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INHIBITION OF *SALMONELLA* BY BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA DERIVED FROM U.S. KIMCHI AND BROILER CHICKEN

JI YEUN KIM[1], JANAY A. YOUNG[1], NEREUS W. GUNTHER IV[2] and JUNG-LIM LEE[1,3]

[1]Department of Human Ecology, Food Science Program, College of Agricultural and Related Sciences, Delaware State University, Dover, DE 19901
[2]Agricultural Research Service, Eastern Regional Research Center, Molecular Characterization of Foodborne Pathogens Research Unit, United States Department of Agriculture, Wyndmoor, PA

[3]Corresponding author.
TEL: 1-302-857-6448;
FAX: 1-302-857-6441;
EMAIL: jlee@desu.edu

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ABSTRACT

In this study, lactic acid bacteria (LAB) were isolated from kimchi prepared in the U.S.A. and from broiler chickens. The resulting isolates were examined for potential probiotic characteristics. A total of 488 LAB strains were isolated, and among them, 16 originating from kimchi and 14 from broilers produced bacteriocin-like substances, which showed antimicrobial effects against *Salmonella* Enteritidis, Heidelberg, Newport and Typhimurium. These strains were investigated with regard to additional probiotic properties such as tolerance to gastric juice and bile, resistance to enzymes, and antibiotics susceptibilities. *Lactobacillus casei* or *paracasei* Cab-18, *Lb. saniviri* Cuc-1, and *Leuconostoc mesenteroides* Com-54 isolated from kimchi and *Lb. johnsonii* F-6 and *Lb. crispatus* F-59 isolated from broiler chicken demonstrated promising probiotic properties with the ability to produce bacteriocin-like substances with relatively strong antimicrobial activity against *Salmonella*. These strains could potentially be applied as supplements to poultry in order to reduce *Salmonella* contamination.

PRACTICAL APPLICATIONS

Salmonellosis associated with consumption of contaminated meats, especially poultry products, is a major concern due to the common outbreaks in the U.S.A. Although it has been shown that lactic acid bacteria (LAB) could reduce *Salmonella* contaminations, LAB isolated from kimchi have not been studied for the potential to inhibit the persistence of *Salmonella* spp. in live poultry. The present study screened LAB, which showed antimicrobial effects against four clinically important serotypes of *Salmonella*. Additionally, we selected LAB that had additional probiotic properties, which would allow for the strains use as potential feed additives. Therefore, the LAB strains isolated in this study could be applied as feed supplements to poultry for the purpose of reducing colonization of *Salmonella* in poultry as well as contamination during processing.

INTRODUCTION

In 2010, *Salmonella* were the most common reported foodborne pathogen and were responsible for the largest number of hospitalizations and deaths (CDC 2011). In the U.S.A., the major serotypes responsible for *Salmonella* infection were Enteritidis, Newport and Typhimurium from 1996 to 2010 (CDC 2011). Humans are usually infected by *Salmonella* via contaminated animal foods, including poultry products. In 2013, 416 people were infected by *Salmonella* Heidelberg associated with contaminated name-brand chicken throughout 23 states in the U.S.A. (CDC 2013). Additionally, live poultry and their environment can be a source of *Salmonella* infections of humans. Since 1991, live poultry have been associated with 45 *Salmonella* outbreaks (CDC 2013).

Efforts to reduce the amounts of *Salmonella* present in poultry have utilized antimicrobials, antibiotics, macronutrient constituents, chlorate and nitro-based compounds, and immune-enhancing reagents as feed supplements and water additives (Doyle and Erickson 2006). Studies have reported that oral administration of probiotic lactic acid bacteria (LAB) might reduce the intestinal colonization of *Salmonella* in chicken (Pascual *et al.* 1999; Higgins *et al.* 2007) and the inflammation caused by *Salmonella* in broiler chickens (Chen *et al.* 2012). Probiotics are live microorganisms that offer an advantage to its host by enhancing the hosts' beneficial microflora (Fuller 1989; Ljungh and Wadstrom 2006). LAB are often used as probiotics and are generally recognized as safe by the Food and Drug Administration. In addition, LAB have been widely studied and used in the food industry because of their preservative effects resulting from the decrease in the pH of the food environment mediated by lactic acid and the production of other antimicrobial metabolites, such as diacetyl, hydrogen peroxide or bacteriocins (Nes *et al.* 1996; Oyetayo *et al.* 2003). Bacteriocins are proteinaceous substances with bactericidal or bacteriostatic activity against specific bacterial species (Klaenhammer 1993; Nes *et al.* 1996).

LAB are involved in various fermentations of foods, such as dairy, bakery and vegetable products. Kimchi is a traditional Korean food that is fermented using vegetables, herbs and fruits such as Chinese cabbage, cucumber, radish, red pepper, onion, ginger, garlic and pear. Kimchi preparation includes natural fermentation of the mixed ingredients, which leads to outgrowth of various microorganisms during storage for 3–5 weeks. Various dietary benefits of kimchi have been claimed such as antioxidative activity (Hwang *et al.* 2000; Ryu *et al.* 2004), antimutagenic and antitumor activities (Cho *et al.* 1997; Shin *et al.* 1998), immune stimulatory activity (Kim *et al.* 1997), and weight-controlling or lipid-lowering activity (Kwon *et al.* 1999; Sheo and Seo 2004).

