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MINI-REVIEW

Recent research on 3-phenyllactic acid, a broad-spectrum antimicrobial compound

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Abstract 3-Phenyllactic acid (PLA), which is an organic acid widely existing in honey and lactic acid bacteria fermented food, can be produced by many microorganisms, especially lactic acid bacteria. It was proved as an ideal antimicrobial compound with broad and effective antimicrobial activity against both bacteria and fungi. In addition, it could be used as feed additives to replace antibiotics in livestock feeds. This article presented a review of recent studies on the existing resource, antimicrobial activity, and measurement of PLA. In addition, microorganism strains and dehydrogenases producing PLA were reviewed in detail, the metabolic pathway and regulation of PLA synthesis in LAB strains were discussed, and high-level bioproduction of PLA by microorganism fermentation was also summarized.

Keywords 3-Phenyllactic acid · Antimicrobial compound · Dehydrogenase · Metabolic pathway · Fermentation

Introduction

Food safety is generally recognized as a primary public safety issue in the world. Food microbial contamination is an important factor to result in food safety problems, which possibly bring about public health problems and great economic losses. Bacterial contamination, especially by pathogenic species including *Listeria*, *Staphylococcus*, *Escherichia coli*, and *Salmonella*, can cause foodborne illnesses (Kaneko et al. 1999). Fungal contamination by yeasts and moulds, which easily grow and reproduce in food system with feeble oxygen, can cause food spoilage (Schnurer et al. 1999) and even can produce toxic secondary metabolites, namely, mycotoxins, which are capable of causing disease and death in humans and animals (Riley et al. 1993). Application of antimicrobial preservatives, which inhibit the growth of bacteria or fungi, is an effective approach to bring down the food safety hazards by microbial contaminations.

3-Phenyllactic acid (2-hydroxy-3-phenylpropanoic acid or β-phenyllactic acid, PLA), a kind of an organic acid, has been reported as an antimicrobial compound with broad-spectrum activity against bacteria including *Listeria monocytogenes* (Dieuleveux et al. 1998b), *Staphylococcus aureus*, and *Escherichia coli* O157:H7 (Ohhira et al. 2004), and fungi including yeasts (Schwenninger et al. 2008) and a wide range of moulds, such as *Aspergillus ochraceus*, *Penicillium roqueforti*, and *Penicillium citrinu* (Lavermicocca et al. 2003). The present article is a review of recent studies on the properties, measurement, antimicrobial activity, and biosynthesis pathway, as well as its biological production by microbial fermentation and the possible enzymes producing PLA.

PLA

Existing sources

PLA was found widely existing in honey, and its content was commonly much higher than other phenolic acids in honey. PLA was suggested as chemical marker for thistle (*Galactites tomentosa* Moench) unifloral honeys, in which PLA content reached 100–800 mg/kg (Tuberoso et al. 2011). It was also found in high concentration in heather, ling heather, and manuka honeys (820, 875, and 243 mg/kg) (Tan et al. 1988; Dimitrova et al. 2007). Most of other

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honeys had PLA concentration level of less than 100 mg/kg (Wilkins et al. 1993 and 1995; Dimitrova et al. 2007), while it could not be detected in sunflower honey (Dimitrova et al. 2007). Recently, PLA was reported as metabolite of food microorganism, especially lactic acid bacteria (LAB). So, PLA was found existing in fermentation foods using LAB as starter, such as sourdoughs (Van der Meulen et al. 2007; Ryan et al. 2009).

Chemical structure

The molecular formula and molecular weight of PLA are $C_9H_{10}O_3$ and 166 g/mol, respectively. PLA has an asymmetric carbon atom and thus has two chiral isomers: D- and L-PLA (Fig. 1).

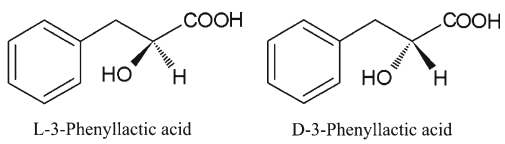
Measurement

Reverse-phase HPLC is a simple and widely applicable method for measuring PLA. It was adopted in most of quantitative PLA measurement of microbial fermentation broth (Armaforte et al. 2006). In addition, it was developed to quantify the PLA content in rumen fluid (Khan et al. 1998) and honey (Tuberoso et al. 2011) (shown in Table 1). Regular gas chromatography–mass spectrometry (GC/MS) and capillary electrophoresis could also be used to measure PLA (Wilkins et al. 1995; Sarkissian et al. 2000). However, the aforementioned methods are usually non-stereospecific and cannot separate and detect the PLA enantiomers, L- and D-PLA.

