

## Production of Phenyl Lactic Acid (PLA) by Lactic Acid Bacteria and its Antifungal Effect

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Phenyllactic acid (PLA) which is known as antimicrobial compound can be synthesized through the reduction of phenylpyruvic acid (PPA) by lactate dehydrogenase (LDH) of lactic acid bacteria (LAB). LAB producing PLA was isolated from Korea Kimchi and identified to *Lactobacillus plantarum* SJ21 by 16 rRNA gene sequence analysis. Cell-free supernatant (CFS) from *L. plantarum* SJ21 was assessed for both the capability to produce the antimicrobial compound PLA and the antifungal activity against four fungal pathogens (*Rhizoctonia solani*, *Aspergillus oryzae*, *Botrytis cinerea*, and *Colletotrichum acutatum*). PLA concentration was investigated to be 3.23mM in CFS when *L. plantarum* SJ21 was grown in MRS broth containing 5mM PPA for 16 h. PLA production also could be promoted by the supplement of PPA and phenylalanine in MRS broth, but inhibited by the supplement of 4-hydroxyphenylpyruvic acid and tyrosine as precursors. Antifungal activity demonstrated that all fungal pathogens were sensitive to 5% CFS (v/v) of *L. plantarum* SJ21 with average growth inhibitions ranging from 27.32% to 69.05% ( $p < 0.005$ ), in which *R. solani* was the most sensitive to 69.05% and followed by *B. cinerea*, *C. acutatum*, and *A. oryzae*. The minimum inhibitory concentration (MIC) for commercial PLA was also investigated to show the same trend in the range from 0.35 mg mL<sup>-1</sup> (2.11 mM) to 0.7 mg mL<sup>-1</sup> (4.21 mM) at pH 4.0. The inhibition ability of CFS against the pathogens was not affected by heating or protease treatment. However, pH modification in CFS to 6.5 caused an extreme reduction in their antifungal activity. These results may indicate that antifungal activities in CFS were caused by acidic compounds like PLA or organic acids rather than proteins or peptides molecules.

**Key words:** Phenyllactic acid, *Lactobacillus plantarum* SJ21, Supplement, Antifungal effect

**Percentage of growth inhibition (I) of selected fungi by CFS obtained from *Lactobacillus plantarum* SJ21 fermentation with some modifications.**

Fungal pathogens	CFS (a)	pH6.5 (b)	121°C (c)	Trypsin (d)
<i>Aspergillus oryzae</i>	27.3±5.8	0	28.8±3.5	27.8±2.2
<i>Botrytis cinerea</i>	47.9±3.1	0	54.4±4.5	51.4±3.5
<i>Colletotri chumaculatum</i>	41.7±4.2	0	50.3±5.9	48.3±3.9
<i>Rhizoctonia solani</i>	69.1±1.3	0	71.6±1.8	70.1±4.5

(a) PDA treated with CFS, (b) PDA treated with pH-modified CFS (pH=6.5), (c) PDA with heat-treated CFS (121°C, 20 min), and (d) PDA treated with CFS + Trypsin. All plates were prepared with a 5% CFS (v/v). The percentage of growth inhibition (I) was calculated as  $I = 100(A_C - A_T)/A_C$ , where  $A_T$  and  $A_C$  are the area of mycelial growth in treated and control plates, respectively.

