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Isolation of Lactic Acid Bacteria Showing Antioxidative and Probiotic Activities from *Kimchi* and Infant Feces

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The purpose of this study was to investigate lactic acid bacteria with antioxidative and probiotic activities isolated from Korean healthy infant feces and *kimchi*. Isolates A1, A2, S1, S2, and S3 were assigned to *Lactobacillus* sp. and isolates A3, A4, E1, E2, E3, and E4 were assigned to *Leuconostoc* sp. on the basis of their physiological properties and 16S ribosomal DNA sequence analysis. Most strains were confirmed as safe bioresources through non-hemolytic activities and non-production of harmful enzymes such as β-glucosidase, β-glucuronidase and tryptophanase. The 11 isolates showed different resistance to acid and bile acids. In addition, they exhibited antibacterial activity against foodborne bacteria, especially *Bacillus cereus*, *Listeria monocytogenes*, and *Escherichia coli*. Furthermore, all strains showed significantly high levels of hydrophobicity. The antioxidant effects of culture filtrates of the 11 strains included 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging capacity, 2.2′-azino-*bis* (2-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity, and superoxide dismutase activity. The results revealed that most of the culture filtrates have effective scavenging activity for DPPH and ABTS radicals. All strains appeared to have effective superoxide dismutase activity. In conclusion, the isolated strains A1, A3, S1, and S3 have significant probiotic activities applicable to the development of functional foods and health-related products. These strains might also contribute to preventing and controlling several diseases associated with oxidative stress, when used as probiotics.

Keywords: Antioxidant, *kimchi*, lactic acid bacteria, probiotics

Introduction

Lactic acid bacteria (LAB) are widely used in fermented food production and considered as generally recognized as safe (GRAS) organisms that can be safely applied for medical and veterinary functions [22]. LAB constitute a heterogeneous group of industrially important bacteria that are used to produce, using various substrates, fermented foods and beverages such as milk, vegetables, cereals, meat cocoa beans, *etc*. [12]. Yogurt, cheese, and fermented milk products are mentioned as the main food sources of probiotics [43]. Probiotics are defined as "living micro-organisms which, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" [21, 26]. The beneficial effects of probiotics include the prevention and treatment of diarrheal disease, prevention of systemic infections, management of inflammatory bowel disease, immunomodulation, prevention and treatment of allergies, anticancer effects, treatment of cholesterolemia, and alleviation of lactose intolerance [15, 48, 50].

Free radicals and active oxygen have been recognized as an important factor in the pathogenesis of several human diseases [19]. Reactive oxygen metabolites (ROM), generated through normal reactions within the body during respiration in aerobic organisms, can cause damage to proteins, mutations in DNA, oxidation of membrane phospholipids, and modification of low-density lipoproteins. Excessive amounts of ROM lead to cellular damage, which, in turn, promotes chronic diseases, including atherosclerosis, arthritis, diabetes, neurodegenerative diseases, cardiovascular disease,

and cancer [1]. To neutralize the oxidant molecules, the human body synthesizes antioxidant enzymes and molecules that, together with the antioxidants contained in food, form the biological antioxidant barrier. However, in certain circumstances, the defense system fails to protect the body against oxidative stress; consequently, the possibility of increasing antioxidant defenses is considered to be important in the maintenance of human health and disease prevention [46].

The aim of this study was to isolate lactic acid bacteria with high probiotic potentiality in exerting antioxidant activity for counteracting oxidative stress in the host. It can be used as probiotics, which can improve the total antioxidant status and decrease markers of oxidative stress in healthy people. Although the ability of probiotic bacteria to exert antioxidant activity has attracted some attention, so far, no rigorous comparative studies of this feature have been published. Therefore, in this study, we investigated lactic acid bacteria from *kimchi* and infant feces, showing both probiotic potentiality and antioxidative properties when subjected to exogenous oxidative stress.

Materials and Methods

Isolation of Microorganisms

All strains were obtained from *kimchi* and from feces samples of newborn infants at a postnatal care center of Busan, Korea. Each sample was diluted with sterilized phosphate-buffered saline (PBS) by 10-fold serial dilution. A 0.1 ml aliquot of each dilution was plated onto Man Rogosa Sharpe (MRS) agar (Difco, Detroit, MI, USA) for screening of LAB strains. The plates were aerobically incubated at 30°C for 36 h. Cells were grown under a diurnal light (cool-white light). After incubation, white colonies that formed were selected for single-colony isolation.

Identification of Microorganism

16S rDNA analysis was carried out as described by Moyer *et al*. [35]. The 16S rDNA was selectively amplified from purified genomic DNA using the polymerase chain reaction (PCR) with oligonucleotide primers designed to anneal to conserved positions in the 3′ and 5′ regions of bacterial 16S rDNA. The forward primer corresponded to positions 8 to 28 of *E. coli* 16S rDNA (5′-AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer corresponded to the complement of positions 1492 to 1473 (5′-GGTTACCTTGTTACGACTT-3′). The recombinant DNA was tested by PCR, and the nucleotide sequences were analyzed using the software programs BLASTN and BLASTX of GenBank.