It is well known that the microbiome in fermented kimchi is dominated by LAB such as *Leuconostoc*, *Lactobacillus* and *Weissella* (Kim and Chun 2005; Jung *et al.* 2011; Park *et al.* 2012). Species thought to be involved in kimchi fermentation are *Leuconostoc (Leu) mesenteroides*, *Lactobacillus (Lb) brevis*, *Lb. curvatus*, *Lb. plantarum*, *Lb. sakei*, *Lactococcus (Lc) lactis*, *Pediococcus pentosaceus*, *Weissella confusa* and *Weissella kimchii* (Kim *et al.* 2000; Kim and Chun 2005; Lee *et al.* 2005; Shin *et al.* 2008; Park *et al.* 2010). Additionally, several studies reported that some kimchi-isolated LAB strains could produce bacteriocins. Bacteriocin Nisin Z-producing *Lc. lactis* ssp. *lactis* was isolated from kimchi (Park *et al.* 2003) as well as bacteriocin-producing *Leu. mesenteroides* and *Lb. plantarum* (Kim 1995; Chung *et al.* 2010).

This is the first study to isolate LAB from kimchi made with all U.S. ingredients and to screen those LAB strains for production of *Salmonella*-inhibiting bacteriocin. Additional LAB strains from broiler chicken intestines and feces collected in the Delmarva region, a major area of poultry production, were isolated and screened for bacteriocin production. Finally, the screened LAB isolates were evaluated for probiotic characteristics to determine their potential application as probiotics toward the inhibition of *Salmonella* growth in poultry.

MATERIALS AND METHODS

Preparation of Kimchi

Three types of homemade kimchi were prepared using ingredients originating only in the U.S.A., predominately from the Delmarva region: red cabbage, white cabbage and cucumber kimchi were prepared in the food demonstration facility at Delaware State University. Eight minor ingredients were commonly added in all three kimchi; radish, chives, boiled rice flour, salt, pear, onion, garlic and ginger. Red pepper powder was added only in the red cabbage and cucumber kimchi. The cut cabbages were soaked in brine for 5 h at room temperature and then rinsed with cold water. In order to make seasonings, onions, garlic, ginger and pear were blended with other minor ingredients. The cabbages and seasonings were mixed together and placed into a closed container. Cucumber kimchi was made with salted cucumber and followed the same recipe as the cabbage kimchi.

In addition to the homemade kimchi, commercial kimchi, which was manufactured in a local Asian market, was purchased in Newark, DE. The four kinds of kimchi were kept at refrigeration temperatures (4C) for fermentation.

Isolation of LAB

LAB were isolated from kimchi and broiler chickens. Twenty-five grams of each kimchi sample was taken at 0, 10, 20 and 30 days of storage. The kimchi samples were homogenized with 225 mL of MRS+ broth, MRS (Difco, BD, Sparks, MD) broth supplemented with L-cysteine hydrochloride (Fisher Science, Fair Lawn, NJ), using a stomacher (Interscience, St. Nom, France) for 10 min.

Broiler chicken materials were provided by the poultry research center at the University of Maryland Eastern Shore. Ileum, cecum and fecal materials were taken from broilers at 7, 28 and 49 days of age. Ten grams of each sample was homogenized with 90 mL of MRS+ broth using a stomacher (Interscience).

The homogenized kimchi and broiler samples were 10-fold serially diluted with MRS+ broth, plated on MRS agar (Difco) supplemented with L-cysteine hydrochloride

(MRS+ agar) and cultured anaerobically for 48 h at 37C. The colonies that showed LAB morphologies were re-streaked on MRS+ agar and then colonies were selected. The selected isolates were cultured in MRS+ broth for 24 h at 37C from which glycerol (Fisher Science) stocks were prepared and stored at −80C for the following studies.

Screening of *Salmonella* Inhibition by Bacteriocin-Producing LAB

A modified assay of the well diffusion method, adopted from Schillinger and Lucke (1989), was used to test the *Salmonella* spp. inhibitory abilities of LAB isolates. *Salmonella* enterica serotype Enteritidis, Heidelberg, Newport and Typhimurium were cultured in tryptic soy broth (Carolina Biological Supply Co., Burlington, NC) for 24 h at 37C. The cultures were adjusted to optical density (OD) = 0.3 at 600 nm and 0.5% of each strain was inoculated into 0.7% of molten nutrient agar (Carolina Biological Supply Co.) cooled to 40C. Five milliliters of the inoculated soft agar was poured on the nutrient agar plates and left for 20 min to harden. On the nutrient agar, 3-mm wells were made using a sterile cork borer and 15 μL of each isolate was loaded into the wells. After the plates were incubated for 48 h at 37C, the clear zone diameters around each well were measured.

The isolates that showed inhibitory effects on the growth of *Salmonella* were further screened for production of bacteriocins using the well diffusion method. The isolates that showed clear zones were grown in the MRS+ broth for 24 h and the supernatants were obtained by centrifugation at 5,000 × g for 10 min. The supernatants were adjusted to pH 6.5 with 2 N sodium hydroxide (Fisher Scientific) and supplemented with 1 mg/mL catalase to eliminate the antimicrobial effects of any organic acids or hydrogen peroxide produced by the isolates. The supernatants were filter-sterilized using 0.22-μm pore size syringe filters (Millipore, Billerica, MA) and used as crude bacteriocin preparations. On the *Salmonella*-inoculated nutrient agar mentioned earlier, 6-mm wells were made and 100 μL of a crude bacteriocin preparation was added to each well. After incubation for 12 h at 37C, the size of the resulting clear zones was measured. Bacteriocin-producing LAB that inhibited the growth of *Salmonella* spp. were selected for probiotic characterization.

