J. Microbiol. Biotechnol. (2019), 29(11), 1729–1738
https://doi.org/10.4014/jmb.1907.07039

jmb

Suitability of *Lactobacillus plantarum* SPC-SNU 72-2 as a Probiotic Starter for Sourdough Fermentation

Da Min Park[1], Jae-Han Bae[1], Min Soo Kim[1], Hyeontae Kim[1], Shin Dal Kang[2], Sangmin Shim[2], Deukbuhm Lee[2], Jin-Ho Seo[3], Hee Kang[4], and Nam Soo Han[1]*

[1]*Brain Korea 21 Center for Bio-Resource Development, Division of Animal, Horticultural, and Food Sciences, Chungbuk National University, Cheongju 28644, Republic of Korea*
[2]*Research Institute of Food and Biotechnology, SPC Group, Seoul 08826, Republic of Korea*
[3]*Department of Agricultural Biotechnology, Center for Food and Bioconvergence, Seoul National University, Seoul 08826, Republic of Korea*
[4]*Humanitas College, Kyung Hee University, Yongin 17104, Republic of Korea*

Received: July 17, 2019
Revised: October 7, 2019
Accepted: October 11, 2019

First published online:
October 13, 2019

**Corresponding author*
Phone: +82-43-261-2567
Fax: +82-43-271-4412
E-mail: namsoo@cbnu.ac.kr

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872

In sourdough fermentation, lactic acid bacteria perform important roles in the production of volatile and antimicrobial compounds, and exerting health-promoting effects. In this study, we report the probiotic properties and baking characteristics of *Lactobacillus plantarum* SPC-SNU 72-2 isolated from kimchi. This strain is safe to use in food fermentation as it does not carry genes for biogenic amine production (*i.e.*, *hdc*, *tdc*, and *ldc*) and shows no β-hemolytic activity against red blood cells. The strain is also stable under simulated human gastrointestinal conditions, showing tolerance to gastric acid and bile salt, and adheres well to colonic epithelial cells. Additionally, this strain prevents pathogen growth and activates mouse peritoneal macrophages by inducing cytokines such as tumor necrosis factor-α, interleukin (IL)-6, and IL-12. Furthermore, the strain possesses good baking properties, providing rich aroma during dough fermentation and contributing to the enhancement of bread texture. Taken together, *L. plantarum* SPC-SNU 72-2 has the properties of a good starter strain based on the observation that it improves bread flavor and texture while also providing probiotic effects comparable with commercial strains.

Keywords: *Lactobacillus plantarum*, sourdough, starter, probiotics, baking

Introduction

Sourdough is made by mixing flour, water, and salt with a starter culture of fermenting lactic acid bacteria (LAB) and yeasts. Sourdough fermentation improves the sensory characteristics of baked bread, such as volume, texture, and flavor, while also improving its nutritional value [1]. In addition, sourdough extends the shelf-life of breads by preventing staling and microbial growth; thus, it reduces the requirement of chemical preservatives in breadmaking [2, 3]. However, it is difficult to guarantee bread quality in terms of reproducibility because the microbiota in naturally fermented sourdough is influenced by a number of factors including raw materials and incubation environment [4]. Therefore, researchers are continually working to develop sourdough starters to bake breads with even better flavors and textures than those of bread made with doughs fermented with only baker's yeast [5].

Sourdough bread making involves the use of LAB for acidification and flavor production in combination with yeasts for leavening and also to add flavor [6]. LAB produce organic acids that inhibit the action of endogenous amylase, retard bread staling during storage, protect the bread from bacterial and fungal spoilage, and enhance the gas-holding and water-binding abilities of the dough [7, 8]. LAB often produce exopolysaccharides that improve the rheological properties of bread by increasing volume and loftiness and decreasing firmness [9, 10]. Different types of LAB contribute to bread characteristics in different ways. For instance, proteolytic LAB contribute to bread flavor

and the production of volatile compounds [11]; antimicrobial compound-producing LAB contribute to shelf-life extension [12], and volatile compound-producing LAB provide better flavor [13].

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [14]. Probiotics are granted the "generally recognized as safe" status (GRAS) [15] and are known to offer a variety of health benefits including prevention of pathogen growth, immunomodulation, anticancer effects, relief from lactose intolerance symptoms, reduction of serum cholesterol levels, prevention and alleviation of diarrhea, and mucosal preservation [16, 17]. Owing to these health-promoting effects, probiotics have received much attention to date for their application in food fermentation. However, there is a significant issue with developing a probiotic starter for sourdough fermentation: the probiotics become inactivated during the baking process. Recent studies have reported that dead or inactivated probiotics maintained their beneficial effects on the host in terms of immunomodulatory activity [18–20]. This prompted us to pursue the development of a probiotic starter for sourdough fermentation that would ensure health benefits and high quality of the baked products. In our laboratory, we have established a plant-adapted LAB library after isolating various LAB from traditionally fermented foods such as jeung-pyun (a rice sourdough cake) [21, 22], makgeolli (a rice wine) [23, 24], nuruk (solid wheat preculture for alcohol fermentation), sauerkraut [25], and kimchi [26, 27]. To examine various LAB as potential sourdough starters, we measured the growth rates and gas productivity of plant-adapted LAB (25 strains) in wheat dough. We then analyzed their probiotic characteristics, such as the presence of biogenic amine genes, and their hemolytic activity, tolerance for acid and bile salt, adherence to epithelial cells, and immunostimulatory activity in mouse peritoneal macrophages. Among them, *Lactobacillus plantarum* SPC-SNU 72-2, isolated from kimchi, was selected as a candidate starter, as it showed superior properties for sourdough fermentation, and the heat-killed form was able to stimulate macrophages. In this study, we report the probiotic characteristics of *L. plantarum* SPC-SNU 72-2 and its potential as a starter culture for sourdough fermentation.

Materials and Methods

Microorganisms and Culture Conditions

L. plantarum SPC-SNU 72-2 was isolated from kimchi and deposited in the Korean Collection for Type Cultures (KCTC, Korea, accession no., KCTC13315BP). *L. rhamnosus* GG was obtained from the KCTC (strain number KCTC 5033), where the strain has been sub-deposited from the American Type Culture Collection (ATCC) with the strain number ATCC 53103. *L. brevis* KCTC 12777BP, *L. curvatus* KCTC 12778BP, and *L. sanfranciscensis* KCTC 12779BP were also obtained from KCTC. A type strain *L. plantarum* KACC11451 was obtained from KACC (Korean Agricultural Culture Collection). The commercial sourdough starter strain *L. plantarum* L73 (Flora Pan) was obtained from Lallemand (Saint-Simon, France). *L. reuteri* ATCC23272, *Enterococcus faecium* ATCC19434, *Enterobacter cloacae* ATCC23373, and all the pathogen strains including *Escherichia coli* O157 ATCC11775, *Listeria monocytogenes* ATCC19115, *Salmonella* Typhimurium ATCC14028, and *Helicobacter pylori* ATCC 700392 were obtained from ATCC. The LAB strains were cultured in MRS broth (Difco, USA) at 30°C, while *E. faecium*, *Enterobacter cloacae*, *E. coli*, *L. monocytogenes*, and *S.* Typhimurium were cultured aerobically at 37°C using brain heart infusion (BHI) broth (Oxoid Ltd., UK). *H. pylori* were cultivated in BHI broth supplemented with 5% inactivated fetal horse serum (HyClone, USA). For dough fermentation, dried yeast (*Saccharomyces cerevisiae* KCTC 12776BP) isolated from nuruk, a traditional Korean fermentation starter, was used.