Acid and Bile Salt Tolerance

For the acid tolerance assay, tested bacteria were incubated in MRS broth at 30°C for 18 h, and 1 ml of culture was transferred into 9 ml of PBS adjusted to pH 2.5 with 5 M HCl and then incubated at 30°C. The number of viable bacteria were counted after 0 h and 3 h incubation periods on an MRS agar plate. Triplicates of each sample were created. For the bile salt tolerance assay, tested bacteria were incubated in MRS broth at 30°C for 18 h, and 1 ml of culture was transferred into 9 ml of MRS broth containing 0.3% (w/v) bile salt, and then incubated at 30°C. The number of viable bacteria was counted after 0 h and 24 h incubation periods on a MRS agar plate. Triplicates of each sample were performed [51].

Cell Surface Hydrophobicity

Cell surface hydrophobicity was carried out as described by Dolye and Fosenberg [13]. Tested bacteria cultured at 30°C for 18 h in MRS broth were harvested by centrifugation (5,000 ×*g*, 10 min, 4°C) and washed twice with quarter-strength Ringer's solution. These bacteria were resuspended with the same solution. Supernatant and either *n*-hexadecane or chloroform was added at a ratio of 1:1, respectively. The mixture was vortexed for 2 min. After vortexing, the solution was left at room temperature for 30 min to separate the layer. The suspension of the test bacteria (reading 1) and separated water-soluble layer (reading 2) was measured at 580 nm absorbance for the calculation of hydrophobicity as follows. Adhesion assays were performed in three replicates.

$$\text{Hydrophobicity (\%)} = \left[\frac{(OD_{580nm}\ \text{reading 1} - OD_{580nm}\ \text{reading 2})}{OD_{580nm}\ \text{reading 1}}\right] \times 100$$

Hemolytic Activity and Enzymatic Activity

Hemolysis was evaluated using sheep blood agar (Asan Pharmaceutical, Korea) plates, and incubated at 30°C for 48 h. Recorded characteristics of hemolysis on blood agar were β-hemolysis (clear zones around colonies), α-hemolysis (a green zone around colonies), and γ-hemolysis (no halo around colonies) [31]. The assay was performed in duplicate. To investigate the enzymatic activity of the test bacteria, tryptophanase, urease, and gelatinase tests were carried out with an API ZYM kit (bioMerieux, France). Each of the strains was incubated on an MRS agar plate, and cells were suspended in 0.85% NaCl solution (McFarland turbidity adjusted to 5–6). A 65 µl quantity of each suspension was inoculated in each API ZYM kit cupule, and after incubating for 4 h at 37°C, zym test reagent was added. Changes of color were observed and the enzyme activity was investigated [28].

Antimicrobial Activity

To investigate the antimicrobial activity, the strains were tested for their potential inhibitory effects against some food-poisoning pathogenic organisms, using the paper disc method (6 mm; Toyo Roshi Kaisha, Japan) with culture supernatant filtrate. The pathogenic bacteria used as indicators were gram-negative and gram-positive reference strains, including *Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus*

cereus, and *Listeria monocytogenes*. Each pathogen was cultured for 24 h. The concentration was adjusted to 10^6~10^7 CFU/ml, plated to MRS agar, and dried. Fresh overnight MRS culture strains were harvested by centrifugation at 5,000 ×*g* for 10 min. Cell-free supernatants and supernatants that had been neutralized with 1 mol/l NaOH (pH 6.8 ± 0.2) were used after being filter-sterilized (0.22 μm pore size; Satorious, France). A 60 μl aliquot of each supernatant (pH adjusted to 6.8 ± 0.2, and non-treated supernatant) was absorbed to a 6 mm paper disc and laid on the plate smeared with pathogenic bacteria. This was incubated at 37°C for 8–12 h and checked for the appearance of a clear inhibition zone. Each assay was performed in triplicate.

Antibiotic Susceptibility Assay

Antibiotic susceptibility was investigated by measuring the OD_{600} values compared with control (MRS broth with antibiotic not added). The antibiotic agents used were ampicillin, chloramphenicol, tetracycline, streptomycin, and erythromycin. Overnight-cultured colonies of each strain were suspended into MRS broth to approximately OD_{600} at 1 density. A volume of 100 μl of the obtained suspension was inoculated into each MRS broth containing antibiotic agents and incubated at 30°C for 24 h. To perform the analysis, tests were conducted in duplicate for each bacteria strains.

