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Change in *Lactobacillus brevis* GS1022 and *Pediococcus inopinatus* GS316 in Gajami Sikhae Fermentation

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Lactic acid bacteria are widely known to prevent and treat intestinal health conditions, heart disease, depression, and obesity. In Korea, such bacteria are commonly consumed through various fermented foods, although most are isolated from kimchi, and research on the lactic acid bacteria in fermented seafood is insufficient. This study was therefore conducted to observe changes in bacterial flora according to the culture date of lactic acid bacteria in the fermentation of traditional Korean Gajami Sikhae produced in Pohang and to isolate the bacteria of probiotic value. The bacteria were periodically isolated and identified from date of preparation to 50 days after preparation to investigate which *Lactobacillus* are involved in Gajami Sikhae. As fermentation progressed, it was confirmed that *Pediococcus* sp. and *Lactobacillus* sp. participate predominantly in the early and later periods of fermentation, respectively. During the entire fermentation period, 170 isolates were screened, and the following five species were found to be involved: *Pediococcus pentosaceus, Pediococcus inopinatus, Leuconostoc mesenteroides, Lactobacillus brevis,* and *Lactobacillus plantarum*. Five strains of these species were selected through acid and bile tolerance tests, and their coaggregation, autoaggregation, hydrophobicity, antibacterial, and antioxidant activities were then evaluated. As a result, it is thought that *L. brevis* GS1022, which has excellent digestive fluid resistance, and *P. inopinatus* GS316, which has excellent cohesiveness, may be useful as probiotic strains.

Key words : Antimicrobial activity, Gajami Sikhae, lactic acid bacteria, probiotics

Introduction

Flounder is widely used as a food item in various products, such as dried products, raw fish, and frozen food, because it is abundant in nutrients such as proteins and unsaturated fatty acids [12]. Sikhae is prepared by dehydrating seafood such as squid, flounder, and pollack with salt and fermenting them with grains, red pepper powder, and vegetables. Organic acids produced by lactic acid bacteria (LAB) prohibit decay of the food, and yeast provides a texture and flavor that makes the food suitable for consumption [23]. As fermentation progresses, the fishbone becomes tender and the unsavory smell of raw marine products disappears.

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A previous study assessed the microbiological and physicochemical characteristics of flounder [11]. Because its fermentation principle and ingredients are similar to those of kimchi, sikhae has similar physiological characteristics. The anticancer effects of LAB, fibrin, as well as the blood pressure-lowering effects of γ-aminobutyric acid (GABA), have been previously reported [17]. Lee [16] reported that *Weissella* sp. isolated from kimchi demonstrated antibacterial and anticancer effects.

According to Guarner and Schaafsma [10], probiotics is a generic term for living organisms that are beneficial for the body when consumed in moderate amounts. Probiotics generally comprise LAB, including some *Bacillus* sp., as well as *Saccharomyces*, and have diverse health functions [5]. Probiotics have functions such as relieving lactose intolerance and allergic reactions, immunomodulation, decreasing blood cholesterol levels, suppressing colorectal cancer, alleviating atopic dermatitis, and alleviating Crohn's disease [24]. However, essential conditions must be met for these probiotics to perform these functions: safety and stability in the human body, possibility of colonization, epithelial cell

adhesion, self-aggregation, and stability in the production process must be ensured [1]. Because LAB are tolerant to low pH conditions and bile acid, they are considered high-value probiotics [13]. LAB are generally recognized as safe and can produce organic acids by metabolizing sugar. LAB also suppress constipation and prevent harmful bacteria in the intestines by inhibiting intestinal decay [29]. Additionally, probiotics have beneficial effects, such as reduction of blood cholesterol levels, promotion of anticancer activities, and enhancement of immunity, when ingested in appropriate amounts [6]. Because many LAB isolated from kimchi are probiotic, it is expected that LAB isolated from Gajami Sikhae will also be probiotic. The purpose of this study was to investigate the types and dominant species of LAB involved in the fermentation of Gajami Sikhae. LAB were isolated every five days during the fermentation process, and changes in the microflora were examined. The most basic function of a probiotic is perhaps the ability to survive in the digestive tract, which was evaluated using acid solution, bile acid solution, artificial gastric fluid, and artificial small intestine fluid. Acid tolerance was evaluated by measuring the survival rate of the isolated LAB with different adaptation times for each solution. In addition, autoaggregation and coaggregation abilities were evaluated to determine the beneficial effect of intestinal colonization. We also evaluated the function of these bacteria to exclude the formation of harmful bacterial colonies. Antioxidant activities, cell surface hydrophobicity, and antimicrobial activities were evaluated to assess the potential of the isolated bacterial strains as probiotics. Morphological, physiological, and molecular biology techniques were used to identify strains exhibiting high probiotic activity.

Materials and Methods

Isolation and preservation of LAB

LAB strains were isolated from a home-made Gajami Sikhae sample produced in Pohang, Gyeongsangbuk-do province, South Korea. The sample was stored at 10℃ for 50 days from the day of preparation. To determine the types of LAB involved in the fermentation process, 30 g of sikhae and 30 ml of sterilized, distilled water were mixed, homogenized, and centrifuged at 3,100× g for 10 min (1248R, GYROZEN Co., Ltd., Korea). The supernatant was diluted and inoculated on *Lactobacilli* de Man, Rogosa and Sharpe (MRS) (Difco, USA) agar medium. After incubation at 37℃ for 24 hr, colonies were first selected based on shape, color, and size. Each of the selected colonies was separated by single-colony isolation. The total glycerol concentration was adjusted to approximately 20%, and samples were stored at -80℃. The isolation of LAB was conducted every 5 days for a total of 50 days. For secondary selection of isolated LAB, MRS agar medium supplemented with 0.1%(w/v) $CaCO_3$ was used, and strains that produced a clear zone of ≥35 mm were selected.