Microbial Characteristics

The growth rates of microorganisms in Lactobacilli MRS medium were measured by optical density at 600 nm using a spectrophotometer. The biochemical characteristics of microorganisms were analyzed using the API Kit CHL (BioMériux Co., France) according to the manufacturer's instruction. The analysis results of *L. plantarum* SPC-SNU 72-2 were compared with those of a type strain, *L. plantarum* KACC 11451, and a commercial sourdough starter strain, *L. plantarum* L73.

Evaluation of Probiotic Properties

Identification of biogenic amine genes. To verify the safety of *L. plantarum* SPC-SNU 72-2, we used a multiplex PCR method to detect each gene related to biogenic amine production and the 16S rRNA gene in the genomic DNA. The *hdc* (histidine decarboxylase), *tyrdc* (tyrosine decarboxylase), and *ldc* (lysine decarboxylase) genes were amplified using the following primer pairs: *hdc*: HDC3 (5′-GATGGTATTGTTTCKTATGA-3′) and HDC4 (5′-CAAACACCA GCATCTTC-3′) [28]; *tyrdc*: TD2 (5′- ACATAGTCAACCATRTTGAA-3′) and TD5 (5′-CAAATGGAAGAAGAAGTAGG-3′) [28]; *ldc*: LDCf (5′-TATGGGATCGCAACTCGGC-3′) and LDCr (5′-AAGATAGCG CAACGCGTCG-3′) [29]; 16S rRNA gene: 27F (5′-AGAGTTTGA TCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). In the multiplex PCR, each biogenic amine gene was amplified simultaneously with 16S rRNA gene in a PCR tube by adding each corresponding primer set. The 16S rRNA gene was used as positive control for PCR reaction and template condition. Genomic DNA of *L. plantarum* 72-2 was used as the template in the PCR reaction. The amplification program was as follows: 95°C for 5 min, followed by 32 cycles of 95°C for 45 sec, 58°C for 45 sec,

and 72°C for 75 sec with a final extension at 72°C for 5 min. The genomic DNAs of *L. reuteri* ATCC23272, *E. facieum* ATCC19434, and *E. cloacae* ATCC23373 were used as positive controls for *hdc*, *tyrdc*, and *ldc* genes, respectively.

Hemolytic activity assay. To verify the safety of *L. plantarum* 72-2, bacterial cells were inoculated into BHI medium supplemented with 7% horse blood (MB CELL, Korea) by referring to Ryu and Chang's method [30]. After an anaerobic incubation at 37°C for 24 h, hemolysis was observed on the medium. *Listeria monocytogenes* was used as a positive control for hemolytic activity.

Acid and bile salt tolerance assay. Resistance to acidic conditions was studied by the method proposed by Conway *et al*. [31] with slight modifications. LAB were cultured in MRS medium overnight and harvested by centrifugation at 6,000 ×*g* for 10 min. Cells were washed three times with phosphate-buffered saline (PBS; pH 7.2) and resuspended in an equal volume of PBS adjusted to pH 3.0 with HCl. Acid tolerance was evaluated by spreading cells on MRS agar medium after 0, 90, and 180 min incubation at 37°C and viable cell counting after incubation at 30°C for 48 h [32]. Tolerance to bile salts was evaluated by suspending cells in PBS solution containing 0.4 % (w/v) bile salt (Sigma, USA) and incubating at 37°C [33]. Samples were then taken at 0, 90, and 180 min and spread onto MRS agar medium. The colonies were counted after incubation at 30°C for more than 48 h and the survival rate was calculated.

Analysis of adhesion to epithelial cells. The adhesion assay was performed as described by Messaoudi *et al*. [34]. Caco-2 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, HycloneTM, USA) supplemented with 10% fetal bovine serum (FBS; HycloneTM), and 1% each of 10,000 U/ml penicillin and 10 mg/ml streptomycin in 0.85% NaCl. Caco-2 cells were seeded in 24-well tissue culture plates (2 cm^2 per well) at a density of approximately 4.7 × 10^5 cells per well. Once the culture reached confluency, the medium was changed to one without antibiotics. After cultivation of *L. plantarum* 72-2 in MRS medium, cells were recovered by centrifugation, washed twice with PBS (pH 7.4), and resuspended in DMEM without serum and antibiotics to a concentration of 10^8 CFU/ml. The cells were then applied to a Caco-2 cell monolayer in each well and incubated at 37°C in 5% CO_2 for 2 h. After incubation, non-adherent bacteria were removed by washing twice with PBS. Cells with adhered bacteria were treated with detachment solution including 0.1% Triton X-100 and 0.1% trypsin-EDTA (Sigma) for 15 min. To calculate the number of adherent bacteria, the suspensions of the detached cells were plated onto MRS agar after being appropriately diluted, and then incubated at 30°C for 48 h.

Antimicrobial activity assay. The inhibitory activity of *L. plantarum* 72-2 cells on pathogenic bacteria was assayed by the disc diffusion method using three cell fractions. The first fraction was a cell-free supernatant (CFS), which was a medium fraction that was centrifuged to remove cells and 5-fold concentrated using a rotary evaporator. The second fraction was a neutralized supernatant (NS), which was CFS adjusted to pH 7.0 with 5 N NaOH. Lastly, whole cells (WC) were prepared by separating cells from culture by centrifugation, washing with the same volume of PBS, and resuspending the cells in PBS. Each fraction (50 µl) was loaded onto a paper disc placed on the surface of MRS agar medium which was then overlaid with 0.7% (w/v) MRS agar medium inoculated with each indicator strain. These fractions were incubated at 37°C for 24 h and their antimicrobial activities were determined by the size of the inhibition zones surrounding the paper disc.

Immunostimulatory activity assay. To prepare the dead cells of *L. plantarum* 72-2, the strain was cultured overnight in MRS medium, recovered by centrifugation, and boiled for 5 min in a PBS suspension. After freeze-drying, the powder was collected and appropriately diluted with distilled water. To evaluate the cytokine-inducing ability of the LAB, we used mouse peritoneal macrophages from seven-week-old male Balb/c mice (Koatech, Korea). Before isolation of peritoneal macrophages, mice were preserved in a temperature- and humidity-controlled pathogen-free animal facility with a 12-h light-dark cycle. The animal protocol (KHUASP(SE)-15-002) was approved by the Kyung Hee University Institutional Animal Care and Use Committee. Mouse peritoneal macrophages were prepared according to the method reported by Kang *et al*. [27]. In brief, 2 ml of 3.5% sterile thioglycolate medium (BD Biosciences, USA) was injected intraperitoneally into healthy Balb/c mice. Four days later, peritoneal cells were collected through peritoneal lavage with DMEM. After centrifugation at 1,000 ×*g* for 10 min, the cells were suspended in DMEM containing 10% FBS and 1% penicillin-streptomycin and incubated overnight in a humidified atmosphere of 5% CO_2 at 37°C. Non-adherent cells were removed. Macrophages at 5 × 10^5 cells/mL were stimulated with dead lactic acid bacteria at 4, 20, or 100 µg/ml for 24 h, and the supernatant was collected for the cytokine release assay. The cytokine content was measured by enzyme-linked immunosorbent assay (ELISA). TNF-α, IL-6 Duoset (R&D Systems, USA), and IL-12 OptEIA set (BD Biosciences) were used. The ELISA was performed according to the manufacturer's protocol.