## Introduction

3-Phenyllactic acid (PLA) and 4-hydroxyphenyllactic acid (HPLA) which can be produced by many lactic acid bacteria (LAB) (Lavermicocca et al., 2000), are known as antifungal compounds. Especially PLA was characterized having a broad inhibitory activity against yeast and bacteria as well as fungi (Dieuleveux and Gueguen, 1998; Dieuleveux et al., 1998). Valerio et al. (2004) reported that both the organic acids could be produced by a wide range of LAB species but their production is strain dependent, and presumed that the behavior of the antifungal activity was positively related to the PLA and HPLA content in the LAB culture filtrate. Although the production of PLA varied greatly based on the strains and species, it was known that PLA can be produced by a wide range of LAB species, such as *Lactobacillus*, *Enterococcus*, *Weissella*, and *Leuconostoc* (Valerio et al., 2004). PLA and HPLA were found as metabolic by-products in LAB strains through the amino acids degradation of phenylalanine and tyrosine, respectively, by which these amino acids were transaminated to 2-keto-carboxylic acids such as phenylpyruvic acid (PPA) and 4-hydroxyphenylpyruvic acid (HPPA) (Yvon et al., 1997), and then the ketoacids further reduced to the 2-hydroxy carboxylic acids (PLA and HPLA) (Li et al., 2007; Mu et al., 2010; Vermeulen et al., 2006). The supplement of the above amino acids and ketoacids in the initial culture broth for LAB fermentation could effectively increase the corresponding 2-hydroxy acids (PLA or HPLA) production, and the supplement of ketoacids was more significant than that of the original amino acids to improve the corresponding 2-hydroxy acids biosynthesis (Li et al., 2007; Mu et al., 2009; Mu et al., 2010).

Furthermore, PLA is an antimicrobial compound with a wide activity spectrum against some yeast such as *Candida pulcherrima* and *Rhodotorula mucilaginosa* (Schwenninger et al., 2008) and molds including some mycotoxigenic species such as *Aspergillus ochraceus*, and *Penicillium citrinum* (Valerio et al., 2004). In addition, PLA has been found to inhibit a range of Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Klebsiella oxycota* (Lavermicocca et al., 2000; Valerio et al., 2004). Recently, several LAB have been screened for their antifungal potential and their ability to produce PLA (Gerez et al., 2010; Gerez et al., 2013; Ryan et al., 2011), but additional studies are required in this field due to the wide diversity of both LAB and food spoilage molds. Therefore, the aim of this work was to isolate the novel LAB strains and to assess the antifungal activity of the isolated LAB against four fungal pathogens (*Rhizoctonia solani*, *Aspergillus oryzae*, *Botrytis cinerea*, and *Collectotricum aculatum*) and then to study their capability to produce the antimicrobial compound 3-phenyllactic acid and its relationship with the

antifungal activity of the LAB.

## Materials and methods

**Microorganism and culture media** Lactic acid bacteria (LAB) were obtained from Korea Kimchi and cultivated in deMan-Rogosa-Sharpe (MRS) medium at 37°C without shaking. The MRS medium contains 10 g L<sup>-1</sup> peptone, 10 g L<sup>-1</sup> beef extract, 5 g L<sup>-1</sup> yeast extract, 20 g L<sup>-1</sup> glucose, 2 g L<sup>-1</sup>, K<sub>2</sub>HPO<sub>4</sub>, 2 g L<sup>-1</sup> triammonium citrate, 5 g L<sup>-1</sup> CH<sub>3</sub>COONa, 1 mL L<sup>-1</sup> Tween-80, 0.58 g L<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.25 g L<sup>-1</sup> MnSO<sub>4</sub>·4H<sub>2</sub>O, and pH was adjusted to 6.2 by 1 M HCl or NaOH. All the microbial fermentation experiments were developed in 10 mL test tubes containing 5 mL MRS broth. LABs producing phenyllactic acid (PLA, Sigma Co, USA) were isolated with 5 mL MRS broth containing 5 mM phenylpyruvic acid (PPA, Sigma Co, USA). To prepare the inoculum for fermentation experiments, the isolated LABs were inoculated into a 10 mL test tube containing 5 mL MRS broth and grown for the seed culture at 37°C for 24 h. A 2% (v/v) inoculum of the seed was aseptically added to 5 mL of MRS for fermentation experiment. In order to evaluate the effect of amino acids and ketoacids as precursors on PLA production, different substrates, phenylpyruvic acid (PPA), 4-hydroxyphenylpyruvic acid (HPPA), phenylalanine and tyrosine purchased from Sigma (St. Louis, USA), were supplemented in the initial MRS broth with various concentrations. The fermentation was carried out in a 10 mL test tube at 37°C for 36 h without shaking. Fungal pathogens, *Rhizoctonia solani*, *Aspergillus oryzae*, *Botrytis cinerea*, *Collectotricum aculatum*, were offered from Chungcheongnam-Do Agricultural Research & Extension Services.

