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# Identification of Bacteriocin-producing Lactic Acid Bacteria from Kimchi and Partial Characterization of their Bacteriocin

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Nineteen strains of bacteriocin-producing lactic acid bacteria were isolated from 432 Kimchi samples, and identified by the comprehensive biochemical and morphological tests verifying their cellular fatty acid composition. Using partially purified bacteriocins from these isolates, their inhibitory activities against other lactic acid bacteria and some pathogens, and sensitivity to enzyme and heat treatments were tested. The isolates were identified as *Lactobacillus plantarum* (2 strains), *L. curvatus* (2 starins), *L. brevis* (2 strains), *Enterococcus faecium* (6 strains), *Leuconostoc mesenteroides* subsp. *mesenteroides* (1 strain) and *Lactobacillus* sp. (6 strains). The bacteriocins produced by *E. faecium* strains provided the broadest spectrum of inhibition, affecting against other Gram-positive bacteria including lactic acid bacteria and health-threatening bacteria such as *Clostridium perfringens* and *Listeria monocytogenes*. The bacteriocins of *Lactobacillus* sp., *L. plantarum* and *L. brevis* strains were capable of inhibiting many strains of the lactic acid bacteria, whereas those of *L. curvatus* and *L. mesenteroides* subsp. *mesenteroides* strains were only inhibitory to a few strains. Generally, the inhibitory activities of both *E. faecium* and *Lactobacillus* sp. strains were greater than those of other producer strains. The bacteriocins from the isolates were sensitive to several proteolytic enzymes, and those of *L. curvatus* and *L. mesenteroides* subsp. *mesenteroides* were also sensitive to lipase and α-amylase as well as to proteolytic enzymes. The bacteriocins from the strains of *Lactobacillus* sp. and a strain of *L. brevis* were resistant to autoclaving.

Lactic acid bacteria are widely associated with the fermentation of a variety of meat, dairy, vegetable and bakery products. Their role is to improve taste and palatability, but they also contribute to the preservation of the fermented products. In Korea, there are many kinds of Kimchi (traditional Korean pickles) to which those characteristics of lactic acid bacteria are applied. Kimchi is prepared with vegetables and it becomes palatable and preservable one through proper fermentation caused mainly by lactic acid bacteria. Kimchi has been served as the indispensable side dish at any Korean meal. Moreover, Koreans tended to rely on Kimchi for its nutrition when fresh vegetables were scarce in winter.

Lactic acid bacteria have long been known to produce antimicrobial proteins called bacteriocins. Certain bacteriocins produced inhibit a variety of food-bome pathogens, including *Bacillus cereus, Clostridium perfringens, Listeria monocytogenes,* and *Staphylococcus aureus* (24). This suggests that bacteriocin-producing lactic acid bacteria may be useful in biocontrol of food either by applying the culture directly or by adding the produced bacteriocin as a natural preservative.

From this point of view, there are many reports on the isolation of bacteriocin- producing lactic acid bacteria from food grade starter cultures, fermented sausage, vacuum-packed meat, retail lamb, cucumber brine, etc. (24). However, there is only one report which describes the bacteriocin-producing bacteria, *i.e. Pediococcus cerevisiae* and *Leuconostoc* sp., from Kimchi (27).

In this study, bacteriocin-producing lactic acid bacteria were isolated from many Kimchi samples and identified. The antimicrobial activity of these isolates against other Gram-positive and negative bacteria including several pathogen were examined, along with some of the characteristics of their bacteriocins.

## MATERIALS AND METHODS

### Bacterial Strains and Media

The strains used in this study are listed in Table 1. All lactic acid bacteria were grown in MRS broth unless otherwise indicated. For the cultivation of non-lactic acid

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Key words: bacteriocin, Kimchi, cellular fatty acid composition, *Lactobacillus plantarum, L. curvatus, L. brevis, Enterococcus faecium, Leuconostoc mesenteroides, Lactobacillus* sp.

**Table 1.** Strains used in this study

| Strain | Other designation | Reference |
|---|---|---|
| *Aeromonas hydrophila* KCTC[a] 2358 | ATCC 7966 | |
| *Bacillus cereus* KCTC 1012 | ATCC 9634 | |
| *Bacillus subtilis* KCCM 11914 | ATCC 21697 | |
| *Clostridium perfringens* ATCC 13124 | | |
| *Escherichia coli* KCTC 1039 | ATCC 9637 | |
| *Listeria monocytogenes* ATCC 19111 | | |
| *Micrococcus luteus* KCCM 11455 | NRRL-B-1018 | |
| *Salmonella typhimurium* KCTC 1925 | | |
| *Staphylococcus aureus* KCTC 1621 | ATCC 25923 | |
| *Vibrio parahaemolyticus* ATCC 27519 | | |
| *Lactobacillus plantarum* IAM 12477 | ATCC 14917[T] | |
| *Lactobacillus plantarum* DU 3003[b] | | (17) |
| *Lactobacillus curvatus* NCFB 2739 | ATCC 25601[T] | |
| *Lactobacillus sake* JCM 1157 | ATCC 15521[T] | |
| *Lactobacillus sake* DU 3011[b] | | (17) |
| *Lactobacillus brevis* JCM 1059 | ATCC 8287[T] | |
| *Lactobacillus brevis* DU 3025[b] | | (17) |
| *Enterococcus faecalis* JCM 5803 | ATCC 19433[T] | |
| *Enterococcus faecalis* DU 2103[b] | | (17) |
| *Enterococcus faecium* JCM 5804 | ATCC 19434[T] | |
| *Enterococcus faecium* KCTC 3095[I] | | |
| *Enterococcus faecium* DU 2102[b] | | (17) |
| *Leuconostoc mesenteroides* subsp. *mesenteroides* JCM 6124 | ATCC 8293[T] | |
| *Leuconostoc mesenteroides* subsp. *mesenteroides* DU 1003[b] | | (17) |
| *Pediococcus pentosaceus* IAM 12300 | | |
| *Pediococcus pentosaceus* DU 2203[b] | | (17) |
| *Pediococcus acidilactici* IAM 1233 | ATCC 8042 | |

