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ORIGINAL ARTICLE

In vitro study of potentially probiotic lactic acid bacteria strains isolated from kimchi

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Abstract The objective of the present study was to investigate lactic acid bacteria (LAB) isolated from kimchi for their potential probiotic use. Ten preselected LAB strains were evaluated for their functionality and safety. Examined characteristics included acid and bile tolerance, cell adhesion, antimicrobial activity against pathogens, hemolytic activity, undesirable biochemical characteristics, and antibiotic resistance. Results indicated that consumption of these 10 strains does not pose any health risk, as they were not hemolytic and exhibited no undesirable biochemical activity or antibiotic resistance. In particular, three strains, *Lactobacillus plantarum* NO1, *Pediococcus pentosaceus* MP1, and *Lactobacillus plantarum* AF1, showed high degrees of acid and bile tolerance, adherence to Caco-2 and HT-29 cells, and antimicrobial activity against four pathogens (*Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella typhi*, and *Listeria monocytogenes*). These results suggest that LAB strains from kimchi may have potential use as novel probiotics.

Keywords Kimchi · Lactic acid bacteria · Safe and functional properties (in vitro) · Probiotic

Introduction

The growing demand for healthier foods is stimulating innovation and new product development in the food industry worldwide (Saarela et al. 2000). For example, the health-promoting effects of probiotics have led to their increased use in fermented dairy foods (Guglielmotti et al. 2007; Maragkoudakis et al. 2006; Bertazzoni et al. 2004). Among these microorganisms, lactic acid bacteria (LAB), especially *Lactobacillus* and *Bifidobacterium* spp., are the most commonly used probiotics in food for human consumption (Foligné et al. 2010). LAB are generally regarded as safe (GRAS), as they have a long history of safe use as starter culture bacteria (Carr et al. 2002). However, it has been frequently reported that some members of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Enterococcus*, and *Bifidobacterium* cause infections that in some patients has led to clinical conditions such as endocarditis and septicemia (Liong 2008). There are many sources of exposure to these bacteria, including probiotics, fermented foods, and the host's own microbiota (Borriello et al. 2003), and it was recently speculated that bacteria in food may act as reservoirs of antibiotic resistance genes (Franz et al. 2005; Ammor et al. 2007; Clementi and Aquilanti 2011; Mathur and Singh 2005). Indeed, although LAB have been accepted as safe, this assessment was not until recently based on any real scientific criteria (Donohue 2006).

It is now recognized that probiotic products exhibit specific properties such as gastric acid and bile tolerance, adherence to epithelial surfaces, and antagonist activity against pathogens (Saarela et al. 2000). They also lack undesirable properties such as expression of virulence factors, harmful biochemical activity, and antibiotic resistance (Donohue 2006; Ammor et al. 2007; Clementi and Aquilanti 2011). These activities offer opportunities for the development of beneficial products for humans and animals. Accordingly, new species and more specific bacterial strains are continuously being sought as novel probiotic candidates. At the same time, the efficacy of these new strains should be carefully assessed. And an evaluation of the new candidates should be applied to all strains of bacteria, including those traditionally used in food fermentation, to confirm their safety status.

Kimchi is a traditional Korean food and has a long history of safe production and consumption (Chang and

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Chang 2010). Kimchi is consumed every day as a side dish in Korea, with Korean people consuming an average of 91.9 g of kimchi per day (World Institute of Kimchi 2011). Kimchi fermentation is a natural process that is initiated by a variety of microorganisms originally present in the raw kimchi materials. Although there are about 200 species of microorganisms involved in kimchi fermentation, the microorganisms primarily responsible are LAB such as *Leuconostoc* spp. and *Lactobacillus* spp. (Chang and Chang 2010; Nam et al. 2009). Consequently, kimchi is a good source of potentially beneficial LAB.

