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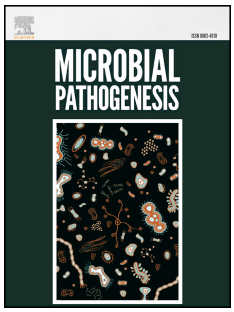

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ORIGINAL ARTICLE

Antioxidant and immunostimulatory effect of potential probiotic *Lactobacillus paraplantarum* SC61 isolated from Korean traditional fermented food, *jangajji*

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ABSTRACT

This study aimed to isolate and demonstrate their antioxidant and immunostimulatory activities of potential probiotics. The isolated strains, S. Pum19, SC28, and SC61 showed potential probiotic properties including stability in artificial gastric and bile conditions, non-production of β-glucuronidase, suitable antibiotic susceptibility, and attachment to intestinal cells. S. Pum19, SC28, and SC61 strains were identified as *Leuconostoc citreum*, *Pediococcus pentosaceus*, and *Lactobacillus paraplantarum*, respectively. Of the 3 potential probiotic LAB strains, intact cells of *L. paraplantarum* SC61 showed higher antioxidant activity, including DPPH radical scavenging, β-carotene bleaching inhibition, reducing power, superoxide anion scavenging, and ABTS radical scavenging activity. In addition, *L. paraplantarum* SC61 produced the most nitric oxide production and its mRNA expression level for iNOS, IL-1β, IL-6, and TNF-α were superior to those of *L. rhamnosus* GG. Therefore, *L. paraplantarum* SC61 was demonstrated to exhibit antioxidant and immunostimulatory activity and to have potential use as a probiotic product.

Keywords: *Lactobacillus*; Probiotic; Safety; Antioxidant activity; Immunostimulatory activity

1. Introduction

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts [1]. Probiotics must be able to survive in and colonize the gastrointestinal tract in order to improve the health of the host [2]. *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Bifidobacterium* species are common probiotic strains and are widely used as probiotics products in industry. Several studies have indicated that probiotics have shown various functional effects, such as antioxidant, anti-inflammatory [3], anti-biofilm, and antidiabetic activities [4].

Reactive oxygen metabolites (ROMs) are created through normal biological processes and can cause damage to proteins, mutations in DNA, oxidation of membrane phospholipids, and modification in low-density lipoproteins [5]. Excessive amounts of ROMs can result in cellular damage, leading to atherosclerosis, arthritis, diabetes, neurodegenerative diseases, cardiovascular diseases, and cancer [6]. The antioxidative properties, including radical scavenging, metal ion chelation, enzyme inhibition, among others, of several *Lactobacilli* and *Bifidobacteria* strains have been reported [7,8].

Immunity describes the state of having sufficient biological defenses to avoid disease, infection, or unwanted biological invasion. Immunity is the natural resistance that provides defense through several physical, chemical, and cellular approaches. Subsequent general defenses include secretion of cytokines and antimicrobial substances, fever, and phagocytic activity related to inflammatory responses. The intestinal microbiota could play a fundamental role in maintaining immune homeostasis [9]. Some probiotics have been shown to enhance proinflammatory cytokines, and *Lactobacillus acidophilus* La205 enhanced natural killer cells [4,10].

Jangajji (pickled vegetable) is a Korean side dish made from garlic, pepper leaves, cucumbers, radishes, and other vegetables using *doenjang*, *ganjang*, or *gochujang*. A study on lactic acid bacteria (LAB) isolated from *jangajji* reported the antimutagenicity of *Lactobacillus paracasei* subsp. *tolerans* JG22 [11]. The aim of this study was to isolate and identify strains which probiotic properties from various foods and to evaluate their antioxidant activity, and immune-enhancing activity.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Leuconostoc citreum S.Pum19, *Pediococcus pentosaceus* SC28, and *Lactobacillus paraplantarum* SC61 were isolated from pumpkin, octopus *jeotgal*, and burdock *jangajji*, respectively and identified by 16S rRNA sequencing. *Lactobacillus rhamnosus* GG, a reference probiotic strain, was obtained from the Korean Collection for Type Cultures (Jeolla-do, Korea). All LAB strains were isolated using lactobacilli MRS agar (BD Biosciences, Franklin Lakes, NJ, USA) and stored in MRS broth with 20% (v/v) glycerol (Sigma-Aldrich, St. Louis, MO, USA) at –80 °C.

2.2. Tolerance to gastric acid and bile salt

The gastric acid and bile salt tolerances of LAB strains were determined according to the method of Jeon *et al*. [12]. To evaluate gastric acid tolerance, LAB strains were incubated in 10 mL MRS broth at 37 °C for 18 h. This cultured medium was inoculated into MRS broth with 0.3% pepsin (Sigma-Aldrich), adjusted to pH 2.5 using 1 N HCl, and incubated at 37 °C

for 3 h. Diluted bacterial cell suspension was spread on MRS agar at 0 and 3 h, and bacterial cell concentration was determined as log CFU/mL.