[a]Abbreviations: ATCC, American Type Culture Collection, Rockville, Md. U.S.A.; DU, Department of Food Technology, Dongguk University, Seoul, Korea; IAM, Institute of Molecular and Cellular Biosciences, University of Tokyo; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan; KCCM, Korean Culture Center of Microorganisms, Department of Food Engineering, Yonsei University, Seoul, Korea; KCTC, Korean Collection for Type Cultures, Genetic Engineering Research Institute, Korea Institute of Science and Technology, Daejeon, Korea; NCFB, National Collection of Food Bacteria, Institute of Food Research, Reading, United Kingdom; NRRL, Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Ill. U.S.A. [b]The strain isolated from Kimchi by authors. [T]Indicates the type strain of the species. [I]Indicates the indicator strain used for the isolation of bacteriocin-producing lactic acid bacteria and measurement of bacteriocin activity in this study.

bacteria, the media used were: APT broth for *L. monocytogenes;* Nutrient broth for *Aeromonas hydrophila, Bacillus subtilis, B. cereus* and *Micrococcus luteus;* Sodium thioglycollate medium for *Clostridium perfringens;* Trypticase soy (TS) broth for *Escherichia coli* and *Staphylococcus aureus;* and Brilliant green broth for *Salmonella typhimurium.* These strains were maintained as stab cultures in agar media at 4°C. The agar media and soft agar were prepared by adding 1.5% and 0.7% agar, respectively, to the appropriate broth listed above. Cultures were incubated at 30°C (lactic acid bacteria) or 37°C (non-lactic acid bacteria). All media used were supplied by Difco Laboratories with the exception of TS broth by BBL Microbiology Systems and blood agar by Korea Media Co.

**Isolation of Bacteriocin-producing Lactic Acid Bacteria**

The strains of bacteriocin-producing lactic acid bacteria were isolated from Kimchi samples which obtained from either home or market, and these samples were at the early stages of fermentation. The juice of sample was diluted with a series of 1% peptone water, spread onto the surface of MRS agar plates, and incubated for 18h at 30°C to allow colonies to develop. Anaerobic incubation (GasPak; Difco) was used to rule out any inhibition due to hydrogen peroxide production. The plates having 30 to 200 cfu were overlayed with approximately 8 ml of soft MRS agar which inoculated at a level of approximately $10^7$ cfu with an ovemight culture of the indicator strain (*Enterococcus faecium* KCTC 3095). After further incubation for 24 h, the colonies showing the clear zone of inhibition were checked. To exclude antagonistic effect caused by lactic acid production, the culture broth of 24 h incubation with positive strains in the test mentioned above was neutralized by adding 3 N NaOH, followed by sterile filtration through a 0.2 μm-pore-size cellulose acetate filter (Millipore Corp.), and then its inhibiting activity was confirmed by the agar well diffusion assay described at the detection of antagonistic activity.

**Identification of Isolates**

To identify isolates, the followings were examined: cell morphology, Gram stain, spore formation, motility, catalase activity, reduction of nitrate, tellurite and tetrazolium, hydrolysis of gelatin, arginine and hippurate, ammonia production from arginine, gas production from glucose, reaction in litmus milk, production of indole and dextran, effects of temperature, pH, and NaCl on growth, and acid production from carbohydrates (7, 33, 39). The configuration of the lactic acid enantiomers was determined enzymatically, using D(−)- and L(+)-lactate-dehydrogenase (Boehringer Mannheim GmbH) (25). A 3 days culture of the test strain grown in GYP-mineral salt broth was used as the sample. The medium contained double strengthened concentration of glucose, yeast extract and peptone, but sodium acetate was omitted.

The peptidoglycan type of cell wall was also examined. *meso*-Diaminopimelic acid in peptidoglycan was detected by the methods of Komagata and Suzuki (14). Three mg of freeze-dried cells was hydrolysed with 1 ml of 6N HCl at 100°C for 18 h, and the hydrolysate was

applied to TLC.

Enterococci strains were also monitored for their ability to produce hemolysins. The strains were grown in MRS broth for 18 h and then transferred onto blood agar plates which contain fresh defibrinated sheep blood (5%). Following incubation for 18 to 24 h, a clear zone around the colonies showing hemolysin activity was checked.

The identification of isolates was confirmed by fatty acid analysis of cells using gas chromatography (GC). Methyl esters of cellular fatty acids were prepared from lyophilized cells by using a 5% methanolic hydrochloride acid solution (9). After extraction with hexane, methyl esters of fatty acids were identified on a model GC-8A gas chromatography system (Shimazu Corp.) equipped with a flame ionized detector. GC was performed with a glass column (3 m by 2.6 mm) packed with 15% DEGS on Chromosorb WAW, DMCS 100/120. The temperature at both the injector and detector was 240°C and column temperature was 195°C. The carrier gas was nitrogen (flow rate, 20 ml/min). The chromatograms were analyzed with a Chromatopac C-R1B analyzer (Shimazu) and the peak of each fatty acid was identified by comparing the retention time with those from the standard mixture (Sigma Chemical Co.).

**Partial Purification of Bacteriocin**

A partially purified bacteriocin preparation was made by ammonium sulfate precipitation. Bacteriocin-producing strains were cultured in MRS broth for 18 h. Culture broth was centrifuged and sterile filtered. The cell-free supernatant fluid was decanted into a beaker set in an ice bucket, added stepwise solid ammonium sulfate to achieve 75% saturation, and allowed to stir for an additional 30 min. The precipitated suspension was centrifuged for 30 min at 10,000 x g at 5°C and decanted. The pellet was dissolved in 5 mM phosphate buffer, pH 7.0, to 5% of the starting volume. The concentrated solution was dialyzed against the same buffer at 5°C overnight. Dialyzed material was freeze-dried and stored at -20°C until use. This lyophilisate was regarded as crude bacteriocin.

**Detection of Antagonistic Activity**

For the detection of antagonistic activity, an agar well diffusion assay was used (37). MRS agar plates were overlayed with MRS soft agar lawns which contain approximately $10^7$ cfu of log-phase culture of an indicator strain (*Enterococcus faecium* KCTC 3095). Wells were made in MRS agar plates with a sterile 6-mm cork borer and the bottom was sealed by the addition of 1 to 2 drops of agar. Portions (0.1 ml) of culture broth or partially purified bacteriocin solution were placed in the wells and allowed to diffuse into the agar at 4°C for an hour. The plates were then incubated anaerobically for 18 to 24 h and examined for inhibition of the indicator lawn. For an activity assay, twofold serial dilutions of the test solution were used (21). The titer was defined as the reciprocal of the highest dilution showing a definite inhibition of the indicator lawn, and expressed in arbitary units (AU) per milliliter.