Tekewe et al. (2008) reported the stereospecific determination of PLA using chiral HPLC method, in which Chiralcel OJ-H column based on cellulose tris-(4-methyl benzoate) chiral stationary phase was used to separate PLA enantiomers. In addition, using modified cyclodextrins as chiral additive during chromatography or capillary electrophoresis process could achieve the separation of PLA enantiomers (Nardi and Eliseev 1993; Heil et al. 1998).

Antibacterial activity

Dieuleveux et al. (1998b) firstly reported the antibacterial activity of PLA. They purified and identified the novel anti-*Listeria* compound, PLA, produced and excreted from *Geotrichum candidum*, and found that D-PLA showed slightly higher anti-*Listeria* activity than L-PLA. Then,



L-3-Phenyllactic acid D-3-Phenyllactic acid

Fig. 1 Chemical structure of PLA

PLA was proved to be able to inhibit *Listeria monocytogenes* growth in culture medium, milk, and cheese. It could reduce the bacteria population by 4.5 log in ultra-high-temperature treatment whole milk, to give fewer cells than in the control after 5 days of culture (Dieuleveux and Gueguen 1998).

In addition, PLA could inhibit a range of Gram-positive bacteria, such as *Staphylococcus aureus*, *Enterococcuss faecalis*, and *Bacillus cereus*, and Gram-negative bacteria, such as *Salmonella enterica*, *Escherichia coli*, *Providencia stuartii*, and *Klebsiella oxytoca* (Dieuleveux et al. 1998a; Ohhira et al. 2004). PLA could show higher inhibitory effect in acidic pH (Ohhira et al. 2004). The mechanism of antibacterial action is not clear yet, but it was suggested that the bacterial cell wall should be an action site of PLA. Scanning electron microscope studies showed that the bacteria exposed to PLA had damaged, even broken cell wall structure. The bacteria formed aggregates and secreted polysaccharides; then, the cell wall lost rigidity, causing the cells to swell, even collapse (Dieuleveux et al. 1998a).

Antifungal activity

In addition to antibacterial activity, the inhibitory properties of PLA have also been demonstrated against yeasts, such as *Candida pulcherrima*, *Candida parapsilosis*, and *Rhodotorula mucilaginosa* (Schwenninger et al. 2008) and a wide range of mould species isolated from bakery products, flour, and cereals, including some mycotoxigenic species, namely, *Aspergillus ochraceus*, *Penicillium roqueforti*, *Penicillium citrinu*, etc. (Lavermicocca et al. 2000 and 2003). PLA has relatively high MIC value for antiyeast activity (50 to more than 500 mM at pH 4.0 to 6.0), and the value of PLA decreased with decreasing pH (Schwenninger et al. 2008). And MIC value against moulds at pH 4.0 is 45 mM (Strom et al. 2002).

The fungal inhibitory activity of PLA was firstly characterized by Lavermicocca et al. (2000), who purified the antifungal compounds from *Lactobacillus plantarum* Strain 21B, a lactic acid bacterium (LAB) with high antifungal activity. Using the PLA-producing strain, *L. plantarum* 21B, as a starter during sourdough bread fermentation process, the fungal growth could be delayed for 7 days (Lavermicocca et al. 2000). PLA has been considered as antifungal compound marker (Schnürer and Magnusson 2005) and been widely purified and characterized from various LABs, such as *L. plantarum* 21B (Lavermicocca et al. 2000), *L. plantarum* MiLAB 393 (Strom et al. 2002), *L. plantarum* IMAU10014 (Wang et al. 2012), *L. plantarum* (Prema et al. 2010), and *Weissella cibaria* FMF4B16 (Ndagano et al. 2011). And it was presumed that the behavior of the antifungal activity of LAB strains was positively related to the metabolic content of PLA (Valerio et al. 2004).