Analysis of Sourdough Fermentation

Analysis of maltose fermentability of the lactic acid bacteria. MRS broth medium containing 2% maltose was prepared, and then 10% (v/v) of the preculture of *L. plantarum* 72-2, *L. plantarum* KACC 11451, and *L. plantarum* L73 were inoculated. Cell growth was observed by measuring absorbance at 600 nm for 10 h.

Sourdough fermentation and breadmaking. The 72-2 strain, the type strain KACC11451, and the commercial strain L73 were used to ferment sourdough and white pan bread was made by the sponge and dough method. The type strain KACC11451 is the taxonomically representative strain of *L. plantarum*, and the commercial strain L73 is a strain used worldwide as a sourdough starter. Ingredients constituting sponge dough, as shown in Supplementary Table S1, were mixed and fermented for 4 h to prepare the sponge dough. Ingredients for dough including the sourdough and sponge dough were mixed and fermented at 38°C

in 85% humidity for 100 min. The dough after the second fermentation process was baked for 37 min in a technical oven at 210°C in the upper part, and 250°C in the bottom part, and cooled down at room temperature to reach 32°C internal temperature.

Analysis of the physicochemical properties of breads. The physicochemical properties (pH, total titratable acidity, and chromaticity) of the bread made of dough without LAB inoculation (control bread), the breads made of sourdoughs fermented by the type strain KACC11451 (KACC11451-bread), the commercial strain L73 (L73-bread), and the isolate 72-2 strain (72-2-bread) were measured. Measurement of the specific volume of bread was conducted using a benchtop laser-based scanner (Volscan Profiler 600, Stable Micro Systems, Godalming, UK). The specific volume was calculated as ml/g. Measurement of the moisture content was performed using an infrared moisture analyzer (FD-720, Kett Electric Laboratory, Japan).

Analysis of volatile compounds in breads. Volatile compounds were analyzed using a GC/MS system (Agilent 7890A/5975C, Agilent Technologies, USA) equipped with a DB-WAX column (Agilent) to compare aromatic metabolites in the breads made using the tested microorganisms. The samples were prepared with 1 g of each bread. Helium was used as the carrier gas and the flow rate was 1 ml/min. GC/MS analysis conditions were as follows: holding at 40°C for 5 min, increasing temperature at a rate of 8°C/min up to 230°C, and holding at 230°C for 10 min. After GC/MS analysis, the overall quantitative values of alcohols, aldehydes, ketones, esters, and acids were compared using MSD Chemstation software (Agilent).

Statistical Analysis

Each experiment was conducted in duplicate or triplicate as indicated, and the data were presented as the mean value ± standard deviation. Statistical analysis was performed to determine significant difference ($p < 0.01$ or 0.05) using ANOVA in SPSS software.

Results

Biochemical Characteristics of *L. plantarum* 72-2

L. plantarum 72-2 was analyzed with the API Kit CHL to characterize its biochemical properties with reference to the type strain and the commercial strain. The 72-2 strain showed differences in sugar availability (Table S2), being incapable of utilizing L-arabinose and D-turanose, whereas the type strain and the commercial strain could utilize these sugars. In addition, all the strains tested utilized D-maltose, which is the dominant sugar in wheat flour.

Probiotic Properties

Biogenic amine genes and hemolytic activity. Previous reports have indicated that some LAB in fermented foods produce harmful biogenic amines that can lead to negative health effects such as food poisoning. To evaluate the safety of the 72-2 strain, the presence of genes related to biogenic amine production were examined by PCR. As shown in Fig. 1A, the 72-2 strain does not possess the *hdc*, *tyrdc*, and *ldc* genes, which produce tyramine, histamine, and cadaverine, respectively. Meanwhile, the PCR products for those genes were detected in the positive control strains, *L. reuteri* ATCC23272, *E. faecium* ATCC19434, and *E. cloacae* ATCC23373.

Another important property to consider when looking for LAB suitable for food production is the absence of hemolytic activity of the microorganisms. To assess the hemolytic activity of the 72-2 strain, we inoculated the cells on horse blood agar medium and hemolysis was

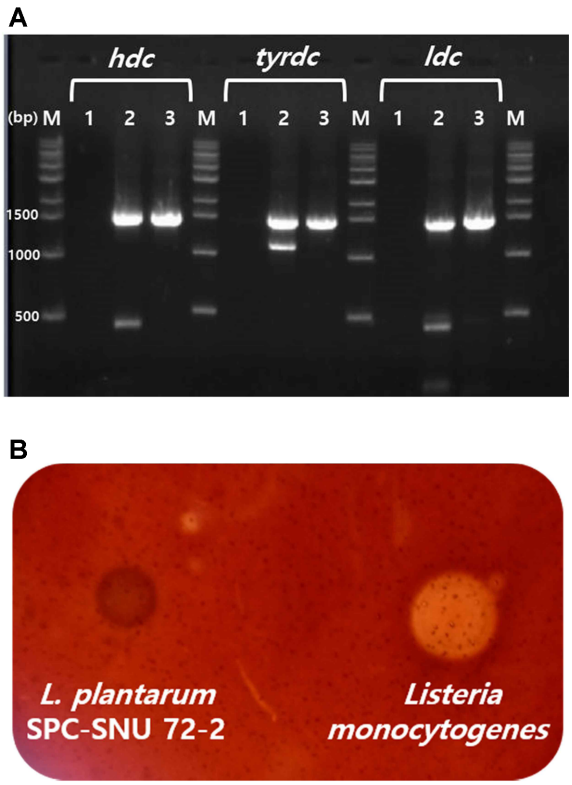

Fig. 1. Safety evaluation of *Lactobacillus plantarum* SPC-SNU 72-2.

(**A**) Identification of genes related to biogenic amine production. Lane M, 1 kb DNA marker; lane 1, negative control which has no template DNA; lane 2, positive controls having *hdc* (histidine decarboxylase, 440 bp), *tyrdc* (tyrosine decarboxylase, 1,100 bp), and *ldc* (lysine decarboxylase, 422 bp) genes from *L. reuteri* ATCC23272, *Enterococcus faecium* ATCC19434, and *Enterobacter cloacae* ATCC23373, respectively. The DNA of 16S-rRNA gene (1,530 bp) was also amplified. Lane 3, the 72-2 strain. (**B**) Hemolytic activity analysis of *L. plantarum* SPC-SNU 72-2. Left, the 72-2 strain; right, *Listeria monocytogenes* showing a clear zone around the cell drop.

investigated after incubation at 37°C for 24 h. The 72-2 strain did not show any clear zones around the cell drop, while *L. monocytogenes* revealed a clear zone that could be interpreted as hemolytic activity (Fig. 1B). Therefore, the 72-2 strain was confirmed to be non-hemolytic.

