ORIGINAL ARTICLE

Production of natural antimicrobial compound D-phenyllactic acid using *Leuconostoc mesenteroides* ATCC 8293 whole cells involving highly active D-lactate dehydrogenase

L. Li[1], S.-Y. Shin[1], K.W. Lee[2] and N.S. Han[1]

1 Brain Korea 21 Center for Bio-Resource Development, Division of Animal, Horticultural, and Food Sciences, Chungbuk National University, Cheongju, Korea
2 Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Seoul National University, Seoul, Korea

Significance and Impact of the Study: This is the first study on the production of D-phenyllactic acid, which is a natural antimicrobial compound, from phenylpyruvate using *Leuconostoc mesenteroides* cells. The strain, ATCC 8293, that was used in the study, possesses high stereoselectivity and delivers a high yield. Therefore, it might be a promising candidate for use in large-scale production facilities and in fermented foods.

Abstract

Phenyllactic acid (PLA) is an antimicrobial compound naturally synthesized in various fermented foods and its D-form of PLA is known to be more active than the L-isomer. In this study, *Leuconostoc mesenteroides* ATCC 8293 cells, elaborating D-lactate dehydrogenase (D-ldh) were used to produce D-PLA from phenylpyruvic acid (PPA). When cultured in the presence of PPA (≤ 50 mmol l^{-1}), growing cells produced a maximum yield of 35 mmol l^{-1} of D-PLA, and the yields were between 75·2 and 83·3%. Higher conversion yields were obtained at pH 6·0–7·0 when growing cells were used, while the optimum pH range was broader for resting cells. The time required for the complete conversion of PPA into PLA could be shortened to 3 h using resting cells. D-ldh, an enzyme encoded by the LEUM_1756 gene of *Leuc. mesenteroides* ATCC 8293, was found to be responsible for the conversion of PPA into PLA. The K_m and k_{cat} values of the enzyme for PPA were found to be 15·4 mmol l^{-1} and 5645 s^{-1}, respectively. The conditions required for the efficient production of D-PLA were optimized for both growing and resting cells of *Leuc. mesenteroides*, with special emphasis on achieving high stereoselectivity and conversion yield.

Introduction

Dieuleveux *et al.* (1998a) found that phenyllactic acid (PLA), an antimicrobial compound, inhibits the growth of *Listeria monocytogenes* in milk and cheese. PLA has been shown to inhibit the growth of yeasts such as *Candida pulcherrima*, *Candida parapsilosis* and *Rhodotorula mucilaginosa*, and a wide range of mould species isolated from bakery products, flour and cereals, including some mycotoxigenic species such as *Aspergillus ochraceus*, *Penicillium roqueforti* and *Penicillium citrinu* (Lavermicocca *et al.*

2000, 2003; Schwenninger *et al.* 2008). In addition, PLA was found to inhibit a range of Gram-negative bacteria such as *Salmonella enterica*, *Escherichia coli*, *Providencia stuartii* and *Klebsiella oxytoca,* and Gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcuss faecalis* and *Bacillus cereus* (Dieuleveux *et al.* 1998a; Ohhira *et al.* 2004). Between the two optical isomers, D-PLA shows higher antibacterial activity than L-PLA (Dieuleveux *et al.* 1998a). PLA has a low molecular mass (166 Da) and is hydrophilic. Therefore, it diffuses into food and feed more easily than bacteriocin, which is known as universal

antimicrobial agent (Dieuleveux *et al.* 1998a). In addition, PLA has been used as a pharmaceutical agent in 'Danshen-su' medicine in China as a platelet-aggregation inhibitor (Mu *et al.* 2009).

In the catabolism of lactic acid bacteria (LAB), phenylalanine is converted to phenylpyruvate (PPA) by aminotransferase. The PPA is subsequently converted to PLA by lactate dehydrogenase (ldh). PPA is considered a feasible precursor for the large-scale production of PLA because it can be synthesized easily from hydantoin at a low cost. In addition, PPA is used to produce phenylalanine, which is in great demand for the production of the sweetener, aspartame (Christidis and Schouteeten 1985; Matsunaga *et al.* 1987). Large-scale production of PLA from PPA using the enzymatic method requires large amounts of nicotinamide adenine dinucleotide phosphate (NADH) and therefore has limited applications in the industry. In this aspect, microbial conversion of PPA to PLA is regarded a more cost-efficient technique. PLA has been produced by the action of a variety of bacteria, including *Lactobacillus*, *Leuconostoc*, *Bacillus*, *Weissella*, *Pediococcus*, *Aspergillus* and *Geotrichum* (Dieuleveux *et al.* 1998b; Valerio *et al.* 2004; Mu *et al.* 2009, 2012; Ndagano *et al.* 2011; Zheng *et al.* 2011). However, with the exception a few studies on *Lactobacillus* sp. SK007 and *Bacillus coagulans* SDM, detailed studies to optimize the production and yield of PLA have not been carried out in the other bacteria (Mu *et al.* 2009; Zheng *et al.* 2011). Due to the higher antibacterial effect of D-PLA than L-PLA, a microorganism that produces highly active D-ldh would be the ideal candidate for use in a large-scale setup. Among the D-lactic acid-producing LAB, *Leuconostoc* is a representative genus that is used as a starter culture in the manufacture of various fermented foods such as cheese, sauerkraut and kimchi. It has been reported that *Leuc. mesenteroides* mainly produces the D-form of lactic acid (Garvie 1980; Jin *et al.* 2006).