Identification of LAB

Morphological characterization

Prior to molecular biological identification, Gram staining was performed. First, selected LAB were cultured for 12-16 hr in $CaCO_3$-supplemented MRS medium and stained with a Gram staining kit (Difco, USA). The staining results were viewed under a microscope to determine the cell wall structure and shape and size of the strain. (Data not shown.)

Molecular biological identification

16S rDNA sequencing was performed on the initially selected strains to identify the change of LAB during Gajami Sikhae fermentation. The phenol–chloroform method [13] was used to isolate the chromosomal DNA of the strains. For this, 2 μl of 10× Taq buffer, 1.6 μl of 2.5 mM dNTPs, 1 μl of primers (forward and reverse), 20 ng of template DNA, and 0.2 U Taq polymerase (LPS solution, Korea) were mixed to prepare a reaction mixture for polymerase chain reaction (PCR). After initial denaturation at 95℃ for 5 min, denaturation at 95℃ for 1 min, annealing at 55℃ for 30 s, and extension at 72℃ for 1 min were repeated 35 times, followed by a final extension at 72℃ for 5 min. Amplification products were identified using 1.5% agarose gel electrophoresis. The primers used in the PCR reaction were prepared according to Petri [20], and are shown in Table 1.

Biochemical identification

Isolated LAB were subcultured on MRS broth, and the availability of 49 carbon sources was confirmed using a Api 50 CHL kit (Biomérieux, France). The Api web program (http://apiweb.biomerieux.com) was used to read standard species and confirm the results.

Evaluation of the probiotic characteristics of the isolated LAB

Evaluation of acid tolerance

Table 1. Primers used in this study

Species		Sequence	*Target size (bp)*
L. lactis sp.	F	TTG CAT GGA ATG AGC GGA AAC	248
	R	TAT CCT CCC ATT GAT AAA CCA GCG	
L. brevis sp.	F	GGA AGA TCA AGA ATA TCG GTG	1361
	R	GCG TCT CTA ATT CAC TGA GC	
L. buchneri sp.	F	CTA TCT TTA ACC GCA TTG CCG	1007
	R	GAC ACG CTT CTC ATG ATT GTC	
L. curvatus sp.	F	CCA GAT CCA TCA GAA GAT ACG	480
	R	GCT AAC TTA CCA CTA ACG ACC	
L. hilgardii sp.	F	TTC CTT GGT AAT GTG CTT GC	684
	R	AAT GGC AAT CGC AAT GGA CG	
L. plantarm sp.	F	GAA GAT TTG CCC ATC GGT G	1113
	R	CGT TTG ATG GTA GCG TTG C	
Lc. mesenteroides sp.	F	GTG GTC ATG GGT CTT AGC	886
	R	GGA TCA AGA CTA GCC AAT GG	
O. oeni sp.	F	GGT AGA TTA ACC CGC GAC G	1588
	R	GGA ATC GGT AGC ATC CTG	
P. acidilactici sp.	F	ATG ATG GAC AGA CTC CCT G	776
	R	CGA GCT GCG TAG ATA TGT C	
P. damnosus sp.	F	GTC TAA ACT GGT GGT TAA ACG	470
	R	ATC GCA CCT GGT TCA ATG C	
P. inopinatus sp.	F	CTA TCC TTA CAA TGT GCA TCG	567
	R	TGG TGC GTC AGT AAA TGT AAG	
P. parvurus sp.	F	GCA TGA ATC ACT TTT CGC TC	331
	R	CAA AGA TTG TGA CCC AGT TG	
P. pentosaceus sp.	F	GGG AAC GGT TTT AGT TTT ATA CG	396
	R	CTA AGA GCG GTG ATG ATA AG	
W. paramesenteroides sp.	F	GCT GAT GAA CCC ATA CCT C	641
	R	GAC CTG ATT CGC TCG TTG	

To evaluate the acid tolerance of the isolated LAB, the method described by Tokatlı [29] was used. LAB cultured at 37℃ for 24 hr were centrifuged at 6,000× g for 15 min to collect the cells. The cells were washed twice with phosphate-buffered saline (PBS) and resuspended in PBS, with the adjusted to 2.5. The suspension was incubated at 37℃ for 4 hr, and the suspensions at 0 and 4 hr of culture were diluted with sterilized saline and cultured on MRS agar. The survival rate of LAB was calculated as shown in equation (1).