To evaluate bile salt tolerance, LAB strains were grown in 10 mL MRS broth at 37 °C for 18 h. This cultured medium was inoculated into MRS broth added with 0.3% oxgall (BD), and incubated at 37 °C for 24 h. Diluted bacterial cell suspension was spread on MRS agar at 0 and 24 h, bacterial cell concentration was determined as log CFU/mL.

2.3. Enzyme activity and antibiotic susceptibility

The enzyme activity was determined using the API ZYM kit (BioMerieux, Lyons, France). Cultured bacterial cells were harvested by centrifugation at 8,000 × *g* for 10 min at 4 °C. Cell pellets were resuspended in phosphate-buffered saline (PBS; Invitrogen, Carlsbad, CA, USA). Sixty-five microliters of cell suspension (10^5 CFU/mL) were added to each cupule and incubated at 37 °C for 4 h. Enzyme activity of LAB strains was recorded on a scale of 0 to 5 according to color intensity.

One hundred microliters of LAB culture were plated on MRS agar, and commercial antibiotic paper discs (ampicillin (10 μg), gentamicin (10 μg), kanamycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), and doxycycline (30 μg)) were placed onto the MRS plates. After incubation at 37 °C for 24 h, the diameter (mm) of the inhibition zone was evaluated according to the guidelines of the Clinical and Laboratory Standards Institute [13].

2.4. Adhesion ability to intestinal cells

HT-29 cells (Korean Cell Line Bank, Seoul, Korea) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Hyclone, Logan, UT, USA) supplemented with 10%

(v/v) fetal bovine serum (FBS) (Hyclone) and 1% (v/v) penicillin/streptomycin (Hyclone). Overnight cultures of LAB strains were harvested by centrifugation at 8,000 × *g* at 4 °C for 10 min and resuspended in PBS. Then, 0.1 mL of bacterial suspension was added to HT-29 cells seeded at 1×10^5 cells/mL in 24-well tissue culture plates. After incubation at 37 °C for 2 h under 5% CO_2, non-adhesive bacteria were removed by washing with PBS 3 times, and adhesive bacteria were detached by 1% (v/v) Triton-X100 solution (Sigma-Aldrich). Adhesive bacteria were by the plate count method using MRS agar.

The adhesion ability of the LAB strains to HT-29 cells was calculated as the percentage of adhered bacterial cells ($N_{2\,h}$) divided by the initial number of bacterial cells ($N_{0\,h}$).

$$\text{Adhesion ability (\%)} = \frac{N_{2\,h}}{N_{0\,h}} \times 100$$

2.5. Cell surface hydrophobicity and autoaggregation

For cell surface hydrophobicity, overnight cultures of LAB strains were harvested by centrifugation at 8,000 × *g* for 10 min at 4 °C and washed twice with PBS, and resuspended in PBS adjusted to an absorbance of 0.5 ± 0.02 at 600 nm ($OD_{Initial}$). Then, 3 mL of bacterial suspension and 1 mL of xylene were mixed and preincubated at 37 °C for 10 min. The mixture was kept at 37 °C for 20 min to allow separation into water and xylene phases. The aqueous phase was collected and its absorbance measured at 600 nm (OD_{Time}).

The cell surface hydrophobicity of the LAB strains was calculated using the following formula:

$$\text{Cell surface hydrophobicity (\%)} = (1 - \frac{OD_{Time}}{OD_{Initial}}) \times 100$$

For autoaggregation, overnight cultures of LAB strains were harvested by centrifugation at 8,000 × *g* for 10 min at 4 °C, washed twice with PBS, and resuspended in PBS adjusted to an absorbance of 0.3 ± 0.05 at 600 nm ($OD_{Initial}$). Then, 4.5 mL of bacterial suspension was vortexed and incubated at 4 °C for 4 and 24 h, respectively. One milliliter of bacterial suspension was collected and its absorbance at 600 nm measured (OD_{Time}).

The autoaggregation of the LAB strains was calculated using the following formula:

$$\text{Autoaggregation (\%)} = (1 - \frac{OD_{Time}}{OD_{Initial}}) \times 100$$

2.6. Antioxidant activity

2.6.1. Preparation of intact cells

Overnight LAB cultures were centrifuged at 8,000 × *g* for 10 min at 4 °C, and the resulting bacterial cell pellets were washed three times with PBS, and resuspended in PBS at a final concentration of 1×10^8 CFU/mL for antioxidant activity.

2.6.2. 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the LAB strains was determined according to a previously described method [14]. Two milliliters of intact cells and 2 mL of 0.4 mM DPPH solution in methanol were mixed and incubated at 37 °C for 30 min in the dark. After incubation, the reaction mixture was centrifuged at 8,000 × *g* for 10 min at 4 °C, and the absorbance of the supernatant at 517 nm was measured. The DPPH radical scavenging activity of the LAB strains was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = (1 - \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}}) \times 100$$

Where OD_{Sample} and $OD_{Control}$ are absorbance of samples and of distilled water, respectively, mixed with DPPH solution.