**Identification of LAB** The partial sequencing of 16S rRNA for the LAB strain was done with the help of DNA sequencing service, SOLGENT, Daejeon, South Korea using universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The online program BLAST was used in identifying the related sequences with known taxonomic information available at the databank of NCBI (<http://www.ncbi.nlm.nih.gov/BLAST>). A Phylogenetic tree was constructed using CLUSTAL X program (Thompson et al., 1997), which involved sequence alignment by neighbor joining method (Saitou and Nei, 1987) and maximum parsimony using the MEGA4 program (Kumar et al., 2001). Grouping of sequences was based on confidence values obtained by bootstrap analysis of 1,000 replicates. Gaps were edited in the BioEdit program and evolutionary distances were calculated using Kimura two parameter model. Reference sequences were retrieved from GenBank under the accession numbers indicated in the trees.

**Preparation of cell-free supernatants (CFS)** LAB colonies were precultured for 18 h at 37°C in 10 mL of MRS broth. An aliquot (200  $\mu$ L) of the preculture was then inoculated into fresh sterile MRS broth (20 mL) and allowed to grow at 37°C without shaking for 72 h. Cell free supernatants (CFS) were recovered by centrifugation (7200 $\times$ g, 10 min), and the CFS filtered with 0.45- $\mu$ m Millipore membrane were used for identifying PLA and screening their antifungal activity.

**Determination of antifungal activity** Antifungal activity was tested with cell-free supernatants of LAB by modification method of Wang et al. (2012). Briefly, CFS was mixed with the sterilized PDA (pH 4) to achieve a final concentration of 5-10% for *Rhizoctonia solani*, *Aspergillus oryzae*, *Botrytis cinerea*, *Collectotricum aculatum* (v/v) and the mixture was poured into Petri dishes. Resulting media was centrally inoculated with 5  $\mu$ L of the previously prepared fungal pathogen spore suspensions and incubated at 25°C. The sterile MRS broth instead of CFS in the same proportions was also used for control plates. During 8-days incubation period to grow the pathogens onto the plate, the area of mycelial growth in both treated and control plates were determined from the mean perpendicular diameter measurements assuming a circular growth. The percentage of growth inhibition (I) was calculated as  $I = 100(A_C - A_T)/A_C$ , where  $A_T$  are the area of mycelial growth in both treated and control plates, respectively. CFS were also subjected to heating (121°C, 15 min) treatment to evaluate the possible antifungal compounds present in each CFS besides PLA and the remaining antifungal activity of treated CFS was further assessed as described above.

**Identification of PLA** PLA in CFS was identified by HPLC as described by Valerio et al. (2004) and Gerez et al. (2010) with minor modifications. CFS were adjusted to pH 2.0 with 10 M formic acid and extracted three times with 20