Determination of 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH) Scavenging Activity

The antioxidant activity of strain culture filtrate was measured using the DPPH assay [6] as modified by Sanchez-Moreno *et al*. [44]. Bacterial culture filtrates were added to 2 ml of DPPH (0.4 mM) solution. When DPPH interacts with an antioxidant that can donate hydrogen, it is reduced, and this decreases the absorbance; readings at 514 nm were recorded using a UV-Vis spectrophotometer. In this study, L-ascorbic acid 0.5% (w/v) (Sigma Chemical Co., St. Louis, MO, USA) was used as a positive antioxidant standard. Electron donating ability (EDA) was expressed as percentage of absorbance gap between the sample-added and non-added mixture [5]. All determinations were carried out in triplicate.

$$\text{EDA (\%)} = \frac{(\text{Absorbance of standard} - \text{Absorbance of sample})}{\text{Absorbance of standard}} \times 100$$

Determination of 2, 2′-Azino-*Bis* (3–Ethylbenzthiazoline–6-Sulfonic Acid) (ABTS) Radical

The experiments were conducted using an improved ABTS decolorization assay [42]. This involves the generation of the $ABTS^+$ chromophore by the oxidation of ABTS with potassium persulfate. The ABTS radical cation ($ABTS^+$) was produced by reacting 7 mM stock solution of ABTS with 5.5 mM potassium persulfate and allowing the mixture to stand in the dark for at least 6 h at room temperature before use. Before using the $ABTS^+$ solution, it was diluted with distilled water to an adjusted absorbance of approximately 1 (±0.02) at 734 nm. The absorbance at 734 nm was measured 10 min after mixing the strains that were culture filtrated with 1 ml of $ABTS^+$ solution. The $ABTS^+$ scavenging capacity of the filtrate was compared with that of L-ascorbic acid 0.5% (w/v) used as a negative control. ABTS scavenging ability (%) is expressed by the following formula. All determinations were carried out in triplicate.

$$\text{ABTs scavenging ability (\%)} = \left(1 - \frac{\text{Sample absorbance}}{\text{Control absorbance}}\right) \times 100$$

Determination of Superoxide Radical (O_2^-) Scavenging Activity

SOD activity was determined as described by Marklund and Marklund [32]. First, 2.6 ml of 50 mM Tris-HCl buffer and 0.2 ml of 7.2 mM pyrogallol were added to 0.2 ml of bacterial culture filtrates and left at room temperature for 10 min. After reacting time had elapsed, 0.1 ml of 1 N HCl was added to the mixture to halt the reaction, and the amount of oxidized pyrogallol at OD_{420} was measured. The superoxide radical scavenging capacity of the filtrate was expressed as a percentage of the absorbance gap between the sample-added and non-added mixtures.

$$\text{SOD scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of sample-added mixture}}{\text{Absorbance of non-added mixture}}\right) \times 100$$

Results

Isolation and Identification of Lactic Acid Bacteria

Thirty strains of lactic acid bacteria were isolated from *kimchi* and healthy infant feces. After culturing for 48 h, 11 strains were selected as forming wide and white colonies on the MRS agar plate; all strains were gram positive with rod shape. These were cultured on MRS with glycerol broth and stored at –70°C. The isolated strains were named A1, A2, A3, A4, E1, E2, E3, E4, S1, S2, and S3. The A1, A2, S1, S2, and S3 genes were confirmed by sequence analysis and proved to have over 98% homology with the sequence of *Lactobacillus* sp. Isolates A3, A4, E1, E2, E3, and E4 were found to be over 98% homologous to *Leuconostoc* sp. by sequence alignment (Fig. 1).

Resistance to Bile Salts and Acid

LAB survival in low pH is very important for tolerating initial stress in the stomach [16]. At the application level, when LAB enters the human body, the first constraint is gastric acid, with a very low pH level of approximately 2–3 [4]. The results indicated that some tested strains tolerated a simulated PBS (pH 2.5) solution (Fig. 2). A1, A3, A4, E2, S1, S2, and S3 showed an over 50% survival rate, and E2 and S3 showed an over 90% survival rate after 3 h