2.6.3. β-Carotene bleaching inhibitory activity

The β-carotene bleaching inhibitory activity of LAB strains was determined according to the method of Kachouri *et al*. [15]. A β-carotene-linoleic acid mixture was prepared as follows: 66 μL of linoleic acid, 3 mg of β-carotene, 300 μL of Tween 80, and 10 mL of chloroform were mixed and evaporated using a vacuum evaporator. The mixture was then diluted with 75 mL of distilled water. Two hundred microliters of intact cells and 4 mL of β-carotene-linoleic acid mixture were mixed and incubated at 37 °C for 2 h in the dark. The absorbance of the samples at 470 nm was measured, and the β-carotene bleaching inhibitory activity was calculated according to the following formula:

$$\text{β-Carotene bleaching inhibitory activity (\%)} = (\frac{\text{OD}_{\text{Sample,2 h}} - \text{OD}_{\text{Control,2 h}}}{\text{OD}_{\text{Control,0 h}} - \text{OD}_{\text{Control,2 h}}}) \times 100$$

Where OD_{Sample} and $OD_{Control}$ are the absorbance of samples and of distilled water, respectively, mixed with β-carotene-linoleic acid solution.

2.6.4. Reducing power activity

The reducing power activity of LAB strains was determined according to the method of Das and Goyal [16]. Two hundred microliters of intact cells were mixed with 0.2 mL of 0.2

M sodium phosphate buffer (pH 6.6) and 0.2 mL of 1% potassium ferricyanide, and the reaction mixture was incubated at 50°C for 20 min. After incubation, 0.2 mL of 10% trichloroacetic acid was added and centrifuged at 8,000 × *g* for 10 min at 4 °C. Five hundred microliters of the supernatant were mixed with 0.1 mL of 0.1% ferric chloride (Sigma-Aldrich) and 0.4 mL of distilled water and reacted for 10 min. The absorbance of the samples at 700 nm was measured, and L-cysteine (Sigma-Aldrich) was used as a standard.

2.6.5. Superoxide anion scavenging activity

The superoxide anion scavenging activity of LAB strains was determined according to the method of Das and Goyal [16]. The reaction mixture was prepared as follows: 0.2 M sodium phosphate buffer, 50 μM nitrobluetetrazolium (NBT), 75 μM nicotinamide adenine dinucleotide (NADH), and 15 μM phenazine methosulfate (PMS) were mixed. Fifty microliters of intact cells were added to 1 mL of reaction mixture and incubated at 37 °C for 10 min. The absorbance of the samples at 560 nm was measured, and the superoxide anion scavenging activity was measured according to the following formula:

$$\text{Superoxide anion scavenging activity } (\%) = \left(\frac{\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}}\right) \times 100$$

Where $\text{OD}_{\text{Sample}}$ and $\text{OD}_{\text{Control}}$ are the absorbance of samples and of distilled water, respectively, mixed with reaction mixture.

2.6.7. 2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity

The ABTS radical scavenging activity of LAB strains was determined according to the

method of Ji *et al.* [18]. The ABTS radical cation ($ABTS^+$) was produced by allowing an ABTS (Sigma-Aldrich) stock solution with 5.5 mM potassium persulfate (Sigma-Aldrich) to stand in the dark for at least 6 h before use. Before use, the $ABTS^+$ solution was diluted with distilled water to an adjusted absorbance of 0.7 at 734 nm. One hundred fifty microliters of intact cells were mixed with 1.35 mL of $ABTS^+$ solution, incubated at 37 °C for 10 min, and centrifuged at 8,000 × *g* for 10 min at 4 °C. The absorbance of the samples at 734 nm was measured, and the ABTS radical scavenging activity was calculated according to the following formula:

$$\text{ABTS radical scavenging activity (\%)} = (1 - \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}}) \times 100$$

Where OD_{Sample} and $OD_{Control}$ are the absorbance of the samples and of distilled water, respectively, mixed with $ABTS^+$ solution.

2.7. Immunostimulatory activity

2.7.1. Nitric oxide (NO) production activity

Raw 264.7 cells were obtained from the Korean Cell Line Bank. Raw 264.7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Hyclone) supplemented with 10% (v/v) fetal bovine serum (FBS) (Hyclone), and 1% (v/v) penicillin/streptomycin (Hyclone).

The NO production activity of LAB strains was determined according to a previously reported method with minor modifications [7]. A suspension of 2×10^5 cells/well in a final volume of 1 mL was added to 96-well tissue culture plate. Raw 264.7 cells were maintained under 5% CO_2 at 37 °C for 2 h to allow adhesion of peritoneal macrophages in the plate. Raw 264.7 cells were stimulated with 1 μg/mL lipopolysaccharide (LPS; Sigma-Aldrich) or LAB

strains (1×10^6 CFU/mL) under 5% CO_2 at 37 °C for 24 h. One hundred microliters of cell culture supernatant and 100 μL of Griess reagent were mixed and incubated for 10 min at room temperature. The absorbance of the sample at 540 nm was measured. The NO concentration was obtained by measuring the nitrite concentration in the cell culture supernatant using the Griess reagent system.