Acid and bile salt tolerance. The 72-2 strain was incubated in an acid (pH3.0) buffer solution for 0, 90, and 180 min, and the number of viable cells was counted. Fig. 2A shows that the 72-2 strain could tolerate the acidic condition and survived even after 90 or 180 min, just like the control probiotic strain, *L. rhamnosus* GG (LGG). For analysis of bile tolerance, viable cells were counted after 0, 90, and 180 min of incubation in 0.4% bile salts (Fig. 2B). The viability of both the LGG and 72-2 strains decreased significantly to a similar degree after a 90 min incubation with bile salts. However, LGG did not show any viable cells after a 180 min incubation, while the viability of the 72-2 strain was maintained from 90 min to 180 min. This result showed the higher viability of the 72-2 strain as compared to the LGG strain.

Adhesion to Caco-2 cells. The ability of 72-2 cells to adhere to Caco-2 cells was measured by incubating the 72-2 strain on a Caco-2 cell monolayer for 2 h. As shown in Fig. 3, approximately 800 adherents of the 72-2 strain per 100 cells of Caco-2 were observed. The 72-2 strain showed significantly higher adhesion ability to colonic epithelial cells than LGG as well as other lactobacilli used as controls.

Antimicrobial activity. Inhibitory activity of the 72-2 strain against representative pathogens was determined by the disc diffusion method using three fractions of cells: cell-free supernatant (CFS), neutralized supernatant (NS), and whole cells (WC) (Table 1). The CFS fraction of the 72-2 strain showed inhibitory activity against all pathogens tested, *E. coli* O157, *L. monocytogenes*, *S.* Typhimurium, and *H. pylori*. However, the NS fraction did not show any inhibitory activity against the tested strains and this result indicated that the 72-2 strain produced pH-dependent antimicrobial compounds such as organic acids.

Immunostimulatory activity. Table 2 shows the ability of the heat-killed cells of 72-2 strain to stimulate the secretion of immune cytokines by mice peritoneal macrophages. LGG strain was used as a reference lactic acid

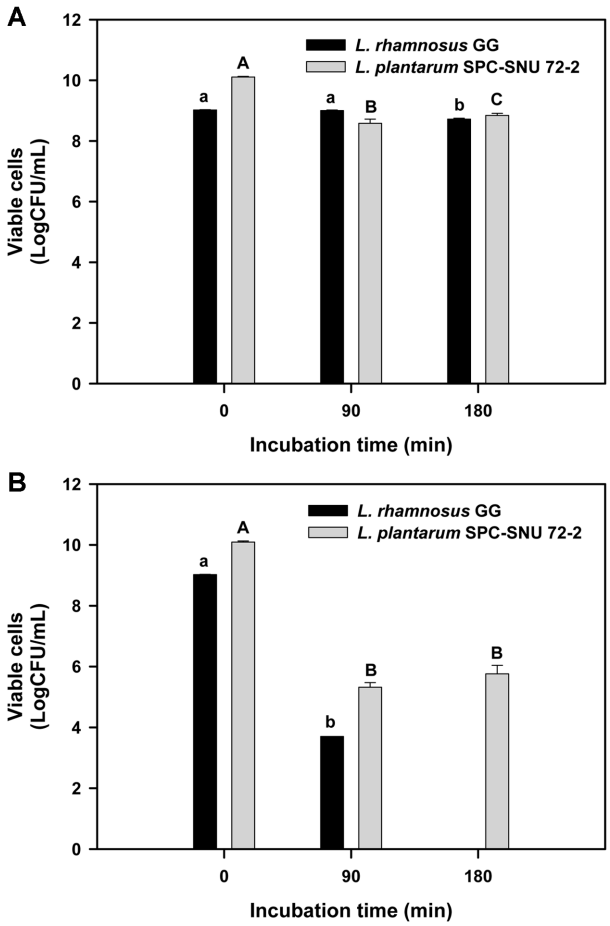

Fig. 2. Viability of *Lactobacillus plantarum* SPC-SNU 72-2 in the gastrointestinal tract.
(**A**) Acidic condition (pH 3.0), (**B**) 0.4% bile salt condition. Error bars represent the standard deviations, and different lowercase and uppercase letters on the error bars indicate significant differences in *L. rhamnosus* GG and *L. plantarum* SPC-SNU 72-2, respectively ($p < 0.05$).

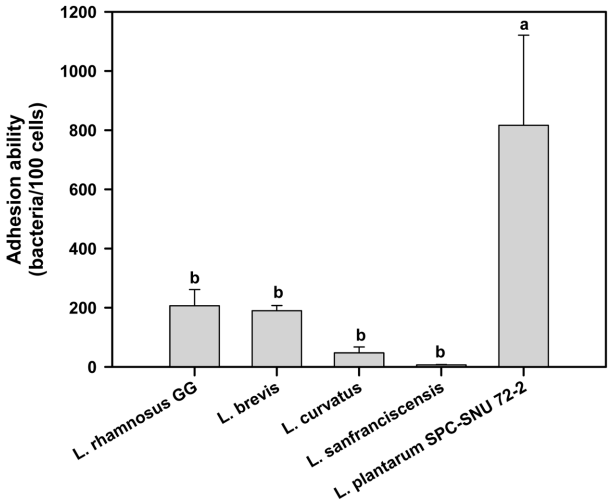

Fig. 3. Intestinal adhesion ability of *Lactobacillus plantarum* SPC-SNU 72-2.
Intestinal adhesion ability of the strains were determined using Caco-2 cells as colonic epithelial cells. Bars are means ($n = 3$). Error bars represent the standard deviations, and different letters on the error bars indicate significant differences ($p < 0.05$).

Table 1. Antimicrobial activities of *Lactobacillus plantarum* SPC-SNU 72-2.

Pathogens	WC	CFS (cm)	NS
Escherichia coli O157:H7 ATCC 11775	ND	0.95 ± 0.05	ND
Listeria monocytogenes ATCC 19115	ND	1.15 ± 0.05	ND
Salmonella Typhimurium ATCC 14028	ND	1.00 ± 0.00	ND
Helicobacter pylori ATCC 700392	ND	2.60 ± 0.00	ND

The antimicrobial activities of each fraction were determined by the diameter size (cm) of the inhibition zones surrounding the paper disc (0.6 cm). WC, whole cells; CFS, cell free supernatant; NS, neutralized supernatant; ND, non-detected.

bacterium. While LGG strain induced TNF-α and IL-6 secretion in a dose-dependent manner, 72-2 strain induced cytokine secretion highly, even in the treatment of a low cell concentration. IL-12 induction was observed in macrophages treated with 4–20 μg/ml of 72-2 strain. Generally, heat-killed 72-2 cells showed significantly higher cytokine-inducing abilities than heat-killed LGG cells.

