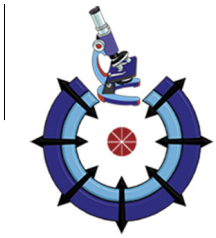
Journal of Genetic Engineering and Biotechnology (2013) **11**, 53–59



Academy of Scientific Research & Technology and
National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ARTICLE

1,2 Propanediol utilization by *Lactobacillus reuteri* DSM 20016, role in bioconversion of glycerol to 1,3 propanediol, 3-hydroxypropionaldehyde and 3-hydroxypropionic acid

Heba M. Amin [a,*], Abdelgawad M. Hashem [b], Mohamed S. Ashour [a], Rajini Hatti-Kaul [c]

[a] *Department of Microbiology and Immunology, MSA University, Cairo, Egypt*
[b] *Department of Microbiology and Immunology, Cairo University, Cairo, Egypt*
[c] *Department of Biotechnology, Chemical Center, Lund University, Lund, Sweden*

Received 12 June 2012; revised 22 November 2012; accepted 15 December 2012
Available online 28 January 2013

KEYWORDS

1,2 Propanediol (1,2 PD);
Lactobacillus reuteri;
1,3 Propanediol (1,3 PD);
Propionic acid;
Propionaldhyde;
3-Hydroxypropionaldehyde (3-HPA)

Abstract The objective of the presented work is to demonstrate the metabolism of 1,2 propandiol by *Lactobacillus reuteri* and to elucidate the metabolites produced during the process. This Metabolic pathway is crucial for biotechnological applications using *L. reuteri* in bioconversion of glycerol to industrially important plate-form chemicals. *L. reuteri* grown on minimal media containing 1,2 propanediol was able to utilize the compound as a sole carbon and energy source. The growth of the bacteria was linear with time; however the specific growth rate was significantly low compared to bacteria grown on the same media in the presence of glucose.

The fermentation of 1,2 propanediol by *L. reuteri* in presence and absence of glucose was followed for 72 h and the metabolites produced during the process were detected using HPLC. 1,2 Propanediol was completely converted to propionaldhyde in a time dependent fashion, this process had a higher rate in presence of glucose. Consequently the produced propionaldhyde was converted to propionic acid and propanol in a skewed equimolar manner. In presence of glucose: acetic acid, lactic acid, succinic acid and ethanol were detected while in absence of glucose only minute amounts of acetic acid and lactic acid were detected which indicates presence of different metabolic pathways for glucose and 1,2 propanediol metabolism. Resting cells of *L. reuteri* induced in presence of 1,2

* Corresponding author. Tel.: +20 1001153645.
E-mail addresses: hebamagdy1412@hotmail.com (H.M. Amin), gogo hashem5@yahoo.com (A.M. Hashem), siefashour@hotmail.com (M.S. Ashour), Rajni.Hatti-Kaul@biotek.lu.se (R. Hatti-Kaul).

Peer review under responsibility of National Research Center, Egypt.

http://dx.doi.org/10.1016/j.jgeb.2012.12.002

propanediol have shown significant capabilities to convert aqueous glycerol to 1,3 propanediol, 3-hydroxypropionaldhyde and a compound proposed to be 3-hydroxypropionic acid as detected by gas chromatographic technique.

1. Introduction

Degradation of the common plant cell wall sugars fucose and rhamnose under anaerobic conditions produces 1,2 PD, which is used as a carbon and energy source by a number of enteric bacteria and by bacteria that grow in environments such as aquatic sediments where 1,2 PD is readily available due to the breakdown of plant material [13]. Catabolism of 1,2 PD requires a complex pathway that is ultimately provides the cell with propionyl-CoA, an electron sink, and ATP [13]. The proteins involved in 1,2 PD degradation are encoded by the genes of 1,2 PD utilization (*pdu*) operon [11]. These genes code for diol dehydratase and other proteins which have been involved in production of 3-HPA and 1,3 PD from glycerol [13,18,26].

Natural producers of 1,3 PD and 3-HPA from glycerol are of genera *Klebsiella* [10,19,37], *Clostridia* [6,18,24], *Citrobacter* [28], *Enterobacter* [38] and *Lactobacilli* [27]. Several Clostridial species such as non-pathogenic *Clostridium butyricum* [26], *Clostridium pasteurianum* [1] grow on glycerol and form 1,3 PD. Facultative anaerobes such as *Klebsiella pneumoniae* and *Citrobacter freundii* also appeared to be suitable for 1,3 PD and 3-HP production. Although it might be easier to handle facultative anaerobes, but since all these strains are classified as opportunistic pathogens, special safety precautions are required to grow them. Along with *K. pneumoniae*, other species of same genera have also been exploited for 1,3 PD and 3-HP production, such as *Klebsiella oxytoca* [37], *Lactobacillus brevis*, *Lactobacillus buchneri* [34].