2.7.2. Reverse transcription-polymerase chain reaction (PCR) analysis

Raw 264.7 cells were seeded at 5×10^5 cells/mL into a 6-well tissue culture plate with treated LPS (1 μg/mL) or LAB strains (1×10^6 CFU/mL) under 5% CO_2 at 37 °C for 20 h. Total RNA was extracted using the RNeasy Mini total RNA isolation Kit (Qiagen Korea, Seoul, Korea). Reverse transcription-polymerase chain reaction (RT-PCR) was performed using a reverse transcription master mix (5 ×) (Lamda Biotech, Ballwin, MO, USA). The following primers were used: β-actin sense, 5'-GTCGGCCGCCCTAGGCACCAG-3'; β-actin antisense, 5'-GGAGGAAGAGGATGCGGCAGT-3'; iNOS sense, 5'-CCCTTCCGAA-GTTTCTGGCAGCAG C-3'; iNOS antisense 5'-GGCTGTCAGAGTCTCGTGGCTTTGG-3'; TNF-α sense, 5'-GCAGAAGAGGCACTCCCCCA-3'; TNF-α antisense, 5'-GATCCAT-GCCGTTGGCCAGG-3'; IL-1β sense, 5'-CAGGATGAGGACATGAGCACC-3'; IL-1β antisense, 5'-CTCTGCAGACTCAAACTCCAC-3'; IL-6 sense, 5'-AGTTGCCTTCTTG-GGACT GA-3'; and IL-6 antisense, 5'-TTCTGCAAGTGCATCATCGT-3'. The PCR products were separated using a 1.5% agarose gel.

2.7.3. Semi-quantitative real time PCR analysis

Semi-quantitative real-time PCR was performed using the SYBR Green PCR Master Mix in a real-time PCR (Thermo Fisher Scientific, Waltham, MA, USA). The reaction mixture comprised SYBR Green master mix (2 ×), primer, cDNA, and RNase free water.

Twenty microliters of mixture were amplified as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 5 sec, 60 °C for 30 sec, and 60 °C for 30 sec. The reaction was followed by a melt curve analysis to determine its reaction specificity.

2.8. Statistical analysis

All experiments were repeated three times with duplicate samples, and data are presented as means ± standard deviations. SPSS 18.0 was used for all statistical analysis. Significant differences among the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Gastric acid and bile salt tolerance of LAB strains

When passing through the gastrointestinal tract, LAB strains encounter the low pH (2.5–3.5) environment of the stomach and 0.3% bile salt concentrations in the intestinal tract [3,7]. The tolerance of LAB strains to gastric acid and bile salt is presented in Table 1. The bacterial cell counts of *L. citreum* S.Pum 19 and *P. pentosaceus* SC28 decreased after 3 h of incubation in gastric acid conditions (0.3% pepsin at pH 2.5), but the bacterial cell counts of *L. rhamnosus* GG and *L. paraplantarum* SC61 did not decrease after 3 h of incubation. The bacterial cell counts of isolated three strains slightly decreased after 24 h of incubation in bile salt conditions (0.3% oxgall), while the *L. rhamnosus* GG cell count did not. Our findings agreed with those in a report on *L. plantarum* EM and its high survival rate in acidic (pH 2.5, 1 h) and bile salt conditions (0.3% oxgall, 3 h) [19]. Decrease of cell viability in gastric

conditions originated from low pH, bactericidal component of pepsin and bile, and these circumstance influenced disruption the cell membrane [9].

3.2. Enzyme production and antibiotic susceptibility of LAB strains

β-Glucuronidase can induced negative effects by affecting carcinogenic enzyme a in the intestinal tract [20]. β-Glucuronidase activity was not detected in any of the tested LAB strains; various enzymes, such as leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase, were detected (data not shown).

Antibiotic susceptibility of LAB strains is considered when assessing the safety of probiotics, because resistance genes of probiotic strains can be transferred to pathogenic strains in the intestinal tract [21]. All the LAB strains were sensitive to ampicillin, tetracycline, chloramphenicol, and doxycycline, while they were all resistance to kanamycin, streptomycin, and ciprofloxacin (data not shown). CLSI guidelines suggested the bacterial susceptibility in a view of the possibility of transfer of antibiotic resistance gene to pathogenic bacteria related to drug-resistant strains [13]. In addition, some bacteria was known as intrinsic resistance. The resistance of these results suggested that the tested LAB strains are safe according to the CLSI guidelines [13].

3.3. Adhesion ability of LAB strains to intestinal cells

Adhesion ability of LAB strains relates to the colonization of probiotic strains in the intestinal tract, which provides beneficial effects, such as exclusion of pathogenic strains and host immunomodulation [7,10]. The adhesion ability of the LAB strains is presented in Fig. 1. *L. paraplantarum* SC61 and *P. pentosaceus* SC28 showed high adhesion abilities of 6.26% and 4.03%, respectively; these adhesion abilities were superior to that of *L. rhamnosus* GG (2.74%) (Fig. 1). In another study, *L. fermentum* LP2 (14.4%) showed a higher adhesion

ability to Caco-2 cells than did *L. rhamnosus* GG (7%) [22].