**Bacteriocin Characterization**

The partially purified bacteriocins in 5 mM phosphate buffer, pH 7.0, (1 mg/ml) were tested for sensitivity to heat and enzyme treatments. Heat treatments were carried out for 10, 30 and 60 min at 100°C or 10, 20 and 30 min at 121°C. Sensitivities to the various enzymes were tested by treating the bacteriocin solutions with catalase (bovine liver, Sigma), α-chymotrypsin (bovine pancreas, Sigma), trypsin (porcine pancreas, Sigma), pepsin (porcine gastric mucosa, Wako Pure Chemical Ind.), protease (*Aspergillus oryzae*, Marshal Co.), α-amylase (*Bacillus subtilis*, Junsei Chemical Co.), lipase (wheat germ, Type 1, Sigma) and lysozyme (chicken egg white, Sigma). Enzymes were dissolved in 5 mM phosphate buffer, pH 7.0, (except for the pepsin which was dissolved in 0.02N HCl) to obtain a final concentration of 1 mg/ml. Samples with and without the enzyme were sterile filtered and incubated at 37°C for 1 h. Before testing for residual activity, the sample was neutralized and boiled for 5 min to destroy any enzyme activity. Residual activity was determined by the agar well diffusion assay.

## RESULTS AND DISCUSSION

**Isolation and Identification of Bacteriocin-producing Strains**

From a total of 432 Kimchi samples, 19 strains of bacteriocin-producing lactic acid bacteria were isolated and each isolate was obtained from a different Kimchi sample. Phenotypic characteristics of these isolates are shown in Table 2-3.

All isolates were Gram-positive, nonmotile and catalase-negative. Of 19 isolates, 12 were rod-shaped, 10 were homofermentative and 2 were heterofermentative. The remaining 7 isolates were sphere-shaped, 6 were homofermentative and 1 was heterofermentative.

Rod-shaped isolates, strains DU 0162, 0180, 0181, 0182, 0183, 0238, 0240, 0241, 0242, 0247, 0256 and 0259, appeared singly, in pairs or in short chains. Cells were non-sporing and acid tolerant. They were negative to growth at 45°C, liquefaction of gelatin, hydrolysis of casein, and formation of indole and $H_2S$. Therefore, these strains were included in the genus *Lactobacillus*.

Among the rod-shaped isolates, strains DU 0162, 0180, 0181, 0182, 0183, 0238, 0240, 0247, 0256 and 0259 were homofermentative. Strains DU 0247 and 0256 produced acid from all tested carbohydrates with the exception of rhamnose and glycerol, DL-lactic acid from glucose,

**Table 2.** The general characteristics of strains of bacteriocin-producing lactic acid bacteria isolated from Kimchi

| Characteristics | Species and strain No. | | | | | |
|---|---|---|---|---|---|---|
| | *Lactobacillus plantarum* DU 0247, 0256 | *Lactobacillus curvatus* DU 0162, 0182 | *Lactobacillus* sp. DU 0180, 0181, 0183, 0238, 0240, 0259 | *Lactobacillus brevis* DU 0241, 0242 | *Enterococcus faecium* DU 0216, 0230, 0237, 0253, 0255, 0267 | *Leuconostoc mesenteroides* subsp. *mesenteroides* DU 0243 |
| Cell form | Rod | Rod | Rod | Rod | Cocci | Cocci |
| Cell size (μm) | 0.8−1.1×3−6 | 0.7−0.9×2−3 | 0.6−0.7×2−3 | 0.7−0.8×2−3 | 0.5−1.0 | 0.5−1.5 |
| Cell arrangement | singly, short chains | singly, short chains | singly, short chains | singly, short chains | pairs, chains | pairs, chains |
| Gram reaction | +[a] | + | + | + | + | + |
| Motility | − | − | − | − | − | − |
| Spore formation | − | − | − | − | − | − |
| Gas from glucose | − | − | − | + | − | + |
| Catalase | − | − | − | − | − | − |
| Reaction in litmus milk: | | | | | | |
| Reduction | + | + | + | + | + | − |
| Peptonization | − | − | − | − | − | − |
| Acid curd | + | + | + | + | + | − |
| Ammonia from arginine | − | − | − | + | + | − |
| Hydrolysis of gelatin | − | − | − | − | − | NT |
| Hydrolysis of esculin | + | + | + | + | − | − |
| Hydrolysis of arginine | NT | NT | NT | NT | + | − |
| Hydrolysis of hippurate | NT | NT | NT | NT | − | NT |
| Nitrate reduction | − | − | − | − | − | − |
| Reduction of tellurite | NT | NT | NT | NT | − | NT |
| Reduction of tetrazolium | NT | NT | NT | NT | +[w] | NT |
| Formation of indole | − | − | − | − | NT | − |
| Dextran formation | − | − | − | − | − | + |
| Growth at 10°C | + | + | + | + | + | + |
| Growth at 15°C | + | + | + | + | + | + |
| Growth at 40°C | + | + | + | + | + | + |
| Growth at 45°C | − | − | − | − | + | − |
| Growth at 50°C | − | − | − | − | − | − |
| Growth at pH 3.6 | + | − | + | + | − | − |
| Growth at pH 3.9 | + | + | + | + | − | − |
| Growth at pH 4.2 | + | + | + | + | − | − |
| Growth at pH 4.8 | + | + | + | + | − | − |
| Growth at pH 8.6 | + | + | + | + | + | + |
| Growth at pH 9.6 | + | + | + | + | + | + |
| Growth at 6.5% NaCl | + | + | + | + | + | +[w] |
| DAP in peptidoglycan | + | − | − | − | − | − |
| Isomer of lactic acid | DL | DL | DL | DL | L(+) | D(−) |
| Sheep blood hemolysis | NT | NT | NT | NT | − | NT |

[a]Symbols; +, positive; +[w], weakly positive; −, negative; NT, not tested.