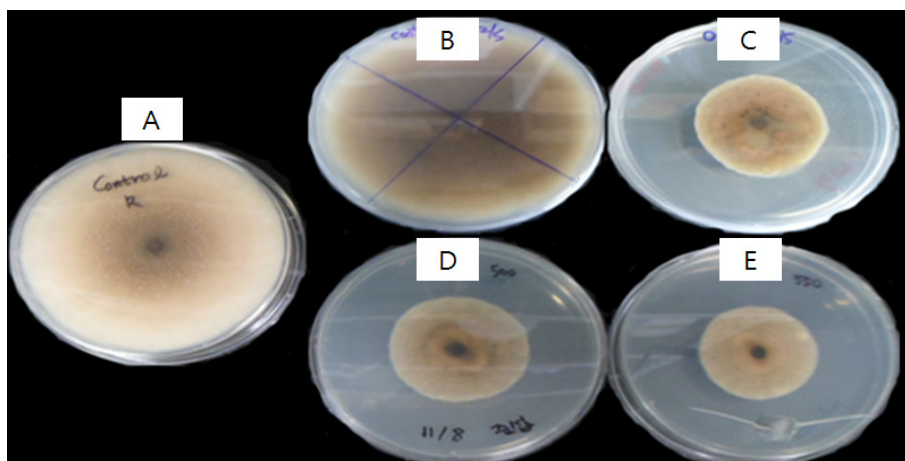
mL of ethyl acetate. The extracts were dried using  $\text{Na}_2\text{SO}_4$  and concentrated in a rotary evaporator (Büchi model R210/215, Flawil, Switzerland). The dried residues were reconstituted with 5 mL of 2.5 mM  $\text{H}_3\text{PO}_4$  and the PLA component filtered with 0.45  $\mu$ m pore-size membrane was analyzed in an HPLC system (Agilent, 1260 infinity, USA) fitted with an CAPCELL PAK C18 column (4.6 $\times$ 250 mm, 5 $\mu$ m, Shiseido Co, Japan) at 30°C, using (A) 0.5%  $\text{H}_3\text{PO}_4$ (v/v) and (B) 0.5%  $\text{H}_3\text{PO}_4$ - $\text{CH}_3\text{CN}$  (V/V) as the mobile phases and a UV-visible detector set at 210 nm. Linear gradient elution was used with solvent A and solvent B at 1 mL  $\text{min}^{-1}$  and A/B ratios of 80:20, 80:20, 0:100 and 0:100 with run times of 0, 12, 13, and 15 min, respectively (Li et al., 2007; Mu et al., 2010). Commercial PLA was used as reference (retention time = 11.66 min).

**Statistical analysis** Three independent replicates of each experiment were performed, and their results were expressed as mean values  $\pm$  standard deviation. The data were subjected to analysis of variance (ANOVA) using SAS package (SAS, 1999). The Duncan's Multiple Range Test (DMRT) was applied to test the significance of treatment means at  $P \leq 0.05$ .