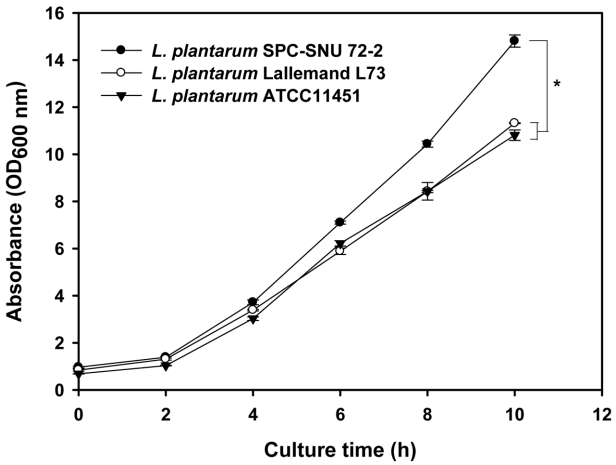

Fig. 4. Maltose fermentability of *Lactobacillus plantarum* SPC-SNU 72-2.

Cell growth was measured in MRS broth containing 2% maltose (*$p < 0.01$, $n = 3$).

Application of *L. plantarum* SPC-SNU 72-2 to Bread Making

Maltose fermentability of *L. plantarum* SPC-SNU 72-2 in MRS medium. Maltose is the most abundant sugar in wheat flour, so the consumption rate of this sugar is an important factor in selecting a sourdough starter. Therefore, each strain (*i.e.*, the 72-2 strain, the KACC11451 strain, and the L73 strain) was cultured in MRS broth containing 2% maltose and cell growth was measured at 600 nm. The 72-2 strain showed the highest growth rate among the tested strains in the medium (Fig. 4). This result reveals that the 72-2 strain is well adapted to the wheat dough environment.

Microbial and chemical characteristics of sourdough fermented with the tested strains. To prepare sourdough, flour, *L. plantarum* cultures, and water were mixed (Table S1) and fermented at 30°C until the pH reached 4.0 ± 0.2. After cooling, the number of LAB, the pH, and the acid content of the sourdough were measured (Table 3). All three strains grew to sizable populations (10^9 CFU/g) with no difference in population size being evident between the strains ($p < 0.05$). However, the lactic acid content produced in both 72-2-dough and L73-dough was significantly lower ($p < 0.05$) than that of the KACC11451-dough. This result shows that the 72-2 and L73 strains would be appropriate to produce bread with a mild acidic taste.

Physicochemical properties of breads baked using three sourdoughs. The sourdoughs fermented by each *L. plantarum* strain were used to make bread after secondary dough fermentation. After bread production, the physicochemical properties of the breads were analyzed (Table 4). The control bread was produced with dough in which only commercial yeast, *Saccharomyces cerevisiae* KCTC 12776BP, was applied as a starter. As a result, no differences were observed in the specific volume, texture, moisture content, or color value between all of the breads tested. However, a lower pH and higher titratable acidity were measured in the three LAB-breads as compared to the yeast-bread. Among LAB-breads, 72-2 and L73 strains resulted in higher pH values than the KACC11451 strain. Generally, the bread made by the 72-2 strain showed similar physicochemical properties as the bread made with the L73 stain.

Table 2. Cytokine induction ability of *Lactobacillus plantarum* SPC-SNU 72-2 (pg/ml).

Cytokines	*L. rhamnosus* GG (μg/ml)			*L. plantarum* SPC-SNU 72-2 (μg/ml)		
	4	20	100	4	20	100
TNF-α	0.00 ± 0.00^{c}*	807.33 ± 856.71^{c}	6,330.67 ± 1641.65^{b}	12,570.00 ± 4,077.91^{a}	16,620.00 ± 3,276.98^{a}	16,999.17 ± 2,017.51^{a}
IL-6	30.83 ± 51.26^{c}	13.33 ± 23.09^{c}	485.83 ± 104.83^{bc}	1,724.17 ± 777.82^{abc}	2,945.00 ± 1,290.47^{ab}	2,724.17 ± 1,831.23^{a}
IL-12	17.00 ± 29.44^{b}	25.44 ± 44.07^{b}	109.44 ± 62.21^{b}	3,918.00 ± 1318.75^{a}	3,965.50 ± 1,205.62^{a}	915.50 ± 449.01^{ab}

*Data are mean ± SD ($n = 3$). Different superscript letters indicate significant differences in same cytokine ($p < 0.05$).

Table 3. Microbial and chemical analysis of sourdough fermented by tested strains.

Types of dough	pH	Titratable acidity (ml)		Number of LAB (CFU/g)	Lactic acid (mM)	Acetic acid (mM)
		pH 6.6	pH 8.5			
72-2-dough	3.87 ± 0.01[a]	8.6 ± 0.21[a]	11.01 ± 0.16[a]	1.67 ± 0.05 × 10^{9}[a]	35.52 ± 1.57[a]	6.74 ± 1.53[a]
L73-dough	3.88 ± 0.02[a]	8.75 ± 0.1[a]	11.14 ± 0.13[a]	1.38 ± 0.59 × 10^{9}[a]	38.85 ± 3.14[a]	8.49 ± 2.36[a]
KACC11451-dough	3.84 ± 0.01[a]	9.35 ± 0.26[a]	11.7 ± 0.28[a]	1.54 ± 0.21 × 10^{9}[a]	83.81 ± 13.34[b]	12.57 ± 3.18[a]

Data are mean ± SD (n = 3). Different superscript letters indicate significant differences in a column ($p < 0.05$). Titratable acidity was measured on 15 g of sourdough.

Volatile compounds in the breads. Volatile compounds were analyzed using a GC/MS system to compare flavor characteristics of LAB-breads and the control bread. Table 5 shows that 1-hexanol, 2-octanone, and 2-pentylfuran were present in higher amounts ($p < 0.05$) in the bread made by the 72-2 strain than in the bread made by the L73 strain or the KACC11451 strain. Overall, the bread made with the 72-2 strain contained higher amounts of alcohols and ketones in comparison to the breads made with L73 and KACC11451 strains (Fig. S1).

Discussion

The beneficial effects of sourdough fermentation by LAB have been extensively studied to promote human health. Breads made by various sourdoughs resulted in a lower glycemic index [5], improved mineral bioavailability [35], and reduced gluten sensitivity [36]. Additionally, during sourdough fermentation, LAB often play important roles in increasing the levels of bioactive compounds such as antioxidant, anticancer, or anti-inflammatory peptides, γ-amino butyric acid, and phytochemicals in baked products [37–39]. In this study, we characterized a starter strain based on the observation that it improves bread flavor and texture while also playing a probiotic role like the commercial strain LGG. This study shows that the 72-2 strain could tolerate the acidic conditions and survived after 90 or 180 min as well as or better than the control. These results indicate the potential tolerance of 72-2 strain to the acid environment of the stomach [32]. Priyadarshani and Rakshit reported that two *Lactobacillus* spp., *L. casei* (TISTR 389) and *L. delbrueckii* subsp. *bulgaricus* (TISTR 895), out of fifteen LAB strains studied produced biogenic amines and the production was not species-dependent but strain-dependent [40]. Interestingly, many *L. plantarum* strains lack capability to produce biogenic amines, can degrade these compounds or even can reduce their production by other producers [41–44]. Clearly, the strain isolated in this study belongs to this majority, as it lacks the genes necessary for biogenic amine production. In addition, the 72-2 strain also exhibited significantly higher intestinal adhesion ability and cytokine release activity as compared to LGG (Fig. 3 and Table 2). Macrophages and dendritic cells, the efficient phagocytes that recognize the invading and colonizing microbes, play an important role in host defense by producing TNF-α, IL-6, and IL-12. The induction level of the cytokines was enhanced by treatment of probiotic strains such as *E. faecalis*, *Weissella cibaria*, *Bifidobacterium*

Table 4. Physicochemical properties of white pan breads.

