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Isolation of *Leuconostoc mesenteroides* subsp. *mesenteroides* DU-0608 with Antibacterial Activity from Kimchi and Characterization of Its Bacteriocin

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A bacteriocin-producing strain, DU-0608, was isolated from Kimchi and identified as *Leuconostoc mesenteroides* subsp. *mesenteroides*. The bacteriocin from isolate was inhibitory against *Listeria monocytogenes*, *Micrococcus luteus* and several strains of lactic acid bacteria. The bacteriocin was inactivated by pepsin, trypsin, α-chymotrypsin, protease, α-amylase and lipase, but not by catalase or by heating at 100°C for 60 min. The molecular weight of the bacteriocin was estimated approximately 6 kDa. The inhibitory effect was bactericidal and rapid. Following treatment with isolate bacteriocin, cells of indicator strain (*Lactobacillus sake* JCM 1157) were damaged at the end regions of the cell wall, whereas the cells treated with nisin were damaged at many places around the cell wall.

Lactic acid bacteria are inhibitory to other bacteria because of organic acids, carbon dioxide, hydrogen peroxide, and other chemicals, including bacteriocins, which are produced during their growth. As a result, fermented foods containing lactic acid bacteria extended their shelf-life and improved the product safety.

Among the lactic acid bacteria, heterofermentative facultative anaerobic leuconostoc are commonly associated with fermented foods. They are important for the production of flavor components, such as diacetyl formed from citrate. It is also known that early predominance of heterofermentative lactic acid bacteria is essential in the production of high-quality cabbage fermented products such as Kimchi and sauerkraut (16, 18, 26). These bacteria play an important role not only in preserving the fermented products and product safety, but also in improving taste and palatability.

Bacteriocin production by *Leuconostoc* sp. was first observed in the 1950s, but more extensive studies of bacteriocins have been done since 1984, when the antagonistic activity of *Leuconostoc* sp. was reported (20). Bacteriocins produced by *Leuconostoc* sp. including mesentericin 5 (3) produced by *Leuconostoc mesenteroides* subsp. *mesenteroides*; leucocin A (10) produced by *L. gelidum* UAL 187; carnosin 44A (22) produced by *L. carnosum* LA44A; leuconocin S (2) produced by *L. paramesenteroides* OX; and mesenterocin 52 (17) produced by *L. mesenteroides* subsp. *mesenteroides* FR 52 have all been examined, however, these bacteriocin-producing bacteria were isolated from meat and dairy products (27). There are few reports on bacteriocin-producing *Leuconostoc* sp. isolated from Kimchi (6, 13).

In this study, a bacteriocin-producing leuconostoc was isolated from Kimchi and identified. The characteristics of its bacteriocin and its mode of action were examined. The structural change of the cell wall of indicator cells after treatment with isolate bacteriocin was also observed by scanning electron microscope.

MATERIAL 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 bacteria, the media used were: APT broth for *Listeria monocytogenes*, nutrient broth for *Aeromonas hydrophila, Bacillus subtilis, B. cereus* and *Micrococcus luteus*; sodium thioglycollate medium for *Clostridium perfringens*; trypticase soy broth for *E. coli* and *Staphylococcus aureus*. All media used were purchased from Difco Laboratories with the exception of TS broth which was purchased from BBL Microbiology Systems. Agar medium was prepared by the addition of 1.5 or 0.75% granulated agar to broth medium for basal or soft overlay agar, respectively. The strains were maintained as stab cultures in agar media at 4°C. Cultures were transferred

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Table 1. Strains used in this study.