Evaluation of bile acid tolerance

To evaluate the bile acid tolerance of isolated LAB, the method described by Tokatlı [29] was used. Using the same preparations as those for evaluating acid tolerance, cells were washed twice and inoculated in 1% MRS broth containing 0.3%(w/v) oxgall (Difco, USA). The inoculum was incubated at 37°℃ for 4 hr, and the cultures at 0 and 4 hr were diluted with sterile saline and cultured on MRS agar. The survival rate of LAB was calculated as shown in equation (1).

$$\%\ \text{survival} = \frac{\text{logCFU of viable cells survited}}{\text{logCFU of initial viable cells inoculated}} \times 100 \quad (1)$$

Autoaggregation and coaggregation assays

The method described by Tareb [26] was used to investigate the autoaggregation of LAB and coaggregation with pathogenic bacteria. The following indicator strains were used: *Candida albicans* ATCC 10231, cultured at 28℃-30℃ using Yeast extract Peptone Dextrose medium; *Escherichia coli* KCTC 2571, cultured at 37℃ using Luria Bertani (LB) medium; *Helicobacter pylori* HPKCTC B0150, cultured at 37℃ using brucella medium containing 10%(v/v) fetal bovine serum; *Staphylococcus aureus* KCTC 1916, cultured at 35℃-40

℃ using LB medium, and *Listeria monocytogenes* KCTC 13064, cultured at 30℃ - 37℃ using listeria enrichment medium. The LAB and pathogens were cultured under optimal culture conditions, washed twice with PBS, and mixed in the same buffer, and the optical density $(OD)_{600}$ value was adjusted to 0.3. The OD_{600} was measured at 0, 1, 2, 3, 4, 5, 20, and 24 hr while each suspension was kept at 25℃. Autoaggregation (%) was calculated using the equation $(1 - OD_{time} / OD_{T0}) \times 100$. OD_{time} is the OD_{600} value for each hour (0, 1, 2, 3, 4, 5, 20, or 24), and OD_{T0} is represented by the OD_{600} value at 0 hr. To investigate coaggregation, LAB and pathogens were cultured and suspended under the same conditions as that used in the autoaggregation test. The OD_{600} was measured at 0, 1, 2, 3, 4, 5, 20, and 24 hr after mixing the LAB and the pathogen suspension at a ratio of 1:1. The coaggregation (%) was calculated using the equation $[(OD_{Patho} + OD_{Lb}) / 2 - (ODmix) / (OD_{Patho} + OD_{Lb}) \times 100]$, where OD_{Patho} and OD_{Lb} were the OD_{600} values of each strain and OD_{mix} was the value of the mixed-strain culture medium. The indicator strain *C. albicans* ATCC 10231 was procured from American Type Culture Collection (ATCC). *Escherichia coli* KCTC 2571, *Listeria monocytogenes* KCTC 13064, and *Staphylococcus aureus* KCTC 1916 were procured from Korean Agricultural Culture Collection. *Helicobacter pylori* HPKCTC B0150 was procured from the microbiology class of Gyeongsang National University.

Antimicrobial activity assay

The antimicrobial activities of the isolates were determined using the disc diffusion method [4]. The indicator strain was the same as that used in the autoaggregation and coaggregation test. Each indicator strain was cultivated under optimal culture conditions and washed twice with PBS, the OD_{600} value was adjusted to 0.1 using the same buffer, and 1% was inoculated on Mueller Hinton (Difco, USA) agar medium. LAB culture supernatants (60 µl) were dispensed into of sterilized paper discs (8 mm, Toyo Roshi Kaisha, Ltd., Japan) and incubated at 30℃ for 24 hr. Ampicillin and amphotericin B were used as positive controls. The antimicrobial activity was measured by the size of the inhibition zone in mm.

Evaluation of cell surface hydrophobicity

To indirectly confirm the adhesion ability of the LAB, the cell surface hydrophobicity was evaluated using the method described by Doyle [7]. LAB incubated at 37℃ for 18 hr were centrifuged (5,000× g, 15 min) to collect the cells. After washing twice with PBS, a suspension having OD_{600} = 1.0 was prepared using PBS. The same quantity of ethyl acetate, chloroform, and xylene was added to the suspension, and the mixture was allowed to stand for 30 min at room temperature after vortexing for 1 min. The OD_{600} value of the suspension and the separated layer were measured, and the hydrophobicity (%) was calculated according to the following formula: hydrophobicity (%) = [(absorbance of sample at 600 nm − absorbance of solvent at 600 nm) / absorbance of solvent at 600 nm ×100].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability

The isolated LAB were cultured, and the supernatants were then subjected to centrifugation (3,100× g, 10 min). The supernatants were filtered using a syringe filter (0.45 µm, echromscience, Korea). The DPPH free radical scavenging activity was measured according to the method of Brand-Williams [2]. A 1-ml volume of DPPH solution (200 µM in methanol) was mixed with 800 µl of LAB culture supernatant, and the reaction was terminated at room temperature for 30 min. After the reaction was terminated, the absorbance was measured using a spectrophotometer (Libra S22, Biochrom Ltd., England) at 517 nm. Distilled water was used as a control, and butylated hydroxytoluene (BHT, Sigma Co., USA), a synthetic antioxidant, was used as a positive control. DPPH radical scavenging activity (%) was calculated according to the equation [1 − (absorbance of sample at 517 nm / absorbance of solvent at 517 nm) ×100].

Statistical analysis

All values are expressed as mean ± standard deviation. Data were analyzed using one-way analysis of variance using Statistical Package for Social Science, version 23 (SPSS; Chicago, USA) for Windows. The differences among groups were assessed using Duncan's multiple range test. Statistical significance was considered at $p<0.05$.

