbes: carotenoids and their applications. Adv Biol 2014:1–13
Kushwaha K (2014) Detection of carotenoids in psychrotrophic bacteria by spectroscopic approach. J Biosci Biotechnol 3:253–260
Kang B, Zhang X, Wu Z, Wang Z, Park S (2014) Production of citrinin-free Monascus pigments by submerged culture at low pH. Enzym Microb Technol 55:50–57

Kanpiengjai A, Mahawan R, Pengnoi P, Lumyong S, Khanongnuch C (2018) Improving the monacolin K to citrinin production ratio in red yeast rice by an X-ray-induced mutant strain of Monascus purpureus. Biotechnologia 99(2):109–118

Kim SK (2013) Marine biomaterials: characterization, isolation and applications. CRC Press, Taylor & Francis, Boca Raton

Kraboun K, Tochampa W, Chatdamrong W, Kongbangkerd T (2013) Effect of monosodium glutamate and peptone on antioxidant activity of monascal waxy corn. Int Food Res J 20(2):623–631

Lee DS, Lee I (2012) Development of monacolin K-enriched ganghwayakssuk (Artemisia princeps Pamp.) by fermentation with Monascus pilosus. J Microbiol Biotechnol 22(7):975–980

Li DL, Li XM, Wang BG (2009) Natural anthraquinone derivatives from a marine mangrove plant-derived endophytic fungus Eurotium rubrum: structural elucidation and DPPH radical scavenging activity. J Microbiol Biotechnol 19(7):675–680

Lin YL, Wang TH, Lee HM, Su NW (2008a) Biologically active components and nutraceuticals in the *Monascus*-fermented rice: a review. Appl Microbiol Biotechnol 77(5):965–973

Lin YL, Wang TH, Lee MH, Su NW (2008b) Biologically active components and nutraceuticals in the Monascus-fermented rice: a review. Appl Microbiol Biotechnol 77(5):965–973

Lisboa HCF (2003) Influence of culture conditions on the production of melanin pigment by Aspergillus fungus [thesis]. Sao Paulo State University, Araraquara

Liu DH, Wei L, Guo T (2014) Detection of DOPA-melanin in the dimorphic fungal pathogen Penicillium marneffei and its effect on macrophage phagocytosis in vitro. PLoS One 9:e92610

Lu LP, Zhang BB, Xu GR (2013) Efficient conversion of high concentration of glycerol to Monacolin K by solid-state fermentation of Monascus purpureus using bagasse as carrier. Bioprocess Biosyst Eng 36(3):293–299

Li YG, Zhang F, Wang ZT, Hu ZB (2004) Identification and chemical profiling of monacolins in red yeast rice using high performance liquid chromatography with photodiode array detector and mass spectrometry. J Pharm Biomed Anal 35(5):1101–1112

Li-Chan ECY (1996) The application of Raman spectroscopy in food science. Trends Food Sci Technol 7:361–370

Limón MC, Rodríguez-Ortiz R, Avalos J (2010) Bikaverin production and applications. Appl Microbiol Biotechnol 87:21–29

Lin YR, Lo CT, Li SY, Peng KC (2012) Involvement of pachybasin and emodin in selfregulation of *Trichoderma harzianum* mycoparasitic coiling. J Agric Food Chem 60:2123–2128

Manan MA, Mohamad R, Ariff A (2017) Monascus spp. A source of natural microbial color through fungal biofermentation. J Microbiol Exp 5:00148

Manikandan M, Hasan N, Wu H (2013) Rapid detection of haloarchaeal carotenoids via liquid—liquid microextraction enabled direct TLC MALDI-MS. Talanta 107:167–175

Mansi M, Gaurav S (2016) Incorporation of biocolours in textiles: an eco- friendly approach. Int J Appl Res 2:214–218

Mapari SAS, Meyer AS, Thrane U, Frisvad JC (2009) Identification of potentially safe promising fungal cell factories for the production of polyketide natural food colorants using chemotaxonomic rationale. Microb Cell Factories 8:24–28

Martinez AT, Ramirez C (1978) Penicillium fagi sp. nov., isolated from beech leaves. Mycopathologia 63:57–59

Mcewen CN, Mckay RG, Larsen BS (2005) Analysis of solids, liquids and biological tissues using solids probe introduction at atmospheric pressure on commercial LC/MS instruments. Anal Chem 77:7826–7831

Medentsev AG, Akimenko VK (1998) Naphthoquinone metabolites of the fungi. Phytochemistry 47:935–959

Mohamed GF, Shaheen MS, Khalil SKH, Hussein AMS, Kamil MM (2011) Application of FT-IR spectroscopy for rapid and simultaneous quality determination of some fruit products. Nat Sci 9:21–31

Malmstrom BG, Ryden L (1968) The copper-containing oxidases. In: Singer TP (ed) Biological Oxidations. Interscience, New York, pp 415–438

Mapari Sameer AS, Thrane U, Meyer AS (2010) Fungal polyketide azaphilone pigments as future natural food colorants? Trends Biotechnol 28:300–307
Martin JP, Haider K (1969) Phenolic polymers of Stachybotrys atra, Stachybotrys chartarum and Epicoccum nigrum in relation to humic acid formation. Soil Sci 107:260–270
Morresi L, Ficcadenti M, Pinto N, Murri R, Cuccioloni M, Angeletti M (2010) Optical and electrical behavior of synthetic melanin thin films spray-coated. Energy Procedia 2(1):177–182
Mueller DR, Vincent WF, Bonilla S, Laurion I (2004) Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. FEMS Microbiol Ecol 53(1):73–87
Neilands JB (1952) A crystalline organo-iron pigment from a rust fungus Ustilago sphaerogena. J Am Chem Soc 74(19):4846–4847
Nosanchuk JD, Stark RE, Casadevall A (2015) Fungal melanin: what do we know about structure? Front Microbiol 6:1–7
Molnar P, Deli J, Tanaka T, Kann Y, Tani S, Gyemant N, Molnar J, Kawase M (2010) Carotenoids with anti-*Helicobacter pylori* activity from Golden delicious apple. Phytother Res 24:644–648
Osman MY, Sharaf AI, Osman MY, El-Khouly AZ, Ahmed IE (2004) Synthetic organic food colouring agents and their degraded products: effects on human and rat cholinesterases. Br J Biomed Sci 61:128–132
Onofri S, Selbmann L, Zucconi L, Pagano S (2004) Antarctic microfungi as models for exobiology. Planet Space Sci 52:229–237
Orozco SFB, Kilikian BV (2008) Effect of pH on citrinin and red pigments production by Monascus purpureus CCT3802. World J Microbiol Biotechnol 24(2):263–268
Pagano MC, Dhar PP (2015) Fungal pigments: an overview. In: Gupta VK, Mach RL, Sreenivasaprasad S (eds) Fungal bio-molecules: sources, applications and recent developments, 1st edn. Wiley, London
Pal AK, Gajjar DU, Vasavada AR (2014) DOPA and DHN pathway orchestrate melanin synthesis in Aspergillus species. Med Mycol 52:10–18
Pandey N, Rahul J, Anita P, Sushma T (2018) Optimisation and characterization of the orange pigment produced by a cold adapted strain of Penicillium sp. (GBPI_P155) isolated from mountain ecosystem. Mycology 9(2):81–92
Park HG, Stamenova EK, Jong SC (2004) Phylogenetic relationships of Monascus species inferred from the ITS and the partial b-tubulin gene. Bot Bull Acad Sin 45:325–330
Pawelek JM, Platt JT (1998) Cosmetic melanins. Patent No. 5,744,125
Pezzella A, Barra M, Musto A, Navarra A, Alfè M, Manini P (2015) Stem cell-compatible eumelanin biointerface fabricated by chemically controlled solid state polymerization. Mater Horiz 2(2):212–220
Plonka PM, Grabacka M (2006) Melanin synthesis in microorganisms – biotechnological and medical aspects. Acta Biochim Pol 53:429–443
Prado-Cabrero A, Scherzinger D, Avalos J, Al-Babili S (2007) Retinal biosynthesis in fungi: characterization of the carotenoid oxygenase CarX from *fusarium fujikuroi*. Eukaryot Cell 6:650–657
Rao N, Prabhu M, Xiao M, Li WJ (2017) Fungal and bacterial pigments: secondary metabolites with wide applications. Front Microbiol 8:1113
Ratna PBS (2012) Pollution due to synthetic dyes toxicity and carcinogenicity studies and remediation. Int J Environ Sci 3:940–955
Robinson N, Wood K, Hylands PJ, Gibson TM (1992) Blue pigments of Penicillium herquei. J Nat Prod 55:814–817
Rodríguez-Ortiz R, Mehta B, Avalos J, Limón M (2010) Stimulation of bikaverin production by sucrose and by salt starvation in *Fusarium fujikuroi*. Appl Microbiol Biotechnol 85:1991–2000
Rohrig B (2016) Eating with your eyes: the chemistry of food colorings. Chemistry 2015:2015
Rajagopal K, Kathiravan G, Karthikeyan S (2011) Extraction and characterization of melanin from Phomopsis: a phellophytic fungi isolated from Azadirachta indica A. Juss. Afr J Microbiol Res 5:762–766

Ramesh C, Vinithkumar NV, Kirubagaran R, Venil CK, Dufossé L (2019) Multifaceted applications of microbial pigments: current knowledge, challenges and future directions for public health implications. Microorganisms 7(7):186

Rebecca G, Jorge AF, Magnus L, Mohammad JT, Patrik RL (2018) Pigment production by the edible filamentous fungus Neurospora intermedia. Fermentation 4(1):11

Romero-Martinez R, Wheeler M, Guerrero-Plata A, Rico G, Torres-Guerrero H (2000) Biosynthesis and functions of melanin in Sporothrix schenckii. Infect Immun 68(6):3696–3703

Sanchez S, Rutz B, Rodríguez-Sanoja R (2013) Microbial production of carotenoids. In: Brian M, David A, Ioannis G, Linda H (eds) Microbial production of food ingredients, enzymes and nutraceuticals. Elsevier Science, Burlington, pp 194–223

Santos MA, Mateos L, Stahmann KP, Revuelta JL (2005) Insertional mutagenesis in the vitamin B2 producer fungus Ashbya gossypii. In: Barredo JL (ed) Methods in biotechnology, microbial processes and products, vol 18. Humana Press, Totowa, NJ, pp 283–300

Sarang H, Rajani P, Vasanthakumari MM et al (2017) An endophytic fungus, *Gibberella moniliformis* from *Lawsonia inermis* L. produces lawsone, an orange-red pigment. Antonie Van Leeuwenhoek 110:853–862

Schnitzler N, Peltroche-Llacsahuanga H, Bestier N, Zündorf J, Lütticken R, Haase G (1999) Effect of melanin and carotenoids of Exophiala (Wangiella) dermatitidis on phagocytosis, oxidative burst, and killing by human neutrophils. Infect Immun 67(1):94–101

Seppa H (2001) Superconducting transition-edge bolometer in a resistive and in an inductive mode. IEEE Trans Appl Supercond 11(1):759–761

Simova ED, Frengova GI, Beshkova DM (2004) Synthesis of carotenoids by Rhodotorula rubra GED8 co-cultured with yogurt starter cultures in whey ultrafiltrates. J Ind Microbiol Biotechnol 31:115–121

Singgih M, Andriatna W, Damayanti S et al (2015) Carotenogenesis study of Neurospora intermedia N-1 in liquid substrate fermentation. J Chem Pharm Res 7(5):842–847

Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 8:6–9

Stackebrandt E, Goebel B (1994) Taxonomic note: a place for DNA-DNA Reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bact 44:846–849

Stahmann KP, Revuelta JL, Seulberger H (2000) Three biotechnical processes using Ashbya gossypii, Candida famata, or Bacillus subtilis compete with chemical riboflavin production. Appl Microbiol Biotechnol 53(5):509–516

Suh SH, Rheem S, Mah JH, Lee W, Byun MW, Hwang HJ (2007) Optimization of production of monacolin K from gamma-irradiated Monascus mutant by use of response surface methodology. J Med Food 10(3):408–415

Sun S, Zhang X, Chen W, Zhang L, Zhu H (2016) Production of natural edible melanin by Auricularia auricula and its physicochemical properties. Food Chem 196:486–492

Suraiya S, Kim JH, Tak JY, Siddique MP, Young CJ, Kim JK et al (2018) Influences of fermentation parameters on lovastatin production by Monascus purpureus using Saccharina japonica as solid fermented substrate. Lebensm Wiss Technol 92:1–9

Suwannarach N, Kumla J, Watanabe B, Matsui K, Lumyong S (2019) Characterization of melanin and optimal conditions for pigment production by an endophytic fungus, Spissiomyces endophytica SDBR-CMU319. PLoS One 14(9):e0222187

Samanta AK, Agarwal P (2009) Application of natural dyes in textiles. Ind J Fib Text Res 34(4):384–399

Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (2002) Introduction to food- and airborne fungi. Centraalbureau voor schimmelcultures, Utrecht

Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CHW et al (2014) Phylogeny, identification and nomenclature of the genus Aspergillus. Stud Mycol 78:141–173

Samson RA, Yilmaz N, Houbraken J, Spierenburg H, Seifert KA, Peterson SW et al (2011) Phylogeny and nomenclature of the genus *Talaromyces* and taxa accommodated in *Penicillium* subgenus *Biverticillium*. Stud Mycol 70:159–183

Sardaryan E: Strain of the microorganism *Penicillium oxalicum* var. Armeniaca and its application. 2002 US Patent 6,340,586 B1

Sardaryan E: Food supplement. 2004 US Patent 0105864 A1

Sardaryan E, Zihlova H, Strnad R, Cermakova Z (2004) Arpink Red: meet a new natural red food colorant of microbial origin. In: Dufossé L (ed) Pigments in food, more than colours. Université de Bretagne Occidentale, Quimper, pp 207–208

Schrader B, Dippel B, Erb I, Keller S, Lochte T, Schulz H, Tatsch E, Wessel S (1999) NIR Raman spectroscopy in medicine and biology: results and aspects. J Mol Struct 481:21–32

Selvi BT (2014) Natural dyeing of cotton fabrics with pigment extracted from *Roseomonas fauriae*. Univers J Environ Res Technol 4:54–59

Simonovska B, Albreht A, Vovk I, Rodi Z (2012) Determination of lutein by high-performance thin-layer chromatography using densitometry and screening of major dietary carotenoids in food supplements. J Chromatogr A 1231:59–65

Souza PN, Grigoletto TL, de Moraes LA, Abreu LM, Guimarães LH et al (2015) Production and chemical characterization of pigments in filamentous fungi. Microbiology 162:12–21

Tobie WC (1934) The pigment of *Bacillus violaceus*: the production, extraction and purification of violacein. J Bacteriol 29:223–227

Toju H, Tanabe AS, Yamamoto S, Sato H (2012) High-coverage its primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PLoS One 7:e40863

Torres FAE, Zaccarim BR, de Lencastre Novaes LC, Jozala AF, Santos CA, Teixeira MF, Santos-Ebinuma VC (2016) Natural colorants from filamentous fungi. Appl Microbiol Biotechnol 100:2511–2521

Tudor D, Robinson SC, Cooper PA (2013) The influence of pH on pigment formation by lignicolous fungi. Int Biodeterior Biodegrad 80:22–28

Unagul P, Wongsa P, Kittakoop P, Intamas S, Srikitikulchai P, Tanticharoen M (2005) Production of red pigments by the insect pathogenic fungus *Cordyceps unilateralis* BOC 1869. J Ind Microbiol Biotechnol 32:135–140

Velmurugan P, Kamala Kannan S, Balachandar V, Lakshmanaperumalsamy P, Chae JC, Oh BT (2010) Natural pigment extraction from five filamentous fungi for industrial application and dyeing of leather. Carbohydr Polym 79:262–268

Venkatachalam M, Zelena M, Cacciola F, Ceslova L, Girard-Valenciennes E, Clerc P, Dugo P, Mondello L, Fouillaud M, Rotondo A, Giuffrida D, Dufossé L (2018) Partial characterization of the pigments produced by the marine-derived fungus *Talaromyces albobiverticillius* 30548. Towards a new fungal red colorant for the food industry. J Food Compos Anal 67:38–47

Vinale F, Ghisalberti EL, Sivasithamparam K, Marra R, Ritieni A, Ferracane R, Woo S, Lorito M (2009) Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. Lett Appl Microbiol 48:705–711

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) Trichoderma–plant–pathogen interactions. Soil Biol Biochem 40:1–10

Vining LC, Kelleher WJ, Schwarting AE (1962) Oosporein production by a strain of *Beauvaria bassiana* originally identified as *Amanita muscaria*. Can J Microbiol 8:931–933

Visalakchi S, Muthumary J (2009) Antimicrobial activity of the new endophytic *Monodictys castaneae* SVJM139 pigment and its optimization. African J Microbiol Res 3:550–556

Wang L, Han-Bai Z, Frisvad JC, Samson RA (2004) Penicillium persicinum, a new griseofulvin, chrysogine and roquefortine C producing species from Qinghai province, China. Antonie Van Leeuwenhoek 86:173–179

Watt C-K, McInnes AG, Smith DG, Wright JLC, Vining LC (1977) The yellow pigments of *Beauvaria* species. Structures of tenellin and bassianin. Can J Chem 55:4090–4098

White T, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR-protocols a guide to methods and applications. In: Innis MA, Gelfand DH, Sninski JJ, White TJ (eds). Academic press, San Diego, pp 315–322

Wiemann P, Willmann A, Straeten M, Kleigrewe K, Beyer M, Humpf H-U, Tudzynski B (2009) Biosynthesis of the red pigment bikaverin in fusarium fujikuroi: genes, their function and regulation. Mol Microbiol 72:931–946

Wheeler MH, Bell AA (1988) Melanins and their importance in pathogenic fungi. Curr Top Med Mycol 2:338–387

Woo PC, Lam CW, Tam EW, Lee KC, Yung KK, Leung CK, Sze KH, Lau SK, Yuen KY (2014) The biosynthetic pathway for a thousand-year-old natural food colorant and citrinin in Penicillium marneffei. Sci Rep 4:6728

Xia XK, Huang HR, She ZG, Shao CL, Liu F, Cai XL, Lin YC (2007) 1H and 13C NMR assignments for five anthraquinones from the mangrove endophytic fungus Halorosellinia sp. (no. 1403). Magn Reson Chem 45(11):1006–1009

Yang Y, Liu B, Du X et al (2015) Complete genome sequence and transcriptomics analyses reveal pigment biosynthesis and regulatory mechanisms in an industrial strain, Monascus purpureus YY-1. Sci Rep 5:8331

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi. Volume 1: diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 2: perspective for value-added products and environments. Springer International Publishing, Cham

Yu J-H, Keller N (2005) Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol 43:437–458

Zhang BB, Xing HB, Jiang BJ, Chen L, Xu GR, Jiang Y et al (2018) Using millet as substrate for efficient production of monacolin K by solid-state fermentation of Monascus ruber. J Biosci Bioeng 125(3):333–338

Zou Y, Hou X (2017) Optimization of culture medium for production of melanin by Auricularia auricular. Food Sci Technol 37:153–157

Zou Y, Tian M (2017) Fermentative production of melanin by Auricularia auricula. J Food Process Preserv 41(3):e12909

Zhan J, Burns AM, Liu MX, Faeth SH, Gunatilaka AAL (2007) Search for cell motility and angiogenesis inhibitors with potential anticancer activity: beauvericin and other constituents of two endophytic strains of Fusarium oxysporum. J Nat Prod 70:227–232

Chapter 20
Bioprospecting of Industrially Important Mushrooms

Harpreet Kour, Satwinder Kour, Yashpal Sharma, Shaveta Singh, Isha Sharma, Divjot Kour, and Ajar Nath Yadav

Contents

H. Kour · S. Kour · Y. Sharma
Department of Botany, University of Jammu, Jammu, India

S. Singh · I. Sharma
Department of Microbiology, Shoolini University, Vill-Bhajhol Solan, Himachal Pradesh, India

D. Kour · A. N. Yadav (✉)
Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Himachal Pradesh, India
e-mail: ajar@eternaluniversity.edu.in

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_20

20.1 Introduction

Bioprospecting is defined as a systematic and cataloged search for useful products derived from bioresources including plants, microorganisms, animals, etc. along with their associated traditional knowledge (Pandey et al. 2020). Humans have been capitalizing on naturally derived products for thousands of years. Even though as cutting edge technologies have practically revolutionized drug discovery, modern therapeutics is still largely dependent on compounds derived from natural products. There are ample natural resources including mushrooms, still lying unfathomed and underexploited. The enormity of global fungal diversity is estimated to be between 2.2 and 3.8 million (Hawksworth and Lücking 2017), but only 1,20,000 species have been elucidated so far. This number keeps on spiking with the addition of new taxa by reports coming from less explored and uncharted regions across the globe, so it is coherent to anticipate that the application of new technologies to the investigation of the currently unidentified and vast majority of mushroom species will yield many more benefits for humankind.

As the Father of Medicine, Hippocrates said more than 2000 years ago "Let food be thy medicine and medicine be thy food." To place this in perspective, mushrooms have plethora of medicinal food, drug, and mineral attributes, hence they are priceless asset for the human basis in the world. In addition to this, they also help in the bioconversion of waste organic materials of great benefit to both man and nature (Zhu et al. 2013). Mushrooms have been held dear as edible and medical provisions for humankind. As a protein-rich food it may be an enticing alternative to conventional protein sources and of great prominence to vegetarians (Cheung 2013; Yadav et al. 2019a). The nutritional value of mushrooms derives from the considerable amount of proteins, vitamins and minerals (iron, zinc, selenium, sodium), essential fatty acids, chitin, fibers (Painuli et al. 2020). Although many hundreds of species of edible mushrooms dwell in the wild, less than 20 species are used extensively as food and only 8–10 species are regularly cultivated to any significant extent and are consumed either in fresh or processed form (Ghorai et al. 2009; Abdel-Azeem et al. 2021).

Mushrooms portray a largely untapped medicine cabinet of many as yet unknown therapeutics (Aly et al. 2011; Kour et al. 2019). Since archiac times, mushrooms have been used as a primary source of medicines. Natives and ancestors knew the traditional importance of edible and wild mushrooms and great deal of research has been carried out and is increasingly being undertaken to identify and characterize mycochemicals and to delineate their actions and mechanisms, due to the growing interest in the use of mushroom based products in treating various ailments including as adjuvants in traditional therapies (Venturella et al. 2021). Mushrooms are stockpiles of several bioactive constituents including polysaccharides, complexes (polysaccharide-protein and polysaccharide-peptide), proteases, polyphenols, terpenoids, steroids, polyketides, ribonucleases, lectins etc. that evinces splendid pharmacological activities such as metabolic activation, maintenance of homeostasis and immune balance, decreasing cholesterol levels, as anti-oxidants with

revitalizing and energy-boosting properties, and their role in the prevention and improvement of life-threatening diseases such as cancer, neurodegenerative disorders, diabetes, and metabolic syndrome (Roupas et al. 2012; Sánchez 2017; Zeb and Lee 2021; Singh and Yadav 2020; Yadav et al. 2019b). A large number of mushroom species including *Agaricus bisporus*, *A. blazei*, *Agrocybe cylindracea*, *Coprinus plicatilis, Collybia dryophila*, *Collybia radicata, Collybia peronata, Coriolus versicolor*, *Cordyceps sinensis*, *C. militaris, Fomes fomentarius, Ganoderma lucidum*, *Inonotus obliquus*, *Lentinus edodes*, *Pholiotina appendiculata, Pleurotus ostreatus, Suillus bovinus, Tremella fociformis*, *Tricholoma mongolicum*, *Leucopaxillus giganteus* are being unraveled extensively for their pharmaceutical utility. This upholds their candidature as natural resources for novel drug discovery. More recently, several species are used as dietary supplements (DSs), prebiotics, functional foods, and nutraceuticals (Elkhateeb 2020; Nowak et al. 2018).

During the last few decades, consumption and cultivation of mushrooms that are considered to have potential in the functional food market have burgeoned enormously (Badalyan and Singh 2014). There has been ongoing research to scrutinize the elemental advantages of mushrooms including identification of new metabolites, which could be used as multispectral therapeutics. These have evoked interests to bioprospect mushrooms for nutritional, pharmaceutical, and nutraceuticals value (Yadav 2020). Keeping in view the growing insistence in exploring the natural resources with health-promising effects as nutraceuticals and pharmaceuticals, we endeavored to highlight the significance of macrofungi in human health.

20.2 Nutritional Composition of Mushrooms

Mushrooms have been associated with humankind since ages with profound biological and economical impact. Diverse species of wild mushrooms have been hunted and consumed by man from ancient times for delicacy, taste, and pleasing flavor. Mushrooms are highly nutritious with well-balanced proteins, vitamins, carbohydrates, minerals, low fat concentrations, trace elements, and fibers. There are approximately 1400 known species of mushrooms among which 2000 have been categorized to be safe for human consumption and around about 650 of these have medicinal properties. Protein is an important constituent of mushrooms (Agrahar-Murugkar and Subbulakshmi 2005; Wani et al. 2010). The composition of the substratum, harvest time size of pileus, and species of mushrooms greatly defines the content of protein in the mushrooms. *Pleurotus* sp. has been documented to consist of about 8.9% and 38.7% protein on dry weight basis (Thatoi and Singdevsachan 2014).

Lipid content is estimated to be less than 5% on dry weight basis in edible mushrooms. The fatty acid profile generally favors unsaturated fatty acids particularly linoleic acids. The level of linoleic acids is low and contributes to flavor of the mushrooms (Yilmaz et al. 2006). *Agaricus bisporus, Lentinula edodes,* and *Pleurotus ostreatus* have been reported to contain 2700–4700 mg of potassium,

500–1400 mg of phosphorus, 20–200 mg of magnesium, 4.7–9.2 mg of zinc, and 0.50–3.5 mg of copper on dry weight basis (Cheung 2010). The information regarding the vitamin content of wild mushrooms is limited but cultivated ones have been shown to contain folates, niacin, and riboflavin varying between the range 0.30 and 0.64, 31 and 65 and 1.8 and 5.1 mg/100 g of dry weight, respectively (Mattila et al. 2001).

Carbohydrates typically account for the existing component of fruiting bodies with glucose, mannitol, and trehalose being the main representatives of monosaccharides, their derivatives and oligosaccharide groups, respectively (Kalač 2009). Mannitol is important for volume growth and firmness of the fruiting bodies and differs widely. The values 13.7%, 6.5%, and 1.0% were found in *Lactarius deliciosus*, *Agaricus arvensis,* and *Tricholoma portentosum* (Barros et al. 2007) and 0.2%, 0.8%, 11.7%, and 13.9% of dry matter in *Lycoperdon perlatum, Lepista nuda, Ramaria botrytis, and Cantharellus cibarius*, respectively (Barros et al. 2008). Khan et al. (2008) studied nutritional profile of *P. cystidiosus*, *P. florida*, *P. geestaranus*, *P. highking*, *P. ostreatus*, and *P. sajor-caju*. *P. sajor-caju* showed highest content of protein, viz. 24.5 g/100 g of dry weight, *P. cystidiosus* showed highest lipid content of 5.5 g/100 g dry sample, *P. geestaranus* showed carbohydrate content of 45.9 g/100 g dry sample, *P. highking* showed highest fiber content of 30.3 g/100 g dry sample and total ash content of *P. florida* was 8.3 g/100 g dry sample highest among all the studied species.

The study of Khan et al. (2009) reported protein, carbohydrate, total lipid, crude fiber, and total mineral contents in different oyster mushrooms in different ranges per 100 g of the mushroom species. The study suggested that these mushrooms may be used as low energy, healthy food stuff with protein supplementing properties. The study of Khan and Tania (2012) suggested the nutraceutical potential of mushrooms belonging to genus *Pleurotus* due to their rich nutritional composition which includes essential amino acids, essential fatty acids, polysaccharides, dietary fibers, and proteins. Palazzolo et al. (2012) suggested *Fistulina hepatica, Infundibulicybe geotropa*, *Laetiporus sulphureus*, *Macrolepiota procera* var. *procera* and *Suillus granulatus* to be source of nutritional elements for the human diet. Ahmed et al. (2013) evaluated yield and chemical composition of *P. highking*, *P. ostreatus,* and *P. geesteranus* among which *P. geesteranus* showed better chemical composition, especially in terms of protein and mineral contents. Sudheep and Sridhar (2014) studied the nutritional qualities of *Termitomyces globulus* and *Agaricus abruptibulbus*. The study showed high quantity of crude protein, crude fiber, calorific value, and low quantity of crude lipid in both the mushrooms. The contents of K and Se were observed to be high, while Na, Ca, and P contents were low. The essential amino acids except leucine, lysine, and tyrosine were comparable to soybean and wheat. Oleic acid constituted a major unsaturated fatty acid and which also showed a significant increase in cooked *A. abruptibulbus*.

The study of Liu et al. (2016) reported high crude protein content, total carbohydrate, and low crude fat contents in different mushroom species of Southwest, China. Mushrooms also showed the accumulation of copper, magnesium, potassium, sodium, and zinc from the soil.

20.3 Bioactive Compounds from Mushrooms

20.3.1 Immunomodulating Compounds

Immunomodulators are key parts for the human wellbeing and health ventures, mirroring the way that the immune system is the main boundary for illness avoidance. In any living being, the insusceptible immune framework creates a wide scope of immunomodulators to keep up homeostasis inside the body (El Enshasy and Hatti-Kaul 2013). In clinical practice, immunomodulators are generally characterized into immunosuppressants, immunostimulants, and immunoadjuvants. The role of immunomodulator has expanded quickly in the course of recent years due to wide-running clinical applications for incitement and concealment of the safe immune system (Fig. 20.1). They are even utilized as prodrugs or prophylactic medication for prevention of infections and other diseases (Ayeka 2018).

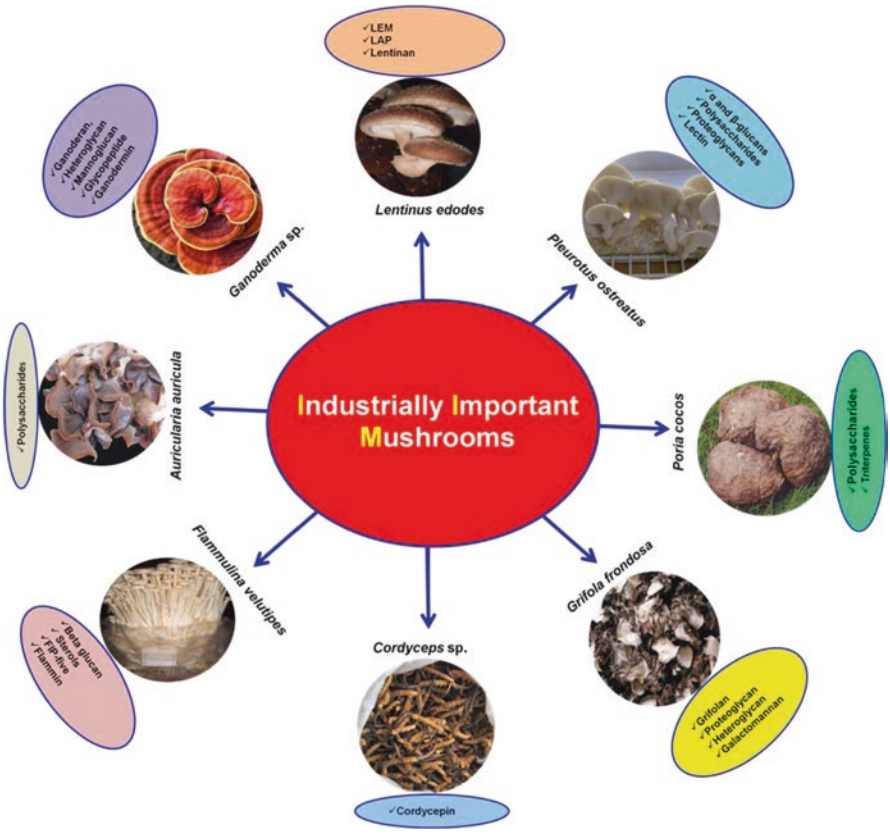


Fig. 20.1 Depicts the bioactive compounds from industrially important mushrooms

Synthetically incorporated mixtures and monoclonal antibodies of anti-proliferative and anti-metabolic drugs produce the most elevated incomes among all classes of immunomodulators. As of late, there has been developing interest in characteristic immunomodulators as options in contrast to the presently utilized substance medicates that have a health risk (Shukla et al. 2014). Mushrooms are among the most fascinating having wide range of compounds for drug applications and are main parts of conventional medication around the world. Approximately 700 mushrooms are reported for their medicinal value. Mushrooms are alternative for current drug used as immunomodulator as they provide safe enhancement of immune system (Ooi and Liu 1999). Various mushrooms have showed significant increase of immune cells in vivo and in vitro studies. Mushrooms have reported actives for both inborn and adaptive immune system (Minato 2010). They multiply and enact intrinsic safe framework parts like characteristic executioner (NK) cells, neutrophils, and macrophages, and animate cytokines articulation and discharge. These cytokines thusly actuate versatile insusceptibility through the advancement of B cells for antibodies creation and incitement of T cell separation to T assistant (Th) 1 and Th2 cells, which intervene cell and humoral insusceptibilities, separately (Wasser 2017).

The organic solvent extracts of *Ganoderma lucidum* inhibited the release of IL-8, IL6, MMP-2, and MMP-9 in cancer cells. Also significant decrease in the viability of melanoma and triple negative breast cancer was shown by this mushroom. Polysaccharides separated from *Ganoderma lucidum* have been demonstrated to be immunomodulating through anti-tumor activity (Ikekawa 2001). A human preliminary including 34 progressed stage disease patients explored the impacts of a 12-week oral supplementation of Ganopoly (*G. lucidum* polysaccharide remove) of a sum of 1800 mg split into 3 day by day doses. At the finish of the 12 weeks, there was a significant reduction in plasma TNF and interleukin-1 (IL-1) and a high increase in the mean natural killer cell movement contrasted with pattern, showing an improved invulnerable reaction (Gao et al. 2003). Human hepatoma Hep3B cell-transplanted mice when administered with *Phellinus linteus* mushroom extract daily for 8 weeks had shown a significant increase in T cell numbers; IL-12, IFN-c, and TNF-a secretion along with reduction in tumor size (Huang et al. 2011a).

Methanolic extracts of *Phellinus adamantinus* had shown a strong cytotoxic effect against EAC cells (Rajeshwari and Kriushnapriya 2011). *Piptoporus betulinus* also known as Birch bracket or birch polypore is cultivated in many Western and Asian countries due to its medicinal properties. Dried fruiting bodies fraction of *P. betulinus* was evaluated for their anti-cancer properties and found to have an effect against human lung carcinoma (A549), colon adenocarcinoma (HT-29), and rat glioma (C6). This fraction showed decrease in the proliferation of tumor cells along with induction of morphological changes in cancer cell lines (Lemieszek et al. 2009). *Phellinus baumii* is a folk medicinal mushroom also called as oak bracket has cytotoxic activity against human melanoma cells A375 (Yang et al. 2020). *Suillus placidus* also known as slippery white mushroom, methanolic extract of this mushroom had shown growth inhibition of MCF-7 cell lines. This extract induces apoptosis and increases the p53 level. Thus it can be used in curing breast cancer (Vaz et al. 2012). In another study, *Funlia trogii* called *Trametes trogii* had

also shown anti-cancer activity. The aqueous extract of this mushroom had shown anti-cancer activity against HT29, LNCaP, PC3, MCF-7, and MDA-MB-231 tumor cells (Rashid et al. 2011). *Pyropolyporus fomentarius* also known as tinder polypore, petroleum ether fraction of this mushroom had shown cytotoxic activity against murine sarcoma S180 cells. This mushroom had also reported to shown apoptosis in leukemia L1210 cells (Li et al. 2016).

Fomitopsis pinicola also known as red belt conk mushroom had also known to have anti-cancer activity. The ethanolic and methanol extracts of this mushroom had shown anti-cancer activity against HeLa and Hep3B cancer cell lines (Choi et al. 2007). Karst chloroform extract of this mushroom had shown suppression of proliferation on S180, HL-60, K562, 09375, Eca 109, and MMC-7721 cell lines (Gao et al. 2017). *Irpex lacteus* also called as white tooth mushroom had been reported to use for the treatment of chronic glomerulonephritis in traditional Chinese medicinal system (Han et al. 2020). Hot water extract of this mushroom contains seven water-soluble polysaccharide fractions. These fractions had shown significant inhibition effects on human hepatocellular liver carcinoma (HepG2) and Henrietta lack (HeLa) tumor cells with IC50 values of 60.95 and 99.95 μg/mL, respectively. These findings suggest that the polysaccharide fractions of *I. lacteus* have significant anti-tumor activities (Na et al. 2012).

G. frondosa had shown indirect cytotoxicity against HepG-2 cells along with inhibition of Heps cells growth. This compound stimulated tumoricidal activity along with production of various other factors such as interleukin-1β, NO, and TNF-α. It was concluded that the anti-tumor activity of GP11 polysaccharide was due to the enhancement of the host immune system by the TLR-4-mediated up-regulation of the production of nitric oxide and TNF-α (Mao et al. 2015). *Agaricus blazei* is the most studied medicinal mushroom and β-(1–6) and β-(1–3) glucan have been isolated from *A. blazei. A. blazei* had shown the increase of interferon (IFNγ) and synthesis of antibodies especially IgE and reduction of histamine from mast cells (Hetland et al. 2011). Triterpenes isolated from fruiting bodies of *Fomitopsis pinicola* and *F. officinalis* were evaluated against MCF7, HeLa, HepG2, A549 cell lines. In this study, these triterpenes had shown significant effect of cancer cells by inhibition of VEGF expression, cytokines IL4, and IFN gamma tumor factor (Shi et al. 2017).

Lectin is a polysaccharide isolated from *Volvariella volvacea* and *Pleurotus* have shown immunomodulating effect and also have anti-tumor properties (Devi et al. 2013). Lectin isolated from other mushroom has shown the activity of interferon (IFN)-γ and other cytokines, activated through up-regulation of inducible nitric oxide synthase (NOS), interleukin (IL)-1β, and transforming growth factor-b (Singh et al. 2010). Ganoderic acids isolated from *Ganoderma* sp. have also reported as excellent immunomodulator and showed the activation of nuclear factor (NF)-kB pathway and mitogen-activated protein kinases (Liang et al. 2019). Compound PKS purified from *Trametes versicolor* was administered in thirty patients having cT3/T4 adenocarcinoma of rectum and the result showed a significant increase of NK cell counts along with decrease of serum immunosuppressive acidic protein level in the cancer patients (Sadahiro et al. 2010). Adjuvant effect of PKS in cancer patients was

studied in year 2007 through randomized controlled trials on 4037 patients. The result indicated a significantly improved survival of cancer patients with the addition of PKS. Fifteen preclinical trials of the compound supported the anti-cancer activity of PKS through immunomodulation (Oba et al. 2007). In another study PSK had shown the increase in secretion of IL-2 and lymphocyte-activated killer (LAK) (Jiménez-Medina et al. 2008).

GL-B a polysaccharide isolated from *G. lucidum* has reported to induce immune response by secretion of TNF-α and INF γ and inhibit the growth of Sarcoma 180 cells (Zhang and Lin 1999). Fraction D of polysaccharide isolated from maitake mushroom (*G. frondosa*) had shown anti-cancer activity. When human breast cancer cells (MCF7) were treated with maitake (D-fraction) extract at different concentrations, significant decrease in cell viability of cancer cell line has been observed. There was a significant increase in the apoptotic activity in dose-dependent manner due to up-regulation of BAK-1 and cytochrome c transcripts (Soares et al. 2011). In a similar study, D-fraction of polysaccharides has also shown direct effect on canine and human tumor cancer cell lines. Grifolin, a compound extracted from the fresh fruiting bodies of *Albatrellus confluen*ce, had shown to inhibit growth of some cancer cell lines in vitro by upregulation of death associated protein kinase 1 DAPK1 (Luo et al. 2011). Polysaccharide GFP isolated from *G. frondosa* has reported to increase in TNF-, IL-6, IFN, MIP-1, and MIP-2 levels (Chen et al. 2012). GFP-A compound also purified from *G. frondosa* has shown the activation IL-1β, IL-2, IL-6, IFN-γ, and T cells ($CD4^+$ and $CD8^+$) (Xiao-Lei et al. 2015). β-glucan is polysaccharide isolated from various mushrooms and had been used as commercial immunostimulant products. It consists of glucose monomers forming polymer by β-glycosidic bonds (Ngwuluka et al. 2016). Lentinan and schizophyllan isolated from shiitake (*L. edodes*) and *Schizophyllum commune,* respectively, are two main glucan isolated and have used as immunomodulators in cancer patients (Li et al. 2019a). Fungal immunomodulatory proteins (FIPs) are other group of bioactive compounds obtained from edible mushrooms like *G. lucidum*, *Pleurotus citrinopileatus*, *Flammulina velutipes*, and *Auricularia polytricha.* These bioactive compounds activate the production of IL-4, TNF-α, and lymphotoxin (Liu et al. 2020).

20.3.2 Proteins and Peptides

Proteins and peptides have myriad of vital functions and processes in the body, essential for life. These include cell adhesion ligands, signaling molecules, anticoagulants, high-affinity effectors, and catalysts. There are more than 20,000 functionally distinct proteins and peptides. During the last decades, the potential of mushroom proteins for human health has attracted the interest of the scientific community. Further, there is a great opportunity to innovate and utilize these molecules and pathways for benefit of the patients. Mushrooms produce many bioactive proteins and peptides with pharmaceutical potential that act as significant bioactive nutraceuticals in mushrooms with multiple health benefits. These primarily include

lectins, fungal immunomodulatory proteins (FIPs), ribosome inactivating proteins (RIPs), ribonucleases, and laccases. Of all the mushroom proteins, lectins are probably the most extensively investigated group of mushroom compounds with multidirectional health-promoting effects including antiproliferative, anti-tumor, immunomodulatory, anti-fungal, HIV-1 reverse transcriptase inhibitory activities (Hassan et al. 2015; Wang and Ng 2000b). Lectins are nonimmune proteins that hydrophobically bind to specific carbohydrates inducing cell agglutination; include polysaccharide-protein and polysaccharide-peptide complexes (Santos et al. 2014). Diversity of bioactivity is attributed to lectins isolated from mushrooms like *Agaricus* species, *Amanita pantherina*, *Boletus satanas*, *Coprinus cinereus*, *Ganoderma lucidum*, *Flammulina velutipes*, *Grifola frondosa*, *Hericium erinaceus*, *Lactarius deterrimus*, *Laetiporus sulphureus*, *Pleurotus* sp., *Volvariella volvacea*, *Tricholoma mongolicum* and has been accepted for clinical application in immunomodulating and cancer therapy (Sonawane et al. 2014). The lectins produced by the species *Ganoderma carpense* and *Pleurotus ostreatus* have shown anti-proliferative activity on tumor cells (Ngai and Ng 2004). Wu et al. (2011) isolated a protein extract from *P. ostreatus* that exhibited a therapeutic effect towards the SW 480 and monocytic leukemia THP-1, colorectal cancer cell line by inducing their apoptosis. Lectins from *Pleurotus citrinopileatus* exhibit anti-neoplastic and anti-viral effects (Li et al. 2008). In addition, the proteins of *P. eryngii* and *P. ostreatus*, eryngin and pleurostrin show anti-bacterial and anti-fungal properties (Erjavec et al. 2012).

Fungal immunomodulatory proteins are a novel family of protein immunomodulators, obtained from mushrooms in the recent years. FIP LZ-8 was the first fungal immunomodulatory protein that was isolated from *Ganoderma lucidum*. Since then, number of mushrooms FIPs has been extracted from *Ganoderma lucidum*, *Ganoderma microsporum*, *Poria cocos*, *Flammulina velutipes*, *Volvariella volvacea*, *Antrodia camphorata*, *Trametes versicolor,* etc. with immunomodulatory activity. They also prevent the invasion and metastasis of tumor cells and thus can be used as adjuvants for treating tumor (El Enshasy and Hatti-Kaul 2013; Lin et al. 2010). Bioactive protein "RIPs," a category of enzymes extracted from several mushroom species including *Pleurotus tuber-regium*, *Flammulina velutipes*, *Lyophyllum shimeji*, *Calvatia caelata*, and *Hypsizygus marmoreus* (Lam and Ng 2001; Wang and Ng 2001) are capable of inactivating ribosomes, inhibiting the HIV-1 reverse transcriptase activity and fungal proliferation (Puri et al. 2012). Like RIPs, laccases also belong to the enzyme category with a huge potential for biotechnological and biomedical applications (Agrawal et al. 2018). Also known as green tool in the field of biotechnology, they are mainly isolated from *Pleurotus eryngii*, *Pleurotus ostreatus*, *Tricholoma mongolicum,* and *Clitocybe maxima*. Well known for their anti-viral and anti-proliferative activity, in the human body this protein can confer activity against HIV (Wang and Ng 2006a).