Lactobacillus reuteri is a heterofermentative lactic acid bacterium and is frequently found in the gastrointestinal tract of humans and other animals. *L. reuteri* has been reported to exhibit "probiotic" properties. It has been extensively analyzed for probiotic applications, including its safe administration to healthy individuals, its ability to colonize the intestine, as a diarrhea therapeutic agent, as an inhibitor of bacterial pathogens, and the immunological modulation of the gastrointestinal mucosa [3,20,25,29,33,36]. Some strains of *L. reuteri* have the ability to produce and excrete the broad-spectrum antimicrobial compound reuterin, which is structurally identical to 3-HPA, during anaerobic metabolism of glycerol [5,31,32]. The probiotic effects of *L. reuteri* have been proposed to be largely associated with the production of reuterin, and this antimicrobial substance is also an effective food preservative agent [16,35].

L. reuteri have been also shown to use glycerol as an external hydrogen acceptor source during fermentation [22]. This study was undertaken to investigate the correlation between the utilization of 1,2 PD by *L. reuteri* and its potential for bioconversion of glycerol to 1,3 PD, 3-HPA and 3-HP.

2. Materials and methods

2.1. Bacteria and growth conditions

L. reuteri DSM 20016 was obtained from DSMZ culture collection (Germany). The stock culture was kept at −20 °C in solution containing MRS broth (Defco, England) and 20% glycerol. For inoculum preparation, 0.25 ml of stock culture was added to 100 ml MRS broth supplemented with 20 mM 1,2 propanediol, incubated at 37 °C for 8 h under anaerobic condition. This induction process was done once more before the culture being used for inoculation of the fermentation media.

2.2. 1,2 Propanediol metabolism

In a 500 ml conical flask, 250 ml Modified MRS (MOD-MRS) containing 100 mM 1,2 propanediol were inoculated with 1% of the pre-cultured *L. reuteri*. The micro-organism was left to grow anaerobically for 72 h at 37 °C. MOD-MRS composition (per liter) 5 g Bactopeptone, 4 g Lab-lemco (Meat Extract), 2 g Yeast Extract, 0.5 ml Tween 80, 1 g K_2HPO_4, 3 g $NaH_2PO_4 \cdot H_2O$, 0.6 g CH_3COONa, 0.3 g $MgSO_4 \cdot 7H_2$ and 0.04 g $MnSO_4 \cdot H_2O$. Ten milliliters samples were removed at different time intervals and pelleted. Supernatant was stored at −20 °C until tested for presence of metabolic products.

2.3. Effect of glucose addition on 1,2 PD metabolism

To the previously mentioned MOD-MRS media supplemented with 1,2 PD glucose were added (40 mM) at zero time. In a second set of experiments 40 mM glucose were added prior to 1,2 PD depletion from the media. In the third set of experiments glucose solution was fed batched so that 30 mM were added at zero time and the remaining 10 mM were added prior to 1,2 PD depletion from the media. Control experiments were performed by culturing *L. ruteri* cells on the same media without glucose addition. Samples (10 ml) were taken every 12 h and OD was measured to calculate cell density. Cells were centrifuged and supernatant was tested for production of propionic acid, propanol and propionaldehyde.

Biotransformation of glycerol to 1,3 propanediol by resting cells of *L. reutri* Biotransformation was performed anaerobically in a cooling thermomixer KTMR-133 (HLC-Biotech, Germany) set at 800 rpm and 37 °C unless mentioned otherwise reaction mixture (2 ml) volume contained resting cells of *L. reuteri* (20 mg) and glycerol (100 mM) in distilled water. At different time intervals samples were taken and quantitative determination of glycerol l,3-hydroxypropionaldyde and 1,3 propandiol was carried out.

