International Microbiology (2019) 22:217–225
https://doi.org/10.1007/s10123-018-00041-5

ORIGINAL ARTICLE

Modulation of laccase transcriptome during biodegradation of naphthalene by white rot fungus *Pleurotus ostreatus*

Shaza M. Elhusseiny[1] · Heba M. Amin[2] · Rania I. Shebl[1]

Received: 18 August 2018 / Revised: 5 October 2018 / Accepted: 29 October 2018 / Published online: 15 November 2018
© Springer Nature Switzerland AG 2018

Abstract

Biodegradation of polycyclic aromatic hydrocarbons (PAHs) using *Pleurotus ostreatus* was investigated in the current study along with the expression levels of laccase genes involved in biodegradation under variable conditions. Biodegradation of PAHs (naphthalene, anthracene, and 1,10-phenanthroline) was detected spectrophotometrically. Recorded data revealed that biodegradation of the tested PAHs was time dependent. Elevated level of naphthalene biodegradation (86.47%) was observed compared to anthracene (27.87%) and 1,10-phenanthroline (24.51%) within 3 days post incubation. Naphthalene was completely degraded within 5 days. Further incubation enhanced the biodegradation of both anthracene and 1,10-phenanthroline until reaches 93.69% and 92.00% biodegradation of the initial concentration within an incubation period of 11 and 14 days, respectively. Naphthalene was selected as a PAH model. HPLC and thin layer chromatography of naphthalene biodegradation products at time intervals proposed that naphthalene was first degraded to α- and β-naphthol which was further metabolized to salicylic and benzoic acid. The metabolic pathway of naphthalene degradation by this fungus was elucidated based on the detected metabolites. The expression profile of six laccase isomers was evaluated using real-time PCR. The transcriptome of the fungal laccase isomers recorded higher levels of transcription under optimized fermentation conditions especially in presence of both naphthalene and Tween 80. The accumulation of such useful metabolites from the biodegradation of PAH pollutants recommended white rot fungus as a potential candidate for production of platform chemicals from PAH wastes.

Keywords Polycyclic aromatic hydrocarbons · White rot fungus · Biodegradation · *Pleurotus ostreatus*

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical compounds of low hydrophobicity and low water solubility consisting of carbon and hydrogen, arranged in the form of two or more aromatic rings (Jacques et al. 2008). PAHs are considered ubiquitous environmental contaminants that are primarily generated during the incomplete combustion of organic materials in automobile exhaust (Abdel-Shafy and Mansour 2016), petrochemical industry, or accidentally spilled during the transportation of petroleum (Sartoros et al. 2005). PAHs are of critical public health concern due to their carcinogenic, mutagenic, and teratogenic properties as well as their persistent toxicity and bioaccumulation properties (Ukiwe et al. 2013).

Microbial degradation of PAHs is still the major potential degradation pathway, although PAHs may undergo adsorption, volatilization, photolysis, and chemical degradation (Ghosal et al. 2016). Biodegradation is one of the most essential methods of bioremediation that rely on achieving benefit from the biodegradation efficiency of microorganisms in detoxifying environmental pollutants and to treat soils, water, or sediments contaminated with PAHs (Ukiwe et al. 2013). Moreover, biodegradation techniques to date are the most

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10123-018-00041-5) contains supplementary material, which is available to authorized users.

✉ Rania I. Shebl
Shbel.rania@gmail.com

Shaza M. Elhusseiny
shazaelhusseiny@gmail.com

Heba M. Amin
htmagdy@msa.eun.eg

[1] Department of Microbiology and Immunology, Faculty of Pharmacy, Ahram Canadian University (ACU), 4th Industrial Area, 6th of October City, Cairo 12566, Egypt

[2] Department of Microbiology and Immunology, Faculty of Pharmacy, Modern Sciences and Arts University (MSA), 6th of October City, Cairo, Egypt

economic and eco-friendly strategies for removal of organic and inorganic contaminant due to the wide range of microbial-mediated catabolic reactions which are clearly associated with their enzymes (Bisht et al. 2015). It was also reported that many isolated bacterial and fungal species were capable of effectively biodegrading petroleum hydrocarbons and even polynuclear aromatic hydrocarbons (Fathepure 2015).

The biodegradation process of PAHs involves many factors among which are the existence of microbial strains capable of degrading the pollutant as well as the bioavailability of the contaminant to microbial attack. Other environmental factors such as the type of soil, temperature, pH, oxygen level, the presence of surfactants, and the nutrient content of soil are also contributing in biodegradation performance (Gratia et al. 2009). It was also reported that fungal laccases are useful biocatalysts in a wide range of biotechnological application, including detoxification of environmental pollutants and waste water treatment (Piscitelli et al. 2010). Laccase synthesis and secretion are strictly influenced by nutrient levels, culture conditions, developmental stage, as well as by the addition of a wide range of inducers to the culture media. Most of these factors have been shown to act at transcriptional level, producing different responses among different enzyme isoforms in the same strain and between different fungal species (Piscitelli et al. 2011).

Accordingly, current study aimed to investigate the application of white rot fungus in biodegradation of PAHs and to evaluate the expression level of laccase genes involved in biodegradation under variable conditions. Elucidation of the metabolic pathway for naphthalene biodegradation by *Pleurotus ostreatus* was also discussed.

Materials and methods

Microorganism and culture

Pleurotus ostreatus (*P. ostreatus*) was kindly supplied from the Pharmaceutical Microbiology Laboratory of the National Center for Radiation Research and Technology (NCRRT - Egypt) (El-Batal et al. 2015). The strain was periodically sub-cultured on potato dextrose agar (PDA) and preserved at 4 °C.