Ribonucleases, another bioactive protein isolated, purified and characterized from the fruiting bodies or mycelia of a variety of mushroom species including *Boletus griseus* (Wang and Ng 2006b), *Hypsizygus marmoreus* (Guan et al. 2007), *Pleurotus pulmonarius* (Ye and Ng 2002), *Pleurotus ostreatus* (Ye and Ng 2002), *Volvariella volvacea* (Wang and Ng 1999), *Ganoderma lucidum* (Wang and Ng

2004), *Clitocybe maxima* (Wang and Ng 2004), display multitude of biological activities other than ribonucleolytic activity such as anti-mitogenic, immunosuppressive, anti-bacterial, anti-fungal, antiproliferative, HIV-1 reverse transcriptase inhibiting, translation inhibitory and angiogenic activities (Jose Alves et al. 2013).

20.4 Industrially Important Mushrooms and their Nutraceutical Potential

Mushroom cultivation has a long tradition especially in Asian countries like China and Japan where it started centuries ago. Edible mushrooms constitute an important part of human diet in many countries around the globe. Approximately 12,000 species of fungi have been reported as mushrooms, of which 2000 species are documented as edible or medicinally (O'Neil et al. 2013). Among the 2000 edible mushroom species found across the world, only 35 are grown on a commercial scale and 20 are cultivated on an industrial scale (Akwaowo et al. 2000). According to different usages by the consumers, the commercial mushroom species are divided into edible mushrooms such as *Agaricus bisporus* (button mushroom), the most extensively cultivated mushroom worldwide, *Lentinus edodes* (shiitake), *Pleurotus* sp. (oyster mushrooms), *Auricularia auricula-judae* (wood ear mushroom), *Flammulina velutipes* (winter mushroom), and medicinal mushrooms mainly *Ganoderma lucidum*, *Cordyceps* sp., *Poria cocos*, *Trametes versicolor*, *Grifola frondosa,* etc. The edible class of mushrooms has shown potential functional and medicinal properties.

20.4.1 Agaricus

Agaricus is a genus of saprobic mushrooms that includes economically and commercially important species such as *A. bisporus*, the button mushroom (Savoie et al. 2013). It is the most extensively cultivated species of edible mushroom accounting for 31.8% of the world market (Thawthong et al. 2014). It is the predominant mushroom cultivated in Western countries and the cultivation area is expanding fast in China (Zhang et al. 2014). Also, it is the most important mushroom of commercial significance in India (Maheshwari 2013) as most species are edible, some are delicious and largely palatable, and others have considerable medicinal properties. There is an increasing number of scientific reports which confirm the significant ecological, high dietary value, and a wide range of biologically active health-giving elements of mushrooms of the genus *Agaricus* (Dai et al. 2009). The high-quality nutrients like carbohydrates, proteins, lipids, dietary fibers, minerals, and vitamins present this mushroom as potent healthy food. Moreover, due to the presence of high amount of some active ingredients such as polysaccharides (including acidic 424 polysaccharides), lipopolysaccharides, essential amino acids, vitamins (C,

B12, D), anti-oxidants (folate, ergothioneine, polyphenol), peptides, glycoproteins, nucleosides, triterpenoids, lectins, fatty acids and their derivatives, these mushrooms have always been appreciated to have anti-microbial, anti-cancer, anti-diabetic, antihypertensive, anti-inflammatory, hypocholesterolemic, hepatoprotective and anti-oxidant activities (Atila et al. 2021; Bhushan and Kulshreshtha 2018; Mattila et al. 2001).

Significant attention has been laid on the immune modulating and anti-tumor properties of *A. bisporus* (Chen et al. 2006; Karunarathna et al. 2016). However, studies into the anti-cancer effects of *Agaricus bisporus* are limited. Much of the research has focused on the anti-cancer effects of carbohydrate fractions of this species. For example, lectins isolated from the white button mushroom have been shown to increase the sensitivity of lung, colon, and glioblastoma cancer cells to chemotherapeutic drugs (Goto et al. 2002), inhibit colon cancer cell proliferation (Yu et al. 1993), and enhance cellular anti-oxidant defense mechanisms (Shi et al. 2002).These lectins have also been shown to lower blood glucose and cholesterol (Jeong et al. 2010a; Ismaya et al. 2020). It also suggests that a high intake of button mushrooms may promote innate immunity against tumors and viruses (Adotey et al. 2011; De Silva et al. 2012; Sinha et al. 2020).

Another species, *Agaricus subrufescens*, the almond mushroom, is presently cultivated in different countries for use as foods and nutraceuticals. It produces various bioactive compounds with many health-promoting properties such as cytokine induction activity, lymphocyte activation properties, anti-cancer and tumor suppressive effects, anti-microbial, anti-mutagenic, anti-clastogenic properties, anti-allergic effect, anti-genotoxic activity, and biological responses on the immune system (de Oliveira et al. 2002; Luiz et al. 2003; Wisitrassameewong et al. 2012).

Agaricus blazei popularly known as "sun mushroom" is an immensely popular edible medicinal mushroom native to Brazil and it has been cultivated mainly in Japan, particularly due to traditional beliefs that it has anti-tumor properties, and the ability to stimulate the immune system. *A. blazei* nutraceuticals, which are commercially available mainly in the form of tablets or teas containing pulverized dried fruiting bodies, or liquid extracts are widely used in China and Japan, and are exported to many other countries around the world. The majority of scientific research carried out on *A. blazei* has demonstrated that their polysaccharides and polysaccharide-protein complexes are the active agents for the purported anti-cancer properties. Various in vivo and in vitro studies have reported effectiveness of polysaccharides from *A. blazei* against sarcomas or cancers of connective tissue (Gonzaga et al. 2009; Hetland et al. 2016; Itoh et al. 1994; Kawagishi et al. 1989; Mizuno et al. 1990; Jiang et al. 2018). In addition, clinical studies in humans have reported the role of *A. blazei* in reducing the adverse side effects of chemotherapy (Ahn et al. 2004; Okamoto 2007). Furthermore, many studies have reported that this fungus can be used as a healthy food for the prevention of a range of illnesses including diabetes, arteriosclerosis, and chronic hepatitis (Biedron et al. 2012; Varghese et al. 2019). In addition, powdered formulations from *A. blazei* with proteins, carbohydrates, and unsaturated fatty acids can be used in low-calorie diets and have shown high anti-oxidant activity with high content of tocopherols and phenolic compounds (Carneiro et al. 2013).

20.4.2 Lentinus

Lentinus edodes commonly known as the "shiitake mushroom" is the second most popular edible mushroom cultivated worldwide after *Agaricus bisporus* (Bisen et al. 2010). Large-scale commercialization of *L. edodes* is due to its flavor, aroma, taste, and several nutritional and medicinal attributes. More than 130,000 tons of *L. edodes* are produced annually, of which 45% is sold fresh, the rest being sold dried. Commercially it is available in many forms such as a sugar-coated tablet, capsules, concentrate, powdered extract, syrup, tea, wine, or elixirs, as a medicinal dish or may be injected as a solution (1 mg/vial) and are at hand in most Asian countries and are increasingly accessible in USA, New Zealand, Australia, and Europe. *L. edodes* is among the most valuable medicinal mushrooms studied since Ming Dynasty (1369–1644), has a long history in oriental folklore for treatment of diseases involving depressed immune system, tumors, flu, cardiac diseases, hypertension, obesity, problems related to sexual dysfunction and aging, diabetes, hepatic diseases, bronchial inflammation, and environmental allergies (Kües and Liu 2000). It is a source of several well studied polysaccharides, viz. LEM (a glycoprotein from *Lentinus edodes* mycelia), LAP (a glycoprotein from *L. edod*es culture media), and Lentinan, a β-D glucan extracted from both the fruiting body and mycelium. LEM also contains various nucleic acid derivatives; vitamin B compounds (B-1 and B-2), ergosterol, and eritadenine (an anti-cholesterolemic agent) (Breene 1990). Again, both LEM and LAP possess immunostimulatory effects (Mizuno and Zhuang 1995). Chihara et al. (1970) first isolated and studied lentinan and reported that it has appreciably stronger anti-tumor effects than other mushroom polysaccharides. It was reported to have prolonged the survival of patients with gastric and colorectal carcinoma. Both lentinan and LEM possess strong anti-tumor activity by enhancing various immune system functions rather than affecting the tumor cells (or viruses) directly. In Japan, lentinan is currently classified as a medicine, whereas LEM and LAP are considered food supplements (Wasser 2005). Lentinan is often used to help support immune function in immunocompromised cancer patients during chemotherapy. It is also known to lower the exhausting effects of the chemotherapy such as hair loss, nausea, and lowered immune status to a great extent.

Additionally, *L. edodes* has also been reported to harbor components that are effective against hypertension, hyperlipidemia and cardiovascular complications, hepatic disorders, act as anti-oxidants, inhibit blood aggregation and cancer (Friedman 2016; Fukushima-Sakuno 2020; Ma et al. 2018). Further, the protein Lentin present in *L. edodes* employs anti-fungal activity against *Botrytis cinerea*, *Mycosphaerella arachidicola,* and *Physalospora pyricola*. This protein also has an inhibitory activity on reverse transcriptase of HIV1 and multiplication of leukemia cells (Ngai and Ng 2003). Moreover, *L. edodes* possesses anti-atherosclerotic biofunctionality that can be used as functional food-based therapeutics against cardiovascular diseases (Rahman et al. 2018).

20.4.3 Pleurotus

The mushrooms of the genus *Pleurotus* rank second in the world mushroom market which belongs to a group known as "white rot fungi" (Tsujiyama and Ueno 2013) and produce oyster shaped mushrooms, accordingly they are called as oyster mushrooms. It is an edible mushroom that grows saprophytically on logs and tree stumps in shelf like pattern (Johnny and Okon 2013) and enjoys a worldwide distribution from temperate to tropical areas. Various species of *Pleurotus* are commercially cultivated on non-composted lignocellulosic agro wastes (Johnny and Okon 2013) such as *P. ostreatus* (oyster mushroom), *P. eryngii* (king oyster or Cardoncello), *P. pulmonarius* (phenix mushroom), *P. djamor* (pink oyster mushroom), *P. sajor-caju* (Indian oyster), *P. cystidiosus* (abalone oyster), *P. citrinopileatus* (golden oyster mushroom), and *P. cornucopiae* (Knop et al. 2015; Pérez-Martínez et al. 2015; Knop et al. 2015; Zhang et al. 2017). *Pleurotus* sp. are renowned for—nutrition, taste, and physiological functions thus possessing both sensory characteristics and outstanding nutritional profile (Bhardwaj et al. 2020). They contain amino acids, carbohydrates, lactones, and terpenes, that provides the fruiting body and mycelial biomass with valuable aromas and flavor characteristic (Smiderle et al. 2012). *P. ostreatus* is used in soup preparation, stir-fry recipes or eaten stuffed. Another species of culinary value is *P. eryngii* (DC.) Quél. is considered best for vegetarian dishes, being served sautéed, grilled, braised, stewed, or boiled (Reis et al. 2012). Oyster mushrooms contain very lower amount of carbohydrates, sugars, and no or lesser amount of cholesterol, good amount of dietary fiber, and high value proteins which include most of amino acids, minerals, and vitamins (Ahmed et al. 2013; Kumar 2020).

Different bioactive metabolites including glycoproteins, peptides, polysaccharides, lipids phenolics, and hydrolytic and oxidative enzymes are known from *Pleurotus* sp. that possess numerous pharmaceutical properties. Two of the most potent bioactive compounds derived from *Pleurotus* sp. are the immune stimulant polysaccharides and the natural statins, the latter are hypocholesterolemic that too with milder side effects and higher activity than the synthetic ones (Inácio et al. 2015). The polysaccharide-rich extracts by triggering the complement system also act on innate immunity via the pathway of adaptive immunity and by stimulating macrophage function. Methanolic extracts of *Pleurotus* species were reported to inhibit the growth of *Staphylococcus aureus*, *Bacillus megaterium*, *Klebsiella pneumoniae*, *E. coli*, *Candida glabrata*, *C. albicans,* and species of *Trichophyton* and *Epidermophyton* to different degrees (Akyuz et al. 2010). Also, fruiting bodies of Oyster mushrooms have been reported to possess a novel ubiquitin-like protein having HIV-1 reverse transcriptase inhibitor activity (Wang and Ng 2000b). Further a laccase extracted from *Pleurotus ostreatus* inhibits the replication of HCV (hepatitis C virus) (M EL-Fakharany et al. 2010). In addition, Roy et al. (2020) highlighted that the extracts from *Pleurotus* sp. can be used as an alternative natural anti-human cytomegalovirus (HCMV) compounds.

The presence of a large number of bioactive components in oyster mushrooms makes them able to possess potential antihypertensive, anti-diabetic, anti-obesity, anti-aging activities in addition to the hepatoprotective action (Chaturvedi et al. 2018; Patel et al. 2012; Selegean et al. 2009; Zhang et al. 2020). Fruiting bodies of oyster mushrooms contain higher degrees of anti-oxidants and are the important source of vitamins and selenium content which are potent natural anti-oxidants in biological systems (Sifat et al. 2020). This property is due to the presence of pleuran (the best known β-glucan), a polysaccharide isolated from *P. ostreatus* (Atri et al. 2012). It is composed of D-glucose molecules linked with bonds of the type (1, 3)-β and (1 → 6)-β-glucans. The compound exhibits anti-inflammatory activities and anti-neoplastic properties against various cells, including colorectal cancer cells HT-29, prostate cancer cells, and breast cancer cells (Martin and Brophy 2010; Sarma et al. 2018). In addition, the two-water soluble heteropolysaccharide fractions, PSPO-1a and PSPO-4a isolated from *P. ostreatus* have been reported to exhibit the stronger DPPH and superoxide anion radical scavenging activity. Also, the bioactive components like β-glucan, vitamin C, and phenolics present in different species of *Pleurotus* increase the activity of anti-oxidant enzymes, viz. superoxide dismutase, catalase, etc. which are responsible for the fall off of hepatic cell necrosis (Bobek et al. 1997; Fu et al. 2002). Similarly, Sumy et al. (2010) reported the hepatoprotective effect of *Pleurotus florida* against paracetamol induced liver damage in Wistar albino rats. Dietary supplements of *P. ostreatus* have been found to reduce the accumulation of cholesterol in the serum and liver of adult humans and diabetic patients to a considerable extent (Jayakumar et al. 2006).

20.4.4 Auricularia

Auricularia Bull. is a very important genus of wood-decaying fungi with gelatinous, ear- to shell-shaped fruiting bodies. It is a key genus accounting for approximately 17% of world's mushroom production and is the third most cultivated mushroom genus after *Lentinus* (22%) and *Pleurotus* (19%) (Royse et al. 2017). Species from the genus *Auricularia* have been the subject of research into possible medicinal applications since 1960s, in large part, due to their anti-tumor activity (Sekara et al. 2015). They have been highly valued in Asian cuisine and in natural medicine for ages. The bioactive compounds of *Auricularia* species are known for their anti-tumor, anticoagulant immunomodulating, and anti-oxidant properties (Ma et al. 2018).

The genus *Auricularia* comprised of eighteen species (Bandara et al. 2017b) that are recognized worldwide, having intercontinental to cosmopolitan distributions of which *Auricularia auricula-judae*, *A. cornea*, *A. delicata*, *A. fuscosuccinea*, *A. heimuer*, *A. thailandica,* and *A. villosula* have been reported as edible species (Bandara et al. 2019). *Auricularia cornea* and *A. heimuer* are commercially cultivated in different countries including Vietnam, Thailand, Philippines, Malaysia, Indonesia, and China (Bandara et al. 2020; Chang and Lee 2004; Duc 2005; Irawati et al. 2012; Reyes et al. 2009; Thongklang et al. 2020; Wu et al. 2014). In 2017, China produced

approximately 6.3 billion kg of *Auricularia* which made it the second most widely cultivated mushroom in that country. In fact, *Auricularia auricula-judae* has a history of ~2100 years of cultivation in China (Yao et al. 2018).

The East Asian culture has valued these *Auricularia* mushrooms for their gelatinous texture and characteristic flavor. These mushrooms readily rehydrate from a dried state in soups and sauces, imparting meals with a unique slippery and pleasant crunchy texture. Nutritionally, dried *Auricularia* contains carbohydrates (79.9–93.2%), crude proteins(6.5–13%), total soluble sugars (9.9–17.9%), crude fat (0.48–4.5%), crude fiber (3.5–12.5%), macro- and micro-elements such as Ca, Cu, Cr, Fe, Mg, Mn, Ni, K, P, Na, and Zn and have been shown to have a 50% higher fiber content than do the other edible mushrooms (Bandara et al. 2017a; Shin et al. 2007). Reports on the medicinal potential and pharmacological properties of isolated compounds or different solvent extracts from *Auricularia* have shown the anticoagulant, anti-inflammatory, anti-oxidant, anti-tumor, anti-viral and immunomodulatory, hypoglycemic, and hypolipidemic as well as anti-hyper cholesterolemic activities (Sun et al. 2010; Damte et al. 2011; Oli et al. 2020; Reza et al. 2015; Zhao et al. 2015). Zhao et al. (2018) reported that *Auricularia* mushrooms have the ability to modulate intestinal microbial community composition. Further, *Auricularia auricular-judae* increased the abundance of *Bifidobacteriales* and *Bacteroidales* while decreased the abundance of *Fusobacteriales,* two of the most important probiotics.

Auricularia polysaccharides with (1 → 3)-β-D glucan molecules have anti-tumor properties which is mediated through cancer cell apoptosis (Meng et al. 2014). *Auricularia* extracts have been used in anti-gastrointestinal cancer therapy such as treatments for colorectal, stomach, liver, pancreatic, and esophageal cancer. Yu et al. (2014) reported the anti-cancer activities of *Auricularia polytricha*. In another report polysaccharides from *Auricularia* have been shown to inhibit S80 Sarcoma cancer transplanted in mice. Fermentation followed by hot water extraction of *A. auricula-judae* produces Huaier cream. Huaier granule is formed by mixing Huaier cream with powdered sugar, dextrin, and auxiliary materials. Huaier granules possess anti-cancer, anti-oxidant, and anti-coagulant activities and are effective against esophageal, pancreatic, gastric, and colorectal and hepatocellular cancers (Ma et al. 2018). Cai et al. (2015) reported that hot water extracted polysaccharides from *Auricularia auricula-judae* inhibited the activities of *S. aureus* and *E. coli*. Additionally, high degree of deacetylation of *Auricularia* chitosan polysaccharides reduced the growth of *E. coli* and *S. aureus* (Chang et al. 2019). Sulfated AAPt (total *A. auricula* polysaccharides) has been shown to inhibit the activity of Newcastle Disease Virus on cultured chicken embryo fibroblasts measured using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay, provides evidence for its anti-viral activity (Nguyen et al. 2012a). In a study conducted by Nguyen et al. (2012b), it was reported that sulfated polysaccharides (AAPt) from *A. auricula-judae* showcase immunoenhancing activity. Similarly, in a submerged culture of *A. auricula-judae*, exopolysaccharides (CEPSN-1 and CEPSN-2) with (1 → 4)-α-D-glucose back bones demonstrated immunomodulatory activities (Zhang et al. 2018).

20.4.5 Flammulina

Flammulina velutipes also named golden needle mushroom (China) and enokitake (Japan) is one of the most popular edible mushrooms commonly available in the market or groceries stores sold in vacuum packages. It is found throughout the north-temperate regions including Asia, North America, and Europe (Ingold 1980). It has long been recognized for its nutritional value and delicious taste. As a culinary and medicinal mushroom, *F. velutipes* has been cultivated and widely used as a restorative drug and as a tonic food in China since 800 A.D (Yang et al. 2012). Currently, *F. velutipes* due to its desirable taste, aroma, and high nutritional value is among the four most widely cultivated mushrooms globally. It is a highly nutritious food being rich in proteins, vitamins, carbohydrates, and crude fiber. Many bioactive constituents from different families have been isolated from different parts of the mushroom such as polysaccharides (especially Beta-glucan), sterols, fungal immunomodulatory protein, FIP-fve, ribosome inactivating proteins such as flammin, velin, velutin, and flammulin which have been reported to possess many health-promoting properties such as anti-cancer and anti-tumor activities, antihypertensive and hypocholesteromic activity, anti-atherosclerotic and thrombosis inhibition, anti-aging and anti-oxidant properties, ability to aid with restoring memory and overcoming learning disabilities, anti-inflammatory, anti-proliferative, antibacterial, ribosome inactivation, and melanosis inhibition (Chang et al. 2014; Lee et al. 2013; Ng and Wang 2004; Rahman et al. 2015; Tang et al. 2016; Wang and Ng 2000a; Yang et al. 2015; Yi et al. 2013; Zhao et al. 2020). This mushroom is also well known for its curative properties for liver diseases and gastroenteric ulcers (Tang et al. 2016).

20.4.6 Huitlacoche

Corn smut or boil smut are the young, fleshy, edible galls produced mainly in the ears of the plant host *Zea mays* when they are infected by the dimorphic fungus, *Ustilago maydis* (Valverde et al. 2012). Huitlacoche or Cuitlacoche is the Aztec name given to these ear galls that has been used traditionally as human food being highly priced as an interesting dish or condiment in Mexico and some other Latin American countries since pre-Columbian times (Vanegas et al. 1995). Huitlacoche or cuitlacoche comes from the word Nahuatl (the language of the Mexicas or Aztecs) "cuitlacochin" or "cuitlacuchtli" that means "degenerate corn on the cob" (Juárez-Montiel et al. 2011). The popular press in the USA has termed Huitlacoche as the "Maize Mushroom," "Mexican Truffle," "Maizteca Mushroom," or "Caviar Azteca" (Valverde and Paredes-López 1993). Recent interest in developing Huitlacoche as a cash crop has increased due to emerging acceptance of Huitlacoche by the North American public who notice it as a gourmet food item that is a part of a growing

market for haute Mexican cuisine. Its edible popularity has gained wider acceptance, and has expanded to other countries like Japan, China, and some of the European community as France, Spain, and Germany where it is now consumed as an exotic delicacy. It is currently a culinary delight of international chefs and has now been introduced into the "nouveau cuisine" of luxury restaurants as it offers a very unique flavor, aroma, and organoleptic characteristics (Lizárraga-Guerra et al. 1997; Aydoğdu and Golukcu 2017). In addition to its unique flavor, Huitlacoche has been identified as a high-quality functional food due to its attractive characteristics, nutritional value, and nutraceutical potential.

The nutritional value of this mushroom is of great importance for human diet. Being rich in protein, Huitlacoche could be suggested as an alternative protein source for vegetarian diets. (Valverde and Paredes-López 1993) reported that protein content ranged from 11.5% to 16.4%. In addition, Huitlacoche contains all the essential amino acids, lysine (6.3–7.3 g/100 g protein) being the most abundant. Other abundant amino acids include serine, glycine, aspartic and glutamic acid. Further, fatty acids, oleic and linoleic acids (54.5–77.5%) are present in considerable amount adding to its nutritional attributes (Valverde et al. 2015).

Lizarraga-Guerra and Lopez (1998) identified 16 carbohydrates in Huitlacoche. The most abundant monosaccharides, glucose and fructose, are followed by glycerol, sorbitol, and mannitol. Valdez-Morales et al. (2010) reported eight monosaccharides and eight alditols in Huitlacoche, with glucose and fructose being the most abundant carbohydrates. Further, Huitlacoche contains also homoglycans and heteroglycans as found in other edible mushrooms. Considerable amount (11% of the total dry matter) of crude cellulose and a very low crude fat (1.8%) (dry basis) was detected from the Huitlacoche by (Aydoğdu and Golukcu 2017). However, Vanegas et al. (1995) reported that fat content of Huitlacoche ranged from 2.7% to 6.5% (dry basis). Low fat content of Huitlacoche means that it has low-calorie and accordingly it is useful as a low-calorie diet for human. β-glucans content in Huitlacoche is higher (20-120 mg/g of Huitlacoche, in dry weight) than that reported in corn (0.5–3.8 mg/g) and is similar to other edible mushrooms (Valdez-Morales et al. 2010). β-glucans stimulate the complement system and improve the response of the macrophages and killer cells. Other compounds reported in the fungus are anti-oxidants which are useful for preventing diseases such as arteriosclerosis. Valdez-Morales et al. (2010) also reported anti-mutagenic activity in Huitlacoche but without identifying the compound exhibiting the potential. As a consequence of this, it has been included in what are now known as nutraceuticals (León-Ramírez et al. 2014; Martinez-Medina et al. 2021).

Huitlacoche has been marked as a high-quality nutraceutical having numerous nutritional traits and even superior bioactive substances than other mushrooms. Since, it has been gaining acceptance in international cuisines mainly due to its extraordinary flavor and unique quality, further studies are required to promote its nutritional and health benefits as well as its production, storage, and trade.

20.4.7 Ganoderma

Ganoderma P. Karst. is a group of wood degrading, dark mushrooms with hard fruiting bodies having a glossy exterior and a woody texture. This genus is comprised of more than 200 species; however, only *G. lucidum* and *G. sinense* (GS) are recognized in the Chinese Pharmacopoeia as "Ling-zhi" having similar pharmacological effects (Xie et al. 2012). In Chinese, "Lingzhi" is designated as *G. lucidum* or red "Lingzhi," whereas "Zizhi" as *G. sinense* or purple "Lingzhi." It is also known as "Reishi," "Munnertake," or "Sachitake" in Japan and "Youngzhi" in Korea (Bulam et al. 2019; Zeng et al. 2018; Zhang et al. 2019; Kumar and Yadav 2019). Ancient Chinese believed that it could cure various diseases and worshipped it as "Mushroom of Immortality," "Herb of spiritual potency," and "Celestial Herb" that symbolizes happiness, sanctity, success, goodness, and longevity (Lin 2009).

Reishi mushrooms are unique in that its pharmaceutical rather than nutritional value is paramount and are commercialized under different nutraceutical brands, crude drugs, dietary supplements, health drinks and powders, teas, as well as specific functional agents (Bijalwan et al. 2020; Galor et al. 2011; Jong and Birmingham 1992). The pharmacologically active products prepared from different parts of the mushroom including mycelia, spores, and fruiting body are marketed worldwide as traditional medicines as well as dietary supplements in the USA (Yuen and Gohel 2005).

Ganoderma is a genus of highly prized vitality-enhancing herb that has been used particularly in China, Japan, and Korea for more than 2000 years to improve longevity and health (Cheng et al. 2013). *Ganoderma lucidum* comprises probably 400 different biologically active constituents which have been proved to have several therapeutical properties (Ahmad 2018; Hapuarachchi et al. 2016). The bioactive constituents are reported to be responsible for the anti-tumor, immunomodulatory, anti-cancer, anti-inflammatory, anti-oxidant, anti-hypertensive, immunodeficiency, anti-diabetic, anti-viral, anti-bacterial, anti-fungal, anti-atherosclerotic, anti-aging, anti-androgenic, anti-hepatotoxic, radical scavenging property, neuroprotection, sleep promotion, cholesterol synthesis inhibition, hypoglycemia, inhibition of lipid peroxidation, hepatoprotective properties, maintenance of gut health, prevention of obesity, and stimulation of probiotics (Baby et al. 2015; Bishop et al. 2015; Cao and Yuan 2013; Cheng et al. 2012a, b, 2011; De Silva et al. 2013; González et al. 2020; Wang et al. 2020). Traditional preparations from *Ganoderma* have been used as functional food to prevent and treat anorexia, arthritis, asthma, bronchitis, cardiovascular problems, constipation, diabetes, dysmenorrhea, gastritis, hypercholesterolemia, hypertension, insomnia, migraine, nephritis, neurasthenia, neoplasia, and tumorigenesis (Cheng et al. 2010; Paterson 2006; Tan et al. 2015; Wang et al. 2012).

The polysaccharides or glycans extracted from both *G. lucidum* and *G. sinense* have been developed into clinical drugs and recorded in Chinese Pharmacopeia of 2015 (Chan et al. 2017; Zhou et al. 2014). *G. lucidum* polysaccharide (GLPS) has been developed into a drug named "Ji 731 Injection" in China since 1973 which has been approved by the Chinese FDA as "Polysacharidum of *G. lucidum* Karst

Injection in 2000", which is applied intramuscularly. It is one of a few non-hormonal drugs used for treating neurosis, polymyositis, dermatomyositis, atrophic myotonia, and muscular dystrophy in China during the past 40 years. In contrast, *G. sinense* polysaccharide (GSP) tablet has been approved in China by the State Food and Drug Administration (SFDA) in 2010 for treating leukopenia and hematopoietic injury caused by concurrent chemo/radiation therapy. β-glucan, an established immunostimulating polysaccharide, is one of the components in GSP (Zhang et al. 2019).

Triterpenes such as ganoderic acid T, ganoderic acid D, and ganoderiol F exhibit significant anti-cancer properties. Various in vitro as well as in vivo studies reported that ganoderic acid T prevents tumor invasion and produces anti-cancerous results by inhibiting matrix metalloproteinase-9 expression (Chen et al. 2010). In another in vitro study, ganoderiol F has revealed cytotoxic effect against Lewis lung carcinoma, T-47D cell lines, and sarcoma-180 (Gao et al. 2002; Min et al. 2000). Nithya et al. (2015) reported that *G. lucidum* through its anti-oxidant and enzymatic action has potential effect against mammary carcinoma.

Polysaccharides, polysaccharide-peptide complex, triterpenes, and phenolic components of *G. lucidum* have been shown to exhibit various anti-oxidant properties (Kan et al. 2015; Obodai et al. 2017). Polysaccharide components of *G. lucidum* have been reported to possess various anti-microbial properties (Smania et al. 2007). Aqueous extract of the carpophores of *Ganoderma lucidum* has been reported to inhibit 15 different types of gram-positive and gram-negative bacteria. Methanol extract of *G. lucidum* exhibited anti-microbial action against *E. coli*, *S. aureus*, *B. cereus*, *E. aerogenes,* and *P. aeruginosa* (Gaylan et al. 2018; Siwulski et al. 2015). Ganodermin obtained from mycelium inhibited growth of *Fusarium oxysporum*, *Botrytis cinerea,* and *Physalospora piricola*. *Ganoderma lucidum* toothpaste exhibited anti-fungal results against *Candida albicans* (Nayak et al. 2010).

20.4.8 Cordyceps

An entomopathogenic fungus, *Cordyceps sinensis* (CS) is a rare and exotic medicinal mushroom which has been known to have numerous pharmacological and therapeutic implications to promote human health and longevity. It has been recognized as the most famous tonic and health supplement in traditional Chinese medicine (TCM) for centuries. It is also known as "Dong Chong Xia Cao," which means "winter worm summer grass" in China (Li et al. 2006a, b). It is also called as caterpillar fungus as it parasitizes *Lepidopteran* larvae. It is widely distributed in China, Tibetan Plateau, Bhutan, Nepal, and north east regions of India at an altitude of 3500–5000 meters above sea level. Both dead larva and fruiting body have been used as a traditional medicine and health supplement for hundreds of years for "lung invigoration and kidney nourishment" in China and other Asian countries (Dong and Yao 2008; Kuo et al. 1994).

Cordyceps have been reported to contain numerous bioactive components such as proteins, fat, carbohydrates, exopolysaccharides, cordycepin, phenolic

compounds, polysaccharides, cordycepic acid, adenosine, proteoglucans, terpenoids, amphenol, steroids, ergosterol, lectins, etc. (Ashraf et al. 2020; Elkhateeb et al. 2019; Tuli et al. 2013; Yue et al. 2013). Of these, main active constituent which is more widely studied for its medicinal potential/value along with its nutraceutical potential comprises a novel bio-metabolite called as Cordycepin (3′deoxyadenosine) which has a very wide variety of pharmacological actions such as anti-oxidant, immunological, hypolipidemic, anti-inflammatory, hepatoprotective, nephroprotective, anti-apoptotic properties, anti-cancer, anti-microbial, anti-viral, and hypoglycemic properties (Guo et al. 2020; Jordan et al. 2008; Kuo et al. 2007; Lo et al. 2006; Nakamura et al. 2003; Qin et al. 2019; Yamaguchi et al. 1990).

Moreover, various mechanisms have been reported for the pharmacological actions of cordycepin such as inhibition of DNA and RNA synthesis, post-transcriptional processing of hnRNA and activation of adenylate cyclase, inhibition of chemotaxis and particular protein synthesis of macrophage cell lines, anti-tumorigenic activity on some cell lines, and enhancement of cell differentiation (Guo et al. 2020). Nutritionally potent *Cordyceps* are considered a powerhouse of energy, because of their ability to revitalize several organ systems. Various pharmaceutical as well as nutraceutical preparations made from *Cordyceps* dry powder are marketed (Kopalli et al. 2019). The Chinese Pharmacopoeia records that the main functions of *C. sinensis* are replenishing the kidney, soothing the lung, staunching bleeding, and dispersing phlegm. It can also be used to treat continuous cough caused by fatigue, asthma, hemoptysis, impotence, spermatorrhoea, and aches in abdomen and knee.

Another species, *Cordyceps militaris* is phylogenetically related to *Cordyceps sinensis* and has been reported to have the similar biochemical components as *C. sinensis*. Both *C. sinensis* and *C. militaris* are traditionally used mushrooms to improve lung functions and regulate the immune system in China. *However, C. militaris* is less expensive and more easily available than *C. sinensis* and has been reported to possess multiple health benefits, including anti-tumor, immunomodulatory, anti-inflammatory, anti-oxidative, and antibiotic effects (Hsu et al. 2017; Jeong et al. 2010b; Jin et al. 2018; Lee et al. 2019; Oh et al. 2011; Park et al. 2009; Wang et al. 2013; Wong et al. 2011; Zeng et al. 2017). According to a recent report, *C. sinensis* and *C. militaris* can be effective agents for the prevention and treatment of COVID-19. Moreover, they can also be used either to maintain the lung functions after overcoming the disease.

20.4.9 Poria Cocos

Poria cocos (Polyporaceae) or *Poria*, referred to as “Fuling” in Chinese and Hoelen in Japan is a fungus that has been used in clinical Chinese medicine for thousands of years. The dried sclerotia of *P. cocos* has frequently been used as a tonic to benefit the internal organs and is one of the most important crude drugs in traditional Oriental medicine with a wide range of applications in ameliorating phlegm and

edema, relieving nephrosis and chronic gastritis and improving uneasiness of minds (Nie et al. 2020; Xia et al. 2014). It is an ingredient in biscuits, bread, cakes, tea, soups, and dishes in China, Korea, India, and other southeast Asian and European countries (Zhu et al. 2018). Pharmacological studies reveal that polysaccharides and triterpenes are the two major components in *Poria cocos* with a broad range of biological activities including diuretic (Feng et al. 2013), anti-tumor (RuiDian et al. 2010), anti-bacterial (Wang et al. 2013), anti-inflammatory (Lee et al. 2014), antioxidant, cytotoxic (Zhou et al. 2008), anti-emetic (Tai et al. 1995), anti-hyperglycemic (Li et al. 2010), anti-hemorrhagic fever effects (Li et al. 2019b). PCP based product named "compound polysaccharide oral solution" was developed and sold as an over the counter health supplement since 1970s. In 2015, "Polysaccharidum of *Poria cocos* oral solution" was approved as a drug by Chinese Food and Drug Administration for treating multiple types of cancers, hepatitis, and other diseases alone or during chemo- or radiation therapy for patients with cancer.

20.4.10 Grifola Frondosa

Grifola frondosa, a Basidiomycetes fungus that belongs to the family Grifolaceae, order Polyporales, is a premier culinary as well as a medicinal mushroom commonly known as Maitake, "hui-shu-hua," king of mushrooms, sheep's head, hen-of-the-woods and cloud mushroom. It is increasingly being recognized as a potent source of polysaccharide compounds with dramatic health-promoting potential (Mayell 2001; Wu et al. 2021).

The first large-scale commercial production was developed in Japan in 1981 (Takama et al. 1981). Ten years later, USA and China also began large-scale artificial cultivation. It is a widely consumed edible and medicinal fungus. Its fruiting bodies are rich in a variety of nutrients, such as proteins, carbohydrates, dietary fiber vitamin D2 (ergocalciferol), and minerals with low fat content and caloric value (He et al. 2017). Due to its delicious and special taste, the umami or palatable taste which is mainly attributed to its high trehalose, glutamic and aspartic amino acid, and 5′ -nucleotide content, *G. frondosa* is not only used as a food ingredient, but also as a flavoring substance (Huang et al. 2011b). *G. fondosa* has been used for several decades due to its significant pharmacological properties. Numerous nutraceutical preparations of *G. frondosa* are emerging in different forms of capsules, tablets, and additives to food formulations (Gregori et al. 2016).

In addition to the fruiting body, there is also an increasing demand for *G. frondosa* mycelium and its bioactive metabolites. The major bioactive component has been found to be β-glucans (He et al. 2018; Zhao et al. 2017). The D-fraction, a β-glucan complex with about 30% protein has been widely studied after its discovery by Nanba's group in the late 1980s (Hishida et al. 1988). It has been commercially developed into complementary medicine and health care products against breast and prostate cancers. In addition to the D-fraction, there are many other bioactive polysaccharide fractions that are obtained from *G. frondosa*, such as the

MD-fraction (Nanba and Kubo 1998), X-fraction (Kubo et al. 1994), Grifolan (Adachi et al. 1994), MZ-fraction (Masuda et al. 2006), and MT-α-glucan (Hong et al. 2007) which often in combination with whole maitake powder, have shown promising bioactivities including immunomodulation, anti-oxidation, hepatoprotection, anti-diabetic, anti- inflammation and as an adjunct to cancer and HIV therapy (Chen et al. 2018; Hetland et al. 2021; Valverde et al. 2015).

They may also provide some benefit in the treatment of hyperlipidemia, hypertension, and hepatitis. "Hui Shu Hua Capsules" approved by SFDA are Maitake glycan-based drugs (β-glucan is the main component of maitake glycan) with no less than 40% maitake glycans isolated from cultured mycelium of *G. frondosa*. This drug is clinically used against cancer, polycystic ovary syndrome, and impaired glucose tolerance conditions (Deng et al. 2009; Ren et al. 2002; Chen et al. 2020). Additionally, maitake glycans are also used for cosmetic purposes (Lee et al. 2003).

20.4.11 Trametes Versicolor

Coriolus versicolor (L.) Quél. which is now known by its accepted scientific name as *Trametes versicolor* (L.) Lloyd (family Polyporaceae) is the most common wood rotting species on dead hardwoods (Hobbs 2004). This species, whose folk names are Turkey Tail in western cultures, Yun-Zhi (cloud-like mushroom) in China, or Kawaratake (mushroom by the river bank) in Japan, is thought to have had a long history of use in traditional medicine, particularly in Asia (Chu et al. 2002; Hobbs 1995). Turkey tail is one of the most potent and the best studied of all medicinal mushrooms, including shiitake (*Lentinus edodes*), and reishi (*Ganoderma lucidum*) (Hobbs 2004). Two polysaccharides, polysaccharopeptide (PSP) and Polysaccharopeptide Krestin (PSK), isolated from *T. versicolor* are effective immunostimulants which are used to supplement chemotherapy and radiotherapy of cancers and various infectious diseases (Habtemariam 2020; Jiménez-Medina et al. 2008). Furthermore, it seems that these polysaccharides may also act as prebiotics by stimulating the growth and/or activity of probiotic bacteria in the colon (Yu et al. 2013).

Additionally, strong anti-viral effects of some polysaccharopeptides isolated from *T. versicolor* and significant anti-oxidant activity of *Trametes* sp. fruiting body extracts have been reported (Hsu et al. 2013; Que et al. 2014) due to the production of Polysaccharide Krestin (PSK) which is an approved drug paid for by National Health Care in Japan and various polysaccharide-peptide complexes, compounds which reduce cancer metastases, stimulate the production of interleukin-1 in human cells, and also display anti-fungal and anti-neurodegenerative effects (Arockiasamy et al. 2008; Deng et al. 2013; Knežević et al. 2018; Lin et al. 2003). Till date, about thirteen different types of *T. versicolor* based drugs and health products are available in various commercial and clinical brands which are authorized by the China State Food and Drug Administration (Dou et al. 2019) (Fig. 20.1).

20.5 Conclusion and Future Perspective

Mushrooms, with high level of biodiversity are palmary and creditable sources of nutrition and bioactive compounds with pharmaceutical attributes which is currently alluring scientific research worldwide. Neoteric approaches and techniques are providing effectual ways to identify utile chemical compounds and synthesize them readily while upholding the biodiversity of our planet. The collated information and suggested research have lucidly expounded that a number of mushrooms have been investigated, nevertheless a significant number of novel species still remain unexplored inhabiting various niches which need to be unraveled. In this context, the scientific community, society, and all concerned bodies should operate in concert and bolster their relationships in a shared vision for the effective conservation, bioprospecting and fair and equitable sharing of benefits out of the use of these genetic resources. Since bioprospecting, of mushrooms heavily entrusts on the knowledge of local healers and practitioners. So participation and collaboration of traditional healers are highly solicited. Since mushroom biodiversity is crucial to mankind, additional work should also be carried out on indigenous mushrooms with a huge potential to be commercialized as food source or to be used as a source of functional ingredients. Furthermore, comprehensive research, interdisciplinary collaboration, involvement of the pharmaceutical industry, time and money are imperative to delve into this potential not only in the form of dietary supplements but also in the form of approved drugs.