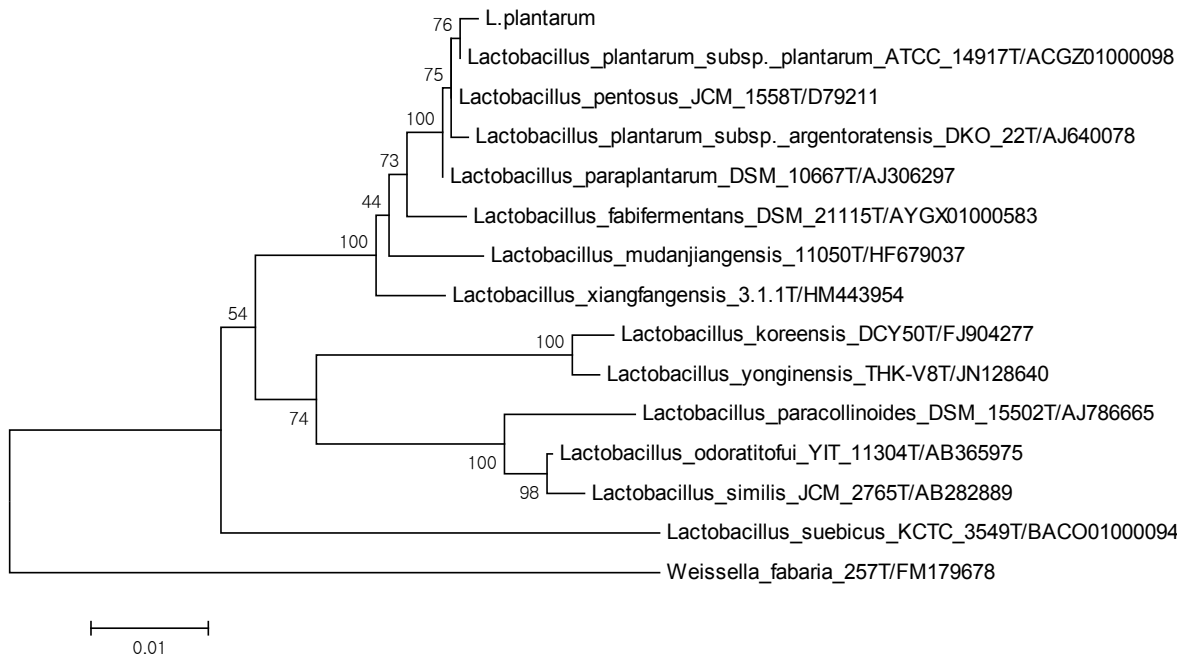
## Results and discussion

### Isolation and identification of PLA producing LAB

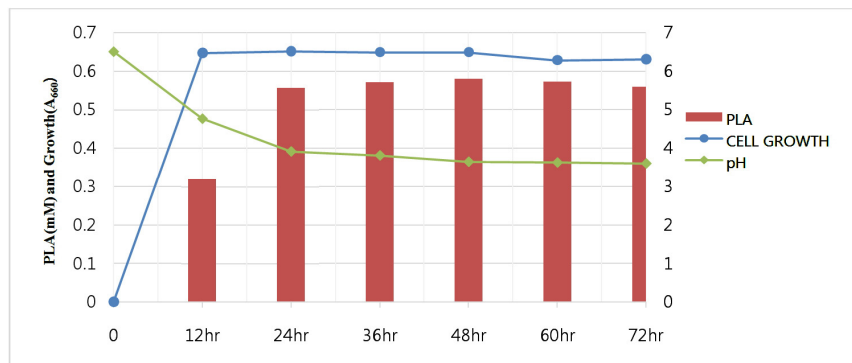
A PLA producing strain was selected by assessing the mycelial growth inhibition of *Rhizoctonia solani* on MRA media with culture filtrates of lactic acid bacteria isolated from Korea Kimchi and by finally confirming PLA in the culture filtrate through HPLC analysis. The selected strain showed a marked inhibition for mycelial growth of fungal pathogen and the perpendicular diameter of a circular growth was similar to commercial PLA used as control after 7 days of incubation (Fig. 1). According to 16S rRNA sequence analysis,



**Fig. 1.** Growth of *Rhizotonia solani* in PDA after 10 days at 25°C in presence of CFS from *Lactobacillus plantarum* SJ21 fermentation in MRS broth : (A) PDA, (B) PDA + 5% MRS broth (v/v), (C) PDA + 5% CFS (v/v), (D) PDA + PLA(Sigma, 500 ppm), (E) PDA + PLA(Sigma, 550 ppm).



**Fig. 2.** Phylogenetic tree based on 16S rRNA gene sequences, showing the position of *Lactobacillus plantarum* SJ21 strain with respect to related species. The scale bar indicates 0.005 substitutions per nucleotide position and accession numbers are given in parenthesis.



**Fig. 3.** Cell growth, pH variation and PLA production by *Lactobacillus plantarum* SJ21 in MRA broth. All the fermentations were developed at 37°C for 72 h in MRA broth without the precursors.

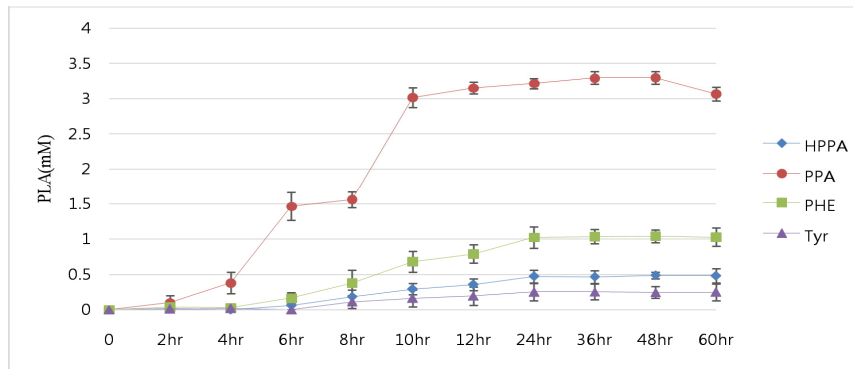
the strain was identified as *Lactobacillus plantarum*. Comparison of the 16S rRNA sequence among available strains of *Lactobacillus* species showed high homology (>99%) to *Lactobacillus plantarum* ATCC14917<sup>T</sup>. Neighbor-joining method was employed to construct the phylogenetic tree which illustrates the relationships of 16S rRNA sequence of strain and other *Lactobacillus* species (Fig. 2).