Biodegradation of PAHs

Each of the tested PAHs (naphthalene, anthracene, and 1,10-phenanthroline) (Sigma-Aldrich, USA) was added to two conical flasks containing 400 ml of sterile salt solution (SS) (NH_4Cl 0.1 g/L, $MgSO_4$ H_2O 0.2 g/L, KH_2PO_4 0.2 g/L, K_2HPO_4 0.38 g/L, $FeCl_3$ 0.05 g/L, and pH was adjusted to 6.8) at final concentration of 50 μg/ml. Fresh fungal isolate precultured in potato dextrose broth (PDB) (5% *v*/*v*) was inoculated into one flask containing each of the tested PAH and SS. Another control flask was inoculated with an equal volume of PDB but free from the fungal isolate to assess for physical degradation of the tested PAHs by the effect of shaking. Flasks were incubated in dark at 28 °C on a rotatory shaker (RAJ Scientific Industries, India) at 150 rpm (Meckenstock et al. 2000). Samples were analyzed at 24-h intervals to determine the residual PAHs' concentration. Residual PAHs were extracted by mixing culture media with an equal volume of n-hexane (Tedia, USA) using a shaker at 28 °C for 30 min to increase the extraction efficiency (Yuan et al. 2000).

After separation of two phases, 1 ml of organic phase was measured spectrophotometrically at λmax 275 nm, 356 nm, and 264 nm for naphthalene, anthracene, and 1,10-phenanthroline, respectively. Calibration curve was performed by plotting different concentrations of each PAH against its corresponding optical density to assess the concentration of the tested PAH in presence and in absence of fungi (Meckenstock et al. 2000).

Detection and characterization of naphthalene metabolites

High pressure liquid chromatography

P. ostreatus was incubated with naphthalene as a sole carbon and energy source. Detected metabolites were characterized using high pressure liquid chromatography (HPLC), where 1 ml of acetonitrile was added to the residue obtained post an overnight evaporation of 1 ml of n-hexane extract. Remaining naphthalene concentration was determined by the aid of HPLC (Shimadzu, Japan) using Alltech C18 (reverse phase column), acetonitrile/water (70/30) as a mobile phase at a flow rate of 1 ml/min using UV-Vis detector at 221 nm. Peaks as well as retention times of naphthalene metabolites were compared to that of standards. The concentrations of naphthalene and its metabolites were determined by calculating the area under the curve (Silva et al. 2009).

Thin layer chromatography

Salt solution sample was acidified to reach a pH value of 2 using 1 N HC1. Aromatic metabolites were extracted four times using 10 ml ethyl acetate (Sigma-Aldrich, USA). The organic layer was pooled, allowed to evaporate at 50 °C, then the residue was dissolved in 1 ml methanol (Sigma-Aldrich, USA). Methanolic extract as well as standards were spotted on thin layer chromatographic plates and allowed to develop in chloroform-methanol (9:1). Plates were visualized using UV lamp (254 nm) and the R_f values (distance traveled by sample/distance traveled by solvent) were determined. All thin layer chromatography (TLC) plates were sprayed with 5% ferric chloride solution (Mumbai, India) for visualization of α-naphthol and salicylic acid spots (Bestetti et al. 1994).

Expression of laccase genes using real-time PCR

The effects of different environmental factors, surfactants, and variable concentrations of malt extract, glucose, $CuSO_4$, EDTA, and $CdSO_4$ were evaluated in a previous study to determine the best biodegradation conditions (Elhusseiny et al. 2017). Expression of laccase genes was assessed under different biodegradation conditions in order to determine the relation between the conditions that influence naphthalene biodegradation and the expression levels of different laccases.

Expression level of six laccase isomers was assessed according to the method of Castanera et al. (2012). The fungal isolate was inoculated into three flasks, one containing salt solution and naphthalene only, the second contained salt solution and Tween 80, while the third one contained salt solution in presence of both naphthalene and Tween 80. Additional two flasks were also considered, where the fungal isolate was inoculated into a flask containing salt solution, naphthalene, and high concentration of inducers (malt extract, glucose, and $CuSO_4$) along with low concentration of inhibitors (EDTA and $CdSO_4$). The other flask contains salt solution, naphthalene, and low concentration of inducers with high concentration of inhibitors. Negative control flask was prepared by inoculating the fungal isolate in salt solution alone. Another flask containing PDB was inoculated with the same volume of the fungal isolate. All flasks were incubated at 28 °C on a rotary shaker at 150 rpm for 5 days.

Post incubation period, RNA was extracted from all flasks using High Pure miRNA Isolation Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. Extracted RNA (4 μl) was mixed with SYBR Green PCR Master Mix (2 μl), enzyme (0.2 μl), and forward (1 μl) and reverse primers (1 μl) in presence of resolution buffer (1 μl) and $MgCl_2$ (0.8 μl). Expression levels of six laccase isomers and β-actin (internal control) were assessed for each biodegradation condition using the primer sequences that were described in the Supplementary Table (1) (Pezzella et al. 2013).

Statistical analysis

For all experiments, results were presented as the mean ± standard error of three independent experiments. Statistical significance was determined using unpaired *t* test and one way ANOVA. Differences at *P* values less than 0.05 were considered significant.