Probiotic Characterization

Gastric Juice and Bile Tolerance. The method used by Lim and Im (2009) was modified for the gastric juice and bile assays. For the gastric juice assay, 3 mg/mL pepsin (Acros Organics, Fair Lawn, NJ) was added into phosphate buffered saline adjusted to pH 2.5. To test bile tolerance, 0.3% bile salt (Fisher Science) was added to the MRS broth. Selected isolates were adjusted to OD = 0.4 at 600 nm and 1% of each isolate was inoculated into the artificial gastric juice and bile solution. The inoculated solutions were incubated at 37C and viable bacterial cells were counted at 0 and 2 h for the gastric juice tolerance and 0 and 24 h for the bile tolerance.

Enzyme Resistance. To assess the enzyme resistance of selected isolates, four enzyme–MRS broths were prepared containing 1 mg/mL α-amylase (MP Biomedicals, Santa Ana, CA), 1 mg/mL proteinase K (Promega, Madison, WI), 1 mg/mL trypsin (Fisher Science) and 0.5 mg/mL lysozyme (Fisher Science). In the enzyme–MRS broth and control–MRS broth, without the addition of any enzymes, 0.5% of selected isolates was inoculated and incubated for 24 h at 37C. ODs (600 nm) of cultured MRS broth were measured using a microplate reader (Biotech Instruments, Inc., Winooski, VT) at 0, 4 and 24 h during incubation. Isolates that showed enzyme resistance against all four enzymes compared with control were screened and their viable bacteria were enumerated at 0 and 24 h. This test was repeated three times to establish mean values and standard deviations.

Antibiotic Susceptibility. The susceptibility of selected isolates to antibiotics was evaluated using the well diffusion method. MRS top agar was prepared by inoculation of 0.3% selected LAB strains into 0.7% soft MRS agar, poured on hard MRS agar, and 3-mm wells were made on the MRS top agar. Ten microliters of six different antibiotics, 3 mg/mL ampicillin (Alfa Aesar, Ward Hill, MA), 3 mg/mL chloramphenicol (Alfa Aesar), 1 mg/mL erythromycin (MP Biomedicals), 5 mg/mL kanamycin (Boston Bioproduct, Ashland, MA), 5 mg/mL streptomycin (MP Biomedicals), and 3 mg/mL tetracycline (MP Biomedicals), was placed into the wells. The plates were incubated anaerobically for 48 h at 37C and the diameter of clear zones around the wells was measured. This test was repeated three times to verify data consistency.

Sequencing Analysis of Probiotic LAB Isolates.

Sixteen LAB isolates were chosen based on the results from both the *Salmonella*-inhibiting bacteriocin production and the probiotic characterization assays. These isolates were identified using 16S rDNA sequencing that was performed at United States Department of Agriculture (USDA) Eastern Regional Research Center (PA) and Genewiz, Inc. (South Plainfield, NJ). To identify the genus and species of the isolates, the sequences were analyzed with the BLAST program of the National Center for Biotechnology Information (NCBI, Bethesda, MD).

The sequence data were optimized using the BioEdit v.7.2 program (Tom Hall Ibis Therapeutics, Carlsbad, CA), and sequence alignments were performed using CLUSTAL

W (http://www.genome.jp/tools/clustalw/). The neighbor-joining method was used for phylogenetic analysis by the MEGA v. 6 program (Tamura *et al.* 2013). Confidence values for individual branches were determined by 1,000 replication bootstrap analyses.

RESULTS AND DISCUSSION

Isolation and Screening of Bacteriocin-Producing LAB

A total of 488 LAB candidates were isolated from kimchi and Broiler samples (Table 1). Culture and morphological characteristics were examined by microscope observations. Different types of microorganisms were observed and the majority of them appeared as gram-positive rods and cocci-shaped bacteria. Also, catalase negative reactions were observed in all isolates.

These bacteria were screened for their antimicrobial capacities against four kinds of *Salmonella* enterica serotypes: *S*. Enteritidis, *S*. Heidelberg, *S*. Newport and *S*. Typhimurium, and the isolates that showed clear zones of 6 mm or more in diameter were assigned as positive strains. Using the well diffusion assay against *Salmonella* spp. (Fig. 1), a total of 132 isolates were shown to possess antimicrobial activities (Table 1). The inhibitory effect of crude bacteriocin preparations from culture supernatants of those 132 isolates were assessed by agar diffusion method against the same four *Salmonella* serotypes. Table 1 shows that crude supernatants from 50 total isolates produced significant clear zone, with 27 kimchi isolates and 23 broiler isolates producing bacteriocin-like substances. Ten isolates, Cab-37, W-51, Cuc-66, Cuc-77, I1-57, I2-31, I3-3, C3-13, C3-15 and F-50, showed *Salmonella* inhibition against all four serotypes (Table 2). From this study, 16 kimchi isolates and 14 broiler isolates were selected by satisfying the following criteria: (1) triple positive inhibitory effect (13 mm or greater clear zone) against at least one serotype or (2) inhibition effect against two or more *Salmonella* serotypes as well as double positive inhibition (10–12 mm of clear zone) against at least one of the serotypes (Table 2); these selected isolates were tested for further probiotic characteristics.