**Cell growth** *L. plantarum* fermentation was performed at 37°C with MRS broth without PPA to assess PLA production and pH change. Growth and PLA production curves for *L. plantarum* are shown in Fig. 3. Cell growth reached to stationary phase after 12 h and PLA production progressively increased thereafter by the end of this stage. The highest production was reached to 0.58 mM after 36 h, in which pH

was changed to lower the initial pH 6.5 to pH 4.0, indicating that the acidification of the media was directly related to PLA production by LAB. A similar results were described by Vermeulen et al. (2006) who reported PLA formation of *L. sanfranciscensis* DSM20451 continues to process by stationary phase cells. Valerio et al. (2004) also reported the production of PLA in *L. plantarum* ITM21B was found in synthetic media with a maximum production of 0.165 mM after 72 h. Recently, the growth conditions on LAB fermentations has become an actual topic in recent studies to achieve a maximum production of a given metabolite with antifungal properties such as PLA (Mu et al., 2012; Rodríguez et al., 2012).

#### Effect on PLA production by precursors supplement

The effect of ketoacids (PPA and HPPA) and amino acids



**Fig. 4.** The effects of ketoacids and amino acids and supplement on PLA production during *L. plantarum* SJ21 fermentation process. All the fermentations were developed at 37°C for 60 h in MRA broth with each 5 mM of PPA, HPPA, Phe and Tyr, respectively. Values are means of three replications  $\pm$  standard deviation.

**Table 1.** Effect of various supplement amounts (HPPA, PPA, PHE, Tyr) for PLA Production by *Lactobacillus plantarum* SJ21.

Supplement amount (mM)	PLA Production (mM)			
	PPA	HPPA	PHE	Tyr
0	0.56 $\pm$ 0.01	0.56 $\pm$ 0.01	0.56 $\pm$ 0.01	0.56 $\pm$ 0.01
2	1.38 $\pm$ 0.04	0.58 $\pm$ 0.01	0.91 $\pm$ 0.02	0.41 $\pm$ 0.00
4	2.86 $\pm$ 0.07	0.53 $\pm$ 0.02	1.15 $\pm$ 0.02	0.34 $\pm$ 0.01
6	4.40 $\pm$ 0.12	0.51 $\pm$ 0.01	1.33 $\pm$ 0.04	0.30 $\pm$ 0.01
8	6.22 $\pm$ 0.03	0.49 $\pm$ 0.01	1.47 $\pm$ 0.01	0.27 $\pm$ 0.02
10	7.74 $\pm$ 0.14	0.48 $\pm$ 0.02	1.55 $\pm$ 0.08	0.28 $\pm$ 0.06

(Phe and Tyr) supplement on PLA during *L. plantarum* SJ21 fermentation process was investigated. The PLA production was effectively improved by the supplement amount of 5 mM PPA and Phenylalanine (Phe). Although the PPA precursors showed more promotion effect on the corresponding 2-hydroxy acids (PLA) production than Phe, the maximum PLA production reached 3.32 and 1.08 mM after 24 h fermentation, respectively, in comparison of the result of 0.58 mM PLA obtained by the fermentation with only MRS broth without the supplement of PPA (Fig. 1). However, when the LBA was fermented in MRS broth supplemented with 5 mM HPPA and Tyr, PLA production appeared to be 0.48 and 0.26 mM, respectively, lower than that of the MRS broth fermentation without the supplement, indicating PLA production by *L. plantarum* SJ21 was rather inhibited by HPPA and Tyr. Several reports suggested that PPA and Phe are the metabolic precursors of PLA, while Tyr and HPPA are the precursors of HPLA in LAB strains (Vermeulen et al., 2006; Zheng et al., 2011). Further, PLA production could be improved by supplement with the corresponding precursors (PPA and Phe) (Valerio et al., 2004). A similar result was identified in this study in which the ketoacid (PPA) and amino acid (Phe) supplements as substrates should have affected the yield of the metabolic precursors such as PLA during fermentation process. In addition, the influence of various supplement amounts of amino acid and ketoacid precursors on PLA production during *L. plantarum* SJ21