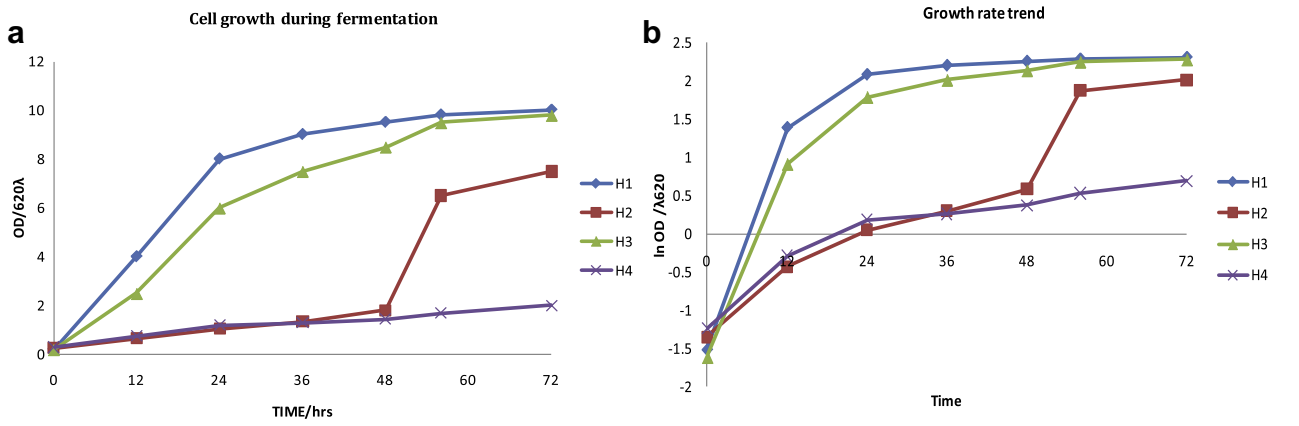


Figure 1 Growth kinetics of *L. reuteri* 20016 on MOD-MRS supplemented with 40 mM 1,2 PD in presence (H_1 ♦, H_2 ▲, H_3 ■) and in absence (H_4 ×) of glucose. (a) Growth measured by optical density, (b) kinetics of growth.

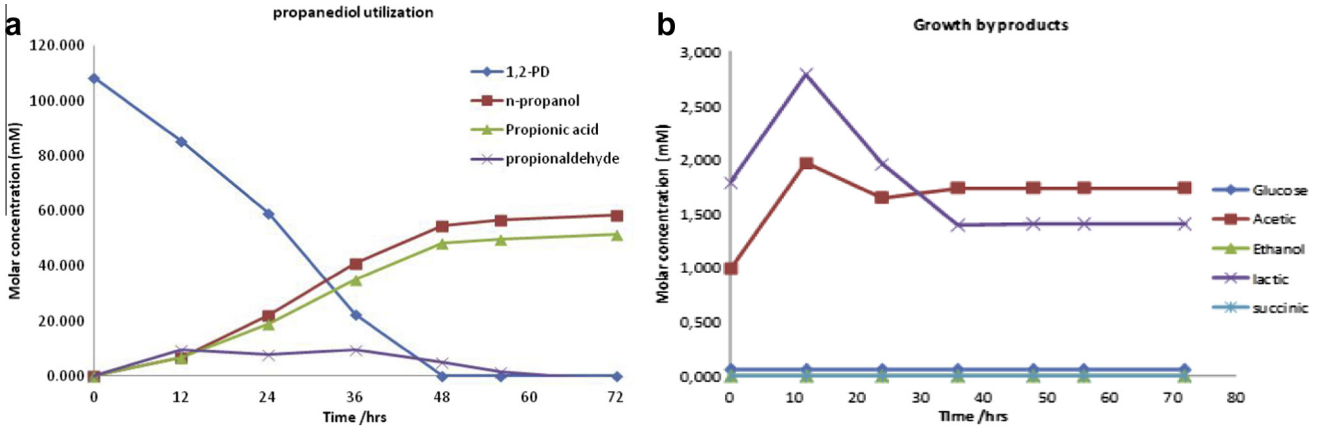


Figure 2 Bioconversion (a) and metabolic products (b and c) during 1,2 propanediol utilization by *L. reuteri* 20016 in absence of glucose.

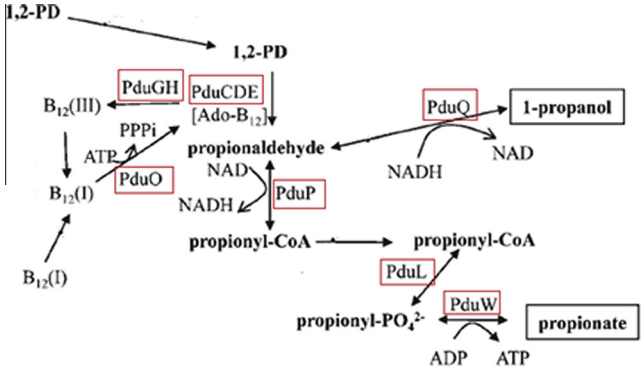


Figure 3 Metabolism of 1,2 propanediol through expression of Pdu operone.

2.4. Analytical methods

Cell growth: Cell growth was followed by measuring OD at 620 nm using spectrophotometer Ultrospec 1000 (Pharmacia Biotech, Uppsala, Sweden) and then correlated with CDW.

For determination of dry cell weight: Cultured cells were recovered by centrifugation (10,000*g* for 5 min), and washed using distilled water and dried overnight in an oven at 85 °C then weighed.

2.5. Measurement of metabolites and substrate

Determination of propionic acid, n-propanol, 1,2 propanediol, glucose, succinic acid, lactic acid, acetic acid and ethanol concentrations were done by HPLC (JASCO, Tokyo, Japan) equipped with RI detector (ERC Inc., Kawaguchi, Japan) and JASCO intelligent autosampler. Separation of the compounds was performed using Aminex HPX 87-H cation exclusion chromatographic column (Length 300 mm, ID 7.8 mm, particle diameter 9 µm, Bio-rad Laboratories, CA, USA) connected to Micro-guard cartridge. The column temperature was kept at 55 °C using oven (Schimadzu, Kyoto, Japan). Samples were diluted with MilliQ water, treated with 20% w/w sulfuric acid (20 µl/ml sample) and then filtered through 0.45 µm polypropylene filters. Fifty microliters of the sample was injected in 5 mM H_2SO_4 as a mobile phase and the flow rate was 0.6 ml/min.