Therefore, in this study, 21 strains of the *Leuconostoc* genus were compared based on their PPA to D-PLA conversion yields and *Leuc. mesenteroides* ATCC 8293 was chosen for further optimization. PLA was produced using both growing and resting cells, and the conditions required for the efficient production of PLA using growing cells were optimized. In addition, the kinetic parameters of D-ldh were determined.

Results and discussion

PLA yields of Leuconostoc spp

To select a potential strain for the production of PLA, 21 *Leuconostoc* strains were cultivated in MRS broth contain-

ing PPA for 24 h at 30°C, and their growth rates and PLA yields were compared. The PLA yields of all 21 *Leuconostoc* spp. were over 75%, and the optical density of the cultures, as estimated at a wavelength of 600 nm, was approx. 1·5 (data not shown). This indicated that *Leuconostoc* spp. might be the most appropriate LAB for the microbial production of PLA. Of the 21 strains tested, *Leuc. mesenteroides* S7, *Leuc. citreum* 95 and *Leuc. mesenteroides* ATCC 8293 showed high PLA yields (81·3, 80·7 and 80·0%, respectively). *Leuc. mesenteroides* ATCC 8293 was chosen for subsequent analyses and optimization, because its whole genome has been sequenced and sequence information for the D-*ldh* gene is available (Li *et al.* 2012).

Leuconostoc mesenteroides ATCC 8293 produced the D-form of PLA

When *Leuc. mesenteroides* ATCC 8293 resting cells were incubated in 50 mmol l^{-1} Tris-HCl buffer (pH 6·0) containing 30 mmol l^{-1} PPA for 4 h, D-PLA was synthesized in the solution (Fig. 1c). This result showed that *Leuc. mesenteroides* ATCC 8293 exclusively produced the D-form of PLA as single product from PPA. For the large-scale production of optically pure D-PLA, an enzymatic method that involves the use of D-ldh is preferred. However, this method has limited application in the industry because the cofactor (NADH) needs to be regenerated at every step, and the cost of cofactor regeneration is prohibitively high. In this study, *Leuconostoc* cells harbouring D-ldh enzyme were employed for the bioconversion of PPA to pure D-PLA; the method resulted in high yields. The use of microbial whole cells provides several other advantages over conventional enzymatic processes: it does not require the use of expensive enzymes and therefore lowers the cost of production, it allows the consumption of high concentrations of substrate, and the cells used for the production may be recycled.