Results and Discussion

Isolation and identification of LAB

A total of 170 LAB strains were isolated over 50 days to assess the LAB involved in the fermentation of Gajami Sikhae. 16S rDNA analysis showed that *P. inopinatus*, *P. pentosaceus*, *Lc. mesenteroides*, *L. brevis*, and *L. plantarum* were present in sikhae over the 50 days of fermentation. *Pediococcus* spp. were dominant at the beginning of fermentation

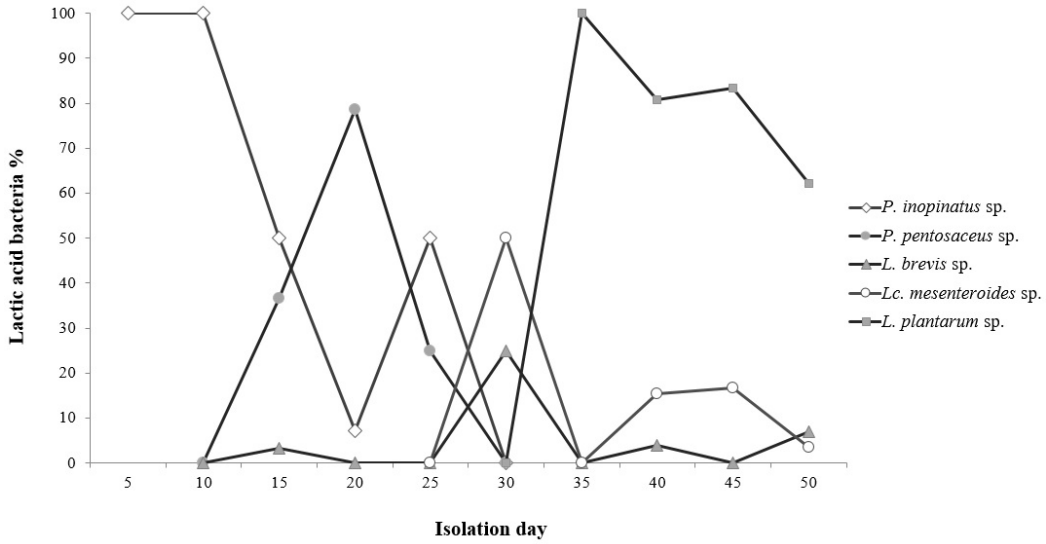

Fig. 1. Changes in lactic acid bacteria during Gajami Sikhae fermentation.

(0-20 days), and *Lactobacillus* spp. were dominant at the middle stage (21-50 days) (Fig. 1). When LAB were cultured in $CaCO_3$-supplemented medium, 29 strains were selected based on the size of the clear zone. According to a study on the distribution of LAB in kimchi, *Lc. mesenteroides*, *P. cerevisiae*, *L. plantarum*, and *L. brevis* were dominant [22], and the distribution of LAB in sikhae showed that *Lc. mesenteroides* and *L. plantarum* were dominant [15]. In the present study, LAB were isolated from the production date to day 50 after production, and the dominant strains were different from those identified in previous studies.

Probiotic activity

Evaluation of acid tolerance

LAB are ingested via the oral cavity and pass through the stomach, where various enzymes are present. They finally reach the small intestine through the bile duodenum. Thus, LAB should be resistant to acid to survive at the low pH of the stomach, which contains gastric acid. Acid tolerance tests showed that of 29 strains selected through acid production, 17 exhibited a survival rate of 20% or more at pH 2.5 (Fig. 2). Eight strains showing a high survival rate of 60% or more were selected, and their tolerance to bile acid was measured.

Evaluation of tolerance to bile acid

LAB must pass through the stomach and are thus exposed to bile secreted from the gallbladder. To survive in the extreme intestinal environment, LAB must display bile acid tolerance. Many LAB have a high survival rate in the in-

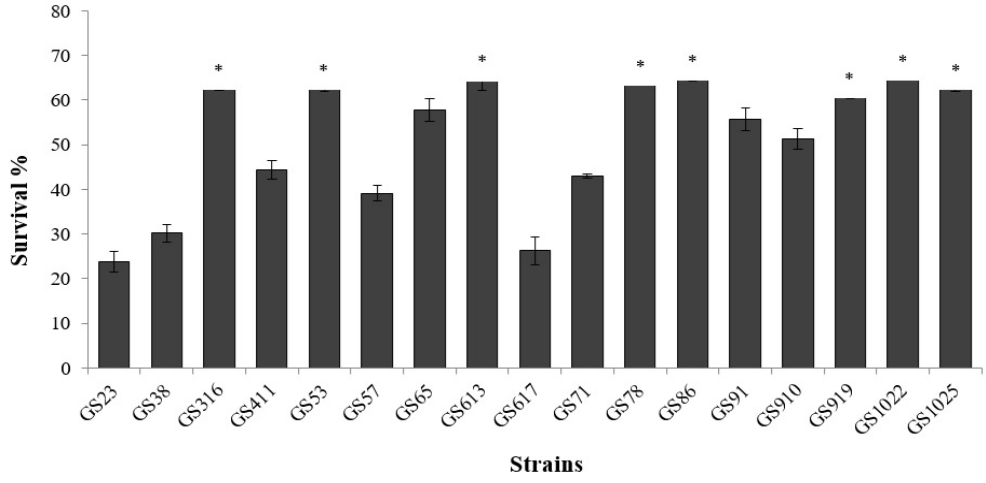

Fig. 2. Survival rates of lactic acid bacteria after 4 hr at a pH of 2.5.