and contained *meso*-diaminopimelic acid in their cell wall. Strains DU 0162 and 0182 produced acid from ribose but not from amygdalin, arabinose, mannitol and sorbitol. They produced DL-lactic acid from glucose, and did not contain *meso*-diaminopimelic acid. The characteristics of these strains agree with the description of *L. plantarum* and *L. curvatus*, respectively (5, 6, 12, 39). The remaining homofermentative isolates, strains DU 0180, 0181, 0183, 0238, 0240 and 0259, shared the same characteristics, and their carbohydrate fermentation patterns were identical with those of strains DU 0247 and 0256 described above, but they did not contain *meso*-diaminopimelic acid in their cell wall. Although these strains seem to belong to *L. sake*, because their characteristics in both the carbohydrate fermentation pattern and peptidoglycan type of cell wall most closely resem-

**Table 3.** Acid formation from carbohydrates of strains of bacteriocin-producing lactic acid bacteria isolated from Kimchi

| Characteristics | Species and strain No. | | | | | |
|---|---|---|---|---|---|---|
| | *Lactobacillus plantarum* DU 0247, 0256 | *Lactobacillus curvatus* DU 0162, 0182 | *Lactobacillus* sp. DU 0180, 0181, 0183, 0238, 0240, 0259 | *Lactobacillus brevis* DU 0241, 0242 | *Enterococcus faecium* DU 0216, 0230, 0238, 0253, 0255, 0267 | *Leuconostoc mesenteroides* subsp. *mesenteroides* DU 0243 |
| Amygdalin | +[a] | − | + | − | + | + |
| Arabinose | + | − | + | + | + | + |
| Arbutin | + | + | + | + | + | + |
| Cellobiose | + | + | + | − | + | + |
| Esculin | + | + | + | + | + | − |
| Fructose | + | + | + | + | + | + |
| Galactose | + | + | + | + | + | + |
| Glucose | + | + | + | + | + | + |
| Gluconate | + | + | + | + | + | + |
| Glycerol | − | − | − | + | + | + |
| Lactose | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + |
| Maltotriose | + | +[w] | + | + | + | + |
| Mannitol | + | − | + | − | + | + |
| Mannose | + | + | + | + | + | + |
| Melezitose | + | − | + | − | − | + |
| Melibiose | + | − | + | + | + | + |
| Raffinose | + | − | + | + | − | + |
| Rhamnose | − | − | − | − | − | + |
| Ribose | + | + | + | + | + | + |
| Salicin | + | + | + | − | + | + |
| Sorbitol | + | − | + | − | − | + |
| Sorbose | + | − | + | − | +[w] | − |
| Sucrose | + | + | + | + | + | + |
| Trehalose | + | − | + | + | + | + |
| Xylose | + | − | +[w] | + | + | + |

[a]Symbols; +, positive; +[w], weakly positive; −, negative; NT, not tested.

ble those of *L. sake*, the reactions for mannitol, melezitose, raffinose and sorbitol were atypical (5, 6, 12, 39). Thus, the present authors include these strains tentatively in "*Lactobacillus*" sp.

The heterofermentative strains of rod-shape isolates, strains DU 0241 and 0242, produced gas from glucose, ammonia from arginine, and DL-lactic acid from glucose. They showed positive reactions for acid production from melibiose, arabinose, fructose, gluconate and ribose, and negative reactions from cellobiose and salicin. Their characteristics agree with the description of *L. brevis* (5, 6, 12, 39).

The homofermentative strains of sphere-shaped isolates, strians DU 0216, 0230, 0237, 0253, 0255 and 0267, were arranged in pairs or in chains. They grew at 10 and 45°C, in GYP broth containing 6.5% of NaCl, and at pH 9.6, and produced L-lactic acid from glucose. They reduced litmus before curd formation in milk and did not reduce nitrate. Positive reaction was for hydrolysis of arginine. Therefore, these strains were included in the genus *Enterococcus*. They produced acid from arabinose, melibiose, and sorbose but not from melezitose, and showed negative responses for reduction of tetrazolium and decarboxylation of tyrosine. These characteristics correspond with the description of *E. feacium* (5, 31, 39).

The heterofermentative strains of sphere-shaped isolates, strain DU 0243, occurred singly, in pairs or in chains, and produced gas and D-lactic acid from glucose. They showed negative reactions for litmus reduction in milk and ammonia formation from arginine. Therefore, these strains belong to genus *Leuconostoc*. They produced acid from sucrose, trehalose, arabinose and melibiose, and formed dextran from sucrose. They did not grow