Acknowledgments The authors are grateful to the Department of Biotechnology, Dr. KSG Akal College of Agriculture, Eternal University, Baru Sahib and Department of Environment, Science & Technology (DEST), Shimla Himachal Pradesh, India for funding the project "Development of Microbial Consortium as Bio-inoculants for Drought and Low Temperature Growing Crops for Organic Farming in Himachal Pradesh" as well as for providing the facilities and financial support, to undertake the investigations. There are no conflicts of interest.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Vol-1: biodiversity and ecological perspective. Springer, Cham

Adachi Y, Okazaki M, Ohno N, Yadomae T (1994) Enhancement of cytokine production by macrophages stimulated with (1→ 3)-β-D-glucan, grifolan (GRN), isolated from *Grifola frondosa*. Biol Pharm Bull 17:1554–1560

Adotey G, Quarcoo A, Holliday J, Fofie S, Saaka B (2011) Effect of immunomodulating and antiviral agent of medicinal mushrooms (immune assist 24/7 TM) on CD4+ T-lymphocyte counts of HIV-infected patients. Int J Med Mushrooms 13:109–113

Agrahar-Murugkar D, Subbulakshmi G (2005) Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. Food Chem 89:599–603

Agrawal K, Chaturvedi V, Verma P (2018) Fungal laccase discovered but yet undiscovered. Bioresour Bioprocess 5:1–12

Ahmad MF (2018) Ganoderma lucidum: persuasive biologically active constituents and their health endorsement. Biomed Pharmacother 107:507–519

Ahmed M, Abdullah N, Ahmed KU, Bhuyan M (2013) Yield and nutritional composition of oyster mushroom strains newly introduced in Bangladesh. Pesq Agrop Brasileira 48:197–202

Ahn W-S, Kim D-J, Chae G-T, Lee J-M, Bae S-M, Sin J-I et al (2004) Natural killer cell activity and quality of life were improved by consumption of a mushroom extract, Agaricus blazei Murill Kyowa, in gynecological cancer patients undergoing chemotherapy. Int J Gynecol Cancer 14:589–594

Akwaowo EU, Ndon BA, Etuk EU (2000) Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis* hook f.). Food Chem 70:235–240

Akyuz M, Onganer A, Erecevit P, Kirbag S (2010) Antimicrobial activity of some edible mushrooms in the eastern and Southeast Anatolia region of Turkey. Gazi Univ J Sci 23:125–130

Aly AH, Debbab A, Proksch P (2011) Fifty years of drug discovery from fungi. Fungal Divers 50:3–19

Arockiasamy S, Krishnan IPG, Anandakrishnan N, Seenivasan S, Sambath A, Venkatasubramani JP (2008) Enhanced production of laccase from *Coriolus versicolor* NCIM 996 by nutrient optimization using response surface methodology. Appl Biochem Biotechnol 151:371–379

Ashraf SA, Abd Elmoneim OE, Siddiqui AJ, Patel M, Awadelkareem AM, Snoussi M et al (2020) Cordycepin for health and wellbeing: a potent bioactive metabolite of an Entomopathogenic medicinal fungus *Cordyceps* with its nutraceutical and therapeutic potential. Molecules 25:1–21

Atila F, Owaid MN, Shariati MA (2021) The nutritional and medical benefits of *Agaricus bisporus*: a review. J Microbiol Biotechnol Food Sci 2021:281–286

Atri N, Sharma S, Joshi R, Gulati A, Gulati A (2012) Amino acid composition of five wild Pleurotus species chosen from north West India. Eur J Biol Res 4:31–34

Aydoğdu M, Golukcu M (2017) Nutritional value of Huitlacoche, maize mushroom caused by Ustilago maydis. Food Sci Technol 37:531–535

Ayeka PA (2018) Potential of mushroom compounds as immunomodulators in cancer immunotherapy: a review. Evid Based Complement Alternat Med 2018:1–9

Baby S, Johnson AJ, Govindan B (2015) Secondary metabolites from *Ganoderma*. Phytochemistry 114:66–101

Badalyan SM, Singh M Potential of mushroom bioactive molecules to develop healthcare biotech products. In: Proceedings of the 8th International Conference on mushroom biology and mushroom products (ICMBMP8), 2014. Citeseer, pp 373–378

Bandara A, Mortimer P, Vadthanarat S, Xingrong P, Karunarathna S, Hyde K et al (2020) First successful domestication of a white strain of *Auricularia cornea* from Thailand. Stud Fungi 5:420–434

Bandara AR, Karunarathna SC, Mortimer PE, Hyde KD, Khan S, Kakumyan P et al (2017a) First successful domestication and determination of nutritional and antioxidant properties of the red ear mushroom Auricularia thailandica (Auriculariales, Basidiomycota). Mycol Prog 16:1029–1039

Bandara AR, Karunarathna SC, Phillips AJ, Mortimer PE, Xu J, Kakumyan P et al (2017b) Diversity of AuriculariaAuriculariaceae, Auriculariales in Thailand. Phytotax 292:19–34

Bandara AR, Rapior S, Mortimer PE, Kakumyan P, Hyde KD, Xu J (2019) A review of the polysaccharide, protein and selected nutrient content of *Auricularia*, and their potential pharmacological value. Mycosphere J 10:579–607

Barros L, Baptista P, Correia DM, Casal S, Oliveira B, Ferreira IC (2007) Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. Food Chem 105:140–145

Barros L, Venturini BA, Baptista P, Estevinho LM, Ferreira IC (2008) Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. J Agric Food Chem 56:3856–3862

Bhardwaj K, Sharma A, Tejwan N, Bhardwaj S, Bhardwaj P, Nepovimova E et al (2020) Pleurotus macrofungi-assisted nanoparticle synthesis and its potential applications: a review. J Fungi 6:1–21

Bhushan A, Kulshreshtha M (2018) The medicinal mushroom *Agaricus bisporus*: review of phytopharmacology and potential role in the treatment of various diseases. J Nat Sci Med 1:4–9

Biedron R, Tangen J, Maresz K, Hetland G (2012) Agaricus blazei Murill-immunomodulatory properties and health benefits. Funct Foods Health Dis 2:428–447

Bijalwan A, Bahuguna K, Vasishth A (2020) Insights of medicinal mushroom (*Ganoderma lucidum*): prospects and potential in India. Biodivers Int J 4:202–209

Bisen P, Baghel RK, Sanodiya BS, Thakur GS, Prasad G (2010) Lentinus edodes: a macrofungus with pharmacological activities. Curr Med Chem 17:2419–2430

Bishop KS, Kao CH, Xu Y, Glucina MP, Paterson RRM, Ferguson LR (2015) From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. Phytochemistry 114:56–65

Bobek P, Ozdín Ľ, Kuniak Ľ (1997) Effect of oyster mushroom and isolated β-glucan on lipid peroxidation and on the activities of antioxidative enzymes in rats fed the cholesterol diet. J Nutr Biochem 8:469–471

Breene WM (1990) Nutritional and medicinal value of specialty mushrooms. J Food Prot 53:883–894

Bulam S, Üstün NŞ, Pekşen A (2019) Health benefits of Ganoderma lucidum as a medicinal mushroom. Turkish J Agric-Food Sci Technol 7:84–93

Cai M, Lin Y, Luo Y-L, Liang H-H, Sun P (2015) Extraction, antimicrobial, and antioxidant activities of crude polysaccharides from the wood ear medicinal mushroom *Auricularia auricula-judae* (higher basidiomycetes). Int J Med Mushrooms 17:591

Cao Y, Yuan H-S (2013) Ganoderma mutabile sp. nov. from southwestern China based on morphological and molecular data. Mycol Prog 12:121–126

Carneiro AA, Ferreira IC, Dueñas M, Barros L, Da Silva R, Gomes E et al (2013) Chemical composition and antioxidant activity of dried powder formulations of *Agaricus blazei* and *Lentinus edodes*. Food Chem 138:2168–2173

Chan K-M, Yue GG-L, Li P, Wong EC-W, Lee JK-M, Kennelly EJ et al (2017) Screening and analysis of potential anti-tumor components from the stipe of *Ganoderma sinense* using high-performance liquid chromatography/time-of-flight mass spectrometry with multivariate statistical tool. J Chromatogr A 1487:162–167

Chang AKT, Frias RR Jr, Alvarez LV, Bigol UG, Guzman JPMD (2019) Comparative antibacterial activity of commercial chitosan and chitosan extracted from *Auricularia* sp. Biocatal Agric Biotechnol 17:189–195

Chang Y-C, Chow Y-H, Sun H-L, Liu Y-F, Lee Y-T, Lue K-H et al (2014) Alleviation of respiratory syncytial virus replication and inflammation by fungal immunomodulatory protein FIP-fve from Flammulina velutipes. Antivir Res 110:124–131

Chang YS, Lee SS (2004) Utilisation of macrofungi species in Malaysia. Fungal Divers 15:15–22

Chaturvedi VK, Agarwal S, Gupta KK, Ramteke PW, Singh M (2018) Medicinal mushroom: boon for therapeutic applications. 3. Biotech 8:1–20

Chen C, Chu H, Hao J, Li Y (2006) Influences of selenium-rich protein and polysaccharide extracted from selenium-rich *Agaricus blazei* on S180 sarcoma bearing mice. Zhongyaocai 29:1215–1217

Chen G-t, Ma X-m, Liu S-t, Liao Y-l, Zhao G-q (2012) Isolation, purification and antioxidant activities of polysaccharides from Grifola frondosa. Carbohydr Polym 89:61–66

Chen N-H, Liu J-W, Zhong J-J (2010) Ganoderic acid T inhibits tumor invasion in vitro and in vivo through inhibition of MMP expression. Pharmacol Rep 62:150–163

Chen S, Yong T, Xiao C, Su J, Zhang Y, Jiao C et al (2018) Pyrrole alkaloids and ergosterols from Grifola frondosa exert anti-α-glucosidase and anti-proliferative activities. J Funct Foods 43:196–205

Chen X, Ji H, Zhang C, Yu J, Liu A (2020) Structural characterization and antitumor activity of a novel polysaccharide from *Grifola frondosa*. J Food Meas Charact 14:272–282

Cheng C, Yang M, Yu K, Guan S, Tao S, Millar A et al (2012b) Identification of metabolites of ganoderic acid D by ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. Drug Metab Dispos 40:2307–2314

Cheng C-R, Li Y-F, Xu P-P, Feng R-H, Yang M, Guan S-H et al (2012a) Preparative isolation of triterpenoids from Ganoderma lucidum by counter-current chromatography combined with pH-zone-refining. Food Chem 130:1010–1016

Cheng CR, Yang M, Wu ZY, Wang Y, Zeng F, Wu WY et al (2011) Fragmentation pathways of oxygenated tetracyclic triterpenoids and their application in the qualitative analysis of Ganoderma lucidum by multistage tandem mass spectrometry. Rapid Commun Mass Spectrom 25:1323–1335

Cheng C-R, Yang M, Yu K, Guan S-H, Wu X-H, Wu W-Y et al (2013) Metabolite identification of crude extract from *Ganoderma lucidum* in rats using ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. J Chromatogr B 941:90–99

Cheng C-R, Yue Q-X, Wu Z-Y, Song X-Y, Tao S-J, Wu X-H et al (2010) Cytotoxic triterpenoids from Ganoderma lucidum. Phytochemistry 71:1579–1585

Cheung P (2010) The nutritional and health benefits of mushrooms. Nutr Bull 35:292–299

Cheung PC (2013) Mini-review on edible mushrooms as source of dietary fiber: preparation and health benefits. Food Sci Human Wellness 2:162–166

Chihara G, Hamuro J, Maeda YY, Arai Y, Fukuoka F (1970) Fractionation and purification of the polysaccharides with marked antitumor activity, especially lentinan, from *Lentinus edodes* (Berk.) sing.(an edible mushroom). Cancer Res 30:2776–2781

Choi D, Park S-S, Ding J-L, Cha W-S (2007) Effects of Fomitopsis pinicola extracts on antioxidant and antitumor activities. Biotechnol Bioprocess Eng 12:516

Chu KK, Ho SS, Chow AH (2002) Coriolus versicolor: a medicinal mushroom with promising immunotherapeutic values. J Clin Pharmacol 42:976–984

Dai Y-C, Yang Z-L, Cui B-K, Yu C-J, Zhou L-W (2009) Species diversity and utilization of medicinal mushrooms and fungi in China. Int J Med Mushrooms 11

Damte D, Reza MA, Lee S-J, Jo W-S, Park S-C (2011) Anti-inflammatory activity of dichloromethane extract of Auricularia auricula-judae in RAW264. 7 cells. Toxicol Res 27:11–14

de Oliveira JM, Jordao B, Ribeiro LR, da Eira AF, Mantovani M (2002) Anti-genotoxic effect of aqueous extracts of sun mushroom (*Agaricus blazei* Murill lineage 99/26) in mammalian cells in vitro. Food Chem Toxicol 40:1775–1780

De Silva DD, Rapior S, Fons F, Bahkali AH, Hyde KD (2012) Medicinal mushrooms in supportive cancer therapies: an approach to anti-cancer effects and putative mechanisms of action. Fungal Divers 55:1–35

De Silva DD, Rapior S, Sudarman E, Stadler M, Xu J, Alias SA et al (2013) Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. Fungal Divers 62:1–40

Deng G, Lin H, Seidman A, Fornier M, D'Andrea G, Wesa K et al (2009) A phase I/II trial of a polysaccharide extract from Grifola frondosa (Maitake mushroom) in breast cancer patients: immunological effects. J Cancer Res Clin Oncol 135:1215–1221

Deng J-S, Huang S-S, Lin T-H, Lee M-M, Kuo C-C, Sung P-J et al (2013) Analgesic and anti-inflammatory bioactivities of eburicoic acid and dehydroeburicoic acid isolated from Antrodia camphorata on the inflammatory mediator expression in mice. J Agric Food Chem 61:5064–5071

Devi KSP, Roy B, Patra P, Sahoo B, Islam SS, Maiti TK (2013) Characterization and lectin microarray of an immunomodulatory heteroglucan from *Pleurotus ostreatus* mycelia. Carbohydr Polym 94:857–865

Dong C-H, Yao Y-J (2008) In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of *Cordyceps sinensis*. LWT-Food Sci Technol 41:669–677

Dou H, Chang Y, Zhang L (2019) Coriolus versicolor polysaccharopeptide as an immunotherapeutic in China. Prog Mol Biol Transl Sci 163:361–381

Duc P (2005) Mushrooms and cultivation of mushrooms in Vietnam. Mushroom Growers' Handbook 2

El Enshasy HA, Hatti-Kaul R (2013) Mushroom immunomodulators: unique molecules with unlimited applications. Trends Biotechnol 31:668–677

El-Fakharany EM, Haroun BM, Ng T, Redwan E-RM (2010) Oyster mushroom laccase inhibits hepatitis C virus entry into peripheral blood cells and hepatoma cells. Protein Pept Lett 17:1031–1039

Elkhateeb WA (2020) What medicinal mushroom can do. J Chem Res 5:106–118

Elkhateeb WA, Daba GM, Thomas PW, Wen T-C (2019) Medicinal mushrooms as a new source of natural therapeutic bioactive compounds. Egypt Pharm J 18:88–101

Erjavec J, Kos J, Ravnikar M, Dreo T, Sabotič J (2012) Proteins of higher fungi–from forest to application. Trends Biotechnol 30:259–273

Feng Y-L, Zhao Y-Y, Ding F, Xi Z-H, Tian T, Zhou F et al (2013) Chemical constituents of surface layer of Poria cocos and their pharmacological properties (I). Zhongguo Zhong Yao Za Zhi Zhongguo zhongyao zazhi China Journal of Chinese Materia Medica 38:1098–1102

Friedman M (2016) Mushroom polysaccharides: chemistry and antiobesity, antidiabetes, anticancer, and antibiotic properties in cells, rodents, and humans. Foods 5:80

Fu H, De S, Ho C (2002) Antioxidant and free radical scavenging activities of edible mushrooms. J Food Lipids 9:35–43

Fukushima-Sakuno E (2020) Bioactive small secondary metabolites from the mushrooms *Lentinula edodes* and *Flammulina velutipes*. J Antibiot 73:687–696

Galor S, Yuen J, Buswell J, Benzie I (2011) Ganoderma lucidum (Lingzhi or Reishi), a medicinal mushroom in herbal medicine: biomolecular and clinical aspects, 2nd edn. CRC Press/Taylor and Francis, Florida

Gao J-J, Min B-S, Ahn E-M, Nakamura N, Lee H-K, Hattori M (2002) New triterpene aldehydes, lucialdehydes A—C, from *Ganoderma lucidum* and their cytotoxicity against murine and human tumor cells. Chem Pharm Bull 50:837–840

Gao Y, Wang P, Wang Y, Wu L, Wang X, Zhang K et al (2017) In vitro and in vivo activity of Fomitopsis pinicola (sw. Ex fr.) karst chloroform (fpkc) extract against s180 tumor cells. Cell Physiol Biochem 44:2042–2056

Gao Y, Zhou S, Jiang W, Huang M, Dai X (2003) Effects of Ganopoly®(a *Ganoderma lucidum* polysaccharide extract) on the immune functions in advanced-stage cancer patients. Immunol Investig 32:201–215

Gaylan CM, Estebal JC, Tantengco OAG, Ragragio EM (2018) Anti-staphylococcal and antioxidant properties of crude ethanolic extracts of macrofungi collected from the Philippines. Pharm J 10:106–109

Ghorai S, Banik SP, Verma D, Chowdhury S, Mukherjee S, Khowala S (2009) Fungal biotechnology in food and feed processing. Food Res Int 42:577–587

Gonzaga MLC, Bezerra DP, Alves APNN, de Alencar NMN, de Oliveira MR, Lima MW et al (2009) In vivo growth-inhibition of sarcoma 180 by an α-(1→ 4)-glucan–β-(1→ 6)-glucan-protein complex polysaccharide obtained from *Agaricus blazei* Murill. J Nat Med 63:32–40

González A, Atienza V, Montoro A, Soriano JM (2020) Use of Ganoderma lucidum (Ganodermataceae, Basidiomycota) as radioprotector. Nutrients 12:1143

Goto S, Kamada K, Soh Y, Ihara Y, Kondo T (2002) Significance of nuclear glutathione S-transferase π in resistance to anti-cancer drugs. Jpn J Cancer Res 93:1047–1056

Gregori A, Švagelj M, Voglar D, Berovic M (2016) Growth characteristics and ergosterol content of *Grifola frondosa* in various solid-state substrates. Chem Biochem Eng Q 30:183–188

Guan G, Wang H, Ng T (2007) A novel ribonuclease with antiproliferative activity from fresh fruiting bodies of the edible mushroom Hypsizigus marmoreus. Bioch Biophys Acta 1770:1593–1597

Guo Z, Chen W, Dai G, Huang Y (2020) Cordycepin suppresses the migration and invasion of human liver cancer cells by downregulating the expression of CXCR4. Int J Mol Med 45:141–150

Habtemariam S (2020) Trametes versicolor (Synn. Coriolus versicolor) polysaccharides in cancer therapy: targets and efficacy. Biomedicine 8:135

Han Y, Ma L, Bao H, Bau T, Li Y (2020) Preventive effect of small molecular fraction of Irpex lacteus (Agaricomycetes) fruiting body against chronic nephritis in mice and identification of active compounds. Int J Med Mushrooms 22:313–323

Hapuarachchi K, Wen T, Jeewon R, Wu X, Kang J, Hyde K (2016) Mycosphere essays 7. *Ganoderma lucidum*-are the beneficial anti-cancer properties substantiated? Mycosphere 7:305–332

Hassan MAA, Rouf R, Tiralongo E, May TW, Tiralongo J (2015) Mushroom lectins: specificity, structure and bioactivity relevant to human disease. Int J Mol Sci 16:7802–7838

Hawksworth DL, Lücking R (2017) Fungal diversity revisited: 2.2 to 3.8 million species. The Fungal kingdom:79–95

He X, Wang X, Fang J, Chang Y, Ning N, Guo H et al (2017) Polysaccharides in *Grifola frondosa* mushroom and their health promoting properties: a review. Int J Biol Macromol 101:910–921

He Y, Li X, Hao C, Zeng P, Zhang M, Liu Y et al (2018) Grifola frondosa polysaccharide: a review of antitumor and other biological activity studies in China. Discov Med 25:159–176

Hetland G, Eide DM, Tangen JM, Haugen MH, Mirlashari MR, Paulsen JE (2016) The Agaricus blazei-based mushroom extract, Andosan™, protects against intestinal tumorigenesis in the A/J Min/+ Mouse. PLoS One 11:e0167754

Hetland G, Johnson E, Bernardshaw SV, Grinde B (2021) Can medicinal mushrooms have prophylactic or therapeutic effect against COVID-19 and its pneumonic superinfection and complicating inflammation? Scand J Immunol 93:e12937

Hetland G, Johnson E, Lyberg T, Kvalheim G (2011) The mushroom *Agaricus blazei* Murill elicits medicinal effects on tumor, infection, allergy, and inflammation through its modulation of innate immunity and amelioration of Th1/Th2 imbalance and inflammation. Adv Pharmacol Sci 2011

Hishida I, Nanba H, Kuroda H (1988) Antitumor activity exhibited by orally administered extract from fruit body of *Grifola frondosa* (maitake). Chem Pharm Bull 36:1819–1827

Hobbs C (1995) Mushrooms, an exploration of tradition, healing and culture. Botanical Press, Santa Cruz, California

Hobbs C (2004) Medicinal value of Turkey tail fungus *Trametes versicolor* (L.: Fr.) Pilat (Aphyllophoromycetideae). A literature review. Int J Med Mushrooms 6:195–218

Hong L, Xun M, Wutong W (2007) Anti-diabetic effect of an α-glucan from fruit body of maitake (*Grifola frondosa*) on KK-ay mice. J Pharm Pharmacol 59:575–582

Hsu P-Y, Lin Y-H, Yeh E-L, Lo H-C, Hsu T-H, Su C-C (2017) Cordycepin and a preparation from Cordyceps militaris inhibit malignant transformation and proliferation by decreasing EGFR and IL-17RA signaling in a murine oral cancer model. Oncotarget 8:93712

Hsu W-k, Hsu T-h, Lin F-y, Cheng Y-k, Yang JP-W (2013) Separation, purification, and α-glucosidase inhibition of polysaccharides from *Coriolus versicolor* LH1 mycelia. Carbohydr Polym 92:297–306

Huang H-Y, Chieh S-Y, Tso TK, Chien T-Y, Lin H-T, Tsai Y-C (2011a) Orally administered mycelial culture of *Phellinus linteus* exhibits antitumor effects in hepatoma cell-bearing mice. J Ethnopharmacol 133:460–466

Huang S-J, Tsai S-Y, Lin S-Y, Liang C-H, Mau J-L (2011b) Nonvolatile taste components of culinary-medicinal maitake mushroom, Grifola frondosa (dicks.: Fr.) SF Gray. Int J Med Mushrooms 13:265–272

Ikekawa T (2001) Beneficial effects of edible and medicinal mushrooms on health care. Int J Med Mushrooms 3:1–8

Inácio FD, Ferreira RO, CAVD A, Brugnari T, Castoldi R, Peralta RM et al (2015) Proteases of wood rot fungi with emphasis on the genus *Pleurotus*. Biomed Res Int 2015:1–10

Ingold C (1980) Flammulina velutipes. Bull Br Mycol Soc 14:112–118

Irawati D, Hayashi C, Takashima Y, Wedatama S, Ishiguri F, Iizuka K et al (2012) Cultivation of the edible mushroom *Auricularia polytricha* using sawdust based substrate made of three

Indonesian commercial plantation species, *Falcataria moluccana, Shorea* sp., and *Tectona grandis*. Micologia Aplicada International 24:33–41

Ismaya WT, Tjandrawinata RR, Rachmawati H (2020) Lectins from the edible mushroom Agaricus bisporus and their therapeutic potentials. Molecules:25

Itoh H, Ito H, Amano H, Noda H (1994) Inhibitory action of a (1→ 6)-β-D-glucan-protein complex (FIII-2-b) isolated from *Agaricus blazei* Murill (" Himematsutake") on meth a fibrosarcoma-bearing mice and its antitumor mechanism. Jpn J Pharmacol 66:265–271

Jayakumar T, Ramesh E, Geraldine P (2006) Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl4-induced liver injury in rats. Food Chem Toxicol 44:1989–1996

Jeong J-W, Jin C-Y, Kim G-Y, Lee J-D, Park C, Kim G-D et al (2010a) Anti-inflammatory effects of cordycepin via suppression of inflammatory mediators in BV2 microglial cells. Int Immunopharmacol 10:1580–1586

Jeong SC, Jeong YT, Yang BK, Islam R, Koyyalamudi SR, Pang G et al (2010b) White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats. Nutr Res 30:49–56

Jiang L, Yu Z, Lin Y, Cui L, Yao S, Lv L et al (2018) Low-molecular-weight polysaccharides from Agaricus blazei Murrill modulate the Th1 response in cancer immunity. Oncol Lett 15:3429–3436

Jiménez-Medina E, Berruguilla E, Romero I, Algarra I, Collado A, Garrido F et al (2008) The immunomodulator PSK induces in vitro cytotoxic activity in tumour cell lines via arrest of cell cycle and induction of apoptosis. BMC Cancer 8:1–10

Jin Y, Meng X, Qiu Z, Su Y, Yu P, Qu P (2018) Anti-tumor and anti-metastatic roles of cordycepin, one bioactive compound of *Cordyceps militaris*. Saudi J Biol Sci 25:991–995

Johnny I, Okon J (2013) Antidiabetic effect of *Pleurotus ostreatus* (Jacq. Ex Fr) kumm. Mushroom on Alloxan-induced diabetic rats. Indian J Pharm Biol Res 1:31–36

Jong S, Birmingham J (1992) Medicinal benefits of the mushroom *Ganoderma*. Adv Appl Microbiol 37:101–134

Jordan J, Hirsch G, Lee T (2008) C. sinensis ablates allograft vasculopathy when used as an adjuvant therapy with cyclosporin a. Transpl Immunol 19:159–166

Jose Alves M, Ferreira ICFR, Dias J, Teixeira V, Martins A, Pintado M (2013) A review on antifungal activity of mushroom (basidiomycetes) extracts and isolated compounds. Curr Top Med Chem 13:2648–2659

Juárez-Montiel M, de León SR, Chávez-Camarillo G, Hernández-Rodríguez C, Villa-Tanaca L (2011) Huitlacoche (corn smut), caused by the phytopathogenic fungus *Ustilago maydis*, as a functional food. Revista iberoamericana de micologia 28:69–73

Kalač P (2009) Chemical composition and nutritional value of European species of wild growing mushrooms: a review. Food Chem 113:9–16

Kan Y, Chen T, Wu Y, Wu J (2015) Antioxidant activity of polysaccharide extracted from Ganoderma lucidum using response surface methodology. Int J Biol Macromol 72:151–157

Karunarathna SC, Chen J, Mortimer P, Xu J, Zhao R, Callac P et al (2016) Mycosphere essay 8: a review of genus *Agaricus* in tropical and humid subtropical regions of Asia. Mycosphere J 7:417–439

Kawagishi H, Inagaki R, Kanao T, Mizuno T, Shimura K, Ito H et al (1989) Fractionation and antitumor activity of the water-in-soluble residue of *Agaricus blazei* fruiting bodies. Carbohydr Res 186:267–273

Khan MA, Amin SR, Uddin MN, Tania M, Alam N (2008) Comparative study of the nutritional composition of oyster mushrooms cultivated in Bangladesh. Bangladesh J Mushroom 2:9–14

Khan MA, Khan LA, Hossain MS, Tania M, Uddin MN (2009) Investigation on the nutritional composition of the common edible and medicinal mushrooms cultivated in Bangladesh. Bangladesh J Mushroom 3:21–28

Khan MA, Tania M (2012) Nutritional and medicinal importance of *Pleurotus* mushrooms: an overview. Food Rev Intl 28:313–329

Knežević A, Stajić M, Sofrenić I, Stanojković T, Milovanović I, Tešević V et al (2018) Antioxidative, antifungal, cytotoxic and antineurodegenerative activity of selected *Trametes* species from Serbia. PLoS One 13:e0203064

Knop D, Yarden O, Hadar Y (2015) The ligninolytic peroxidases in the genus *Pleurotus:* divergence in activities, expression, and potential applications. Appl Microbiol Biotechnol 99:1025–1038

Kopalli SR, Cha K-M, Lee S-H, Hwang S-Y, Lee Y-J, Koppula S et al (2019) Cordycepin, an active constituent of nutrient powerhouse and potential medicinal mushroom *Cordyceps militaris* Linn., ameliorates age-related testicular dysfunction in rats. Nutrients 11:906

Kour D, Rana KL, Yadav N, Yadav AN, Singh J, Rastegari AA et al (2019) Agriculturally and industrially important fungi: current developments and potential biotechnological applications. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi, volume 2: perspective for value-added products and environments. Springer International Publishing, Cham, pp 1–64. https://doi.org/10.1007/978-3-030-14846-1_1

Kubo K, Aoki H, Nanba H (1994) Anti-diabetic activity present in the fruit body of *Grifola frondosa* (maitake). I. Biol Pharm Bull 17:1106–1110

Kües U, Liu Y (2000) Fruiting body production in basidiomycetes. Appl Microbiol Biotechnol 54:141–152

Kumar K (2020) Nutraceutical potential and processing aspects of oyster mushrooms (Pleurotus species). Curr Nutr Food Sci 16:3–14

Kumar V, Yadav HK (2019) Therapeutic potential of an edible macro-fungus: *Ganoderma lucidum* (Curtis) P. karst. Indian J Tradit Knowl 18:702–713

Kuo C-F, Chen C-C, Lin C-F, Jan M-S, Huang RY, Luo Y-H et al (2007) Abrogation of streptococcal pyrogenic exotoxin B-mediated suppression of phagocytosis in U937 cells by Cordyceps sinensis mycelium via production of cytokines. Food Chem Toxicol 45:278–285

Kuo Y-C, Lin C-Y, Tsai W-J, Wu C-L, Chen C-F, Shiao M-S (1994) Growth inhibitors against tumor cells in Cordyceps sinensis other than cordycepin and polysaccharides. Cancer Investig 12:611–615

Lam S, Ng T (2001) First simultaneous isolation of a ribosome inactivating protein and an antifungal protein from a mushroom (*Lyophyllum shimeji*) together with evidence for synergism of their antifungal effects. Arch Biochem Biophys 393:271–280

Lee H-C, Cheng W-Y, Huang BE-G, Hsu Y-H, Huang S-Y (2014) Anti-inflammatory and hypoglycemic efficacy of Poria cocos and Dioscorea opposita in prediabetes mellitus rats. RSC Adv 4:55649–55657

Lee SK, Lee JH, Kim HR, Chun Y, Lee JH, Yoo HY et al (2019) Improved cordycepin production by Cordyceps militaris KYL05 using casein hydrolysate in submerged conditions. Biomol Ther 9:1–11

Lee Y-L, Kim H-J, Lee M-S, Kim J-M, Han J-S, Hong E-K et al (2003) Oral administration of *Agaricus blazei* (H1 strain) inhibited tumor growth in a sarcoma 180 inoculation model. Exp Anim 52:371–375

Lee Y-T, Lee S-S, Sun H-L, Lu K-H, Ku M-S, Sheu J-N et al (2013) Effect of the fungal immunomodulatory protein FIP-fve on airway inflammation and cytokine production in mouse asthma model. Cytokine 61:237–244

Lemieszek MK, Langner E, Kaczor J, Kandefer-Szerszen M, Sanecka B, Mazurkiewicz W et al (2009) Anticancer effect of fraction isolated from medicinal birch polypore mushroom, *Piptoporus betulinus* (bull.: Fr.) P. karst.(Aphyllophoromycetideae): in vitro studies. Int J Med Mushrooms 11:351–364

León-Ramírez CG, Sánchez-Arreguín JA, Ruiz-Herrera J (2014) Ustilago maydis, a delicacy of the Aztec cuisine and a model for research. Nat Res 5:1–12

Li F, Gao X, Rao B, Liu L, Dong B, Cui L (2006a) Effects of cordyceps sinensis alcohol extractive on serum interferon-gamma level and splenic T lymphocyte subset in mice with viral myocarditis. Chin J Cell Mol Immunol 22:321–323

Li Q-Z, Zheng Y-Z, Zhou X-W (2019a) Fungal immunomodulatory proteins: characteristic, potential antitumor activities and their molecular mechanisms. Drug Discov Today 24:307–314

Li S, Yang F, Tsim KW (2006b) Quality control of Cordyceps sinensis, a valued traditional Chinese medicine. J Pharm Biomed Anal 41:1571–1584
Li T-H, Hou C-C, Chang CL-T, Yang W-C (2010) Anti-hyperglycemic properties of crude extract and triterpenes from *Poria cocos*. Evid Based Complement Alternat Med 2011:1–8
Li X, He Y, Zeng P, Liu Y, Zhang M, Hao C et al (2019b) Molecular basis for Poria cocos mushroom polysaccharide used as an antitumour drug in China. J Cell Mol Med 23:4–20
Li Y, Liu Q, Wang H, Ng T (2008) A novel lectin with potent antitumor, mitogenic and HIV-1 reverse transcriptase inhibitory activities from the edible mushroom *Pleurotus citrinopileatus*. Biochim Biophys Acta 1780:51–57
Li Y, Zhou Y, Wang X, Wang P, Xiao Y, Cheng X et al (2016) Fruit extract from *Pyropolyporus fomentarius* (L. ex Fr.) Teng induces mitochondria-dependent apoptosis in leukemia cells but enhances immunomodulatory activities of splenic lymphocytes. Nutr Cancer 68:708–717
Liang C, Tian D, Liu Y, Li H, Zhu J, Li M et al (2019) Review of the molecular mechanisms of *Ganoderma lucidum* triterpenoids: Ganoderic acids a, C2, D, F, DM, X and Y. Eur J Med Chem 174:130–141
Lin C-H, Sheu G-T, Lin Y-W, Yeh C-S, Huang Y-H, Lai Y-C et al (2010) A new immunomodulatory protein from *Ganoderma microsporum* inhibits epidermal growth factor mediated migration and invasion in A549 lung cancer cells. Process Biochem 45:1537–1542
Lin J-p, Lian W, L-m X, P-l C (2003) Production of laccase by Coriolus versicolor and its application in decolorization of dyestuffs:(I) production of laccase by batch and repeated-batch processes. J Environ Sci 15:1–4
Lin ZB (2009) Lingzhi: from mystery to science. Peking University Medical Press
Liu Y, Bastiaan-Net S, Wichers HJ (2020) Current understanding of the structure and function of fungal immunomodulatory proteins. Front Nutr 7:132
Liu Y, Chen D, You Y, Zeng S, Li Y, Tang Q et al (2016) Nutritional composition of boletus mushrooms from Southwest China and their antihyperglycemic and antioxidant activities. Food Chem 211:83–91
Lizárraga-Guerra R, Guth H, López MG (1997) Identification of the most potent odorants in huitlacoche (*Ustilago maydis*) and austern pilzen (*Pleurotus* sp.) by aroma extract dilution analysis and static head-space samples. J Agric Food Chem 45:1329–1332
Lizarraga-Guerra R, Lopez MG (1998) Monosaccharide and alditol contents of huitlacoche (*Ustilago maydis*). J Food Compos Anal 11:333–339
Lo H-C, Hsu T-H, Tu S-T, Lin K-C (2006) Anti-hyperglycemic activity of natural and fermented Cordyceps sinensis in rats with diabetes induced by nicotinamide and streptozotocin. Am J Chin Med 34:819–832
Luiz R, Jordao B, Da Eira A, Ribeiro L, Mantovani M (2003) Mechanism of anticlastogenicity of Agaricus blazei Murill mushroom organic extracts in wild type CHO (K1) and repair deficient (xrs5) cells by chromosome aberration and sister chromatid exchange assays. Mutat Res Fundam Mol Mech Mutagen 528:75–79
Luo X-j, Li L-l, Deng Q-p, Yu X-f, Yang L-f, Luo F-j et al (2011) Grifolin, a potent antitumour natural product upregulates death-associated protein kinase 1 DAPK1 via p53 in nasopharyngeal carcinoma cells. Eur J Cancer 47:316–325
Ma Y, Wang C, Zhang Q, Peng X, Feng Y, Meng X (2018) The effects of polysaccharides from *Auricularia auricula* (Huaier) in adjuvant anti-gastrointestinal cancer therapy: a systematic review and network meta-analysis. Pharmacol Res 132:80–89
Maheshwari S (2013) A guide for white button mushroom (*Agaricus bisporus*) production. Open Access Sci Rep 2:2–3
Mao G-H, Ren Y, Feng W-W, Li Q, Wu H-Y, Zhao T et al (2015) Antitumor and immunomodulatory activity of a water-soluble polysaccharide from Grifola frondosa. Carbohydr Polym 134:406–412
Martin KR, Brophy SK (2010) Commonly consumed and specialty dietary mushrooms reduce cellular proliferation in MCF-7 human breast cancer cells. Exp Biol Med 235:1306–1314

Martinez-Medina GA, Chávez-González ML, Verma DK, Prado-Barragán LA, Martínez-Hernández JL, Flores-Gallegos AC et al (2021) Bio-functional components in mushrooms, a health opportunity: Ergothionine and huitlacohe as recent trends. J Funct Foods 77:104326

Masuda Y, Kodama N, Nanba H (2006) Macrophage J774. 1 cell is activated by MZ-fraction (Klasma-MZ) polysaccharide in Grifola frondosa. Mycoscience 47:360–366

Mattila P, Könkö K, Eurola M, Pihlava J-M, Astola J, Vahteristo L et al (2001) Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. J Agric Food Chem 49:2343–2348

Mayell M (2001) Maitake extracts and their therapeutic potential-a review. Altern Med Rev 6:48–60

Meng L-Z, Xie J, Lv G-P, Hu D-J, Zhao J, Duan J-A et al (2014) A comparative study on immunomodulatory activity of polysaccharides from two official species of Ganoderma (Lingzhi). Nutr Cancer 66:1124–1131

Min B-S, GAo J-J, Nakamura N, Hattori M (2000) Triterpenes from the spores of Ganoderma lucidum and their cytotoxicity against meth-a and LLC tumor cells. Chem Pharm Bull 48:1026–1033

Minato K-i (2010) Mushrooms: Immunomodulating activity and role in health promotion. In: Watson RR, Zibadi S, Preedy VR (eds) Dietary components and immune function. Humana Press, Totowa, NJ, pp 529–539. https://doi.org/10.1007/978-1-60761-061-8_28

Mizuno T, Hagiwara T, Nakamura T, Ito H, Shimura K, Sumiya T et al (1990) Antitumor activity and some properties of water-soluble polysaccharides from "Himematsutake," the fruiting body of *Agavicus blazei* Murill. Agric Biol Chem 54:2889–2896

Mizuno T, Zhuang C (1995) Maitake, Grifola frondosa: pharmacological effects. Food Rev Intl 11:135–149

Na Z, Yan L, Jia-hui L, Juan W, Shuang Y, Nan Z et al (2012) Isolation, purification and bioactivities of polysaccharides from Irpex lacteus. Chem Res Chin Univ 28:249–254

Nakamura K, Konoha K, Yamaguchi Y, Kagota S, Shinozuka K, Kunitomo M (2003) Combined effects of Cordyceps sinensis and methotrexate on hematogenic lung metastasis in mice. Recept Channels 9:329–334

Nanba H, Kubo K (1998) Antitumor substance extracted from Grifola. Google Patents,

Nayak A, Nayak RN, Bhat K (2010) Antifungal activity of a toothpaste containing Ganoderma lucidum against *Candida albicans*-an in vitro study. J Int Oral Health 2:51–57

Ng T, Wang H (2004) Flammin and velin: new ribosome inactivating polypeptides from the mushroom Flammulina velutipes. Peptides 25:929–933

Ngai PH, Ng T (2003) Lentin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukemia cells. Life Sci 73:3363–3374

Ngai PH, Ng T (2004) A mushroom (*Ganoderma capense*) lectin with spectacular thermostability, potent mitogenic activity on splenocytes, and antiproliferative activity toward tumor cells. Biochem Biophys Res Commun 314:988–993

Nguyen TL, Chen J, Hu Y, Wang D, Fan Y, Wang J et al (2012a) In vitro antiviral activity of sulfated Auricularia auricula polysaccharides. Carbohydr Polym 90:1254–1258

Nguyen TL, Wang D, Hu Y, Fan Y, Wang J, Abula S et al (2012b) Immuno-enhancing activity of sulfated Auricularia auricula polysaccharides. Carbohydr Polym 89:1117–1122

Ngwuluka NC, Ochekpe NA, Aruoma OI (2016) Functions of bioactive and intelligent natural polymers in the optimization of drug delivery. In: Hosseini M, Makhlouf ASH (eds) Industrial applications for intelligent polymers and coatings. Springer International Publishing, Cham, pp 165–184. https://doi.org/10.1007/978-3-319-26893-4_8

Nie A, Chao Y, Zhang X, Jia W, Zhou Z, Zhu C (2020) Phytochemistry and pharmacological activities of Wolfiporia Cocos (FA wolf) Ryvarden & Gilb. Front Pharmacol 11

Nithya M, Ambikapathy V, Panneerselvam A (2015) In vivo antioxidant and enzymatic activity of Ganoderma lucidum (Curt. Fr.) P. karst. On mammary cells of DMBA induced Sprague Dawley rats. Int J Curr Microbiol App Sci 4:69–77

Nowak R, Nowacka-Jechalke N, Juda M, Malm A (2018) The preliminary study of prebiotic potential of polish wild mushroom polysaccharides: the stimulation effect on *lactobacillus* strains growth. Eur J Nutr 57:1511–1521

O'Neil E, Nicklas A, Fulgoni III VL (2013) Mushroom intake is associated with better nutrient intake and diet quality: 2001–2010 National Health and Nutrition Examination Survey. Nutrition & Food Sciences

Oba K, Teramukai S, Kobayashi M, Matsui T, Kodera Y, Sakamoto J (2007) Efficacy of adjuvant immunochemotherapy with polysaccharide K for patients with curative resections of gastric cancer. Cancer Immunol Immunother 56:905–911

Obodai M, Narh Mensah DL, Fernandes Â, Kortei NK, Dzomeku M, Teegarden M et al (2017) Chemical characterization and antioxidant potential of wild *Ganoderma* species from Ghana. Molecules 22:196

Oh JY, Choi W-S, Lee CH, Park H-J (2011) The ethyl acetate extract of Cordyceps militaris inhibits IgE-mediated allergic responses in mast cells and passive cutaneous anaphylaxis reaction in mice. J Ethnopharmacol 135:422–429

Okamoto T (2007) Agaricus blazei to improve side effects caused by cancer drugs. Curr Topics Pharmacol 11:27–30

Oli AN, Edeh PA, Al-Mosawi RM, Mbachu NA, Al-Dahmoshi HO, Al-Khafaji NS et al (2020) Evaluation of the phytoconstituents of *Auricularia auricula-judae* mushroom and antimicrobial activity of its protein extract. Eur J Integr Med 38:101176

Ooi VEC, Liu F (1999) A review of pharmacological activities of mushroom polysaccharides. Int J Med Mushrooms 1:195–206

Painuli S, Semwal P, Egbuna C (2020) Mushroom: nutraceutical, mineral, proximate constituents and bioactive component. In: Egbuna C, Dable Tupas G (eds) Functional foods and nutraceuticals: bioactive components. Formulations and Innovations. Springer International Publishing, Cham, pp 307–336. https://doi.org/10.1007/978-3-030-42319-3_17

Palazzolo E, Letizia Gargano M, Venturella G (2012) The nutritional composition of selected wild edible mushrooms from Sicily (southern Italy). Int J Food Sci Nutr 63:79–83

Pandey AT, Pandey I, Hachenberger Y, Krause B-C, Haidar R, Laux P et al (2020) Emerging paradigm against global antimicrobial resistance via bioprospecting of fungi into novel nanotherapeutics development. Trends Food Sci Technol

Park SE, Yoo HS, Jin C-Y, Hong SH, Lee Y-W, Kim BW et al (2009) Induction of apoptosis and inhibition of telomerase activity in human lung carcinoma cells by the water extract of Cordyceps militaris. Food Chem Toxicol 47:1667–1675

Patel Y, Naraian R, Singh V (2012) Medicinal properties of Pleurotus species (oyster mushroom): a review. World J Fungal Plant Biol 3:1–12

Paterson RRM (2006) Ganoderma–a therapeutic fungal biofactory. Phytochemistry 67:1985–2001

Pérez-Martínez AS, Acevedo-Padilla SA, Bibbins-Martínez M, Galván-Alonso J, Rosales-Mendoza S (2015) A perspective on the use of *Pleurotus* for the development of convenient fungi-made oral subunit vaccines. Vaccine 33:25–33

Puri M, Kaur I, Perugini MA, Gupta RC (2012) Ribosome-inactivating proteins: current status and biomedical applications. Drug Discov Today 17:774–783

Qin P, Li X, Yang H, Wang Z-Y, Lu D (2019) Therapeutic potential and biological applications of cordycepin and metabolic mechanisms in cordycepin-producing fungi. Molecules 24:2231

Que Y, Sun S, Xu L, Zhang Y, Zhu H (2014) High-level coproduction, purification and characterisation of laccase and exopolysaccharides by *Coriolus versicolor*. Food Chem 159:208–213

Rahman MA, Abdullah N, Aminudin N (2015) Antioxidative effects and inhibition of human low density lipoprotein oxidation in vitro of polyphenolic compounds in Flammulina velutipes (Golden needle mushroom). Oxidative Med Cell Longev 2015:1–10

Rahman MA, Abdullah N, Aminudin N (2018) Lentinula edodes (shiitake mushroom): an assessment of in vitro anti-atherosclerotic bio-functionality. Saudi J Biol Sci 25:1515–1523

Rajeshwari S, Kriushnapriya S Anticancer activities of phytochemicals from a wood rot Mushroom Phellinus adamantinus-in silico and in vitro approach. In: International conference on bioscience, biochemistry and bioinformatics, 2011. IPCBEE Singapore, pp 198–202

Rashid S, Unyayar A, Mazmanci M, McKeown S, Banat I, Worthington J (2011) A study of anticancer effects of Funalia trogii in vitro and in vivo. Food Chem Toxicol 49:1477–1483

Reis FS, Barros L, Martins A, Ferreira IC (2012) Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: an inter-species comparative study. Food Chem Toxicol 50:191–197

Ren H, Wang H, Yuan F, Zhang J (2002) Clinical observation of Maitake capsules in the treatment of IGT. In: The Third International Conference of Diabetes., Beijing China

Reyes RG, Lopez L, Kumakura K, Kalaw SP, Kikukawa T, Eguchi F (2009) Coprinus comatus, a newly domesticated wild nutriceutical mushroom in the Philippines. J Agric Technol 5:299–316

Reza MA, Hossain MA, Damte D, Jo W-S, Hsu WH, Park S-C (2015) Hypolipidemic and hepatic steatosis preventing activities of the wood ear medicinal mushroom *Auricularia auricula-judae* (higher basidiomycetes) ethanol extract in vivo and in vitro. Int J Med Mushrooms 17:723–734

Roupas P, Keogh J, Noakes M, Margetts C, Taylor P (2012) The role of edible mushrooms in health: evaluation of the evidence. J Funct Foods 4:687–709