The objective of the present study was to investigate LAB isolated from kimchi for their potential probiotic use. Ten preselected LAB isolates from kimchi, including *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp., were evaluated for their functionality and safety. Examined characteristics included acid and bile tolerance, cell adhesion, antimicrobial activity against pathogens, hemolytic activity, undesirable biochemical characteristics, and antibiotic resistance.

Materials and methods

Bacterial strains and media

A total of 10 LAB strains preselected among 409 LAB cultures isolated from kimchi were used. The preselected isolates have been identified and genotipically/phenotipically typed in previous works (see Table 1 for references). The selection of strains was carried out previously based on distinct characteristics, including antimicrobial activity and metabolic characteristics [e.g., production of γ-aminobutyric acid (GABA), exopolysaccharide (EPS), or mannitol]. Bacterial strains and media used in the present work are summarized in Table 1 along with relevant references. *Lactobacillus rhamnosus* GG ATCC 53103 and *Bacillus cereus* ATCC 14579 were used as reference strains for the examination of cell adhesion and hemolysis, respectively. Pathogens were cultured for 12 h at 37 °C in Luria-Bertani (LB) broth (Difco, Sparks, MD, USA) or Brain Heart Infusion (BHI) broth (Difco). LAB were propagated at 30 °C for 24 h in de Man Rogosa and Sharpe (MRS; Difco) and Muller Hinton (MH; Difco) broth without shaking. For EPS production, *Leuconostoc kimchii* GJ2 was cultivated in sucrose medium (1 % tryptone, 0.5 % yeast extract, 0.5 % dipotassium phosphate, 0.5 % diammonium citrate, 5 % sucrose, pH7.0). ATCC strains were purchased from the American Type Culture Collection (Manassas, VA, USA).

Acid and bile tolerance

Tolerances levels of LAB to acid and bile salt were assessed as described previously with modification (Santini et al. 2010; Lian et al. 2003). LAB were first cultivated in 5 ml of MRS broth at 30 °C for 24 h. Cultures were then harvested (9,950*g*, 5 min), after which approximately 8.2–9.6 log CFU/ml of cells were resuspended in 1 ml of phosphate-buffered saline (PBS, pH2.5; Hyclone, Logan, UT, USA) or simulated gastric juice (SGJ; pepsin 3 mg dissolved in 1 ml of 0.5 % saline buffer, pH2.5) and/or bile salt (0.3 % oxgall dissolved in PBS, pH8.0). Suspensions were incubated at 37 °C for 1 h in PBS (pH2.5) or SGJ, or for 3 h in bile salt. Thereafter, the suspensions were harvested (9,950*g*, 5 min) and resuspended in MRS broth, after which viable cell numbers were counted on MRS agar after incubation at 30 °C for 48 h. In parallel, controls were set up in which LAB were suspended in MRS broth without acid or bile salt. To investigate the effects of EPS on acid and bile tolerance, *Leuconostoc kimchii* GJ2 was cultivated in both MRS and sucrose broth media.

In vitro adhesion assay

Adhesion of LAB to Caco-2 and HT-29 cells was assayed according to the method of Fernández et al. (2003) with modification. In brief, monolayers of Caco-2 (American Type Culture Collection, Manassas, VA, USA) and HT-29 cells (American Type Culture Collection) were prepared by inoculating 5.7 log CFU/ml into 24-well tissue culture plates (Corning Costar, Cambridge, MA, USA) containing Dulbecco's Modified Eagle Medium (DMEM; Hyclone) or Rosewell Park Memorial Institute 1640 medium (RPMI; Hyclone), respectively. Both media were supplemented with 10 % (v/v) fetal bovine serum (FBS; Hyclone). Once cells had formed a monolayer, approximately 7.2–9.6 log CFU/ml of viable LAB was added to each well and incubated at 37 °C for 1 h in a 5 % CO_2 incubator (Sci 165D; Astec, Tokyo, Japan). After incubation, monolayers were washed three times with PBS to release unattached bacteria. Total numbers of adherent bacteria in each well were then counted by lysing cells; 1 ml of 0.05 % (v/v) Triton X-100 was added to wells, after which the plate was shaken (Green SSeriker Vison, Gyeonggi-Do, Korea) for 10 min at 160 rpm at room temperature. Counts of viable bacteria were then made on MRS agar after incubation at 30 °C for 24–48 h. *Lactobacillus rhamnosus* GG was used as a positive control for the adhesion assay.