TABLE 1. TOTAL NUMBER OF LACTIC ACID BACTERIA (LAB) ISOLATES AND ANTIMICROBIAL LAB ISOLATES AGAINST *SALMONELLA*

	Sample	Number of isolates	Number of antimicrobial isolates	Number of bacteriocin-producing isolates
Kimchi	Red cabbage	85	26	7
	White cabbage	39	9	3
	Cucumber	72	15	4
	Commercial	58	22	13
Broiler	Ileum	97	36	8
Chicken	Cecum	83	9	4
	Fecal	54	15	11
Total		488	132	50

During the last few decades, a number of new bacteriocins produced by LAB strains have been identified and characterized. Their antimicrobial activities were mostly against gram-positive pathogens (McAuliffe *et al.* 2001; Van Reenen *et al.* 2003). However, several bacteriocins have been described to possess activities against gram-negative bacteria (Messens and De Vuyst 2002), such as plantaricin 35d produced by *Lb. plantarum* (Messi *et al.* 2001), bacteriocin ST34BR produced by *Lc. lactis* ssp. *lactis* (Todorov and Dicks 2004) and bacteriocin BacTN635 produced by *Lb. plantarum* TN635 (Smaoui *et al.* 2010).

Ragazzo-Sanchez *et al.* (2009) reported that bacteriocin-like substances produced by *Lactobacillus* isolated from mango inhibited *Salmonella* species. The most commonly implicated food commodity in *Salmonella* outbreaks in the U.S.A. during 1998–2008 were poultry products, especially eggs (112 outbreaks, 28%), chicken (64 outbreaks, 16%) and turkey (28 outbreaks, 7%) (Jackson *et al.* 2013).

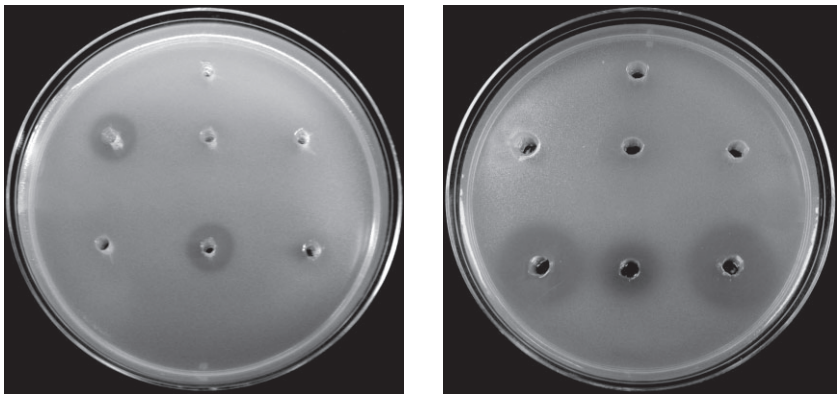

FIG. 1. ANTIMICROBIAL ACTIVITY OF (A) LACTIC ACID BACTERIA (LAB) ISOLATES AND (B) CRUDE BACTERIOCIN PREPARATIONS PRODUCED BY LAB ISOLATES
On top of *Salmonella*-inoculated nutrient agar, (A) 3-mm and (B) 6-mm holes were made and 20 μL of LAB isolates bacterial culture and 100 μL of crude bacteriocin produced by LAB isolates were added into each hole. The clear zones were measured after incubation for 12 h.

TABLE 2. ANTIBACTERIAL ACTIVITIES OF CRUDE BACTERIOCIN PRODUCED FROM KIMCHI AND BROILER CHICKEN ISOLATES AGAINST *SALMONELLA* STRAINS

Isolate	*S.* Enteritidis	*S.* Heidelberg	*S.* Newport	*S.* Typhimurium
Cab-18	+	+	–	+++
Cab-21	+++	+	–	+
Cab-25	++	+++	–	–
Cab-37	++	+	+	+++
Cab-39	–	–	–	+++
Cab-50	–	–	+	+
Cab-78	+	–	–	–
W-51	+	+	+	++
W-53	–	–	–	+
W-71	–	+	–	++
Cuc-1	+	–	++	++
Cuc-52	+++	++	–	+
Cuc-66	+++	++	+++	+
Cuc-77	+++	+++	++	+++
Com-3	+	–	–	–
Com-33	+	–	–	–
Com-34	–	+	–	–
Com-35	+++	+++	–	–
Com-36	+	++	–	–
Com-53	–	+	–	–
Com-54	+++	+	+	–
Com-62	–	+	–	–
Com-72	–	–	–	+
Com-73	+	–	+	++
Com-74	+	+	+	–
Com 75	++	–	–	+
Com 77	–	+	–	–
I1-57	++	++	++	++
I2-1	++	–	+	–
I2-15	–	–	–	+
I2-31	+++	++	+	++
I3-2	++	–	–	++
I3-3	+++	+++	+++	+++
I3-7	++	–	–	–
I3-32	+	–	–	–
C2-30	–	++	–	+++
C3-12	+++	–	–	-
C3-13	+++	++	+	+
C3-15	+++	++	+	+++
F-1	++	++	–	++
F-6	++	++	–	–
F-11	++	–	–	–
F-34	++	++	–	++
F-50	++	+	+	++
F-54	+	+	+	–
F-56	–	+	–	–
F-57	+	+	–	–
F-58	–	–	+	–
F-59	+	+	++	–
F-60	+	–	–	–

+ Shows positive result of clear zone 9 mm or less.
++ Shows positive result of clear zone 10–12 mm.
+++ Shows positive result of clear zone 13 mm or greater.
– Shows negative result.

Egg- and chicken-associated outbreaks were commonly caused by serotypes Enteritidis, Heidelberg and Typhimurium (Jackson *et al.* 2013). We have screened LAB strains that produced bacteriocin-like substances to inhibit the most common four serotypes, including those three serovars.