fermentation process was investigated. Both the promotion effect of the corresponding precursors (PPA and Phe) and the inhibitory effect of the non-corresponding precursors (HPPA and Tyr) on the PLA production were strengthened with increasing the supplement amounts (Table 1). Yvon et al. (1997) reported aminotransferase initiated the degradation of Phe and Tyr to produce the corresponding ketoacids, PPA and HPPA, respectively. Subsequent reports identified that PLA was the hydrogenation-reduction product of PPA (Mu et al., 2010; Zheng et al., 2011). Furthermore, in the PLA metabolic pathway, the supplement of non-corresponding amino acid precursor (Tyr) might inhibit the aminotransferase of the corresponding amino acid precursor (Phe). The similar inhibitory effect on PLA production was identified in the supplement of Tyr, in which Tyr showed more inhibitory effect on non-corresponding 2-hydroxy acid (PLA) production than HPPA.

#### Inhibitory effect of CFS against fungal pathogens

Antifungal activity of CFS from *L. plantarum* SJ21 demonstrated that all fungal pathogens were sensitive to 5% CFS (v/v) with average growth inhibitions ranging from 27.32% to 69.05% ( $p < 0.005$ ), in which *Rhizoctonia solani* was the most sensitive to 69.05% and followed by *B. cinerea*, *C. aculatum*, and *A. oryzae* (Table 1). The apparent results were also confirmed in further MIC experiments with commercial PLA and chemical antimicrobial agents. The fungal pathogens showed

**Table 2. Minimal inhibitory concentrations of commercial phenyllactic acid (PLA) in the studied fungal pathogens\*.**

Antimicrobial	Fungal pathogens			
	<i>Aspergillus oryzae</i>	<i>Botrytis cinerea</i>	<i>Colletotrichum aculatum</i>	<i>Rhizoctonia solani</i>
Sodium benzoate (mg mL <sup>-1</sup> )	0.54	0.61	0.47	0.38
Polyoxin (mg mL <sup>-1</sup> )	0.19	0.11	0.15	0.14
PLA (mg mL <sup>-1</sup> , mM)	0.7, 4.21	0.55, 3.31	0.5, 3.01	0.35, 2.11

\*Minimal inhibitory concentrations were determined at pH 4.0 and 25°C in PDA medium.

**Table 3. Percentage of growth inhibition (I) of selected fungi by CFS obtained from *Lactobacillus plantarum* SJ21 fermentation with some modifications.**

Fungal pathogens	CFS (a)	pH6.5 (b)	121°C (c)	Trypsin (d)
<i>Aspergillus oryzae</i>	27.3±5.8	0	28.8±3.5	27.8±2.2
<i>Botrytis cinerea</i>	47.9±3.1	0	54.4±4.5	51.4±3.5
<i>Colletotri chumaculatum</i>	41.7±4.2	0	50.3±5.9	48.3±3.9
<i>Rhizoctonia solani</i>	69.1±1.3	0	71.6±1.8	70.1±4.5

(a) PDA treated with CFS, (b) PDA treated with pH-modified CFS (pH=6.5), (c) PDA with heat-treated CFS (121°C, 20 min), and (d) PDA treated with CFS + Trypsin. All plates were prepared with a 5% CFS (v/v). The percentage of growth inhibition (I) was calculated as  $I = 100(A_C - A_T)/A_C$ , where  $A_T$  and  $A_C$  are the area of mycelial growth in treated and control plates, respectively.