Strain	Other designation[a]	Reference
Aeromonas hydrophila KCTC 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	
Lactobacillus plantarum IAM 12477	ATCC 14917[T]	
Lactobacillus plantarum DU 3003[b]		(2)
Lactobacillus curvatus NCFB 2739	ATCC 25601[T]	
Lactobacillus sake JCM 1157[c]	ATCC 15521[T]	
Lactobacillus sake DU 3011[b]		(2)
Lactobacillus brevis JCM 1059	ATCC 8287[T]	
Lactobacillus brevis DU 3025[b]		(2)
Enterococcus faecalis JCM 5803	ATCC 19433[T]	
Enterococcus faecalis DU 2103[b]		(2)
Enterococcus faecium JCM 5804	ATCC 19434[T]	
Enterococcus faecium KCTC 3095		
Enterococcus faecium DU 2102[b]		(2)
Leuconostoc mesenteroides subsp. *mesenteroides* JCM 6124	ATCC 8293[T]	
Leuconostoc mesenteroides subsp. *mesenteroides* DU 1003[b]		(2)
Leuconostoc mesenteroides subsp. *dextranicum* KCTC 3530	ATCC 19255[T]	
Pediococcus pentosaceus IAM 12300		
Pediococcus pentosaceus DU 2203[b]		(2)
Pediococcus acidilactici IAM 1233	ATCC 8042	

[a]Abbrevations: 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 strains isolated from Kimchi by authors.
[c]The indicator strain used for isolation of bacteriocin-producing lactic acid bacteria and measurment of bacteriocin activity.
[T]The type strain of the species.

and propagated twice at 37°C before use in experiments.

Isolation of Bacteriocin-producing Strain

Bacteriocin-producing strain was isolated from a total number of 130 samples of Kimchi at the early stage of fermentation. The procedure for isolation was the same as that reported in the previous paper (6). A portion of the Kimchi sample was incubated at 37°C in MRS broth, and then a ten-fold dilution series of 1% Bacto-peptone was plated. Plates containing 50 to 100 colonies were overlayed with approximately 8 ml MRS soft agar containing about 10^7 cells of the indicator strain. In routine tests, *Lactobacillus sake* JCM 1157 was used as the indicator strain. The plate was further incubated at 30°C for 18 to 24 h and was examined for a inhibition zone around the colony.

Identification of Isolate

To identify isolated bacteriocin-producing strain, the following characteristics 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 (9, 25, 30). The range of fermented sugars was determined by using API 50 CHL medium (Biomerieux sa). The inoculated media were incubated at 25°C and read after 24 and 48 h. The configuration of the lactic acid enantiomers was determined enzymatically by using D (-)- and L (+)-lactate-dehydrogenase (Boehringer Mannheim GmbH) (19).

The peptidoglycan type of cell wall was also examined. *meso*-Diaminopimelic acid in peptidoglycan was detected by the method of Komagata and Suzuki, using cellulose TLC (14). Furthermore, the identification of the isolate was confirmed by fatty acid analysis of cells using a gas chromatography system (GC-8A, Shimazu Corp.). Methyl ester of cellular fatty acids were prepared from lyophilized cells by usings a 5% methanolic hydrochloride acid solution.

DNA base composition was estimated. DNAs were isolated from cells grown in GYP-sodium acetate-mineral salt broth for 1 to 2 days, and purified by the method of Saito and Miura (23). The purified DNA was hydrolyzed to nucleotides and nucleosides as described by Tomaoka and Komagata (29), and the hydrolysate was applied to a reversed phase high-performance liquid chromatography (LC-4A, Shimazu Corp.).

Purification of Bacteriocin

The bacteriocin-producing strain was cultured in MRS broth at 30°C for 18 h. The culture broth was centrifuged and sterile filtered. The cell-free supernatant fluid was decanted into a beaker set in an ice bucket and solid ammonium sulfate was added stepwise to achieve 70% saturation and then stirred for an additional 30 min. The precipitated suspension was centrifuged at 10,000×g for 30 min 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.

SDS-PAGE Analysis of Bacteriocin

Partially purified bacteriocin was examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with a

10-20% continuous gradient gel designed to separate low molecular weight proteins (4, 15, 31). Molecular weight marker (Novex Experimental Technology) were used as standard proteins. Electrophoresis was carried out at 12.5 Vcm^{-1}. After electrophoresis, the gel was divided into two parts. One part of the gel was stained with Coomassie brilliant blue R-250 (Fluka Chemie AG) (7). The other part was used for the detection of bacteriocin activity of the separated bands according to the direct method of Bhunia *et al.* (1). The gel was subsequently placed on MRS agar plate and overlayed with MRS soft agar containing the indicator strain at the level of approximately 10^7 cfu/ml. After incubation at 30°C overnight, an inhibition zone was observed and the bacteriocin band in the stained gel was identified.