In glycerol bioconversion experiments by resting cells of *L. reutrei* determination of glycerol, 1,3 propanediol, 3-hydroxyproroionaldhye and 3-hydroxypropionic acid concentrations were performed using gas chromatography (GC, Varian 430-GC,

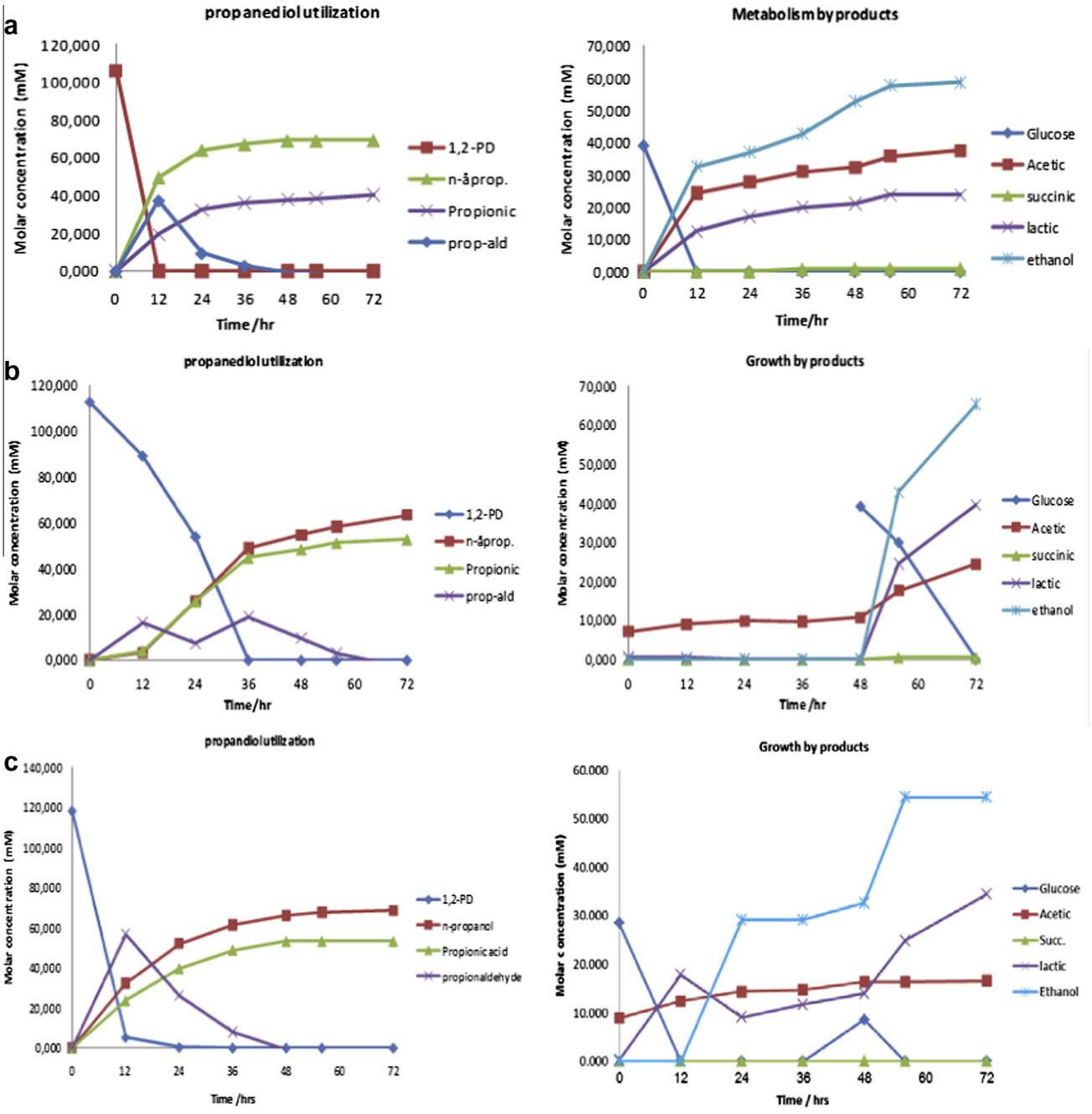


Figure 4 Bioconversion and metabolic products during 1,2 propanediol utilization by *L. reuteri* 20016 in presence of glucose (a) 40 mM glucose added at zero time. (b) 40 mM glucose added prior to 1,2 PD depletion from the media (c) 30 mM glucose added at zero time and 10 mM added prior to 1,2 PD depletion.

Varian, USA) equipped with Factor Four Capillary column, VF-1 ms (Varian, 15 M × 0.25 mm) and a flame ionization detector. The initial column oven temperature was increased from 50 to 250 °C at a rate of 20 °C/min. The samples, diluted with acetonitrile at concentration of 0.1–0.5 mg/ml, were injected in split injection mode of 10% at 275 °C.