Results

Biodegradation of PAHs

Biodegradation results revealed that *P. ostreatus* could potentially degrade naphthalene, anthracene, and 1,10-phenanthroline, where the decrease in the UV absorption pattern of naphthalene, anthracene, and 1,10-phenanthroline indicates a marked decrease in their concentrations relative to time (Supplementary Data 1). The fungal isolate resulted in 86.47% biodegradation of the initial concentration of naphthalene compared to 20.27% degradation in control uninoculated media within an incubation period of 3 days. In the meantime, anthracene and 1,10-phenanthroline showed lower extent of fungal biodegradation during the same incubation period and recorded 27.87% and 24.51% biodegradation of the initial concentration compared to 2.52% and 1.55% degradation in control uninoculated media, respectively ($P < 0.01$). Further biodegradation occurred for both anthracene and 1,10-phenanthroline until reaches 93.69% and 92.00% biodegradation of the initial concentration compared to 14.66% and 13.51% degradation in control uninoculated media within an incubation period of 11 and 14 days, respectively (Fig. 1).

Characterization and detection of naphthalene metabolites

High pressure liquid chromatography

HPLC analysis of acetonitrile extract of the culture at different time intervals detected the presence of naphthalene eluted at 5.1 min as well as four metabolites eluted at 2.56 min, 2.72 min, 2.1 min, and 1.86 min confirmed by standard α, β-naphthol, salicylic and benzoic acid and showed the same retention time, respectively (Fig. 2).

Time course of naphthalene biodegradation showed a drop in the initial naphthalene concentration from 50 to 40 μg/ml due to experimental conditions such as autoclaving and extraction conditions that affects the initial concentration. The first day revealed an obvious decrease in naphthalene

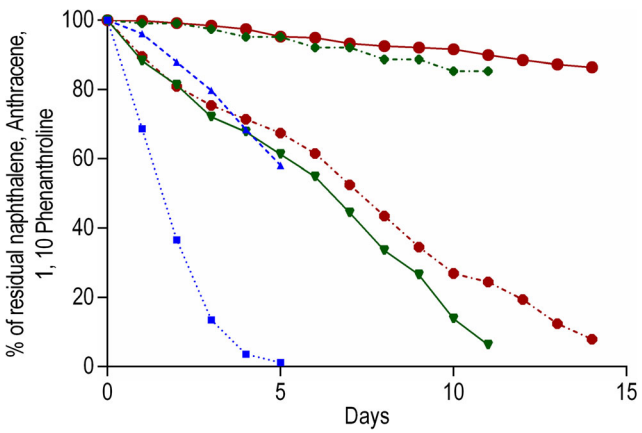

Fig. 1 Biodegradation of PAHs by *P. ostreatus* in SS media (test naphthalene, control naphthalene, test anthracene, control anthracene, test 1,10-phenanthroline, and control 1,10-phenanthroline)