Cell surface hydrophobicity and autoaggregation of LAB strains are connected to adhesion ability and aid colonization of the intestinal tract. An upper limit of 40% cell surface hydrophobicity is a prerequisite for probiotic strains [23]. The cell surface hydrophobicity and autoaggregation of the tested LAB strains are presented in Table 2. *L. paraplantarum* SC61 (56.16%) showed the highest cell surface hydrophobicity in xylene, and its value was superior to that of *L. rhamnosus* GG (42.28%). All LAB strains exhibited a high percentage of autoaggregation ranging from 64% to 70% after incubation at 37 °C for 24 h. *L. plantarum* KJ722784 was reported to have a high hydrophobicity in n-hexadecane [24]. *L. acidophilus* DSM9126 was the highest autoaggregation (69%) after 24 h [25].

3.4. Antioxidant activity of LAB strains

Antioxidants are chemical compounds that play roles in prevention or reduction of oxidative damage by free radicals [26]. Various components, such as NADH, proteins, and antioxidant enzymes produced by LAB strains are related to antioxidant activity [8]. The DPPH radical scavenging activity of LAB strains is presented in Fig. 2A. *L. paraplantarum* SC61 (51.06%) exhibited a DPPH radical scavenging activity higher than that of the other tested LAB strains and of *L. rhamnosus* GG (39.31%); *L. citreum* S.Pum19 (32.73%) exhibited the next highest activity, followed by *P. pentosaceus* SC28 (32.60%) (Fig. 2A). In a previous study, *L. paracasei* FM-LP-4 showed higher DPPH radical scavenging activity than did *L. rhamnosus* GG [14].

The β-carotene bleaching inhibition activity of LAB strains is presented in Fig. 2B. The β-carotene bleaching inhibition activity of *L. paraplantarum* SC61 (35.64%) was similar to that of *L. rhamnosus* GG (35.06%); *L. citreum* S.Pum19 (30.25%) had the next highest activity, followed by *P. pentosaceus* SC28 (29.42%) (Fig. 2B). Another study observed that

intact *L. lactis* KC24 cells showed 58.73% inhibition of β-carotene bleaching activity [7].

The reducing power activity of LAB strains is presented in Fig. 2C. The reducing power of *L. paraplantarum* SC61 (101.41 μM) was similar to that of *L. rhamnosus* GG (105.48 μM) followed by *L. citreum* S.Pum19 (72.52 μM) and *P. pentosaceus* SC28 (72.43 μM) (Fig. 2C). In a previous study, a 10^{10} CFU/mL bacterial cells of *L. plantarum* DM5 (140 μM) exhibited high reducing power activity [16]. In addition, *L. paracasei* FM-LP-4 showed higher reducing power activity than did *L. rhamnosus* GG [14].

The superoxide anion scavenging activity of LAB strains is presented in Fig. 2D. The superoxide anion scavenging activity of *L. paraplantarum* SC61 (30.22%) was similar to that of *L. rhamnosus* GG (27.66%); followed by *P. pentosaceus* SC28 (23.32%) and *L. citreum* S.Pum19 (18.26%) (Fig. 2D). *L. plantarum* DM5 showed 48% superoxide anion scavenging activity at 1×10^{10} CFU/mL of bacterial cells [16]. Ren *et al*. [17] observed that several LAB strains had superoxide anion scavenging activity ranging from 40–74%.

The ABTS radical scavenging activity of LAB strains is presented in Fig. 2E. The ABTS 2radical scavenging activity of *L. paraplantarum* SC61 (17.32%) was similar to that of *L. rhamnosus* GG (19.38%); followed by *L. citreum* S.Pum19 (3.35%) and *P. pentosaceus* SC28 (3.34%) (Fig. 2E). In a previous study, the intracellular extract of *Enterococcus durans* LAB18s (9.4%) was shown to have high ABTS radical scavenging activity [27]. Additionally, Han *et al*. [28] recently showed that intact *P. pentosaceus* R1 cells exhibited the highest ABTS radical scavenging activity (42.4%).

3.5. Immunostimulatory activity of LAB strains

LAB strains can promote the growth of macrophages, which selectively aid in the removal of harmful microorganisms and exterior material, and immunostimulatory activity via production proteins and enzymes related to immune activity [10]. The NO producing

activity of *L. paraplantarum* SC61 (14.77 μM) is highest among the other tested strains and superior to that of *L. rhamnosus* GG (1.61 μM), followed by *L. citreum* S.Pum19 (0.64 μM) and *P. pentosaceus* SC28 (0.53 μM) (data not shown). In a previous study, *Leuconostoc mesenteroides* DH34 (7.90 μM) and *Leuconostoc kimchii* WK18 (4.69 μM) exhibited higher NO production activity than did *L. rhamnosus* GG (2.47 μM) [29].