3. Results and discussion

Biofuel has increasingly become a reliable alternative for energy production. At the meantime depletion of fossil fuels, environmental concerns and the concept of sustainability made the chemical industries seriously exploring bio-based renewable feed stock for producing bulk chemicals and the first half of the 21st century may witness this dramatic shift from petroleum production to bio-based renewable products [23]. For every 100 gal of biodiesel that is produced, 5–10 gal of the less glamorous crud glycerol are left behind with a sparking debate about the best uses of this waste [7,21]. *L. reuteri* have been shown to use glycerol and thus could be employed for production of useful bulk chemicals from glycerol [22].

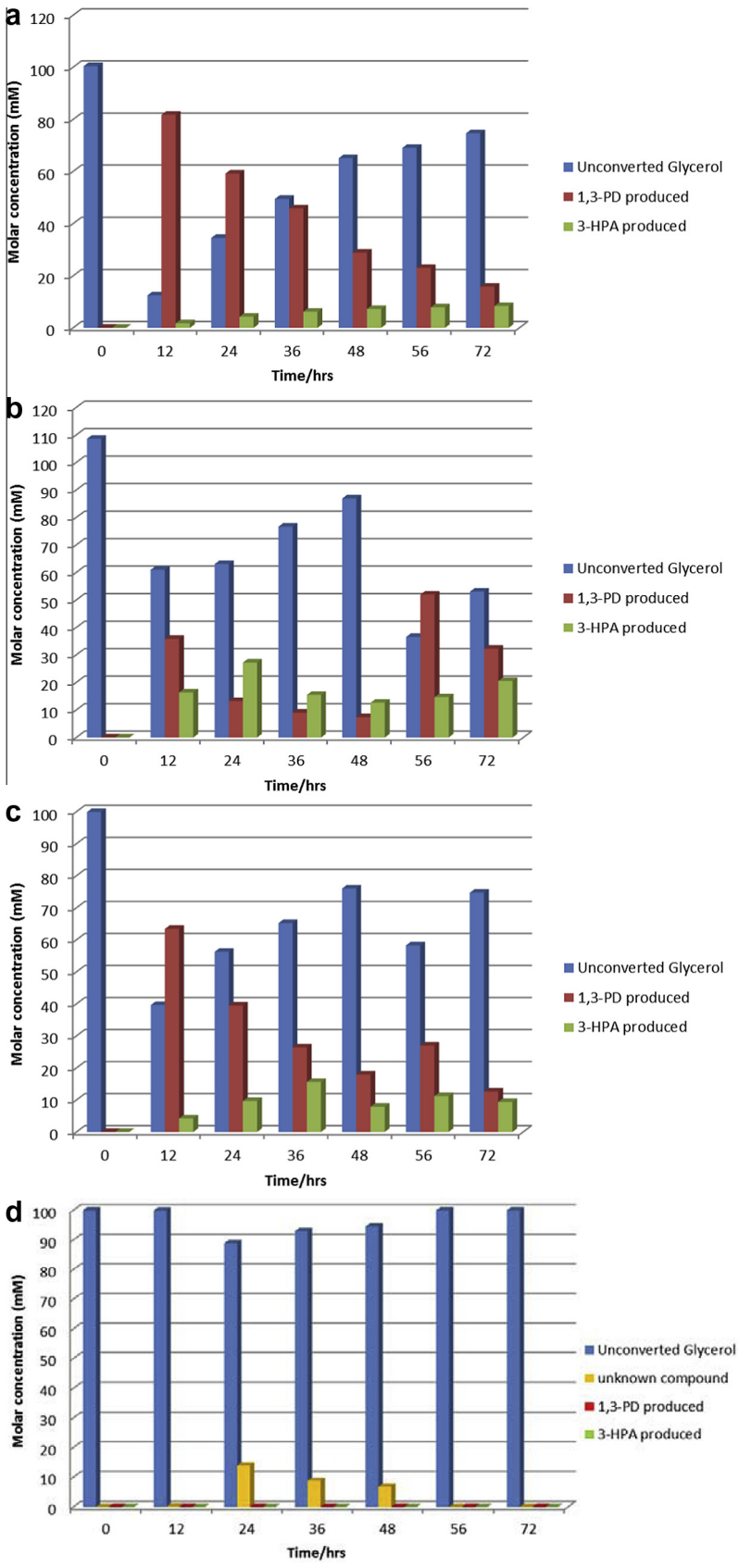


Figure 5 Bioconversion of glycerol using resting cells of *L. reuteri*: resting cells induced in presence of glucose; 40 mM glucose added at zero time (a), 40 mM glucose added prior to 1,2 PD depletion from the media (b), 30 mM glucose added at zero time and 10 mM added prior to 1,2 PD depletion (c). Resting cells induced in absence of glucose (d).