**Table 4.** Cellular fatty acid composition of bacteriocin-producer strains from Kimchi and reference strains

| Strain | Straight-chain acids | | | | | | | | Cy | Unknown |
|---|---|---|---|---|---|---|---|---|---|---|
| | $C_{12:0}$[a] | $C_{14:0}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{17:0}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{20:1}$ | $C_{19:0}$ | |
| *Lactobacillus plantarum* | | | | | | | | | | |
| DU 0247 | 0.94 | 2.96 | 29.41 | 5.44 | 0.26 | 4.45 | 22.50 | 11.80 | 14.54 | 7.35 |
| DU 0256 | 0.49 | 3.87 | 31.68 | 5.52 | 0.25 | 4.39 | 20.16 | 13.84 | 12.07 | 7.41 |
| IAM 12477[c] | 1.19 | 4.05 | 30.92 | 4.91 | 0.39 | 4.46 | 23.79 | 9.57 | 13.26 | 7.45 |
| *Lactobacillus curvatus* | | | | | | | | | | |
| DU 0162 | 1.47 | 3.72 | 7.83 | 10.30 | 0.31 | 3.81 | 48.76 | 9.72 | 12.64 | 7.30 |
| DU 0182 | 1.30 | 3.70 | 8.52 | 8.43 | 1.59 | 4.80 | 41.14 | 9.07 | 12.68 | 8.22 |
| NCFB 2739[c] | 0.72 | 2.70 | 11.51 | 6.98 | 0.41 | 5.59 | 54.78 | 4.28 | 5.31 | 8.72 |
| *Lactobacillus brevis* | | | | | | | | | | |
| DU 0241 | 0.47 | 4.45 | 22.75 | 9.31 | 0.33 | 4.21 | 40.41 | 5.34 | 6.55 | 6.17 |
| DU 0242 | 0.20 | 5.55 | 20.24 | 10.42 | 0.10 | 4.81 | 45.45 | 3.76 | 4.00 | 5.42 |
| JCM 1059[c] | 0.07 | 1.62 | 22.35 | 5.47 | 0.29 | 3.19 | 49.72 | 5.11 | 5.79 | 5.83 |
| *Lactobacillus* sp. | | | | | | | | | | |
| DU 0180 | 1.40[b] | 3.52 | 9.37 | 8.66 | 0.39 | 3.98 | 46.12 | 6.89 | 12.52 | 6.92 |
| DU 0181 | 1.25 | 3.51 | 10.44 | 8.44 | 0.42 | 4.76 | 43.39 | 6.03 | 14.06 | 7.38 |
| DU 0183 | 0.99 | 3.36 | 10.59 | 8.97 | 0.39 | 4.78 | 47.09 | 6.77 | 9.21 | 7.38 |
| DU 0238 | 1.14 | 3.15 | 7.99 | 9.92 | 0.43 | 5.27 | 33.72 | 13.16 | 16.24 | 8.38 |
| DU 0240 | 1.13 | 2.96 | 7.98 | 8.99 | 0.32 | 2.44 | 53.28 | 6.78 | 8.52 | 7.27 |
| DU 0259 | 0.61 | 2.97 | 8.88 | 8.91 | 0.35 | 3.07 | 46.89 | 8.38 | 10.61 | 8.63 |
| *Lactobacillus sake* | | | | | | | | | | |
| JCM 1157[c] | 1.24 | 5.88 | 35.75 | 7.44 | 1.36 | 2.73 | 19.25 | 12.62 | 8.21 | 5.52 |
| *Enterococcus faecium* | | | | | | | | | | |
| DU 0216 | 0.50 | 6.79 | 28.74 | 5.10 | 2.58 | 3.96 | 18.39 | 10.85 | 14.08 | 8.40 |
| DU 0230 | 1.19 | 6.74 | 28.23 | 4.58 | 4.55 | 8.90 | 19.52 | 8.35 | 10.31 | 7.33 |
| DU 0237 | 0.98 | 5.85 | 38.12 | 4.38 | 1.63 | 5.70 | 20.62 | 8.18 | 9.86 | 4.65 |
| DU 0253 | 0.63 | 5.96 | 33.46 | 4.63 | 1.68 | 1.97 | 16.37 | 7.79 | 17.59 | 9.02 |
| DU 0255 | 1.30 | 6.41 | 33.00 | 4.76 | 1.15 | 4.73 | 19.56 | 9.02 | 11.05 | 8.40 |
| DU 0267 | 0.70 | 6.82 | 35.43 | 5.27 | 1.07 | 7.02 | 16.38 | 9.31 | 10.84 | 7.16 |
| JCM 5804[c] | 1.36 | 6.72 | 30.48 | 6.92 | 0.91 | 4.55 | 16.39 | 10.17 | 16.70 | 5.70 |
| *Leuconostoc mesenteroides* subsp. *mesenteroides* | | | | | | | | | | |
| DU 0243 | 0.23 | 7.75 | 33.35 | 4.85 | 2.04 | 3.13 | 20.72 | 7.11 | 12.20 | 8.19 |
| JCM 6124[c] | 0.67 | 6.76 | 32.73 | 7.01 | 1.63 | 4.99 | 23.18 | 6.72 | 10.98 | 5.31 |

[a]The number before the colon indicates the number of carbon atoms of the fatty acid, and the number after the colon indicates the number of double bonds. Cy indicates cyclopropane acid. [b]The number refers to the percentage of an acid to a total acid. [c]Reference strain, type strain of the species.

at pH 4.8. The characteristics of the strain correspond with the description of *L. mesenteroides* subsp. *mesenteroides* (5, 31, 39).

On the basis of these data, the isolates were identified as *Lactobacillus plantarum* (2 strains), *L. curvatus* (2 strains), *L. brevis* (2 strains), *Enterococcus faecium* (6 strains), *Leuconostoc mesenteroides* subsp. *mesenteroides* (1 strain) and *Lactobacillus* sp. (6 strains). Furthermore, these identifications were confirmed by their cellular fatty acid composition as shown in Table 4.

*L. plantarum* DU 0247 and 0256 had straight-chain fatty acids of $C_{16:0}$, $C_{18:1}$, and $C_{20:1}$, and cyclopropane acid of $C_{19:0}$ as major fatty acids. The straight-chain fatty acids of $C_{16:0}$ and $C_{18:1}$ were the most predominant among them. Their profiles were found to be similar to those of *L. plantarum* IAM 12477 (type strain) and corresponded with the result appearing in other literature (38). The strains of *Lactobacillus curvatus* DU 0162 and 0182 contained a straight-chain fatty acid of $C_{18:1}$ as a major fatty acid. A relatively large amount of the straight-chain fatty acids of $C_{16:0}$, $C_{16:1}$, $C_{18:1}$ $C_{20:1}$ and cyclopropane acid of $C_{19:0}$ were also present. These strains showed almost the same profile as those of *L. curvatus* NCFB 2739 (type strain). *L. brevis* DU 0241 and 0242 had a large amount of straight-chain fatty acids of $C_{18:1}$ and $C_{16:0}$, and both the fatty acids comprised over 60% of the total fatty acids in its amount. Their profiles were identical with those of *L. brevis* JCM 1059 (type strain). Unidentified

strains of *Lactobacillus* sp. DU 0180, 0181, 0183, 0238, 0240 and 0259 showed almost the same fatty acid profile with each other. They contained a large amount of straight-chain fatty acid of $C_{18:1}$ as a major fatty acid, and a considerable amount of straight-chain fatty acids of $C_{16:0}$, $C_{16:1}$, $C_{20:1}$ and cyclopropane acid of $C_{19:0}$ were also found. Fatty acid profiles of these strains were significantly distinct from those of *L. sake* JCM 1157 (type strain) and almost identical with those of *L. curvatus* NCFB 2739 (type strain).

*E. faecium* DU 0216, 0230, 0237, 0253, 0255 and 0267 contained straight-chain fatty acids of $C_{16:0}$ and $C_{18:1}$ as major fatty acids, and larger amount of straight-chain fatty acid of $C_{20:1}$ and cyclopropane acid of $C_{19:0}$ was also contained. Each fatty acid profile of these strains was found to be identical with those of *E. faecium* JCM 5804 (type strain).