Tolerance of the LAB Isolates Stimulated with Gastric Juice and Bile Salt

Health-promoting effects of probiotic bacteria can be expected only when they are able to survive passage through the stomach and gastrointestinal tract and colonize the gut (Dunne *et al.* 2001). Gastric juice of approximate pH 2.0 is secreted in the stomach, which causes the destruction of most microorganisms ingested (Vizoso Pinto *et al.* 2006). However, probiotic strains are likely to be buffered by food or other carrier matrix molecules after consumption and are not likely to be exposed to the extremes of pH in the stomach (Prasad *et al.* 1998). In this study, 11 of 16 strains previously selected for antimicrobial activity isolated from kimchi and 10 of 14 strains isolated from broiler survived in artificial gastric juice (pH 2.5) for 2 h (Table 3).

Additionally, tolerance of LAB strains to bile salts is considered an important characteristic for the colonization and metabolic activity of the strains in the gut of the host (Strompfova and Laukova 2007). Resistance to bile salt is also a prerequisite for maintaining the equilibrium of the gut microflora as well as lowering of the host's serum cholesterol (Taranto *et al.* 1996; Liong and Shah 2005). The concentrations of human bile ranged from 0.3 to 0.5% (Dunne *et al.* 1999). In the present study, it was observed that most of the screened isolates (28 of 30 strains) tolerated 0.3% bile salts for 24 h effectively (Table 4).

As a result, 20 of the LAB strains, comprised 11 Kimchi isolates and 9 broiler isolates, showed tolerance to both gastric acid and bile salts. Among them, Cab-18, Cab-39, Cuc-1, Com-54 and Com-75 kimchi strains expressed relatively high tolerances and I3-3, C2-30, F-6 and F-59 broiler strains were superiorly resistant to simulated gastric juice and bile (Tables 3 and 4) compared with the other LAB strains. Additional probiotic characteristics were evaluated using those 20 strains tolerant to both gastric acid and bile salts.

Resistance of LAB Strains against Enzymes

Bacteria used as probiotics are delivered into the digestive system via the mouth. Thus, LAB isolates for use as probiotics should have resistances to the decomposition enzymes such as lysozyme and α-amylase in the oral cavity and trypsin in the intestine (Shukla *et al.* 2010). Most isolates survived in the presence of α-amylase, trypsin, proteinase K, and lysozyme except Com-26, which was sensitive to trypsin and lysozyme

TABLE 3. TOLERANCE OF SCREENED LACTIC ACID BACTERIA ISOLATES TO ARTIFICIAL GASTRIC JUICE AT 0 AND 2 H

	Tolerance to gastric juice (log cfu/mL)	
Isolates	Initial population (0 h)	After 2 h
Cab-18	6.81 ± 0.00	2.47 ± 0.04
Cab-21	6.69 ± 0.07	1.77 ± 0.1
Cab-25	6.43 ± 0.00	1.58 ± 0.15
Cab-37	6.97 ± 0.01	–
Cab-39	6.79 ± 0.03	3.45 ± 0.21
W-51	7.33 ± 0.05	1.00 ± 0.00
W-71	7.19 ± 0.00	–
Cuc-1	6.67 ± 0.07	3.84 ± 0.00
Cuc-52	7.21 ± 0.00	–
Cuc-66	6.51 ± 0.05	1.47 ± 0.00
Cuc-77	6.32 ± 0.00	–
Com-35	6.63 ± 0.06	–
Com-36	6.34 ± 0.19	3.92 ± 0.1
Com-54	7.08 ± 0.00	4.02 ± 0.17
Com-73	6.92 ± 0.11	2.22 ± 0.37
Com-75	7.16 ± 0.01	3.45 ± 0.21
I1-57	5.84 ± 0.09	–
I2-1	5.30 ± 0.00	–
I2-31	6.01 ± 0.15	2.48 ± 0.48
I3-2	6.03 ± 0.11	–
I3-3	6.36 ± 0.11	5.91 ± 0.19
C2-30	5.93 ± 0.04	5.95 ± 0.00
C3-12	6.42 ± 0.14	2.46 ± 0.04
C3-13	5.81 ± 0.05	1.45 ± 0.21
C3-15	6.45 ± 0.04	–
F-1	6.05 ± 0.14	2.71 ± 0.03
F-6	5.94 ± 0.34	6.20 ± 0.04
F-34	6.05 ± 0.38	3.15 ± 0.21
F-50	6.35 ± 0.07	2.74 ± 0.06
F-59	6.15 ± 0.11	4.03 ± 0.11
Enterococcus sp.	6.68 ± 0.11	2.59 ± 0.05

Values are means ± standard deviation.
–, none detected.

