Isolation of lactic acid bacteria starters from *Jeung-pyun* for sourdough fermentation

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Abstract Lactic acid bacteria (LAB) are key for the fermentation of sourdoughs to improve the quality and nutritive value of bread. The aim of this study was to isolate the LAB starter for sourdough fermentation from *Jeung-pyun*, a Korean traditional rice cake. Among the twenty two LAB screened, five isolates were selected based on exo-polysaccharide production. Among them, three isolates showed cell growth greater than 8.5 Log CFU/g, maximum increase in the volume of dough, and dextran concentration up to 0.16%. During the sourdough fermentation, pH and total titratable acidity (TTA) were changed, as the three isolates synthesized lactic acid and acetic acid with fermentation quotients less than 2.0. They were identified as *Leuconostoc lactis* EFEL005, *Lactobacillus brevis* EFEL004, and *Le. citreum* EFEL006. They displayed good fermentation properties (growth, dextran production, pH, and TTA) in dough and they are regarded as potential starters to be used in sourdough fermentation.

Keywords *Jeung-pyun* · Exo-polysaccharides · Lactic acid bacteria · Fermentation · Sourdough

Introduction

Fermentation of cereal flour by yeast and lactic acid bacteria (LAB) is commonly regarded as sourdough, which helps in improving the texture, flavor, and shelf life of baked products [1]. Among the cereals, wheat is the most widely used worldwide to produce sourdough products [2, 3]. Depending on the type of flour used, approximately 10–40% of sourdough is mixed with the final bread dough [4]. Thus, selection of suitable starters for dough fermentation is very important. The quality of bread depends on the type of microorganisms involved in fermentation [5]. The major qualities required by sourdough starters are: production of exopolysaccharide (EPS), organic acid, release of carbon dioxide, and displaying good growth profiles in the dough. EPS produced by LAB in sourdough improve the quality of bread by enhancing the rheological properties by increasing the volume, loffyness and decreasing the firmness of bread [6]. Acidification of fermented doughs by LAB inhibits the action of endogenous amylase, retards bread staling during storage, protects bread from bacterial and fungal spoilage, and increases the gas-holding and water-binding abilities of dough [7, 8]. Hetero-fermentative LAB produce EPS, carbon dioxide, and organic acids and thus they are regarded as good starter candidates [9]. Additionally, sourdough lowers the post-prandial glucose response, increases mineral bioavailability in whole meal bread, and reduces gluten intolerance [10]. Because the metabolic properties of LAB are strain-specific, the selection of the starter LAB for sourdough is

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one of the most important factors in the making of sourdough bread.

Jeung-pyun is a fermented rice cake produced by mixing rice flour, water, sugar, salt, and *makgeolli* (a traditional Korean rice beer). *Makgeolli* is rich in essential nutrients such as amino acids, sugars, and vitamins, which support the growth of numerous LAB species such as *Lactobacillus*, *Weissella*, and *Pediococcus* [9]. Recently, probiotic LAB were isolated from *makgeolli* and considered as a starter for the fermentation of rice dough *Jeung-pyun* [11]. Currently, baker's yeast is the only standard starter used commercially for making bread. Few studies have examined the development of LAB starters for potential application in the fermentation of cereal based dough [4, 9]. Thus, there is a need to develop ideal starters for the fermentation of different cereal based sourdoughs to produce bread enriched in nutrients and accepted by consumers.

In the present study, we isolated LAB starters from *Jeung-pyun* for sourdough fermentation. First, twenty two LAB strains were screened based on EPS production in wheat peptone medium. The screened isolates were grown individually in wheat dough and characterized based on growth profile, gas formation by volume change, dextran production, pH change, and organic acids release during dough fermentation.

Materials and methods

LAB strains and culture conditions

The LAB strains used for the fermentation of dough were as follows: 22 isolates from *Jeung-pyun* were used (Table 1) and they were cultured in MRS broth for 24 h at 30 °C prior to the experiments [12].

Quantification of EPS produced in wheat peptone medium

All the LAB strains were cultured in wheat peptone agar or broth media as described by Korakli et al. [12] with some modifications. For preparation of modified wheat peptone broth, wheat grain extract was prepared by adding 30 g of whole wheat to 500 mL of tap water and boiled for 1 h. The sample was decanted and filtered through several thicknesses of cheese cloth padded with cotton. To the above filtrate 20 g peptone, 10 g yeast extract, 10 g glycerine, and 20 g sucrose were added and the final volume was made up to 1.0 L after adjusting the pH to 6.8–7.0. Production of EPS on wheat peptone agar medium was carried out by spreading the LAB strains (100 µL) on wheat peptone agar medium followed by incubation for 48 h at 30 °C. After 2 days, 30 mL of sterile saline

solution (NaCl 0.85% w/v) was added to the respective agar plates and EPS gels formed on media were scrubbed using glass rods followed by transferring to 50 mL tubes. The production of EPS in wheat peptone broth medium was carried out by inoculating the LAB strains (100 µL) in to 10 mL of wheat peptone broth medium and followed by incubation at 30 °C for 48 h. The resulting tubes were centrifuged at 4500×g at 30 °C for 15 min. After the supernatant was discarded, the pellet was mixed with cold ethanol (98%) and incubated overnight at 4 °C. After the samples were centrifuged at 4500×g at 4 °C for 15 min, the pellet was dried in an oven at 55 °C.