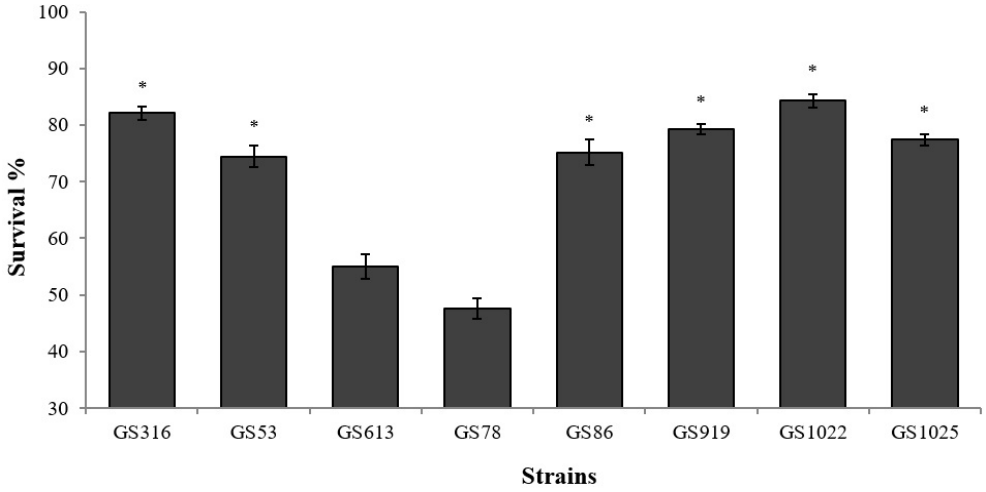

Fig. 3. Survival rates of lactic acid bacteria after 4 hr in 0.3% (w/v) oxgall.

testinal environment, and resistance to bile has been reported to be due to a bile acid-degrading enzyme [8]. Gilliland [9] reported that cholesterol-degrading strains have a strong resistance to bile salts and bile acid-degrading activity. Six of the eight strains with high resistance to acid also had a high survival rate of >70% in the 0.3%(w/v) oxgall-supplemented medium (Fig. 3). Six strains with cholesterol degradation potential were used in a simulated digestive fluid tolerance test.

Autoaggregation and coaggregation assays

For the LAB used as probiotics to function continuously in the body, they must have the ability to attach to the intestinal cells. The autoaggregation ability is a method to indirectly confirm the cell adhesion ability. It is generally understood that strains with a high aggregation ability also have a high cell adhesion ability. In this study, 11 strains showed a aggregation ability of 19% – 89% after 24 hr (Table 2). Among these strains, *C. albicans* ATCC 10231 showed the most effective autoaggregation ability. Among the LAB, *P. inopinatus* GS316 showed a higher autoaggregation rate and better adhesion ability than the other strains. The ability to coaggregate with pathogenic strains prevents the clustering of disease-causing microorganisms. Therefore, LAB strains can prevent infection by competitively binding to pathogenic microorganisms and intestinal cells. The coaggregation abilities of five pathogenic strains and six LAB were investigated, and *P. inopinatus* GS316 showed the highest coaggregation ability (>60%), outperforming all other experimental groups (Table 3). *L. plantarum* GS86 and *L. plantarum*

Table 2. Autoaggregation percentages for probiotic strains of Gajami Sikhae origin after incubation

Probiotic strains	% of autoaggregation (25℃)			
	2 hr	4 hr	20 hr	24 hr
Pathogenic strains				
C. albicans ATCC 10231	6.28±0.17^{d}	15.39±0.34^{e}	70.08±0.81^{h}	89.43±1.54^{g}
E. coli KCTC 2571	3.42±0.37^{c}	10.82±0.31^{bc}	51.10±0.92^{g}	61.61±1.39^{f}
H. pylori HPKCTC B0150	3.45±0.36^{c}	12.54±0.80^{cd}	47.61±0.89^{f}	50.27±1.61^{e}
L. monocytogenes KCTC 13064	2.47±0.28^{ab}	10.26±0.35^{b}	21.73±1.23^{b}	34.46±2.14^{c}
S. aureus KCTC 1916	3.39±0.64^{c}	12.20±1.92^{bc}	17.41±0.25^{a}	19.07±2.70^{a}
Probiotic strains				
P. inopinatus GS316	5.87±0.77^{d}	14.33±1.66^{de}	47.45±1.41^{f}	58.84±1.49^{f}
P. pentosaceus GS53	2.09±0.95^{a}	6.79±0.71^{a}	20.18±1.27^{b}	26.14±1.22^{b}
L. plantarum GS86	3.45±0.36^{c}	11.17±0.76^{bc}	31.37±1.59^{d}	37.20±2.31^{c}
Lc. mesenteroides GS919	2.47±0.28^{ab}	7.73±0.75^{a}	24.33±0.83^{c}	28.24±1.07^{b}
L. brevis GS1022	3.99±0.64^{c}	11.05±2.13^{bc}	31.57±0.67^{d}	37.64±1.95^{c}
L. plantarum GS1025	3.06±0.14^{bc}	11.12±0.78^{bc}	33.44±1.36^{e}	41.99±1.74^{d}

$^{(a-h)}$Significant differences (p<0.05) among all bacteria strains tested at same time. The data are expressed as the mean ± SD (n=3).

GS1025 showed >35% activity in all experimental groups.

Evaluation of cell surface hydrophobicity

High hydrophobicity among the cell surface properties of microorganisms is strongly related to their ability to adhere to the intestinal cell surface [27]. *L. rhamnosus* GG, one of the representative probiotic LAB, exhibited a hydrophobicity of 53.3% in a study by Todorov [28]. The six LAB selected in this study showed a hydrophobicity of approximately 25% for ethyl acetate, 70% for chloroform, and 95% for xylene (Fig. 4). According to Perez [19], bacterial cell surface hydrophobicity >85% becomes beneficial for adhering to epithelial cells. All the six LAB showed high hydrophobicity to xylene at approximately 90%, suggesting that they have high epithelial cell adhesion ability.