Roy D, Ansari S, Chatterjee A, Luganini A, Ghosh S, Chakraborty N (2020) In vitro search for antiviral activity against human cytomegalovirus from medicinal mushrooms Pleurotus sp. and Lentinus sp. J Antivir Antiretrovir 12:201

Royse DJ, Baars J, Tan Q (2017) Current overview of mushroom production in the world. Edible Med Mushrooms Technol Appl:5–13

RuiDian K, ShunFa L, Yi C, ChuRong J, QiaGuang S (2010) Analysis of chemical composition of polysaccharides from Poria cocos wolf and its anti-tumor activity by NMR spectroscopy. Carbohydr Polym 80:31–34

Sadahiro S, Suzuki T, Maeda Y, Tanaka A, Kamijo A, Murayama C et al (2010) Effects of preoperative immunochemoradiotherapy and chemoradiotherapy on immune responses in patients with rectal adenocarcinoma. Anticancer Res 30:993–999

Sánchez C (2017) Bioactives from mushroom and their application. In: Puri M (ed) Food bioactives: extraction and biotechnology applications. Springer International Publishing, Cham, pp 23–57. https://doi.org/10.1007/978-3-319-51639-4_2

Santos AF, Da Silva M, Napoleão T, Paiva P, Correia MS, Coelho L (2014) Lectins: function, structure, biological properties and potential applications. Curr Topics Peptide Protein Res 15:1–22

Sarma D, Saha AK, Datta BK (2018) Bioactive compounds with special references to anticancer property of oyster mushroom *Pleurotus ostreatus*. J Pharmacogn Phytochem 7:2694–2698

Savoie J-M, Foulongne-Oriol M, Barroso G, Callac P (2013) 1 genetics and genomics of cultivated mushrooms, application to breeding of agarics. In: Kempken F (ed) Agricultural Applications. Springer, Berlin Heidelberg, Berlin, Heidelberg, pp 3–33. https://doi.org/10.1007/978-3-642-36821-9_1

Sekara A, Kalisz A, Grabowska A, Siwulski M (2015) Auricularia spp.-mushrooms as novel food and therapeutic agents-a review. Sydowia 67:1–10

Selegean M, Putz MV, Rugea T (2009) Effect of the polysaccharide extract from the edible mushroom *Pleurotus ostreatus* against infectious bursal disease virus. Int J Mol Sci 10:3616–3634

Shi Y-l, Benzie IF, Buswell JA (2002) Role of tyrosinase in the genoprotective effect of the edible mushroom, *Agaricus bisporus*. Life Sci 70:1595–1608

Shi Z-T, Bao H-Y, Feng S (2017) Antitumor activity and structure-activity relationship of seven lanostane-type triterpenes from *Fomitopsis pinicola* and *F. officinalis*. China J Chin Materia Medica 42:915–922

Shin CK, Yee CF, Shya LJ, Atong M (2007) Nutritional properties of some edible wild mushrooms in Sabah. J Appl Sci 7:2216–2221

Shukla S, Bajpai VK, Kim M (2014) Plants as potential sources of natural immunomodulators. Rev Environ Sci Biotechnol 13:17–33

Sifat N, Lovely F, Zihad SNK, Hossain MG, Shilpi JA, Grice ID et al (2020) Investigation of the nutritional value and antioxidant activities of common Bangladeshi edible mushrooms. Clin Phytosci 6:1–10

Singh J, Yadav AN (2020) Natural bioactive products in sustainable agriculture. Springer, Singapore

Singh RS, Bhari R, Kaur HP (2010) Mushroom lectins: current status and future perspectives. Crit Rev Biotechnol 30:99–126

Sinha SK, Upadhyay TK, Sharma SK (2020) Investigations on immunomodulatory effect of white button mushroom medicinal properties against COVID-19. SGVU Int J Environ Sci Technol 6:32–51

Siwulski M, Sobieralski K, Golak-Siwulska I, Sokół S, Sękara A (2015) Ganoderma lucidum (Curt.: Fr.) karst.–health-promoting properties. A review. Herba Polonica 61:105–118

Smania EFA, Delle Monache F, Yunes RA, Paulert R, Smania Junior A (2007) Antimicrobial activity of methyl australate from Ganoderma australe. Rev Bras 17:14–16

Smiderle F, Olsen L, Ruthes A, Czelusniak P, Santana-Filho A, Sassaki G et al (2012) Exopolysaccharides, proteins and lipids in Pleurotus pulmonarius submerged culture using different carbon sources. Carbohydr Polym 87:368–376

Soares R, Meireles M, Rocha A, Pirraco A, Obiol D, Alonso E et al (2011) Maitake (D fraction) mushroom extract induces apoptosis in breast cancer cells by BAK-1 gene activation. J Med Food 14:563–572

Sonawane H, Bhosle S, Bapat G, Vikram G (2014) Pharmaceutical metabolites with potent bioactivity from mushrooms. J Pharm Res 8:969–972

Sudheep NM, Sridhar KR (2014) Nutritional composition of two wild mushrooms consumed by the tribals of the Western Ghats of India. Mycology 5:64–72

Sumy AK, Jahan N, Sultana N (2010) Study on the hepatoprotective effect of oyster mushroom (*Pleurotus Florida*) against paracetamol induced liver damage in Wistar albino rats. J Bangladesh Soc Physiologist 5:46–52

Sun Y-X, Liu J-C, Kennedy JF (2010) Purification, composition analysis and antioxidant activity of different polysaccharide conjugates (APPs) from the fruiting bodies of Auricularia polytricha. Carbohydr Polym 82:299–304

Tai T, Akita Y, Kinoshita K, Koyama K, Takahashi K, Watanabe K (1995) Anti-emetic principles of Poria cocos. Planta Med 61:527–530

Takama F, Minomiya S, Yoda R, Ishii H, Muraki S Parenchyma cells, chemical components of maitake mushroom (*Grifola frondosa* SF Gray) cultured artificially, and their changes by storage and boiling. In: Proceedings of the Eleventh International Scientific Congress on the Cultivation of Edible Fungi, Australia, 1981/edited by NG Nair, AD Clift, 1981. Sydney:[sn], 1981.,

Tan W-C, Kuppusamy UR, Phan C-W, Tan Y-S, Raman J, Anuar AM et al (2015) Ganoderma neo-japonicum Imazeki revisited: domestication study and antioxidant properties of its basidiocarps and mycelia. Sci Rep 5:1–10

Tang C, Hoo PC-X, Tan LT-H, Pusparajah P, Khan TM, Lee L-H et al (2016) Golden needle mushroom: a culinary medicine with evidenced-based biological activities and health promoting properties. Front Pharmacol 7:474

Thatoi H, Singdevsachan SK (2014) Diversity, nutritional composition and medicinal potential of Indian mushrooms: a review. Afr J Biotechnol 13

Thawthong A, Karunarathna SC, Thongklang N, Chukeatirote E, Kakumyan P, Chamyuang S et al (2014) Discovering and domesticating wild tropical cultivatable mushrooms. Chiang Mai J Sci 41:731–764

Thongklang N, Keokanngeun L, Taliam W, Hyde K (2020) Cultivation of a wild strain of *Auricularia Cornea* from Thailand. Curr Res Environ Appl Mycol 10:120–130

Tsujiyama S, Ueno H (2013) Performance of wood-rotting fungi-based enzymes on enzymic saccharification of rice straw. J Sci Food Agric 93:2841–2848

Tuli HS, Sharma AK, Sandhu SS, Kashyap D (2013) Cordycepin: a bioactive metabolite with therapeutic potential. Life Sci 93:863–869

Valdez-Morales M, Barry K, Fahey GC Jr, Domínguez J, de Mejia EG, Valverde ME et al (2010) Effect of maize genotype, developmental stage, and cooking process on the nutraceutical potential of huitlacoche (*Ustilago maydis*). Food Chem 119:689–697

Valverde M, Paredes-López O (1993) Production and evaluation of some food properties of huitlacoche (Ustilago maydis). Food Biotechnol 7:207–219

Valverde ME, Hernández-Pérez T, Paredes-Lopez O (2012) Huitlacoche–a 21st century culinary delight originated in the Aztec times. In: Hispanic foods: chemistry and bioactive compounds. ACS Publications, pp 83–100

Valverde ME, Hernández-Pérez T, Paredes-López O (2015) Edible mushrooms: improving human health and promoting quality life. Int J Microbiol 2015

Vanegas PE, Valverde ME, Paredes-Lopez O, Pataky JK (1995) Production of the edible fungus huitlacoche (*Ustilago maydis*): effect of maize genotype on chemical composition. J Ferment Bioeng 80:104–106

Varghese R, Dalvi YB, Lamrood PY, Shinde BP, Nair C (2019) Historical and current perspectives on therapeutic potential of higher basidiomycetes: an overview. 3 Biotech 9:1–15

Vaz JA, Ferreira IC, Tavares C, Almeida GM, Martins A, Vasconcelos MH (2012) Suillus collinitus methanolic extract increases p53 expression and causes cell cycle arrest and apoptosis in a breast cancer cell line. Food Chem 135:596–602

Venturella G, Ferraro V, Cirlincione F, Gargano ML (2021) Medicinal mushrooms: bioactive compounds, use, and clinical trials. Int J Mol Sci 22:1–30

Wang H, Ng T (2000a) Flammulin: a novel ribosome-inactivating protein from fruiting bodies of the winter mushroom *Flammulina velutipes*. Biochem Cell Biol 78:699–702

Wang H, Ng T (2000b) Isolation of a novel ubiquitin-like protein from Pleurotus ostreatus mushroom with anti-human immunodeficiency virus, translation-inhibitory, and ribonuclease activities. Biochem Biophys Res Commun 276:587–593

Wang H, Ng T (2004) Isolation of a new ribonuclease from fruiting bodies of the silver plate mushroom Clitocybe maxima. Peptides 25:935–939

Wang H, Ng T (2006a) A laccase from the medicinal mushroom Ganoderma lucidum. Appl Microbiol Biotechnol 72:508–513

Wang H, Ng T (2006b) A ribonuclease from the wild mushroom boletus griseus. Appl Microbiol Biotechnol 72:912–916

Wang H, Ng TB (1999) Isolation of a new ribonuclease from fresh fruiting bodies of the straw mushroom. Biochem Biophys Res Commun 264:714–718

Wang H, Ng TB (2001) Isolation and characterization of velutin, a novel low-molecular-weight ribosome-inactivating protein from winter mushroom (*Flammulina velutipes*) fruiting bodies. Life Sci 68:2151–2158

Wang X, Wang B, Zhou L, Wang X, Veeraraghavan VP, Mohan SK et al (2020) Ganoderma lucidum put forth anti-tumor activity against PC-3 prostate cancer cells via inhibition of Jak-1/STAT-3 activity. Saudi J Biol Sci 27:2632–2637

Wang X-C, Xi R-J, Li Y, Wang D-M, Yao Y-J (2012) The species identity of the widely cultivated Ganoderma,'G. lucidum'(Ling-zhi), in China. PLoS One 7:e40857

Wang Y-Z, Zhang J, Zhao Y-L, Li T, Shen T, Li J-Q et al (2013) Mycology, cultivation, traditional uses, phytochemistry and pharmacology of *Wolfiporia Cocos* (Schwein.) Ryvarden et Gilb.: a review. J Ethnopharmacol 147:265–276

Wani BA, Bodha R, Wani A (2010) Nutritional and medicinal importance of mushrooms. J Med Plants Res 4:2598–2604

Wasser SP (2005) Reishi or ling zhi (Ganoderma lucidum). Encycl Dietary Suppl 1:603–622

Wasser SP (2017) Medicinal mushrooms in human clinical studies. Part I. anticancer, oncoimmunological, and immunomodulatory activities: a review. Int J Med Mushrooms 19

Wisitrassameewong K, Karunarathna SC, Thongklang N, Zhao R, Callac P, Moukha S et al (2012) Agaricus subrufescens: a review. Saudi J Biol Sci 19:131–146

Wong JH, Ng TB, Wang H, Sze SCW, Zhang KY, Li Q et al (2011) Cordymin, an antifungal peptide from the medicinal fungus *Cordyceps militaris*. Phytomedicine 18:387–392

Wu F, Yuan Y, Malysheva VF, Du P, Dai Y-C (2014) Species clarification of the most important and cultivated Auricularia mushroom "Heimuer": evidence from morphological and molecular data. Phytotaxa 186:241–253

Wu J-Y, Chen C-H, Chang W-H, Chung K-T, Liu Y-W, Lu F-J et al (2011) Anti-cancer effects of protein extracts from *Calvatia lilacina, Pleurotus ostreatus* and *Volvariella volvacea*. Evid Based Complement Alternat Med 2011:1–10

Wu J-Y, Siu K-C, Geng P (2021) Bioactive ingredients and medicinal values of *Grifola frondosa* (Maitake). Foods 10:1–28

Xia B, Zhou Y, Tan HS, Ding LS, Xu HX (2014) Advanced ultra-performance liquid chromatography–photodiode array–quadrupole time-of-flight mass spectrometric methods for simultaneous screening and quantification of triterpenoids in *Poria cocos*. Food Chem 152:237–244

Xiao-Lei M, Meng M, Li-Rong H, Zheng L, Xiao-Hong C, Chun-Ling W (2015) Immunomodulatory activity of macromolecular polysaccharide isolated from Grifola frondosa. Chin J Nat Med 13:906–914

Xie J, Zhao J, Hu D-J, Duan J-A, Tang Y-P, Li S-P (2012) Comparison of polysaccharides from two species of *Ganoderma*. Molecules 17:740–752

Yadav AN (2020) Recent trends in mycological research, volume 1: agricultural and medical perspective. Springer, Switzerland

Yadav AN, Mishra S, Singh S, Gupta A (2019a) Recent advancement in white biotechnology through fungi. Volume 1: diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 2: perspective for value-added products and environments. Springer International Publishing, Cham

Yamaguchi N, Yoshida J, Ren L, Chen H, Miyazawa Y, Fujii Y et al (1990) Augmentation of various immune reactivities of tumor-bearing hosts with an extract of *Cordyceps sinensis*. Biotherapy 2:199–205

Yang W, Pei F, Shi Y, Zhao L, Fang Y, Hu Q (2012) Purification, characterization and antiproliferation activity of polysaccharides from *Flammulina velutipes*. Carbohydr Polym 88:474–480

Yang W, Yu J, Zhao L, Ma N, Fang Y, Pei F et al (2015) Polysaccharides from *Flammulina velutipes* improve scopolamine-induced impairment of learning and memory of rats. J Funct Foods 18:411–422

Yang Y, Zhang L, Chen Q, Lu W-L, Li N (2020) Antitumor effects of extract of the oak bracket medicinal mushroom, *Phellinus baumii* (Agaricomycetes), on human melanoma cells A375 in vitro and in vivo. Int J Med Mushrooms 22

Yao F-J, Lu L-X, Wang P, Fang M, Zhang Y-M, Chen Y et al (2018) Development of a molecular marker for fruiting body pattern in *Auricularia auricula-judae*. Mycobiology 46:72–78

Ye X, Ng T (2002) A novel and potent ribonuclease from fruiting bodies of the mushroom Pleurotus pulmonarius. Biochem Biophys Res Commun 293:857–861

Yi C, Fu M, Cao X, Tong S, Zheng Q, Firempong CK et al (2013) Enhanced oral bioavailability and tissue distribution of a new potential anticancer agent, Flammulina velutipes sterols, through liposomal encapsulation. J Agric Food Chem 61:5961–5971

Yilmaz N, Solmaz M, Türkekul İ, Elmastaş M (2006) Fatty acid composition in some wild edible mushrooms growing in the middle Black Sea region of Turkey. Food Chem 99:168–174

Ying Z, Yan Z, Yan M, Jiangang Z, Hanmin L, Yuanxia S (2018) Structural characterization and immunomodulatory activity of exopolysaccharides from submerged culture of Auricularia auricula-judae. International Journal of Biological Macromolecules 115:978–984

Yu J, Sun R, Zhao Z, Wang Y (2014) Auricularia polytricha polysaccharides induce cell cycle arrest and apoptosis in human lung cancer A549 cells. Int J Biol Macromol 68:67–71

Yu L, Fernig DG, Smith JA, Milton JD, Rhodes JM (1993) Reversible inhibition of proliferation of epithelial cell lines by *Agaricus bisporus* (edible mushroom) lectin. Cancer Res 53:4627–4632

Yu Z-T, Liu B, Mukherjee P, Newburg DS (2013) Trametes versicolor extract modifies human fecal microbiota composition in vitro. Plant Foods Hum Nutr 68:107–112

Yue K, Ye M, Zhou Z, Sun W, Lin X (2013) The genus Cordyceps: a chemical and pharmacological review. J Pharm Pharmacol 65:474–493

Yuen JW, Gohel MDI (2005) Anticancer effects of *Ganoderma lucidum*: a review of scientific evidence. Nutr Cancer 53:11–17

Zeb M, Lee CH (2021) Medicinal properties and bioactive compounds from wild mushrooms native to North America. Molecules 26:251

Zeng P, Guo Z, Zeng X, Hao C, Zhang Y, Zhang M et al (2018) Chemical, biochemical, preclinical and clinical studies of *Ganoderma lucidum* polysaccharide as an approved drug for treating myopathy and other diseases in China. J Cell Mol Med 22:3278–3297

Zeng Y, Lian S, Li D, Lin X, Chen B, Wei H et al (2017) Anti-hepatocarcinoma effect of cordycepin against NDEA-induced hepatocellular carcinomas via the PI3K/Akt/mTOR and Nrf2/HO-1/NF-κB pathway in mice. Biomed Pharmacother 95:1868–1875

Zhang L, Lu Y, Feng X, Liu Q, Li Y, Hao J et al (2020) Hepatoprotective effects of Pleurotus ostreatus protein hydrolysates yielded by pepsin hydrolysis. Catalysts 10:595

Zhang Q-H, Lin Z-B (1999) The antitumor activity of Ganoderma lucidum (Curt.: Fr.) P. karst. (Ling Zhi)(Aphyllophoromycetideae) polysaccharides is related to tumor necrosis factor-α and interferon-γ. Int J Med Mushrooms 1

Zhang X, Wang L, Ma F, Yang J, Su M (2017) Effects of arbuscular mycorrhizal fungi inoculation on carbon and nitrogen distribution and grain yield and nutritional quality in rice (*Oryza sativa* L.). J Sci Food Agric 97:2919–2925

Zhang Y, Geng W, Shen Y, Wang Y, Dai Y-C (2014) Edible mushroom cultivation for food security and rural development in China: bio-innovation, technological dissemination and marketing. Sustainability 6:2961–2973

Zhang Y, Jiang Y, Zhang M, Zhang L (2019) Ganoderma sinense polysaccharide: an adjunctive drug used for cancer treatment. Prog Mol Biol Transl Sci 163:165–177

Zhao F, Wang Y-F, Song L, Jin J-X, Zhang Y-Q, Gan H-Y et al (2017) Synergistic apoptotic effect of d-fraction from *Grifola frondosa* and vitamin C on hepatocellular carcinoma SMMC-7721 cells. Integr Cancer Ther 16:205–214

Zhao R, Yang W, Pei F, Zhao L, Hu Q (2018) In vitro fermentation of six kinds of edible mushrooms and its effects on fecal microbiota composition. Lwt 96:627–635

Zhao S, Gao Q, Rong C, Wang S, Zhao Z, Liu Y et al (2020) Immunomodulatory effects of edible and medicinal mushrooms and their bioactive Immunoregulatory products. J Fungi 6:269

Zhao S, Rong C, Liu Y, Xu F, Wang S, Duan C et al (2015) Extraction of a soluble polysaccharide from Auricularia polytricha and evaluation of its anti-hypercholesterolemic effect in rats. Carbohydr Polym 122:39–45

Zhou L, Zhang Y, Gapter LA, Ling H, Agarwal R, K-y N (2008) Cytotoxic and anti-oxidant activities of lanostane-type triterpenes isolated from *Poria cocos*. Chem Pharm Bull 56:1459–1462

Zhou Z, Han Z, Zeng Y, Zhang M, Cui Y, Xu L et al (2014) Chinese FDA approved fungal glycan-based drugs: an overview of structures, mechanisms and clinical related studies. J Transl Med 4:1–11

Zhu L, Xu J, Zhang S, Wang R, Huang Q, Chen H et al (2018) Qualitatively and quantitatively comparing secondary metabolites in three medicinal parts derived from Poria cocos (Schw.) wolf using UHPLC-QTOF-MS/MS-based chemical profiling. J Pharm Biomed Anal 150:278–286

Zhu M-J, Du F, Zhang G-Q, Wang H-X, Ng T-B (2013) Purification a laccase exhibiting dye decolorizing ability from an edible mushroom *Russula virescens*. Int Biodeterior Biodegrad 82:33–39

Chapter 21
Bioactive Attributes of *Xylaria* Species from the Scrub Jungles of Southwest India

Bijavara Ramakrishnappa Jagadish, Kandikere Ramaiah Sridhar, Hosamane Ramesh Dattaraj, Nagabhushana Chandramohana, and Shivannegowda Mahadevakumar

Contents

B. R. Jagadish · H. R. Dattaraj
Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

K. R. Sridhar (✉)
Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India

Centre for Environmental Studies, Yenepoya (Deemed to be University), Mangalore, Karnataka, India

N. Chandramohana
Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore, Karnataka, India

S. Mahadevakumar
Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, Karnataka, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_21

21.1 Introduction

Xylaria Hill ex Schrank (Class, *Sordariomycetes*; Subclass, *Xylariomycetidae*; Order, *Xylariales*) is the largest ascomycetous genus in the family *Xylariaceae*. Being cosmopolitan genus distributed worldwide (Li et al. 2017). Kirk et al. (2008) have predicted the global existence up to 300 species of *Xylaria*. The Index Fungorum documented up to 817records of *Xylaria* (accessed on May 17, 2020). The main distinguishing features of *Xylaria* include characteristic carbonaceous erect cylindrical-clavate-globoid-irregular multi-perithecial stromata (Roger 1979; Trierveiler-Pereira et al. 2009). Other specific morphological traits are stromal features (shape, size and pigmentation), nature of ascus (apical apparatus) and ascospore characters (shape, size and surface ornamentation) (Whalley 1996; Stadler 2011; Maharachchikumbura et al. 2016).

A wide difference has been seen in the morphology and size of stromata of *Xylaria*. For instance, tiny hair-like stromata (~0.5 mm diameter in *X. filiformis*) to bulb-like stromata (~55 mm diameter in *X. acuminatilongissima*) (Rogers and Samuels 1986; Latha et al. 2015). Besides, they preferentially grow on different substrates like dead tree trunk, stubs, woody litter, bark, leaf litter, soil, termite mound, fruits, and seeds (Roger et al. 2008; Karun and Sridhar 2015; Ju et al. 2018). In addition to dead plant substrates, *Xylaria* is also known to perpetuate as endophytes in a wide range of live tissues (e.g. trees, palms, orchids, aroids, bromeliads and liverworts) (Bayman et al. 1998; Davis et al. 2003; U'ren et al. 2009; Linnakoski et al. 2012; Rajulu et al. 2013; Jin et al. 2017). To overcome uncertainty among several closely resembling species of *Xylaria*, phylogenetic studies have been undertaken using different sequences (ITS, α-actin, β-tubulin and rpb2) and resolved that *Xylaria* is a paraphyletic genus (Lee et al. 2000; Hsieh et al. 2010).

Xylaria is well known for their potential to control pathogens (bacteria and fungi), production of enzymes, cytotoxic potential, biologically potent metabolites, and antioxidant activities (Table 21.1). Different species of *Xylaria* are useful for medicinal and industrial applications (Tudor et al. 2014; Divate et al. 2017). They are also the target species for ethnomedicinal applications by traditional healers as well as tribals (Latha et al. 2015). *Xylaria* is known for anticancer and cytotoxic potential (Schüffler et al. 2007; Ramesh et al. 2015; Ahmed and Jahan 2017). They produce many value-added natural products and bioactive metabolites (e.g. alkaloids, cytochalasins, xylaramide, xylarin and xyloketals) (Boonphong et al. 2001; Lin et al. 2001; Ma et al. 2013; Rajulu et al. 2013). *Xylaria* possesses broad spectrum of antibacterial and antifungal properties (Ramesh et al. 2012, 2014, 2015; Elias et al. 2018; Devi et al. 2020).

Xylaria hypoxylon and *X. polymorpha* capable to inhibit the bacterial plant pathogen *Xanthomonas campestris* in mango (Thirumalesh et al. 2014). They also produce antifungal compound called piliformic acid to control the fungal pathogen *Colletotrichum gloeosporioides* (Elias et al. 2018). *Xylaria hypoxylon* and *X. polymorpha* have been assessed for their proximal and bioactive components by Adeduntan (2014) (Table 21.2). They possess highest quantity of crude fibre

Table 21.1 Bioactive metabolites of four *Xylaria* species

	Compound class	Metabolite	Activity	Reference
Xylaria escharoidea	Antimicrobial compound	4,8-dihydroxy-3,4-dihydronaphthalen-1(2H)-one	Antibacterial and anti-*Candida*	Nagam et al. (2020)
Xylaria hypoxylon	Cytochalasins	19,20-Epoxycytochalasin Q	Prevent microfilament polymerization, inhibit cell motility, inhibit phagocytosis and depolymerize actin filaments	Espada et al. (1997)
		Deacetyl-19,20-epoxycytochalasin Q		
		18-Deoxy-19,20-epoxycytochalasin Q		
		19,20-Epoxycytochalasin C		
		19,20-Epoxycytochalasin R		
		18-Deoxy-19,20-epoxycytochalasin R		
		Cytochalasin R		
		19,20-Epoxycytochalasin D		
		19,20-Epoxycytochalasin N		
		Cytochalasin Q		
	Polyketide	2-hexylidene-3-methylsuccinicacid (piliformic acid)	Antibacterial and antifungal	Chesters and O'Hagan (1997)
	Pyranone derivatives	Xylarone	Cytotoxic	Schüffler et al. (2007)
		8,9-Dehydroxylarone		
	Naphthalene derivatives	Xylariol A	Cytotoxic	Gu and Ding (2008)
		Xylariol B		
Xylaria longipes	N-containing compound	Xylaramide	Antifungal	Schneider et al. (1996)
	Phenolic derivative	Tyrosol	Antioxidant	
Xylaria polymorpha	Benzofuran derivative	Xylaral	Antifungal	Gunawan et al. (1990)
	Polyketide	2-hexylidene-3-methylsuccinicacid (piliformic acid)	Antibacterial and antifungal	Chesters and O'Hagan (1997)
	Polyketides	Xylarinic acid A	Antifungal	Jang et al. (2007)
		Xylarinic acid B		
	Diterpenoids	15-Hydroxy-16-(a-d-mannopyranosyloxy) isopimar-7-en-19-oic-acid	Antibacterial and antioxidants	Shiono et al. (2009)
		16-(a-d-Mannopyranosyloxy) isopimar-7-en-19-oic acid		Shiono et al. (2009); Yan et al. (2011)
		16-(a-d-Glucopyranosyloxy) isopimar-7-en-19-oic acid		
	Phenolic derivative	Xylarinol B	Photocatalytic	Lee et al. (2009)

Table 21.2 Nutritional composition and bioactive components of two *Xylaria* species (Adeduntan 2014)

Nutritional composition (%)		
	Xylaria hypoxylon	*Xylaria polymorpha*
Crude protein	6.44	7.77
Crude fibre	36.81	29.16
Total lipids	2.46	1.87
Ash	3.26	1.52
Bioactive components (mg/g dry mass)		
Tannins	0.04	0.07
Flavonoids	1.69	1.18
Phytate	4.12	9.88
Oxalate	0.32	0.63

followed by crude protein, lipids, and ash. Among the four bioactive compounds, phytate was the highest followed by flavonoids (Singh and Yadav 2020).

Xylaria produces several multifunctional enzymes (e.g. cellulases, laccases, lignocellulases and xylanases) and pigments (e.g. melanin, phlegmacin and wood colouring agents) of biological, industrial and bioremediation significance (Wang et al. 2005; Robinson et al. 2011; Nghi et al. 2012; Chaurasia et al. 2014; Tudor et al. 2014; Abdel-Azeem et al. 2021). Enzymes like polyphenol oxidase (laccase) from *X. polymorpha* have been purified by Chaurasia et al. (2014) at ambient room temperature without stringent reaction conditions as well as expensive reagents. *Xylaria polymorpha* was cultivated by solid substrate fermentation using wheat straw to produce multifunctional enzyme called GH78 protein, which has the capacity to efficiently degrade lignocelluloses (Nghi et al. 2012).Antioxidant potential of some *Xylaria* has also been investigated recently (Yue et al. 2013; Fernando et al. 2016; Rebbapragada and Kalyanaraman 2016; Zhou et al. 2018).

Xylaria is the one widely distributed genus throughout the Indian subcontinent (Debnath et al. 2018). Deccan Plateau of the Indian subcontinent possesses nearly 60 species of *Xylaria*, which accounts up to 10% of known species worldwide (Kshirsagar et al. 2009; Hsieh et al. 2010; Mohanan 2011; Patil et al. 2012; Ramesh et al. 2012; Karun and Sridhar 2015; Latha et al. 2015; Patel and Krishnappa 2017; Dattaraj et al. 2020; www.fungifromindia.com). *Xylaria hypoxylon* and *X. longipes* are widespread in different regions of the Western Ghats and the west coast of India. *Xylaria hypoxylon* has been reported to occur on a wide range of substrates like decaying wood, logs, twigs, bark, leaf litter, pods and seeds. *Xylaria escharoidea* grows mainly in association with termite mounds, while *X. longipes* also known to be associated with termite mounds and capable to establish on mainly on woody substrates (rotting stubs, logs and twigs). *Xylaria polymorpha* prefers to grow mainly on decaying wood, logs and bark.

Spectroscopic techniques have been employed to understand the versatility of metabolites and molecular structures of various biological samples as future diagnostic tools (Sahu and Mordechai 2016). In addition, Fourier transformed infrared (FTIR) spectroscopy is another effective tool to understand the overall chemical composition of biological samples in the mid-infrared wavelengths (4000–400 cm^{-1}). The metabolic fingerprint created by FTIR represents authentic quality of biological samples (Cheng et al. 2010; Vodnar et al. 2010; Erwanto et al. 2016). The current chapter aims to review the metabolites, proximal properties, bioactive components, elemental composition by EDS spectra, functional groups by FTIR and antioxidant potential of four dominant *Xylaria* spp. (*Xylaria escharoidea, X. hypoxylon, X. longipes* and *X. polymorpha*) occurring in different substrates and habitats in the scrub jungles of southwest India.

21.2 Sporocarps of *Xylaria* and Processing

During macrofungal diversity surveys in the scrub jungles of the southwest of Karnataka (12°48′N, 74°55′E; 115 masl), several species of *Xylaria* inhabiting different habitats were recorded. Mature whole sporocarps of commonly occurring *Xylaria* species inhabiting in termite mound (*X. escharoidea*), woody litter (*X. hypoxylon*), wood stub (*X. longipes*) and dead bark (*X. polymorpha*) were collected from three locations (as replicates), brought to the laboratory and cleaned to eliminate debris using a soft brush. Each replicate sample was oven-dried (55 ± 3 °C) until attaining constant weight, powdered and preserved in glass vials for analysis.

21.3 FTIR and EDS Analysis

The Fourier transformed infrared spectroscopy (FTIR) analysis was performed to follow the functional groups present the sporocarps of *Xylaria* spp. using Perkin Elmer Spectrum 1000 (Waltham, MA 02451 USA) at the wavelength range 4500–500 cm^{-1} with a resolution 4 cm^{-1}.The energy dispersive spectroscopy (EDS) analysis (HITACHI Noran System 7, USA) was carried out to detect the elemental composition of sporocarps.

21.4 Antioxidant Assays

Methanol extract of *Xylaria* powder was prepared by extraction of 0.5 g powder in 30 ml methanol and shaken on a rotary shaker (150 rpm, 48 h). The mixture was

centrifuged to collect the supernatant in pre-weighed Petri plates followed by drying at laboratory temperature (25 ± 3 °C). The dry weight of the extract was determined gravimetrically and dissolved in methanol to obtain the required concentration (1 mg/ml) to test the antioxidant activity. Three assays of antioxidant activity evaluated were: (a) Mo(VI) to Mo(V) reduction by antioxidant compounds as total antioxidant activity (TAA); (b) Fe(III) ion to Fe(II) ion reduction as Fe^{2+} chelation capacity (FCC); (c) the DPPH radical-scavenging activity.

21.4.1 Total Antioxidant Activity

The total antioxidant activity (TAA) of sporocarps of *Xylaria* was assessed according to the method by Prieto et al. (1999). The sporocarp extracts of *Xylaria* in methanol (0.1 ml) were mixed with reagent mixture (Sulphuric acid, 0.6 M + sodium phosphate, 28 mM + ammonium molybdate, 4 mM) (1 ml). Incubated the mixture in a water bath (95 °C; 90 min) and cooled to room temperature to measure the absorbance (695 nm) against methanol as blank. The TAA was expressed as μM equivalent of ascorbic acid per gram dry mass of sporocarps (μM AAEs/g).

21.4.2 Ferrous Ion-Chelation Capacity

The ferrous ion-chelation capacity (FCC) of sporocarps was assessed by the method outlined by Hsu et al. (2003). The sporocarp extracts in methanol (1 ml) were treated with a mixture of ferrous chloride (2 mM) (0.1 ml) and ferrozine (5 mM) (0.2 ml) followed by making up the final volume to 5 ml with methanol. After incubation of mixture at room temperature (10 min), the absorbance was measured at 562 nm. The reagents devoid of the sample served as control.

$$\text{Ferrous ion} - \text{chelation capacity } \% = 1 - (As_{562}/Ac_{562}) \times 100 \tag{21.1}$$

(where, A_c, absorbance of control; A_s, absorbance of sample)

21.4.3 DPPH Radical-Scavenging Activity

Methanol extracts of sporocarps were assessed to follow the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging activity using the protocol by Singh et al. (2002). The sporocarp sample in different concentrations (0.2–1.0 ml; 0.2–1.0 mg) was made up to final volume 1 ml in methanol. The DPPH (0.01 mM) (4 ml) was added

followed by incubation at room temperature (20 min) and the absorbance was measured (517 nm). The reagents without the sample served as control.

$$\text{Free radical} - \text{scavenging activity}(\%) = [(Ac_{517} - As_{517}) / (Ac_{517})] \times 100 \quad (21.2)$$

(where, A_c, absorbance of control; A_s, absorbance of sample)

21.4.4 Data Analysis

The TAA, FCC, and DPPH radical-scavenging activities between four species of *Xylaria* were assessed by one-way ANOVA (SigmaPlot # 11; Systat Software Inc. USA).

21.5 FTIR Spectra

The FTIR spectra provided a broad metabolic fingerprint of *Xylaria* spp. The band between wavelengths 3500 and 3200 cm^{-1} mainly represents the O–H stretching caused by strong water absorption as seen in *X. escharoidea* and *X. longipes* (Hirri et al. 2016) (Figure 21.1a, b). The region of wavelength 3100–2800 cm^{-1} is mainly related to fatty acids and the absorption peak at 2921.6274 cm^{-1} in *X. longipes* expresses stretch of methylene group of lipid as seen in *Boletus* sp. (Zhao et al. 2015). The bands observed between wavelengths 1200–950 cm^{-1} correspond to polysaccharides in the form of chitin. As chitin is the main structural polysaccharide in mushrooms, the region at wavelength 1200–950 cm^{-1} mainly corresponds to the absorptions of carbohydrate (Qi et al. 2017). Similar strong peaks were seen among the four *Xylaria* between the wavelengths 1085 and 1030 cm^{-1} correspond to C–C stretching, which is attributed as structure of chitin.

The spectra of *X. escharoidea* reveal a broad peak at wavelength 3312.90 cm^{-1} indicating concentration-dependent intermolecular hydrogen bond and C–H deformation vibration at wavelength 1029.69 cm^{-1}, respectively (Figure 21.1a). The broad peak of *X. hypoxylon* with a medium intensity corresponding to 3327.19 cm^{-1} shows the presence of an amine group for NH_2 stretching vibration (Figure 21.2a). The absorption peaks at 1375, 1644.99 and 1039.36 cm^{-1} correspond to C–H deformation vibration (sym.), C=C stretching vibration and C–O stretching vibration, respectively. The spectra of *Xylaria longipes* revealed absence of amine group (NH) as well as concentration-dependent intramolecular hydrogen bonding (Figure 21.1b). Similarly, the spectra of *X. polymorpha* reveal peaks at 3302.94 cm^{-1} and 2169.91 cm^{-1} indicating NH_2 stretching vibration and azide N_3-groups, respectively suggesting the presence of amine group (Figure 21.2b). The absorption peak at 1628 and 1038.74 cm^{-1} corresponds to C=C stretching vibration and C–C skeleton vibration, respectively.

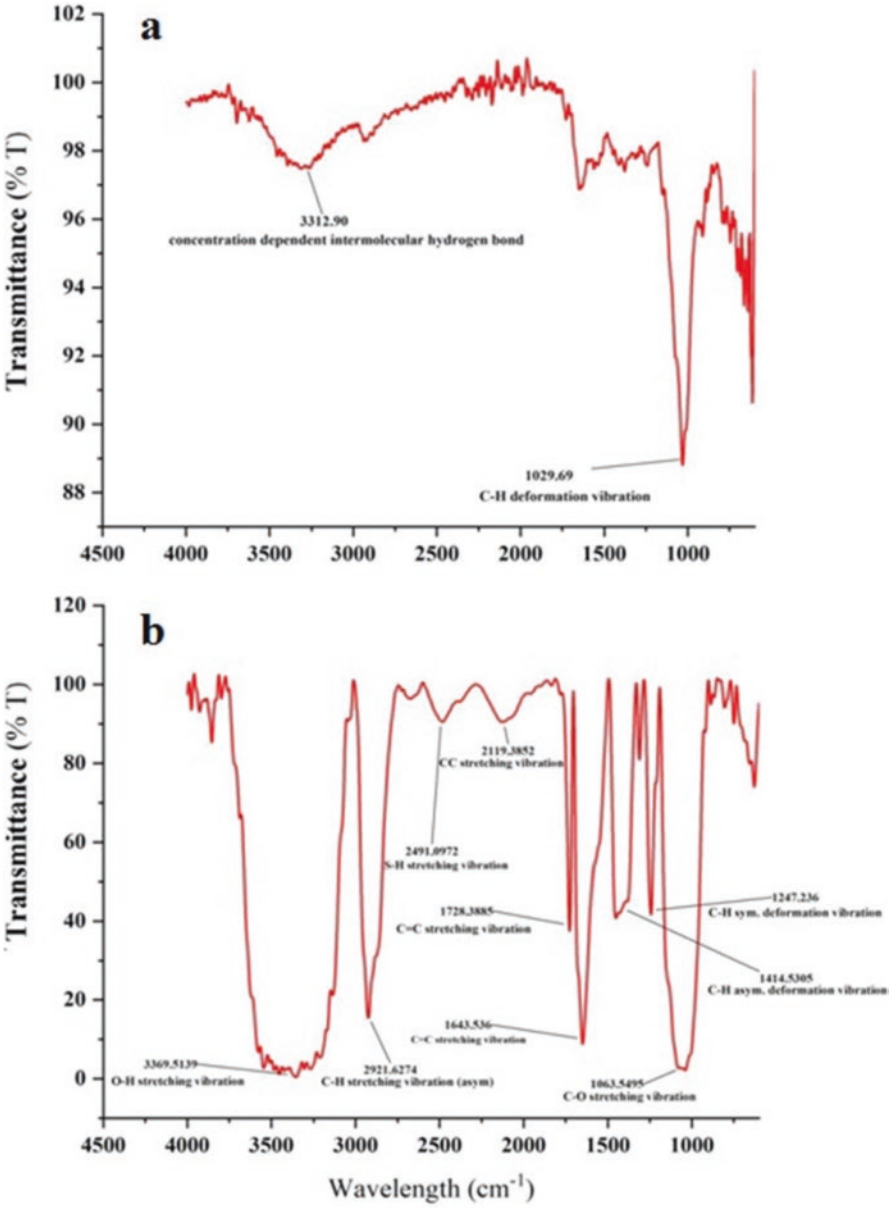


Fig. 21.1 The FTIR spectra showing various functional groups in *Xylaria escharoidea* (**a**) and *X. longipes* (**b**)

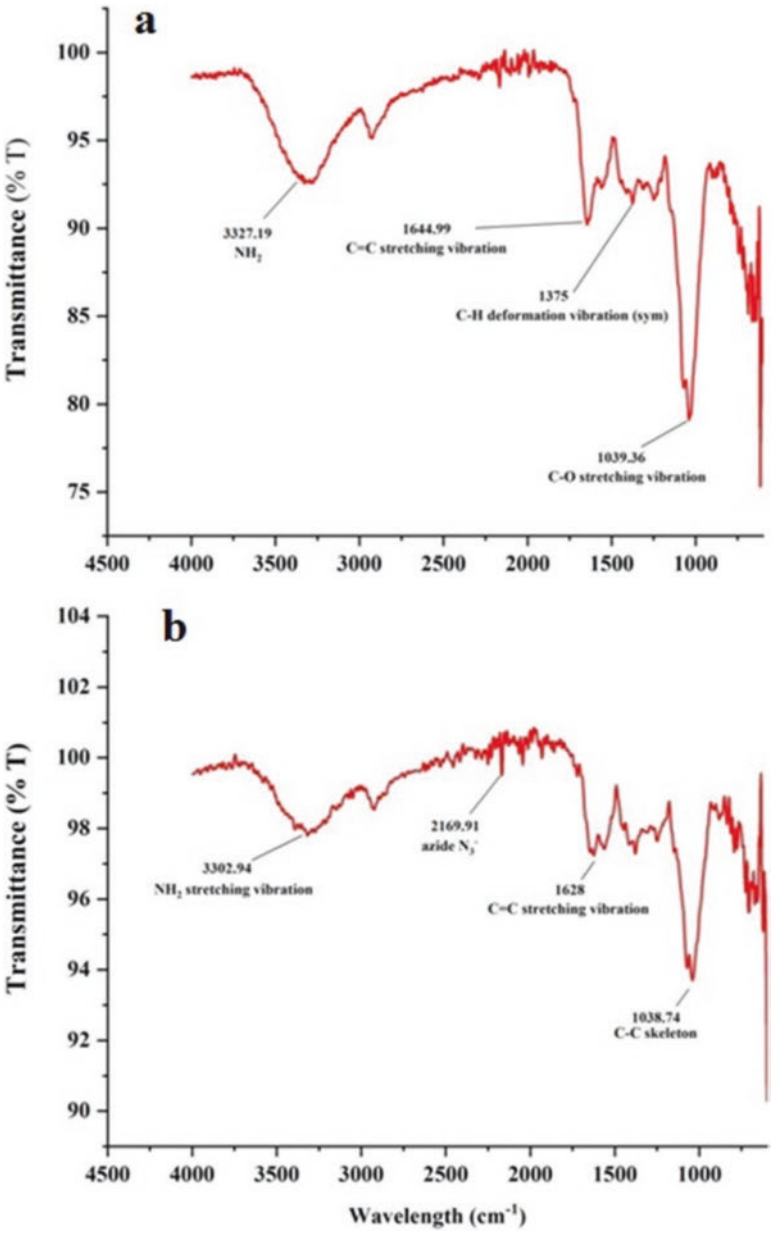


Fig. 21.2 The FTIR spectra showing various functional groups in *X. hypoxylon* (**a**) and *X. polymorpha* (**b**)

21.6 EDS Spectra

Among the four *Xylaria*, the EDS spectra of *X. escharoidea* showed as high as eight distinct elemental compositions (Figs. 21.3 and 21.4). In *X. hypoxylon* and *X. longipes* five elemental compositions exist, while in *X. polymorpha* four different elements were seen (Table 21.3). Among the *Xylaria*, the carbon content was highest in *X. polymorpha* (63.45%) followed by *X. hypoxylon* (32.89%), *X. escharoidea* (28.68%) and *X. longipes* (23.20%). Similarly, the elemental oxygen was highest in *X. longipes* (51.61%) followed by *X. escharoidea* (36.81%), *X. polymorpha* (35.68%) and *X. hypoxylon* (31.95%). The N element was confined to *X. hypoxylon* (35.08%) as well as *X. escharoidea* (33.56%). The *X. escharoidea* had the highest rate of absorption of P, S, K along with Al and Si suggesting the possibilities to use this species as bioindicator of heavy metals (Figure 21.3a). The *X. longipes* showed absorption of Cu suggesting its role in metabolic pathways as well as possibilities of Cu bioremediation (Figure 21.3b), while unlike *X. hypoxylon* (Figure 21.4a), *X. polymorpha* (Figure 21.4b) showed the highest specific absorption of K and Ca.