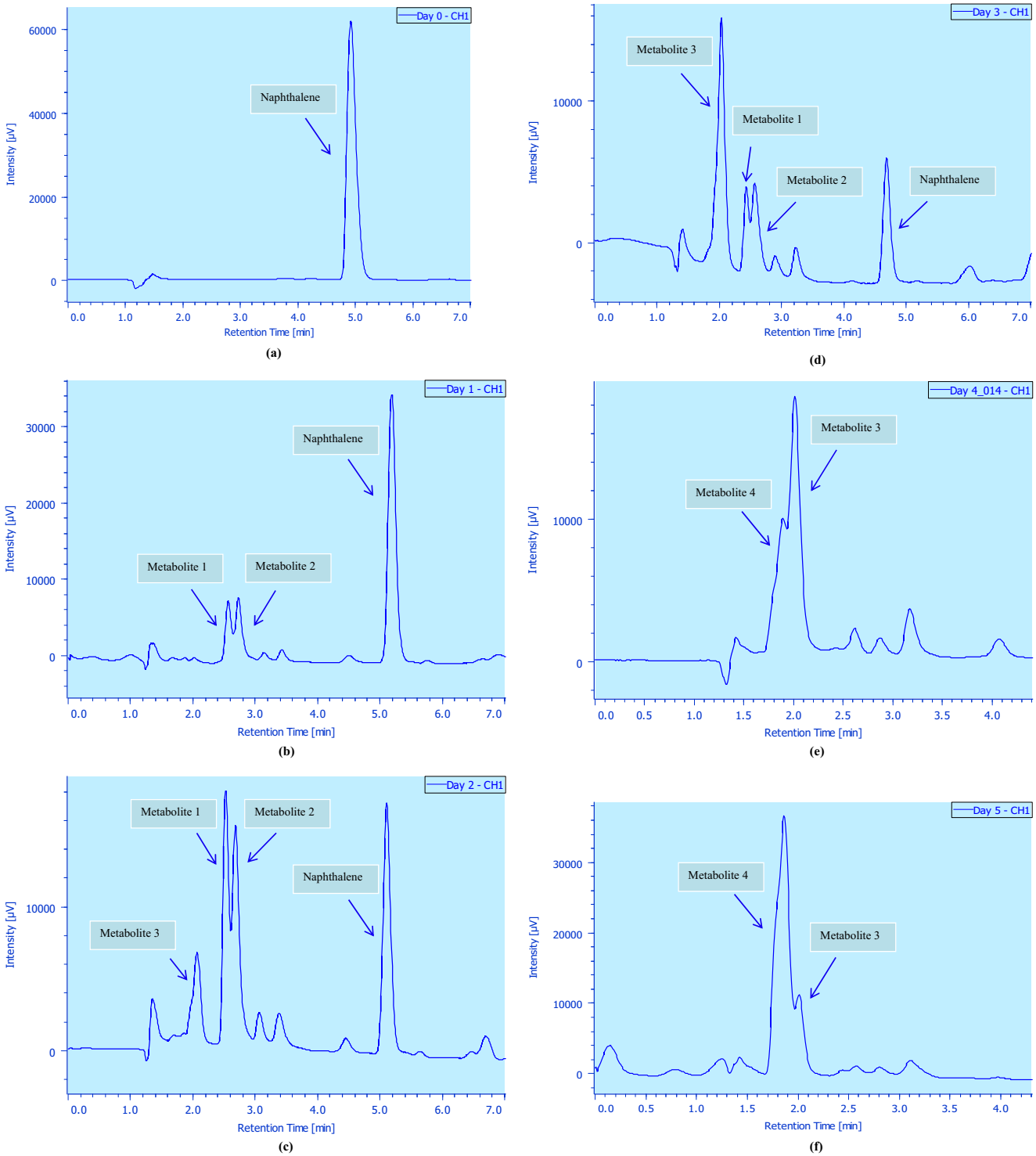

Fig. 2 HPLC elution profile of samples eluted at 24 h interval of **a** naphthalene; **b** naphthalene, α-naphthol, and β-naphthol; **c** naphthalene, α-naphthol, β-naphthol, and salicylic acid; **d** naphthalene, α-naphthol, β-naphthol, and salicylic acid; **e** Salicylic acid and benzoic acid; and **f** salicylic acid and benzoic acid as metabolites resulting from biodegradation of naphthalene by *P. ostreatus*. Metabolite 1 (α-Naphthol), Metabolite 2 (β-Naphthol), Metabolite 3 (salicylic acid), and Metabolite 4 (benzoic acid)

concentration by 0.5-fold and the appearance of nearly equal concentrations of both α, β-naphthol recording 5.41 and 5.39 µg/ml, respectively. The second day showed a further decrease in naphthalene concentration by a value of 50% compared to the first day, with a simultaneous increase in the concentrations of α- and β-naphthol (13.66 and 12.41 µg/ml, respectively) as well as the detection of salicylic acid (4.6 µg/ml). In the third day, the recorded concentrations

of naphthalene, α-naphthol, and β-naphthol decreased, while the concentration of salicylic acid was elevated by more than 1-fold. Naphthalene, α-naphthol, and β-naphthol disappeared in the fourth day, with an increase in the concentration of salicylic acid in addition to the appearance of benzoic acid. The fifth day recorded a decrease in salicylic acid concentration by 1.6-fold and an increase in benzoic acid concentration by about 2.9-fold (Fig. 3).

Thin layer chromatography

Detection of naphthalene metabolites using thin layer chromatography revealed the presence of two spots of α-naphthol and β-naphthol with R_f values of 0.77 and 0.76, respectively. Salicylic acid and benzoic acid were detected as another two spots with R_f values of 0.13 and 0.34, respectively. Post spraying with ferric chloride solution, α-naphthol, and salicylic acid spots appeared as blue and violet spots, respectively (Fig. 4).

Recorded results indicated that *P. ostreatus*-induced biodegradation of naphthalene is accompanied by the formation of four metabolites (α-naphthol, β-naphthol, salicylic acid, and benzoic acid) as indicated by HPLC and TLC (Supplementary Table 2).

Expression of laccase genes using real-time PCR

Data revealed that salt solution medium supplemented with Tween 80 could induce the expression of the six laccase isomers (laccase 1, 2, 3, 4, 10, and12) in the order of 2.5-, 1.3-, 2.9-, 3.4-, 4.4-, and 3.5-fold. Also, supplementation of the salt solution medium with naphthalene only showed greater increase in the expression of laccase genes compared to that in presence of Tween 80 only, where it recorded an increase by 4.9-, 2.0-, 5.6-, 5.8-, 7.7-, and 6.0-fold. On the other hand, the combination between Tween 80 and naphthalene resulted in maximum increase in the expression of laccase genes by 37.4-, 10.1-, 30-, 56.7-, 70.6-, and 67.8-fold in case of laccase 1, 2, 3, 4, 10, and 12, respectively ($P < 0.05$) (Fig. 5).

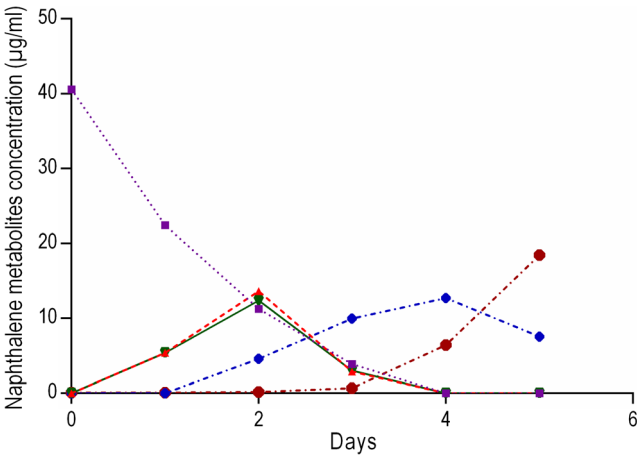

Fig. 3 Time course for naphthalene biodegradation by *P. ostreatus* (naphthalene, α-naphthol, β-naphthol, salicylic acid, and benzoic acid)

Higher concentration of inducers (malt extract, glucose, and $CuSO_4$) and lower concentration of inhibitors (EDTA and $CdSO_4$) stimulated the expression of the tested laccase genes by 13.6-, 6-, 18.2-, 20.6-, 26.3-, and 29.9-fold. While, lower induction in the expression of laccase genes was recorded in presence of low concentration of inducers and high concentration of inhibitors by 1.6-, 1.2-, 1.9-, 1.9-, 1.6-, and 1.6-fold in case of laccase 1, 2, 3, 4, 10, and12, respectively. Moreover, the presence of potato dextrose broth resulted in a marked elevation in the expression level of laccase 2 by 17-fold compared to a minor increase in the expression level of laccase 4 and 12 by a value of 2.1- and 1.8-fold, respectively. However, the expression of other laccase wasn't significantly influenced in presence of PDB (Fig. 5).