Table 1 PLA measurement methods reported

Assay method	Stereospecific determination	Test sample	Reference
Reverse-phase HPLC	No	Rumen fluid	Khan et al. 1998
Reverse-phase HPLC	No	Microbial fermentation broth	Armaforte et al. 2006
Reverse-phase HPLC	No	Honey	Tuberoso et al. 2011
GC/MS	No	Mouse brain	Sarkissian et al. 2000
GC/MS	No	Sourdough	Ryan et al. 2009
GC/MS	No	Honey	Wilkins et al. 1995
Capillary electrophoresis	No	Urine	Zhang et al. 2010
Enantioselective multidimensional GC/MS (using modified cyclodextrins as chiral additives)	Yes	Urine	Heil et al. 1998
Capillary zone electrophoresis (using modified cyclodextrins as chiral additives)	Yes	Standards	Nardi and Eliseev 1993
Chiral HPLC	Yes	Enzymatic catalysis product	Tekewe et al. 2008

Other applications

Like other organic acids, PLA can be used as feed additives to replace antibiotics in livestock feeds. It may exert some positive effects to the immune system of laying hens and then effectively improve production performance and egg quality (Wang et al. 2009b). When supplemented in long-term diet of chick feeds, PLA improves the growth performance, has antipathogen effect in large intestine, and reduces yellowness of meat (Wang et al. 2010). Also, it was reported that PLA may increase immune-related blood cell counts and potentially reduce *E. coli* numbers in weanling and growing pigs (Wang et al. 2009a).

In addition, PLA has potential as a pharmaceutical agent to treat coronary disease since its analogue "Danshensu" from Chinese medicine is applied presently (Wang et al. 1991). And it also has been patented to be used as a skin-protecting ingredient to reduce skin wrinkles (Yu and Van Scott 1997).

Biological PLA production

Microorganisms producing PLA

Lavermicocca et al. (2000) reported the production of PLA from *L. plantarum* 21B, which was the first report showing the production of PLA by lactic acid bacteria (LAB) (2000). Subsequently, it was found that PLA could be generally produced by a wide range of LAB species, such as *Lactobacillus*, *Enterococcus*, *Weissella*, and *Leuconostoc*, but the production varied greatly among strains and species. When grown in DeMan–Rogosa–Sharpe (MRS) medium, most of LAB strains produced less than 1-mM PLA; however, *L. plantarum* 1081, *L. plantarum* 778, *L. plantarum* 1073, *L. acidophilus* 1063 (Gerez et al. 2010), and *L. plantarum* CECT-221 (Rodriguez et al. 2012) could produce 5.2-, 4.1-, 2.6-, 1.1-, and 1.38-mM PLA, respectively (shown in Table 2).

PLA could also be produced by a series of dairy propionic acid bacteria (PAB) strains, such as *Propionibacterium jensenii* DSMZ 20535, *P. thoenii* DSMZ 20276, *P. acidipropionici* DSMZ 4900, and *P. freudenreichii* ssp. *freudenreichii* DSMZ 2027, with the production of 0.01–0.1 mM (Lind et al. 2007). And cofermentation with *P. jensenii* SM11 and *Lactobacillus paracasei* subsp. *paracasei* SM20 produced 1-mM PLA (Schwenninger et al. 2008).

In addition, PLA could be produced by other microorganism strains, such as bacillaceae (*Bacillus coagulans*) (Zheng et al. 2011), fungus (*Geotrichum candidum*) (Dieuleveux et al. 1998b), and Brevibacteriaceae (*Brevibacterium lactofermentum*) (Kamata et al. 1986). And both *G. candidum* and *B. lactofermentum* could produce much higher PLA than LAB and PAB strains (shown in Table 2).