21.7 Antioxidant Potential

21.7.1 Total Antioxidant Activity

Total antioxidant activity of *X. hypoxylon* was highest followed by *X. escharoidea, X. polymorpha* and *X. longipes* (Fig. 21.5a). Although the TAA was highest in *X. hypoxylon*, other antioxidant activities were relatively low, while it was opposite for the *X. escharoidea* and *X. polymorpha* (Figs. 21.5b and 21.6).

21.7.2 Ferrous Ion-Chelation Capacity

The FCC was highest in *X. escharoidea* followed by *X. polymorpha*, *X. hypoxylon* and *X. longipes* (Figure 21.5b). The FCC of *X. escharoidea* and *X. polymorpha* was higher than the activities of purified water-soluble intra- and extra-cellular polysaccharides of termite mound inhabiting *X. escharoidea* in China (Yue et al. 2013). The FCC of *X. hypoxylon* and *X. longipes* is comparable with termite mound inhabiting *X. escharoidea* of China. However, the FCC of water-soluble intra-cellular polysaccharides of *X. escharoidea* of China was weaker compared to another termite mound inhabiting *Xylaria gracillima* in submerged cultures (Li and Wen 2008).

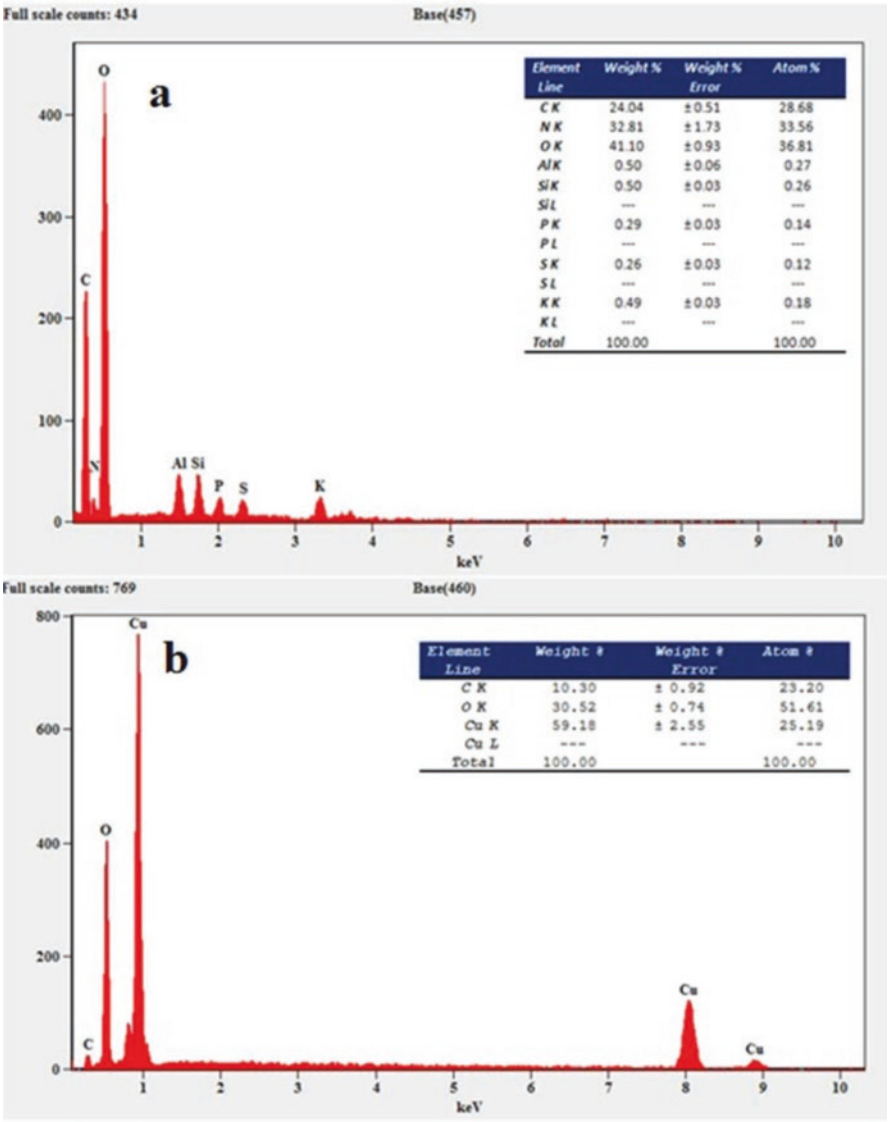


Fig. 21.3 The EDS spectra showing elemental composition of *Xylaria escharoidea* (**a**) and *X. longipes* (**b**)

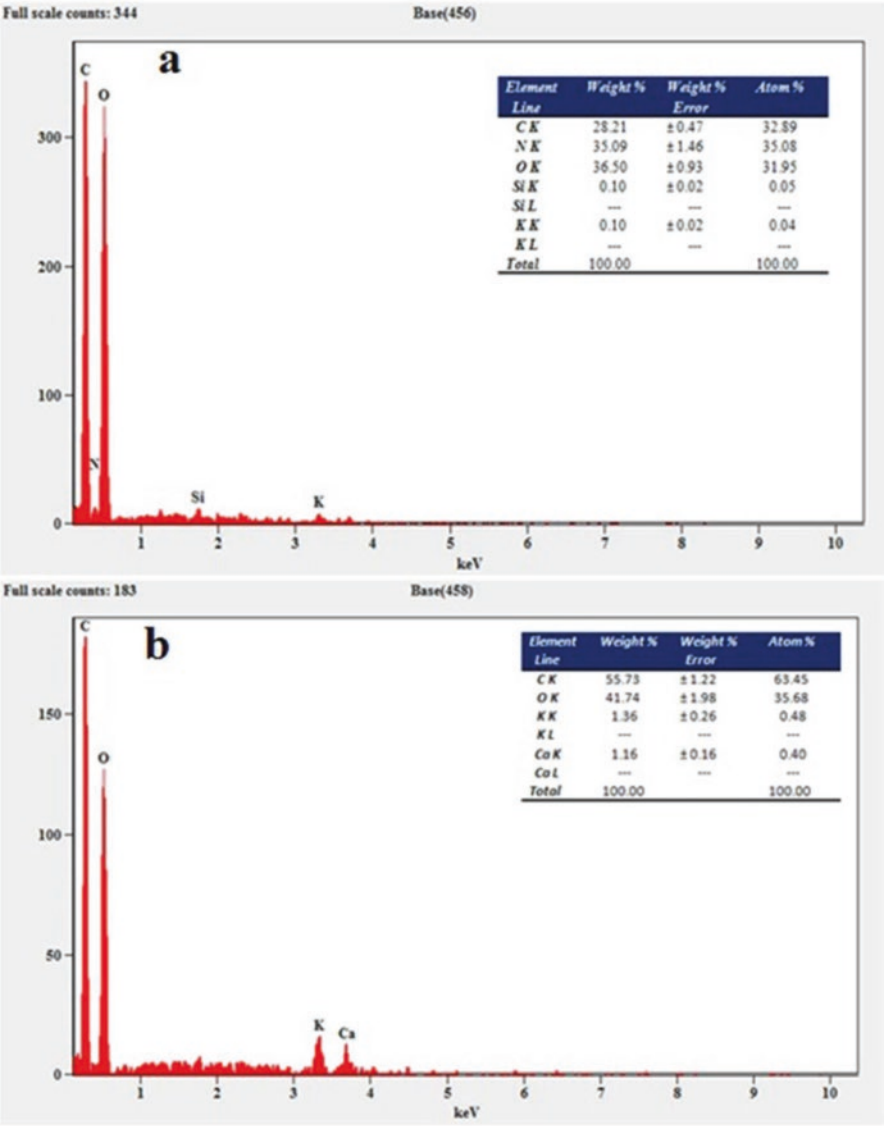


Fig. 21.4 The EDS spectra showing elemental composition of *X. hypoxylon* (**a**) and *X. polymorpha* (**b**)

Table 21.3 Mineral composition of *Xylaria* spp. (g/100 g dry mass)

	Xylaria escharoidea	*Xylaria hypoxylon*	*Xylaria longipes*	*Xylaria polymorpha*
Phosphorus	0.30	–	–	–
Sulphur	0.30	–	–	–
Potassium	0.50	0.10	–	1.40
Copper	–	–	59.20	–
Aluminium	0.50	–	–	–
Cobalt	–	–	–	1.20
Silicon	0.50	0.10	–	–

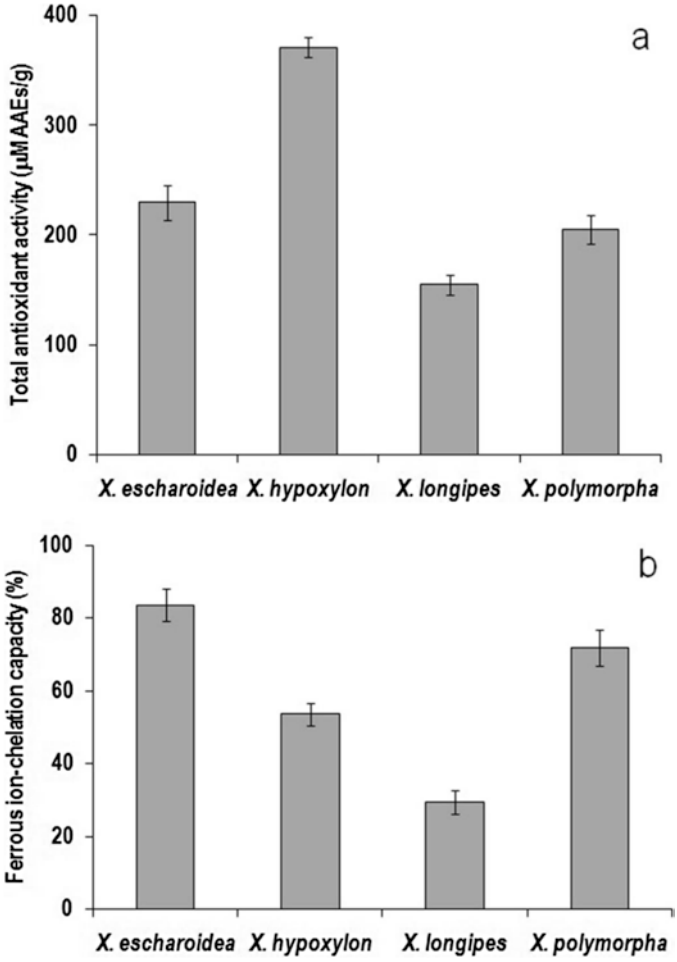


Fig. 21.5 Total antioxidant activity (**a**) and ferrous ion-chelation capacity (**b**) of *Xylaria escharoidea*, *X. hypoxylon*, *X. longipes* and *X. polymorpha* ($n = 3 \pm$ SD)

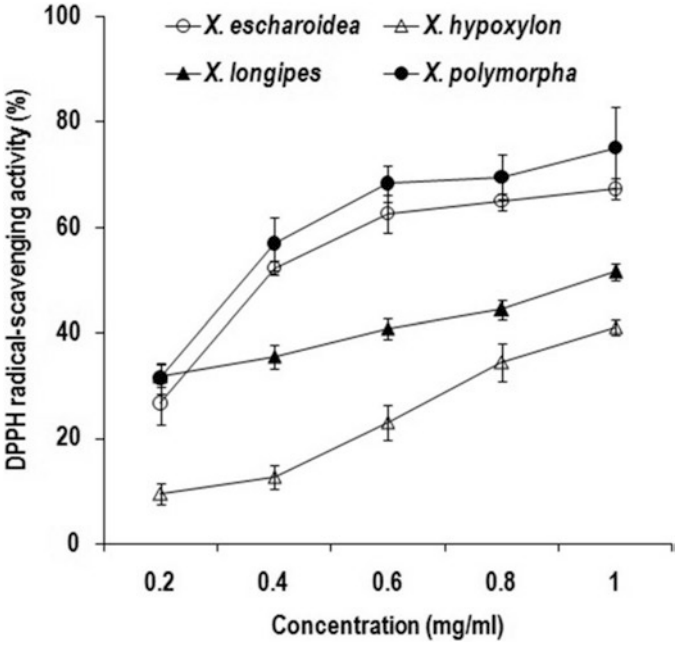


Fig. 21.6 The DPPH radical-scavenging activity of *Xylaria escharoidea*, *X. hypoxylon*, *X. longipes* and *X. polymorpha* ($n = 3 \pm SD$)

21.7.3 DPPH Radical-Scavenging Activity

The DPPH radical-scavenging activity was highest in *X. polymorpha* followed by *X. escharoidea*, *X. longipes* and *X. hypoxylon* (Fig. 21.6). The radical-scavenging activities of methanol extract of *X. escharoidea* and *X. polymorpha* are higher compared to the activities of purified water-soluble intra- and extra-cellular polysaccharides of termite mound inhabiting *X. escharoidea* of China (Yue et al. 2013). However, the hydroxyl radical-scavenging activity of water-soluble intra-cellular polysaccharides of *X. escharoidea* of China was weaker compared to another termite mound inhabiting *Xylaria gracillima* in submerged cultures (Li and Wen 2008). The DPPH radical-scavenging activities of *X. escharoidea* and *X. polymorpha* are comparable to *Xylaria feejeensis* of Sri Lanka (Fernando et al. 2016). According to Fernando et al. (2016), the total phenolics and flavonoid contents of *X. feejeensis* are responsible for the potent antioxidant activity.

Rebbapragada and Kalyanaraman (2016) studied the DPPH radical-scavenging activity of endophytic *X. feejeensis* of the commercial tree *Tectona grandis* at different conditions (culture media, time of incubation, temperature and pH). The activity was highest in two culture broths (potato dextrose and potato dextrose yeast extract) during 15-25 days of incubation (temperature, 20-35 °C; pH, 5–7). The activities of *X. escharoidea* and *X. polymorpha* are comparable to the optimum conditions set

for *X. feejeensis* (Rebbapragada and Kalyanaraman 2016). However, the radical-scavenging activities of *X. hypoxylon* and *X. longipes* are comparable with activities of *X. feejeensis* in two broths (Czapek-Dox broth and Sabouraud dextrose broth) during 5–10 days incubation, higher temperature (40 °C) as well as pH (8–9) (Rebbapragada and Kalyanaraman 2016).

21.7.4 One-Way ANOVA

One-way ANOVA revealed significant differences in TAA, FCC and DPPH activity among four *Xylaria* spp. with Holm-Sidak method ($p < 0.001$). Significant differences in the TAA were seen between *X. hypoxylon* vs. *X. escharoidea, X. longipes* and *X. polymorpha* ($p < 0.001$). Significant differences in the FCC were also seen among all the species of *Xylaria* ($p < 0.001$). Significant differences in the DPPH activity were seen in *X. hypoxylon* vs. *X. polymorpha* and *X. longipes*, so also between *X. escharoidea* and *X. hypoxylon* ($p < 0.001$) but not for other combinations. This reflects that the *Xylaria* spp. studied possess potent antioxidant potential of health importance.

21.8 Outlook and Prospects

Xylaria being one of the largest ascomycetous genera with worldwide distribution provides excellent avenues to evaluate morphological, phylogenetic, bioactive compounds and therapeutic properties. *Xylaria escharoidea, X. hypoxylon, X. longipes* and *X. polymorpha* assessed are predominant in the scrub jungles of southwestern India. *Xylaria escharoidea* is a common inhabitant in almost all the termite mounds in scrub jungles. In some of termite mounds, along with *X. escharoidea* fruit bodies of edible termitomycete, *Termitomyces umkowaan* is also seen. It is interesting to evaluate the specific association of *X. escharoidea* with termites. Do the termites deliberately grow or accommodate *X. escharoidea* (similar to *T. umkowaan*) for their benefits like prevention of invaders by their metabolites or to supply nutrients or for dissemination through termites? *Xylaria hypoxylon* is common on the twigs than logs and stubs, it also grow on the soil as well as compost, while occasionally on fallen seeds in scrub jungles, thus it is likely endophytic in seeds for horizontal transmission. Among the four *Xylaria* spp. *X. hypoxylon* possesses the highest number of metabolites (apoptotic, antimicrobial and cytotoxic) followed by *X. polymorpha* (antioxidant, antimicrobial and photocatalytic) (see Table 21.1). These species are also known for high quantity of crude fibre, high quantity of phytate, moderate quantities of crude protein and flavonoids (see Table 21.2).Owing to presence of high quantity of phytate, *X. polymorpha* seems to be consistent in total antioxidant activity, ferrous ion-chelating capacity and DPPH radical-scavenging activity (see Figs. 21.5 and 21.6). Some of the recent developments on fermentation of *Xylaria*

include submerged fermentation to generate biomass of *Xylaria* spp. by Ramesh et al. (2014) to control drug-resistant bacterial pathogens, while solid substrate fermentation of *X. polymorpha* was carried out to generate multifunctional lignocellulases by Nghi et al. (2012). Similarly, optimum cultural conditions on semi-defined nutrient media have been achieved by Ahmed and Jahan (2017) to study the cytotoxic and other bioactive compounds by *X. hypoxylon*.

Functional group and elemental composition analysis of four *Xylaria* spp. occurring in the scrub jungles of southwest India revealed rich in secondary metabolites as well as elemental composition. However, the FTIR spectral fingerprints cannot recognize metabolites directly by visual inspection owing to a large number of variables, thus needs further sophisticated analysis. As macrofungi are always rich in proteins and carbohydrates, they serve as important source of secondary metabolites of pharmaceutical significance and demands validation by other spectroscopic analysis (e.g. GC-MS, LC-MS, HPLC and NMR). Based on the earlier studies on *Xylaria* and the present study, it is predicted that the antioxidant properties of *Xylaria* are dependent on the species, habitat and geographic location. One-way ANOVA of three antioxidant properties among the four *Xylaria* species studied revealed a significant difference among them. Considering the abundance of *Xylaria* spp. in varied habitats of the scrub jungles of southwest India, further studies on their bioactive components and therapeutic potential will be highly beneficial.

Acknowledgements We are thankful to Dr. M. Pavithra, Department of Biosciences, Mangalore University and Dr. Sudeep Ghate, Yenepoya Research Centre, Yenepoya University for helpful suggestions. One of us (SM) is grateful to the Council of Scientific and Industrial Research, New Delhi for the award of Research Associate Fellowship.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Vol-1: biodiversity and ecological perspective. Springer, Cham

Adeduntan SA (2014) Nutritional and anti-nutritional characteristics of some dominant fungi species in south western Nigeria. Int J Eng Sci 4:18–24

Ahmed FA, Jahan N (2017) Optimization of culture conditions, phytoconstituents and cytotoxicity of *Xylaria hypoxylon* (L.)Grev. Jahangirnagar Univ J Biol Sci 6:1–9

Bayman P, Angulo-Sandoval P, Báez-Ortiz Z, Lodge DJ (1998) Distribution and dispersal of *Xylaria* endophytes in two tree species in Puerto Rico. Mycol Res 102:944–948

Boonphong S, Kittakoop P, Isaka M, Pittayakhajonwut D, Tanticharoen M, Thebtaranonth Y (2001) Multiplolides a and B, new antifungal 10-membered lactones from *Xylaria multiplex*. J Nat Prod 64:965–967

Chaurasia PK, Yadava S, Bharati SL, Singh SK (2014) Selective oxidation and N-coupling by purified laccase of *Xylaria polymorpha* MTCC-1100. Rus J Bioorg Chem 40:455–460

Cheng C, Liu J, Wang H, Xiong W (2010) Infrared spectroscopic studies of chinese medicines. Appl Spectrosc Rev 45:165–178

Chesters NCJE, O'Hagan D (1997) Biosynthesis of the fungal metabolite, piliformic acid(2-hexylidene-3-methylsuccinicacid). J Chem Soc Perkin Trans 1:827–834

Dattaraj HR, Sridhar KR, Jagadish BR (2020) Diversity and bioprospect significance of macrofungi in the scrub jungles of Southwest India. In: Öztürk M, Egamberdieva D, Pešić M (eds) Biodiversity and biomedicine. Elsevier, Academic Press, New York, pp 235–246. https://doi.org/10.1016/B978-0-12-819541-3.00012-8

Davis EC, Franklin JB, Shaw AJ, Vilgalys R (2003) Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: phylogenetics, distribution and symbiosis. Am J Bot 90:1661–1667

Debnath S, Majumdar K, Das P, Saha AK (2018) New distribution record of five species of *Xylaria* from Tripura, Northeast India. Res Rev J Life Sci 8:1–10

Devi R et al (2020) Fungal secondary metabolites and their biotechnological application for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and biomedicine systems: perspectives for human health. Elsevier, Amsterdam, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Divate RD, Wang P-M, Want C-C, Chou ST, Chang CT, Chung YC (2017) Protective effect of medicinal fungus *Xylaria nigripes* mycelia extracts against hydrogen peroxide-induced apoptosis in PC12 cells. Int J Immunopathol Pharmacol 30:105–112

Elias LM, Fortkamp D, Sartori SB, Ferreora MC, Gomes LH, Azevedo JL et al (2018) The potential of compounds isolated from *Xylaria* spp. as antifungal agents against anthracnose. Braz J Microbiol 49:840–847

Erwanto Y, Muttaqien AT, Sugiyono S, Rohman A (2016) Use of Fourier transform infrared (FTIR) spectroscopy and chemometrics for analysis of lard adulteration in "Rambak" crackers. Int J Food Prop 19:2718–2725

Espada A, Rivera-Sagredo A, Fuente JM, Hueso-Rodriguez JA, Elson SW (1997) New cytochalasins from the fungus *Xylaria hypoxylon*. Tetrahedron 53:6485–6492

Fernando MDM, Wijesundera RLC, Soysa SSBDP, de Silva ED, Nanayakkara C (2016) Antioxidant potential and content of the polyphenolic secondary metabolites of white rot macrofungi; *Flavodon flavus* (Klotzsch.) and *Xylaria feejeensis* (Berk.). SDRP J Plant Sci 1:1–6

Gu W, Ding H (2008) Two new tetralone derivatives from the culture of *Xylaria hypoxylon* At-028. Chin Chem Lett 19:1323–1326

Gunawan S, Steffan B, Steglich W (1990) Xylaral, ein Hydroxyphthalid-Derivat aus Fruchtkoerpern von *Xylaria polymorpha* (Ascomycetes). Liebigs Ann Chem 8:825–827

Hirri A, Bassbasi M, Platikanov M, Tauler R, Qussama A (2016) FTIR spectroscopy and PLS-DA classification and prediction of four commercial grade virgin olive oils from Morocco. Food Anal Methods 9:974–981

Hsieh HM, Lin CR, Fang MJ, Rogers JD, Fournier J, Lechat C, Ju YM (2010) Phylogenetic status of *Xylaria* subgenus *Pseudoxylaria* among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. Mol Phylogenet Evol 54:957–969

Hsu CL, Chen W, Weng YM, Tseng CY (2003) Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. Food Chem 83:85–92

Jang YW, Lee IK, Kim YS, Lee SK, Lee HJ, Yu SH et al (2007) Xylarinic acids A and B, new antifungal polypropionates from the fruiting body of *Xylaria polymorpha*. J Antibiot 60:696–699

Jin Z, Li D, Liu T, Yu F, Zhang Z, Su C, Wang Y, Guo Q, Liu Z (2017) Cultural endophytic fungi associated with *Dendrobium officinale*: identification, diversity estimation and their antimicrobial potential. Curr Sci 112:1690–1697

Ju Y-M, Rogers JD, Hsieh H-M (2018) Xylaria species associated with fallen fruits and seeds. Mycologia 110:726–749

Karun NC, Sridhar KR (2015) Xylaria complex in the South Western India. Plant Pathol Quar 5:83–96

Kirk PF, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi, 10th edn. CABI, Egham, UK

Kshirsagar AS, Rhatwal SM, Gandhe RV (2009) The genus *Xylaria* from Maharashtra, India. Indian Phytopathol 62:54–63

Latha KPD, Veluthoor S, Manimohan P (2015) On the taxonomic identity of a fungal morph used in traditional medicine in Kerala State, India. Phytotaxa 201:287–295

Lee IK, Jang YW, Kim YS, Yu SH, Lee KJ, Park SM et al (2009) Xylarinols A and B, two new 2-benzoxepin derivatives from the fruiting bodies of *Xylaria polymorpha*. J Antibiot 62:163–165

Lee JS, Ko KS, Jung HS (2000) Phylogenetic analysis of *Xylaria* based on nuclear ribosomal ITS1–5.8S–ITS2 sequences. FEMS Microbiol Lett 187:89–93

Li QR, Liu LL, Zhang X, Shen XC, Kang JC (2017) Xylaria spinulosa sp nov and *X. atrosphaerica* from southern China. Mycosphere 8:1070–1079

Li SF, Wen HA (2008) Antioxidant activities of bioactive components from *Xylaria gracillima* in submerged culture. Mycosystema 4:487–593

Lin Y, Wu X, Feng S, Jiang G, Luo J, Zhou S, Vrijmoed LLP, Jones EBG, Krohn K, Steingröver K, Zsila F (2001) Five unique compounds: xyloketals from mangrove fungus *Xylaria* sp from the South China Sea coast. J Org Chem 66:6252–6256

Linnakoski R, Puhakka-Tarvainen H, Pappinen A (2012) Endophytic fungi isolated from *Khaya anthotheca* in Ghana. Fungal Ecol 5:298–308

Ma YP, Mao DB, Geng LJ, Zhang WY, Wang Z, Xub CP (2013) Production optimization, molecular characterization and biological activities of exopolysaccharides from *Xylaria nigripes*. Chem Biochem Eng 27:177–184

Maharachchikumbura SSN, Hyde KD, Jones EBG, McKenzie EHC et al (2016) Families of sordariomycetes. Fungal Divers 79:1–317

Mohanan C (2011) Macrofungi of Kerala. Handbook no. 27. Kerala Forest Research Institute, Peechi

Nagam V, Aluru R, Shoaib M, Dong G-R, Li Z, Pallaval VR, Ni J-F (2020) Diversity of fungal isolates from fungus-growing termite *Macrotermes barneyi* and characterization of bioactive compound from *Xylaria escharoidea*. Insect Sci. https://doi.org/10.1111/1744-7917.12799

Nghi DH, Bittner B, Kellner H, Jehmlich N, Ullrich R, Pecyna MJ et al (2012) The wood rot ascomycete *Xylaria polymorpha* produces a novel GH78 glycoside hydrolase that exhibits α-L-rhamnosidase and feruloyl esterase activities and releases hydroxycinnamic acids from lignocelluloses. Appl Environ Microbiol 78:4893–4901

Patel KJN, Krishnappa M (2017) Diversity of Xylariaceae members in Sagara taluk, Karnataka, India. J Mycol Plant Pathol 47:447–452

Patil A, Patil MS, Dangat BT (2012) Three giant ascomycetes (Pyrenomycetes) from Maharashtra, India. Mycosphere 3:353–356

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269:337–341

Qi L, Ji Z, Liu H, Li T, Wang Y (2017) Fourier transform mid-infrared spectroscopy and chemometrics to identify and discriminate *boletus edulis* and *boletus tomentipes* mushrooms. Int J Food Prop 20(Sup 1):S56S68

Rajulu MBG, Thirunavukkarasu N, Babu AG, Aggarwal A, Suryanarayanan S, Reddy MS (2013) Endophytic Xylariaceae from the forests of Western Ghats, southern India: distribution and biological activities. Mycology 4:29–37

Ramesh V, Karunakaran C, Rajendran A (2012) Evaluation of synergistic and antibacterial activity of *Xylaria curta* against drug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Mycology 3:252–257

Ramesh V, Karunakaran C, Rajendran A (2014) Optimization of submerged culture conditions for mycelial biomass production with enhanced antibacterial activity of the medicinal macro fungus *Xylaria* sp strain R006 against drug resistant bacterial pathogens. Curr Res Environ Appl Mycol 4:88–98

Ramesh V, Santosh K, Pavunraj M, Karunakaran C, Rajendran A (2015) In vitro antifungal and anticancer potential of *Xylaria curta* fruiting body fractions against human fungal pathogen and cancer cell lines. Curr Res Environ Appl Mycol 5:20–26

Rebbapragada D, Kalyanaraman R (2016) Evaluation and optimization of antioxidant potentiality of *Xylaria feejeensis* hmjau22039. Asian J Pharm Clin Res 9:269–273

Robinson SC, Tudor D, Cooper PA (2011) Utilizing pigment-producing fungi to add commercial value to American beech (*Fagus grandifolia*). Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-011-3576-9

Roger JD (1979) The Xylariaceae: systematic, biological and evolutionary aspects. Mycologia 71:1–41

Roger JD, Yeomans R, Adams MJ (2008) The relationship of *Xylaria oxyacanthae* to seeds of *Crataegus monogyna*. North Am Fungi 3:1–5

Rogers JD, Samuels DJ (1986) Ascomycetes of New Zealand 8: *Xylaria*. N Z J Bot 24:615–650

Sahu RK, Mordechai S (2016) Spectroscopic techniques in medicine: the future of diagnostics. Appl Spectrosc Rev 51:484–499

Schneider G, Anke H, Sterner O (1996) Xylaramide, a new antifungal compound, and other secondary metabolites from *Xylaria longipes*. Z Naturforsch 51c:802–806

Schüffler A, Sterner O, Anke H (2007) Cytotoxic α-pyrones from *Xylaria hypoxylon*. Z Naturforsch 62c:169–172

Shiono Y, Motoki S, Koseki T, Murayama T, Tojima M, Kimura K (2009) Isopimarane diterpene glycosides, apoptosis inducers, obtained from fruiting bodies of the ascomycete *Xylaria polymorpha*. Phytochemistry 70:935–939

Singh J, Yadav AN (2020) Natural bioactive products in sustainable agriculture. Springer, Singapore

Singh RP, Murthy CKN, Jayaprakasha GK (2002) Studies on antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro methods. J Agric Food Chem 50:81–86

Stadler M (2011) Importance of secondary metabolites in the Xylariaceae as parameters for assessment of their taxonomy, phylogeny and functional biodiversity. Curr Res Environ Appl Mycol 1:75–133

Thirumalesh BV, Thipeswamy B, Krishnappa M (2014) Antibacterial activity of *Xylaria* species *in vitro* against *Xanthomonas campestris* pv *mangiferaeindicae* isolated from bacterial black spot of mango fruit. Int J Life Sci Biotechnol Pharm Res 3:125–130

Trierveiler-Pereira L, Romero AI, Baltazar JM, Loguercio-leite C (2009) Addition to the knowledge of *Xylaria* (Xylariaceae, Ascomycota) in Santa Catarina, Southern Brazil. Mycotaxon 107:139–156

Tudor D, Robinson SC, Krigstin TL, Cooper PA (2014) Microscopic investigation on fungal pigment formation and its morphology in wood substrates. Open Mycol J 8:174–186

U'ren JM, Dalling JW, Gallery RE, Maddison DR, Davis EC, Gibson CM, Arnold AE (2009) Diversity and evolutionary origins of fungi associated with seeds of a neotropical pioneer tree: a case study for analysing fungal environmental samples. Mycol Res 113:432–449

Vodnar DC, Socaciu C, Rotar AM, Stanila A (2010) Morphology, FTIR fingerprint and survivability of encapsulated lactic bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* Subsp *bulgaricus*) in simulated gastric juice and intestinal juice. Int J Food Sci Technol 45:2345–2351

Wang X-N, Tan R-X, Want F, Steglich W, Liu J-K (2005) The first isolation of a Phlegmacin type pigment from the ascomycete *Xylaria euglossa*. Z Naturforsch 60b:333–336

Whalley AJS (1996) The xylariaceous way of life. Mycol Res 100:897–922

Yan S, Li S, Wu W, Zhao F, Bao L, Ding R, Gao H, Wen HA, Song F, Liu HW (2011) Terpenoid and phenolic metabolites from the fungus *Xylaria* sp associated with termite nests. Chem Biodivers 8:1689–1700

Yue S, Li S, Wen H, Fan L (2013) Isolation, identification and antioxidant properties of *Xylaria escharoidea* associated with termite nest. J Anim Vet Adv 12:330–336

Zhao Y, Zhang J, Jin H, Zhang J, Shen T, Wang Y (2015) Discrimination of *Gentiana rigescens* from different origins by Fourier transform infrared spectroscopy combined with chemometric methods. J AOAC Int 98:22–26

Zhou J, Diao X, Want T, Chen G, Lin Q, Yang Z, Xu J (2018) Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species *Rhizophora stylosa* and *R. mucronata* in the South China Sea. PLoS One 13:e0197359. https://doi.org/10.1371/journal.pone.0197359

Chapter 22
Fungicide as Potential Vaccine: Current Research and Future Challenges

Garima Verma, Bimlesh Kumar, Amarish Kumar Sharma, and Ajar Nath Yadav

Contents

G. Verma · A. K. Sharma (✉)
Department of Bioengineering & Biosciences, Lovely Professional University, Phagwara, Punjab, India

B. Kumar
School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

A. N. Yadav
Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_22

22.1 Introduction

With increasing number of groups (high risk) exposed to invasive fungal infections, the importance of clinical intervention or treatment methods is the highly imminent for the cancer patients undergoing chemotherapy, transplantation of bone marrow, and other diseases caused due to chronic immune-deficiency (De Amorim et al. 2013, Edwards Jr and John 2012). Invasive fungal infections can attack patients on continuous antibiotics intake and intravenous catheter treatment in premature infants, respectively (Schaller 1975). Even immune-compromised patients suffering with haematologic malignancies are highly sensitive to invasive fungal infections (Medici and Del Poeta 2015). It has also been noted that the infants with very low birth weight are highly sensitive to fungal infections (invasive) and may require prolonged anti-mycotic treatment or post-natal steroid therapy. *Aspergillus* spp., *Candida* spp. and Zygomycetes are prime fungal culprits responsible for invasive fungal infections in human system (Hsieh et al. 2012). It has been noted earlier that the main causative agent of nosocomial bloodstream infection once spread in USA and European countries was Candida species. It was observed that invasive candidiasis despite experimental clinical therapy, the mortality rate was around 40% (Barchiesi et al. 2016).

Cryptococcus neoformans is seen as the most opportunistic fungal infection affecting HIV patients coupled with high mortality rate. A significant decrease ranging in 90–92% has been observed in HIV infection patients, once they are treated with anti-retroviral therapy. Furthermore, it has been noticed during clinical investigations in half of the total mortality cases due to invasive fungal infections (IFI).

Considering an ever increasing population of patients with weak immune systems and further exposed with immune-suppressive treatments in certain chronic diseases, we are experiencing a new kind of ever increasing, life-threatening infections mostly from fungal family. In reference to the above concern, vaccine against invasive fungal diseases, an ever growing medical attention in the recent years, has increased in the recent years. Due to rapid surge in medical complications arisen due to invasive fungal complications, during patient's treatment for any complications such as diabetes, cancer or HIV/AIDS, there is a great demand to invest in immunological tools or integrate with chemotherapeutics, thereby minimizing the use of antibiotics and subsequently reducing antibiotic resistance.

22.2 History of Vaccines

It has been reported that the use of vaccination was observed for the first time by Thucydides in 430 BC and people during that period were able to survive from lethal contagious diseases. In China, during the period of Middle Ages the people use to practice a procedure known as variolation, in which air dried pustules of smallpox were used and healthy people were exposed to them. It was observed that

this practice was very dangerous though effective also. Later, Jenner discovered that inoculation of agent which was responsible for the mimicking of the disease like pustule derived from cowpox was an efficient way rather than exposing the people directly to the disease. After a century, the microbial origin of infectious diseases was discovered and Pasteur opened up a new way in the development of vaccine. He isolated the agent or micro-organisms responsible for causing a particular disease and converts them into dead or attenuated forms so that they lose their ability to cause disease but can mimic the infectious agent (Rappuoli et al. 2014). Later in 1940s, in-vitro cultures of viruses were grown on animal cells to develop vaccines against several diseases like poliomyelitis, mumps, varicella, measles, rubella, hepatitis A, influenza and rotavirus.

22.3 Evolution of Vaccines

Recently, more sophisticated technologies having efficient way to mimic the disease causing agent and to induce the immunological response (Rappuoli et al. 2014). Currently, different types of vaccines are being used like live attenuated vaccines, sub-unit vaccines or recombinant vaccines which are synthesized in the laboratories.

Due to the development of such vaccines, the eradication of diseases like smallpox is possible. But lately the vaccines against lethal viral or bacterial disease were getting more popularity and there were no vaccines available against fungal diseases. The reason behind this was lack of market appeal, absence of better quality vaccine formulations and expensive clinical investigations.

As the time passes by and the medical need of vaccines against fungal diseases increased rapidly and catches the attention of both investigators and representatives of various industries. With the advancement in biotechnology field, the development of vaccines against fungal pathogens took place. There is huge difference between how vaccine industry sees the fungal vaccines back then and now. The combination of increase in medical need and advanced technologies results in the formation of novel fungal vaccines which are not only capable of preventing us from lethal diseases but also prevents their occurrence and improves the quality of our life (Cassone and Casadevall 2012).

22.4 Microbiome Impact on the Fungal Infection Pathophysiology

With the evolution of our scientific developments, we have witnessed an in-depth understanding of a host's health status is greatly affected by the composition of microbiome residing inside (Pflughoeft and Versalovic 2012). Their population is

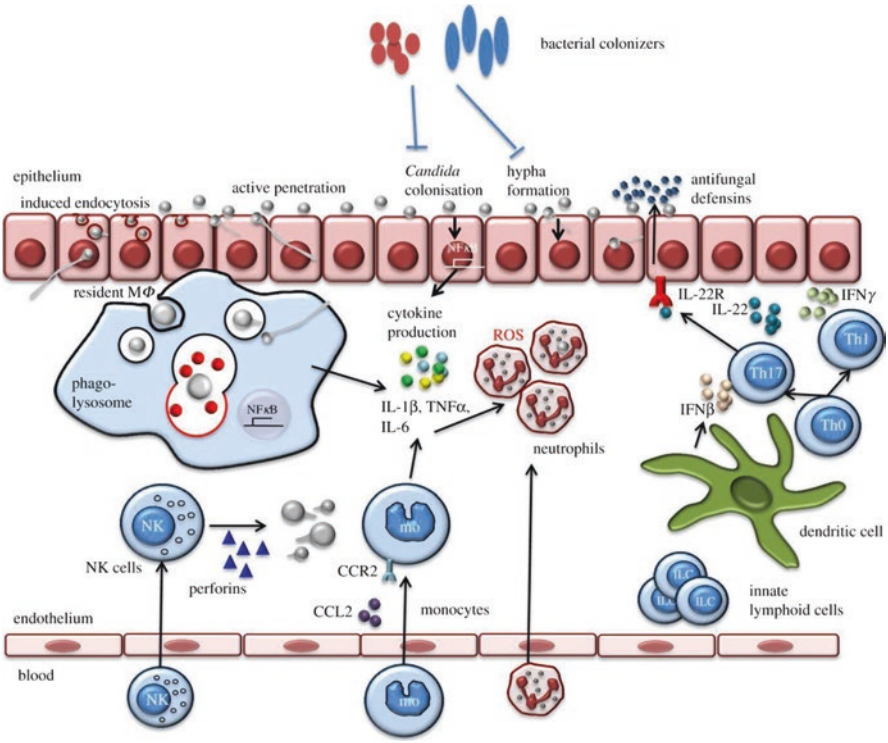


Fig. 22.1 Fungal growth physiology and specific innate immune response under direct and indirect microbiome influence. Production of chemokine and cytokines is stimulated upon invasion of *Candida* into host system thereby activating tissue microphages. Furthermore, neutrophils and monocytes are recruited to ingest and kill the invading pathogens. Helper T cells are activated by antigen presenting cells, which aids in elimination of pathogens.; *CCL2* chemokine (C–C motif) ligand 2; *ROS* reactive oxygen species, *NK* natural killer cell, *CCR2* chemokine receptor type 2

highly controlled by diet and can influence colonization of mucosae with fungi (Fig. 22.1). In the recent experimental study on mice, it was observed that the dietary coconut oil helped to control the colonization of *Candida albicans* of the gastrointestinal tract (Gunsalus et al. 2016). The qualitative and quantitative aspects of microbiome are also greatly influenced by fungus colonization especially Candida. For example, hydrogen peroxide and bacteriocins produced by *Lactobacillus* (Kennedy and Volz 1985) reduces fungal adhesion and growth (Noverr and Huffnagle 2004). Similarly *Pseudomonas aeruginosa* inhibits *Candida albicans* hypha formation thereby restricts tissue infiltration and future infection (Hogan et al. 2004). Furthermore, *Enterococcus faecalis* inhibits morphogenesis of *Candida albicans* hyphae in the host gut system.

Candida colonization is not only affected by microbiome composition but also alter the immune response as well upon fungal infection (Romani et al. 2015; Oever and Netea 2014; Kolwijck and van de Veerdonk 2014) (Fig. 22.1). Several immune

alterations have been observed, including enhanced fluxing of macrophage and dendritic cell precursors during experimental observation on mice treated with short chain fatty acid propionate (Trompette et al. 2014). Tryptophan is used as energy precursor by *Lactobacilli* residing in the gut microsystem and produces indole-3-aldehyde.

Aryl hydrocarbon receptor is stimulated upon exposure to indole-3-aldehyde, which further induces natural killer protein, which acts as a protective agent against *Candida* colonization and infection, respectively (Zelante et al. 2013). Hence it can be strongly concluded that the microbiome's bacterial components can influence the immunological response in the gastrointestinal and respiratory tract, respectively. In an immune-compromised host system, the pathogenic microbes especially the skin fungi can enormously expand and colonize (Huffnagle and Noverr 2013). In an experimental study, it was observed that the mice treated with antibiotics had altered gut microbiome that overlapped with outgrowth of commensal *Candida* species in the gastrointestinal track (Kim et al. 2014). Simultaneously, it is also observed that in Dectin-1 deficient mice model, there is an increased probability of altered gut microbiome with high susceptibility to experimental colitis (Iliev et al. 2012).

22.5 Need of Fungal Vaccines

To the healthy population, the infections caused by these opportunistic fungi remain a substantial threat particularly to immunocompromised hosts (Flavia De Bernardis et al. 2012). The prime targets of fungal infections are the patients having broad spectrum of anti-bacterial therapy, exposed to invasive procedures, undergone organ transplantation or taking anti-cancer drugs (Swaleha Zubair et al. 2017). Even premature infants are at high risk from suffering from fungal infections and are referred as high risk groups.

To treat severe diseases like cancer, the patient has to undergo a very complex and critical type of treatment which can increase the survival rate but on the other hand disrupts the natural barriers of the body and reduces the immunity of the individual. This makes the patient vulnerable to agents or micro-organisms responsible for fungal infections. The problems mentioned above emerge the need to develop the fungal vaccines.

22.6 Challenging Vaccine Targets

The concept of vaccination or the development of fungal vaccine is one of the most interesting and challenging tasks in the medical fraternity, in the current scenario. Many fungal diseases pose a severe obstacle to the concept of vaccination, at least

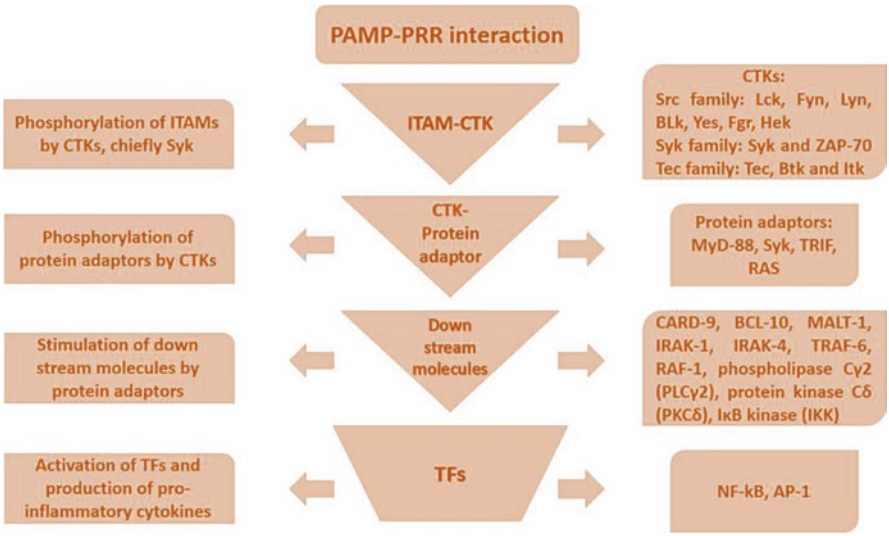


Fig. 22.2 Illustration of fungal sensing and processing signalling pathway. Production of pro-inflammatory cytokines and soluble mediators is initiated on activation of transcription factors (TFs) through phosphorylation of cytoplasmic tyrosine kinase*s* (CTKs) and protein adaptors, respectively

in its active immunization modality. Diseases such as coccidioidomycosis, aspergillosis and candidiasis are the most common fungal infections which typically occur in the immunocompromised host system (Fig. 22.2).