In this work we present evidence that 1,2 PD metabolism by *L. reuteri* 20016 resulted in production of commercially important chemicals including propionaldehyde, 1-propanol and propionic acid. The results presented in Fig. 1 shows the time dependent growth of *L. reuteri* 20016 metabolizing 1,2 PD as a sole carbon and energy source. The pathway of 1,2 PD metabolism necessitates the expression of *Pdu* operon [2,9,15,16], such operon is a complex gene cluster containing number of gene that code for carboxysome formation and B12 dependent 1,2 PD degradation [4,12,13,30].

In this work HPLC analysis of metabolic products of 1,2 PD utilization by *L. reuteri* DSM 20016. Fig. 2a confirmed that 1,2 PD was converted first to propionaldhyde by vit B12 dependent diol dehydratase as reported by several authors [2,9,15,16]. Subsequently, propionaldhyde is catabolized to n-propanol and propionic acid presumably by co-enzyme A dependent aldehyde dehydrogenase, phosphotransacylase, propionate kinase and alcohol dehydrogenase [30]. This pathway provides a source of ATP and carbon compounds such as acetic acid and lactic acid Fig. 2b that can be diverted to central metabolism of known pathways [13]. Moreover, Krooneman et al. [14] reported the isolation of novel strains of *Lactobacillus diolivorans* sp. from maize silage. These *Lactobacilli* were able to degrade 1,2 PD under anoxic conditions to 1-propanol and propionic acid via propionaldehyde intermediate. The results in this work and those reported elsewhere [13,14] supported the idea that 1,2 PD metabolism proceeded through expression of the pdu operone (Fig. 3).

Optimization for the production of specific compounds from 1,2 PD might require batch, fed batch or continuous addition of glucose to the production medium in order to build enough biomass and/or providing the required energy source [18,31,32]. In this paper the effect of glucose addition on metabolism of 1,2 PD by *L. reuteri* DSM 20016 was studied, Fig. 4 illustrated the degradation of 1,2 PD and metabolism of glucose added at different times of fermentation. Biomass production was increased after glucose addition. This increase in biomass accelerated the metabolism of 1,2 PD, however the products of glycerol bioconversion remain the same in presence and/or absence of glucose. As shown in Fig. 4, glucose was metabolized to ethanol, such assumption was deducted from the co-incidence between the onset of ethanol production and glucose addition (as in Fig. 4a and b). the results illustrated in Fig. 4c indicates a lag period between ethanol production and addition of glucose (30 Mm), this lag period could be attributed to low biomass production after glucose (30 mM) addition as shown in Fig. 1, and moreover no ethanol was detected in absence of glucose. These results suggest that glucose addition was not essential for 1,2 PD metabolism and that the metabolism of glucose and 1,2 PD followed different metabolic pathways which are neither interactive nor repressive.

L. reuteri has been utilized in several processes for production of 3-HPA from glycerol [31,32]. The catalytic ability of this bacterium to convert 1,2 PD and/or glycerol to commodity chemicals such as propionaldehyde, 1-propanol, propionic acid and their hydroxyl derivatives 3-HPA, 1,3 PD, 3-hydroxypropionic acid was attributed to the successful expression of their pdu genes. Thus, Morita et al. [39] performed comparative genomic analysis for *Lactobacilli*. The results of this investigation revealed that *L. reuteri* has a unique gene cluster (pdu) that could be utilized for the conversion of glycerol into 3-HPA via the expression of diol (glycerol) dehydratase. 3-HPA could be metabolized further by dehydrogenase to produce 1,3 PD and hydroxypropionic acid.

The expression of *Pdu* gene cluster of *L. reuteri* 20016 might be exploited for bioconversion of glycerol or glycerol–water to 3-HPA, 1,3 PD and 3-HP by resting cells induced by growth on 1,2 PD. The work of Hartlep et al. [17] in 2002 employed a two-step fermentation process that was used for production of 1,3 PD and 3-HPA from glycerol. In the first step cell mass was produced in presence of inducer (glycerol or 1,2 PD), the second step involved the conversion of glycerol to 3-HPA and 1,3 PD by the resting cells. Such techniques might reduce the cost for downstream processing, eliminate the inhibition by metabolic products such as acetic acid and ethanol and would allow for the development of cost efficient continuous production of plat-form chemicals from glycerol waste water in this work. The resting cells of *L. reuteri* grown on MOD-MRS supplemented with glucose and in absence of 1,2 PD or glycerol was not able to produce any bioconversion product when incubated with glycerol. Meanwhile Fig. 5 shows the results of utilizing resting cells of *L. reuteri* induced by 1,2 PD in presence and in absence of glucose for bioconversion of glycerol–water to 1,3 PD, 3-HPA and 3-HP. It is evident that induction condition for obtaining the resting cells such as the presence of glucose, oxygen level and temperature might play an important role in optimization of the production process. Yet the presence of inducer to the *Pdu* operon such as 1,2 PD and/or glycerol is an essential requirement. Process development for optimization of the production of 1,3 PD by resting of *L. reuteri* has been accomplished [8]. Bioconversion products of glycerol are valuable intermediates in green chemistry and renewable resources which have wide range of applications that will be the target of our future work.