When foreign substances enter the body, macrophages modulates the immune system through production of cytokines including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α [10]. The immunostimulatory activity of LAB strains as measured by reverse-transcription PCR is presented in Fig. 3. All samples manifested the reference gene, β-actin, but *L. paraplantarum* SC61 manifested immunostimulatory activity related to mRNA expression of iNOS, IL-1β, IL-6, and TNF-α, equal to that of *L. rhamnosus* GG (Fig. 3). *L. paraplantarum* SC61 manifested higher mRNA expression levels (iNOS (69.62-fold), IL-1β (541.05-fold), IL-6 (3743.05-fold), TNF-α (5.01-fold)) than did *L. rhamnosus* GG (iNOS (26.55-fold), 1L-1β (125.77-fold), IL-6 (525.58-fold), and TNF-α (3.30-fold) (Fig. 4). In a previous study, *L. kimchii* WK18, *L. mesenteroides* DH34, and *L. mesenteroides* GY17 increased production of IL-1β (7.6 fold, 3.46 fold, and 2.26 fold) [29]. In addition, kimchi LAB (*Lactobacillus* sp., *Leuconostoc* sp., and *Bifidobacterium* sp.) increase IL-1β, IL-6, and TNF-α [30]. These result suggested that probiotic strain could stimulate to macrophage.

4. Conclusions

L. citreum S.Pum 19, *P. pentosaceus* SC28, and *L. paraplantarum* SC61 met the criteria for probiotics including gastric acid and bile salt tolerance, enzyme activity, intestinal cell

adhesion, and antibiotic susceptibility. Among these LAB strains, *L. paraplantarum* SC61 isolated from *jangajji*, showed the highest autoaggregation and cell surface hydrophobicity, and was superior to *L. rhamnosus* GG. Intact *L. paraplantarum* SC61 cells exhibited the best antioxidant as measured by DPPH radical scavenging, β-carotene bleaching inhibition, reducing power, superoxide anion scavenging, and ABTS radical scavenging activity. In addition, *L. paraplantarum* SC61 showed higher NO production and higher expression of iNOS, IL-1β, IL-6, and TNF-α genes than did *L. rhamnosus* GG. Therefore, *L. paraplantarum* SC61 showed potential for probiotics use through antioxidant and immunostimulatory activity; this strain could be used in functional foods and pharmaceutical products.

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Table 1.

Gastric acid and bile salt tolerance of LAB strains isolated from Korean fermented foods.

LAB strains	Gastric acid tolerance with pH 2.5 with 0.3% pepsin (log CFU/mL)		Bile salt tolerance with 0.3% oxgall (Log CFU/mL)	
	0 h	3 h	0 h	24 h
Lactobacillus rhamnosus GG	8.56 ± 0.02[a]	8.51 ± 0.05[a]	8.56 ± 0.02[a]	8.58 ± 0.03[a]
Leuconostoc citreum S. Pum19	7.94 ± 0.11[c]	7.35 ± 0.07[b]	7.94 ± 0.11[c]	7.39 ± 0.04[d]
Pediococcus pentosaceus SC28	8.18 ± 0.03[b]	7.52 ± 0.68[b]	8.18 ± 0.03[b]	7.44 ± 0.01[c]
Lactobacillus paraplantarum SC61	8.17 ± 0.07[b]	8.33 ± 0.07[a]	8.17 ± 0.07[b]	7.55 ± 0.03[b]

All values are mean ± standard deviation.

[a-c]Values with different superscript letters in the same column are significantly different ($p < 0.05$).

Table 2.

Cell surface hydrophobicity and autoaggregation of LAB strains.

LAB strains	Cell surface hydrophobicity (%)	Autoaggregation (%)	
		4 h	24 h
Lactobacillus rhamnosus GG	42.28±0.76[b]	22.39±2.15[a]	64.21±3.17[a]
Leuconostoc citreum S.Pum19	11.60±2.81[c]	16.00±1.28[b]	66.94±6.57[a]
Pediococcus pentosaceus SC28	10.28±2.91[c]	15.83±0.75[b]	69.17±5.95[a]
Lactobacillus paraplantarum SC61	56.16±1.01[a]	22.09±1.96[a]	70.06±3.35[a]

All values are mean ± standard deviation.

[a-c]Values with different superscript letters in the same column are significantly different ($p < 0.05$).

Figure legends

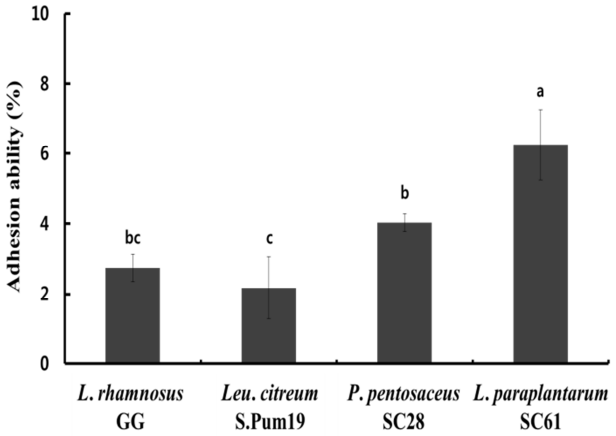
Figure 1 Adhesion ability of LAB strains to human intestinal HT-29 cells. The letters at the bases of the bars denote statistical significance ($p < 0.05$) as determined by Duncan's multiple range test. Error bars indicate the standard deviation from three independent experiments.