Metabolic pathway and regulation of PLA synthesis in LAB strains

The synthesis of PLA in LAB strains results from the catabolism of phenylalanine, in which phenylalanine is transaminated to phenylpyruvic acid (PPA) and PPA further reduced to PLA (shown in Fig. 2) (McSweeney and Sousa 2000; Yvon and Rijnen 2001; Li et al. 2007; Vermeulen et al. 2006). The amino acid phenylalanine had remarkable effect on PLA production in LAB strains. When cultured in synthetic medium without phenylalanine, *L. plantarum* ITM21B did not produce PLA at all; however, the PLA fermentation amounts could be observed and gradually increased if phenylalanine was supplemented with concentrations ranging from 0.1 to 0.4 g/L in initial medium (Valerio et al. 2004). The direct correlation between PLA production

Table 2 PLA production by select microorganism strains

	Strain	PLA (mM)	Source	References
LAB strains[a]	*L. plantarum* 21B	NR	Sourdough	Lavermicocca et al. 2000
	L. plantarum MiLAB 393	NR	Grass silage	Strom et al. 2002
	L. plantarum VLT01	NR	DIPROVAL collection (Bologna University)	Armaforte et al. 2006
	Lactobacillus sp. SK007	0.55	Chinese traditional pickles	Li et al. 2007
	L. plantarum CECT-221	1.38	Spanish Collection of Type Cultures (Valencia, Spain)	Rodriguez et al. 2012
	L. plantarum CRL 778	0.1	Wheat dough	Dallagnol et al. 2011
	L. plantarum	NR	Grass silage	Prema et al. 2010
	L. plantarum IMAU10014	NR	Koumiss	Wang et al. 2012
	L. plantarum VE56	0.48	Fermented cassava	Ndagano et al. 2011
	Weissella cibaria FMF4B16	0.1	Mill flour	Ndagano et al. 2011
	Weissella paramesenteroides LC11	0.13	Fermented cassava	Ndagano et al. 2011
	L. plantarum 1081	5.2	Culture Collection (CRL) of the Centro de Referencia para Lac tobacilos (CERELA-CONICET)	Gerez et al. 2010
	L. plantarum 778	4.1	CRL of CERELA-CONICET	Gerez et al. 2010
	L. plantarum 1073	2.6	CRL of CERELA-CONICET	Gerez et al. 2010
	L. acidophilus 1063	1.1	CRL of CERELA-CONICET	Gerez et al. 2010
	L. paracasei 1501	0.6	CRL of CERELA-CONICET	Gerez et al. 2010
	L. alimentarius ATCC29643	0.37	Fish products	Valerio et al. 2004
	L. mesenteroides subsp. *mesenteroides* ITMY30	0.57	Olive phylloplane	Valerio et al. 2004
	Leuconostoc citreum ITM22A	0.43	Sourdough	Valerio et al. 2004
	Weissella confusa ITM14A	0.06	Sourdough	Valerio et al. 2004
	Enterococcus faecium ATCC882	0.09	Cheese	Valerio et al. 2004
Non-LAB strains[b]	*B. coagulans* SDM	NR	Soil	Zheng et al. 2011
	Propionibacterium jensenii DSMZ 20535	0.014[c]	Dairy	Lind et al. 2007
	P. thoenii DSMZ 20276	0.09[c]	Dairy	Lind et al. 2007
	P. acidipropionici DSMZ 4900	0.07[c]	Dairy	Lind et al. 2007
	P. freudenreichii ssp. *freudenreichii* DSMZ 20271	0.02[c]	Dairy	Lind et al. 2007
	Brevibacterium lactofermentum	11.6[c]	NR	Kamata et al. 1986
	Geotrichum candidum	3.6–6.0[c]	Cheese	Dieuleveux et al. 1998b

NR not reported

[a] Production of PLA in regular MRS broth

[b] Production of PLA in medium without PPA

[c] The values were converted from the unit mg/L

and phenylalanine content in medium was also reported in many other references (Li et al. 2007; Dallagnol et al. 2011; Rodriguez et al. 2012).