Antimicrobial activity

Antimicrobial activities against four pathogens were assessed using the agar well diffusion method with modification (Magnusson and Schnürer 2001). BHI or LB plates were spread with each pathogen at a concentration of 6.0 log CFU/ml. A well with a diameter of 5.0 mm was then punched out from each agar plate, after which LAB

Table 1 Bacterial strains used in this study

Strain	Medium	Source	Property	Reference	GenBank Accession No.
LAB					
Lactobacillus buchneri MS	MRS, MH	Kimchi	GABA-production	Cho et al. (2007)	JX490159
Lactobacillus plantarum AF1	MRS, MH	Kimchi	Antifungal activity	Yang and Chang (2010)	FJ386491
Lactobacillus plantarum NO1	MRS, MH	Kimchi	Antagonistic activity against *H. pylori*	Lee and Chang (2008)	JX490160
Leuconostoc citreum GJ7	MRS, MH	Kimchi	Bacteriocin-production	Chang et al. (2007)	EF121354
Leuconostoc citreum GR1	MRS, MH	Kimchi	Mannitol-production	Chang et al. (2011)	JX490161
Leuconostoc citreum C2	MRS, MH	Kimchi	Mannitol-production	Jung and Chang (2011)	JX490162
Leuconostoc mesenteroides PH1	MRS, MH	Kimchi	Mannitol-production	Jung and Chang (2011)	JX490163
Leuconostoc mesenteroides DM1	MRS, MH	Kimchi	Mannitol-production	Jung and Chang (2011)	JX490158
Leuconostoc kimchii GJ2	MRS, MH, Sucrose media	Kimchi	EPS-production	Kim and Chang (2006)	FJ040198
Pediococcus pentosaceus MP1	MRS, MH	Kimchi	Mannitol-production	Jung and Chang (2011)	JX490164
Lactobacillus rhamnosus GG ATCC 53103	MRS, MH	ATCC	Adherence to epithelium	Lebeer et al. (2007)	
Pathogens					
Staphylococcus aureus ATCC 29213	LB	ATCC	Food born pathogen	Trampuz et al. (2007)	
Escherichia coli O157:H7 ATCC 43895	LB	ATCC	Food born pathogen	Stasic et al. (2012)	
Salmonella typhi ATCC 14028	LB	ATCC	Food born pathogen	Tindall et al. (2005)	
Listeria monocytogenes ATCC 19113	BHI	ATCC	Food born pathogen	Park et al. (2009)	
Bacillus cereus ATCC 14579	LB	ATCC	β-Hemolysis positive strain	Hornstra et al. (2006)	

(9.0 log CFU/ml) in 70 μl of MRS soft agar were deposited in each well. After incubation at 37 °C for 24 h, the diameter of the clear zone around the well was measured.

Enzymatic activities

Enzymatic activities were assayed using an API-ZYM kit (BioMérieux, Lyon, France) according to the manufacturer's instructions. LAB cultures were harvested and resuspended in sterile distilled water, after which 65 μl of suspension (Mcfarland standard 1) was deposited into each well, and the plate was incubated at 37 °C for 4 h. Then, one drop of ZYM-A and ZYM-B reagent was added to each well, and enzyme activity was read after allowing the reaction to run for 5 min.

Hemolysis

Hemolysis was detected by streaking bacterial cells on blood agar containing 7 % horse blood (Oxoid, Hampshire, UK). The plate was then incubated at 30 °C for 24–48 h, after which the clear zone around the colony was observed.