Figure 2 Antioxidant activity of LAB strains as measured by (A) DPPH radical scavenging activity, (B) β-carotene bleaching activity, (C) reducing power activity, (D) superoxide anion scavenging activity, and (E) ABTS radical scavenging activity. The letters at the bases of the bars denote statistical significance ($p < 0.05$) as determined by Duncan's multiple range test. Error bars indicate standard deviation from three independent experiments.

Figure 3 Immunostimulatory activity of *L. paraplantarum* SC61 on gene expression using reverse transcription PCR. C, untreated; CL, lipopolysaccharide-treated; GG, *L. rhamnosus* GG-treated; SC61, *L. paraplantarum* SC61-treated.

Figure 4 Immunostimulatory activity of *L. paraplantarum* SC61 on gene expression using real time PCR. (A) iNOS, (B) IL-1β, (C) IL-6, (D) TNF-α. The quantification results are expressed relative to the control (untreated) after being normalized to β-actin levels. The letters at the bases of the bars denote statistical significance ($p < 0.05$) as determined by Duncan's multiple range test. Error bars indicate standard deviation from three independent experiments.

518 **Figure 1**



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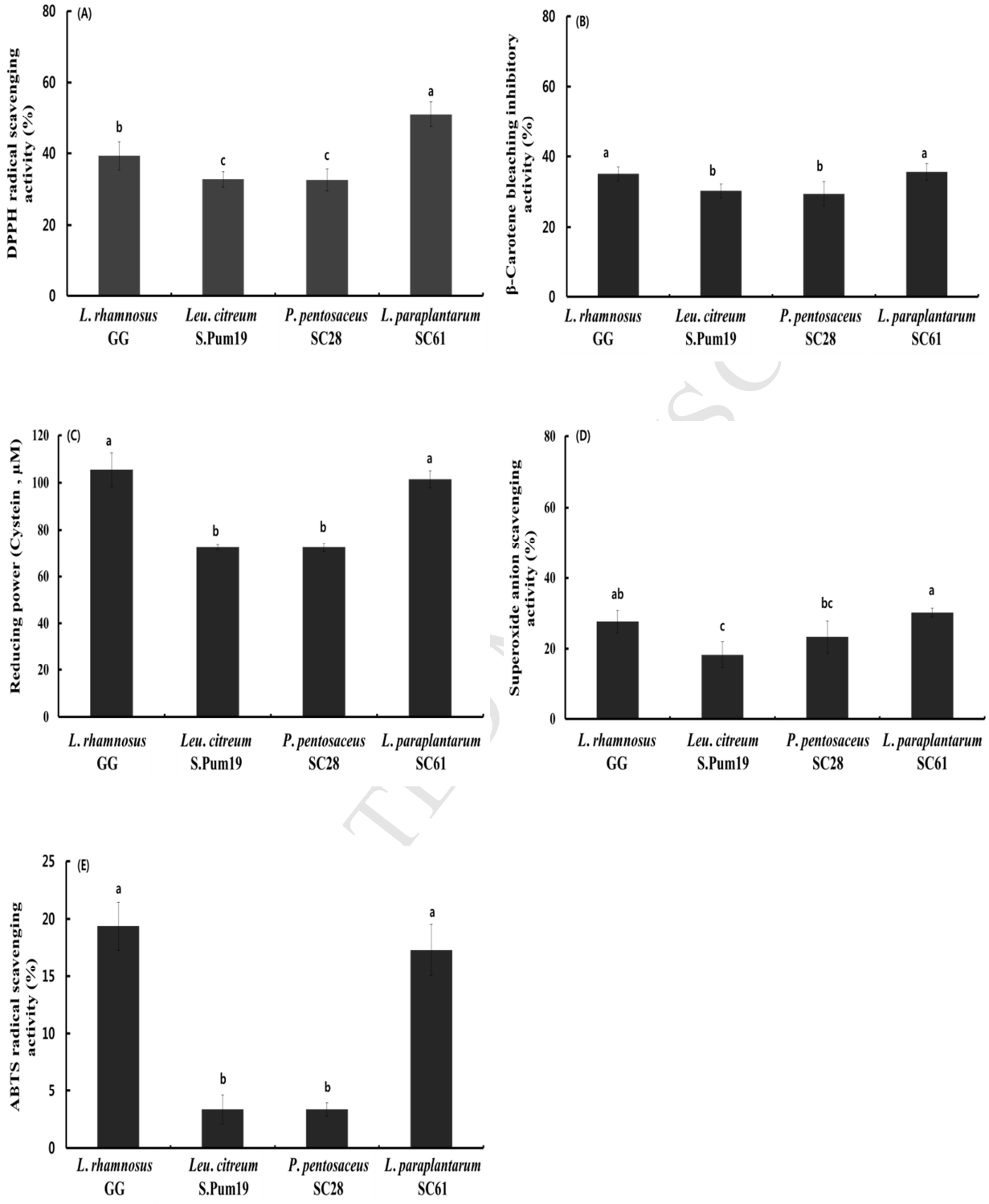
521 **Figure 2**