Parameters tested		Control bread	72-2-bread	L73-bread	KACC11451-bread
Specific volume (ml/g)		4.88 ± 0.28[a]	4.85 ± 0.19[a]	4.85 ± 0.26[a]	4.87 ± 0.24[a]
Texture (N)		7.4 ± 0.31[a]	7.8 ± 0.38[a]	7.8 ± 0.41[a]	7.7 ± 0.35[a]
pH		5.53 ± 0.01[a]	5.41 ± 0.01[b]	5.35 ± 0.02[bc]	5.29 ± 0.01[c]
TTA (ml)		2.27 ± 0.11[b]	3.25 ± 0.14[a]	3.29 ± 0.16[a]	3.25 ± 0.13[a]
Moisture content (%)		41.65 ± 0.30[a]	41.51 ± 0.42[a]	41.49 ± 0.13[a]	41.54 ± 0.18[a]
Lactic acid (mM)		1.61 ± 0.08[b]	16.65 ± 1.26[a]	16.82 ± 1.02[a]	17.10 ± 1.73[a]
Acetic acid (mM)		0.037 ± 0.004[a]	6.08 ± 0.59[a]	7.16 ± 0.47[a]	6.74 ± 1.77[a]
Hunter lab color values	L^*	84.11 ± 0.81[a]	84.54 ± 0.92[a]	85.01 ± 0.9[a]	84.91 ± 0.78[a]
	a^*	-2.16 ± 0.23[a]	-2.35 ± 0.21[a]	-2.11 ± 0.23[a]	-2.19 ± 0.20[a]
	b^*	17.72 ± 0.35[a]	17.22 ± 0.37[a]	17.32 ± 0.30[a]	17.51 ± 0.23[a]

Data are mean ± SD (n = 3). Different superscript letters indicate significant differences in a row ($p < 0.05$). L^*, a^*, and b^* represent lightness, redness, and yellowness, respectively.

Table 5. Quantitative values of 19 volatiles from dough fermented with *Lactobacillus plantarum*.

Groups	Volatiles	72-2 strain	L73 strain	KACC11451 train
Alcohols	Ethyl alcohol	$1.46 \times 10^{6} \pm 1.81 \times 10^{5}$	$9.36 \times 10^{5} \pm 1.73 \times 10^{5}$	$1.07 \times 10^{6} \pm 3.70 \times 10^{5}$
	1-Pentanol	$1.55 \times 10^{7} \pm 1.55 \times 10^{6}$	$1.18 \times 10^{7} \pm 1.81 \times 10^{5}$	$1.68 \times 10^{7} \pm 3.00 \times 10^{6}$
	3-Methyl-1-butanol	$3.70 \times 10^{6} \pm 1.12 \times 10^{6}$	$3.29 \times 10^{6} \pm 1.73 \times 10^{5}$	$3.94 \times 10^{6} \pm 1.58 \times 10^{6}$
	1-Hexanol	$2.39 \times 10^{8} \pm 2.32 \times 10^{7a}$	$1.01 \times 10^{8} \pm 3.45 \times 10^{6b}$	$2.16 \times 10^{8} \pm 1.86 \times 10^{7a}$
	1-Heptanol	$1.24 \times 10^{7} \pm 1.74 \times 10^{6}$	$5.14 \times 10^{6} \pm 5.32 \times 10^{4}$	$8.35 \times 10^{6} \pm 4.25 \times 10^{6}$
	2-Ethylhexanol	$1.05 \times 10^{7} \pm 1.68 \times 10^{6}$	$8.11 \times 10^{6} \pm 5.90 \times 10^{5}$	$1.20 \times 10^{7} \pm 2.58 \times 10^{6}$
	1-Octanol	$8.89 \times 10^{6} \pm 7.49 \times 10^{5}$	$4.42 \times 10^{6} \pm 6.27 \times 10^{4}$	$6.13 \times 10^{6} \pm 2.93 \times 10^{6}$
	1-Nonanol	$7.99 \times 10^{6} \pm 7.13 \times 10^{5}$	$7.91 \times 10^{6} \pm 3.93 \times 10^{5}$	$6.55 \times 10^{6} \pm 3.29 \times 10^{6}$
Aldehydes	Hexanal	$6.28 \times 10^{7} \pm 4.61 \times 10^{6}$	$9.13 \times 10^{7} \pm 2.56 \times 10^{7}$	$1.02 \times 10^{8} \pm 1.49 \times 10^{7}$
	(E)-2-Heptenal	$2.36 \times 10^{7} \pm 8.91 \times 10^{5}$	$1.76 \times 10^{7} \pm 3.74 \times 10^{6}$	$1.99 \times 10^{7} \pm 0.00 \times 10^{0}$
	2-Octenal	$2.33 \times 10^{7} \pm 3.65 \times 10^{6}$	$1.76 \times 10^{7} \pm 1.97 \times 10^{6}$	$3.35 \times 10^{7} \pm 7.38 \times 10^{6}$
	Nonenal	$3.04 \times 10^{6} \pm 2.00 \times 10^{5}$	$2.63 \times 10^{6} \pm 1.30 \times 10^{6}$	$5.22 \times 10^{6} \pm 3.84 \times 10^{5}$
	Benzaldehyde	$2.30 \times 10^{6} \pm 4.33 \times 10^{3}$	$2.46 \times 10^{6} \pm 7.67 \times 10^{3}$	$2.52 \times 10^{6} \pm 1.02 \times 10^{6}$
Ketones	2-Heptanone	$3.01 \times 10^{6} \pm 2.03 \times 10^{6}$	$5.26 \times 10^{6} \pm 1.53 \times 10^{6}$	$2.16 \times 10^{6} \pm 9.38 \times 10^{5}$
	Acetoin	$3.57 \times 10^{6} \pm 1.66 \times 10^{6}$	$3.91 \times 10^{6} \pm 1.45 \times 10^{5}$	$2.53 \times 10^{6} \pm 8.50 \times 10^{5}$
	2-Octanone	$6.68 \times 10^{6} \pm 9.22 \times 10^{5a}$	$3.37 \times 10^{6} \pm 1.97 \times 10^{5ab}$	$2.09 \times 10^{6} \pm 1.03 \times 10^{6b}$
	Phenyl methyl ketone	$6.93 \times 10^{6} \pm 9.14 \times 10^{5}$	$4.92 \times 10^{6} \pm 4.50 \times 10^{5}$	$4.38 \times 10^{6} \pm 5.58 \times 10^{5}$
Acids	Hexanoic acid	$3.99 \times 10^{7} \pm 1.18 \times 10^{6}$	$3.29 \times 10^{7} \pm 8.21 \times 10^{6}$	$4.15 \times 10^{7} \pm 8.71 \times 10^{6}$
Misc.	2-Pentylfuran	$9.89 \times 10^{6} \pm 5.59 \times 10^{5a}$	$5.02 \times 10^{6} \pm 2.83 \times 10^{5b}$	$7.54 \times 10^{6} \pm 5.39 \times 10^{5c}$
Sum		4.85×10^{8}	3.29×10^{8}	4.95×10^{8}