Discussion

Polycyclic aromatic hydrocarbons are environmental pollutants that became a remarkable universal issue due to their potential bioaccumulation ability and their resistance to environmental degradation (Hadibarata et al. 2013). Fungal biodegradation is one of the approaches that was studied in the recent years, where various fungal species were reported to metabolize different PAHs (Ghosal et al. 2016). Fungal biodegradation is hindered by many obstacles such as PAH molecule stability and hydrophobicity, pH, temperature, and the presence of sufficient nutrients (Antízar-Ladislao et al. 2006).

This study aimed to investigate the application of white rot fungus *P. ostreatus* in biodegradation of naphthalene as a model for PAHs, to elucidate the pathway for degradation, and to evaluate the expression level of laccases participating in the degradation process under different fermentation conditions.

P. ostreatus potentially degraded naphthalene, anthracene, and 1,10-phenanthroline (Fig. 1). A higher level of naphthalene degradation was observed compared to anthracene and 1,10-phenanthroline where they showed lower extent of fungal biodegradation. That was in agreement with other studies which reported that high molecular weight PAHs were degraded after the low molecular weight PAHs due to the lower solubility of high molecular weight PAHs (Haritash and Kaushik 2009), which in turn reduces their bioavailability (Ghosal et al. 2016). It was also reported that phenanthrene is not generally degradable by lignin-modifying enzymes, such as those of white rot fungi, because of its high ionization potential (Gasecka et al. 2012). Similar observations were also demonstrated by Pozdnyakova et al. (2006) and Bhattacharya et al. (2014).

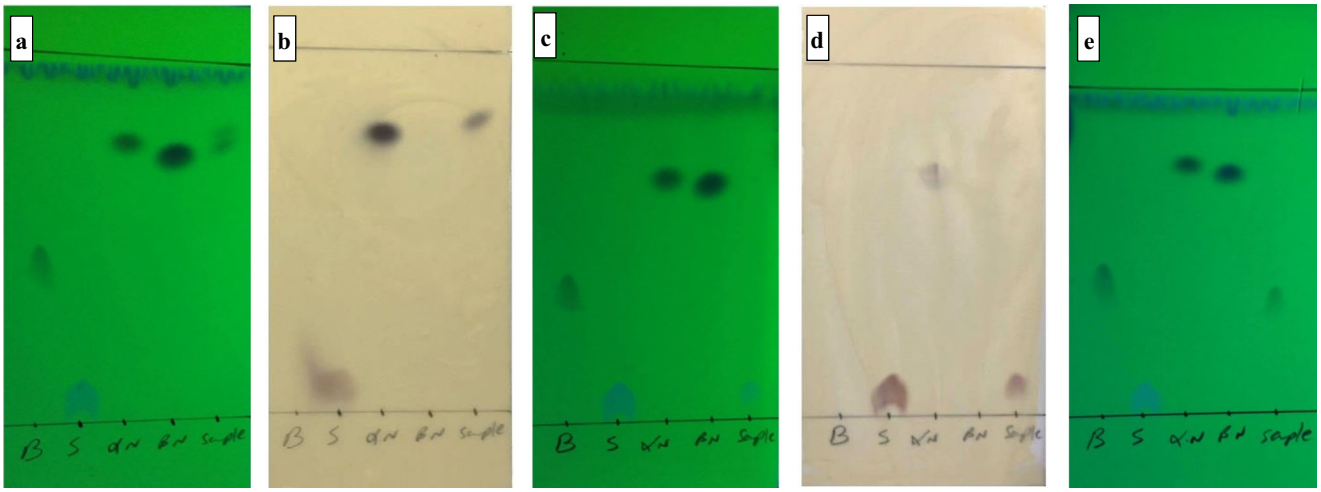

Fig. 4 **a** Thin layer chromatogram showing standard α- and β-naphthol and naphthalene metabolites (α- and β-naphthol) with R_f values of 0.77 and 0.76. **b** Ferric chloride solution was used as a spraying reagent for visualization α-naphthol spot (blue color). **c** Standard salicylic acid and naphthalene metabolite (salicylic acid) with R_f value of 0.13. **d** Visualization of salicylic acid spot (violet color) post ferric chloride spraying. **e** Appearance of benzoic acid standard and benzoic acid as a naphthalene metabolite with an R_f value of 0.34

The elevated extent of fungal biodegradation of naphthalene could be explained by the fact that fungal mycelium had a high surface area, which could maximize both mechanical and enzymatic contact with naphthalene and invade a larger area of media (Furuno et al. 2010). In addition, the extracellular nature of the fungal degradative enzymes enables the fungi to attack high concentrations of toxic chemicals, although these compounds cannot pass through the fungal cell membrane (Bisht et al. 2015).

The decrease in naphthalene concentration along with the detection of newly formed metabolites (Fig. 3) ensures the occurrence of fungal biodegradation of naphthalene rather than its physical degradation. Fungal biodegradation may have occurred by the effect of ligninolytic enzymes, where it was reported that ligninolytic enzyme system played a key role in PAH degradation by white rot- and litter-decomposing fungi (Pozdnyakova 2012). Participation of additional enzymes such as laccase, manganese peroxidase, lignin peroxidase, and 1,2-dioxygenase was also demonstrated in the degradation of PAHs (Hwang et al. 2008). Degradation of naphthalene by the aid of *P. ostreatus* is a complicated enzymatic process, which is accompanied by consumption and production of intermediate products (Hadibarata et al. 2012). It is also worth to point out that identifying the resulting biodegradation metabolites could potentially aid in elucidating the metabolic pathway of this biodegradation process (Balachandran et al. 2012). Thus, detecting naphthalene metabolites in the current study in addition to determination of the time course of naphthalene biodegradation was carried out to aid in proposing a metabolic pathway for fungal biodegradation of naphthalene using *P. ostreatus*.