Antimicrobial activity assay

According to Reid [21], LAB inhibit pathogenic microorganisms via various mechanisms, often causing antimicrobial action through metabolites such as lactic acid, hydrogen peroxide, and bacteriocin. In the present study, the antimicrobial activities of LAB isolated from Gajami Sikhae were measured. To measure the antimicrobial activity exhibited by substances other than bacteriocin, the pH of the

Table 3. Coaggregation of probiotic and pathogenic strains as determined by spectrophotometry

Probiotic strains	% of coaggregation (25℃)			
	2 hr	4 hr	20 hr	24 hr
Coaggregation with *C. albicans* ATCC 10231				
P. inopinatus GS316	3.98 ± 0.42^{c}	10.49 ± 0.83^{e}	78.85 ± 0.88^{e}	83.92 ± 1.88^{e}
P. pentosaceus GS53	1.05 ± 0.22^{a}	2.80 ± 0.93^{a}	26.69 ± 0.97^{a}	38.18 ± 0.70^{c}
L. plantarum GS86	2.79 ± 1.06^{b}	9.00 ± 0.57^{d}	47.60 ± 2.42^{d}	63.43 ± 2.09^{d}
Lc. mesenteroides GS919	0.75 ± 0.20^{a}	4.52 ± 0.39^{b}	31.90 ± 1.75^{b}	25.75 ± 0.85^{a}
L. brevis GS1022	3.10 ± 0.14^{bc}	9.29 ± 0.39^{de}	44.76 ± 1.45^{c}	34.15 ± 2.07^{b}
L. plantarum GS1025	3.36 ± 0.34^{bc}	6.66 ± 1.20^{c}	43.46 ± 0.97^{c}	34.46 ± 0.76^{b}
Coaggregation with *E. coli* KCTC 2571				
P. inopinatus GS316	5.31 ± 0.55^{c}	11.09 ± 0.69^{d}	50.15 ± 1.53^{e}	65.84 ± 1.64^{c}
P. pentosaceus GS53	1.44 ± 0.55^{a}	4.56 ± 0.66^{a}	16.12 ± 0.32^{a}	23.84 ± 0.88^{a}
L. plantarum GS86	4.33 ± 0.58^{bc}	9.24 ± 0.60^{c}	32.06 ± 1.02^{d}	42.00 ± 1.65^{b}
Lc. mesenteroides GS919	2.36 ± 0.66^{a}	7.32 ± 0.37^{b}	18.50 ± 0.60^{b}	23.45 ± 1.76^{a}
L. brevis GS1022	4.31 ± 0.56^{bc}	8.90 ± 0.65^{c}	28.67 ± 1.10^{c}	40.02 ± 2.48^{b}
L. plantarum GS1025	3.62 ± 0.64^{b}	8.82 ± 0.64^{c}	30.54 ± 1.31^{cd}	40.96 ± 2.11^{b}
Coaggregation with *H. pylori* HPKCTC B0150				
P. inopinatus GS316	4.60 ± 0.26^{c}	10.91 ± 0.90^{b}	27.65 ± 0.66^{c}	60.37 ± 0.41^{f}
P. pentosaceus GS53	2.49 ± 0.36^{a}	12.38 ± 0.66^{cd}	30.35 ± 0.39^{d}	27.64 ± 0.50^{b}
L. plantarum GS86	3.47 ± 0.49^{b}	9.15 ± 0.26^{a}	24.62 ± 0.50^{b}	45.02 ± 0.80^{e}
Lc. mesenteroides GS919	3.65 ± 0.49^{b}	12.17 ± 0.29^{c}	22.18 ± 0.29^{a}	23.69 ± 0.47^{a}
L. brevis GS1022	4.47 ± 0.30^{c}	13.29 ± 0.50^{d}	25.05 ± 0.36^{b}	34.72 ± 0.76^{c}
L. plantarum GS1025	3.47 ± 0.32^{b}	9.89 ± 0.67^{ab}	31.90 ± 0.73^{e}	40.57 ± 0.37^{d}
Coaggregation with *L. monocytogenes* KCTC 13064				
P. inopinatus GS316	4.71 ± 1.50^{b}	11.39 ± 0.55^{d}	49.06 ± 0.87^{d}	61.89 ± 1.74^{c}
P. pentosaceus GS53	2.56 ± 0.78^{a}	5.51 ± 0.56^{a}	18.25 ± 0.55^{a}	25.87 ± 1.39^{a}
L. plantarum GS86	3.70 ± 0.53^{ab}	9.64 ± 0.23^{c}	31.09 ± 1.47^{c}	41.88 ± 1.03^{b}
Lc. mesenteroides GS919	3.47 ± 0.64^{ab}	7.54 ± 0.41^{b}	20.64 ± 0.73^{b}	25.74 ± 2.07^{a}
L. brevis GS1022	3.91 ± 0.42^{ab}	8.70 ± 1.40^{bc}	31.99 ± 0.90^{c}	40.99 ± 0.74^{b}
L. plantarum GS1025	3.83 ± 0.76^{ab}	9.08 ± 1.21^{bc}	30.82 ± 0.94^{c}	42.89 ± 2.27^{b}
Coaggregation with *S. aureus* KCTC 1916				
P. inopinatus GS316	5.03 ± 0.81^{d}	11.32 ± 0.66^{c}	44.82 ± 0.96^{c}	60.53 ± 2.13^{d}
P. pentosaceus GS53	6.31 ± 0.60^{e}	6.28 ± 0.23^{a}	20.50 ± 0.62^{a}	27.46 ± 0.65^{a}
L. plantarum GS86	4.16 ± 0.43^{cd}	7.99 ± 0.47^{b}	28.77 ± 1.05^{b}	38.63 ± 0.85^{b}
Lc. mesenteroides GS919	2.13 ± 0.24^{a}	6.52 ± 0.41^{a}	22.08 ± 0.69^{a}	24.73 ± 2.10^{a}
L. brevis GS1022	3.36 ± 0.49^{bc}	8.44 ± 0.88^{b}	27.18 ± 1.55^{b}	37.15 ± 1.32^{b}
L. plantarum GS1025	2.81 ± 0.79^{ab}	8.45 ± 0.44^{b}	29.17 ± 1.48^{b}	41.75 ± 1.94^{c}