*L. mesenteroides* subsp. *mesenteroides* DU 0243 and IAM 1046 (type strain) shared similar fatty acid profiles. Both strains contained straight-chain fatty acids of $C_{16:0}$ and $C_{18:1}$ as dominant fatty acids and larger amount of cyclopropane acid of $C_{19:0}$ was also found.

With the exception of unidentified 6 strains which belonged to genus *Lactobacillus*, the cellular fatty acid compositions of all the strains of bacteriocin-producing lactic acid bacteria fully support the identifications of the strains based on phenotype, fermentation type, isomer of lactic acid, and peptidoglycan type. While these *Lactobacillus* strains were similar to *L. sake* in their characteristics, their fatty acid profiles were different from those of *L. sake*, but they were almost the same with those of *L. curvatus* as mentioned above. There are indications that *L. sake* and *L. curvatus* are related to each other (40~50% homology), but not to other lactobacilli species (12). It seems to be complicated to differentiate between the two species for their relatedness, and needs further study for clear distinction.

**Antimicrobial Spectrum and Characteristics of the Bacteriocins**

The spectrum of antimicrobial activity and physiochemical characteristics of the bacteriocins produced by the isolate strains are shown in Table 5 and 6, respectively. The antimicrobial activity of all isolates could not be prevented by the addition of catalase, indicating it was not caused by the action of hydrogen peroxide (Table 6). Some proteolytic enzymes partially or completely prevented the activity of producer strains (Table 6). This strongly suggests that the inhibitory molecules are proteins. Konisky (15) defined bacteriocins as proteins or protein complex not active against the producer strain. According to this definition, the molecules produced by the isolates are bacteriocins.

The bacteriocins produced by *L. plantarum* DU 0247 and 0256 were capable of inhibiting several strains of lactic acid bacteria. These bacteriocins were sensitive to most of the proteolytic enzymes and stable to heat. Similar results were reported for the *L. plantarum* bacteriocins, such as plantaricin A (4), plantaricin S (11), plantaricin T (11) and plantacin B (40). But, plantaricin S and plantacin B were also sensitive to the lipase and α-amylase (11, 40).

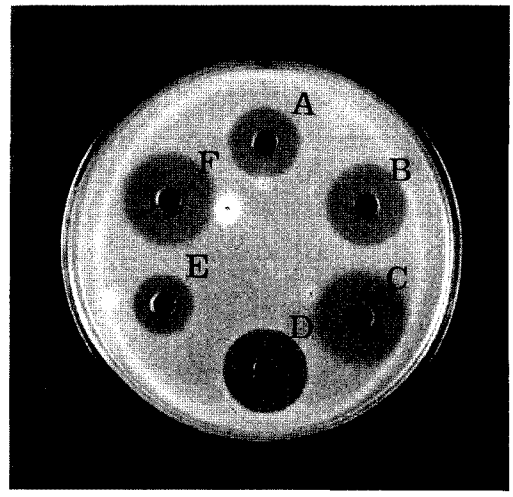


**Fig. 1.** Agar well diffusion showing the bacteriocin activities from representative isolates against indicator *Lactobacillus sake* JCM 1157.
The isolates are *Lactobacillus plantarum* DU 0247 (A), *L. curvatus* DU 0162 (B), *L. brevis* DU 0241 (C), *Enterococcus faecium* DU 0267 (D), *Leuconostoc mesenteroides* subsp. *mesenteroides* DU 0243 (E) and *Lactobacillus* sp. DU 0238 (F). Each well contained 1 mg of crude bacteriocins which were prepared by ammonium sulfate precipitation from culture supematants of the isolates, dialysis and lyophilization.

Inhibitory activity of the bacteriocins produced by *L. curvatus* DU 0162 and 0182 was restricted to a few strains of lactic acid bacteria. Their bacteriocin activity was substantially reduced by the treatment with lipase and α-amylase along with proteolytic enzymes. It seems probable that a glyco and/or lipid moieties are associated with antagonistic activity from the strains of *L. curvatus* as well as of *L. mesenteroides* subsp. *mesenteroides* mentioned below. Their bacteriocins were stable to heat.

The bacteriocins produced by *L. brevis* DU 0241 and 0242 were inhibitory to many strains of the lactic acid bacteria. Proteolytic enzyme digestions resulted in the inactivation of their bacteriocin activity. Their bacteriocins were heat-stable and the bacteriocin activity from *L. brevis* DU 0241 remained even after autoclaving. Similar result was reported for brevicin 37 from *L. brevis* B 37 (28).

*E. faecium* strains, as *Lactobacillus* sp. strains described below, showed the highest number (6 strains) among the bacteriocin-producing strains. The bacteriocins produced by *E. faecium* DU 0216, 0230, 0237, 0253, 0255 and 0267 exhibited the broadest spectrum of inhibition,