Effect of PPA concentration

To determine the optimal substrate concentration, PLA productions were analysed in MRS medium containing PPA at varying concentrations (10, 20, 30, 40, 50 and 60 mmol l^{-1}) for 24 h at 30°C. The results showed that PLA production increased with PPA concentration and reached a maximum level (35 mmol l^{-1}) at a PPA concentration of 50 mmol l^{-1} (Fig. 2a). This value was higher than that reported in a previous study, in which *B. coagulans* SDM exhibited a maximum yield of 25 mmol l^{-1} PLA from 40 mmol l^{-1} PPA (Zheng *et al.* 2011). As illustrated in Fig. 2b, the cell density decreased with increasing PPA concentration and showed a huge
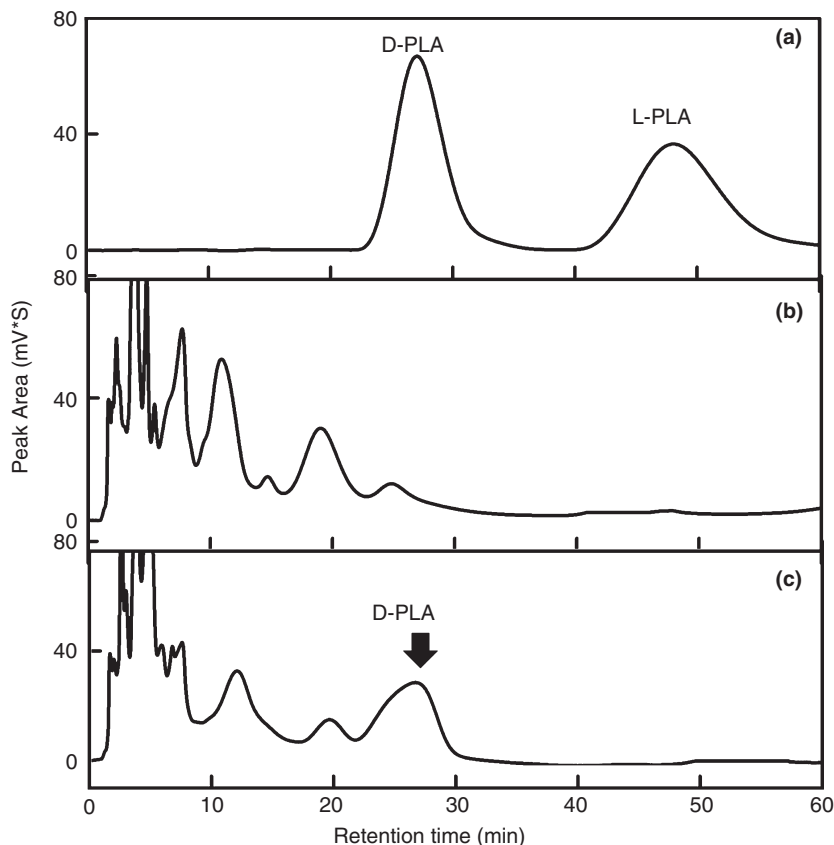
Figure 1 HPLC analysis of the product, PLA, produced by *Leuconostoc mesenteroides* ATCC 8293 in 50 mmol l^{-1} Tris-HCl buffer (pH 6·0) containing 30 mmol l^{-1} PPA. (a) D-PLA and L-PLA standards; (b) reaction product at 0 h (control); and (c) reaction product after 4 h (sample). The experiments were performed in triplicate.

reduction at concentrations above 60 mmol l^{-1}. The highest yield of PLA (83·3%) was obtained at a PPA concentration of 20 mmol l^{-1}. In addition, *Leuc. mesenteroides* ATCC 8293 retained its high conversion efficiency (75–83·3%) even at higher PPA concentrations ($\leq$50 mmol l^{-1}). In the case of *Lactobacillus* sp. SK007, the conversion yield was over 80% at PPA concentrations <20 mmol l^{-1}; however, it decreased to <40% when the PPA concentration was above 20 mmol l^{-1} (Li *et al.* 2007). The same result was observed for *B. coagulans* SDM; the PLA yield was approx. 80% at a PPA concentration of 15 mmol l^{-1}; however, it decreased greatly with increasing PPA concentration (Zheng *et al.* 2011). This unique characteristic of *Leuc. mesenteroides* ATCC 8293 might permit the development of bioconversion processes that can tolerate high substrate concentrations, which is preferred in the industry (Collins and Daugulis 1997).

Inhibition of cell growth by PLA

In the experiment described in the previous section, the total cell mass was found to decrease at high PPA concentrations, and the PLA production did not exceed 35 mmol l^{-1}. Therefore, to determine whether PLA

affects cell growth, the growth rate of *Leuc. mesenteroides* ATCC 8293 was monitored under various concentrations of PLA. The results revealed that the cell growth of *Leuc. mesenteroides* ATCC 8293 was strongly inhibited at PLA concentrations above 30 mmol l^{-1} (data not shown), showing that the PLA elaborated by growing cells inhibits cell itself. Although the mechanism underlying the antibacterial action of PLA is not clearly known, PLA has been reported to damage the bacterial cell wall. Scanning electron microscope studies have shown that *L. monocytogenes* cells agglomerated, lost their rigidity to swelling and secreted polysaccharides following treatment with PLA (Dieuleveux *et al.* 1998a).