$^{(a-f)}$ Significant differences (p<0.05) among all bacteria strains tested at same time. The data are expressed as the mean ± SD (n=3).

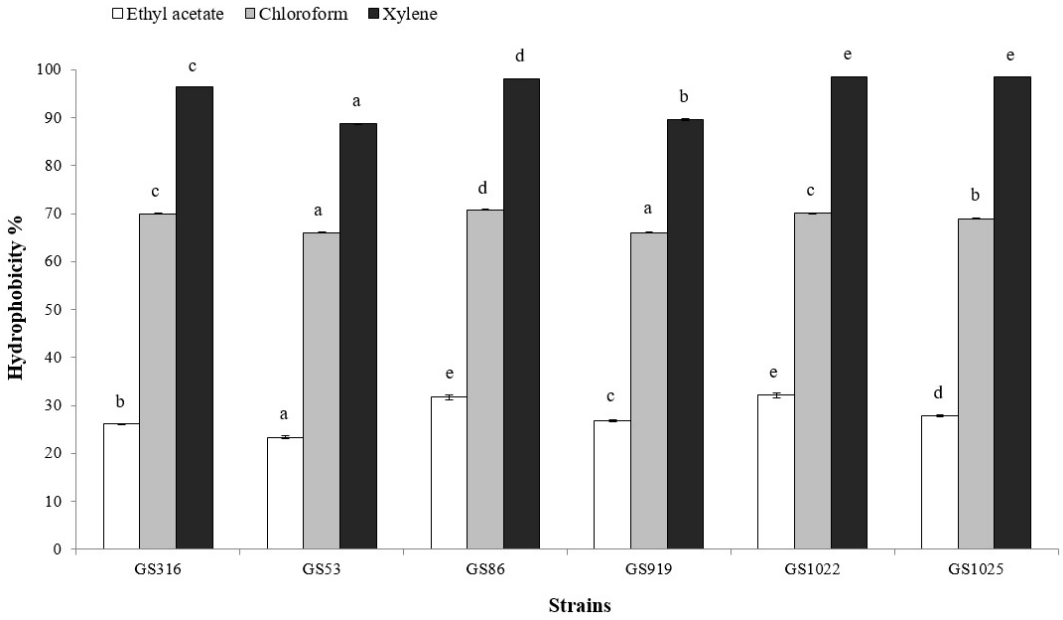

Fig. 4. Cellular hydrophobicity of lactic acid bacteria. Data are expressed as the mean ± SD (n=3). Means with different letters (a-e) above the bars for the same strain are significantly different at $p<0.05$ by Duncan's multiple range test.

culture was not controlled. No LAB showed antimicrobial activity against *C. albicans* ATCC 10231. All strains except *Lc. mesenteroides* GS919 and *L. brevis* GS1025 showed higher antimicrobial activity than ampicillin 10 μg/ml against *E. coli* KCTC 2571. All LAB showed lower antimicrobial activity against *H. pylori* HPKCTC B0150, *L. monocytogenes* KCTC 13604, and *S. aureus* KCTC 1916 than ampicillin 10 μg/ml (Table 4).

Measurement of DPPH free radical scavenging ability

Oxidative stress causes harmful biochemical reactions and various vascular diseases, mutations, cancer, and aging in living organisms [3]. Recently, studies on the antioxidant activity measurement using natural products have been actively performed. Spices such as herbs, oregano, and cinnamon have a high antioxidant ability, similar to those of medicinal materials [25]. LAB producing superoxide dismutase (SOD), such as *L. lactis* and *L. plantarum*, were found to have

Table 4. Antimicrobial activity of probiotic strains present in Gajami Sikhae

Indicator strain	Inhibition zone (mm)				
	C. albicans ATCC 10231	*E. coli* KCTC 2571	*H. pylori* HPKCTC B0150	*L. monocytogenes* KCTC 13064	*S. aureus* KCTC 1916
Probiotic strains					
P. inopinatus GS316	ND	23.1 ± 0.36^{d}	16.4 ± 0.60^{c}	12.1 ± 0.36^{a}	11.1 ± 0.42^{a}
P. pentosaceus GS53	ND	24.4 ± 0.60^{e}	16.1 ± 0.31^{bc}	ND	10.7 ± 0.53^{a}
L. plantarum GS86	ND	20.7 ± 0.57^{bc}	16.3 ± 0.70^{bc}	15.9 ± 0.60^{b}	15.9 ± 0.71^{b}
Lc. mesenteroides GS919	ND	10.5 ± 0.76^{a}	15.3 ± 0.61^{ab}	ND	10.3 ± 0.64^{a}
L. brevis GS1022	ND	21.2 ± 0.76^{c}	18.2 ± 0.70^{d}	18.8 ± 0.31^{c}	15.9 ± 0.83^{b}
L. plantarum GS1025	ND	ND	14.8 ± 0.50^{a}	ND	ND
Probiotic strains					
Ampicillin*	ND	19.7 ± 0.25^{b}	31.9 ± 0.40^{e}	34.0 ± 0.20^{d}	32.7 ± 0.35^{c}
Amphotericin B†	11.9 ± 0.40	ND	ND	ND	ND