TABLE 4. TOLERANCE OF SCREENED LACTIC ACID BACTERIA ISOLATES TO STIMULATED BILE SALT AT 0 AND 24 H

	Tolerance to bile salt (log cfu/mL)	
Isolates	Initial population (0 h)	After 24 h
Cab-18	6.84 ± 0.05	7.09 ± 0.00
Cab-21	6.64 ± 0.08	6.40 ± 0.01
Cab-25	6.97 ± 0.08	7.00 ± 0.33
Cab-37	6.88 ± 0.02	5.23 ± 0.00
Cab-39	6.78 ± 0.02	6.04 ± 0.00
W-51	7.18 ± 0.04	6.25 ± 0.03
W-71	7.18 ± 0.05	5.94 ± 0.13
Cuc-1	6.44 ± 0.06	6.01 ± 0.15
Cuc-52	6.67 ± 0.01	5.77 ± 0.01
Cuc-66	6.21 ± 0.05	5.92 ± 0.16
Cuc-77	5.95 ± 0.06	5.62 ± 0.21
Com-35	5.62 ± 0.21	–
Com-36	6.20 ± 0.13	4.85 ± 0.04
Com-54	6.92 ± 0.01	5.80 ± 0.28
Com-73	7.13 ± 0.04	6.43 ± 0.03
Com-75	7.19 ± 0.01	6.02 ± 0.02
I1-57	6.47 ± 0.00	4.48 ± 0.17
I2-1	6.21 ± 0.01	4.80 ± 0.01
I2-31	6.06 ± 0.15	4.06 ± 0.11
I3-2	6.50 ± 0.15	5.75 ± 0.17
I3-3	6.09 ± 0.02	5.38 ± 0.12
C2-30	6.16 ± 0.16	5.14 ± 0.03
C3-12	6.37 ± 0.07	5.81 ± 0.04
C3-13	6.37 ± 0.07	6.17 ± 0.00
C3-15	6.48 ± 0.03	4.11 ± 0.00
F-1	6.53 ± 0.02	4.70 ± 0.22
F-6	6.05 ± 0.13	5.47 ± 0.00
F-34	6.21 ± 0.05	–
F-50	6.41 ± 0.00	5.03 ± 0.00
F-59	6.41 ± 0.01	6.22 ± 0.07
Enterococcus sp.	6.64 ± 0.29	5.15 ± 0.21

Values are means ± standard deviation.
–, none detected.

treatments and I2-31 and C3-12, which were sensitive to lysozyme (Fig. 2). Also, 16 of the strains grew in a similar manner with the untreated control demonstrating differences in growth of the strains between the control and enzyme treatment of less than 1 log cfu/mL (Fig. 2). Therefore, the resistant LAB isolates may be able to colonize and inhabit the host gut in the presence of those enzymes.

Susceptibility of LAB Isolates to Antibiotics

Antibiotic susceptibility of screened LAB isolates is shown in Table 5. Antibiotic resistance can pose a significant danger for people with common bacterial diseases and is a growing problem that complicates the treatment of nosocomial and community-acquired infections (Mathur and Singh 2005; Moellering *et al.* 2007).

Com-54, Com-73 and Com-75 strains showed susceptibility to all six antibiotics, ampicillin, chloramphenicol, erythromycin, kanamycin, streptomycin and tetracycline. The other 17 strains were not sensitive to kanamycin and/or streptomycin, which are both aminoglycosides. Similar resistance to aminoglycosides, gentamycin and streptomycin, was reported in previous study and that was considered as an intrinsic property among LAB (Hummel *et al.* 2007). Com-36 was the only strain that showed resistance to tetracycline, but all others were sensitive. There was no broad resistance response in this study, which continues to suggest the safety of these strains as probiotics.

Identification of the Selected Probiotic LAB Isolates

Sixteen probiotic LAB strains, 10 kimchi isolates and 6 broiler isolates, were screened that had potential probiotic

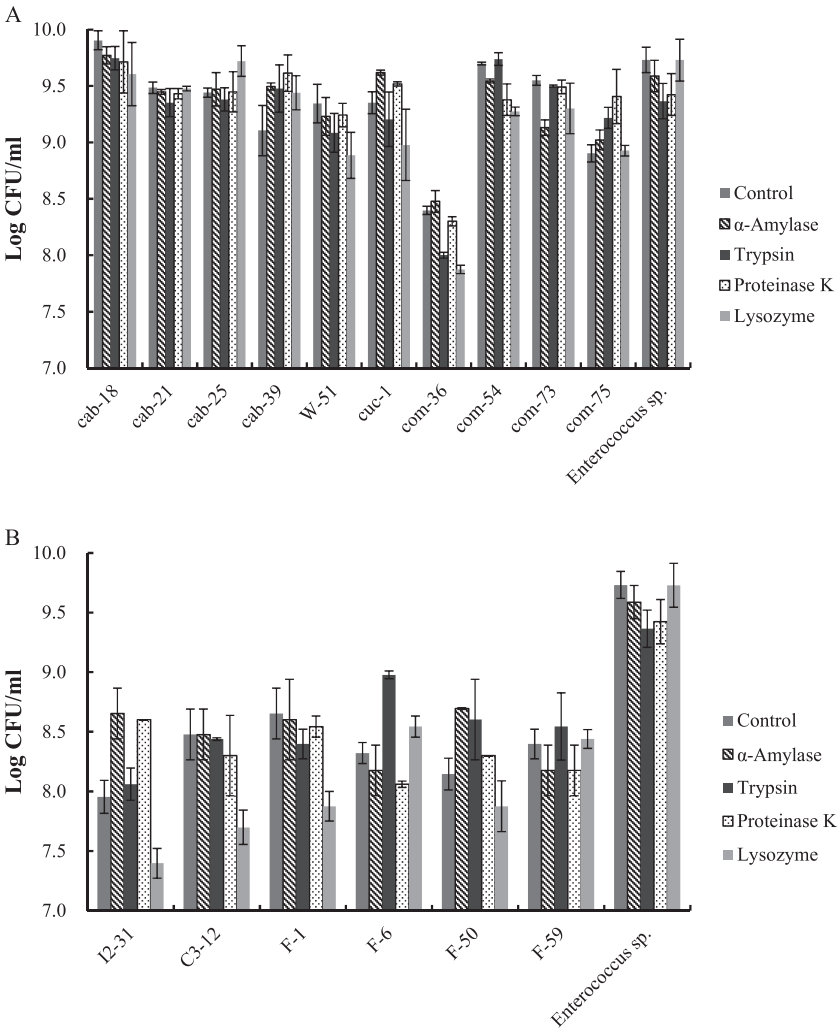

FIG. 2. ENZYME RESISTANCE OF SCREENED LACTIC ACID BACTERIA ISOLATES DERIVED FROM (A) KIMCHI AND (B) BROILER CHICKENS Bacterial counts cultured for 24 h in MRS medium containing α-amylase, trypsin, proteinase K and lysozyme are shown as mean ± SD and compared with the counts from the control medium in which no enzyme was present.