The patients who are ineligible for the active immunization due to some immunological deficit are prone to maximum fungal lethality and increased mortality. The above venerable status is the most influential determinant of vaccine efficacy, despite infection diagnosis and its effective treatment (Atkinson et al. 2002). It is observed that only efficacy trials with a formulation antigenic in nature have been proven safe and immunogenic in animals. In the case of passive vaccination with antibodies, the hosts complement and phagocytic assets are more relevant because the specific antibodies owe their effectiveness to opsonisation and/or complement fixation. As per invasive fungal infections are concerned, in case of Aspergillosis, there exist a number of target populations for vaccination. Mainly (a) bone marrow transplant of patient pre- and post-engraftment, (b) solid organ transplant, inpatients, who could be immunized to develop suitable engraftment, (c) acute myeloid leukaemia patients post-cytostatic chemotherapy in immunocompromised state, (d) inflammatory bowel disease patients untreated with TNF blockers or corticosteroids, which are generally immune-suppressors, (e) patients undergone critical gastrointestinal surgery (Wormley et al. 2007; Barchiesi et al. 2016; Borghi et al. 2014).

22.7 Immunological Basis of Fungal Vaccine

Dual immunological mechanisms for achieving protection are one of the most intriguing aspects related to fungal vaccines. The main mechanisms are-Th1 and Th17 based response and antibody mediated immunity. Although, for the final protective outcome, there is a cooperation of other immunological mechanisms. For both formulation of vaccine and for establishment of protection during the clinical trials, the distinction remains substantial. In T cell based mechanisms, the protection is mediated indirectly, which means initially the induction of inflammatory response takes place that further recruits the soluble effectors such as, cytokines, anti-microbial peptides, chemokines and cellular effectors such as neutrophils, macrophages. At the site of infection, these effectors eliminate or control the fungal pathogen.

In second mechanism, the protection is mediated by antibodies via opsonophagocytosis and complement activation but is dependent on the number of phagocytes and activation state. It also involves neutralization of factors like adhesins or enzymes, hindering the escape of fungal pathogen from the immune system of host or direct elimination or killing of fungus. The studies show that specific antibodies for *C. neoformans* modulate the metabolism of invaded fungi. This opens other mechanism through which humoral immunity can change the result of infection caused by fungus. The mechanism mentioned above shows the importance of vaccines in protecting prospective immunocompromised host system with malfunctioned cellular effectors.

Protection against Candidiasis through vaccines is provided through interaction of Th17 cells with Th1 cellular response. This happens mostly against aspergillosis and endemic mycoses. In the natural history of fungal infections, this mechanism has a counter part where cytokines and receptors important for Th1 and Th17 responses are affected by DNA polymorphisms to influence different types of mucosal candidiasis and aspergillosis.

In the field of vaccines for humans, the vaccine made of the basis of Th1/Th17 response is a novel preparation. The existing vaccines work on the principle of attaining protection by neutralizing bodies. But this immune-protective mechanism has drawback also, which is the requirement of capacity to activate and recruit the phagocytes, as in immune-compromised patients the phagocytes are deficient in number and can cause inflammatory disease like vaginal candidiasis and mucosal infections. In fungal vaccines, Th17 cells play a very intense role by interacting with subsets of CD4 cells and other antibodies. T lymphocytes generate both IFN-g and IL-17, the cytokine signatures of Th1 and Th17 cells, respectively, against the *C. albicans*. In vaccines that rely on Th17 for protection, antibodies can predict the achievement of protective state.

Most of the historic achievements in the field fungal vaccine are based on the neutralization by antibodies. But this field has been neglected for so long by the fungal vaccinologists due to lack of evidence to prove the role of antibodies against fungal infections. The cryptococcal vaccine is the exception involving capsular

polysaccharide antibodies. In the pathogenic species of fungi like *C. albicans* and *C. neoformans*, there is no proof of the existence of factors like, capsule, virulent enzymes, adhesins, which are responsible for mediating virulence of fungi and promoting the disease. By neutralizing the virulence factors, antibodies act as mediators of vaccine-induced protection. In humans, antibodies are not the main component of pre-existing immunity. To induce the adaptive immunity by glycol-conjugate vaccines, new technological improvements should be done to enhance the broad-spectrum protective efficacy of the vaccine and its anti-fungal conjugates.

22.7.1 Immunological (Antigen–Antibody) Interaction in Fungal Infection

The external physical barrier which safeguards the host body continuously from intruding pathogens in the aerosols is basically composed of skin, mucosal epithelial surfaces, gastrointestinal and gastrourinary tract, respectively (Borghi et al. 2014). Pathogenic and non-pathogenic fungal spores suspended in aerosols are predominantly checked by the host's epithelial cell system, through their effective anti-fungal response (Dühring et al. 2015; Dambuza et al. 2017).

Immune responses referred to as pathogen associated molecular patterns (PAMPs) are initiated spontaneously as soon as the host recognizes any component of fungi, specifically recognized by pattern recognition receptors(PRR) (Fig. 22.2). The vast majority of PRRs includes dendritic cells, macrophages, NOD-like receptors, etc., which plays a crucial role in host's innate immunity (Patin et al. 2019). Toll-like receptors (TLR) and Dectins are the prominent PRRs which play a significant role in detecting the intruding fungal moiety in the host system. Hyper expression of gene coding toll-like receptor proteins has been screened in experimental trials in mice infected with systemic candidiasis immunocompromised using cyclophosphamide-dependent (Morovati et al. 2016). Some experimental trials in the recent past have also proved that toll-like receptors 2 are non-responsive in immunosuppressed mice models (Dehghan et al. 2018).

In an innate immunological host's combat system, the phagocytic cells, the dendritic cells and the macrophages, respectively, are able to recognize and target the fungi's primary stages of infection with the help of their specific PRRs and start killing and phagocytising them through their intracellular anti-microbial secretions fluxed with oxygen free radicals. The induction and release of cytokines (CD4+ T cells) are increased during phagocytosis (Salazar and Brown 2018; Romani 2011; Roy and Klein 2012). Simultaneously factors such as defensins, anti-fungal peptides or mannose binding lectins promote opsonisation of fungal moiety, which serves as an additional defence immunological mechanism (Borghi et al. 2014; Romani 2011; Hajishengallis et al. 2017).

Opsonophagocytosis is a very peculiar process of phagocytic cells through which it effectively eliminates pathogens from the host's system, through recognition of

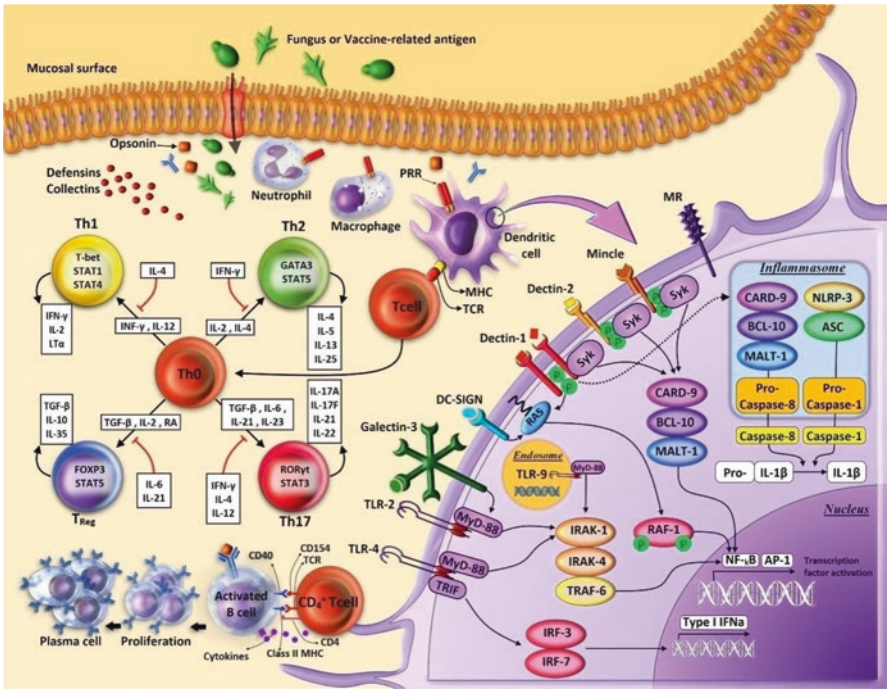


Fig. 22.3 Fungal antigens induce immune response in the host system

beta-1,6-glucans complement particles, expressed on the fungal surface by a specific receptor protein, known as complement receptor 3, majorly expressed on immune cells such as macrophages and neutrophils (Romani 2011). Simultaneously, the helper T cells (T_H -17) are induced by the secretion of defensins and collectins during the opsonisation process of fungal moiety (Fig. 22.3) (Polesello et al. 2017).

When the external epithelial system fails as effective barrier or the first line of defence and the pathogens gets success to intrude into the host's system, the immune system reboots the whole process through a new way through the stimulation of antigen presenting cells and successive production of cytokines, to target and eliminate the fungal particles intruded inside. During this process the immune factors such as PRRs and PAMPs play their specific roles, as they did earlier, as first line of defence. The downstream protein adaptors are triggered as soon as they are connected to the cytoplasmic stimulatory domain of PRRs, leading to upregulation of transcription factors and release of cytotoxic cytokines. In this immunological response, the activation and production of interleukin-1 beta from pro-interleukin-1 beta, respectively, are triggered by the release of Caspase-1 and Caspase-8. Inflammasomes, a cytokine secretion, stimulates Dectin-1, is solely responsible for the activation of caspase molecules, which conducts the above-mentioned process. Subsequently, the antigen presenting cells process the fungal antigen and produce before the naïve T cells. These T cells are formed specifically from the cytokine post

PAMP-PPR interaction. For example, the helper T cell, TH-1 is activating by the exposure of interleukin-6 and interleukin-23 thereby inducing T_H-17 cytokines such as interleukin-17 & 21, respectively.

Fungal antigens are targeted by B immune cells predominantly through two different ways. The first one is through targeting on non-protein antigens by independent T cells. This is the very fact that no memory response is auto saved in the immune system for the respective antigenic protein due to the absence of T cell response, which eventually leads to production of low-affinity IgM isotype antibodies. Secondly, the antibody producing B cell lymphocytes responds to fungal antigens as soon as it is presented by antigen presenting cells (MHC Class 2) to the CD4+ helper T cell, which play a major role in adaptive immunity. Antigen presentation to the B cells, trigger specific immune signals, resulting in production of antigen specific antibody production, which delivers high affinity and increased shelf life. Simultaneously, during this process, immunological memory is created in the host's system which helps in antigen tackling in future attack.

22.7.2 Acquired Immunological Response against Fungal Antigen

Adaptive or acquired type of immunity is developed in the host system through exposure of antigen captured by antigen presenting cells (Major Histocompatibility Complex, MHC class I & II) and brought before B cell lymphocytes through CD4+ and CD8+ helper T cells, which reads the antigenic protein and activates production of the fungal antigen specific antibodies. Furthermore the immunological memory is developed in the host's immune system (Dehghan et al. 2018; Verma et al. 2017; Snarr et al. 2017) (Fig. 22.3). CD+4 T cells differentiation is favoured through antigen presenting cells binding to T_H -helper T cells type 1 thereby promoting release of cytokines, cytotoxic to intruding fungal pathogens. T_H -helper T cells type 1 development requires factors like STAT1/STAT4 while T_H -helper T cells type 17 requires STAT3/ROR-γt transcription factors (Borghi et al. 2014; Salazar and Brown 2018; Romani 2011; Conti et al. 2018) (Fig. 22.3).

The CD+4T cells get differentiated to the T_H-1 cells, upon the secretion of interleukin-12(IL-12) from antigen presenting cells, specifically the Dectins (Romani 2011). Gamma-interferon (IFN-γ) and IL-17 served the purpose of protection against inflammatory response, which is specifically secreted by helper T cells type1 (Górski 1984; Carvalho et al. 2012). Stimulation of phagocytes incites secretion of interferons (IFN), especially IFN-γ, which promotes cell mediated immunity in the host system. Furthermore, cytokines like interleukin-17 and interleukin-22 initiates neutrophilic response thereby releasing peptide toxins, like defensins, bearing anti-microbial property, at the infection site (Matsuzaki and Umemura 2018). The regulatory Treg cells, like Foxp3+/CD4+ T cells, supress the inflammatory responses through the release of transforming growth factor (TGF)-β (Iannitti et al. 2012).

22.8 Fungal Vaccines

In the last few years, a numerous amount of candidates for fungal vaccine have been reported. To check its safety, immunogenicity and efficacy of the vaccine, they are being refined and tested on animal models and later on humans (Table 22.1).

Table 22.1 Fungal vaccine candidates

Fungal species	Vaccine candidate	Immunity	Model
Aspergillus	Asp 16 f	Th1	Murine
Aspergillus	Asp 3 f	Th1	Murine
Aspergillus	Pep1p, Gel1p, Crfl, glucans	Th1	Murine
Aspergillus, Candida	Cell wall glucanase, Crfl	Th1	Murine
Aspergillus, Coccidioides, Cryptococcus, Candida	Heat killed Saccharomyces cerevisiae	Th1, Th2, Th17, antibodies	Murine
Blastomyces	Attenuated mutant	Th1, Th17	Murine
Candida	Agglutinin-like sequence adhesins	Th1, Th17, antibodies	Murine/ Simian
Candida	Hyr1p	Antibodies	Murine
Candida	Secreted aspartyl proteinase protein, Sap2p PEV-7	Antibodies	Murine
Candida, Cryptococcus	Laminaran	Antibodies	Murine
Candida	Mannan linked to human serum albumin	Th1/antibodies	Rabbit
Candida	Live attenuated	T cells	Murine
Candida	Fba peptide	Antibodies	Murine
Coccidioides	Attenuated mutant	Th1, Th17, Th2	Murine
Coccidioides	T cell epitopes Antigen 2/proline rich Ag(Ag2/PRA)	Th1, Th17, Th2	Murine simian
Cryptococcus	Glucuronoxylomannan capsule Galactoxylomannan protein	Antibodies	Murine
Cryptococcus	Peptide mimotopes of GXM capsule (P13)-linked to tetanus or diphtheria toxoid	Antibodies	Murine
Histoplasma	Live, heat killed	Th1, Th17	Murine
Histoplasma	Cell wall membrane fractions/HSP	Th1	Murine
Paracoccidioides	RPb27	Antibodies	Murine
Paracoccidioides	HSP60	Th1	Murine
Paracoccidioides	P10	Th1	Murine
Pneumocystis	55 kDa DNA/p-55	Antibodies	Murine
Pneumocystis	Kexin	Antibodies, $CD8^+$ T cell	Murine

Source: Som G. Nanjappa and Klein (2014)

22.9 Development of Vaccines against Opportunistic Fungal Pathogens

Recently, we have witnessed the global rise of dangerous fungal infection particularly related to high risk groups including premature infants, cancer patients, diabetic patients or immunocompromised patients. But the improved technology in medical field has made it possible to recognize and diagnose fungal infections more efficiently. In patients undergoing invasive surgical procedures, using prosthetic treatment devices, such as Catheters, due to excessive administration of nutrition through parenteral routes, invites entry of fungal pathogens inside the body in substantial amount. It can also lead to defects in the defence mechanism of an individual. It is observed that in 30% cases, the fungal infection may lead to the death of the patient.

According to certain reports, the chance of an individual getting resistant of anti-fungal agents is less than getting resistant to anti-bacterial agents. The incorrect prescription or over use of these anti-fungal agents is the probable reasons behind resistance. Recently, extensive increase has been seen in the number of drug resistant *Candida* species, a type of fungal species. Since 1980s, approximately 500% increase in the occurrence of infections of bloodstream because of Candida species has been observed. The majority of the systemic fungal infections are caused by Candida species, *Aspergillus* species and *Cryptococcus* species. In leukemic, approximately 25% cases lead to death of the patient due to systemic fungal infections. Severe infections caused by fungal agents can cause 5–10% deaths of patients undergoing transplantation of organs like liver, pancreas or liver. After birth, 13% of infant having lower body weight are at high risk of getting affected from acquired fungal sepsis. The factors responsible for the establishment of the fungal pathogens inside the host are immune-suppression, dysfunction of T cells or B cells, diabetic condition, AIDS, organ transplantation, high dosages of corticosteroids, cytotoxic chemotherapy and persistent intake of antibodies or long-term hospitalization.

Generally, the fungal species that can survive at room temperature are exposed continuously to the host in order to establish different interactions from symbiotic to pathogenic. There are many fungal species which may act as primary pathogens and infect the immunocompetent hosts. In case of immunocompromised patients, the pathogen damages the immune system completely and spores or conidia of fungal agent invade inside the body and the infection progresses further.

22.10 *Candida* Species

The Candida pathogens show commensal interactions and the infections caused by these species is the foremost cause for occurrence of nosocomial bloodstream infections, and tentatively around 52–63% infections are caused by C. albicans itself.

Infections are caused by *C. albicans*. Since the last three decades, this species has been considered as the major human pathogen. It is observed that in case of immunocompromised mammalian hosts, *Candida albicans* is a life-threatening pathogen and can cause severe diseases.

According to the reports of Banerjee and colleagues, the rate of candidemia ranges between 0.28 and 0.61 in each 1000 discharges in a hospital under National Nosocomial Infection Surveillance system. This report shows fivefold increase in the rate of candidemia among the members National Nosocomial Infection Surveillance system and even after years the number keep on increasing. This species is the sixth most common type of pathogen that has been isolated and fourth most prevalent in the bloodstream. The factors which control the exponential rise in the isolation of *Candida* species are prolonged exposure of patient to broad-spectrum antibiotics, usage of intravascular catheter devices, development of AIDS, organ transplantation protocols. With the advancement of medical technologies, we have observed the large number of opportunistic infections in patients in critical condition. It has been estimated that in future times, the infections caused by *Candida* species would become more pre-dominant.

22.10.1 Most Common Types of Infections Caused by C. albicans

22.10.1.1 Oropharyngeal Candidiasis

It is commonly known as oral thrush and is the most common type of infection which occurs in oral cavity involving mucosal buccal membranes. Generally, it occurs in infants and patients affected by AIDS. When the person is having this infection then white adherent patches would appear and the membrane gets eroded.

22.10.1.2 Vaginal Candidiasis

It is also known as vulvovaginitis. In features it somewhat resembles with thrush except the irritation, extreme itching and discharge accompanying it. The establishment of this particular infection occurs when the loss of normal bacterial flora takes place due to excessive anti-biotic therapy. The role of normal bacterial flora is to maintain the required acidic pH inside the vagina in order to inhibit the growth of *C. albicans* and also hinders the formation of pseudo hyphae which may lead to the establishment of the infection. Generally, skin infections occur at most or warm parts of the body like axilla, groin and infra-mammary folds. Diabetes and obesity are considered as pre-disposing conditions for topical (skin) infections caused by fungal pathogen.

22.10.1.3 Chronic Mucocutaneous Candidiasis

It is a type of immune-deficiency disease. It presents debilitating, persistent and refractory infections. This infection can be mild or lethal for the patient. It occurs in mucosal membranes, skin or nails.

22.10.2 Secondary Infections

Fungemia is a very common type of fungal infection present in immunosuppressed individuals. It shows the presence of fungal agents in the bloodstream. This infection is not localized to any particular organ or site. Through the bloodstream it gets distributed in the whole body. *Candida albicans* is fungal organism which is ubiquitous in nature. It often colonizes at mucosal surface and skin and does not cause any disease. When the defence or immune mechanism of the host gets impaired then this organism becomes pathogen. Usually, cell mediated immunity protects the host from fungi and controls the infection. The deficiency of cell mediated immunity offers an opportunity to the fungi to spread the infection. It is difficult to demonstrate the humoral immunity but it can be done by either correlating titres of antibodies with protection or by transferring the immune sera. Although due to inconsistent results, the notion that antibodies play the role of protection against the fungi was in controversy.

There were certain studies which proved that cell mediated immunity is the primary defence mechanism along with that antibodies also play important role in the removal of intracellular fungi pathogens completely. The innate immune system of an individual recognizes a variety of micro-organisms and helps in initiating and modulating some adaptive responses which are delivered by the T cells as well as B cells due to its interaction with antigen presenting cells. As soon as the recognition of the pathogen is done then the body starts activating the immune response and both these events are accomplished by pattern recognition receptors. These receptors recognize the pathogen associated molecular patterns, which are the conserved microbial chemical signatures. The skeletal elements and matrix components of cell wall of *Candida albicans* are recognized by the innate immune system. The outermost part of the cell wall consists of mannoprotiens and mannans. The first step in the recognition of *C. albicans* done by the immune system of host is to recognize mannans.

By the induction of particular cytokine profiles, a degree of specificity is observed in the innate response by the pattern recognition receptors. Several opsonic and non-opsonic receptors mediate the phagocytosis of *C. albicans.* The alternative pathway mediates the complement activation and complement binding process. For the chemotaxis and opsonisation of the *C. albicans*, complement activation is necessary.

The uptake of fungal agent is not mediated by toll-like receptors, but they are involved in the presentation of antigen and phagosome maturation. We can use both oxidative and non-oxidative mechanism for the killing of *Candida albicans*. On invasion of fungi, in response Dectin-1 receptor induces the respiratory burst response and this activity is further enhanced by the toll-like receptor signalling. Respiratory burst results in the formation of toxic oxidants and also activates the granule proteases which can kill *Candida albicans*.

The formation of various cytokines and chemokines is induced by Dectin 1, including macrophage inflammatory protein 2, TNF-α, macrophage inflammatory protein 1α, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interleukins-10, IL-2, IL-6, IL-1α, IL-1β, and IL-23. NF- κB or MAPK pathway gets activated by the toll-like receptors and also stimulates the formation of pro-inflammatory cytokine. According to recent studies, there are certain surface components of the fungal pathogen which occupies different toll-like receptors, particularly toll-like receptor 4 and toll-like receptor 2 which plays important role in protecting the fungus.

The antibody formation against the virulent traits of fungi and the ability to tip the protective enhancer balance towards the former which is likely to have convincing response by the defence system against Candida species. According to some studies it is evident that specific antibodies against *Candida* species can perform this task by deactivating the factors responsible for virulence and therefore suppresses the potential fungal pathogen.

For a hyphal cell wall moiety, a specific monoclonal antibody inhibits germ tube formation. A study demonstrated the adherence inhibition of *C. albicans* to polystyrene by using monoclonal antibodies against antigens of cell wall of *C. albicans*. Other study showed the ability of monoclonal antibodies directed to stress mannoprotein present in the outer cell wall of *C. albicans* to perform three distinct activities, i.e. inhibition of adhesion, inhibition of germination and direct killing. These activities work against the pathogen and induce protection. The above-mentioned studies prove that antibodies may show some therapeutic effects and play role under natural of infection. Experimentations on animal models were also conducted to prove the protective role of antibodies.

In order to have effective protection, opsonisation and complement deposition are important factors in both types of vaccination models, i.e. active and passive models. To exert their antibiotic activity, antibodies need the presence of cellular effectors. But in case of immunocompromised patients, this can be a limiting factor. After the vaccination process, antibody production takes place by inhibiting enzyme activity and toxicity. There are many relevant antibodies that can neutralize the cognate adhesins. It is one of the virulent traits of various fungi.

The infectious dichotomy of mucocutaneous and disseminated form of candidiasis has led to very simple explanation of immunological response. T cell immunity is required to prevent mucocutaneous candidiasis and to avoid disseminated disease,

proper neutrophil functioning is required. The above observations were made on the basis of studies conducted on patients suffering from chronic mucocutaneous candidiasis. But these studies are limited in number, but in patients with rare anomaly of thymic aplasia which can reduce the population of T cells but results in predicting that with congenital thymic aplasia chronic mucocutaneous candidiasis can be induced in mice. In results, mice resisted the induction of mucosal disease and exhibits enhanced resistance towards disseminated candidiasis. Against mucosal candidiasis, T cells induce the immune response inside the host system. But in women with normal thymic functions, bouts of the vaginal candidiasis suggest that non-T cell factors play role in inducing the immune response. The models of mucosal candidiasis having high severity levels are not that much satisfactory and are very complicate the sorting out of immunological defects than the patients of chronic mucocutaneous candidiasis. In experimental studies on mice, the host defence against disseminated candidiasis is Thl-dependent cell mediated immunity. Like humans, mice are also susceptible to lethal disease when rendered neutropenic.

The anti-fungal treatment against candidiasis consists of a limited armamentarium primarily of less number of strategies depending on the differences between the eukaryotic cells of fungi and mammals. Since the last three decades in the treatment of fungal infections, we have witnessed the emergence of various efficient strategies. The treatment includes intake of amphotericin B formulations which targets the membrane ergosterol and made membrane spanning channels, various derivatives of azole having broad-spectrum activities like voriconazole, fluconazole, itraconazole, posaconazole and allylamines which can target the synthesis of ergosterol, inhibitors of cell wall echinocandins which include micafungin, caspofungin. These inhibitors can interfere with the synthesis of polysaccharide of cell wall, p-1, 3-glucan, flucytosine, which is an anti-metabolite of DNA or RNA synthesis.

The therapy success rates against mycoses, such as candiasis, are relatively very low and is very much restricted to issues like, toxicity and drug resistance respectively. There are many reasons which can make this disease fatal, such as, challenges during diagnosis, resistance of pathogen towards the drug and poor immunologic condition of patient respectively. Majority of patients suffering from muco-cutaneous diseases does not respond to anti-fungal drugs or medicines. But in patients undergoing invasive medical procedures, candidiasis can be prevented. For significant reduction of *C. albicans* population, anti-fungal treatment has become the most common practice. The notion of preventing various forms of candidiasis gained popularity when weekly therapy with fluconazole becomes successful by reducing the reoccurrence of vulvovaginal candidiasis in women.

The above discussion is related to concept of vaccination against fungal pathogens. But in past, this notion does not get proper attention due to the fewer occurrences of the fungal infections and limited distribution of geography for various fungi in comparison with bacterial and viral infections. The beginning of AIDS and extensive use of potential immunosuppressive therapies to overcome auto-immune disease, transplantation rejection, malignancies have gained interest in the development of effective vaccines against potent fungal pathogens. The fungal diseases are

now considered as major reasons for mortality and morbidity. An ideal vaccine should induce a long-lasting adaptive immune response and prevents the fungal invasion or the multiplication of the pathogen and does not cause any harmful effects to the host. The efficacy of the vaccine depends on the type of pathogen responsible for infection and immune-competence of host. While considering the range of candidal infections in humans, which have innate and adaptive type of immunity then immune protection is one of the major concerns.

Induction of active immunity in immune-compromised patients is precluded as they are vulnerable to mucocutaneous and disseminated candidiasis. In such patients, passive immunity can be used as a beneficial form of immunotherapy because it offers immediate protection to the host body. On the other hand, active immunity is beneficial in those patients whose defence system is immune-competent in nature but also vulnerable to candidiasis. The major targets of candidiasis are women, therefore for vaccination they are the best target subjects. The cells involved in protection against candidal vaginitis are not known. But to prevent vulvovaginal candidiasis, immune-prophylactic vaccine can come into play as it facilitates the transfer and colonization of *Candida* species in infants. The individuals having diabetic condition, denture wearers, having pre-disposing iatrogenic factors or hospital workers should benefit from active immunity. Irrespective of the type of immunity (innate or adaptive) that protects against *Candida*, there is a need to develop an immune-protective agent. But the development of vaccine against any infectious disease is very challenging task and it requires prior considerations. So, for initial considerations following is the rationale.

The arguments that favour the development of vaccine against *Candida* species are:

(a) On considering the problems related to public health, this disease causes sufficient morbidity and mortality.
(b) The currently available prevention and cure treatments are not that much efficient.
(c) On the basis of history of the disease, it can be stated that acquired immunity can only prevent or reduce the severity of the disease but cannot eradicate it from the host body.
(d) Aetiology of candidal infection is limited to a less number of closely related species and most of them can be affected by an appropriate formulation of antifungal vaccine.

There are many limitations in understanding the mechanisms responsible for protection against haematogenously disseminated candidiasis, but latest and beneficial information is surfacing. During clinical observations, neutropenia acts as a risk factor and to support these experimental studies on animal models of disseminated candidiasis were conducted. Deficiency of T cells or its dysfunction is not referred as risk factors. The animal models of disseminated disease prove that T cell dependent cell mediated immunity triggers the host defence system against candidiasis. The studies conducted on mice helps in understanding the mechanism behind protection mechanism which is important for *C. albicans* defence in these agents, but

the conclusions may be over-stated and misleading. Till date, there is no correlation found between occurrence of disseminated candidiasis in HIV/AIDS in humans and severity of T cell deficiency in cell mediated immunity. Specific antibodies, humoral factors, phagocytic cells, cytokines and cell mediated immune response are included in adaptive and innate immunity. If any element of the host defence system prevents the multiplication of fungal agent, then it reduces the deep invasion of the pathogen inside the organ systems and also reduces the morbidity related to mucosal tissue disease.

To develop an effective vaccine against *Candida* species, one needs to understand the dichotomy of *C. albicans* and the interaction of *C. albicans* with human beings. To explain the interaction between host and fungal agent there are two postulates. The first postulate is based on the idea that the pathogen is present as a latent pathogen inside the host system and is controlled by the immune system. If the environment inside the host body gets devastated, then occurrence of mucocutaneous and disseminated disease takes place. The idea behind this postulate arises from the transitory existence of colonization in healthy individuals by *C. albicans* and increased development of candidiasis in patients who are colonized before the immunosuppressive therapy. According to second postulate, the interaction of host and *C. albicans* is a mutualistic type of relationship. The host and pathogen derive reciprocal benefit from each other. If the state of host becomes debilitated, then the interaction favours to pathogen and facilitates its invasion.

In the development of fungal vaccine there are two different approaches. The first approach includes the complete removal of pathogen from the host body, i.e. immunoprophylaxis. The second approach involves induction and maintenance of immune response against controlled antigens that correlate with the diseased state, therefore permitting the maintenance of mutual interaction between the host and *Candida*.

By rejuvenating the immune system of host with liposomized immune-modulator, viz. Tuftsin tetra peptide along with intercalated formulation of Amphotericin B, disseminated candidiasis has been tackled in the mouse model. The cytosolic proteins of *C. albicans* were found in escheriosome based vaccines and this vaccine was found to be effective against disseminated candidiasis in mouse model (Swaleha Zubair et al. 2017).

22.11 *Aspergillus* Species

The fungal disease caused by this species is known as aspergillosis. For individuals having weak immune system, this disease can be life-threatening because they are easy targets of systemic fungal infections. Conventional chemotherapy does not show promising results. For better treatment, one should go for immune-stimulating agents along with anti-fungal drugs (Swaleha Zubair et al. 2017). The vaccines developed against *Aspergillus* can be categorized into four types. They are crude, sub-unit, pan fungal and therapeutic.

22.11.1 Pan Fungal Vaccines

The pan fungal vaccines take benefit from the antigens controlled by the fungal pathogen to fight against other fungal species. These vaccines catch the attention of commercial market because if they are formulated successfully, then they can protect the individual against different types of mycoses. In an experimental study, conjugation of diphtheria toxoid with β-1,3-D-glucan is done. Then this glycoprotein conjugate is injected to mice. As a result, antibody response is produced to β-1 3-D-glucan and it can protect the mice against *Aspergillus*. In another study, the mice were protected by immunizing them with heat killed *Saccharomyces cerevisiae*, from five different genera of fungi including species *Aspergillus*. The one possible mechanism behind the protection could be the stimulation of memory innate defences, which is also known as trained immunity. It was observed that β-1.3-D-glucans can induce the non-specific protection and epigenetic reprogramming against infections. In calnexin protein, T cell epitope was discovered, which is extremely conserved in the members of Ascomycota phylum. The researchers proved that calnexin is a surface protein and if it is injected in mice, then it results in the increase of antigen specific $CD4^{+}$T cells. To identify the proteins effective against *Aspergillus*, proteomic approaches were used.

22.11.2 Crude Vaccines

The crude vaccines contain the whole living or killed organism, i.e. *Ammophilus fumigatus* or fraction of pathogen derived from culture filtrates. But there is light possibility that it leads to incidence of auto-immune responses and excessive amount of reactogenicity due to the presence of antigens. In an experimental study, mice is immunized with live *A. fumigatus*, heat killed *A. fumigatus* or crude culture filtrate via intranasal inhalations. Then the mice were immune-suppressed by using cyclophosphamide and challenged with intravenous or intranasal administration of *A. fumigatus conidia*. In mice which were immunized with heat killed pathogen, increased survival rate was not there. On the other hand, mice that received live *A. fumigatus* or crude filtrate showed significant protection. $CD4^{+}$ T cells, IFN-γ and IL-2 were necessary for protection of the mice which lack IL-4, and show most resistance towards *A. fumigatus*.

The mice with live and not heat killed *conidia* can induce the production of IFN-γ and humoral responses. The heat killed conidia produce $CD4^{+}$ T cells, which further produce IL-4 and IL-13. The mechanism behind this remains speculative, but can be related to certain live conidia germinating into hyphae. It is the morphtype found in invasive disease. Hyphae stimulate the responses by pulmonary dendritic cells and a particular set of antigens.

22.11.3 Sub-Unit Vaccines and Monoclonal Antibodies

The Aspergillus sub-unit vaccines contain the purified form of one or more components of the pathogen. They were used to test on pre-clinical models. In aspergillosis models, recombinant *Aspergillus* proteins were used to produce vaccine mediated protection. These proteins include Asp f3, Asp f16, Asp f9 and Pep1. Purified forma of glycans from the cell wall can be used as immunogens. After the intranasal vaccination with α- and β-1,3-D-glucans, immune protection was observed. But same does not happen with galactomannan vaccination. Generally, T cells recognize peptides and not the carbohydrates. In a study it was observed that mannans can elicit protection against *A. fumigatus conidia*. If mannans are conjugated with bovine serum albumin, it shows much better response. Earlier, it was believed that antibodies protect against mycoses by initiating opsonisation and complement deposition.

22.11.4 Therapeutic Vaccines

In majority cases, where patients receive allogeneic haematopoietic transplants, after the recovery of neutrophil, the invasion of aspergillosis occurs. This suggests the importance of role played by T cell defences. T cells get expanded with antigens in ex-vivo environment and they injected back to the patient. In a study on mouse model, after the invasion of pathogen and transfer of *Aspergillus* specific $CD4^+$ T cells, the survival of mouse gets extended. This study lays the foundation to conduct study in humans, who are invading by *Aspergillus* following haplo-identical haematopoietic transplantation. T cells produce specific antibodies for antigens of *Aspergillus*.

The expansion of antibodies can be done by the incubation of heat killed conidia with blood mononuclear cells. After receiving immunotherapy, 9 out of 10 patients show positive results against the infection. For the expansion of T cell, a technique was developed, which was based on the activation-dependent expression of CD154 and CD137. After, which recombinant *A. fumigatus* is incubated with peripheral blood mononuclear cells? The generated T cell lines produce strong IFN-γ and IL-17 responses against the fungal species including *Aspergillus, Fusarium, Scedosporium, Candida* and *Mucorales*. The expansion of $CD8^+$ T cell clones and identification of antigens are responsible for the stimulation of human $CD4^+$ T cells. The genetically modified human T cells were used to express the B-glucan recognition receptor Dectin-1. These bioengineered T cells can be used to kill *A. fumigatus* germlines.

22.11.5 Systems Used for Delivery of Vaccine and Adjuvants

If the antigens are administered along with stimulatory adjuvants through delivery systems, it produces high immune response. B-1, 3-D-glucan, a carbohydrate antigen has very poor immunogenicity but its conjugation with protein carrier can enhance the response by antibodies. By using delivery systems and adjuvants, we can boost the natural immune response. In most of the commercial vaccines, alum is used as adjuvant, which elicit Th2 cell and antibody response. Other approach used dendritic cells transduced with adenovirus vector. If the mice are infected with dendritic cells pulsated heat killed *A. fumigatus*, then survival rate of the patient increases too many folds.

But too much labour is required in the manufacturing of dendritic vaccines and also its production is way much expensive for a large population. In other successful vaccine against *Aspergillus*, unmethylated CpG-rich oligonucleotides and TiterMax were used as adjuvants. The use of an appropriate adjuvant can induce a strong protective response for aspergillosis. The vaccines having adjuvants can be used in patients having allergies to elicit a balanced response (Stuart M. Levitz 2017) (Table 22.2).

Table 22.2 Problems which can occur during the development of vaccine against *Aspergillus* species

Problems during the development of vaccine	Possible solutions
Immunocompromised patients	• Infusion of donor lymphocytes • Improved forms of adjuvants • Target arms of defence system should be least affected • Vaccination prior to anticipated immune-suppression
Conduction of human trials after animal trials	• In-vitro studies along with phase one human studies • Usage of multiple animal models before conducting human trials
Allergic responses	• By shifting immune response to protective
Humans and fungi are eukaryotes	• Avoid using homologous protein sequences to reduce the chance of incidence of auto-immunity
Need of large number of patients	• Perform satisfactorily clinical trials
Glycosylation of protein by aspergillus	• To stimulate antibody protection, use native proteins
Commercialization	• Open bio-pharmaceutical companies • Try to attract interests through NGOs

Source: Stuart M. Levitz (2017)

22.12 *Cryptococcus* Species

C. neoformans is also one of the important opportunistic pathogenic species of fungi which can reside on both plants and animals. The teleomorph of this species is known as *Filobasidiella neoformans*, which is a filamentous fungus and belongs to Tremellomycetes class. It is often found in the excretions of pigeon. Its genomic sequence was published in 2005. According to recent studies, these fungi by growing on the remains of melted reactor of Chernobyl Nuclear Power Plant utilize the energy of primary beta radiations for radio-trophic growth. They grow as unicellular yeast and then multiply by budding process. During mating, the hyphae begins to form and at the telomeric end of hyphae develops into basidio spores, just before spore formation. If the host body has less glucose concentration, 5% carbon dioxide, serum, less iron content, then the cells start producing a specific capsule of polysaccharide.

When *C. neoformans* grows as yeast, it contains a bulbous capsule mainly made up of polysaccharides. Indian ink stain is used to visualize the capsule under microscope. The ink particles surround the yeast cell and does not penetrate inside the capsule and result in the formation of zone of clearance or halo near the cells. It helps in identifying the *C. neoformans*. On exposure by radiations like gamma-radiation, they show rapid growth. Such radiations enhance the electron transfer capacity of melanin pigment in the fungal species and hence results in increased metabolic activity.

The infection caused by *C. neoformans* in known as *cryptococcosis*. Such infections mostly result in lung infections. *C. neoformans* is the causative agent of fungal meningitis, which is a type of secondary infection in patients suffering from AIDS. Therefore, *C. neoformans* is considered as dangerous fungus. People having fully functioning immune systems are rarely infected by this fungus. It is a facultative intracellular pathogen and occasionally referred as opportunistic fungus.

When *Cryptococcus* does not affect the central nervous system, then it can be cured by in taking fluconazole. The person suffering from cryptococcal meningitis, then it should be treated with oral administration of flucytosine 100 mg/kg/day and intravenous administration of Amphotericin B 0.7–1.0 mg/kg/day for 2 weeks consecutively. Then the treatment should be followed by the intake of 200 mg of fluconazole for 10 days and then 200 mg of the same drug daily until the CD4 count of patient reaches above 100 for 3 months.

The patients who cannot tolerate Amphotericin B can use intravenous Ambisome 4 mg/kg/day instead. The oral administration of flucytosine is not that much effective and has many side effects. These do not cure the infection or kills the pathogen instead it only suppresses the fungal growth. Fungal remains viable and grows from cerebrospinal fluid of the patient. The data from Uganda suggests that 400 mg dosage of drug does not show better outcomes instead 1200 mg/day can be effective. The time duration of treatment and post treatment is not known.

C. neoformans leads to the occurrence of *cryptococcosis*, which is a type of primary pulmonary infection and can cause fatal type of meningitis. The detection of

capsular polysaccharide or isolation of yeast is done through serum or cerebrospinal fluid via latex agglutination test in order to diagnose the disease.

In culture filtrate, three species are defined as cryptococcal exo-antigens which are:

- High molecular weight glucuronoxylomannan.
- Mannoproteins.
- Galactoxylomannan.

In species like, Saccharomyces and Kluyveromyces, mannoproteins are the main structural and/antigenic elements of cell wall. The agglutination and immunological data supports the location of cell wall mononuclear phagocyte system on surface of the non-pathogenic yeasts. From the supernatant of culture containing pathogenic yeast *C. neoformans*, we can recover mononuclear phagocytes but only in small quantities. In cryptococcal infections, mononuclear phagocytes trigger the immune response as antibodies against them are found in the sera of patients affected by these infections. In a study on mice, it was observed that mononuclear phagocytes secreted by *C. neoformans* provoke a delayed and strong hypersensitivity response than capsular polysaccharide does. In cryptococcal cell, the cellular location and exact function of mononuclear phagocytes are not known. But on the basis of study mentioned above, it can be assumed that mononuclear phagocytes arise from the outer surface of cell wall.

To develop the prophylactic vaccines against *C. neoformans*, cytosolic proteins of the pathogen itself can be used. These proteins consist of T cell antigens and show immunogenic response and induction of antibodies inside the host system. The conversion of fibrinogen into fibrin takes place during blood coagulation, which is followed by the polymerization and formation of 3D network. Inside this network, many blood cells and plasma constituents are captured. The above process is catalysed by thrombin protein and cleavage of fibrinogen into small polypeptides takes place. These polypeptides further undergo aggregation and form the clot.

For the past many years, fibrin is used in antibiotic delivery systems; likewise, it can be used for antigen delivery system also. Through in-vitro experiments, fibrin clots can be made in different forms like glues, beads, foams or it can be combined with other polymers to form more complex formulations. When the cytosolic proteins vaccine entrapped in fibrin bead is immunized to mice, and then high antibody titre was observed in the sera of mice. This observation proves the effectiveness of fibrin as potential antigen delivery carriers.

The fibrin beads release the entrapped antigen slowly and therefore contribute in the activation of defence system of body. The antigen delivery mediated by Fib+PLGA-Cp results in upregulation of Type 1 cytokines which further facilitates the induction of CD8+ T lymphocyte response. Therefore, the higher levels of IFN-γ were obtained on immunization with Fib+PLGA-Cp (here Fib stands for fibrin, PLGA stands for poly-lactic-co-glycolic acid and Cp stands for cytosolic proteins) than the immunization with PLGA-Cp and Fib+Cp. The formation of both Th1 and Th2 cells occurs because of the use of fibrin based liposomized antigen. To optimize the activation of Th cells, the required conditions are T cell receptors

occupancy by presented major histocompatibility complex–antigens complex, particular co-stimulatory signals delivered by antigen presenting cells. The fibrin beads act as an ideal antigen delivery system because they are both biodegradable and biocompatible. The usage of bovine thrombin can cause many immunological complications. This problem arises the need of using the commercial fibrin, thrombin and factor 3 (Swaleha Zubair et al. 2017).

22.12.1 Coccidioides

The disease caused by this fungal species is known as coccidioidomycosis. It is also known as valley fever. It is generally caused by *Coccidioides immitis* and *Coccidioides posadasii*. It resides in soil and forms arthroconidia through hyphal growth. If the soil gets disturbed, then it becomes air borne and thereby infects humans or animals by entering inside the respiratory tract. Inside the respiratory tract and at the site of infected tissue, the arthroconidia becomes endosporulating spherules. Coccidioidomycosis has two clinical forms. One form is mild one and causes influenza-like infection. It leads to occurrence of other severe disease, which is followed by the resolution of the infection completely from host system. Then the defence system establishes the immunity to avoid reinfection by the pathogen.

The second form is a rare type. In this form, first the infection occurs and then it followed by chronic progressive disease or acute, fatal dissemination to the meninges, joints, bones, cutaneous tissues and subcutaneous tissues. This form is characterized by the generation of burrowing abscesses progressing to granulomatous reaction. After the patient recovers from coccidioidomycosis, he attains a lifelong immunity. To develop protective host resistance, vaccines are required so that it can induce response through type 1 helper T cell against coccidioidomycosis. In patients suffering from this disease, only 40% show symptoms and rest 60% remain asymptomatic.