Antibiotic susceptibility

LAB were evaluated for their susceptibility to antibiotics according to the technical guidelines of the European Food Safety Authority (EFSA 2008). The minimal inhibitory concentrations (MIC) of nine antibiotics, including ampicillin, vancomycin, gentamycin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline and chloramphenicol (Sigma, St. Louis, MO, USA), were determined. After culturing LAB in MRS broth for 24 h, cells were centrifuged (9,950*g*, 5 min) and resuspended in MH broth containing 0.5 % dextrose. Resultant cell suspensions were then further diluted in the same medium to a density of 5.0 log CFU/ml. Each antibiotic was added to aliquots of the diluted cell suspension, which were incubated at 30 °C for 24–48 h without shaking. Cell growth was observed visually and measured based on the turbidity of the suspensions at 600 nm (Ultrospec 2100 pro; Amersham Biosciences, Uppsala, Sweden). MIC values were determined using the serial antibiotic dilution procedure in MH broth containing 0.5 % dextrose.

Statistical analysis

Data are presented as the means and standard deviations (means ± SD) of three independent experiments performed in triplicate. All statistical analyses on the data were performed using SPSS v.18.0 for Windows (SPSS, Chicago, IL, USA) with statistical significance determined at $P<0.05$.

Table 2 Acid and bile tolerance of LAB

Strain	Initial mean counts log CFU/ml	Survival after1 h at pH 2.5				Survival after 3 h at pH8.0	
		PBS		SGJ		Oxgall	
		log CFU/ml	% [a]	log CFU/ml	%	log CFU/ml	%
Lactobacillus buchneri MS	9.46±0.11	6.81±0.59 b	72.0	8.85±0.35 a	93.6	8.10±0.02 c	85.6
Lactobacillus plantarum AF1	9.32±0.08	8.79±0.19 a	94.3	9.28±0.09 a	99.6	7.95±0.04 c	85.3
Lactobacillus plantarum NO1	9.58±0.30	9.44±0.25 a	98.5	9.55±0.14 a	99.7	8.14±0.03 c	85.0
Leuconostoc citreum GJ7	9.39±0.13	3.74±0.14 c,d	39.8	5.02±0.27 b	53.5	8.01±0.11 c	85.3
Leuconostoc citreum GR1	9.39±0.01	4.33±1.07 c	46.1	4.19±0.12 b,c	44.6	7.56±0.32 d	80.5
Leuconostoc citreum C2	9.38±0.01	3.59±0.62 c,d	38.3	2.73±0.57 d,e	29.1	7.42±0.07 d	79.1
Leuconostoc mesenteroides PH1	9.42±0.01	4.16±0.83 c	44.2	2.76±0.41 d	29.3	8.21±0.08 b,c	87.2
Leuconostoc mesenteroides DM1	9.39±0.01	4.75±0.35 c	50.6	4.24±0.89 b,c	45.2	8.13±0.03 c	86.6
Leuconostoc kimchii GJ2 (non-EPS-producing)	9.09±0.12	2.24±0.06 d	24.6	1.50±0.06 e	16.5	5.83±0.02 e	64.1
Leuconostoc kimchii GJ2 (EPS-producing)	8.21±0.01	3.57±0.14 c	43.5	3.15±0.46 c,d	38.4	8.16±0.01 a	99.4
Pediococcus pentosaceus MP1	9.27±0.01	6.84±0.67 b	73.8	8.91±0.27 a	96.1	8.37±0.04 b	90.3

All Values are means ± standard deviation

Means in the same column with different lowercase letters are significantly different ($P<0.05$)

[a] % Percent inhibition: final (CFU/ml)/control (CFU/ml)×100. Tolerance 100 % indicates that the growth rate of the strain was not affected by the treatment