The transamination reaction is the first catabolic step of phenylalanine and is initiated by an aromatic aminotransferase (AAT) which has broad substrate spectrum, including leucine, tyrosine, tryptophan, and methionine (Yvon et al. 1997). AAT catalyzes transferring the amino group from the amino acid to a suitable α-keto acid acceptor, which commonly is α-ketoglutarate in most LAB strains (Fig. 2) (Yvon et al. 1998; Rijnen et al. 2000). Therefore, α-ketoglutarate has important effect phenylalanine catabolism and impacts on the regulation of PLA biosynthesis (Vermeulen et al. 2006; Dallagnol et al. 2011). Vermeulen et al. reported that the addition of α-ketoglutarate strongly increased PLA formation in *L. plantarum* TMW1.468 by 5 to >30 % (2006). On the other hand, α-ketoglutarate is produced from glutamate by glutamate dehydrogenase, and

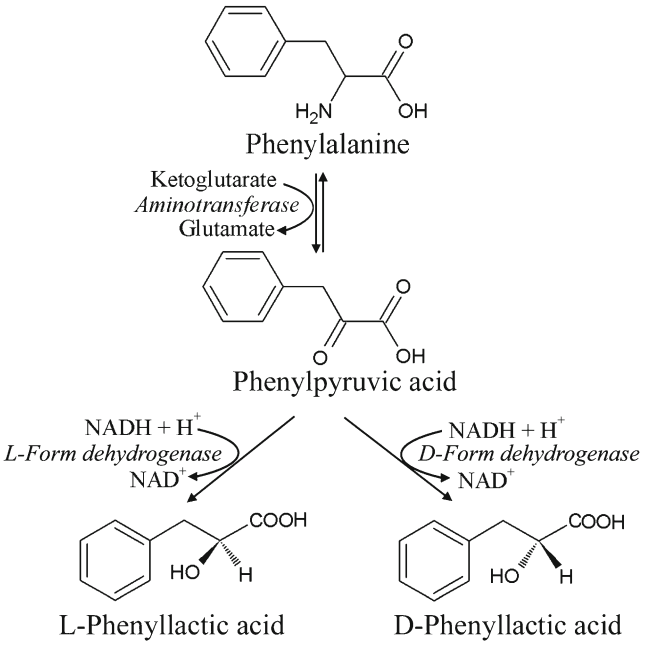


Fig. 2 Possible pathway of PLA biosynthesis in LAB strains

the glutamate dehydrogenase activity is influenced by the redox state of the cell, so PLA formation can be up-regulated indirectly by adding those compounds, which act as alternative electron acceptors to increase $NAD(P)^+$ level, such as citrate, fructose, and glucose (Vermeulen et al. 2006; Li et al. 2007; Dallagnol et al. 2011).

Actually, phenylpyruvic acid (PPA) is the direct precursor of PLA in PLA biosynthesis of LAB strains (Fig. 2) and shows much higher effect on PLA production than phenylalanine. When PPA was used to replace phenylalanine as supplemented substrate at the same concentration, PLA production increased 14-fold during *Lactobacillus* sp. SK007 fermentation (Li et al. 2007). It was suggested that phenylalanine transamination was the limiting factor in PLA production in LAB strains, and the bottleneck could be overcome using PPA as substrate (Li et al. 2007; Mu et al. 2009a and 2009b; Zheng et al. 2011).

Dehydrogenases converting PPA to PLA

There have been several different kinds of dehydrogenases that were characterized to convert PPA to PLA, and lactate dehydrogenase (LDH) is the main kind. In nature, there are two forms of LDH with different catalytic stereospecificity, L-LDH (EC 1.1.1.27) and D-LDH (EC 1.1.1.28). They have the highest catalytic activity for pyruvic acid and wide substrate specificity for α-ketonic acids, such as 2-ketobutyrate, α-ketoglutaric acid, etc. (el Hawrani et al. 1996). So far, it was found that LDHs from many LAB strains have the substrate specificity for PPA, including L-LDH from *Pediococcus acidilactici* DG302 (Garmyn et al. 1995), *L. plantarum* SK002 (Jia et al. 2010), and *Lactobacillus helveticus* 53/7 (Savijoki and Palva 1997), and D-LDH from *Pediococcus pentosaceus* ATCC 25745 (Yu et al. 2012), *P. acidilactici* DSM 20284 (Mu et al. 2012), *L. plantarum* SK002 (Jia et al. 2010), *Lactobacillus pentosus* JCM1558 (Tokuda et al. 2003) (previously called *L. plantarum* ATCC 8041 (Taguchi and Ohta 1991)), and *Lactobacillus confusus* 20196 (Hummel et al. 1983). Also, it was reported that some LDHs from non-LAB organisms could also transform PPA into PLA, such as *Thermoanaerobacter ethanolicus* JW200 (Zhou and Shao 2010), *B. coagulans* SDM (Zheng et al. 2011), and *Clonorchis sinensis* (Yang et al. 2006).