Fermentation of wheat doughs with screened LAB starters

The wheat doughs were prepared by mixing 250 g of wheat flour (Beksul strong flour, CJ, Seoul, Korea), 300 g of water, and 11 g of table sugar. The respective doughs were mixed with 10⁷ cfu/g of screened LAB starters which were grown at the late exponential phase (centrifugation at 4500×g, and 4 °C for 15 min), and harvested by washing twice with sterile saline solution and re-suspension in fresh saline. The doughs were mixed with a hand mixer (Model JU07408-4001, Sanshui Hop Shing Metal & Plastic Ltd., Kwai Chung, Hong Kong). The samples were fermented at 28 °C for 20 h, and then stored at –80 °C until further analysis. Un-inoculated control dough was prepared and incubated under the same conditions.

Enumeration of LAB

Fermented wheat dough samples of 5 g each were homogenized with 45 mL sterile saline solution. The above homogenate was serially diluted in sterile saline solution (10⁻¹ to 10⁻⁷) and aliquots of 20 µL were added to MRS agar plates to incubate at 30 °C for 2 days. Colony numbers between 30 and 300 on each petri dish were counted as total LAB.

Measurement of dough volume during fermentation

To a 50 mL measuring cylinder 30 g of wheat dough was added, and the change in volume during the fermentation was measured at 0, 4, 9, 14, and, 20 h respectively. The expansion ratio was calculated as described by Kang et al. [13].

$$\text{Expansion ratio (\%)} = \frac{\text{Volumes of sourdough after fermentation}}{\text{Volumes of sourdough before fermentation}} \times 100$$

Table 1 EPS profile of LAB isolates on wheat peptone media

Strain no.	LAB isolates	EPS contents in wheat agar media (g/100 mL)	EPS contents in wheat broth media (g/100 mL)
I4	<i>Lactobacillus brevis</i>	0.03	0.62
S2-5		0.20	0.43
S3-4		0.06	0.47
L10	<i>Leuconostoc mesenteroides</i>	0.15	0.91
L21		0.03	0.70
S5-9		0.17	0.78
S5-1	<i>Le. pseudomesenteroides</i>	0.09	0.74
M3-8	<i>Lb. crustorum</i>	0.03	0.51
MC4-1	<i>Lb. fermentum</i>	0.03	0.57
MC5-2	<i>Lb. harbinensis</i>	0.02	0.44
L14	<i>Le. citreum</i>	0.08	0.92
L48		0.07	0.92
L50		0.07	0.92
L51		0.15	0.90
S4-5		0.14	0.77
L27	<i>Le. gasicomitatum</i>	0.14	0.74
L7	<i>Le. gelidum</i>	0.01	0.97
I2	<i>Lb. reuteri</i>	0.03	0.50
I7	<i>Lb. curvatus</i>	0.13	0.77
I13	<i>Lactococcus lactis</i>	0.02	0.50
I15	<i>Le. lactis</i>	0.14	0.69
I20	<i>Weissella confusa</i>	0.02	0.43

Enzyme-assisted dextran assay

The dextran concentration in fermented dough was determined as described by Katina et al. [4]. The sourdough samples were diluted (1:20) and homogenized. The samples were vortexed after adding 10 mL of chilled ethanol (98%) and then centrifuged (4500×g, 4 °C, 15 min). Finally, the dextran pellet was re-suspended in 10 mL of 0.1 M sodium citrate buffer (pH 5.5). The resulting dextrans were hydrolyzed by commercial grade dextranase from *Chaetomium erraticum* and α -glucosidase from *Aspergillus niger* purchased from Sigma-Aldrich (St. Louis, MO, USA). The amount of reducing sugar released was measured by using the dinitrosalicylic acid method [4].

Analysis of acidity and organic acid contents

To determine the change in pH and total titratable acidity, the above suspension was titrated against 0.01 N NaOH, using phenolphthalein as an indicator. TTA was expressed as percent lactic acid. All fermented dough samples (10 g each) were blended with 90 mL of distilled water and centrifuged at 13,000×g for 10 min. The supernatant was boiled for 5 min and centrifuged again. The resulting clear

broth was filtered through a 0.2- μ m syringe filter. The filtrate was used to analyze of organic acids by high-performance liquid chromatography (Young-Lin, Acme 9000, Anyang, Korea) using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). Sulfuric acid (0.008 N) was used as the mobile phase at a flow rate of 0.6 mL/min.