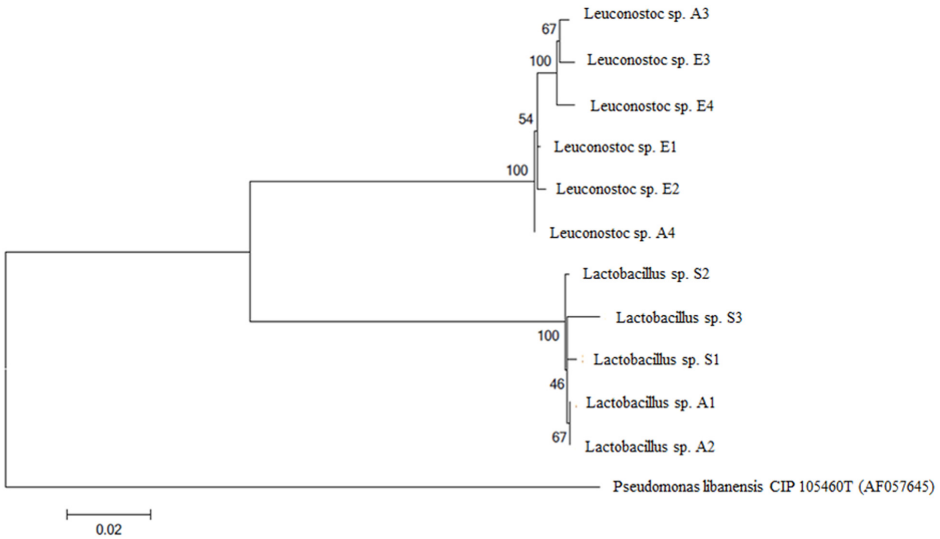

Fig. 1. Phylogenetic tree of 11 strains.
Maximum likehood tree showing the phylogenetic position of isolated strains based on 16S rDNA gene sequences. Evolutionary distance were computered using the Tamura-Nei model. Bootstrap values (>50%) based on 1,000 replications are shown.

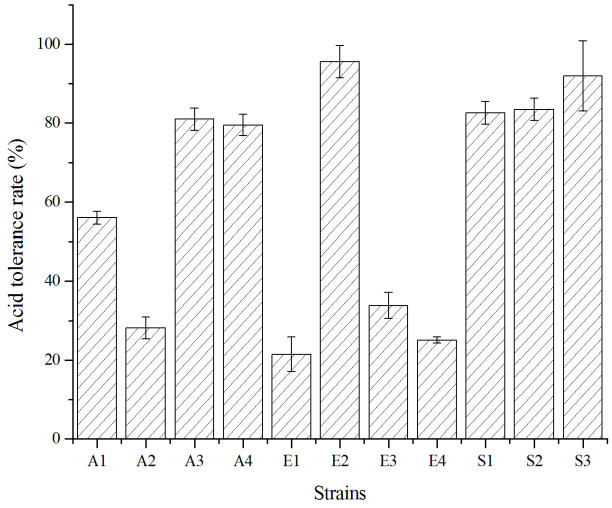

Fig. 2. Survival rate of 11 isolates in PBS.
We inoculated 1 ml of each strain culture to 9 ml of PBS (pH 2.5) solution and counted the viable bacteria at 0 h and 3 h after incubation.

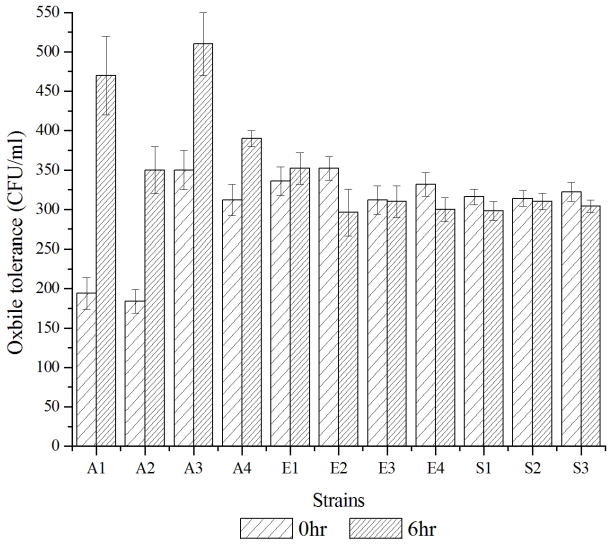

Fig. 3. Ox bile tolerance of 11 strains.
One milliliter of culture was transferred into 9 ml of MRS broth containing 0.3% (w/v) bile salt, and then incubated at 30°C. The number of viable bacteria was determined at 0 h and 24 h of incubation on an MRS agar plate.

incubation with the PBS (pH 2.5) solution. Additionally, the LAB isolates were tested for bile tolerance in MRS broth containing 0.3% ox bile. The obtained results indicated that all strains were resistant to 0.3% ox bile after 24 h incubation. (Fig. 3). Therefore, except for A2, E1, E3, and E4, most strains qualified as a probiotic.

Cell Surface Hydrophobicity

The property of adhesion can provide information about the possibility of probiotics to colonize and may modulate the host immune system. Several mechanisms have been reported about the adhesion of microorganisms to intestinal epithelial cells [45]. Cell hydrophobicity is one of the factors that may contribute to the adhesion of bacterial cells to host tissues [41]. In order to complete the