The optimum pH and temperature for LDH from LAB strains are in the range of 5.5–7.0 and 30–45 °C, respectively, probably because most of LAB strains are mesophile and acidophile (Table 3). The LDH from LAB strains generally showed weak thermostability, especially under more than 45 °C (Li et al. 2008; Jia et al. 2010; Yu et al. 2012; Mu et al. 2012). Some LDHs were characterized from thermophilic non-LAB strains, which showed higher optimum and thermostability, such as *T. ethanolicus* JW200 (Zhou and Shao 2010) and *B. coagulans* SDM (Zheng et al. 2011). In all of the LDHs reported, *P. acidilactici* D-LDH showed the highest catalytic efficiency (k_{cat}/K_m) value of 105 mM^{-1} s^{-1} in D-LDHs, and the k_{cat}/K_m of L-LDH was only reported in *B. coagulans* L-LDH with 110 mM^{-1} s^{-1}.

Although natural LDH showed less substrate specificity to PPA than pyruvic acid, the substrate specificity could be improved by modification of enzyme. Tokuda et al. (2003) constructed a mutant *L. pentosus* D-LDH in which the single amino acid of Tyr52 was replaced by Leu, and this mutant D-LDH (Y52L) showed much higher substrate specificity and catalytic efficiency to PPA than the wild-type D-LDH.

In addition to LDH, some other dehydrogenases were also reported exhibiting catalytic hydrogenation activity to phenylpyruvate, in which D-form dehydrogenases included D-hydroxyisocaproate dehydrogenase (D-HicDH) from *Lactobacillus casei* (Hummel et al. 1985) and the D-mandelate dehydrogenase (D-ManDH) from *Enterococcus faecalis* (Tamura et al. 2002; Wada et al. 2008) and *Lactobacillus curvatus* (Hummel et al. 1988). These D-form dehydrogenases have different optimum substrate; however, interestingly, all of them show broad substrate specificity to 2-ketoacids including phenylpyruvate (Table 4). Especially, *L. casei* D-HicDH (Hummel et al. 1985) and *L. curvatus* D-ManDH (Hummel et al. 1988) show relatively high specificity to phenylpyruvate with K_m of 0.15 mM. In L-form dehydrogenases, *L. confuses* L-

Table 3 Biochemical properties and kinetics parameters of various LDH

Strain		D/L-LDH	Optimum temperature (°C)	Optimum pH	K_m (mM)		k_{cat} (s^{-1})		k_{cat}/K_m (mM^{-1} s^{-1})		References
					Pyruvate	Phenylpyruvate	Pyruvate	Phenylpyruvate	Pyruvate	Phenylpyruvate	
LAB strains	*P. pentosaceus* ATCC 25745	D	45	5.5	0.49	1.73	320	173	658	100	Yu et al. 2012
	P. acidilactici DSM 20284	D	30	5.5	0.091	2.92	287	305	3,157	105	Mu et al. 2012
	L. plantarum SK002	D	30	6.0	0.06	5.4	NR	NR	NR	NR	Jia et al. 2010
	L. pentosus JCM1558	D	NR	NR	0.12	0.8	321	40	2,675	50	Tokuda et al. 2003
	L. confusus 20196	D	45	6.0	0.68	3	NR	NR	NR	NR	Hummel et al. 1983
	L. plantarum SK002	L	40	6.0	0.23	3.96	NR	NR	NR	NR	Jia et al. 2010
	L. pentosus JCM1558	L	30	7.0	1.8	15	NR	NR	NR	NR	Arai et al. 2001
	Lactobacillus sp. SK007	NR	40	6.0	0.32	1.69	NR	NR	NR	NR	Li et al. 2008
Non-LAB strains	*B. coagulans* SDM	D	NR	NR	2.2	4.4	23.6	16.5	11	3.9	Zheng et al. 2011
	B. coagulans SDM	L	NR	NR	2.6	4.3	1,172.2	47.2	450	110	Zheng et al. 2011
	T. ethanolicus JW200	L	60	5.8	0.18	NR	NR	NR	NR	NR	Zhou and Shao 2010