Optimal pH condition for PLA production

The effect of pH on PLA production from PPA was determined for both growing and resting cells of *Leuc. mesenteroides* ATCC 8293. In the case of growing cells, a high PLA yield was obtained at pH 6·0, while a high cell density was obtained at pH 8·0 (Fig. 3a). Because *Leuconostoc* sp. is susceptible to acidic stress, low pH ($\leq$5·0) resulted in a low growth rate and reduced PLA production (Kim *et al.* 2012). This indicated that the
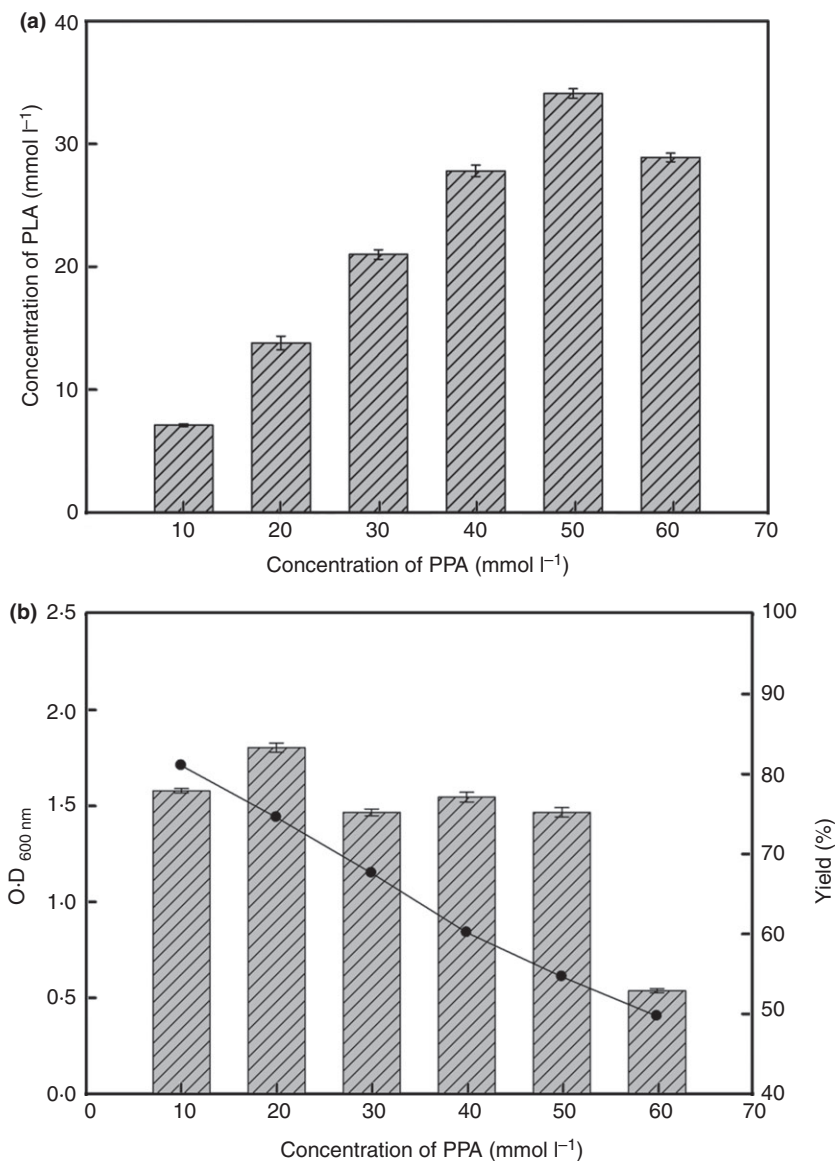
Figure 2 Effects of PPA concentrations on (a) PLA production and (b) PLA yields by *Leuconostoc mesenteroides* ATCC 8293. a, PLA production at various PPA concentrations (▨); b, PLA yields (▨) and cell density (●). The experiments were performed in triplicate.

initial pH of the medium might be an important factor that affects PLA production. Interestingly, PLA production by *B. coagulans* SDM, which is not sensitive to acids, was not affected by the pH of the medium (Zheng *et al.* 2011). In the case of resting cells, high PLA yields were obtained in a broad range of pH and the highest level was achieved at pH 7·0 (Fig. 3b) (Li *et al.* 2012). This suggested that resting cells were able to produce PLA in both acidic and alkaline conditions.

PLA production by growing cells and resting cells

To monitor the production of PLA by growing cells, *Leuc. mesenteroides* ATCC 8293 was cultivated in MRS broth with varying PPA concentrations (30 and 40