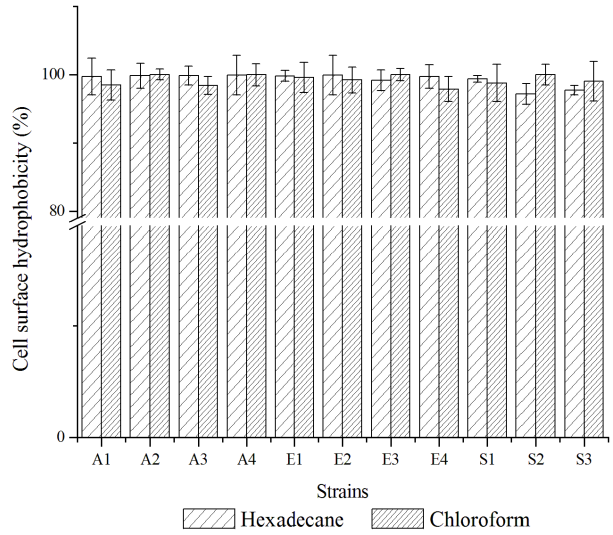

Fig. 4. Hydrophobicity percentage of 11 strains.
Supernant and either *n*-hecadecane or chloroform was added at a ratio of 1:1, respectively. The suspension of the test bacteria and separated layers of water-soluble were measured at OD_{550} nm absorbance.

investigation of probiotic criteria, *in vitro* determination of microbial adhesion to hexadecane and chloroform droplets was performed. This method has been reported to be qualitatively valid to estimate the ability of a strain to adhere to epithelial cells [24]. The results revealed that most strains showed a high value of hydrophobicity over 90%. This indicated that all strains could be good potential probiotics, adhering to gut epithelial cells of human intestine (Fig. 4).

Hemolysis test and Enzymatic Activity Test

To determine the hemolytic activity of the bacteria, blood hemolysis was evaluated on sheep blood agar. Of note, no strain tested in this work exhibited α- and β-hemolytic activity when cultured in sheep blood agar. In contrast, all strains had γ-hemolytic activity. Microbial enzyme production is also one of the key indicators when selecting probiotics. In this experiment, to observe any enzyme activity, tryptophanase, urease, and gelatinase tests were carried

Table 1. Enzymatic activities of 11 strains.

Enzyme	Strains										
	A1	A2	A3	A4	E1	E2	E3	E4	S1	S2	S3
Hemolysis	-	-	-	-	-	-	-	-	-	-	-
Tryptophanase	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-
Gelatinase	-	-	-	-	-	-	-	-	-	-	-
Phosphate alcaline	0	0	0	0	0	0	0	0	0	0	0
Esterase (C4)	2	4	0	0	0	0	0	0	4	3	3
Esterase lipase (C8)	4	3	0	0	0	0	0	0	4	4	1
Lipase (C14)	0	0	0	0	0	0	0	0	0	0	0
Leucine arylamidase	4	4	2	0	0	0	0	0	5	4	5
Valine arylamidase	4	4	1	0	0	0	0	0	5	4	4
Crystine arylamidase	1	1	0	0	0	0	0	0	1	0	1
Trypsin	0	0	0	0	0	0	0	0	0	0	0
α-Chymotrypsin	0	0	0	0	0	0	0	0	0	0	0
Phosphatase acid	2	3	1	0	0	0	1	1	2	3	3
Naphol-AS-BI-phosphohydrolase	3	1	1	0	0	0	1	1	1	1	3
α-Galactosidase	0	0	0	0	1	1	0	2	0	1	0
β-Galactosidase	1	1	1	0	0	0	0	1	0	2	1
β-Glucuronidase	0	0	0	0	0	0	0	0	0	0	0
α-Glucosidase	1	2	2	0	2	2	0	3	3	3	3
β-Glucosidase	0	0	3	5	5	5	4	5	0	5	0
N-Acetyl-β-glucosaminidase	0	0	3	0	0	0	0	0	0	0	0
α-Mannosidase	0	0	0	0	0	0	0	0	0	0	0
α-Fucosidase	0	0	0	0	0	0	0	0	0	0	0

Note: 0 to 5, activity range; 0, no activity; 5, strong activity.

Table 2. Antimicrobial activities of 11 strains against food pathogens.

Pathogens	Isolated strains										
	A1	A2	A3	A4	E1	E2	E3	E4	S1	S2	S3
Culture supernatant with neutralized pH 6.8											
Salmonella typhimurium	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-
Bacillus cereus	-	-	-	-	-	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-	-	-	-	-	-
Culture supernatant with non-adjust											
Salmonella typhimurium	+	+	+	+	++	++	+	++	+	+	+
Escherichia coli	++	++	++	++	++	++	++	++	++	++	++
Klebsiella pneumonia	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	+	+	-	+	++	++	++
Bacillus cereus	+	+	+	+	+	+	+	+	+	+	+
Listeria monocytogenes	++	++	++	++	+	+	+	+	++	++	++

Note: -, No inhibition; +, inhibition zone between 2 and 6 mm; ++, inhibition zone larger than 6 mm.

out using an API ZYM kit. The results of tryptophanase, urease, and gelatinase tests were all negative. As shown in Table 1, each strain showed different enzyme activity. Most significantly, A4, E1, E2, E3, E4, and S2 had high activity for β-glucosidase, which can cause colorectal cancer. A1, A2, A3, S1, and S2 showed no harmful enzyme activity; therefore, these strains could be used as probiotics.