Results and discussion

Effects of acid and bile on cell survival

We initially tested the abilities of the 10 selected LAB strains to survive acid or bile stress. We found that treatment of LAB with acid or bile reduced viable cell numbers (Table 2). Following acid treatment, counts of viable *Lactobacillus plantarum* strains (AF1, NO1) and *Pediococcus pentosaceus* MP1, a homofermentative LAB, were clearly higher than counts of other strains (heterofermentative LAB). It has been previously shown that *Leuconostoc kimchii* GJ2 can produce 21.49±0.46 mg/ml and 0.14±0.09 mg/ml of EPS (in crude form) in sucrose and MRS media, respectively (Kim and Chang 2006). In this study, to investigate the effect of EPS production on bacterial cell viability following acid and bile treatment, *Leuconostoc kimchii* GJ2 was cultivated in sucrose media for EPS production as well as in MRS media as a control. Acid tolerance level of *Leuconostoc kimchii* GJ2 producing EPS in sucrose media was twice that in MRS media. Bile salt had a smaller effect on LAB viability than did acid. Bile treatment resulted in a 1–2 log reduction in viable cell numbers whereas acid reduced viable cell numbers by 1–6 log. EPS production further reduced the effect of bile on bacterial cell viability. These findings are consistent with earlier investigations, which reported that EPS production reduces the effects of low pH and bile on the cell viability of various strains (Sabir et al. 2010; Yuksekdag and Aslim 2010).

Adhesion properties to human cell lines

As shown in Fig. 1, *Lactobacillus plantarum* NO1, *Pediococcus pentosaceus* MP1, and EPS-producing *Leuconostoc kimchii* GJ2 all showed a higher percentage of adhesion to Caco-2 cells than did *Lactobacillus rhamnosus* GG. Based on these data, we selected *P. pentosaceus* MP1 and EPS-producing *Leuconostoc kimchii* GJ2, which showed the highest adhesion property to Caco-2 cells in Fig. 1, and examined the efficiency of their adhesion to HT-29 cells (Fig. 2). Both *P. pentosaceus* MP1 and EPS-producing *Leuconostoc*