mmol l^{-1}). The PPA was completely consumed after 18 h of cultivation, and 21·7 and 27·5 mmol l^{-1} of PLA was produced from 30 and 40 mmol l^{-1} of PPA, respectively (Fig. 4a). During the growth period, about 70% of the PPA consumed was converted to PLA. The rest might have been converted to phenylalanine and subsequently metabolized. These results suggested that the rate of PLA production correlates with the rate of growth-associated product formation in batch fermentation; PLA was mainly synthesized during the growth period at a rate proportional to the growth rate. To monitor the production of PLA by resting cells, 0·16 g ml^{-1} of cell mass (harvested after cultivation) was treated with 30 mmol l^{-1} PPA in 50 mmol l^{-1} Tris-HCl buffer (pH 6·0) at 30°C for 8 h. As illustrated in Fig. 4b, the sub-
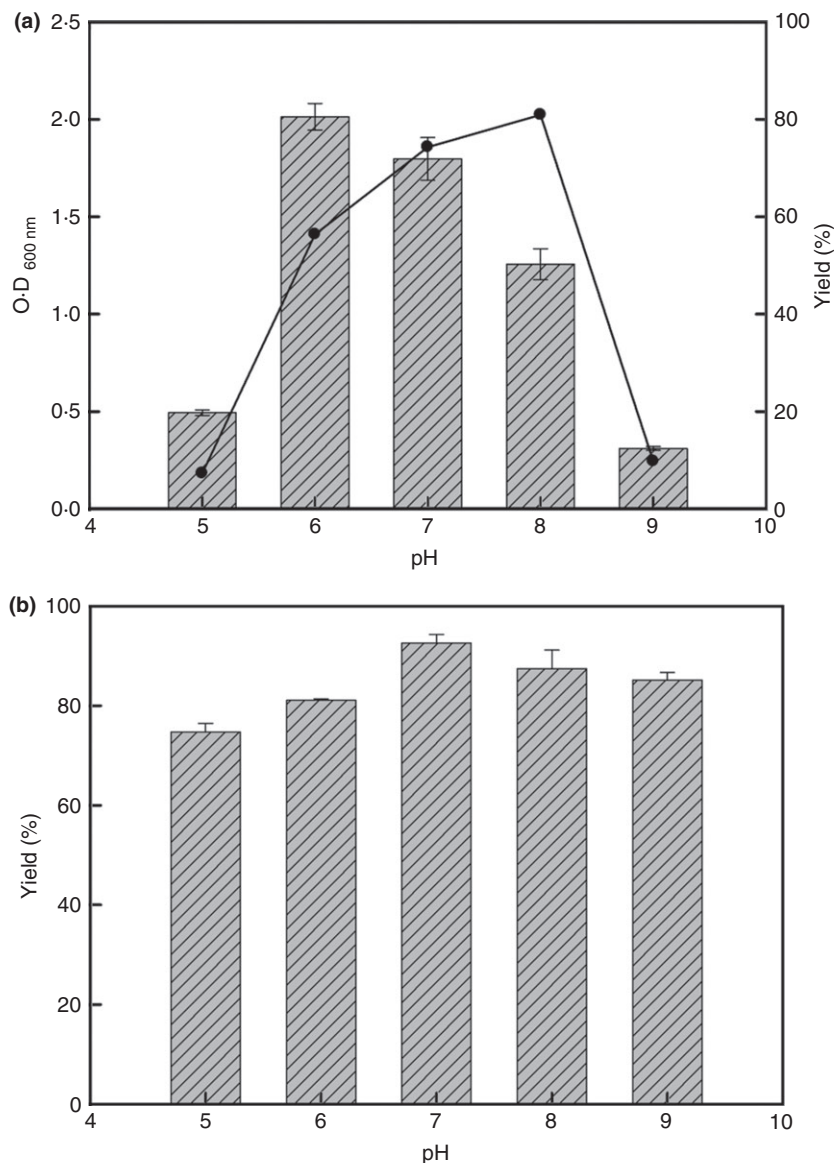
Figure 3 Effects of initial pH on PLA yield (▨) and cell density (●) of growing cells (a) and resting cells (b). The experiments were performed in triplicate.

strate (PPA) was consumed within 3 h, and the PLA concentration and conversion yield reached maximum levels (24·66 mmol l^{-1} and 83·3%, respectively), suggesting that resting cells converted PPA to PLA in a shorter time span (3 h) than the growing cells (18 h) did. Li *et al.* (2007) reported that 6·8 mmol l^{-1} of PLA could be obtained from 12 mmol l^{-1} of PPA using resting cells of *Lb.* SK007. However, the conversion yield decreased gradually when the concentration of PPA was increased to 30·5 mmol l^{-1}. Conversely, both PLA production and yield were higher for *Leuc. mesenteroides* ATCC 8293 resting cells than for *Lb.* SK007 resting cells. These results suggested that resting cells may be reused for multiple bioconversion reactions.