For immune response to *Coccidioides*, T cell mediated immunity is important factor. In innate immunity, polymorphonuclear leukocytes can eliminate the pathogen and show effective results in inhibiting the growth of arthroconidia than mature spherules. To enhance the ability of host to kill arthroconidia, we can perform the in-vitro pre-treatment of macrophages with tumour necrosis factor-α and interferon-γ. Th2 response is considered as non-protective, whereas Th1 response is protective in nature. During the course of infection, *Coccidioides* antigens can induce antibodies in response, which are less beneficial as high antibody titres correlate with poor outcomes. Humoral immunity is also important in subsidizing to host resistance against fungal pathogen.

The subsets of particular antibody act like opsonins against endospores and arthroconidia. After the inhalation of arthroconidia, they reach to alveolus and interact with pulmonary dendritic cells. The dendritic cells further process the antigens, which migrate to lymph nodes and present antigen. They also activate the lymphocytes and lymphocytes move back to the site of infection and release inflammatory

cytokines to initiate a granulomatous response. If the response is insufficient, then arthroconidia undergoes some morphological alterations to spherules and endospores. These forms get the access to blood stream and result in dissemination. At the site of dissemination, antigen processing cells are recruited and repetition of pro-inflammatory response may happen to boost the response. Dendritic cells as antigen presenting cells process the antigen. Then it presents the epitopes of antigen to T cells.

Dendritic cells have pattern recognition receptors which can interact with pathogen associated molecular patterns, like β-glucan and mannoproteins and transduce signals for non-specific and early inflammatory responses. These pathogens associated molecular patterns are highly conserved and surface-exposed molecules. They include toll-like receptors, mannose receptors, Dectin-1 and complement receptor 3. In anti-fungal responses, toll-like receptor 2 and toll-like receptor 4 are involved. The interaction between pattern recognition receptors and pathogen associated molecular patterns triggers the initiation of a cascade of intracellular signalling, which leads to the formation of cytokines like interleukin-12 and interleukin-23, activation of T cells and their further differentiation into antigenic specific $CD4^+$ Th or $CD8^+$ T cells and the expression of anti-fungal activity by the humoral or cell mediated immunity.

Till now many vaccines have been developed against *Coccidioides*. The very first vaccine was made by using the killed vaccines. In animal models, formalin-killed spherule provides a strong protection but in phase 3 trials it failed in the reduction of severity of the disease due to insufficient dosing of the vaccine and the occurrence of side effects like local irritating pain. There is a need to eliminate the irritants and to preserve the immunogens by fractionation. The second vaccine uses the auxotrophic mutants or isolated pathogen with less virulence. Avirulent types are prepared by either deleting the gene mutants of a chitin synthase gene CHS5 or by generating double mutants by disrupting both CTS2 and CTS3. The avirulent types are unable to produce the endosporulate and produce good protection against pulmonary infection. These increase the survival rate but does not results in the complete elimination of the fungi from the infected tissue. The third preparation uses the live cell vaccines because of possible reversion to virulence of an attenuated mutant. The fourth preparation contains sub-cellular vaccines and proteins. Another preparation was prepared by using recombinant technology, which contains purified antigens. They include urease, Pmp1, gel1, SOW, ELI1, antigen 2/proline rich antigen, aspartyl protease, heat shock 60, Coccidioides specific antigen.

The candidates for protein vaccine including PRA, multivalent vaccine comprising of Pep1, Amn1 and P1b, chimeric protein containing CSA and PRA were proposed. The dendritic cells were also used to develop a vaccine. The transformation of dendritic cells was done with plasmid encoding for AG2/PRA protein and introduced inside the mice model before the occurrence of infection. It reduces the fungal burden and causes the induction of IFN-γ in high levels and causes pathology of less severity in mice.

The sixth formulation is glycan-protein conjugate vaccine. The glucan is an immune-modulator and it binds with Dectin-1 receptor to induce the immunity to

parasites, viruses, bacteria and fungi. This vaccine can enhance the process of phagocytosis and killing of antimicrobials, activated complement, increased production of IL-17, stimulated nitric oxide formation, primed spleen cells for production of cytokine, amplified activity of natural killer cells, increase in number of co-stimulatory responses, activation of dendritic cells, maturation of stimulated dendritic cells, mobilization of stem cells at the periphery, increased matrix metalloproteinases, induced haematopoiesis and anti-inflammation. Antigens are delivered by mannans to major histocompatibility complex. This can boost the maturation of dendritic cells, increase in surface expression of CD40 and increased generation of cytokines. The oral glucan can act as an immune-stimulant and induce resistance against the infection. The fungal species like *Aspergillus* and *Candida* share the glucan epitopes and with fungal antigens, glucans act like adjuvants.

Various studies reveal the importance of glycans in inducing a protective antifungal response and it can lead to stimulation of immune protection. Mannan is a pleiotropic immune-modulator which can bind to toll-like receptors and mannose receptors. It has species specific composition and structure. From the cell wall of Candida, mannose trisaccharide glycan-protein produces an antibody in response to both the elements and the required immune response against candidiasis. The effective way to develop a fungal specific vaccine is to produce conjugate vaccines which can combine the glycan to a specific immunogenic protein. Some proteins are cross-immunogenic in nature and if combined with appropriate glycan it can develop a pan fungal vaccine (Yoon and Clemons 2013).

The immunity induced against coccidioidomycosis by vaccine is a feasible method as asymptomatic infections with *Coccidioides* species are very rare. For a successful vaccine, T cell mediated immune protection is necessary by both Th1 and Th17. But there is no proof that in human system antibody produces immune response because there is no relation between disseminated coccidioidomycosis and acquired immunity. But in mouse models, there is conflicting evidence that whether B cells are required in immunity induced by vaccine. An ideal vaccine would prevent or reduce the expansion of infection. It should prevent he symptomatic infection and protect the patients which are at high risk by this disseminated disease (Kirkland and Fierer 2018).

22.13 Conclusion

Vaccines research and development against pathogenic fungi affecting human have gained impetus in recent times due to increased awareness of the medical threat due to invasive fungal infections and consistent failure of chemotherapy to reduce treatment lethality and fatality. Recent developments in the area of proteomics and genomics have motivated the scientific fraternity to strategize new models for vaccine development. Understanding of immunological response against fungi affecting human system, as well as selection of adjuvants which can stimulate innate immunity can help in formulating effective vaccine.

At present times, fungicides, growth inhibiting antibodies, antibody formulations have been proven as affective anti-fungal moieties. For example, echinocandin derived antimycotic acts as effective antibody formulation, by inhibiting enzymes like glucan synthase. The selection of right antibody isotype with same antigenicity against cell surface polysaccharides is very necessary. This is important criteria for passive vaccination therapy.

Elaborate knowledge of host's immune system upon fungus invasion coupled with the use of modern scientific technology is the first step in the developing effective strategy for vaccination. Development of immunological products for the effective diagnosis and treatment of invasive fungal infections can be made possible by understanding the concept of immunotherapy. Monoclonal antibodies and immune adjuvants are the best example of immunotherapeutic. Establishment of pan fungal antigen library could be helpful in the development of universal fungal vaccines. Furthermore, application of proteomics, genomics and vaccinomics using the modern scientific tools and techniques can be very helpful in the development of highly specific DNA vaccines and other important immunotherapeutic products.

References

Atkinson W, Iskander JK, Pickering LK, Schwartz B, Watson JC, Weniger BG (2002) General recommendations on immunization; recommendations of the advisory committee on immunizations practices (ACIP) and the American Academy of family physicians (AAFP). MMWR Recomm Rep 51:1–35

Barchiesi F, Orsetti E, Osimani P, Catassi C, Santelli F, Manso E (2016) Factors related to outcome of bloodstream infections due to *Candida parapsilosis* complex. BMC Infect Dis 16:1–7

Borghi M, Renga G, Puccetti M, Oikonomou V, Palmieri M, Galosi C et al (2014) Antifungal Th immunity: growing up in family. Front Immunol 5:506

Carvalho A, Cunha C, Bozza S, Moretti S, Massi-Benedetti C, Bistoni F et al (2012) Immunity and tolerance to fungi in hematopoietic transplantation: principles and perspectives. Front Immunol 3:156

Cassone A, Casadevall A (2012) Recent progress in vaccines against fungal diseases. Curr Opin Microbiol 15:427–433

Conti P, Tettamanti L, Mastrangelo F, Ronconi G, Frydas I, Kritas SK et al (2018) Impact of fungi on immune responses. Clin Ther 40:885–888

Dambuza IM, Levitz SM, Netea MG, Brown GD (2017) Fungal recognition and host defense mechanisms. Microbiol Spectr 5:887–902

De Amorim J, Magalhaes A, Munoz JE, Rittner GM, Nosanchuk JD, Travassos LR et al (2013) DNA vaccine encoding peptide P10 against experimental paracoccidioidomycosis induces long-term protection in presence of regulatory T cells. Microbes Infect 15:181–191

De Bernardis F, Amacker M, Arancia S, Sandini S, Gremion C, Zurbriggen R et al (2012) A virosomal vaccine against candidal vaginitis: immunogenicity, efficacy and safety profile in animal models. Vaccine 30:4490–4498

Dehghan P, Tolouie S, Baradaran B, Nami S, Morovati H (2018) TLR-2, IL-10 and IL-17-mediated immunity in experimental chemotherapy murine model of systemic candidiasis; cyclophosphamides' impact and roles. Microb Pathog 119:183–192

Dühring S, Germerodt S, Skerka C, Zipfel PF, Dandekar T, Schuster S (2015) Host-pathogen interactions between the human innate immune system and *Candida albicans*—understanding and modeling defense and evasion strategies. Front Microbiol 6:625

Edwards Jr, John E (2012) Fungal cell wall vaccines: an update. J Med Microbiol 61:895

Górski A (1984) Monitoring of the humoral response in clinical immunology. Pol Arch Med Wewn 71:145–148

Gunsalus KT, Tornberg-Belanger SN, Matthan NR, Lichtenstein AH, Kumamoto CA (2016) Manipulation of host diet to reduce gastrointestinal colonization by the opportunistic pathogen Candida albicans. mSphere 1:1

Hajishengallis G, Reis ES, Mastellos DC, Ricklin D, Lambris JD (2017) Novel mechanisms and functions of complement. Nat Immunol 18:1288–1298

Hogan DA, Vik A, Kolter R (2004) A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol Microbiol 54:1212–1223

Hsieh E, Smith PB, Jacqz-Aigrain E, Kaguelidou F, Cohen-Wolkowiez M, Manzoni P et al (2012) Neonatal fungal infections: when to treat? Early Hum Dev 88:S6–S10

Huffnagle GB, Noverr MC (2013) The emerging world of the fungal microbiome. Trends Microbiol 21:334–341

Iannitti RG, Carvalho A, Romani L (2012) From memory to antifungal vaccine design. Trends Immunol 33:467–474

Iliev ID, FunariVA TKD, Nguyen Q, Reyes CN, Strom SP et al (2012) Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science 336:1314–1317

Kennedy MJ, Volz PA (1985) Ecology of *Candida albicans* gut colonization: inhibition of *Candida* adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. Infect Immun 49:654–663

Kim YG, Udayanga KGS, Totsuka N, Weinberg JB, Núñez G, Shibuya A (2014) Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE2. Cell Host Microbe 15:95–102

Kirkland TN, Fierer J (2018) Coccidioidesimmitis and *posadasii*; a review of their biology, genomics, pathogenesis, and host immunity. Virulence 9:1426–1435

Kolwijck E, van de Veerdonk FL (2014) The potential impact of the pulmonary microbiome on immunopathogenesis of *aspergillus*-related lung disease. Eur J Immunol 44:3156–3165

Levitz SM (2017) Aspergillus vaccines: hardly worth studying or worthy of hard study? Med Mycol J 55:103–108

Matsuzaki G, Umemura M (2018) Interleukin-17 family cytokines in protective immunity against infections: role of hematopoietic cell-derived and non-hematopoietic cell-derived interleukin-17s. Microbiol Immunol 62:1–13

Medici NP, Del Poeta M (2015) New insights on the development of fungal vaccines: from immunity to recent challenges. Mem Inst Oswaldo Cruz 110:966–973

Morovati H, Tolouei S, Dehghan P, Baradaran B, Mohammadifard A, Yousefi Z (2016) The effect of cyclophosphamide on *TLR2* gene expression in Balb/c mice with systemic candidiasis. J Isfahan Med Sch 34:771–776

Nanjappa SG, Klein BS (2014) Vaccine immunity against fungal infections. Curr Opin Immunol 28:27–33

Noverr MC, Huffnagle GB (2004) Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. Infect Immun 72:6206–6210

Oever JT, Netea MG (2014) The bacteriome–mycobiome interaction and antifungal host defense. Eur J Immunol 44:3182–3191

Patin EC, Thompson A, Orr SJ (2019) Pattern recognition receptors in fungal immunity. In: Gordon D, Brown, Duncan W (eds) Seminar in cell & development biology, vol 89. Academic Press, pp 1–16

Pflughoeft KJ, Versalovic J (2012) Human microbiome in health and disease. Ann Rev Pathol Mech 7:99–122

Polesello V, Segat L, Crovella S, Zupin L (2017) Candida infections and human defensins. Protein Pept Lett 24:747–756

Rappuoli R, Pizza M, Del Giudice G, De Gregorio E (2014) Vaccines, new opportunities for a new society. Proc Natl Acad Sci U S A 111:12288–12293

Romani L (2011) Immunity to fungal infections. Nat Rev Immunol 11:275–288

Romani L, Zelante T, Palmieri M, Napolioni V, Picciolini M, Velardi A et al (2015) The cross-talk between opportunistic fungi and the mammalian host via microbiota's metabolism. Semin Immunopathol 37:163–171

Roy RM, Klein BS (2012) Dendritic cells in antifungal immunity and vaccine design. Cell Host Microbe 11:436–446

Salazar F, Brown GD (2018) Antifungal innate immunity: a perspective from the last 10 years. J Innate Immun 10:373–397

Schaller JG (1975) Immunodeficiency and autoimmunity. Birth Defects Arig Artic Ser 11:173–184

Snarr BD, Qureshi ST, Sheppard DC (2017) Immune recognition of fungal polysaccharides. J Fungi 3:47

Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C et al (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med 20:159–166

Verma A, Gaffen SL, Swidergall M (2017) Innate immunity to mucosal Candida infections. J Fungi 3:60

Wormley FL, Perfect JR, Steele C, Cox GM (2007) Protection against *cryptococcosis*by using a murine gamma interferon-producing *Cryptococcus neoformans* strain. Infect Immun 75:1453–1462

Yoon HJ, Clemons KV (2013) Vaccines against *Coccidioides*. Korean J Intern Med 288:403–407

Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G et al (2013) Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 39:372–385

Zubair S, Azhar A, Khan N, Ahmad E, Ajmal M, Owais M (2017) Nanoparticle-based mycosis vaccine. In: Markus K, Margarita S (eds) Vaccines for invasive fungal infections. Springer, pp 169–211

Chapter 23
Bioprospecting for Biomolecules from Industrially Important Fungi: Current Research and Future Prospects

Ajar Nath Yadav, Tanvir Kaur, Rubee Devi, Divjot Kour, Neelam Yadav, Ahmed M. Abdel-Azeem, Ashok Yadav, and Amrik Singh Ahluwalia

Contents

A. N. Yadav (✉) · T. Kaur · R. Devi · D. Kour
Department of Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

N. Yadav
Gopi Nath P.G. College, Veer Bahadur Singh Purvanchal University, Ghazipur, Uttar Pradesh, India

A. M. Abdel-Azeem
Botany and Microbiology Department, Faculty of Science, University of Suez Canal, Ismailia, Egypt

A. Yadav
Department of Botany, Institute of Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

A. S. Ahluwalia
Department of Botany, Akal College of Basic Sciences, Eternal University, Baru Sahib, Sirmour, Himachal Pradesh, India

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8_23

23.1 Introduction

Industrial sector is one of the three sectors (agriculture and environment) on which human livelihood and country economy depend. This is also known as secondary sector. Industries are of certain types such as brewing, pharmaceutical, textile, automobile, steel, and many others that produce or manufacture various types of products like food products, medicines, and cosmetics, steel, automobile, and cloth. These products have become basic and major necessity of the humans. Since eighteenth century industries have totally swap the life of humans being, but on the same hand industries also transpose the condition of the environment in a negative way. Industries, the major emitter of greenhouse gas (GHG) are completely ruin the condition of the earth and arose the problems like global warming, pollution and loss of air, water and soil quality, and biodiversity due to the use of harmful chemicals. Now keeping in view of earth health also UN 2030 have assign technologies to be used in the industries for the sustainable development (Fritzsche et al. 2018).

In twentieth century, natural products or biomolecules are now been used empirically as before the setting of industries. Biomolecules are being used in industries like pharmaceutical, cosmetics, food and textile industries for the goods production. Natural products are either obtained from the plant sources or from other organisms like animals and microbes. Microbes like bacteria and fungi are known to be the one of the best sources of the natural products that can be industrially exploited. Fungal biomolecules are currently used in many industries at large scale for the product formation. According to review article, fungi can be exploit in 50 different ways in the industries (Hyde et al. 2019) as fungi have an ability to produce different sort of biomolecules like secondary metabolites, enzymes, organic acids, pigment, and fatty acid.

Fungal biomolecule, secondary metabolites are produced by the fungi for themselves to survive in the harsh conditions. The release of such biological molecules has wide array of applications in industries such as pharmaceuticals as many secondary metabolites exhibit property of inhibiting the growth of predators and pathogens (Devi et al. 2020). Another fungal biomolecule, enzymes are also one of the significant industrial raw material. Enzymes also known as catalyst are used to fast up the chemical as well as the biological reaction to the product formation. Fungi are known to produce various sorts of enzymes such as amylase, laccase, cellulose, protease, pectinase, and xylanase that majorly used in the food as well as pharmaceutical industries (Singh et al. 2016; Tuli et al. 2015; Yadav et al. 2016). The appearance in the first thing is to observe and to make look good earlier different color of chemically synthesized dyes that were being used which are known as environmental hazard (Fig. 23.1).

Fungal biomolecule, pigments also replaced the synthetic dyes. These natural pigments produced by fungi are used as natural coloring agent in the industries now (Copetti 2019; Rana et al. 2019a, b). Further, another known type of biomolecule, i.e., organic acid is also having significant applications in industries. Organic acids like citric acid, lactic acid, itaconic acid, fumaric acid, and succinic acid are used as

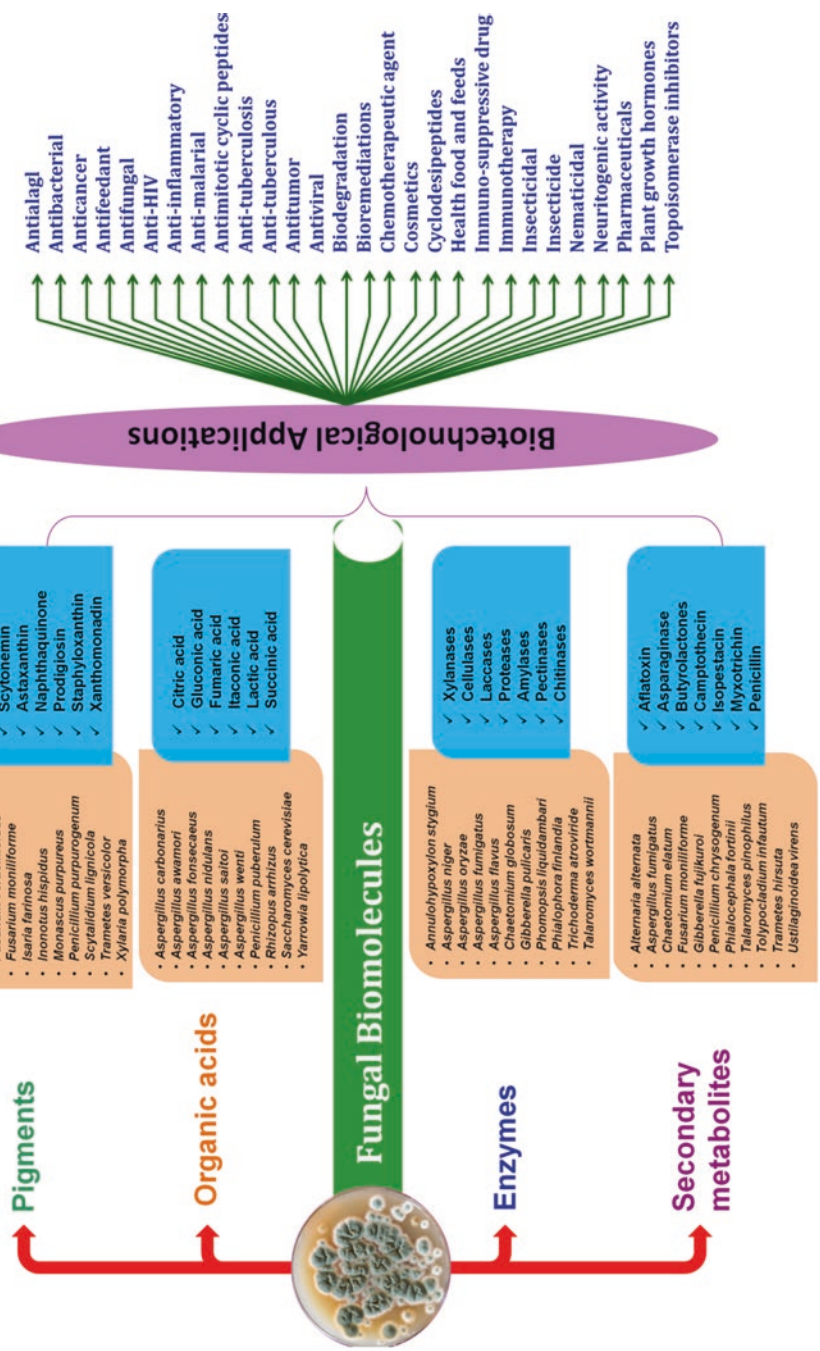


Fig. 23.1 Bioprospecting for biomolecules from industrially important fungi

pH adjuster, taste enhancer, and many other (Hyde et al. 2019; Yadav et al. 2019c). These all biomolecules have great biotechnological applications also in both agriculture and environments. The present chapter complies the different industrially important fungal biomolecules and their role. Moreover the biotechnological applications also have been detailed.

23.2 Types of Fungal Biomolecules

Fungi, an important part of the ecosystem are known to produce several types of biomolecules which have great industrial applications. Secondary metabolites, enzymes, pigments, organic acids, and fatty acid are some of the biomolecules types (Singh and Yadav 2020; Yadav 2019). This all types of biomolecules have numerous applications in industries like cosmetic, food, pharmaceutical, textile, and biorefineries.

23.2.1 Secondary Metabolites

Secondary metabolites are one of the types of biomolecule which are organic in nature. This compound plays a significant role in the industries, environment as well as in agriculture. In industries, these compounds are mostly used in the production drugs in pharmaceutical industries as some of the metabolites have antimicrobial, antiviral, and anticarcinogenic activity (Rastegari et al. 2019a; Rastegari et al. 2019b; Yadav et al. 2019a). On the other hand, secondary metabolites in agro-environment can be used to control the pest on the crops and increase crop yield without harming the environment system. Secondary metabolites are produced by fungi especially endophytic fungi. Fungi are the rich source of thousands of secondary metabolites and some are polyketides, terpenes, and nonribisomal peptides (Devi et al. 2020). Many studies have reported different strains of fungi that have produced several secondary metabolites types. In a study, an endophytic fungus named, *Allantophomopsis lycopodina* was reported for the producing a new α-pyrone metabolite, i.e., Allantopyrone A. This secondary metabolite was known to have cytotoxicity (Shiono et al. 2010).

Endophytic fungi isolated from *Picea rubens* (red spruce) were reported for the production of the various sort of metabolites, namely 15-Hydroxy-3-epiisopetasol, 7-(4-But-1-enyl-2,5-dioxo-2,5-dihydro-furan-3-ylmethyl)-4,5- dicarboxy-non-5-enoic acid and 7-(4-But-1-enyl-2,5-dioxo-2,5-dihydro-furan-3-ylmethyl)-4,5-dicarboxy-non-5-enoic acid. These metabolites were reported for having antimicrobial activity against *Choristoneura fumiferana* (Sumarah et al. 2010). In another investigation, endophytic fungi, *Massrison* sp. isolated from terraneous plant was reported for releasing secondary metabolite Massarigenin D, Spiromassaritone, Paecilospirone, Griseofulvin, and Ketoconazole. These all fungal

metabolites were reported for having an antifungal activity against pathogens like *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, and *Trichophyton rubrum*. Along with antifungal activity, these compounds were also having cytotoxicity activity (Sun et al. 2011). Two bioactive metabolites, benzopyranones and diaportheone A were obtained from endophytic fungi, *Diaporthe* sp. isolated from *Pandanus amaryllifolius* leaves. These compounds were recognized for the antimicrobial activity in the study against *Mycobacterium tuberculosis* (Bungihan et al. 2011).

In a study, secondary metabolites, namely 8-dihydroramulosin, dechlorogriseofulvin, griseofulvin, and mellein were isolated from the endophytic fungus, *Nigrospora* sp. of medicinal plant, *Moringa oleifera*. These metabolites were shown to have antifungal activity against eight pathogenic fungal cultures, i.e., *Botrytis cinerea, Colletotrichum orbiculare, Fusarium oxysporum* f.sp. cucumerinum, *F. oxysporum* f.sp. melonis, *Pestalotia diospyri*, *Pythium ultimum, Rhizoctonia solani,* and *Sclerotinia sclerotiorum* (Zhao et al. 2012). In another report, two fungal endophytes from Panax ginseng roots identified as *Penicillium melinii* and *P. janthinellum* were known to produce secondary metabolites, namely methyl 2,4-dihydro xy-3,5,6-trimethylbenzoate, 3,4,5-trimethyl-1,2-benzenediol, brefeldin A penicillic acid, ergosterol, ergosterol peroxide, and mannitol (Zheng et al. 2013). Secondary metabolite, oosporein known to have characteristic property of antioxidant, antimicrobial, and cytotoxic was isolated from endophytic fungus named *Cochliobolus kusanoi* from *Nerium oleander* L. (Alurappa et al. 2014).

Another secondary metabolite, 3-O-methylfunicone, which was found as efficient cytotoxic against pea aphid *Acyrthosiphon pisum* was obtained from a strawberry endophytic fungi named *Talaromyces pinophilus* (Vinale et al. 2017). In another investigation, a fungal endophyte *Phialemonium curvatum* from *Passiflora edulis* was reported for producing bioactive compounds including uracil, uridine, glycerol, 3-indole acetic acid (IAA), and 4-hydroxybenzoic acid. All these secondary metabolites were having antifungal activity against fungus *Cladosporium cladosporioides* (Rathnayake et al. 2018). Fungal endophyte of *Rauwolfia macrophylla,* namely *Curvularia* sp. was also reported for producing secondary metabolite, i.e., 2′-deoxyribolactone, hexylitaconic acid, and ergosterol which are efficient antioxidants and also exhibits antimicrobial activity (Kaaniche et al. 2019). Antimicrobial metabolites, Cladosporin, Isocladosporin, 5′-hydroxyasperentin, Di (2-ethylhexyl) phthalate, 1-acetyl-17-methoxyaspidospermidin-20-ol, and 3-phenylpropionic acid are efficient in inhibiting the growth of phytopathogens. Among all the metabolites 3-phenylpropionic acid was most efficient and all these strains were obtained from a fungal endophyte *Cladosporium cladosporioides* isolated from plant *Zygophyllum mandavillei* (Yehia et al. 2020). Metabolites, epidithiodiketopiperazine and pretrichodermamide were also reported in a study to be produced by fungi known as *Trichoderma harzianum* and *Epicoccum nigrum* which are the fungal isolates of plant *Zingiber officinale* and *Salix* sp., respectively (Harwoko et al. 2021).

23.2.2 Enzymes

Fungal enzymes have been widely appreciated worldwide for their extensive uses in a variety of sectors, including pharmaceutical, agricultural, chemical, food and petroleum industries. These enzymes are used in the field of pharmaceutical, and are used to treat health associated with the lack of human enzyme due to genetic problem (Abdel-Azeem et al. 2021; Singh et al. 2016). These processes mediated by enzymes are accelerating the food, pharmaceutical, textile, leather, paper, detergent, wastewaters treatment, and petroleum industries; these industries are gaining interest due to some advantages like reduced process time, cost effective, nontoxicity, higher quality products, greater efficiency, less energy input intake and eco-friendly. Industrial enzymes carried high molecular weight of compounds. The most widely used enzymes of operational importance (a-amylase, cellulose, and xylanase) are derived from fungi of *Aspergillus* (Shekher et al. 2011). In order to produce purified and well characterized enzyme on a large scale, fermentation processes have been optimized through the use of specially selected *Aspergillus* strains. Different types of industrial fungal enzymes are available that are used in diverse industrial areas for different purposes.

23.2.2.1 Xylanases

The B-1,4-glycosidic bonds of xylem, the major plant cell wall polysaccharide components of hemicelluloses, were randomly hydrolyzed by xylanases. Xylan has a complex structure in the backbone consisting of β-1,4-linked xylose residues to which short side chains are attached with O-acetyl, alpha-L-arabinofuranosyl, D-alpha-glucuronic, and phenolic acid residues (Coughlan and Hazlewood 1993). The xylem cell wall is the main component of hemicellulose, composed of both hardwood and monocot. Currently, xylan degrading enzymes have gained much essential in the industrial dealing with agriculture, food, pulp, paper, and textile. A number of microbes consisting primarily of fungi and bacteria are reported to produce xylanases which can randomly degrade β-1,4-xylan resulting in a series of oligosaccharides fragments that are linear and branched. Xylenes has specific applications in the upgrading of jute fiber (Knob et al. 2010). When grown on xylan as the substrate, fungi have been reported to be a good producer of xylanases. These have potential function in various industrial processes like improving animal feedstock digestibility and juice clarification, facilitating the release of lignin from pulp, and reducing the amount of chlorine required for paper and pulp bleaching (Saxena et al. 2015).

23.2.2.2 Cellulases

Cellulose is one of the most copious renewable polymers composed of p-1, 4 linked glucose molecules. The hydrolysis of cellulose is the responsibility of endoglucanases and cellobiohydrolases, also called cellulases. Cellulases have a complex system of enzymes involving endo-1, 4-D-D-glucanase, exo-1,4-β-glucanase, and exo-1,4-D-glucosidase. These enzymes together with hemicelluloses and pectinases are used to process lignocellulosic material (Nigam and Singh 1995). However, cellulose and xylanases are used in several other fields including in the textile industries for fiber treatment processes. Cellulases are synthesized by various cellulolytic filamentous fungi such as *Aspergillus*, *Chaetomium*, *Fusarium*, *Myrothecium*, *Penicillium,* and *Trichoderma* species (Yadav et al. 2018). Cellulases and cellulosomes used for cellulose biodegradation produced via various microbes have attracted researchers' interest in finding an eco-friendly tool in several agricultural and waste treatment processes and have been widely used to produce bio-based sustainable products and bioenergy to replace fossil fuels that are depleted. In this research, endophytes such as *Annulohypoxylon stygium*, *Aspergillus niger*, *Alternaria* sp., *Trichoderma atroviride,* and *Talaromyces wortmannii* produced the hemicellulases and other enzymes appropriate for lignocellulosic biomass degradation (Robl et al. 2015).

23.2.2.3 Laccases

Laccase is a type of diamine oxidizing copper-containing polyphenol oxidase, methoxy-substituted phenols, and a substantial range of other compounds. Laccases are polyphenol oxidases glycosylated. These enzymes have significant commercial application in the pulp and paper industries, biotransformation in animal biotechnology, and detoxification of phenolic pollutants. The production of laccase is a common feature of many basidiomycete fungi, especially those associated with and involved in wood decay or terminal decomposition phases. The genus *Trametes* appears to be one of the most effective producers of laccases. Endophytes are a rich and reliable source of bioactive compound with graits potential for medicine, agriculture, and industries. Enzymes like pectinases, xylanases, cellulases, lipases, proteinases, and phenoloxidases, are produce by fungi. These enzymes are essential for penetrating cellwal of host and colonizing inside tissue of plant host (Schulz et al. 2002). In this study, El-Zayat (2008) reported the laccase production from *Chaetomium globosum* isolated from *Glinus lotoides*. Another study (Chen et al. 2011) reported the laccase production from fungi *Pestalotiopsis* sp. isolated from sea mud collected from East China Sea under submerged/solid-state fermentation using diverse lignocellulosic through products as substrates. The fungi *Phomopsis liquidambari* produces ligninolytic enzymes laccase and lignin peroxidase that grow in the presence of submerged fermentation on phenolic 4-hydroxybenzoic acid as the sole carbon and energy (Chen et al. 2013).

23.2.2.4 Proteases

Proteases enzymes are also referred to as proteinases, proteases, and peptidases. These have ability to hydrolyze peptide bond in protein molecules. They are used in several industries and industrial processes like wine and dairy industries for the cheese production, beer preservation, baking industries, meat tenderization, and several other fields like textiles and leather industries and as a preservative to detergents (Vishwanatha et al. 2010). These enzymes have been categorized into endopeptidases and exopeptidases and have been obtained from diverse group of the organism like animals, plants, bacteria, and fungi (Singh and Kumar 2019). At the industrial level, fungal proteases are utilized. Fungi are known to exude intracellular as well as extracellular proteases under solid-state and sub-merged fermentation process. In this study (Singh and Kumar 2019) reported *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger,* and *Aspergillus oryzae* are recognized to be the best sources of protease enzyme. Proteases are used for cheese ripening acceleration and milk proteins modification to reduce the allergenic properties of cow milk products for infants (Qureshi et al. 2015).

23.2.2.5 Amylases

Amylase is another important enzyme used in industries, accounting for about 25% of the worldwide market of enzyme. The a-amylase (a-1,4-glucan-4-glucanohydrolase) belongs to a family of endo-amylases which catalyze the initial hydrolysis of starch into shorter oligosaccharides via the separation of a-D-(1–4) glycosidic bonds. Amylase from fungal sources is widely used in industries because they are more stable than amylases from plants and animals. *Aspergillus oryzae* and *Aspergillus niger* produce a large amount of extracellular enzyme, and amylases are of chief industrial significance. An amylase has been the most common application in the industry for the starch liquefaction process, which converts starch into fructose and glucose syrup. Most amylase has been produced from soil fungi such as *Aspergillus*, *Penicillium,* and *Rhizopus* (Pandey et al. 2000). Caldwell et al. (2000) reported fungi *Phialophora finlandia* and *Phialocephala fortinii* isolated from alpine plant communities its ability to degraded the main polymeric forms of carbon nitrogen and phosphorus found in plants. Another study, (Marlida et al. 2000) fungi *Gibberella pulicaris*, *Acremonium* sp., *Synnematous* sp., and *Nodulisporium* sp. are known to produce starch degrading enzyme. These fungal amylases are used in the process of fermentation, detergent, fuel/alcohol production, processed food or feed pharmaceutical, textile, and paper industries (Sanchez and Cardona 2008).

23.2.2.6 Pectinases

Pectinase is an enzyme that breaks down and converts into pectin which is found in the cell wall of the plant as polysaccharide. This enzyme has shown a robust increase in the market and has also held a leading position among industrial enzyme produced commercially. This enzyme plays an important role in decreasing viscosity and improving yields in the industrial sector. The enzyme helps to eliminate the cloudiness of the juice and stabilize it during citrus juice processing. In the wine process, pectinase is used to promote filtration, increase juice yield, and strengthen flavor and color. Additionally, pectinases used to hydrolyze pectin.

Pectinases used to hydrolyze pectin are also present in biorefineries agro-industrial waste. This agro-waste converted into simple sugars and bioethanol, or fermentable sugars can also be used (Garg et al. 2016; Karthik et al. 2014). In this study, Sunitha et al. (2013) reported fungi *Xylaria* sp., *Talaromyces emersonii*, *Pyllosticta* sp., *Phoma* sp., *Pestalotiopsis disseminata*, *Paecilomyces variotii*, *Nigrospora sphaerica*, *Fusicoccum* sp., *F. oxysporum*, *Fusarium chlamydosporum*, *Drechslera* sp., *Cylindrocephalum* sp., *Coniothyrium* sp., *Colletotrichum gloeosporioides*, *Acremonium implicatum*, *Aspergillus fumigatus*, and producers pectinases enzyme isolated from *Alpinia calcarata*, *Bixa orellana*, *Calophyllum inophyllum*, and *Catharanthus roseus*. Another study (Fouda et al. 2015) reported pectinases producing fungi like *Alternaria alternata*, *Penicillium chrysogenum* isolated from medicinal plant of *Asclepias sinaica*. In addition, Uzma et al. (2016) reported pectinases enzyme producing fungi *Rhizopus* sp., *Phomopsis* sp., *Phoma* sp., *Penicillium* sp., *Mucor* sp., *Fusarium* sp., *Colletotrichum* sp., *Cladosporium* sp., *Aspergillus* sp. (Kaul et al. 2013; Kirti and Reddy 2013).

23.2.2.7 Chitinases

Chitin, a linear homopolymer of β-1,4-linked N acetyl glucosamine, is a component of the exoskeleton of crustacean insects and shells and forms the basic structural component of the fungal wall. Chitinase is an enzyme that degrades this insoluble polymer, also known as chitinolytic enzymes. Fungal chitins play an important role in the ecosystem by degrading and carbon cycling and nitrogen material in chitin (Verma et al. 2015). Chitinase producing fungi also being studied for their potential in biocontrol of nematodes and pathogenic fungi (Gan et al. 2007; Klemsdal et al. 2006). Plant also produces chitinase as a defence response to infection by pathogens (El Gueddari et al. 2002). Chitinase products have many desirable properties and are used to control microbes, tumors, wound healing, wastewater treatment, and drug delivery (Dai et al. 2010). Chitinase has been reported from the fungi of *Neotyphodium* sp. *Colletotrichum musae* (Borges et al. 2009). In this study El Gueddari et al. (2002) reported chitinase producing fungi *Colletotrichum acutatum*, *Fusarium* sp., *Phomopsis* sp., and *Phyllosticta capitalensis* isolated from different plant hosts. Another study, the chitinase produces fungal enzymes like *Trichoderma*

harzianum, *Trichoderma flavofuscum*, and *Trichoderma viride* (Lunge and Patil 2012).

23.2.3 Pigments

Pigments are the chemical structure which is used as a coloring agent in industries of cosmetics, food, and drugs. Earlier, pigments were chemically synthesized in the industries, however, there use had ban due to their allergenic and carcinogenic nature (Tuli et al. 2015). Since there, intense researches were triggered to found the alternative strategy for the pigment production and found microbes as a promising source of pigments. Among microbes, many fungal species were reported for producing the pigments. Fungal pigments are the type of secondary metabolites which is now commercially used (Akilandeswari and Pradeep 2016). In a study, five different fungal isolates, namely *Emericella nidulans, Fusarium verticillioides, Isaria farinosa, Monascus purpureus*, and *Penicillium purpurogenum* were reported to produce pigments (Velmurugan et al. 2010).

Monascus purpureus was also reported in another study for producing pigment of yellow, orange, and red color (Kongruang 2011). In a study, five pigment producing fungi, namely *Arthrographis cuboidea, Inonotus hispidus, Xylaria polymorpha*, and *Trametes versicolor* used to induce color in American beech woods for decorative purpose. Among all the strains, *T. versicolor* were found to efficient pink producing pigments in the beech blocks (Robinson et al. 2012). In another investigation, *Fusarium moniliforme* from paddy field soil was found to be efficient producer of natural pigment (Pradeep et al. 2013). In 2014, a study reported three different fungi, namely *Scytalidium lignicola*, *S. ganodermophthorum,* and *Inonotus hispidus* for producing yellow coloration pigments (Robinson et al. 2014).

Fungal species identified as *Fusarium* sp. were reported for producing bioactive pigments (Mani et al. 2015). Another report has published marine sponge fungi, i.e., *Trichoderma pararessei* for producing yellow pigment and also this strain has reported for inhibiting the growth of *Salmonella typhi* and *Escherichia coli* (Sibero et al. 2016). Fungal species of genera from marine ecosystem, i.e., *Penicillium, Talaromyces,* and *Aspergillus* were reported for producing pigments (Fouillaud et al. 2017). *Penicillium* sp. isolated from Indian Himalayan region was reported for producing orange coloration pigments (Pandey et al. 2018). In another investigation *Aspergillus chevalieri* was reported for producing red pigments (Palacio-Barrera et al. 2019). Another species of *Penicillium,* i.e., *P. mallochii* was also reported for producing orange red pigments (Bouhri et al. 2020).

23.2.4 Organic Acids

Organic acid is another biomolecule to be known to be produced by the fungi, which contains functional group such as having acidic properties such as alcohol, carboxyl, sulfonic, and thiol groups (Yin et al. 2015). This type of biomolecules has a vast array of applications in various fields like agriculture and industries like cosmetics, pharmaceuticals, food and beverages. Fungi are known to produce several types of organic acids such as citric acid, gluconic acid, fumaric acid, itaconic acid, lactic acid, and succinic acid (Hyde et al. 2019).

23.2.4.1 Citric Acid

A tricarboxylic acid, citric acid ($C_6H_8O_7.H_2O$) is one of the common plant and animal metabolites which is present in citrus fruits like lemon, pineapple, oranges, and many more (Angumeenal and Venkappayya 2013). Citric acid is one of the important and versatile acids, which is used in food and beverages, pharmaceutical and cosmetic industries. In food industries, citric acid is extensively used production of cheese and antimicrobial agents. Furthermore it is used as pH adjuster; acidulate in jam and jellies, flavoring agent and emulsifier in ice cream (Copetti 2019). In pharmaceutical and cosmetics industries, citric acid is used as antioxidant additive, buffering agents with metal ion chelating abilities (Lee and Arepally 2012; Soccol et al. 2006).

Citric acid production is now commercially produced by the fungal species *Aspergillus niger*, but various other species are also reported for its production. In a report published, a yeast named *Yarrowia lipolytica* was recognized for the production of citric acid from rapeseed oil (da Silva et al. 2012; Kamzolova et al. 2011). In 2013, *Candida guilliermondii* was reported as an effective species for the production of citric acid (West 2013). *Aspergillus carbonarius* was also found be a citric acid producer (Weyda et al. 2014). Aspergillus species like *A. awamori, A. fonsecaeus, A. nidulans, A. phoenicis, A. saitoi,* and *A. wenti* have also been reported for the producing the citric acid (Hyde et al. 2019).

23.2.4.2 Gluconic Acid

Gluconic acid is a type of mild acid which is non-corrosive, non-volatile, and nontoxic. It also has a vast array of applications in industrial like animals feed, food, leather, pharmaceutical textile (Singh et al. 1999). It is also widely used as a cement additive (Ahmed et al. 2015). Gluconic acid production is known to be produced by many fungal species belonging to genera like *Aspergillus, Penicillium, Fusarium, Mucor,* and *Gliocladium*, but all these species of *Aspergillus* named, *A. niger* is considered as one of the potent fungi species. *A. niger* is commercially used in the industries for the gluconic acid production (Sankpal et al. 2001). In a study, two

species of Penicillium, namely *P. puberulum* and *P. frequentans* were sorted out be an efficient gluconic acid producer (Ahmed et al. 2015). In another study, *Klebsiella pneumonia* was also reported for the production of gluconic acid (Wang et al. 2016).

23.2.4.3 Fumaric Acid

Fumaric acid (FA), a multifunctional chemicals intermediate contains two carbonyl groups and a double bond at α, β position in each molecule. This organic acid is known by other names like allomaleic, boletic, lichenic, and tumaric acid (Das et al. 2016). FA has applications in nearly every field of industrial chemistry such as in food and beverages (to adjust pH, enhance flavor, and to control the growth of microbes); feed industry (as antibacterial agent); resin industry (for polymerization and esterification reactions) and newer applicable in medical sciences such as immunology, neurology, and dermatology (Das et al. 2016; Sharma et al. 2012). Fungal based fumaric acid is generally known to be produced by the species of genera named as *Rhizopus* by using different substrates. *R. oryzae* (Zhang et al. 2012), *Rhizopus arrhizus* (Gu et al. 2013), and *R. nigricans* use glucose as a substrate for the production of FA, whereas *R. Formosa* uses cassava bagasse (Engel et al. 2008; Hyde et al. 2019).