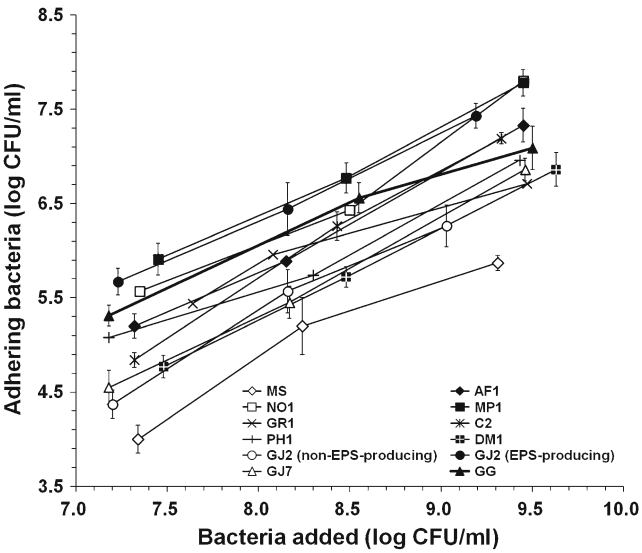

Fig. 1 Adhesion of bacterial cells to Caco-2 cells

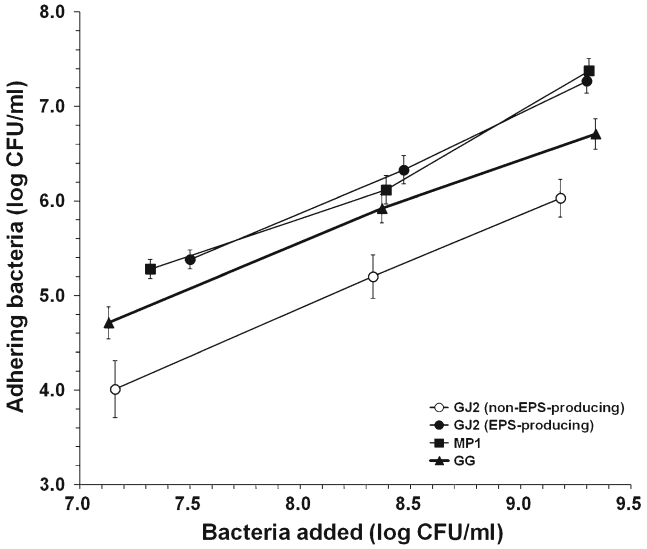

Fig. 2 Adhesion of bacterial cells to HT-29 cells

kimchii GJ2 showed greater adhesion to HT-29 cells than did *Lactobacillus rhamnosus* GG, regardless of bacterial cell density. Adhesion of all LAB isolates to both Caco-2 and HT-29 cells (inoculated bacterial cells into cell lines) was concentration-dependent, with adhesion to HT-29 cells (average adhesion rate of 0.1–1.0 %) being clearly lower than adhesion to Caco-2 cells (average rate of 0.2–2.3 %) (Figs. 1, 2). Previous results have similarly indicated that adhesion of bacterial cells to HT-29 cells is markedly lower than to Caco-2 cells (Laparra and Sanz 2009; Gopal et al. 2001).

Leuconostoc kimchii GJ2 as a control was clearly less adherent to both Caco-2 and HT-29 cells than EPS-producing *Leuconostoc kimchii* GJ2 (Figs. 1, 2). This is consistent with the findings of Russo et al. (2012), who reported that bacterial adhesion increases with EPS production. Bacterial adhesion to the intestinal epithelial mucosa is a complicated process that is influenced by multiple surface biophysical and biochemical properties of both the bacteria and epithelial mucosa (Servin and Coconnier 2003). It has been suggested that the EPS produced by LAB has an ecological function related to cell adhesion (Ruas-Madiedo et al. 2002).

Antimicrobial activity

The 10 selected LAB strains generally exerted growth inhibitory effects on the four tested pathogens, although *Lactobacillus buchneri* MS did not inhibit *Listeria monocytogenes* (Table 3). In particular, *Lactobacillus plantarum* AF1, *Lactobacillus plantarum* NO1, *Leuconostoc mesenteroides* DM1, and *Pediococcus pentosaceus* MP1 showed strong antimicrobial activities against all four tested pathogens, and all LAB strongly inhibited *Staphylococcus aureus*. Antimicrobial compounds from LAB include organic acids, CO_2, H_2O_2, and bacteriocins (Ammor et al. 2006). Organic acids, bacteriocins, δ-dodecalactone, and cyclo (Leu-Leu) are all known to be inhibitory substances released by the LAB isolates (Chang et al. 2007; Kim and Chang 2006; Yang and Chang 2008). Production of these substances, which inhibit the growth of undesirable bacteria and pathogens, is a beneficial feature of probiotics (Dunne et al. 2001).

Enzymatic activities

Enzymatic activities of the selected LAB were measured using an API-ZYM kit (Table 4). None of the isolates showed alkaline phosphatase, α-chymotrypsin, β-glucuronidase, or α-fucosidase activity. It has been reported that β-glucuronidase or α-chymotrypsin activity may have negative effects in the colon (Heavey and Rowland 2004; Delgado et al. 2008). Weak-to-moderate N-acetyl-β-glucosaminidase activity was observed with *Lactobacillus plantarum* NO1, *Lactobacillus plantarum* AF1, *Leuconostoc citreum* GJ7, and *Pediococcus pentosaceus* MP1. Further,

Table 3 Antimicrobial activities of 10 LAB

Strain	Inhibition[a] (pathogen)			
	Staphylococcus aureus	*E. coli* O157:H7	*Salmonella typhi*	*Listeria monocytogenes*
Lactobacillus buchneri MS	++	+	+	–
Lactobacillus plantarum AF1	+++	++	++	++
Lactobacillus plantarum NO1	+++	++	+++	++
Leuconostoc citreum GJ7	+++	+	+	+
Leuconostoc citreum GR1	++	+	+	+
Leuconostoc citreum C2	++	+	+	+
Leuconostoc mesenteroides PH1	+++	+	++	+
Leuconostoc mesenteroides DM1	+++	++	++	+
Leuconostoc kimchii GJ2	++	+	++	+
Pediococcus pentosaceus MP1	+++	++	++	+