NR not reported

Table 4 Comparison of kinetic parameter K_m (mM) of various D-form dehydrogenases producing PLA

Substrate	Recombinant *P. pentosaceus* D-LDH	*L. pentosus* D-LDH	Mutant *L. pentosus* D-LDH (Y52L)	*L. casei* D-HicDH	Recombinant *E. faecalis* D-ManDH2	*E. faecalis* D-ManDH1	*E. faecalis* D-ManDH2	*L. curvatus* D-ManDH
Pyruvate	0.49	0.12	1.8	NR	NR	NR	NR	NR
Phenylpyruvate	1.73	0.8	0.067	0.15	5.7	11	3.4	0.15
Hydroxypyruvate	0.76	0.28	3.0	NR	NR	NR	NR	NR
2-Ketobutyrate	4.25	8.1	0.38	0.17	9.5	27	4.0	0.55
2-Ketovalerate	13.6	17.0	0.15	0.11	0.3	2.8	0.4	0.17
2-Ketoisovalerate	37.2	27.0	11.7	NR	0.18	0.5	0.15	0.18
2-Ketocaproate	34.7	20.9	0.056	0.11	1.5	4.2	0.6	0.10
2-Ketoisocaproate	45.3	31.0	0.12	0.06	0.13	0.6	0.11	0.09
References	Yu et al. 2012	Tokuda et al. 2003	Tokuda et al. 2003	Hummel et al. 1985	Wada et al. 2008	Tamura et al. 2002	Tamura et al. 2002	Hummel et al. 1988

NR not reported

HicDH was characterized to have substrate specificity to PPA with k_{cat}/K_m of 2.81×10^8 s^{-1} M^{-1} (Feil et al. 1997).

High-level bioproduction of PLA by microorganism fermentation

PLA production could be remarkably improved when adding PPA in initial medium or during fermentation process. Commercially, PPA can be obtained easily at relatively low price through organic synthesis from hydantoin (Christidis and Schouteeten 1985) and has been used to produce phenylalanine which is in high demand for the production of an artificial sweetener "aspartame" (Matsunaga et al. 1987; Leng et al. 2006). Therefore, it is an effective approach to produce PLA in large scale using PPA as material. It was reported that PLA content increased 14-fold in *Lactobacillus* sp. SK007 fermentation, which reached 1.12 g L^{-1}, when PPA was added in MRS broth (Li et al. 2007). Using response surface methodology, the medium components containing PPA were optimized for PLA production of *Lactobacillus* sp. SK007, and the PLA fermentation yield increased to 2.30 g L^{-1} (Mu et al. 2009a).

Further, the fed-batch fermentation of *Lactobacillus* sp. SK007 with substrate PPA feeding and pH-control was reported to be able to produce 17.38-g L^{-1} PLA, with the conversion ratio of PPA to PLA of 51.1 % and PLA production rate of 0.241 g L^{-1} h^{-1} (Mu et al. 2009b). Recently, Zheng et al. isolated a thermophilic bacteria, *B. coagulans* SDM, having PLA producing ability at a high temperature, which was helpful to improve the solubility and dissolution rate of substrate PPA. Using whole cells of *B. coagulans* SDM converting PPA, PLA was produced in a high concentration of 37.3 g L^{-1} and high productivity of 2.3 g L^{-1} h^{-1} (Zheng et al. 2011).

Future

So far, the scientific researches for the antimicrobial activity of PLA focused on the PLA producing strains in the form of protective cultures. There were not enough application researches of PLA in food system as a pure antimicrobial agent. More researches are needed to verify the effectiveness of PLA in food system and to compare it with other typical antimicrobial agents in detail.

Although PLA exists in many ordinary foods such as honey and LAB-fermented foods, PLA is still not allowed by legislation to be used as an additive. It is necessary to implement more human trials to study the metabolism pathway, health effects, and possible toxicity effects of PLA. And these data would give a guide to whether it can be approved as a legal additive.

To our best knowledge, there is no reference reporting the downstream process of PLA preparation. Therefore, in addition to optimizing the high-level production of PLA, the downstream process researches should be strengthened in the future.

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