Inhibition of Growth of Pathogenic Bacteria

The 11 isolated strains were tested for their antibacterial activity, using culture filtrate with non-adjusted pH or neutralized to pH 6.8, against six pathogenic bacteria. The results showed that neutralized culture filtrate had no inhibition activity against six pathogens, but all pH non-adjusted culture filtrates showed clear inhibition against most of the tested indicator bacteria. These results demonstrated that the acid cell-free extract produced by the tested strains cause antimicrobial activity like bacteriocin, such as an organic acid and nitric acid (Table 2). These could also exert beneficial effects in the intestine when used as natural bacteriocin.

Antibiotic Susceptibility

A1, A2, S1, S2, and S3 were resistant only to the β-lactam antibiotic ampicillin and exhibited sensitivity to the aminoglycoside antibiotic streptomycin, the chloramphenicol antibiotic chloramphenicol, the tetracycline antibiotic tetracycline, and the macrolide antibiotic erythromycin.

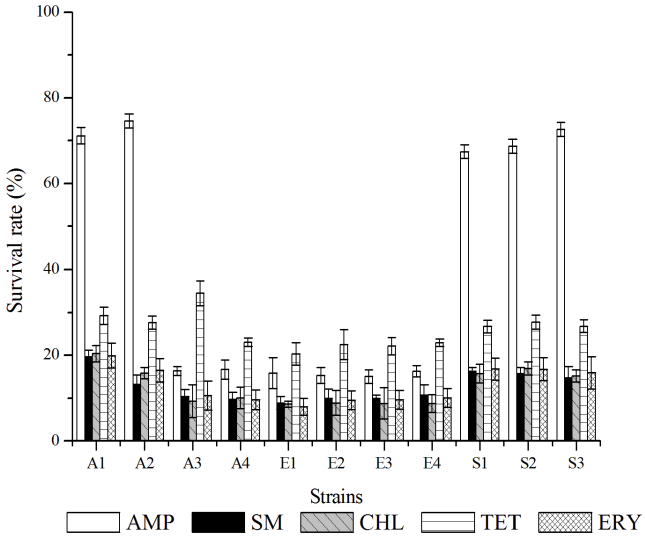

Fig. 5. Antibiotic susceptibility of 11 strains.
Each antibiotic corresponding concentration ranges were as follows: ampicillin (50 μg/ml), chloramphenicol (179 μg/ml), tetracycline (50 μg/ml), streptomycin (50 μg/ml), and erythromycin (20 μg/ml).

Excepting A1, A2, S1, S2, and S3, all strains showed sensitivity to the five antibiotic agents (Fig. 5).

Each Strain Culture Filtrate Scavenged DPPH and ABTs Radicals

Antioxidant substances donate electrons or hydrogen atoms to the free radical to create a complex. DPPH and

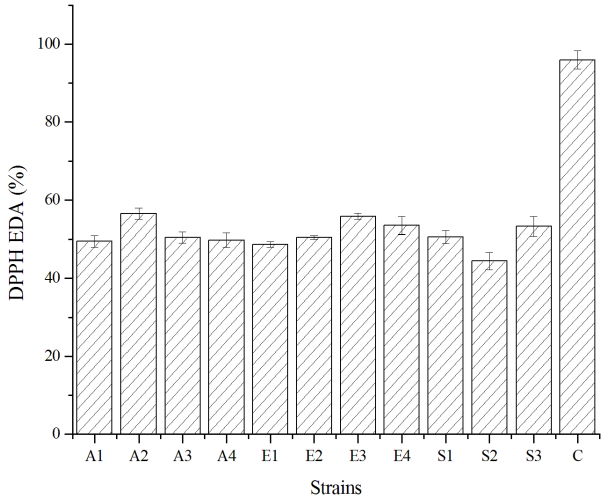

Fig. 6. DPPH radical scavenging activities of 11 strains.
Bacterial culture filtrates were added to 2 ml of DPPH (0.4 mM) solution. DPPH radical scavenging activity was measured by absorbance at 514 nm. In this study, L-ascorbic acid 0.5% (w/v) (Sigma Chemical Co., St. Louis, MO, USA) was used as a positive antioxidant standard.

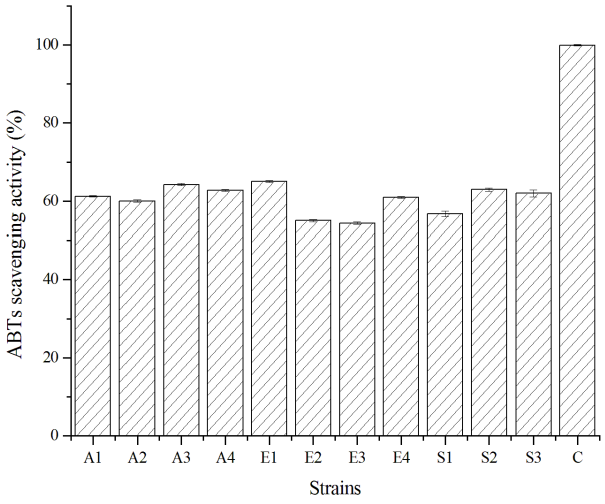

Fig. 7. ABTS radical scavenging of 11 strains.
Absorbance at 734 nm was measured 10 min after mixing of strain culture filtrate with 1 ml of $ABTS^{+}$ solution. The $ABTS^{+}$ scavenging capacity of the filtrate was compared with that of L-ascorbic acid 0.5% (w/v) used as a negative control.