Acknowledgements

This work was supported by grant in aid from the Swidish link and Vennova to whom the authors are entitled.

References

[1] H. Biebl, Fermentation of glycerol by *Clostridium pasteurianum*—batch and continuous culture studies, J. Ind. Microbiol. Biotechnol. 27 (2001) 18–26.

[2] T.A. Bobik, G.D. Havemann, R.J. Busch, D.S. Williams, H.C. Aldrich, The propanediol utilization (*pdu*) operon of *Salmonella enterica* serovar Typhimurium LT2 includes genes necessary for formation of polyhedral organelles involved in coenzyme B(12)-dependent 1,2-propanediol degradation, J. Bacteriol. 181 (1999) 5967–5975.

[3] Y.H. Chang, J.K. Kim, H.J. Kim, W.Y. Kim, Y.B. Kim, Y.H. Park, Selection of a potential probiotic *Lactobacillus* strain and subsequent in vivo studies, Antonie Van Leeuwenhoek 80 (2001) 193–199.

[4] P. Chen, D.I. Andersson, J.R. Roth, The control region of the pdu/cob regulon in *Salmonella typhimurium*, J. Bacteriol. 176 (1994) 5474–5482.

[5] T.C. Chung, L. Axelsson, S.E. Lindgren, W.J. Dobrogosz, In vitro studies on reuterin synthesis by *Lactobacillus reuteri*, Microb. Ecol. Health Dis. 2 (1989) 137–144.

[6] T. Colin, A. Bories, C. Lavigne, G. Moulin, Effects of acetate and butyrate during glycerol fermentation by *Clostridium butyricum*, Curr. Microbiol. 43 (2001) 238–243.