[a]+ 7.42–10.28 mm; ++ 10.29–13.14 mm; +++ 13.15–16.00 mm; – no inhibition zone

Table 4 API ZYM analysis of the enzyme activities of the LAB

Enzyme	LAB strain									
	Lactobacillus buchneri MS	*Lactobacillus plantarum* AF1	*Lactobacillus plantarum* NO1	*Leuconostoc citreum* GJ7	*Leuconostoc citreum* GR1	*Leuconostoc citreum* C2	*Leuconostoc mesenteroides* PH1	*Leuconostoc mesenteroides* DM1	*Leuconostoc kimchii* GJ2	*Pediococcus pentosaceus* MP1
Alkaline phosphatase	0[a]	0	0	0	0	0	0	0	0	0
Esterase (C4)	0	0	10	5	0	0	0	0	0	0
Esterase lipase (C8)	5	5	5	0	0	0	0	0	0	0
Lipase (C14)	0	5	0	5	0	0	0	0	0	0
Leucine arylamidase	10	5	30	5	0	0	0	0	0	≥40
Valine arylamidase	5	5	10	5	0	0	0	0	0	10
Cystine arylamidase	0	0	5	5	0	0	0	0	0	0
Trypsin	5	0	0	0	0	0	0	0	0	0
α-Chymotrypsin	0	0	0	0	0	0	0	0	0	0
Acid phosphatase	5	5	5	10	5	0	0	0	20	0
Naphtol-AS-BI-phophohydrolase	10	10	5	5	10	5	0	5	0	0
α-Galactosidase	5	0	5	0	0	0	0	0	0	0
β-Galactosidase	≥40	≥40	≥40	0	0	0	0	0	0	0
β-Glucuronidase	0	0	0	0	0	0	0	0	0	0
α-Glucosidase	0	10	5	10	10	10	10	10	20	0
β-Glucosidase	10	20	10	5	0	0	10	20	0	10
N-Acetyl-β-glucosaminidase	0	10	20	5	0	0	0	0	0	10
α-Mannosidase	0	0	0	5	0	0	0	0	0	0
α-Fucosidase	0	0	0	0	0	0	0	0	0	0

All values are means (n=3)

[a] *0* No enzyme activity; *5*, *10*, *20*, *30*, *≥40* indicates nanomoles of hydrolyzed substrate after 4 h of incubation at 37 °C

only 3 of the LAB isolates (*Lactobacillus buchneri* MS, *L. plantarum* AF1, *L. plantarum* NO1) showed β-galactosidase activity. β-Galactosidase released by probiotics reportedly contributes to the relief of lactose maldigestion symptoms (Leahy et al. 2005; Ouwehand et al. 2002), since β-galactosidase hydrolyzes lactose to glucose and galactose. When we examined lactose fermentation ability using an API 50 CHL kit (BioMérieux), we found that only 3 (*Lactobacillus buchneri* MS, *L. plantarum* AF1, *L. plantarum* NO1) of the 10 isolates were able to ferment lactose (data not shown). This result was surprising as most LAB can ferment lactose (Liu 2003). However, some LAB isolated from kimchi have been previously shown to have lost that ability (Chang 2010), which is consistent with this study. This loss of lactose fermentation ability suggests a lack of a lactose component in kimchi; consequently, kimchi LAB have no need to metabolize lactose. Among LAB in kimchi, the more evolutionally developed strains might have deleted or turned off the expression of lactose metabolic genes in favor of genes enabling the use of other sugars such as glucose, maltose, or sucrose as energy sources. Indeed, all three of these sugars are present in kimchi.