The data are expressed as the mean ± SD (n=3). ND; Not Detected.
Means with differed letters $^{(a-e)}$ with the value in the same pathogenic strains are significantly different at $p<0.05$ by Duncan's multiple range test.
*Ampicillin concentration: 10 μg/ml.
†Amphotericin B concentration: 50 μg/ml.

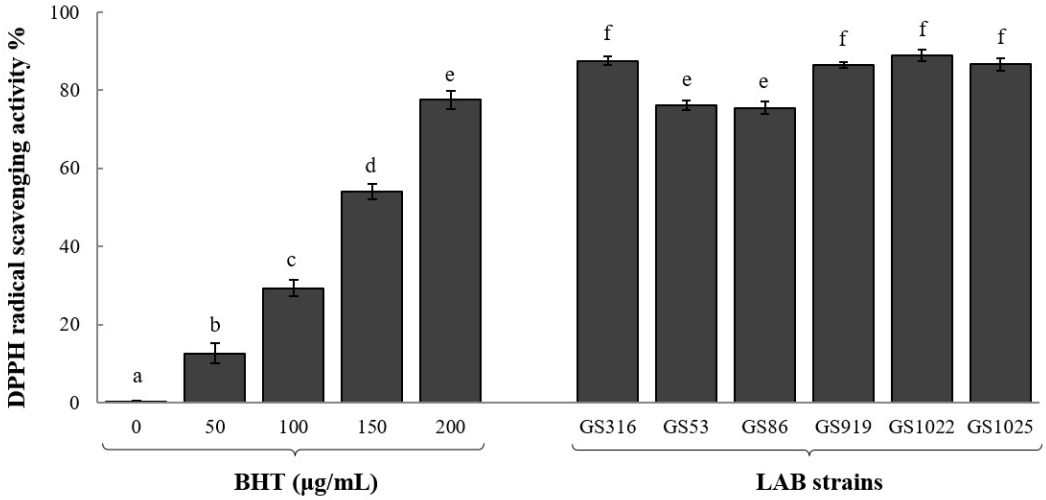

Fig. 5. DPPH radical scavenging activity of lactic acid bacteria. Data are expressed as the mean ± SD (n=3). Means with different letters (a-e) above the bars for the same strains are significantly different at $p<0.05$ by Duncan's multiple range test.

effective antioxidant activity as well as improved colitis. To investigate the antioxidant activity of isolated LAB, DPPH radical scavenging activity of LAB culture was assessed. All six LAB showed over 75% activity (Fig. 5), demonstrating that all the LAB have antioxidant activity similar to that of BHT 200 μg/ml (77.6%), a synthetic antioxidant.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 가자미 식해 발효에서 *Lactobacillus brevis* GS1022과 *Pediococcus inopinatus* GS316의 균총 변화 연구

임수정[1] · 배은영[1] · 설민경[1] · 조영제[1] · 정희영[2] · 김병오[1]*
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유산균은 장 건강, 심장질환, 우울증, 비만 등을 예방하고 치료할 수 있다고 널리 알려져 있고, 특히 한국은 다양한 발효식품에서 유산균을 섭취할 수 있다. 하지만 국내 발효식품에서 분리된 유산균에 대한 연구는 대부분 김치에 한정되어 있기 때문에 대표적인 어류 전통 발효식품인 식해류의 유산균에 대한 연구는 미비한 실정이다. 따라서 본 연구는 한국의 전통발효식품인 가자미 식해의 발효에 관여하는 유산균의 배양일에 따른 균총의 변화를 관찰하고, 가자미 식해로부터 프로바이오틱스로 이용 가치가 있는 균을 분리하기 위해 시행되었다. 포항의 가정집에서 제조된 가자미 식해에서 유산균을 제조일로부터 50일까지 5일 간격으로 분리하고 동정하였고, 동정 결과 *P. pentosaceus, P. inopinatus, Lc. mesenteroides, L. brevis, L. plantarum* 5개 균주가 발효에 관여함을 알 수 있었다. 발효가 진행됨에 따라 초기 발효에는 *Pediococcus* sp.가, 중기 이후부터는 *Lactobacillus* sp.가 우세하게 발효에 관여하는 것을 확인할 수 있었다. 분리한 유산균 중 우수한 프로바이오틱 활성을 가지는 균주를 알아보기 위하여 산 내성, 담즙산 내성 시험을 통해 균주를 선별한 뒤 응집능, 표면 소수성, 항균 활성, 항산화 활성 등을 평가하였다. 그 결과 본 연구의 가자미 식해 분리 균주 중 최종적으로 선택된 소화액 내성이 뛰어난 *L. brevis* GS1022와 응집능이 뛰어난 *P. inopinatus* GS316은 프로바이오틱 균주로 이용할 가치가 충분하다고 사료된다.