Data are mean ± SD (n = 3). Different superscript letters indicate significant differences in a row ($p < 0.05$).

longum, *and B. breve* [45–48]. Particularly, it is reported that heat-killed cells of *E. faecalis*, *Leuconostoc citreum* EFEL2061, and *L. plantarum* KCTC 13314BP showed enhanced production of the cytokines in macrophages [27, 48, 49]. Therefore, the heat-killed cells of *L. plantarum* SPC-SNU 72-2 can be expected to maintain immunostimulatory effect after LAB inactivation during the baking process. To our knowledge, this is the first report in which LAB was applied as a starter strain to ferment sourdough and to provide health-promoting effect as a probiotic in a baked product.

In conclusion, in this study we evaluated the feasibility of using of *L. plantarum* SPC-SNU 72-2 (KCTC13315BP) as a sourdough starter to produce functional breads. For this, the probiotic properties and baking characteristics of 72-2 strain were investigated. The 72-2 strain showed probiotic characteristics such as safety for food use, high tolerance to gastrointestinal stress, and high adhesion to human epithelial cells. Heat-killed cells of 72-2 strain induced high levels of cytokines such as TNF-α, IL-6, and IL-12 from mouse peritoneal macrophages. Meanwhile, the 72-2 strain showed the fast growth rates in the maltose medium. The baking characteristics of the 72-2 strain were also similar to the commercial sourdough starter, Lallemand L73, and the use of this strain yielded better physicochemical results than the single use of yeast. The bread made with the 72-2 strain showed similar physicochemical properties as the bread made with the commercial L73 stain. Therefore, the 72-2 strain has great potential in producing functional breads with immunostimulating effect.

Acknowledgements

The present work was supported by the SPC Group and by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agricultural Microbiome R&D Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (918006-04-2-SB010).

Conflict of Interests

The authors declare no conflict of interest. Shin Dal Kang, Sangmin Shim, and Deukbuhm Lee are employed in the Research Institute of Food and Biotechnology, SPC Group that sponsored this research.

References

1. Katina K, Arendt E, Liukkonen KH, Autio K, Flander L, Poutanen K. 2005. Potential of sourdough for healthier cereal products. *Trends Food Sci. Technol*. **16:** 104-112.
2. Clarke CI, Arendt EK. 2005. A review of the application of sourdough technology to wheat breads, pp. 137-161. *In* Taylor S (eds.), *Advances in Food and Nutrition Research* v. 49, Elsevier, Amsterdam, Netherlands.
3. Mantzourani I, Plessas S, Odatzidou M, Alexopoulos A, Galanis A, Bezirtzoglou E, *et al*. 2019. Effect of a novel *Lactobacillus paracasei* starter on sourdough bread quality. *Food Chem.* **271:** 259-265.
4. Carnevali P, Ciati R, Leporati A, Paese M. 2007. Liquid sourdough fermentation: industrial application perspectives. *Food Microbiol.* **24:** 150-154.
5. Nionelli L, Montemurro M, Pontonio E, Verni M, Gobbetti M, Rizzello CG. 2018. Pro-technological and functional characterization of lactic acid bacteria to be used as starters for hemp (*Cannabis sativa* L.) sourdough fermentation and wheat bread fortification. *Int. J. Food Microbiol.* **279:** 14-25.
6. De Vuyst L, Vancanneyt M. 2007. Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiol.* **24:** 120-127.
7. Gerez CL, Torino MI, Roll G, Devaldez FG. 2009. Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. *Food Control* **20:** 144-148.
8. Loponen J, Kanerva PI, Zhang C, Sontag-Strohm T, Salovaara H, Ganzle MG. 2009. Prolamin hydrolysis and pentosan solubilization in germinated-rye sourdoughs determined by chromatographic and immunological methods. *J. Agric. Food Chem.* **57:** 746-753.
9. Arendt EK, Moroni A, Zannini E. 2011. Medical nutrition therapy: use of sourdough lactic acid bacteria as a cell factory for delivering functional biomolecules and food ingredients in gluten free bread. *Microb. Cell Fact.* **10(Suppl1):** S15.
10. Di Monaco R, Torrieri E, Pepe O, Masi P, Cavella S. 2015. Effect of sourdough with exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) on sensory quality of bread during shelf life. *Food Bioproc. Tech.* **8:** 691-701.
11. Nionelli L, Rizzello CG. 2016. Sourdough-based biotechnologies for the production of gluten-free foods. *Foods* **5:** 65.
12. Jonkuvienė D, Vaičiulytė-Funk L, Šalomskienė J, Alenčikienė G, Mieželienė A. 2016. Potential of *Lactobacillus reuteri* from spontaneous sourdough as a starter additive for improving quality parameters of bread. *Food Technol. Biotechnol*. **54:** 342-350.
13. Petel C, Onno B, Prost C. 2017. Sourdough volatile compounds and their contribution to bread: a review. *Trends Food Sci. Technol.* **59:** 105e123.
14. FAO/WHO. 2002. Guidelines for the evaluation of probiotics in food. Available from http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf. Accessed Jully 2, 2019.
15. Figueroa-González I, Quijano G, Ramírez G, Cruz-Guerrero A. 2011. Probiotics and prebiotics – perspectives and challenges. *J. Sci. Food Agric*. **91:** 1341-1348.
16. Kumar R, Dhanda S. 2017. Mechanistic insight of probiotics derived anticancer pharmaceuticals: a road forward for cancer therapeutics. *Nutr. Cancer* **69:** 375-380.
17. Nazir Y, Hussain SA, Hamid AA, Song Y. 2018. Probiotics and their potential preventive and therapeutic role for cancer, high serum cholesterol, and allergic and HIV diseases. *BioMed Res. Int.* **3428437:** 17.
18. Piqué N, Berlanga M, Miñana-Galbis D. 2019. Health benefits of heat-killed (tyndallized) probiotics: an overview. *Int. J. Mol. Sci.* **20:** 2534.
19. Lee HA, Kim H, Lee KW, Park KY. 2016. Dead *Lactobacillus plantarum* stimulates and skews immune responses toward T helper 1 and 17 polarizations in RAW 264.7 sells and mouse splenocytes. *Int. J. Microbiol. Biotechnol.* **26:** 469-476.
20. Sarkar S. 2018. Whether viable and dead probiotic are equally efficacious? *Nutrition Food Sci.* **48:** 285-300.
21. Lim SB, Tingirikari JMR, Kwon YW, Li L, Kim GE, Han NS. 2017. Polyphasic microbial analysis of traditional Korean Jeung-Pyun sourdough fermented with Makgeolli. *J. Microbiol. Biotechnol.* **27:** 226-233.
22. Lim SB, Tingirikari JMR., Seo JS, Li L, Shim S, Seo JH, *et al.* 2018. Isolation of lactic acid bacteria starters from Jeung-pyun for sourdough fermentation. *Food Sci. Biotechnol*. **27:** 73-78.
23. Kim SY, Yoo KS, Kim JE, Kim JS, Jung JY, Jin Q, *et al.* 2010. Diversity analysis of lactic acid bacteria in Korean rice wines by culture-independent method using PCR-denaturing gradient gel electrophoresis. *Food Sci. Biotechnol*. **19:** 749-755.
24. Park J, Seo JS, Kim SA, Shin SY, Park JH, Han NS. 2017. Microbial diversity of commercial makgeolli and its influence on the organoleptic characteristics of Korean rice sourdough, Jeung-Pyun. *J. Microbiol. Biotechnol.* **27:** 1736-1743.
25. Kim SY, Yoo KS, Kim YJ, Seo EY, Kim BS, Han NS. 2011. Monitoring of *Leuconostoc* population during sauerkraut fermentation by quantitative real-time polymerase chain reaction. *J. Microbiol. Biotechnol.* **21:** 1069-1072.
26. Moon JS, Choi HS, Shin SY, Noh SJ, Jeon CO, Han NS. 2015. Genome sequence analysis of potential probiotic strain *Leuconostoc lactis* EFEL005 isolated from kimchi. *Journal of Microbiology* **53:** 337-342.
27. Kang H, Moon JS, Lee MG, Han NS. 2016. Immunomodulatory effects of *Leuconostoc citreum* EFEL2061 isolated from kimchi, a traditional Korean food, on the Th2 type-dominant immune response *in vitro* and *in vivo*. *J. Funct. Foods* **20:** 79-87.
28. O'Sullivan DJ, Fallico V, O'Sullivan O, McSweeney PLH, Sheehan JJ, Cotter PD, *et al.* 2015. High-throughput DNA sequencing to survey bacterial histidine and tyrosine decarboxylases in raw milk cheeses. *BMC Microbiol.* **15:** 266.