The enzyme, ldh, is responsible for PLA production

A genomic analysis of *Leuc. mesenteroides* ATCC 8293 revealed that seven genes encode lactate dehydrogenases or related enzymes: LEUM_0373, LEUM_1837, LEUM_0445, LEUM_1233, LEUM_0503, LEUM_1756 and LEUM_2043. Our previous study showed that the LEUM_1756 gene was the most highly transcribed and translated during cell growth in MRS medium (Li *et al.* 2012). Therefore, in this study, to analyse the substrate specificity and kinetic parameters of D-ldh enzyme, the LEUM_1756 gene was cloned and expressed in recombinant *E. coli*. The enzymatic activity of the purified D-ldh was assayed at various concentrations of PPA (for PLA) and pyruvate (for
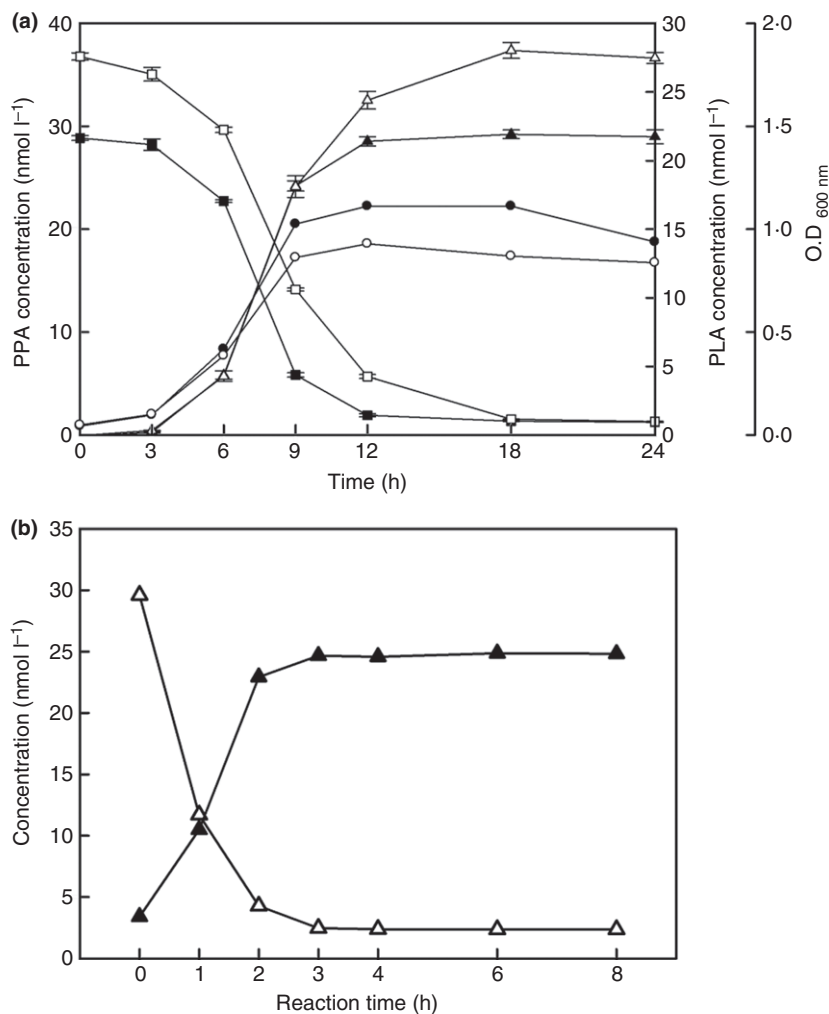
Figure 4 (a) Rates of PPA consumption (squares) and PLA production (triangles) and changes in cell mass (circles) with time in growing cells of *Leuconostoc mesenteroides* ATCC 8293 in 30 mmol l⁻¹ (dark), or 40 mmol l⁻¹ MRS-PPA medium (white) for 24 h. (b) Rates of PPA consumption (△) and PLA production (▲) in 50 mmol l⁻¹ Tris-HCl buffer (pH 6·0) containing 30 mmol l⁻¹ PPA, using 0·16 g ml⁻¹ of *Leuc. mesenteroides* ATCC 8293 resting cells. The experiments were performed in triplicate.

D-lactic acid). Apparent K_m, V_{max} and k_{cat} values for PPA were obtained and compared with those obtained for pyruvate (Table 1). The K_m and k_{cat} values for PPA were 15·4 mmol l⁻¹ and 5646 s⁻¹, respectively, while those for pyruvate were 0·58 mmol l⁻¹ and 2900 s⁻¹, respectively, suggesting that the D-ldh enzyme has a lower substrate affinity but a higher catalytic activity to PPA than pyruvate. The D-ldh enzyme of *Leuc. mesenteroides* ATCC 8293 showed a higher catalytic efficiency (k_{cat}/K_m, $3·67 \times 10^5$) for PPA than the D-ldh enzyme of *B. coagulans* (k_{cat}/K_m, $3·9 \times 10^3$) (Zheng *et al.* 2011). In our previous study (Li *et al.* 2012), the D-ldh enzyme encoded by the LEUM_1756 gene showed a specific activity of 4450 U mg⁻¹ towards pyruvate. To our knowledge, the ldh encoded by the LEUM_1756 gene is the most active D-lactic acid-producing enzyme among all the LAB that have been examined so far. In this study, D-ldh showed a lower substrate affinity but a higher catalytic activity to PPA than to pyruvate. The ldh enzyme in *Leuc. mesenteroides* ATCC 8293 is involved not only in the reduction of pyruvate to lactic acid

but also in the reduction of PPA to PLA. Moreover, its catalytic activity for PLA is higher than that for D-lactic acid.