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525 **Figure 3**

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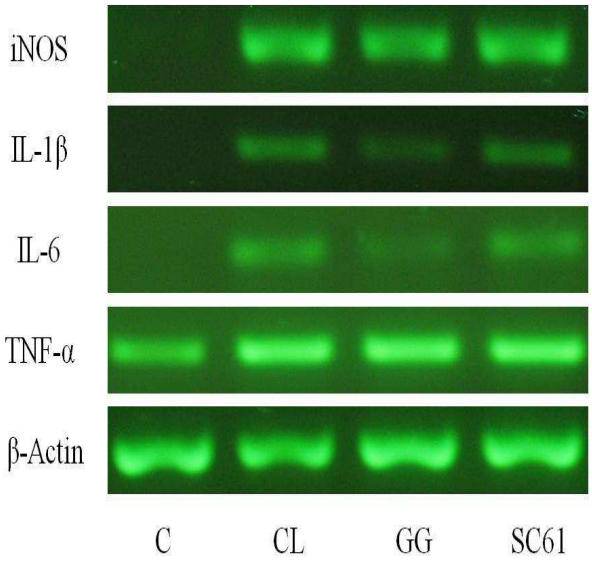

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530 **Figure 4**

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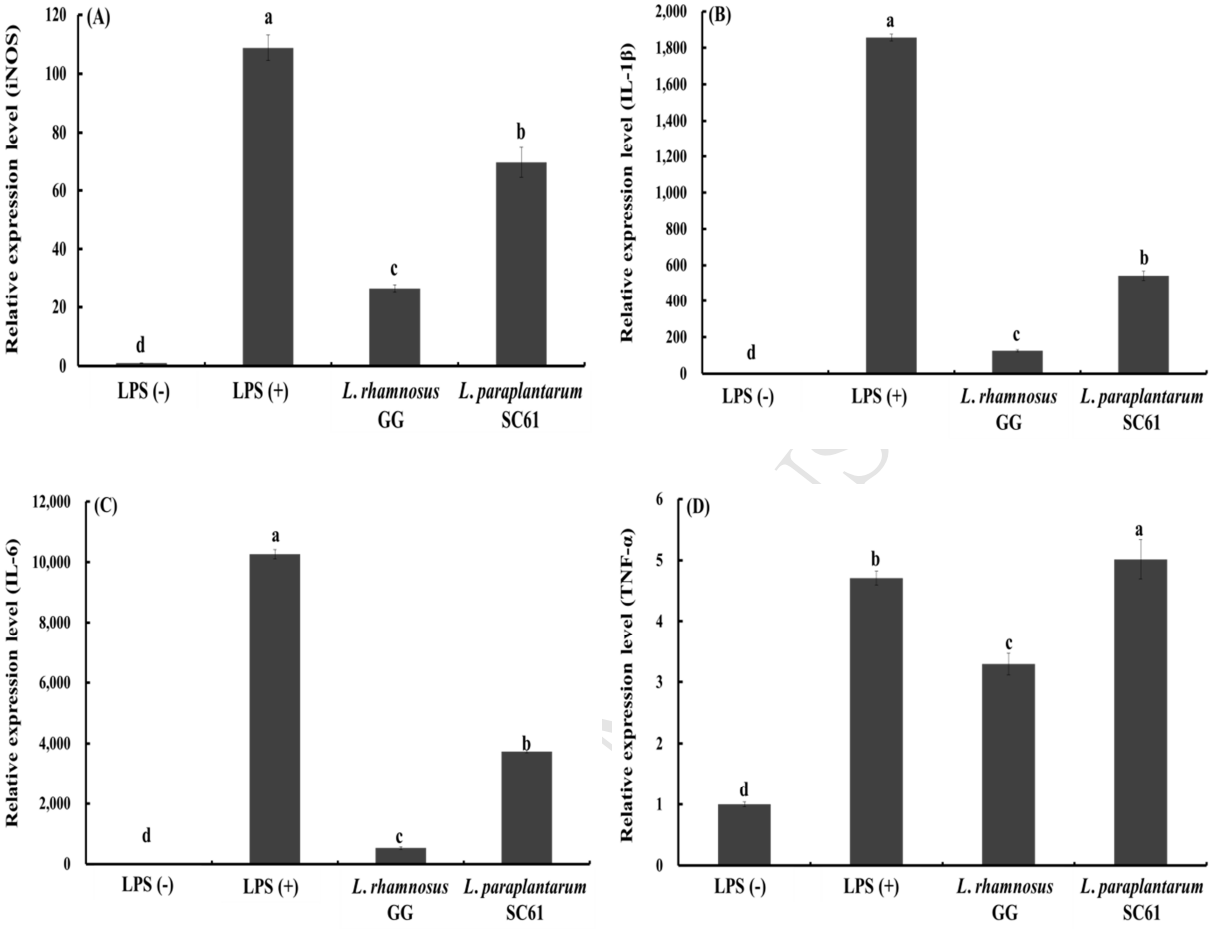

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Highlights

- *Lactobacillus paraplantarum* SC61 was isolated from *jangajji* under harsh conditions.
- *L. paraplantarum* SC61 showed high efficacy, safety, and stability.
- *L. paraplantarum* SC61 showed high antioxidant and immune-enhancing activity.
- *L. paraplantarum* SC61 could be used as a probiotic.