29. Noh SJ. 2017. Physiological characterization of *Leuconostoc lactis* EFEL005 as potential probiotics (master degree thesis). Chungbuk National University, Cheongju, Korea.
30. Ryu EH, Chang HC. 2013. *In vitro* study of potentially probiotic lactic acid bacteria strains isolated from kimchi. *Ann. Microbiol*. **63:** 1387-1395.
31. Conway PL, Gorbach SL, Goldin BR. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J. Dairy Sci*. **70:** 1-12.
32. Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E. 2006. Probiotic potential of *Lactobacillus* strains isolated from dairy products. *Int. Dairy J.* **16:** 189-199.
33. Gilliland SE, Staley TE, Bush LJ. 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct. *J. Dairy Sci*. **67:** 3045-3051.
34. Messaoudi S, Madi A, Prévost H, Feuilloley M, Manai M, Dousset X, *et al.* 2012. In vitro evaluation of the probiotic potential of *Lactobacillus salivarius* SMXD51. *Anaerobe* **18:** 584-589.
35. Cizeikiene D, Juodeikiene G, Bartkiene, E, Damasius J, Paskevicius A. 2015. Phytase activity of lactic acid bacteria and their impact on the solubility of minerals from wholemeal wheat bread. *Int. J. Food Sci. Nutr*. **66:** 736-742.
36. Di Cagno R, De Angelis M, Auricchio S, Greco L, Clarke C, De Vincenzi M, *et al*. 2004. Sourdough bread made from wheat and nontoxic flours and started with selected Lactobacilli is tolerated in celiac sprue patients. *J. Appl. Environ. Microbiol*. **70:** 1088-1096.
37. Coda R, Rizzello CG, Gobbetti M. 2010. Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a functional bread enriched of γ-aminobutyric acid (GABA). *Int. J. Food Microbiol*. **137:** 236-245.
38. Coda R, Rizzello CG, Pinto D, Gobbetti M. 2012. Selected lactic acid bacteria synthesize antioxidant peptides during sourdough fermentation of cereal flours. *Appl. Environ. Microbiol*. **78:** 1087-1096.
39. Gobbetti M, Rizzello CG, Di Cagno R, De Angelis M. 2014. How the sourdough may affect the functional features of leavened baked goods. *Food Microbiol.* **37:** 30-40.
40. Priyadarshani WMD, Rakshit SK. 2014. Growth and biogenic amine (histamine and tyramine) potential of probiotic *Lactobacillus asei* in skim milk. *Am. J. Food Technol.* **9:** 69-79.
41. Arena ME, Manca de Nadra MC. 2001. Biogenic amine production by *Lactobacillus*. *J. Appl. Microbiol.* **90:** 158-162.
42. Capozzi V, Russo P, Ladero V, Fernández M, Fiocco D, Alvarez MA, *et al*. 2012. Biogenic amines degradation by *Lactobacillus plantarum*: toward a potential application in wine. *Front. Microbiol.* **3:** 122.
43. Masson F, Talon R, Montel MC. 1996. Histamine and tyramine production by bacteria from meat products. *Int. J. Food Microbiol*. **32:** 199-207.
44. Tosukhowong A, Visessanguan W, Pumpuang L, Tepkasikul P, Panya A, Valyasevi R. 2011. Biogenic amine formation in Nham, a Thai fermented sausage, and the reduction by commercial starter culture, *Lactobacillus plantar*um BCC 9546. *Food Chem.* **129:** 846-853.
45. Kidd P. 2003. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern. Med. Rev.* **8:** 223-246.
46. Park HE, Um HB, Lee WK. 2018. The immune enhancing effects and characteristics of *Bifidobacterium longum* and *Bifidobacterium breve* for the probiotic use in humans and animals. *J. Biomed. Transl. Res.* **19:** 65-72.
47. Park HE, Kang KW, Kim BS, Lee SM, Lee WK. 2017. Immunomodulatory potential of *Weissella cibaria* in aged C57BL/6J mice. *J. Microbiol. Biotechnol.* **27:** 2094-2103.
48. Sparo M, Delpech G, Batisttell, S, Basualdo JA. 2014. Immunomodulatory properties of cell wall extract from *Enterococcus faecalis* CECT7121. *Braz. J. Infect. Dis.* **18:** 551-555.
49. Jeong MJ, Kim JH, Yang H, Kang SD, Song S, Lee D, *et al.* 2019. Heat-killed *Lactobacillus plantarum* KCTC 13314BP enhances phagocytic activity and immunomodulatory effects via activation of MAPK and STAT3 pathways. *J. Microbiol. Biotechnol.* **29:** 1248-1254.