Materials and methods

Bacterial strains and growth conditions

Total 21 strains of *Leuconostoc* genus were used; among them, 11 were type cultures (*Leuc. lactis* KCTC 3528, *Leuc. mesenteroides* subsp. *dextranicum* KCTC 3530, *Leuc. mesenteroides* subsp. *mesenteroides* ATCC 8293, KCTC 3719, NRRL B-512F, NRRL B742C, and KCTC 3100, *Leuc. fallax* KCTC 3537, *Leuc. fructosum* KCTC 3544, and *Leuc. citreum* KACC 91035 and KCTC 3526) and 9 were isolated from fermented foods (*Leuc. mesenteroides* S7, *Leuc. mesenteroides* S1, and *Leuc. citreum* 75, 95, D3, 87, 74, 61, 63 and 64). To quantify the PLA yield, PPA solution was sterilized using a 0·45-μm membrane filter and added to the MRS broth (Difco, Detroit, MI). The

Table 1 Kinetic parameters of the D-ldh enzyme (LEUM_1756) for pyruvate and PPA

Substrate	K_m (mmol l^{-1})	V_{max} (μmol l^{-1} min^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m [(mol l^{-1})$^{-1}$s^{-1}]
PPA	15·4	555·56	5646	3·67 × 10^5
Pyruvate	0·58	476·19	2900	4·99 × 10^6

cells were cultivated for 24 h at 30°C. Both PPA and PLA were purchased from Sigma (Sigma-Aldrich Co., St. Louis. MO).

Determination of the PLA yields of various Leuconostoc spp

The precultured 21 *Leuconostoc* spp. were inoculated in MRS medium containing PPA (MRS-PPA) and incubated at 30°C for 24 h. Culture media were centrifuged (10 786 **g**, 2 min, 4°C) after boiling for 5 min. The concentration of PPA and PLA in the supernatant was estimated using a high-performance liquid chromatography (HPLC) system, which consisted of a Young Lin M930 solvent delivery pump (Young Lin, Seoul, Korea), a Young Lin M720 absorbance detector and an Aminex® HPX-87H column (Bio-Rad, Hercules, CA). The flow rate of the mobile phase (0·004 mol l^{-1} H$_2$SO$_4$) was 0·6 ml min^{-1} and monitored at 215 nm absorbance using a UV spectrophotometric detector. The PLA yield was defined as the ratio of the concentration of PLA produced (mmol l^{-1}) and the concentration of PPA consumed (mmol l^{-1}).

Analysis of D- and L-PLA isomers

To identify the stereoisomeric form of the PLA product, resting cells of *Leuc. mesenteroides* were treated with 30 mmol l^{-1} PPA in 50 mmol l^{-1} Tris-HCl buffer (pH 6·0) at 30°C for 4 h and the product was analysed by HPLC. The analysis unit was consisted of a solvent delivery pump (Young Lin M930), an absorbance detector (Young Lin M720) and a CRX-G column (Shodex™ OR-pac CRX-853, 8·0 × 50 mm, Showa Denco, Tokyo, Japan). The flow rate of the mobile phase (0·25 mmol l^{-1} CuSO$_4$ in H$_2$O) was 1 ml min^{-1}. The reaction was monitored at 250 nm using a UV spectrophotometric detector. In addition, PPA consumption and PLA production were estimated.

Effects of PPA concentration and pH on PLA yield

To determine the effects of PPA concentration on the total cell mass and PLA production by *Leuc. mesenteroides* ATCC 8293, PLA yields were compared after the addition of various concentrations of PPA (10, 20, 30, 40, 50 and 60 mmol l^{-1}) to the medium. The total cell mass was measured, and the PLA yield was calculated using the method described in the previous section. The effects of pH on the conversion yield were examined in a pH range of 5·0–9·0. To prepare resting cells, *Leuc. mesenteroides* was grown in MRS medium (2 l) at 30°C for 12 h, the culture was centrifuged (4500 **g**, 15 min, 4°C), and the cell pellets were washed twice with 50 mmol l^{-1} Tris-HCl buffer (pH 6·0).

Kinetic analysis of ldh enzyme

The major D-ldh enzyme (encoded by the LEUM_1756 gene) in *Leuc. mesenteroides* ATCC 8293 was cloned and overexpressed in *E. coli* BL21 Star™ (DE3) (Invitrogen, Seoul, Korea) cells from an inducible pET-21a(+) vector. The D-ldh enzyme was purified by Ni-NTA column chromatography (Li *et al.* 2012). To monitor the enzymatic reaction, D-ldh enzyme (0·01 ml with a protein concentration of 0·06 mg ml^{-1}) was incubated in a substrate solution (0·3 ml) containing various concentrations of PPA (1·25, 2·5, 5 and 7·5 mmol l^{-1}) and 0·5 mmol l^{-1} NADH in 0·1 mol l^{-1} Tris-HCl buffer (pH 7·0). One unit of enzyme activity was defined as the amount of enzyme required for the oxidation of 1 μmol l^{-1} NADH per min. The results were analysed using double reciprocal Lineweaver-Burk plots.