Pozdnyakova et al. (2006) reported the inability of *P. ostreatus* degradation towards naphthalene although the fungus was effective in degrading the soluble derivatives such

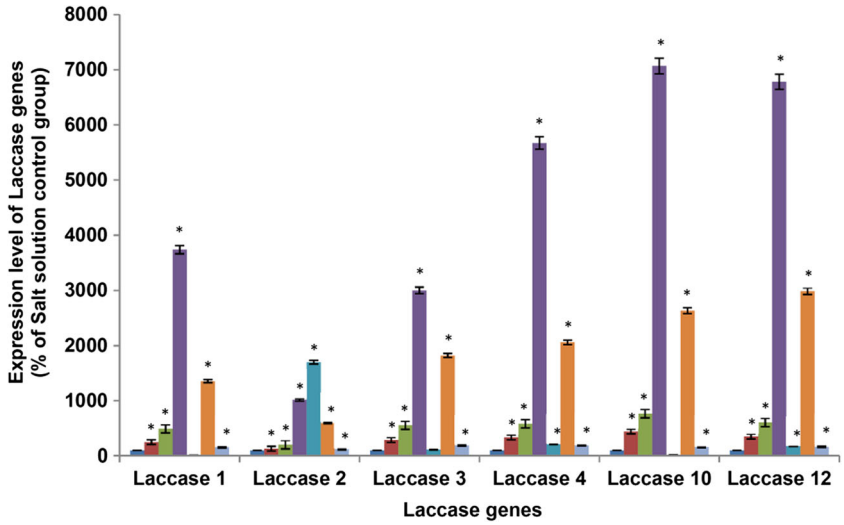

Fig. 5 Expression levels of laccase genes (Laccase 1, Laccase 2, Laccase 3, Laccase 4, Laccase 10, and Laccase 12) in different conditions. Asterisk indicates statistically significant difference compared to control group at $P < 0.05$ (one-way ANOVA, Tukey-Kramer multiple comparisons test) (SS salt solution, N naphthalene, T80 Tween 80, and PDB potato dextrose broth) (SS, SS + T80, SS + N, SS + N + T80, PDB, SS + N+(inducers ↑, inhibitors ↓), and SS + N+(inducers ↓, inhibitors ↑)

as α- and β-naphthols and α-naphthylamine. Another study showed the presence of naphthalene metabolites such as 1,4-naphthoquinone, 2-hydroxybenzaldehyde, salicylic acid, and benzoic acid after incubation with white rot fungus *Armillaria* sp. F022 (Hadibarata et al. 2012). The detection of 1,4-naphthoquinone, benzoic acid, and catechol derivatives was also reported using white rot fungus *Pleurotus eryngii* (Hadibarata et al. 2013). Our results presented an evidence for the first time for the proposed metabolic pathway of naphthalene degradation by white rot fungus *P. ostreatus*. As shown in Fig. 6, the formed α- and β-naphthols were subsequently metabolized to salicylaldehyde which was further oxidized to salicylic acid. Finally, salicylic acid was dehydroxylated into benzoic acid.

Fungi generally produce several laccase isoenzymes encoded by complex multi-gene families (Giardina et al. 2010). Laccase isoenzymes are characterized by slightly different catalytic properties and regulatory mechanisms (Piscitelli et al. 2011). An obvious relationship was also reported between the inducers of biodegradation process and the release of laccase enzymes (Yao and Ji 2014). Thus, the present study investigated the expression profiles of six laccase genes in different conditions relative to the expression of a reference gene. Results revealed that Tween 80 alone could act as an inducer for the expression of laccase enzymes even in absence of naphthalene. In the same context, naphthalene alone could induce the expression of laccase enzymes. Combination of both Tween 80 and naphthalene resulted in maximum increase in the expression of laccase enzymes. Similar results were demonstrated in other studies, where the addition of Tween 80 positively impacted the marine-derived fungal activity of laccase enzyme to around 1300 U L^{-1} (D'Souza-Ticlo et al. 2009). Another study demonstrated that Tween 80 stimulated higher laccase production (3952 U g^{-1} of dry substrate) when it was added to the medium (Patel et al. 2009). It was also reported that the addition of Tween 80 resulted in higher yields of ligninolytic enzymes in fungi as there is evidence that this surfactant resulted in higher permeability of oxygen and extracellular enzyme transport through the cell membranes of fungi and also increased the bioavailability of the PAHs which consequently facilitate its consumption by *P. ostreatus* (El-Batal et al. 2015).

In the present study, higher concentrations of inducers such as malt extract (nitrogen source), glucose (carbon source), and $CuSO_4$, and lower concentrations of inhibitors (EDTA and $CdSO_4$) resulted in elevation in the expression level of laccases. On the other hand, lower induction in laccase expression was recorded in presence of low concentration of malt extract, glucose, and $CuSO_4$ as well as high concentration of EDTA and $CdSO_4$ (Fig. 5). In the same context, laccase activity was elevated by 6-fold due to the synergistic effect between copper and lignin especially when malt extract medium was supplemented with carbon and nitrogen sources (Tinoco et al. 2011). Another study reported that the addition of yeast extract, copper sulfate, and ferulic acid resulted in the highest enzymatic activity (Karp et al. 2012). Moreover, glucose, ammonium nitrate, and $CuSO_4$ demonstrated stimulatory effect on laccase production (Pratheebaa et al. 2013). It is important to point out that fungal laccases are multi-copper oxidoreductase enzymes that catalyze the oxidation of a great variety of phenolic compounds and aromatic amines through simultaneous reduction of molecular oxygen to water (Giardina et al. 2010). Thus, the recorded elevated biodegradation and the increased laccase expression in presence of $CuSO_4$ could be correlated to the activation and stabilization of *P. ostreatus*-produced laccase in case of copper availability (Bhattacharya et al. 2014). Regarding inhibitors, it was reported Cd and Pb induced an inhibitory effect on the fungal laccases (García-Delgado et al. 2013). In addition, chelating agents such as EDTA were found to exert an inhibitory potential on the enzyme activity and Benzo [a] pyrene degradation by *P. ostreatus* (Bhattacharya et al. 2014). Current observations also revealed that the presence of PDB resulted in marked elevation in the expression level of laccase 2 by 17-