16S rRNA gene sequencing of selected starters

Total bacterial DNA was used as a template for PCR amplification of the V3 region of the 16S rRNA gene using the universal primers 338F and 518R [14]. The PCR products were sequenced by SolGent Co., Ltd (SolGent, Daejeon, Korea). The sequences were then compared against type culture using the Ribosomal Database with the BLAST algorithm (<https://rdp.cme.msu.edu>).

Results and discussions

Production of EPS by LAB strains on wheat peptone medium

Among the 22 LAB screened, *Leuconostoc mesenteroides* L10, *Le. mesenteroides* S5-9, *Le. citreum* L51, *Le. citreum* S4-5, *Le. gasicomitatum* L27, *Le. lactis* I15, and *Lactobacillus curvatus* I7 produced high amount of EPS on wheat agar medium (Table 1). In addition, when cultured in wheat broth medium, *Le. mesenteroides* L10, *Le. mesenteroides* S5-9, *Le. citreum* L14, *Le. citreum* L48, *Le. citreum* L50, *Le. citreum* L51, *Le. citreum* S4-5, *Le. gasicomitatum* L27, *Lb. curvatus* I7, and *L. lactis* I15 produced high amount of EPS in the broth (Table 1). Based on the above results, *Le. mesenteroides* S5-9, *Le. citreum* S4-5, *Le. gasicomitatum* L27, *Lb. curvatus* I7, and *Le. lactis* I15 were selected as starter candidates for the fermentation of sourdough. Production of EPS by *Weissella confusa* I20 and *Lb. brevis* S2-5 was comparatively lower than other strains. As a result, *W. confusa* I20 and *Lb. brevis* S2-5 were used as controls. It was reported that LAB isolated from different cereal based sources produce different types of EPS, which enhances the quality of bread by decreasing the firmness and increasing the freshness and volume after storage [6].

Viable cell counts during sourdough fermentation

Adaptation of the screened starters to wheat dough is very important in fermentation. At the end of fermentation (20 h), *Le. lactis* I15 and *Lb. brevis* S2-5 showed maximum cell growth of (≥ 9.0 Log CFU/g). *Le. citreum* S4-5, *Lb. curvatus* I7, and *W. confusa* I20 displayed a growth profile of 8.5 Log CFU/g. The remaining starter strains showed viable cell counts of less than 8.5 Log CFU/g (Table 2). The growth profiles of the above LAB were comparable to those described in previous reports [4]. *Leuconostoc* spp. and *Lb. brevis* are the predominant bacteria in wheat

sourdough [9, 15]. The growth profiles of *Leuconostoc* spp. and *Lb. brevis* S2-5 in wheat dough clearly indicated their high ability to adapt to the natural environment.

Volume change in fermented doughs

Among the screened LAB strains, *Lb. brevis* S2-5, and *Le. lactis* I15 were the prominent strains which displayed a significant increase in the volume of fermented dough by 46.6% during 14 and 9 h respectively. While the *Leuconostoc* spp. (*Le. citreum*, *Le. mesenteroides*, and *Le. gasicomitatum*) showed a 40% increase in volume in 9 h as shown in Table 2. In case of control, 83.3% increase in volume of fermented dough was observed due to naturally existing bacteria in wheat flour. Addition of LAB starters to the dough will help in increasing the volume of fermented sourdough by releasing carbon dioxide during fermentation, and thus enhance the rheological properties; and improve the nutritional quality of bread [16].

Dextran production in fermented doughs

It was observed that *Le. citreum* S4-5, *Lb. brevis* S2-5, *Le. lactis* I15, and *Le. gasicomitatum* L27 produced the highest levels of dextran (0.16%) as shown in Table 2. In uninoculated control, the dextran concentration was 0.008% indicating that the fermentation of dough was mediated by non LAB strains. The production of dextran by the above LAB starters was in agreement with previous reports [4, 9]. Dextran produced during dough fermentation increases the viscosity, improve softness and, shelf life, and act as a prebiotic candidate in bread [17–19].