Acknowledgements

Conflict of Interest

The authors have no conflict of interest.

References

Christidis, Y. and Schouteeten, A. (1985) Process for preparation of crystallized monohydrated sodium phenylpyruvate. US patent no. 4,518,800.

Collins, L.D. and Daugulis, A.D. (1997) Biodegradation of phenol at high initial concentrations in two-phase partitioning batch and fed-batch bioreactors. *Biotechnol Bioeng* **55**, 155–162.

Dieuleveux, V., Lemarinier, S. and Guerguen, M. (1998a) Antimicrobial spectrum and target site of D-3-phenyllactic acid. *Int J Food Microbiol* **40**, 177–183.

Dieuleveux, V., Van Der Pyl, D., Chataud, J. and Gueguen, M. (1998b) Purification and characterization of anti-*Listeria* compounds produced by *Geotrichum candidum*. *Appl Environ Microbiol* **64**, 800–803.

Garvie, E.I. (1980) Bacterial lactate dehydrogenase. *Microbiol Rev* **44**, 106–139.

Jin, Q., Yoon, H.S., Han, N.S., Lee, J. and Han, J.S. (2006) Effect of lactic acid bacteria on D- and L-lactic acid contents of Kimchi. *Food Sci Biotechnol* **15**, 948–953.

Kim, J.E., Eom, H.-J., Kim, Y., Ahn, J.E., Kim, J.H. and Han, N.S. (2012) Enhancing acid tolerance of *Leuconostoc mesenteroides* with glutathione. *Biotechol Lett* **34**, 683–687.

Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A. and Gobbetti, M. (2000) Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. *Appl Environ Microbiol* **66**, 4084–4090.

Lavermicocca, P., Valerio, F. and Visconti, A. (2003) Antifungal activity of phenyllactic acid against molds isolated from bakery products. *Appl Environ Microbiol* **69**, 634–640.

Li, X., Jiang, B. and Pan, B. (2007) Biotransformation of phenylpyruvic acid to phenyllactic acid by growing and resting cells of a *Lactobacillus* sp. *Biotechol Lett* **29**, 593–597.

Li, L., Eom, H.-J., Park, J.-M., Seo, E., Ahn, J.E., Kim, T.-J., Kim, J.H. and Han, N.S. (2012) Characterization of the major dehydrogenase related to D-lactic acid synthesis in *Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8293. *Enzyme Microb Technol* **51**, 274–279.

Matsunaga, T., Higashijima, M., Sulaswatty, A., Nishimura, S., Kitamura, T., Tsuji, M. and Kawaguchi, T. (1987) Repeated batch production of L-phenylalanine from phenylpyruvate and NH₄Cl by immobilized cells of *Nocardia opaca* under hydrogen high pressure. *Biotechnol Bioeng* **31**, 834–840.

Mu, W., Chen, C., Li, X., Zhang, T. and Jiang, B. (2009) Optimization of culture medium for the production of phenyllactic acid by *Lactobacillus* sp. SK007. *Bioresour Technol* **100**, 1366–1370.

Mu, W., Yu, S., Zhu, L., Zhang, T. and Jiang, B. (2012) Recent research on 3-phenyllactic acid, a broad-spectrum antimicrobial compound. *Appl Microbiol Biotechnol* **95**, 1155–1163.

Ndagano, D., Lamoureux, T., Dortu, C., Vandermoten, S. and Thonart, P. (2011) Antifungal activity of 2 lactic acid bacteria of the *Weissella* genus isolated from food. *J Food Sci* **76**, 305–311.

Ohhira, I., Kuwaki, S., Morita, H., Suzuki, T., Tomita, S., Hisamatsu, S., Sonoki, S. and Shinoda, S. (2004) Identification of 3-phenyllactic acid as a possible antibacterial substance produced by *Enterococcus faecalis* TH10. *Biocontrol Sci* **9**, 77–81.

Schwenninger, S.M., Christophe, L., Stefan, T., Christoph, J., Cacilia, S., Laurent, B. and Leo, M. (2008) Characterization of low-molecular-weight antiyeast metabolites produced by a food-protective *Lactobacillus-Propionibacterium* coculture. *J Food Prot* **71**, 2481–2487.

Valerio, F., Lavermicocca, P., Pascale, M. and Visconti, A. (2004) Production of phenyllactic acid by lactic acid bacteria: an approach to the selection of strains contributing to food quality and preservation. *FEMS Microbiol Lett* **233**, 289–295.

Zheng, Z., Ma, C., Gao, C., Li, F., Qin, J., Zhang, H., Wang, K. and Xu, P. (2011) Efficient conversion of phenylpyruvic acid to phenyllactic acid by using whole cells of *Bacillus coagulans* SDM. *PLoS ONE* **6**, 19030–19036.