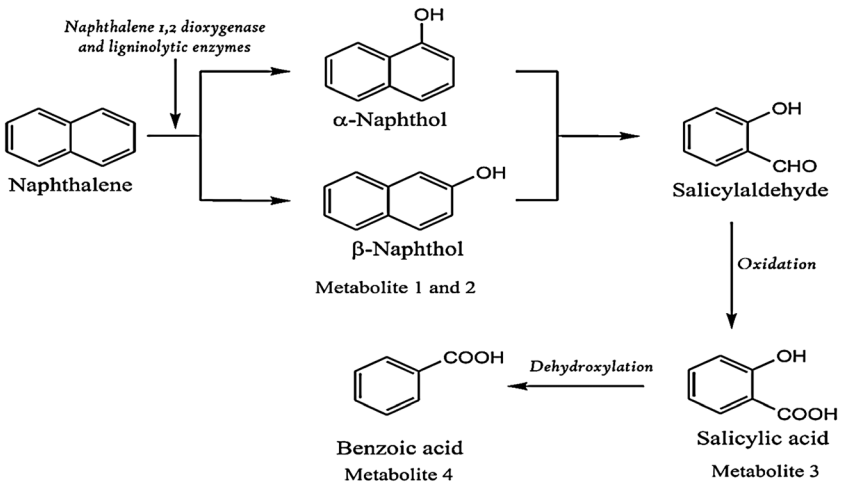

Fig. 6 Elucidation of the proposed metabolic pathway for fungal biodegradation of naphthalene by *P. ostreatus*. Naphthalene dioxygenase and ligninolytic enzymes, attack on the aromatic ring to form α- and β-naphthol (Metabolites 1 and 2). 1,2-Dihydroxynaphthalene is metabolized to salicylaldehyde which is oxidized to salicylic acid (Metabolite 3). Salicylic acid is further dehydroxylated into benzoic acid (Metabolite 4)

fold compared to a minor increase or non-significant effect on the expression level of other tested laccases (Fig. 5). The elevated expression level of laccase 2 in PDB could be justified by the reported induction of laccase 2 transcription in presence of high concentration of carbon (Pezzella et al. 2013) which is available in presence of PDB.

Current study also recorded the highest expression level in case of laccase 10 and 12 in different conditions. While another study reported that, among the produced laccases, laccase 2 and laccase 10 genes are the main sources of laccase activity in the induced *P. ostreatus* submerged fermentation containing different nutrients and high amount of glucose as a carbon source. The difference between this study and current findings may be justified by that the enzymatic activities of different laccases and the laccase gene family transcription profiles greatly differ among different culture conditions and closely related strains (Castanera et al. 2012). These findings could also greatly highlight the significance of investigating the expression profile of laccase enzymes in different fermentation conditions and their related influence on naphthalene biodegradation potential.

Conclusion

Results presented herein provided enough evidence for the biodegradative pathway of naphthalene by white rot fungus *P. ostreatus*, as a model for PAHs. Moreover, it sheds light on how the process stimulation or inhibition might proceed via modulating the expression of laccase isozymes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdel-Shafy HI, Mansour MS (2016) A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Pet 25:107–123

Antízar-Ladislao B, Lopez-Real J, Beck AJ (2006) Investigation of organic matter dynamics during in-vessel composting of an aged coal–tar contaminated soil using fluorescence excitation–emission spectroscopy. Chemosphere 64:839–847

Balachandran C, Duraipandiyan V, Balakrishna K, Ignacimuthu S (2012) Petroleum and polycyclic aromatic hydrocarbons (PAHs) degradation and naphthalene metabolism in Streptomyces sp.(ERI-CPDA-1) isolated from oil contaminated soil. Bioresour Technol 112:83–90

Bestetti G, Di Gennaro P, Galli E, Leoni B, Pelizzoni F, Sello G, Bianchi D (1994) Bioconversion of substituted naphthalenes to the corresponding salicylic acids. Appl Microbiol Biotechnol 40:791–793

Bhattacharya S, Das A, Prashanthi K, Palaniswamy M, Angayarkanni J (2014) Mycoremediation of Benzo [a] pyrene by Pleurotus ostreatus in the presence of heavy metals and mediators. 3 Biotech 4:205–211

Bisht S, Pandey P, Bhargava B, Sharma S, Kumar V, Sharma KD (2015) Bioremediation of polyaromatic hydrocarbons (PAHs) using rhizosphere technology. Braz J Microbiol 46:7–21

Castanera R, Pérez G, Omarini A, Alfaro M, Pisabarro AG, Faraco V, Amore A, Ramírez L (2012) Transcriptional and enzymatic profiling of Pleurotus ostreatus laccase genes in submerged and solid-state fermentation cultures. Appl Environ Microbiol 78:4037–4045

D'Souza-Ticlo D, Garg S, Raghukumar C (2009) Effects and interactions of medium components on laccase from a marine-derived fungus using response surface methodology. Mar Drugs 7:672–688