ABTS accept electrons or hydrogen atoms from antioxidant substances and convert them into irreversible stable molecules. Antioxidant activity can be measured *via* electron donating ability. To improve the validity of the results, both kinds of radical scavenging activity assays were used; DPPH and ABTS.

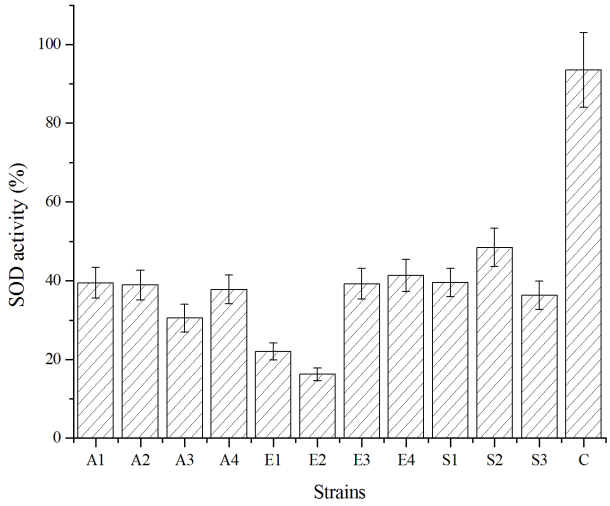

Fig. 8. SOD scavenging activities of 11 strains.
First, 2.6 ml of 50 mM Tris-HCl buffer (50 mM Tris + 10 mM EDTA, pH 8.5) and 0.2 ml of 7.2 mM pyrogallol were added to 0.2 ml of bacterial culture filtrates and left at room temperature for 10 min. After the reacting time, 0.1 ml of 1 N HCl was added to the mixture to halt the reaction and the amount of oxidized pyrogallol was measured at OD_{420}.

The DPPH radical scavenging activities of culture filtrate of the 11 strains were near and over 50% (Fig. 6), and the ABTS radical scavenging activities of culture filtrates were all greater than 50% (Fig. 7). The results showed the synthetic antioxidant (L-ascorbic acid) was lower in value than the control. However, when compared with the concentration of synthetic antioxidant (0.05% (w/v)) typically used in the experiment, these results were thought to have high antioxidant activity.

Superoxide Radical Scavenging Activity

In vivo, the important enzymatic antioxidant defense mechanism is superoxide dismutase (SOD). SOD is an enzyme that catalyzes the dismutation of superoxide (O_2^{-}) into oxygen and hydrogen peroxide to protect the body from oxygen toxicity [30]. In this research, we measured superoxide radical scavenging generated by pyrogallol auto-oxidation and scavenging superoxide anion radicals were confirmed as shown in Fig. 8. Excepting E1 and E2, all strains exhibited over 35%, and S2 showed the highest SOD scavenging activity (Fig. 8).

Discussion

Modern society has a very keen interest in personal health, and as a result, functional foods such as probiotics

are being developed. Probiotics are defined as "living micro-organisms which, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" [18]. Until now, lactic acid bacteria have been recognized for improve intestinal health, but recently the market for products containing this bacterial strain has changed. The *Lactobacillus* market is entering into a new phase, adding new function to conventional intestinal regulation.

Oxidative stress occurs when the supply of the body's antioxidant is insufficient to handle or neutralize free radicals [20]. Free radicals are highly unstable molecules that interact with other molecules in our bodies to destroy cellular membranes, enzymes, and DNA. They accelerate aging and contribute to the development of many diseases, including cancer and heart disease [2]. Antioxidants are chemical compounds that can prevent, stop, or reduce oxidative damage, protecting the human body from free radicals and retarding the progress of many diseases. Therefore, the development and utilization of effective antioxidants are desired [25, 27, 34]. Lactic acid bacteria are widely found in Korean traditional fermented food such as *kimchi*, as well as in the human intestine. Specifically, during breastfeeding, 90% of the intestinal flora of newborn colostrum is composed of lactic acid bacteria. In this study, selection of specific strains can exert a role in the prevention of oxidative stress and related disease. Thus, we isolated potential probiotic lactic acid bacteria from kimchi and infant feces.