[7] G.P. Da Silva, M. Mack, J. Contiero, Glycerol: a promising and abundant carbon source for industrial microbiology, Biotechnol. Adv. 27 (2009) 30–39.
[8] H.M. Amin, T. Dishisha, A.M. Hashem, M.S. Ashour, R. Hatti-Kaul, Biotransformation of glycerol to 1,3 propanediol by *L. reuteri* ATCC 20016 in aqueous system, in: Alex-Biovision conference 2012, 2012.
[9] G.D. Havemann, T.A. Bobik, Protein content of polyhedral organelles involved in coenzyme B12-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2, J. Bacteriol. 185 (2003) 5086–5095.
[10] H. Huang, C.S. Gong, G.T. Tsao, Production of 1,3-propanediol by *Klebsiella pneumoniae*, Appl. Biochem. Biotechnol. 98–100 (2002) 687–698.
[11] R.M. Jeter, Cobalamin-dependent 1,2-propanediol utilization by *Salmonella typhimurium*, J. Gen. Microbiol. 136 (1990) 887–896.
[12] J.M. Shively, C.E. Bradburne, H.C. Aldrich, T.A. Bobik, J.L. Mehlman, et al., Sequence homologs of the carboxysomal polypeptide CsoS1 of the thiobacilli are present in cyanobacteria and entericbacteria that form carboxysomes-polyhedral bodies, Can. J. Bot. 76 (1998) 906–916.
[13] Cheryl A. Kerfeld, Sabine Heinhorst, Gordon C. Cannon, Bacterial microcompartments, Annu. Rev. Microbiol. 64 (2010) 391–408.
[14] J. Krooneman, F. Faber, A.C. Alderkamp, S.J.H.W. Oude Elferink, F. Driehuis, I. Cleenwerck, J. Swings, J.C. Gottschal, M. Vancanneyt, *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage, Int. J. Syst. Evol. Microbiol. 52 (2002) 639–646.
[15] N. Leal, G. Havemann, T. Bobik, PduP is a coenzyme-A-acylating propionaldehyde dehydrogenase associated with the polyhedral bodies involved in B12-dependent 1,2-propanediol degradation by *Salmonella enteric* serovar Typhimurium LT2, Arch. Microbiol. 180 (2003) 353–361.
[16] Q. Luthi-Peng, S. Scharer, Z. Puhan, Production and stability of 3-hydroxypropionaldehyde in *Lactobacillus reuteri*, Appl. Microbiol. Biotechnol. 60 (2002) 73–80.
[17] M. Hartlep, W. Hussman, N. Prayitno, I. Meynial, A.P. Zeng, Study of two stage process for microbial production of 1,3 propanediol from glycerol, Appl. Microbiol. Biotechnol. 60 (2002) 60–66.
[18] H. Malaoui, R. Marczak, Influence of glucose metabolism by wild-type and mutant strains of *Clostridium butyricum* E5 grown in chemostatic culture, Appl. Microbiol. Biotechnol. 55 (2001) 226–233.
[19] A. Nemeth, B. Kupesulik, B. Sevella, 1,3-Propanediol oxidoreductase production with *Klebsiella pneumoniae* DSM2026, World J. Microbiol. Biotechnol. 19 (2003) 659–663.
[20] E. Niv, T. Naftali, R. Hallak, N. Vaisman, The efficacy of *Lactobacillus reuteri* ATCC 55730 in the treatment of patients with irritable bowel syndrome: a double blind, placebo-controlled, randomized study, Clin. Nutr. 24 (2005) 925–931.
[21] S. Papanikolaou, G. Aggelis, Biotechnological valorization of biodiesel derived glycerol waste through production of single cell oil and citric acid by *Yarrowia lipolytica*, Lipid Technol. 21 (4) (2009) 83–87.
[22] Q.L. Peng, F.B. Dileme, Z. Puhan, Effect of glucose on glycerol bioconversion by *Lactobacillus reuteri*, Appl. Microbiol. Biotechnol. 59 (2002) 289–296.
[23] A.J. Ragauskas, C.K. Williams, B.H. Davison, G. Britovsek, J. Cairney, C.A. Eckert, et al., The path forward for biofuels and biomaterial, Science 311 (2006) 484–490.
[24] C. Raynaud, P. Sarcabal, I. Meynial-Salles, C. Croux, P. Soucaille, Molecular characterization of the 1,3-propanediol operon of *Clostridium butyricum*, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 5010–5015.
[25] V. Rosenfeldt, K.F. Michaelsen, M. Jakobsen, et al., Effect of probiotic *Lactobacillus* strains on acute diarrhea in a cohort of nonhospitalized children attending daycare centers, Pediatr. Infect. Dis. J. 21 (2002) 417–419.
[26] S. Papanikolaou, M. Fick, G. Aggelis, The effect of raw glycerol concentration on the production of 1,3-propanediol by *Clostridium butyricum*, J. Chem. Technol. Biotechnol. 79 (2004) 1189–1196.
[27] H. Schutz, F. Radler, Anaerobic reduction of glycerol to 1,3-propanediol by *Lactobacillus brevis* and *Lactobacillus buchneri*, Syst. Appl. Microbiol. 5 (1984) 169–178.
[28] C. Seifert, S. Bowien, G. Gottschalk, R. Daniel, Identification and expression of the genes and purification and characterization of the genes products involved in reactivation of coenzyme B12-dependent glycerol dehydratase of *Citrobacter freundii*, Eur. J. Biochem. 268 (2001) 2369–2378.
[29] A.V. Shornikova, I.A. Casas, E. Isolauri, H. Mykkänen, T. Vesikari, *Lactobacillus reuteri* as a therapeutic agent in acute diarrhea in young children, J. Pediatr. Gastroenterol. Nutr. 24 (1997) 399–404.
[30] D.D. Sriramulu, M. Liang, D. Hernandez-Romero, E. Raux-Deery, H. Lunsdorf, et al., *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-propanediol by disproportionation, J. Bacteriol. 190 (2008) 4559–4567.
[31] T.L. Talarico, W.J. Dobrogosz, Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*, Antimicrob. Agents Chemother. 33 (1989) 674–679.
[32] T.L. Talarico, I.A. Casas, T.C. Chung, W.J. Dobrogosz, Production and isolation of reuterin: a growth inhibitor produced by *Lactobacillus reuteri*, Antimicrob. Agents Chemother. 32 (1988) 1854–1858.
[33] N. Valeur, P. Engel, N. Carbajal, E. Connolly, K. Ladefoged, Colonization and immunomodulation by *Lactobacillus reuteri* ATCC 55730 in the human gastrointestinal tract, Appl. Environ. Microbiol. 70 (2004) 1176–1181.
[34] M. Veiga da Cunha, M.A. Foster, 1,3-Propanediol: NAD+ oxidoreductase of *Lactobacillus brevis* and *Lactobacillus buchneri*, J. Bacteriol. 174 (1992) 1013–1019.
[35] S. Vollenweider, C. Lacroix, 3-Hydroxypropionaldehyde: applications and perspectives of biotechnological production, Appl. Microbiol. Biotechnol. 64 (2004) 16–27.
[36] Z. Weizman, G. Asli, A. Alsheikh, Effect of aprobiotic infant formula on infections in child care centers: comparison of two probiotic agents, Pediatrics 115 (2005) 5–9.
[37] G. Yang, J. Tian, J. Li, Fermentation of 1,3-propanediol by a lactate deficient mutant of *Klebsiella oxytoca* under microaerobic conditions, Appl. Microbiol. Biotechnol. 73 (2007) 1017–1024.
[38] M.M. Zhu, P.D. Lawman, D.C. Cameron, Improving 1,3-propanediol production from glycerol in a metabolically engineered *Escherichia coli* by reducing accumulation of sn-glycerol-3-phosphate, Biotechnol. Prog. 18 (2002) 694–699.
[39] H. Morita, H. Toh, S. Fukuda, H. Horikawa, S. Oshima, T. Suzuki, et al., Comparative genome analysis of *Lactobacillus reuteri* and *Lactobacillus fermentum* reveal a genomic Island for reuterin and cobalamin production, DNA Res. 15 (2008) 151–161.