**Table 5.** Antimicrobial spectrum of bacteriocins[a] produced by isolates

| Bacteriocin producer | Indicator strain | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *L. plantarum* IAM 12477 | *L. plantarum* DU 3003 | *L. sake* JCM 1157 | *L. sake* DU 3011 | *L. brevis* KCTC 3102 | *L. brevis* JCM 1059 | *L. brevis* DU 3025 | *E. facealis* JCM 5803 | *E. faecalis* DU 2103 | *E. faecium* KCTC 3095[b] | *E. faecium* JCM 5804 | *E. faecium* DU 2102 | *P. pentosaceus* IAM 12300 | *P. pentosaceus* DU 2203 | *P. acidilactici* IAM 1233 | *Leuc. mesenteroides* subsp. *mesenteroides IAM 1046* | *Leuc. mesenteroides* subsp. *mesenteroides* JCM 6124 | *Leuc. mesenteroides* subsp. *mesenteroides* DU 1003 | *A. hydrophila* KCTC 2358 | *B. cereus* KCTC 1012 | *B. subtilis* KCCM 11914 | *C. perfringens* ATCC 132124 | *E. coli KCTC 1039* | *L. monocytogenes* ATCC 19111 | *M. luteus* KCCM 11455 | *S. typhimurium* KCTC 1925 | *S. aureus* KCTC 1621 | *V. parahaemolyticus* ATCC 27519 |
| *Lactobacillus plantarum* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DU 0247 | 0[c] | 80 | 5,120 | 0 | 0 | 0 | 0 | 0 | 40 | 640 | 0 | 20 | 0 | 20 | 40 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0256 | 0 | 20 | 81,920 | 0 | 0 | 0 | 0 | 160 | 20 | 20 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lactobacillus curvatus* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DU 0162 | 0 | 0 | 2,560 | 0 | 0 | 0 | 0 | 80 | 0 | 160 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0182 | 0 | 0 | 2,560 | 0 | 0 | 0 | 0 | 160 | 0 | 160 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lactobacillus brevis* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DU 0241 | 0 | 40 | 20,480 | 0 | 0 | 0 | 10 | 160 | 20 | 1,280 | 0 | 20 | 160 | 20 | 160 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0242 | 0 | 80 | 160 | 0 | 0 | 0 | 20 | 0 | 80 | 40 | 0 | 20 | 0 | 160 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Enterococcus faecium* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DU 0216 | 320 | 20 | 10,240 | 40 | 160 | 160 | 20 | 40 | 40 | 80 | 320 | 20 | 80 | 40 | 80 | 320 | 320 | 40 | 0 | 0 | 160 | 320 | 0 | 40 | 320 | 0 | 0 | 0 |
| DU 0230 | 160 | 40 | 10,240 | 40 | 320 | 160 | 20 | 40 | 40 | 160 | 320 | 20 | 160 | 40 | 80 | 1,280 | 320 | 40 | 0 | 0 | 160 | 200 | 0 | 10 | 160 | 0 | 0 | 0 |
| DU 0237 | 320 | 20 | 10,240 | 40 | 160 | 160 | 20 | 40 | 40 | 80 | 320 | 10 | 160 | 20 | 160 | 640 | 160 | 20 | 0 | 0 | 100 | 200 | 0 | 10 | 200 | 0 | 0 | 0 |
| DU 0253 | 320 | 80 | 10,240 | 20 | 320 | 80 | 20 | 20 | 80 | 80 | 320 | 40 | 160 | 320 | 160 | 640 | 80 | 40 | 0 | 0 | 100 | 200 | 0 | 10 | 200 | 0 | 0 | 0 |
| DU 0255 | 320 | 20 | 10,240 | 40 | 160 | 160 | 20 | 40 | 80 | 40 | 160 | 20 | 160 | 20 | 80 | 640 | 80 | 20 | 0 | 0 | 100 | 100 | 0 | 10 | 160 | 0 | 0 | 0 |
| DU 0267 | 320 | 40 | 5,120 | 20 | 320 | 80 | 40 | 20 | 80 | 40 | 160 | 40 | 160 | 160 | 80 | 640 | 80 | 40 | 0 | 0 | 100 | 200 | 0 | 40 | 200 | 0 | 0 | 0 |
| *Leuconostoc mesenteroides* subsp. *mesenteroides* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DU 0243 | 0 | 0 | 320 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lactobacillus* sp. | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| DU 0180 | 0 | 40 | 81,920 | 20 | 160 | 0 | 10 | 320 | 20 | 1,280 | 0 | 20 | 160 | 10 | 320 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0181 | 0 | 40 | 81,920 | 40 | 160 | 0 | 10 | 320 | 20 | 1,280 | 0 | 20 | 160 | 20 | 160 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0183 | 0 | 40 | 40,960 | 40 | 160 | 0 | 10 | 320 | 40 | 5,120 | 0 | 20 | 160 | 20 | 160 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0238 | 0 | 80 | 163,840 | 0 | 320 | 0 | 10 | 320 | 80 | 5,120 | 0 | 40 | 320 | 80 | 160 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0240 | 0 | 80 | 81,920 | 0 | 160 | 0 | 20 | 160 | 40 | 1,280 | 0 | 10 | 320 | 80 | 160 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DU 0259 | 0 | 10 | 5,120 | 0 | 0 | 0 | 0 | 80 | 0 | 640 | 0 | 0 | 40 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Neutralized cell-free supernatant fluid was used for the assay. [b] Indicator strain used for the isolation of bacteriocin-producing lactic acid bacteria in this study. [c] Bacteriocin activity was measured by the agar well diffusion assay and expressed in AU/ml.

**Table 6.** Effect of various enzymes and heat treatments on antimicrobial activity of partially purified bacteriocin of isolates

| Bacteriocin producer | Catalase | Protease | Pepsin | Trypsin | Chymotrypsin | α-Amylase | Lipase | Lysozyme | Heating at 100℃, 10 min | 100℃, 30 min | 100℃, 60 min | 121℃, 10 min | 121℃, 20 min | 121℃, 30 min |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lactobacillus plantarum* | | | | | | | | | | | | | | |
| DU 0247 | 100[a] | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 0 | 0 | 0 |
| DU 0256 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 100 | 50 | 0 | 0 | 0 |
| *Lactobacillus curvatus* | | | | | | | | | | | | | | |
| DU 0162 | 100 | 0 | 0 | 0 | 0 | 25 | 0 | 100 | 100 | 100 | 50 | 0 | 0 | 0 |
| DU 0182 | 100 | 0 | 0 | 0 | 0 | 25 | 0 | 100 | 100 | 50 | 25 | 0 | 0 | 0 |
| *Lactobacillus brevis* | | | | | | | | | | | | | | |
| DU 0241 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 50 | 25 | 6 | 6 |
| DU 0242 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 0 | 0 | 0 | 0 | 0 |
| *Enterococcus faecium* | | | | | | | | | | | | | | |
| DU 0216 | 100 | 25 | 0 | 25 | 0 | 100 | 100 | 100 | 50 | 13 | 0 | 0 | 0 | 0 |
| DU 0230 | 100 | 7 | 0 | 7 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 0 | 0 | 0 |
| DU 0237 | 100 | 13 | 0 | 13 | 0 | 100 | 100 | 100 | 25 | 25 | 13 | 0 | 0 | 0 |
| DU 0253 | 100 | 13 | 0 | 13 | 0 | 100 | 100 | 100 | 50 | 25 | 13 | 0 | 0 | 0 |
| DU 0255 | 100 | 50 | 0 | 50 | 0 | 100 | 100 | 100 | 50 | 25 | 25 | 0 | 0 | 0 |
| DU 0267 | 100 | 25 | 0 | 25 | 0 | 100 | 100 | 100 | 50 | 25 | 25 | 0 | 0 | 0 |
| *Leuconostoc mesenteroides* subsp. *mesenteroides* | | | | | | | | | | | | | | |
| DU 0243 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 50 | 0 | 0 | 0 | 0 |
| *Lactobacillus* sp. | | | | | | | | | | | | | | |
| DU 0180 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 25 | 6 | 6 |
| DU 0181 | 100 | <1 | <1 | 0 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 25 | 6 | 6 |
| DU 0183 | 100 | <1 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 25 | 6 | 6 |
| DU 0238 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 25 | 6 | 6 |
| DU 0240 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 25 | 6 | 6 |
| DU 0259 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 100 | 50 | 50 | 25 | 6 | 6 |