Hemolysis

In this study, none of the tested LAB isolates induced hemolysis on horse blood agar (γ-hemolytic). In contrast, *Bacillus cereus* ATCC 14579 produced a clear zone around its colony on horse blood agar (β-hemolysis).

Antibiotic resistance

The 10 LAB isolates were evaluated for their resistance to nine antibiotics, including those highlighted by EFSA (2008). All the isolates were susceptible to all the antibiotics tested, except vancomycin (Table 5). Bacteria from the genus *Leuconostoc* are known to be intrinsically resistant to vancomycin (Ammor et al. 2007; Clementi and Aquilanti 2011). Moreover, no breakpoint for vancomycin is required for *Lactobacillus reuteri*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus* obligate/facultative heterofermentative, *Pediococcus* spp., or *Leuconostoc* spp. according to the technical guidelines of the EFSA (2008). Therefore, it seems reasonable to conclude that consumption of the LAB isolates examined in the present study does not represent a health risk to humans due to antibiotic resistance.

Conclusion

For the development of novel probiotics, new species and more specific strains of bacteria are being sought. For this purpose, the selection and evaluation of new microorganisms from traditional fermented foods could be a means of

Table 5 Minimum inhibitory concentrations (MIC) of antibiotics for LAB

Strain	MIC (μg/ml)[b]								
	AMP	VAN	GEN	KAN	STR	ERY	CLI	TET	CHL
Break points for facultative heterofermentative lactobacilli[a]	4	n.r.[c]	16	64	64	1	1	8	4
Lactobacillus buchneri MS	1	>512	0.25	4	4	0.06	0.125	8	2
Break points for *Lactobacillus plantarum*[a]	2	n.r.	16	64	n.r.	1	1	32	8
Lactobacillus plantarum AF1	1	>512	0.03	1	0.5	0.03	0.06	4	2
Lactobacillus plantarum NO1	2	>512	0.25	4	2	0.06	1	8	4
Breakpoints for leuconostocs[a]	2	n.r.	16	16	64	1	1	8	4
Leuconostoc citreum GJ7	0.5	>512	2	4	16	0.125	0.06	2	4
Leuconostoc citreum GR1	0.5	>512	2	16	32	0.06	0.015	1	4
Leuconostoc citreum C2	0.5	256	1	16	16	0.06	0.015	1	4
Leuconostoc mesenteroides PH1	2	>512	0.5	16	16	16	0.06	2	4
Leuconostoc mesenteroides DM1	1	>512	0.25	4	4	0.125	0.06	2	2
Leuconostoc kimchii GJ2	2	>512	0.5	8	32	0.03	0.015	2	2
Breakpoints for pediococci[a]	4	n.r.	16	16	64	1	1	8	4
Pediococcus pentosaceus MP1	1	>512	0.5	16	8	0.03	1	4	2

[a] Breakpoints were according to the guidelines of the EFSA (EFSA 2008)

[b] Strains with MICs lower than or equal to the breakpoints are considered susceptible. *AMP* ampicillin; *VAN* vancomycin; *GEN* gentamycin; *KAN* kanamycin; *STR* streptomycin; *ERY* erythromycin; *CLI* clindamycin; *TET* tetracycline; *CHL* chloramphenicol

[c] *n.r* Not required

ensuring safety. Here, we evaluated the functionality and safety of 10 LAB strains isolated from kimchi. By investigating their virulence determinants, undesirable biochemical characteristics, and antibiotic resistance pattern, all the tested isolates were found to be safe for human consumption. In particular, *Lactobacillus plantarum* NO1, *Lactobacillus plantarum* AF1, and *Pediococcus pentosaceus* MP1 appear to meet the functional criteria required to be a beneficial probiotic (in vitro); i.e., acid and bile tolerance, cell adherence, and antagonistic activity against pathogens. We therefore propose that these strains can be considered new probiotic candidates.

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