23.2.4.4 Itaconic Acid

Itaconic acid is an unsaturated di-carbonic acid which is also known as 2-methylidenebutanedioic acid. It is a precursor of chemicals, polymers, and fuels that have broad spectrum of applications in the industries which produces resins, acrylic plastics, acrylate latexes, anti-scaling agents and superabsorbents (Zhao et al. 2018). Itaconic acid is mainly produced by using classic production fungi, *Aspergillus terreus* via fermentation method (Cordes et al. 2015). There are also other fungal species, which have also been reported, such as *Ustilago maydis*, *Aspergillus niger,* and *Saccharomyces cerevisiae* (Becker et al. 2015).

23.2.4.5 Lactic Acid

Organic acid, 2-hydroxycarboxylic acid, i.e., lactic acid is one of the most important and extensively used acids around the globe in various industries due to their wide range of biotechnological applications (Ghaffar et al. 2014). It is mainly used in the production of yoghurt and cheese in food industries, skin care products in cosmetic industries, and oral hygiene products. Moreover, lactic acid also helps in manufacturing of polylactic acid which has applications in the food packaging utensils and textile industry. Lactic acid production is widely produced by the bacteria; however, fungi, *Rhizopus oryzae* have been also reported to produce lactic acid (Martinez et al. 2013).

23.2.4.6 Succinic Acid

Succinic acid is an another commercial organic acid which is also known as amber acid, butanedioic acid, and 1,2-ethanedicarboxylic acid. In nature succinic acid exists in the various forms of its ester, which act as building block for the synthesis of chemicals such as 1,4-butanediol, tetrahydrofuran, γ-butyrolactone, N-methylpyrrolidone, and bio-based polymers such as polybutylene succinate and polyester polyols (Choi et al. 2015). Traditionally, succinic acid in industries was used as pigments, food additives, detergents, toners, cement additive, cosmetics and pharmaceutical intermediates (Nghiem et al. 2017). Succinic acid earlier has small market size, but since 1990 the succinic acid demand has drastically increased. This organic acid is known to produce by fungi *Rhizopus* sp. as an intermediate of biochemical pathways of carboxylic acid formation. *Rhizopus* sp. is commercial strain which was patented by DuPont and known to produce fumaric acid and L-malic acid along with succinic acid (Ling and Ng 1989). Succinic acid production was also reported by the fungi Yarrowia lipolytica (Li et al. 2018).

23.2.5 Fatty Acids

Since prebiblical times, fungi have been used to produce a variety of products including beer, bread, cheese, and wine. The twentieth century has been considered to be golden age of industrial microbiology, which yielded a countless products through fermentation processes such as antibiotics, amino acids, enzymes, polymers, and vitamins. The advancements in molecular biology techniques paved ways to use molds and yeast as microbial cell factories for the production of homologous and heterologous proteins as well as many antibiotics, fatty acids, and pigments (Adrio and Demain 2003).

Fatty acids, esterified to glycerol, are the main constituents of oils and fats. The industrial utilization of oils and fats, either for food or oleochemical products, is based on chemical modification of both the carboxyl and unsaturated groups present in fatty acids. Essential fatty acids (EFAs) are important for optimal human health but simultaneously are not synthesized by the human body and hence must be obtained from the dietary sources. In humans polyunsaturated fatty acids (PUFAs) cannot be synthesized in sufficient quantities due to which they are often referred to as EFAs. There is a little vagueness in deciding which fatty acids should be actually considered as EFAs, since some of them can be synthesized in vivo if their precursors are provided. Nevertheless, some experts consider fatty acids which are directly linked to the eicosanoid pathways as EFAs (Dyal and Narine 2005). The deficiency of EFA can negatively influence the health of humans and researchers are greatly focussing on finding external sources of these fatty acids so that they can be produced cheaply on an industrial scale. On the other hand, the immense environmental pressures demand cleaner processes, and there is a market for new products. Current developments are greatly focusing on green chemistry, using cleaner processes, less

energy, and renewable resources (Scrimgeour et al. 2005). Recently, there has been a striking increase use of various molds as potential producers of EFAs on an industrial scale. Some molds have unique fatty acid compositions with PUFAs in relatively high amounts (Dyal and Narine 2005). There is huge diversity of fungi which can accumulate more than 20% of their biomass as lipids (Passoth 2017). Filamentous fungi, like *Mortierella alpina*, producers of longer polyunsaturated fatty acids (Ochsenreither et al. 2016).

PUFAs are vitamin like compounds which possess structural functions in cell membranes and also serve as precursors for the biosynthesis of hormones. The most known are arachidonic acid and docosahexaenoic acid, which are of commercial interest and produced through fungal synthesis. The study of Sakuradani et al. (2009) suggested that manipulating genes of *Mortierella alpina* strains facilitate improvement of PUFA productivity and elucidation of the functions of enzymes involved in PUFA biosynthesis. The microbial production of PUFAs has been improved through molecular engineering techniques, particularly *Candida lipolytica* and *Saccharomyces cerevisiae* (Copetti 2019) (Stahmann 2011).

Arachidonic acid (ARA) is a 20-carbon chain fatty acid with four methylene-interrupted cis double bonds, the first with respect to the methyl end (omega, ω or n) is located between carbon 6 and 7. ARA thus belongs to the omega-6 (n-6) PUFA (Tallima and El Ridi 2018). Arachidonic acid is obtained from animal organs, eggs, fish, meat, poultry, and seafood (Abedi and Sahari 2014) and is incorporated in phospholipids in the cells' cytosol, adjacent to the endoplasmic reticulum membrane that is studded with the proteins necessary for phospholipid synthesis and their allocation to the diverse biological membranes. A variety of physiological functions of ARA includes the protection of gastric mucosa, treatment of skin psoriasis, reduction of fatty liver, killing of tumor cells, and improvement of lipid metabolism of cirrhotic patients (Higashiyama et al. 2002). On the industrial scale, *M. alpina* is considered as the major source for preparation of ARA (Nisha et al. 2011; Wu et al. 2017; You et al. 2011). In the study of (Bajpai et al. 1991b) *Mortierella alpina* and *Mortierella elongata* were compared with regard to content of arachidonic acid. *M. alpina* was observed to produce 2.1 g of ARA per liter in media consisting of 10% glucose while the highest percentage of ARA in lipid (43.3%) was observed at a glucose concentration of 2%. The study also revealed up to 66% increase in ARA in lipids during storage. The production of ARA has been also reported from *Mortierella alliacea, Mortierella elongata*, *Mortierella schmuckeri,* and *Pythium irregulare* (Aki et al. 2001; Berkeley 1996; Chaudhuri et al. 1998; Cheng et al. 1999).

Docosahexaenoic acid (DHA) is another important PUFA. DHA is important for development of brain tissues of babies. Its content of serum phospholipid, human breast milk, and platelet total lipid as well as phosphatidylcholine is especially sensitive to dietary influence (Bajpai et al. 1991a). The sperm, testis, retina, and cerebral cortex are especially rich in DHA. DHA has a role in nervous, reproductive, and visual systems which have been well cited. Bajpai et al. (1991a) reported production of DHA from *Thraustochytrium aureum*.

The fatty acid, eicosapentaenoic acid (EPA) found in the flesh of cold-water fish, viz. cod liver, halibut, herring, mackerel, salmon, tuna, and whale blubber is taken by mouth for some heart-related conditions including clogged heart arteries, to prevent or treat heart attacks, and to reduce levels of triglycerides in people with very high levels. It is also used for attention deficit-hyperactivity disorder, Alzheimer's disease, depression, personality disorder, and schizophrenia. EPA is further also used to help maintain body weight in people with cancer, and to reduce the side effects of chemotherapy. Women use EPA to reduce symptoms of menopause, high blood pressure during high-risk pregnancies, and to reduce the risk of an infant having delayed growth while still in the uterus. Cheng et al. (1999) investigated the potential production of the EPA and ARA with fermentation process using *Mortierella elongata* and *Pythium irregulare*.

23.3 Biotechnological Applications of Fungal Biomolecules

Fungal biomolecules are known to have huge amount of biotechnological applications in both industrial and agricultural sectors. In agriculture, biomolecules could be used for the enhancement of crops yield by either inhibiting the growth of other pest and pathogens or stimulating the plant growth by fungal growth hormones. On the other hand, different types of biomolecules in industries are used as color additive, pH adjuster, taste enhancer, and many other purposes.

23.3.1 Agriculture Sector

Agriculture has seen a change in recent times. Nowadays more emphasis is being placed on new technologies, with production, mechanization, increased chemical use, and government policies favoring the needs of a growing population, which is expected to reach 9.7 billion by 2050 (DESA U 2015). Intensive farming methods, although they have met many goals and achieved positive effects in reducing risks in farming, are volatile and expensive. Precipitation of the top layer of our soil, depletion and contamination of ground water and biological and abiotic stresses in agriculture are some of the drawbacks to consider. These agrochemical inputs are not economically viable and are not environmentally friendly (Kaur et al. 2021). Until now, these practices have made agricultural yields unpredictable. Modern agricultural practices include the introduction of chemical fertilizers and agriculture to control pests and weeds. The world is facing severe threats due to reduced water supply and increasing soil salinity for crop production (Egamberdieva et al. 2008; Egamberdieva and Lugtenberg 2014).

Currently, 90% of microbial pesticides are available in the market and comprise approximately $ 3 billion the total market of crop protection (Marrone 2014). Fungi are one of the microbial agents that play an important role in agriculture as this

organism can be used as a biofertilizer as well as a biopesticides (Kour et al. 2020; Kumar et al. 2021; Yadav et al. 2019d). Various fungi have been reported as biofertilizer and biopesticides that inhibit the growth of pest and other agricultural microbes like *Beauveria Bassiana* (John et al. 2010), *Cladosporium cladosporioides* (Paul and Park 2013), *Paecilomyces lilacinus* (Jensen et al. 2020), *Metarhizium anisopliae* (Howard et al. 2010), *Nomuraea rileyi* (Perinotto et al. 2012), *Trichoderma asperellum* (El Komy et al. 2015), *Trichoderma harzianum* (Sala et al. 2020), *Trichoderma lignorum* (Mostafa et al. 2012), *Trichoderma virens* (Christopher et al. 2010), *Trichoderma viride* (John et al. 2010), and *Verticillium lecanii* (Aqueel and Leather 2013). Thus, in the future, screening and selection of efficient bio-agents with dual activities of biocontrol and plant growth promotion will be very beneficial for achieving stability in agriculture.

23.3.2 Industrial Sector

Agricultural productivity to meet the growing demands of human populations is a major concern of all countries. Utilization of green compounds to achieve sustainability is the current need. To accomplish this objective, exploring and focussing on fungal microbiome and their products open new doors for the researchers. More than 100 different diseases are known to affect crop plants. These crop plant diseases present the biggest hurdles limiting the productivity of food (Savary et al. 2012). It has been well established that a range of plant diseases at different planting stages and plant pests are responsible for half of the total agricultural production loss in the world (Yadav et al. 2020c).

Fungal species secrete an array of secondary metabolites which display various biological features and are of industrial and economic importance (Yadav et al. 2019b) such as antibiotics possessing inhibitory and antimicrobial activities and pigments which are colored compounds involving antioxidant features (Chiang et al. 2009; Yadav et al. 2020b). There are commercially available *Trichoderma* products as biopesticides which alter the soil properties and enhance plant growth (Sharma et al. 2019; Yadav et al. 2020a). Volatiles from fungi especially endophytic fungi are a promising source of new bioactive compounds for exploitation in agricultural sector due to their antifungal and biocontrol activities. A very potent genus *Muscodor* plays a major role in this respect and is commercially exploited worldwide for their broad spectrum antipathogenic activity due to which it has opened up new areas of agricultural research on microbial volatiles. Mycofumigation with *Muscodor albus* and *M. roseus* has effectively enhanced the *Beta vulgaris* L. stand establishment simultaneously reducing the severity of disease when these two mycofumigating agents were applied in autoclaved sterilized soil prior infested with *Aphanomyces cochlioides, Pythium ultimum,* and *Rhizoctonia solani* (Santra and Banerjee 2020; Stinson et al. 2003). Further there are many possibilities for applying fatty acids with antimicrobial activities in agricultural sector when the

application of conventional antibiotics is undesirable or sometimes forbidden (P Desbois 2012).

Biosurfactants are other low molecular weight surface-active compounds extensively produced by fungi and yeast and have potential commercial applications (Sen 2010). There are diverse sectors of agriculture which require biosurfactants. A potent lipopeptide biosurfactant, surfactin has been reviewed as a versatile bioactive molecule capable of inhibiting fibrin clot formation and is also applied for enhanced oil recovery. Additionally it has been also known to possess antifungal, antiviral, and insecticidal properties. Surfactin can also be used as bioremediation agent for soil and water treatment (Mulligan 2005). In agriculture, biosurfactants can be widely exploited for biodegradation of pollutants to improve the quality of agriculture soil as well as for indirect plant growth promotion as these possess antimicrobial activity and also improve the plant microbe interaction beneficial for plant. In pesticide industry, harsh surfactant presently utilized can be replaced by these biosurfactants as these have been known to be utilized as carbon source by soil inhabiting microbes (Sachdev and Cameotra 2013). The growing demand for eco-friendly and sustainable agricultural practices is driving towards the application of beneficial biological products. Fungi play a marvellous role and serve as significant biological tool in sustainable agriculture with the process of mycocontrol—mycoherbicides, mycoinsecticides, and mycoremediation.

23.4 Conclusion and Future Prospects

Fungi an important organism are known to apply in various fields of industries, environment as well as agriculture for the production of goods, environmental sustainability by removing the harmful pollutants and for the production of plant and enhancement of plant product yield. All fungi used in the various fields are recognized to produce special compounds known as biomolecules. These biomolecules use in the various sectors are considered as a sustainable natural product. Organic acid, pigments, fatty acid, secondary metabolites, and enzymes are the various known biomolecules that have been known. This biomolecules are currently used in several different industries like food, textile, and pharmaceutical for coloring agent, taste enhancer, pH adjuster, and cement additives. This biomolecules are recovered from the fungi with a relative ease. Currently, fungal biomolecules have widely used but still many of the functions are under the blackbox, which should be explored and in future it can be used for various industrial purposes.

References

Abdel-Azeem AM, Yadav AN, Yadav N, Usmani Z (2021) Industrially important fungi for sustainable development, Vol-1: biodiversity and ecological perspective. Springer, Cham

Abedi E, Sahari MA (2014) Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci Nutr 2:443–463

Adrio JL, Demain AL (2003) Fungal biotechnology. Int Microbiol 6:191–199

Ahmed AS, Farag SS, Hassan IA, Botros HW (2015) Production of gluconic acid by using some irradiated microorganisms. J Radiat Res Appl Sci 8:374–380. https://doi.org/10.1016/j.jrras.2015.02.006

Aki T, Nagahata Y, Ishihara K, Tanaka Y, Morinaga T, Higashiyama K et al (2001) Production of arachidonic acid by filamentous fungus, *Mortierella alliacea* strain YN-15. J Am Oil Chem Soc 78:599–604

Akilandeswari P, Pradeep BV (2016) Exploration of industrially important pigments from soil fungi. Appl Microbiol Biotechnol 100:1631–1643. https://doi.org/10.1007/s00253-015-7231-8

Alurappa R, Bojegowda MRM, Kumar V, Mallesh NK, Chowdappa S (2014) Characterisation and bioactivity of oosporein produced by endophytic fungus *Cochliobolus kusanoi* isolated from *Nerium oleander* L. Nat Prod Res 28:2217–2220

Angumeenal AR, Venkappayya D (2013) An overview of citric acid production. LWT - Food Sci Technol 50:367–370. https://doi.org/10.1016/j.lwt.2012.05.016

Aqueel MA, Leather SR (2013) Virulence of *Verticillium lecanii* (Z.) against cereal aphids; does timing of infection affect the performance of parasitoids and predators? Pest Manag Sci 69:493–498

Bajpai P, Bajpai PK, Ward OP (1991a) Production of docosahexaenoic acid by *Thraustochytrium aureum*. Appl Microbiol Biotechnol 35:706–710

Bajpai PK, Bajpai P, Ward OP (1991b) Arachidonic acid production by fungi. Appl Environ Microbiol 57:1255–1258

Becker J, Lange A, Fabarius J, Wittmann C (2015) Top value platform chemicals: bio-based production of organic acids. Curr Opin Biotechnol 36:168–175. https://doi.org/10.1016/j.copbio.2015.08.022

Berkeley W (1996) Method for arachidonic acid production. Japanese Patent H8–214893

Borges WS, Borges KB, Bonato PS, Said S, Pupo MT (2009) Endophytic fungi: natural products, enzymes and biotransformation reactions. Curr Org Chem 13:1137–1163

Bouhri Y, Askun T, Tunca B, Deniz G, Aksoy SA, Mutlu M (2020) The orange-red pigment from *Penicillium mallochii*: pigment production, optimization, and pigment efficacy against glioblastoma cell lines. Biocatal Agric Biotechnol 23:101451. https://doi.org/10.1016/j.bcab.2019.101451

Bungihan ME, Tan MA, Kitajima M, Kogure N, Franzblau SG, de la Cruz TEE et al (2011) Bioactive metabolites of *Diaporthe* sp. P133, an endophytic fungus isolated from *Pandanus amaryllifolius*. J Nat Med 65:606–609

Caldwell BA, Jumpponen A, Trappe JM (2000) Utilization of major detrital substrates by dark-septate, root endophytes. Mycologia 92:230–232

Chaudhuri S, Ghosh S, Bhattacharyya D, Bandyopadhyay S (1998) Effect of mustard meal on the production of arachidonic acid by *Mortierella elongata* SC-208. J Am Oil Chem Soc 75:1053–1055

Chen H-Y, Xue D-S, Feng X-Y, Yao S-J (2011) Screening and production of ligninolytic enzyme by a marine-derived fungal *Pestalotiopsis* sp. J63. Appl Biochem Biotechnol 165:1754–1769

Chen Y, Wang H-W, Li L, Dai C-C (2013) The potential application of the endophyte *Phomopsis liquidambari* to the ecological remediation of long-term cropping soil. Appl Soil Ecol 67:20–26

Cheng MH, Walker TH, Hulbert GJ, Raman DR (1999) Fungal production of eicosapentaenoic and arachidonic acids from industrial waste streams and crude soybean oil. Bioresour Technol 67:101–110

Chiang Y-M, Lee K-H, Sanchez JF, Keller NP, Wang CC (2009) Unlocking fungal cryptic natural products. Nat Prod Commun 4:1505–1510

Choi J-M, Han S-S, Kim H-S (2015) Industrial applications of enzyme biocatalysis: current status and future aspects. Biotechnol Adv 33:1443–1454. https://doi.org/10.1016/j.biotechadv.2015.02.014

Christopher DJ, Raj TS, Rani SU, Udhayakumar R (2010) Role of defense enzymes activity in tomato as induced by *Trichoderma virens* against fusarium wilt caused by *Fusarium oxysporum* f sp. lycopersici. J Biopest 3:158–162

Copetti MV (2019) Fungi as industrial producers of food ingredients. Curr Opin Food Sci 25:52–56. https://doi.org/10.1016/j.cofs.2019.02.006

Cordes T, Michelucci A, Hiller K (2015) Itaconic acid: the surprising role of an industrial compound as a mammalian antimicrobial metabolite. Ann Rev Nutr 35:451–473

Coughlan M, Hazlewood G (1993) B-1, 4-smallcap˜ D-Xylan-degrading enzyme systems: biochemistry, molecular biology and applications. Biotechnol Appl Biochem 17:259–289

da Silva LV, Tavares CB, Amaral P, Coelho MAZ (2012) Production of citric acid by *Yarrowia lipolytica* in different crude glycerol concentrations and in different nitrogen sources. In: Bardone E, Brucato A, Keshavarz T, Pierucci S, Klemes JJ (eds) Ibic2012: international conference on industrial biotechnology, vol 27. Chemical engineering transactions, pp 199–204

Dai C-c, Chen Y, Tian L-s, Shi Y (2010) Correlation between invasion by endophytic fungus *Phomopsis* sp. and enzyme production. Afr J Agric Res 5:1324–1340

Das RK, Brar SK, Verma M (2016) Chapter 8—Fumaric acid: production and application aspects. In: Kaur Brar S, Jyoti Sarma S, Pakshirajan K (eds) Platform chemical biorefinery. Elsevier, Amsterdam, pp 133–157. https://doi.org/10.1016/B978-0-12-802980-0.00008-0

DESA U (2015) United Nations Department of Economic and Social Affairs/Population Division. 2015. World Population Ageing 2015 (ST/ESA/SER.A/390)

Desbois AP (2012) Potential applications of antimicrobial fatty acids in medicine, agriculture and other industries. Recent Pat Antiinfect Drug Discov 7:111–122

Devi R, Kaur T, Guleria G, Rana KL, Kour D, Yadav N et al (2020) Chapter 9—Fungal secondary metabolites and their biotechnological applications for human health. In: Rastegari AA, Yadav AN, Yadav N (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, pp 147–161. https://doi.org/10.1016/B978-0-12-820528-0.00010-7

Dyal SD, Narine SS (2005) Implications for the use of *Mortierella* fungi in the industrial production of essential fatty acids. Food Res Int 38:445–467

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9

Egamberdieva D, Lugtenberg B (2014) Use of plant growth-promoting rhizobacteria to alleviate salinity stress in plants. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses, vol 1. Springer, New York, pp 73–96

El Gueddari NE, Rauchhaus U, Moerschbacher BM, Deising HB (2002) Developmentally regulated conversion of surface-exposed chitin to chitosan in cell walls of plant pathogenic fungi. New Phytol 156:103–112

El Komy MH, Saleh AA, Eranthodi A, Molan YY (2015) Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato fusarium wilt. Plant Pathol J 31:50–60

El-Zayat S (2008) Preliminary studies on laccase production by *Chaetomium globosum* an endophytic fungus in *Glinus lotoides*. Am Eurasian J Agric Environ Sci 3:86–90

Engel CAR, Straathof AJ, Zijlmans TW, van Gulik WM, van der Wielen LA (2008) Fumaric acid production by fermentation. Appl Microbiol Biotechnol 78:379–389

Fouda AH, Hassan SE-D, Eid AM, Ewais EE-D (2015) Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). Ann Agric Sci 60:95–104

Fouillaud M, Venkatachalam M, Llorente M, Magalon H, Cuet P, Dufossé L (2017) Biodiversity of pigmented fungi isolated from marine environment in La Réunion island, Indian ocean: new resources for colored metabolites. J Fungi 3:1–22

Fritzsche K, Niehoff S, Beier G (2018) Industry 4.0 and climate change—exploring the science-policy gap. Sustainability 10:1–17

Gan Z, Yang J, Tao N, Yu Z, Zhang K-Q (2007) Cloning and expression analysis of a chitinase gene *Crchi1* from the mycoparasitic fungus *Clonostachys rosea* (syn. *Gliocladium roseum*). J Microbiol 45:422–430

Garg G, Singh A, Kaur A, Singh R, Kaur J, Mahajan R (2016) Microbial pectinases: an ecofriendly tool of nature for industries. 3. Biotech 6:1–13

Ghaffar T, Irshad M, Anwar Z, Aqil T, Zulifqar Z, Tariq A et al (2014) Recent trends in lactic acid biotechnology: a brief review on production to purification. J Radiat Res Appl Sci 7:222–229

Gu C, Zhou Y, Liu L, Tan T, Deng L (2013) Production of fumaric acid by immobilized *Rhizopus arrhizus* on net. Bioresour Technol 131:303–307. https://doi.org/10.1016/j.biortech.2012.12.148

Harwoko H, Daletos G, Stuhldreier F, Lee J, Wesselborg S, Feldbrügge M et al (2021) Dithiodiketopiperazine derivatives from endophytic fungi *Trichoderma harzianum* and *Epicoccum nigrum*. Nat Prod Res 35:257–265

Higashiyama K, Fujikawa S, Park EY, Shimizu S (2002) Production of arachidonic acid by *Mortierella* fungi. Biotechnol Bioprocess Eng 7:252–262

Howard AF, Koenraadt CJ, Farenhorst M, Knols BG, Takken W (2010) Pyrethroid resistance in *Anopheles gambiae* leads to increased susceptibility to the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. Malar J 9:1–9

Hyde KD, Xu J, Rapior S, Jeewon R, Lumyong S, Niego AGT et al (2019) The amazing potential of fungi: 50 ways we can exploit fungi industrially. Fungal Divers 97:1–136

Jensen RE, Cabral C, Enkegaard A, Steenberg T (2020) Influence of the plant interacting entomopathogenic fungus *Beauveria bassiana* on parasitoid host choice-behavior, development, and plant defense pathways. PLoS One 15:e0238943

John RP, Tyagi R, Prévost D, Brar SK, Pouleur S, Surampalli R (2010) Mycoparasitic *Trichoderma viride* as a biocontrol agent against *fusarium oxysporum* f. sp. adzuki and *Pythium arrhenomanes* and as a growth promoter of soybean. Crop Prot 29:1452–1459

Kaaniche F, Hamed A, Abdel-Razek AS, Wibberg D, Abdissa N, El Euch IZ et al (2019) Bioactive secondary metabolites from new endophytic fungus *Curvularia* sp. isolated from *Rauwolfia macrophylla*. PLoS One 14:e0217627

Kamzolova SV, Lunina JN, Morgunov IG (2011) Biochemistry of citric acid production from rapeseed oil by *Yarrowia lipolytica* yeast. J Am Oil Chem Soc 88:1965–1976

Karthik L, Kumar G, Keswani T, Bhattacharyya A, Chandar SS, Rao KB (2014) Protease inhibitors from marine actinobacteria as a potential source for antimalarial compound. PLoS One 9:e90972

Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK (2013) Prospecting endophytic fungal assemblage of *Digitalis lanata* Ehrh.(foxglove) as a novel source of digoxin: a cardiac glycoside. 3 Biotech 3:335–340

Kaur T, Devi R, Kour D, Yadav A, Yadav AN, Dikilitas M et al (2021) Plant growth promoting soil microbiomes and their potential implications for agricultural and environmental sustainability. Biologia. https://doi.org/10.1007/s11756-021-00806-w

Kirti S, Reddy M (2013) Characterization of thermostable and alkalophilic lipase enzyme from endophytic fungus *Leptosphaerulina* sp. Ph.D. Thesis

Klemsdal SS, Clarke JL, Hoell IA, Eijsink VG, Brurberg M-B (2006) Molecular cloning, characterization, and expression studies of a novel chitinase gene (*ech30*) from the mycoparasite *Trichoderma atroviride* strain P1. FEMS Microbiol Lett 256:282–289

Knob A, Terrasan CF, Carmona E (2010) β-Xylosidases from filamentous fungi: an overview. World J Microbiol Biotechnol 26:389–407

Kongruang S (2011) Growth kinetics of biopigment production by Thai isolated Monascus purpureus in a stirred tank bioreactor. J Ind Microbiol Biotechnol 38:93–99

Kour D, Kaur T, Yadav N, Rastegari AA, Singh B, Kumar V et al (2020) Phytases from microbes in phosphorus acquisition for plant growth promotion and soil health. In: Rastegari AA, Yadav AN, Yadav N (eds) Trends of microbial biotechnology for sustainable agriculture and

biomedicine systems: diversity and functional perspectives. Elsevier, Amsterdam, pp 157–176. https://doi.org/10.1016/B978-0-12-820526-6.00011-7

Kumar M, Yadav AN, Saxena R, Paul D, Tomar RS (2021) Biodiversity of pesticides degrading microbial communities and their environmental impact. Biocatal Agric Biotechnol 31. https://doi.org/10.1016/j.bcab.2020.101883

Lee G, Arepally GM (2012) Anticoagulation techniques in apheresis: from heparin to citrate and beyond. J Clin Apheresis 27:117–125

Li C, Gao S, Yang X, Lin CSK (2018) Green and sustainable succinic acid production from crude glycerol by engineered *Yarrowia lipolytica* via agricultural residue based in situ fibrous bed bioreactor. Bioresour Technol 249:612–619. https://doi.org/10.1016/j.biortech.2017.10.011

Ling LB, Ng TK (1989) Fermentation process for carboxylic acids. Google Patents

Lunge AG, Patil AS (2012) Characterization of efficient chitinolytic enzyme producing *Trichoderma* species: a tool for better antagonistic approach. Int J Sci Environ Technol 1:377–385

Mani VM, Priya MS, Dhaylini S, Preethi K (2015) Antioxidant and antimicrobial evaluation of bioactive pigment from *fusarium* sp. isolated from stressed environment. Int J Curr Microbiol App Sci 4:1147–1158

Marlida Y, Saari N, Hassan Z, Radu S (2000) Raw starch-degrading enzyme from newly isolated strains of endophytic fungi. World J Microbiol Biotechnol 16:573–578

Marrone PG (2014) The market and potential for biopesticides. In: Gross AD, Coats JR, Seiber JN, Duke SO (eds) Biopesticides: state of the art and future opportunities. American Chemical Society, Washington, DC, pp 245–258

Martinez FAC, Balciunas EM, Salgado JM, Gonzalez JMD, Converti A, de Souza Oliveira RP (2013) Lactic acid properties, applications and production: a review. Trends Food Sci Technol 30:70–83

Mostafa FA, Kasem M, Refaei A, Nour El-Deen A, Genena MA (2012) Integrated management of *Meloidogyne incognita* and *Rotylenchulus reniformis* using *Trichoderma lignorum*, *Paecilomyces sulphurellus* and rice straw tea compost. Egypt J Agronematol 11:125–143

Mulligan CN (2005) Environmental applications for biosurfactants. Environ Pollut 133:183–198

Nghiem NP, Kleff S, Schwegmann S (2017) Succinic acid: technology development and commercialization. Fermentation 3:1–14

Nigam P, Singh D (1995) Enzyme and microbial systems involved in starch processing. Enzym Microb Technol 17:770–778

Nisha A, Rastogi NK, Venkateswaran G (2011) Optimization of media components for enhanced arachidonic acid production by *Mortierella alpina* under submerged cultivation. Biotechnol Bioprocess Eng 16:229–237

Ochsenreither K, Glück C, Stressler T, Fischer L, Syldatk C (2016) Production strategies and applications of microbial single cell oils. Front Microbiol 7:1–26

Palacio-Barrera AM, Areiza D, Zapata P, Atehortúa L, Correa C, Peñuela-Vásquez M (2019) Induction of pigment production through media composition, abiotic and biotic factors in two filamentous fungi. Biotechnol Rep 21:e00308. https://doi.org/10.1016/j.btre.2019.e00308

Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000) Advances in microbial amylases. Biotechnol Appl Biochem 31:135–152

Pandey N, Jain R, Pandey A, Tamta S (2018) Optimisation and characterisation of the orange pigment produced by a cold adapted strain of *Penicillium* sp.(GBPI_P155) isolated from mountain ecosystem. Mycology 9:81–92

Passoth V (2017) Lipids of yeasts and filamentous fungi and their importance for biotechnology. In: Sibirny A biotechnology of yeasts and filamentous fungi. Springer, Cham, pp 149–204

Paul D, Park KS (2013) Identification of volatiles produced by *Cladosporium cladosporioides* CL-1, a fungal biocontrol agent that promotes plant growth. Sensors 13:13969–13977

Perinotto W, Terra A, Angelo I, Fernandes ÉK, Golo P, Camargo M et al (2012) Nomuraea rileyi as biological control agents of *Rhipicephalus microplus* tick. Parasitol Res 111:1743–1748

Pradeep FS, Begam MS, Palaniswamy M, Pradeep B (2013) Influence of culture media on growth and pigment production by *fusarium moniliforme* KUMBF1201 isolated from paddy field soil. World Appl Sci J 22:70–77

Qureshi M, Khare A, Pervez A, Uprit S (2015) Enzymes used in dairy industries. Int J Appl Res 1:523–527

Rana KL, Kour D, Sheikh I, Dhiman A, Yadav N, Yadav AN et al (2019a) Endophytic fungi: biodiversity, ecological significance and potential industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi: volume 1: diversity and enzymes perspectives. Springer, Switzerland, pp 1–62

Rana KL, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V et al (2019b) Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh BP (ed) Advances in endophytic fungal research: present status and future challenges. Springer International Publishing, Cham, pp 105–144

Rastegari AA, Yadav AN, Yadav N (2019a) Genetic manipulation of secondary metabolites producers. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 13–29. https://doi.org/10.1016/B978-0-444-63504-4.00002-5

Rastegari AA, Yadav AN, Yadav N, Tataei Sarshari N (2019b) Bioengineering of secondary metabolites. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 55–68. https://doi.org/10.1016/B978-0-444-63504-4.00004-9

Rathnayake GN, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2018) Chemical investigation of metabolites produced by an endophytic fungi *Phialemonium curvatum* from the leaves of *Passiflora edulis*. Nat Prod Res 32:2483–2486

Robinson S, Tudor D, Zhang W, Ng S, Cooper P (2014) Ability of three yellow pigment producing fungi to colour wood under controlled conditions. Int Wood Prod J 5:103–107

Robinson SC, Tudor D, Cooper PA (2012) Utilizing pigment-producing fungi to add commercial value to American beech (*Fagus grandifolia*). Appl Microbiol Biotechnol 93:1041–1048

Robl D, da Silva Delabona P, dos Santos Costa P, da Silva Lima DJ, Rabelo SC, Pimentel IC et al (2015) Xylanase production by endophytic *aspergillus Niger* using pentose-rich hydrothermal liquor from sugarcane bagasse. Biocatal Biotransformation 33:175–187

Sachdev DP, Cameotra SS (2013) Biosurfactants in agriculture. Appl Microbiol Biotechnol 97:1005–1016

Sakuradani E, Ando A, Ogawa J, Shimizu S (2009) Improved production of various polyunsaturated fatty acids through filamentous fungus *Mortierella alpina* breeding. Appl Microbiol Biotechnol 84:1–10

Sala A, Artola A, Sánchez A, Barrena R (2020) Rice husk as a source for fungal biopesticide production by solid-state fermentation using *B. bassiana* and *T. harzianum*. Biores Technol 296:122322

Sanchez OJ, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol 99:5270–5295

Sankpal N, Cheema J, Tambe S, Kulkarni B (2001) An artificial intelligence tool for bioprocess monitoring: application to continuous production of gluconic acid by immobilized *Aspergillus niger*. Biotechnol Lett 23:911–916

Santra HK, Banerjee D (2020) Fungal endophytes: a source for biological control agents. In: Yadav AN, Mishra S, Kour D, Yadav N, Kumar A (eds) Agriculturally Important Fungi for Sustainable Agriculture. Springer, Cham, pp 181–216

Savary S, Ficke A, Aubertot J-N, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. Food Secur 4:519–537

Saxena A, Yadav A, Kaushik R, Tyagi S, Shukla L (2015) Biotechnological applications of microbes isolated from cold environments in agriculture and allied sectors. In: International Conference on Low temperature science and biotechnological advances. Society of low temperature biology

Schulz B, Boyle C, Draeger S, Römmert A-K, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106:996–1004

Scrimgeour C, Gao Y, Oh WY, Shahidi F (2005) Chemistry of fatty acids. In: Shahidi F (ed) Bailey's industrial oil and fat products. Wiley, Hoboken, pp 1–40

Sen R (2010) Surfactin: biosynthesis, genetics and potential applications. In: Sen R (ed) Biosurfactants. Advances in experimental medicine and biology, vol 672. Springer, New York, pp 316–323

Sharma S, Kour D, Rana KL, Dhiman A, Thakur S, Thakur P et al (2019) *Trichoderma*: biodiversity, ecological significances, and industrial applications. In: Yadav AN, Mishra S, Singh S, Gupta A (eds) Recent advancement in white biotechnology through fungi. : Volume 1: diversity and enzymes perspectives. Springer, Cham, pp 85–120

Sharma U, Prakash T, Surendra V, Roopakarki N, Rao N, Goli D (2012) Hepatoprotective activity of *Fumaria officinalis* against CCl4-induced liver damage in rats. Pharmacologia 3:9–14

Shekher R, Sehgal S, Kamthania M, Kumar A (2011) Laccase: microbial sources, production, purification, and potential biotechnological applications. Enzyme Res 2011:1–11

Shiono Y, Yokoi M, Koseki T, Murayama T, Aburai N, Kimura K-i (2010) Allantopyrone A, a new α-pyrone metabolite with potent cytotoxicity from an endophytic fungus, *Allantophomopsis lycopodina* KS-97. J Antibiot 63:251–253

Sibero MT, Triningsih DW, Radjasa OK, Sabdono A, Trianto A (2016) Evaluation of antimicrobial activity and identification of yellow pigmented marine sponge-associated fungi from Teluk Awur, Jepara, Central Java. J Biotechnol 21:1–11

Singh J, Yadav AN (2020) Natural bioactive products in sustainable agriculture. Springer, Singapore

Singh OV, Pereira BM, Singh R (1999) Isolation and characterization of a potent fungal strain *aspergillus niger* ORS-4 for gluconic acid production. J Sci Ind Res 58:594

Singh P, Kumar S (2019) Microbial enzyme in food biotechnology. In: Kuddus M (ed) Enzymes in food biotechnology—production, applications, and future prospects, 1st edn. Academic, New York, pp 19–28

Singh R, Kumar M, Mittal A, Mehta PK (2016) Microbial enzymes: industrial progress in 21st century. 3 Biotech 6:1–15

Soccol CR, Vandenberghe LP, Rodrigues C, Pandey A (2006) New perspectives for citric acid production and application. Food Technol Biotechnol 44:141–149

Stahmann K-P (2011) Production of vitamin B2 and a polyunsaturated fatty acid by fungi. In: Hofrichter M (ed) Industrial applications. Springer, Berlin, Heidelberg, pp 235–247

Stinson A, Zidack N, Strobel G, Jacobsen B (2003) Mycofumigation with *Muscodor albus* and *Muscodor roseus* for control of seedling diseases of sugar beet and Verticillium wilt of eggplant. Plant Dis 87:1349–1354

Sumarah MW, Puniani E, Sørensen D, Blackwell BA, Miller JD (2010) Secondary metabolites from anti-insect extracts of endophytic fungi isolated from *Picea rubens*. Phytochemistry 71:760–765

Sun Z-L, Zhang M, Zhang J-F, Feng J (2011) Antifungal and cytotoxic activities of the secondary metabolites from endophytic fungus *Massrison* sp. Phytomedicine 18:859–862

Sunitha V, Nirmala Devi D, Srinivas C (2013) Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants. World J Agric Sci 9:1–9

Tallima H, El Ridi R (2018) Arachidonic acid: physiological roles and potential health benefits–a review. J Adv Res 11:33–41

Tuli HS, Chaudhary P, Beniwal V, Sharma AK (2015) Microbial pigments as natural color sources: current trends and future perspectives. J Food Sci Technol 52:4669–4678

Uzma F, Konappa NM, Chowdappa S (2016) Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka. Egypt J Basic Appl Sci 3:335–342

Velmurugan P, Lee YH, Venil CK, Lakshmanaperumalsamy P, Chae J-C, Oh B-T (2010) Effect of light on growth, intracellular and extracellular pigment production by five pigment-producing filamentous fungi in synthetic medium. J Biosci Bioeng 109:346–350

Verma P, Yadav AN, Khannam KS, Panjiar N, Kumar S, Saxena AK et al (2015) Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. Ann Microbiol 65:1885–1899

Vinale F, Nicoletti R, Lacatena F, Marra R, Sacco A, Lombardi N et al (2017) Secondary metabolites from the endophytic fungus *Talaromyces pinophilus*. Nat Prod Res 31:1778–1785

Vishwanatha KS, Rao AA, Singh SA (2010) Production and characterization of a milk-clotting enzyme from *Aspergillus oryzae* MTCC 5341. Appl Microbiol Biotechnol 85:1849–1859

Wang D, Wang C, Wei D, Shi J, Kim CH, Jiang B et al (2016) Gluconic acid production by gad mutant of *Klebsiella pneumoniae*. World J Microbiol Biotechnol 32:132. https://doi.org/10.1007/s11274-016-2080-x

West TP (2013) Citric acid production by Candida species grown on a soy-based crude glycerol. Prep Biochem Biotechnol 43:601–611

Weyda I, Lübeck M, Ahring BK, Lübeck PS (2014) Point mutation of the xylose reductase (XR) gene reduces xylitol accumulation and increases citric acid production in *Aspergillus carbonarius*. J Ind Microbiol Biotechnol 41:733–739

Wu W-J, Zhang A-H, Peng C, Ren L-J, Song P, Yu Y-D et al (2017) An efficient multi-stage fermentation strategy for the production of microbial oil rich in arachidonic acid in Mortierella alpina. Biores Bioprocess 4:1–9

Yadav AN (2019) Fungal white biotechnology: conclusion and future prospects. In: Yadav AN, Singh S, Mishra S, Gupta A (eds) Recent advancement in white biotechnology through fungi: volume 3: perspective for sustainable environments. Springer International Publishing, Cham, pp 491–498. https://doi.org/10.1007/978-3-030-25506-0_20

Yadav AN, Kour D, Rana KL, Yadav N, Singh B, Chauhan VS et al (2019a) Metabolic engineering to synthetic biology of secondary metabolites production. In: Gupta VK, Pandey A (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 279–320. https://doi.org/10.1016/B978-0-444-63504-4.00020-7

Yadav AN, Mishra S, Singh S, Gupta A (2019b) Recent advancement in white biotechnology through fungi. Volume 1: diversity and enzymes perspectives. Springer International Publishing, Cham

Yadav AN, Rastegari AA, Yadav N, Kour D (2020a) Advances in plant microbiome and sustainable agriculture: diversity and biotechnological applications. Springer, Singapore

Yadav AN, Rastegari AA, Yadav N, Kour D (2020b) Advances in plant microbiome and sustainable agriculture: functional annotation and future challenges. Springer, Singapore

Yadav AN, Sachan SG, Verma P, Kaushik R, Saxena AK (2016) Cold active hydrolytic enzymes production by psychrotrophic bacilli isolated from three sub-glacial lakes of NW Indian Himalayas. J Basic Microbiol 56:294–307

Yadav AN, Singh J, Rastegari AA, Yadav N (2020c) Plant microbiomes for sustainable agriculture. Springer, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019c) Recent advancement in white biotechnology through fungi. Volume 2: perspective for value-added products and environments. Springer International Publishing, Cham

Yadav AN, Singh S, Mishra S, Gupta A (2019d) Recent advancement in white biotechnology through fungi: volume 3: perspective for sustainable environments. Springer Nature

Yadav AN, Verma P, Kumar V, Sangwan P, Mishra S, Panjiar N et al (2018) Biodiversity of the genus *Penicillium* in different habitats. In: Gupta VK, Rodriguez-Couto S (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 3–18

Yehia RS, Osman GH, Assaggaf H, Salem R, Mohamed MSM (2020) Isolation of potential antimicrobial metabolites from endophytic fungus *Cladosporium cladosporioides* from endemic plant *Zygophyllum mandavillei*. South Afr J Bot 134:296–302. https://doi.org/10.1016/j.sajb.2020.02.033

Yin X, Li J, Shin H-d DG, Liu L, Chen J (2015) Metabolic engineering in the biotechnological production of organic acids in the tricarboxylic acid cycle of microorganisms: advances and prospects. Biotechnol Adv 33:830–841. https://doi.org/10.1016/j.biotechadv.2015.04.006

You J-Y, Peng C, Liu X, Ji X-J, Lu J, Tong Q et al (2011) Enzymatic hydrolysis and extraction of arachidonic acid rich lipids from Mortierella alpina. Bioresour Technol 102:6088–6094

Zhang B, Skory CD, Yang S-T (2012) Metabolic engineering of Rhizopus oryzae: effects of overexpressing pyc and pepc genes on fumaric acid biosynthesis from glucose. Metab Eng 14:512–520

Zhao J, Zhang Y, Wang L, Wang J, Zhang C (2012) Bioactive secondary metabolites from Nigrospora sp. LLGLM003, an endophytic fungus of the medicinal plant Moringa oleifera lam. World J Microbiol Biotechnol 28:2107–2112

Zhao M, Lu X, Zong H, Li J, Zhuge B (2018) Itaconic acid production in microorganisms. Biotechnol Lett 40:455–464

Zheng C-J, Xu L-L, Li Y-Y, Han T, Zhang Q-Y, Ming Q-L et al (2013) Cytotoxic metabolites from the cultures of endophytic fungi from Panax ginseng. Appl Microbiol Biotechnol 97:7617–7625. https://doi.org/10.1007/s00253-013-5015-6

Index

A. M. Abdel-Azeem et al. (eds.), *Industrially Important Fungi for Sustainable Development*, Fungal Biology, https://doi.org/10.1007/978-3-030-85603-8