characteristics such as gastric juice and bile tolerance, enzyme resistance, antibiotic susceptibility as well as strong bacteriocin activity against four major *Salmonella* serotypes. Table 6 shows identification of the selected 16 isolates by 16S rDNA sequencing, and the degrees of relation based on *16S rDNA* gene between the isolates and type strains are represented in the neighbor-joining tree (Fig. 3).

The species of the selected strains were distinctively classified depending on the kimchi and chicken origin. Kimchi isolates belonged to genus *Lactobacillus* and *Leuconostoc*. Specifically, *Lactobacillus*, *Lb. casei* or *paracasei* (Cab-18 and Cab-25), *Lb. plantarum* (Cab-39), *Lb. saniviri* (Cuc-1) and *Lb. sakei* (Com-36) were identified and *Leu. pseudosesenteroides* (Cab-21), *Leu. mesenteroides* (Com-54), and *Leu. mesenteroides* ssp. *mesenteroides* (Com-73 and Com-75) were included in the genus *Leuconostoc*. All identified species from our study showed similar results to other kimchi studies (Kim *et al*. 2000; Kim and Chun 2005; Lee *et al*. 2005; Shin *et al*. 2008; Park *et al*. 2010) except *Lb. saniviri*, which was recently reported as a new species (Oki *et al*. 2012). Even though many studies were conducted for LAB isolations from kimchi in Korea and Asia, *Lb. saniviri* has not been detected in Kimchi yet. It is possible that the presence of this species is the result of the first trial of LAB isolation from kimchi prepared using U.S. origin ingredients.

From the broiler chickens, two species were identified and they were included in *Lactobacillus*. Isolates I2-31, C3-12, F-1, F-50 and F-59 were classified into *Lb. crispatus* and isolate F-6 was identified as *Lb. johnsonii*. These are normal microflora in chicken intestine (Lu *et al*. 2003; Taheri *et al*. 2009; Torok *et al*. 2011) and are commonly suggested as potential probiotic bacteria for chicken (Van Coillie *et al*. 2007; Taheri *et al*. 2009; Majidzadeh Heravi *et al*. 2011). Also, it was reported that they exhibit antimicrobial activities against pathogens, especially *Salmonella* (Van Coillie *et al*. 2007; Taheri *et al*. 2009).

TABLE 5. ANTIBIOTIC SUSCEPTIBILITIES OF SCREENED LACTIC ACID BACTERIA ISOLATES

Isolates	Ampicillin	Chloramphenicol	Erythromycin	Kanamycin	Streptomycin	Tetracycline
Cab-18	+	+++	+++	–	–	+++
Cab-21	+++	+++	+++	–	–	++
Cab-25	++	+++	+++	–	–	++++
Cab-39	++	++	++	–	–	++
W-51	++	+++	+++	–	–	+++
Cuc-1	++	++	++	–	–	+++
Com-36	+	+++	+++	–	–	–
Com-54	++	+++	+++	+	+	+++
Com-73	++	+++	+++	+	+	+++
Com-75	++	+++	+++	+	+	+++
I2-31	++	+++	+	–	++	+++
C3-12	++	+++	+++	–	++	+++
F-1	+++	+++	+++	–	++	++++
F-6	+++	+++	++++	–	++	++++
F-50	+++	+++	+++	–	–	+++
F-59	++	+++	+++	–	++	+++
Enterococcus sp.	+	+++	+++	–	–	++

Susceptibilities are presented in a diameter of inhibitory zones: +, ≤10 mm; ++, 11–20 mm; +++, 21–30 mm; ++++, >30 mm; –, absence of inhibitory zones.

From the results obtained, it can be concluded that *Lb. casei* or *paracasei* Cab-18, *Lb. saniviri* Cuc-1 and *Leu. mesenteroides* Com-54 isolated from kimchi and *Lb. johnsonii* F-6 and *Lb. crispatus* F-59 isolated from broiler chicken are potential probiotic strains with proven ability to produce bacteriocin-like substances inhibiting *Salmonella* spp. Also, it is possible that supplementation of those strains in chicken may contribute to reduction of *Salmonella* infection from farm to table. This study will be applied toward further research in order to evaluate the *in vivo* properties of these strains in live boiler chickens.