[a] The number refers to the percentage of an activity of partially purified bacteriocin after treatment to an activity of the bacteriocin before treatment. Bacteriocin activity was measured by the agar well diffusion assay using *L. sake* JCM 1157 as an indicator strain.

affecting against other Gram-positive bacteria including all lactic acid bacteria tested and some health-threatening bacteria such as *L. monocytogenes* and *C. perfringens*, but none of the Gram-negative bacteria tested were inhibited. Among the indicator strains, *L. sake* JCM 1157, *L. mesenteroides* subsp. *mesenteroides* IAM 1040, *L. brevis* KCTC 3102, *M. luteus* KCCM 11455 and *C. perfringens* 13124 were highly sensitive to the antimicrobial substances produced by these isolates. These bacteriocins were digested completely by pepsin and α-chymotrypsin, whereas resistant to the digestion by trypsin and protease. There have been many reports about *E. faecium* bacteriocins, such as enterocin 1146 (26), enterocin EIA and EIB (16), enterocin 100 (13) and the bacteriocins from *E. faecium* JBL 1061, 1083 and 1351 (1), and each bacteriocins of them inhibited *L. monocytogenes* (1, 13, 16, 20, 26). Among these bacteriocins, those of *E. faecium* JBL 1061, 1083 and 1351 are more heat-tolerant than other bacteriocins, including those of isolates, and they are trypsin-sensitive but pepsin-insensitive. Enterocin-100 are resistant to the digestion by pepsin as well as by trypsin; enterocin EIA and enterocin 1146 are sensitive to pepsin, trypsin and α-chymotrypsin; and enterocin EIB are insensitive to trypsin and α-chymotrypsin. Despite the limitation of the data obtained in this study, the bacteriocins of these isolates can be said to be different from those bacteriocins.

Enterococci are commonly isolated from various foodstuffs (2, 10, 20) as well as from Kimchi (17). They are normal intestinal flora and presumed to contribute to the overall health of the host (36). Although enterococci are generally considered as harmless, recent studies have established the pathogenic potential of these organisms (22). Since the enterococci identified in this study are non-hemolytic (Table 2), *E. faecium* DU 0216, 0230, 0237, 0253, 0255 and 0267 are presumably non-pathogenic and their bacteriocins are not hemolysin-related.

Inhibitory activity of the bacteriocin produced by *L. mesenteroides* subsp. *mesenteroides* DU 0243, the only one strain of the species, was restricted to a few strains of lactic acid bacteria. Similar antimicrobial spectra of the bacteriocins have also been reported for the bac-

teriocins of *Leuconostoc* species, such as mesenterocin 5 (3), mesenterocin 52 (21), and the bacteriocins from *L. gelidum* IN 139 (32) and *L. gelidum* UAL 187 (8). Although most of *Listeria* strains were sensitive to the bacteriocins from *L. mesenteroides* subsp. *mesenteroides* according to those results, *L. monocytogenes* ATCC 19111 was insensitive to the bacteriocin from the isolate. The activity of their bacteriocin was also reduced by the treatment with lipase and α-amylase as well as proteolytic enzymes. Leuconocin S, a bacteriocin produced by *L. paramesenteroides* OX, is inactivated by α-amylase but not by lipase (18). The bacteriocin activity of the isolate was completely inactivated by heating at 100°C for 60 min and by autoclaving at 121°C for 10 min.

Six strains of *Lactobacillus* sp. DU 0180, 0181, 0183, 0238, 0240 and 0259 showed the antimicrobial activity affecting many strains of lactic acid bacteria. Among the indicator strains, *L. sake* JCM 1157, *E. faecium* KCTC 3095, *E. faecalis* JCM 5803, *L. brevis* KCTC 3102, *P. pentosaceus* IAM 12300 and *P. acidilactici* IAM 1233 were highly sensitive to the bacteriocins of these isolates. Their activities were sensitive to proteolytic enzymes and remained even after autoclaving. Similar characteristics have been reported for the bacteriocins from *L. sake* Lb 706 (30), L. *sake* L45 (23) and *L. sake* 148 (34).

It has previously been shown that, among lactic acid bacteria, the genera of *Lactobacillus*, *Leuconostoc* and *Pediococcus* were included to lactobacillus supercluster within so-called clostridia subbranch of the Gram-positive bacteria, and the genera *Enterococcus*, *Bacillus* and *Clostridium* were differentiated from them but belonged to the same subbranch (12). Moreover, the taxonomic position of the genus *Listeria* is not quite clear, but there are indications that this genus is closely associated to the genus *Enterococcus* (35) and genus *Lactobacillus* (19, 29, 41). Therefore, it is not surprising that enterococci and lactobacilli produce bacteriocins effective against species of *Enterococcus, Lactobacillus, Leuconostoc, Pediococcus, Bacillus, Clostridium* and *Listeria*.

Among the bacteriocins obtained in this study, the bacteriocins produced by *E. faecium* strains were inhibitory to the wide range of Gram-positive bacteria including the health-threatening bacteria such as *C. perfringens* and *L. monocytogenes* as well as lactic acid bacteria, and those of unidentified *Lactobacillus* strains were resistant to autoclaving. Moreover, these bacteriocin activities were found to be greater than those of other producer strains. Therefore, antimicrobial substances and/or its producer strains of the both species seem to have a potential value as novel food preservatives. Further investigations are underway to characterize in detail the bacteriocins in our laboratory. We are also studying their novel applications.

Meanwhile, the results reported in this paper suggest that many bacteriogenic lactic acid bacteria occur in Kimchi. These strains are assumed to affect bacterial flora, mainly, lactic acid bacteria, in Kimchi, and consequently, to be related to its taste. From this point of view, more researches on the ecology of lactic acid bacteria and their role in Kimchi, should be carried out to understand fully the science of Kimchi.

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