almost the same sensitivities to pure PLA by following in the order *R. solani*, *C. aculatum*, *B. cinerea* and *A. oryzae* with MIC values in the range of 0.35 mg mL<sup>-1</sup> (2.11 mM) to 0.7 mg mL<sup>-1</sup> (4.21 mM) at pH 4.0 (Table 2). The obtained MIC values are compared to the result by Ryan et al. (2011) reporting an MIC for PLA of 15 mM against *Aspergillus fumigatus* J9. However, sodium benzoate and sodium propionate used as antifungal agents appeared to be more effective than PLA for the fungal pathogens. Although PLA showed the least inhibition effect on mold growth, it can be well worth enough to natural and safe food preservatives (Prema et al., 2010; Ryan et al., 2011). Furthermore, CFS from *L. plantarum* SJ21 was further subjected to heating (121°C, 20 min) and pH modification (6.5) in order to assess the characteristics of the antifungal compounds besides PLA. The inhibition characteristics of CFS against pathogens were not affected by the heating or protease treatment. However, pH modification in CFS to 6.5 caused an extreme reduction in their antifungal activity (Table 2). These results may indicate that antifungal activities in CFS are very likely to be lost by acidic compounds neutralizing pH of the media rather than proteins or peptides molecules being able to suffer by thermal denaturation. It is known that the antimicrobial activity of the organic acids on molds and bacteria is pH-dependent and especially a maximum inhibition activity can be maintained at low pH values favoring the undissociated state of the acid molecule (Gerez et al., 2010; Schillinger and Villareal, 2010). Similar results were obtained by Wang et al. (2012) reported on the antifungal activity of CFS from *L. plantarum* IMAU10014 against *B. cinerea*, *Glomerella cingulate*, *Phytophthora drechsleri*, *Penicillium citrinum*, *P. digitatum*, and *Fusarium oxysporum*. Furthermore,

growth inhibition properties in CFS also can be caused by synergism with other acidic compounds from LAB metabolism such as 4-hydroxyphenyllactic acid (HPLA) and organic acids (Hladiková et al., 2012; Schillinger and Villareal, 2010). In this study even though authors have not identified the existence of HPLA in CFS, conclusively, the inhibition effect was due to acidic metabolites in fermentation broth and PLA in CFS was concerned in the inhibition activity against fungal pathogens.

## Conclusions

Phenyllactic acid (PLA), which is known as antimicrobial compound can be synthesized through the reduction of phenylpyruvic acid (PPA) by lactate dehydrogenase of lactic acid bacteria (LAB). LAB producing PLA was isolated from Korea Kimchi and identified to *Lactobacillus plantarum* SJ21 by 16 rRNA gene sequence analysis. Cell-free supernatant (CFS) from *L. plantarum* was confirmed to possess both the capability to produce the antimicrobial compound PLA and the antifungal activity against four fungal pathogens (*Rhizoctonia solani*, *Aspergillus oryzae*, *Botrytis cinerea*, and *Collectotricum aculatum*). *L. plantarum* SJ21 showed 65% of conversion ratio by converting 3.23mM PLA from 5mM PPA of precursor for 16 h fermentation. PLA production also be promoted by the supplement of the phenylalanine (Phe) in MRS broth, but inhibited by the supplement of the 4-hydroxyphenylpyruvic acid (HPPA) and tyrosine (Tyr) as precursors. A ketoacid precursor (PPA) showed more promotion effect on the corresponding 2-hydroxy acid (PLA) production than amino acid precursor (Phe). However, Tyr precursor showed more inhibitory effect on PLA production than HPPA precursor.

Both the promotion effect of the corresponding precursors (PPA and Phe) and the inhibitory effect of the non-corresponding precursors (HPPA and Tyr) on the PLA production were strengthened with increasing the supplement amounts. Antifungal activity demonstrated that all fungal pathogens were sensitive to 5% CFS (v/v) of *L. plantarum* SJ21 with average growth inhibitions ranging from 27.32% to 69.05% ( $p < 0.005$ ), in which *R. solani* was the most sensitive to 69.05%. The minimum inhibitory concentration (MIC) for commercial PLA also appeared the same trend with the result of growth inhibition in the range from 0.35 mg mL<sup>-1</sup> (2.11 mM) to 0.7 mg mL<sup>-1</sup> (4.21 mM) at pH 4.0. The inhibition ability of CFS against the pathogens was not affected by the heating or protease treatment. However, pH modification in CFS to 6.5 caused an extreme reduction in their antifungal activity. These results may indicate that antifungal activities in CFS were caused by acidic compounds like PLA or organic acids rather than proteins or peptides molecules.

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