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TABLE 6. IDENTIFICATION OF BACTERIOCIN-PRODUCING PROBIOTIC LACTIC ACID BACTERIA STRAINS ISOLATED FROM KIMCHI AND BROILER CHICKEN

Origin	Isolates	Identified species	Accession No.	Identity	Blasted length (bp)
Kimchi	Cab-18	*Lactobacillus casei*	KF263164.1	100	573
		Lactobacillus paracasei	KC967212.1	100	
	Cab-21	*Leuconostoc pseudomesenteroides*	KF263165.1	99	793
	Cab-25	*Lactobacillus casei*	KF263160.1	99	726
		Lactobacillus paracasei	KF263163.1	99	
	Cab-39	*Lactobacillus plantarum*	KF767997.1	100	792
	W-51	*Leuconostoc mesenteroides*	KF149523.1	99	566
	Cuc-1	*Lactobacillus saniviri*	AB602569.1	99	577
	Com-36	*Lactobacillus sakei*	HG798441.1	99	694
	Com-54	*Leuconostoc mesenteroides*	KF149523.1	98	593
	Com-73	*Leuconostoc mesenteroides* ssp. *mesenteroides*	HG799977.1	100	755
	Com-75	*Leuconostoc mesenteroides* ssp. *mesenteroides*	HG799977.1	99	833
Broiler chicken	I2-31	*Lactobacillus crispatus*	KC747723.1	99	849
	C3-12	*Lactobacillus crispatus*	KC166145.1	100	781
	F-1	*Lactobacillus crispatus*	KF661284.1	100	847
	F-6	*Lactobacillus johnsonii*	KC856466.1	100	829
	F-50	*Lactobacillus crispatus*	KC747723.1	99	850
	F-59	*Lactobacillus crispatus*	KC747723.1	99	843

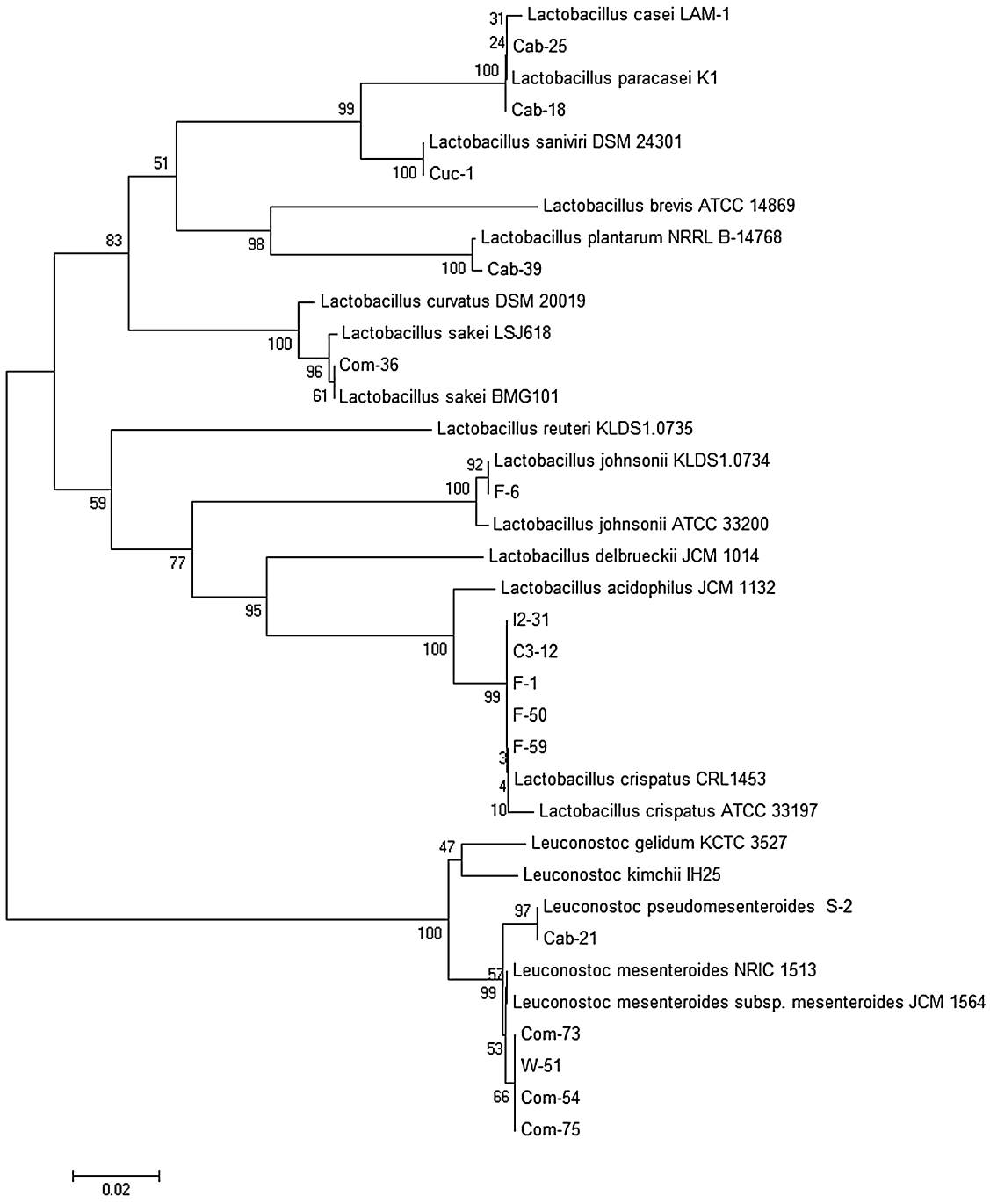

FIG. 3. NEIGHBOR-JOINING TREES RESULTING FROM 16S rDNA GENE SEQUENCES OF THE SCREENED LACTIC ACID BACTERIA ISOLATES DERIVED FROM KIMCHI AND BROILER CHICKEN AND THE TYPE STRAINS
Values on each node are equivalent to percentages of bootstrap confidence level in neighbor-joining analysis.

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