In this study, 11 strains of lactic acid bacteria were isolated from the traditional fermented food *kimchi* and from feces from healthy breast-feeding infants. The A1, A2, S1, and S2 isolates were assigned to *Lactobacillus paracasei*; A3, A4, E1, E2, E3, and E4 isolates were assigned to *Leuconostoc mesenteroides*; and S3 was assigned to *Lactobacillus casei* on the basis of their physiological properties and 16S rDNA sequence analysis. For a strain to be qualified as a probiotics, it must have fulfilled certain physiological characteristics, including survival in the gastrointestinal tract, tolerance to low pH, and tolerance to bile in the form of glycocholic or taurocholic acid and sodium deoxycholate [29, 52], adherence to epithelial cells, demonstration of safety, antagonistic activity against pathogens, and antibiotic properties [23, 49]. It has been reported previously that acidity has the most negative effect on bacterial growth and viability during passage through the stomach [9]. The pH in the human stomach ranges from 1.5 to 4.5 depending on the intervals of feeding, the types of food consumed, and the duration of food digestion, which can take up to 3 h. As the results in Fig. 2 illustrate, A1, A3, A4, E2, S1, S2, and S3 showed high acid tolerance. These important observed results are in agreement with those reported by Burns *et al*. [8, 14, 40]. Studies have previously reported that bile tolerance is also considered an important factor that affects LAB viability [3, 37]. Bile is a result of a digestive secretion that can play a major role in lipid emulsification [8]. The relevant physiological concentrations of human bile salts range from 0.3% to 0.5% [14, 54]. Because of their similarity, the value of 0.3% ox bile (Oxgall) solution is the most used substitute for human bile salts [7, 10, 17, 36]. All 11 strains have tolerance to up to 0.3% ox bile acid. Therefore, these LAB isolates were able to grow and survive at bile salt conditions for up to the tested period of 6 h.

The calculated hydrophobicity value of the 11 strains was consistently around 95%, as shown in Fig. 4. Compared with the characteristic reported by Perez *et al*. [39] of requiring at least 85% hydrophobicity support in order to adhere to the epithelial cell, all isolated strains demonstrated that they possess the capability to adhere to epithelial cells. The capacity for intestinal epithelial cells has to be further verified through *in vivo* experiment, such as Caco 2 cell and mouse empirical studies. The food industry needs to carefully assess the safety and efficacy of all new species before their incorporation into food products [38]. In the present research, A1, A3, S1, and S3 were confirmed as safe bioresources because of their non-hemolytic activities and non-production of harmful β-glucosidase, β-glucuronidase, tryptophanase, and urease. In addition, inhibition of foodborne pathogenic bacteria growth is one of the desirable properties of probiotic bacteria. Pathogens can be antagonized through the production of antimicrobial compounds such as nisin bacteriocin [11], which competes for pathogen binding and receptor sites as well as for available nutrients and growth factors [30, 38, 47]. Under our experiment conditions, 11 strains showed a clear zone for *Salmonella typhimurium, Escherichia coli, Bacillus cereus,* and *Listeria monocytogenes,* and presented different anti-pathogen activities. Through these results, we can expect human healthcare to benefit, improving protection against occurrences of diarrhea, food poisoning, and enteric infection and improvement of our intestinal flora.

As shown in Fig. 5, only A1, A2, S1, S2, and S3 showed resistance to ampicillin. The other strains were susceptible to ampicillin, streptomycin, chloramphenicol, tetracycline, and erythromycin. Intrinsic resistance is not horizontally transferable, and poses no risk in non-pathogenic bacteria [33].

Accumulated evidence suggests that A1, A3, S1, and S3 have good potential for application in functional foods and

health-related products. To augment these results, the present study researched the antioxidant properties of these strains. To elucidate the antioxidant activity of the 11 strains, DPPH and ABTS radical scavenging activities and superoxide radical scavenging were determined as described previously. Fig. 6 shows the DPPH radical scavenging activity of the 11 strains. The scavenging activity of the 11 strain culture filtrates was around 50% and the ABTs radical scavenging activity was all greater than 50% (Fig. 7). The results were lower than that of the control (L-ascorbic), but when compared with the concentration of synthetic antioxidant (0.05% (w/v)) typically used in these experiment, these results are believed to have high antioxidant activity. Additionally, the culture supernatant had 50% antioxidant activity as non-purified solution, demonstrating potential as a natural antioxidant. Increase of the antioxidant capacity by optimizing the environmental factors makes it possible to obtain useful industrial materials. Excepting E1 and E2, all strains exhibited over 35%, and S2 showed the highest, SOD scavenging activity. Yang *et al*. [53] reported that there is a significant positive correlation among the SOD-like activities and the metabolite of LAB contributing to inhibition or activation of the antioxidant activity of SOD. In this study, SOD activity was measured using the culture filtrate. It is considered that purification of the filtrate could exhibit a higher activity.

This study provides support for the formulation of novel probiotic foods or supplements that can play a role in the prevention of oxidative stress and related diseases. The screening of physiological traits to assess the potential of probiotic bacteria for specific functions can be subsequently verified in animal models.

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