Change in total acidity during fermentation of doughs

The final pH of LAB starters decreased to 3.5–3.9, and the total titratable acidity (TTA) values were 0.68–0.99% in all samples after fermentation as shown in Fig. 1(A, B). No

Table 2 Summary of sourdough fermentation profiles by seven screened LAB starters

	Log (CFU/g) dough	Maximum expansion ratio (%)	Dextran (% of DW)	TTA (%)	FQ (fermentation quotient)
Control	ND	83.3	0.008	0.45	0.9
<i>Le. mesenteroides</i> S5-9	8.0	40.0	0.13	0.98	1.1
<i>Le. citreum</i> S4-5	8.5	40.0	0.16	0.85	1.0
<i>Lb. brevis</i> S2-5	9.0	46.6	0.16	0.68	1.5
<i>W. confusa</i> I20	8.5	0.0	0.8	0.99	9.0
<i>Le. lactis</i> I15	9.3	46.6	0.16	0.81	1.0
<i>Lb. curvatus</i> I7	8.5	0.0	0.13	0.71	10.6
<i>Le. gasicomitatum</i> L27	8.1	40.0	0.16	0.91	1.0

significant difference in pH changes were found between sourdough samples. The TTA values of *Le. citreum* S4-5, *Lb. brevis* S2-5, and *Le. lactis* I15 (0.85, 0.68, and 0.81) were relatively lower than other screened LAB as shown in Table 2, and lower acidity of breads are preferred by baking industry as per general consumers. Jayaram et al. [20] reported that differences in pH and TTA values might be related to the ability of LAB to produce different levels of organic acids. Decrease in the pH of sourdough inhibits the activity of endo-amylase and prevent starch hydrolysis during baking, thus enhancing the quality of bread [21]. Reed and Nagodawithana [22] reported that pH is an important parameter for controlling the growth of pathogenic bacteria and preventing microbial contamination. Typically, $\text{pH} \leq 5$ prevents the growth of both undesirable microorganisms and out-competing yeast.

Fig. 1 pH (A) and TTA (B) profiles of seven doughs fermented with seven screened LAB starters along with control. *Le. mesenteroides* S5-9 (black circle), *Le. citreum* S4-5 (white circle), *Le. gasicomitatum* L27 (black square), *Le. lactis* I15 (white diamond), *Lb. curvatus* I7 (triangle), *Lb. brevis* S2-5 (black diamond), *W. confusa* I20 (black down triangle), and control (white square)

Organic acid content in fermented sourdough

The amounts and the ratio of lactic to acetic acid were significantly different among sourdough samples as shown in Fig. 2. The ratio of lactate to acetate is maintained optimum in hetero-fermentative LAB, while lactate is the major end product of fermentation by homo-fermentative LAB [9]. Thus the acetate produced by hetero-fermentative LAB enhances the flavor, aroma, and microbial shelf life [23]. The fermentation quotient (FQ) or the molar ratio of lactate to acetate for sourdoughs should be in the range of 2.0–2.7 [24, 25]. FQ has a considerable effect on the sensory characteristics and shelf life of breads [23]. Accordingly, the molar ratios of lactate to acetate were determined for the seven sourdoughs. The FQ profiles of *Le. citreum* S4-5, *Lb. brevis* S2-5, and *Le. lactis* I15 were 1.0, 1.5, and 1.0, respectively as shown in Table 2. For *Lb. curvatus* I7 and *W. confusa* I20, the FQ values were 9.0 and 10.6, which were fivefold higher than the optimum range and cannot be used for dough fermentation. Thus the above three screened LAB starter candidates with FQ values close the optimum range indicated their hetero fermentative nature and can be considered for the production of sourdough.

16S rRNA gene sequencing of selected starters

Based on the above analyses, *Le. citreum* S4-5, *Lb. brevis* S2-5, and *Le. lactis* I15 were selected for 16S rRNA gene sequencing analysis. The respective strains were deposited in Korean Agricultural Culture Collection Center (KACC). The sequences were compared to the respective type strains in the Ribosome database project at Michigan State

Fig. 2 Organic acid profiles of seven doughs fermented with seven screened LAB starters along with control

University. *Le. lactis* EFEL005 (KACC91922P) showed 99.8% homology with the *Le. lactis* JCM6123 strain, while *Lb. brevis* EFEL004 (KACC91921P) and *Le. citreum* EFEL006 (KACC91923P) showed 97% and 99% homology with *Lb. brevis* ATCC14869 and *Le. citreum* ATCC49370, KCTC3526, and AF111948 strains, respectively.

In conclusion, this study demonstrates the isolation and characterization steps of LAB strains from *Jeung-pyun* to be used as starters for sourdough fermentation. Among the LAB screened on wheat peptone medium, five were selected based on EPS production and were further studied on wheat dough. Finally, *Le. lactis*, *Lb. brevis*, and *Le. citreum* were obtained with their good fermentation properties (growth, dextran production, pH, and TTA). The results suggest that the isolates can be used as potential starters for sourdough fermentation.

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Compliance with ethical standards

Conflict of interest The author, Sangmin Shim, belongs to SPC group.

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