El-Batal AI, ElKenawy NM, Yassin AS, Amin MA (2015) Laccase production by Pleurotus ostreatus and its application in synthesis of gold nanoparticles. Biotechnol Rep 5:31–39

Elhusseiny SM, Amin HM, Shebl RI (2017) Mathematical modelling for optimization of naphthalene degradation by Pleurotus ostreatus. Egypt J Biotechnol 55:46–61

Fathepure BZ (2015) Recent studies in microbial degradation of petroleum hydrocarbons in hypersaline environments. The Proceedings from Halophiles 2013. The International Congress on Halophilic Microorganisms. Frontiers Media SA:35

Furuno S, Päzolt K, Rabe C, Neu TR, Harms H, Wick LY (2010) Fungal mycelia allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading bacteria in water-unsaturated systems. Environ Microbiol 12:1391–1398

García-Delgado C, Jiménez-Ayuso N, Frutos I, Gárate A, Eymar E (2013) Cadmium and lead bioavailability and their effects on polycyclic aromatic hydrocarbons biodegradation by spent mushroom substrate. Environ Sci Pollut Res 20:8690–8699

Gasecka M, Drzewiecka K, Stachowiak J, Siwulski M, Golinski P, Sobieralski K, Golak I (2012) Degradation of polycyclic aromatic hydrocarbons (PAHs) by spent mushroom substrates of Agaricus bisporus and Lentinula edodes. Acta Sci Pol-Hortoru 11:39–46

Ghosal D, Ghosh S, Dutta TK, Ahn Y (2016) Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Front Microbiol 7

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

Gratia E, Weekers F, Margesin R, D'Amico S, Thonart P, Feller G (2009) Selection of a cold-adapted bacterium for bioremediation of wastewater at low temperatures. Extremophiles 13:763–768

Hadibarata T, Yusoff AR, Aris A, Kristanti RA (2012) Identification of naphthalene metabolism by white rot fungus Armillaria sp. F022. J Environ Sci 24:728–732

Hadibarata T, Teh ZC, Zubir MM, Khudhair AB, Yusoff AR, Salim MR, Hidayat T (2013) Identification of naphthalene metabolism by white rot fungus Pleurotus eryngii. Bioprocess Biosyst Eng 36:1455–1461

Haritash A, Kaushik C (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169:1–15

Hwang SS, Choi HT, Song HG (2008) Biodegradation of endocrine-disrupting phthalates by Pleurotus ostreatus. J Microbiol Biotechnol 18:767–772

Jacques RJ, Okeke BC, Bento FM, Teixeira AS, Peralba MC, Camargo FA (2008) Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour Technol 99:2637–2643

Karp SG, Faraco V, Amore A, Birolo L, Giangrande C, Soccol VT, Pandey A, Soccol CR (2012) Characterization of laccase isoforms produced by Pleurotus ostreatus in solid state fermentation of sugarcane bagasse. Bioresour Technol 114:735–739

Meckenstock RU, Annweiler E, Michaelis W, Richnow HH, Schink B (2000) Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. Appl Environ Microbiol 66:2743–2747

Patel H, Gupte A, Gupte S (2009) Effect of different culture conditions and inducers on production of laccase by a basidiomycete fungal

isolate Pleurotus ostreatus HP-1 under solid state fermentation. BioResources 4:268–284

Pezzella C, Lettera V, Piscitelli A, Giardina P, Sannia G (2013) Transcriptional analysis of Pleurotus ostreatus laccase genes. Appl Microbiol Biotechnol 97:705–717

Piscitelli A, Pezzella C, Giardina P, Faraco V, Sannia G (2010) Heterologous laccase production and its role in industrial applications. Bioeng Bugs 1:254–264

Piscitelli A, Giardina P, Lettera V, Pezzella C, Sannia G, Faraco V (2011) Induction and transcriptional regulation of laccases in fungi. Curr Genomics 12:104–112

Pozdnyakova NN (2012) Involvement of the ligninolytic system of white-rot and litter-decomposing fungi in the degradation of polycyclic aromatic hydrocarbons. Biotechnol Res Int 2012:1–20

Pozdnyakova NN, Rodakiewicz-Nowak J, Turkovskaya OV, Haber J (2006) Oxidative degradation of polyaromatic hydrocarbons and their derivatives catalyzed directly by the yellow laccase from Pleurotus ostreatus D1. J Mol Catal B Enzym 41:8–15

Pratheebaa P, Periasamy R, Palvannan T (2013) Factorial design for optimization of laccase production from Pleurotus ostreatus IMI 395545 and laccase mediated synthetic dye decolorization

Sartoros C, Yerushalmi L, Béron P, Guiot SR (2005) Effects of surfactant and temperature on biotransformation kinetics of anthracene and pyrene. Chemosphere 61:1042–1050

Silva IS, Grossman M, Durrant LR (2009) Degradation of polycyclic aromatic hydrocarbons (2–7 rings) under microaerobic and very-low-oxygen conditions by soil fungi. Int Biodeterior Biodegrad 63:224–229

Tinoco R, Acevedo A, Galindo E, Serrano-Carreón L (2011) Increasing Pleurotus ostreatus laccase production by culture medium optimization and copper/lignin synergistic induction. J Ind Microbiol Biotechnol 38:531–540

Ukiwe LN, Egereonu UU, Njoku PC, Nwoko CI, Allinor JI (2013) Polycyclic aromatic hydrocarbons degradation techniques: a review. Int J Chem 5:43

Yao B, Ji Y (2014) Lignin biodegradation with laccase-mediator systems. Front Energy Res 2:12

Yuan S, Wei S, Chang B (2000) Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture. Chemosphere 41:1463–1468