Detection of Antimicrobial Activity

The agar well diffusion method (28) was used to detect the antimicrobial activity against representative Gram-positive and negative indicator organisms. Portions (0.1 ml) of culture broth or partially purified bacteriocin solution were placed in the wells of MRS agar plates which were overlayed with MRS soft agar lawns containing approximately 10^7 cfu of log-phase culture of the indicator strain. The plates were then incubated anaerobically for 18 to 24 h and examined for inhibition of the indicator lawn. For an activity, two-fold serial dilutions of the test solution were used (17). The titer was defined as the reciprocal of the highest dilution showing a definite inhibition on the indicator lawn, and was expressed in arbitrary units (AU) per milliliter.

Characterization of the Bacteriocin

The partially purified bacteriocin in 5 mM phosphate buffer, pH 7.0 (1 mg/ml) was tested for sensitivity to heat and enzyme treatments (6). 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, α-chymotrypsin, trypsin, pepsin, protease, α-amylase, lipase and lysozyme. Enzymes were dissolved in 5 mM phosphate buffer, pH 7.0 (except for the pepsin which was dissolved in 0.02 N 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. The remaining activity in either enzyme or heat-treated solution was determined by agar well diffusion against a lawn of the indicator strain.

Mode of Action

To establish the antimicrobial action of isolate bacteriocin on indicator cells, two batches of indicator strain (*L. sake* JCM 1157) were incubated at 30°C for 3 h and for 18 h to prepare log- and stationary-phase cells, respectively. The cells obtained by centrifugation were suspended in MRS-broth (no glucose added) to an optical density of 0.4 at 660 nm. After addition of the bacteriocin (5,120 AU/ml) to the indicator cell suspensions, the suspensions were incubated at 30°C for 3 h. At each 60 min interval, the viable indicator cells were enumerated by using MRS-agar plates, and the optical density of the cell suspensions at 660 nm was measured by a spectrophotometer (UV-1201, Shimazu Corp.) during incubation.

Scanning Electron Microscopy

Structural changes of the bacteria by bacteriocin treatment were observed with a scanning electron microscope (SEM) (DS-130C Akasi Co.). Both log- and stationary-phase cells obtained as mentioned above, were suspended in saline to an optical density of 0.4 at 660 nm, mixed with the same volume of crude bacteriocin solution (5,120 AU/ml), and incubated for 3 h at 30°C, respectively. Meanwhile, both phase cells were treated with nisin (1,000 IU/ml) in the same way to allow comparison of the structural changes by isolate bacteriocin. After the treatment, the cells obtained by centrifugation were fixed with 2.5% glutaraldehyde at 4°C overnight. The specimens washed with 0.1 M phosphate buffer, pH 7.2-7.4, 3 times, were dehydrated in graded series of ethanol, air-dried at 37°C and ion spatter-coated with 200 nm of gold in a vacuum evaporator (11). They were then examined on a SEM which was operated at an accelerating voltage of 15 kV.

RESULT AND DISCUSSION

Isolation and Identification of Bacteriocin-producing Strain

From a total of 130 Kimchi samples, one strain of bacteriocin-producing leuconostocs was isolated. The isolated strain, DU-0608, was Gram-positive, nonmotile, catalase-negative and heterofermentative. The cells were sphere-shaped and occurred singly, in pairs or in chains. The strain produced gas and D-lactic acid from glucose, and showed negative reactions for litmus reduction in milk and ammonia formation from arginine. Therefore, the strain belongs to genus *Leuconostoc*.

The strain produced acid from sucrose, trehalose, arabinose and melibiose, and formed dextran from sucrose. It did not grow at pH 4.8, and contained 38.1 mol% of G+C content. These characteristics of the strain correspond with the description of *L. mesenteroides* subsp. *mesenteroides* (5, 24, 30). Phenotypic characteristics of the strain are shown in Table 2.

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

Table 2. Characteristics of the bacteriocin-producing strain DU-0608 isolated from Kimchi.

Cell form	Cocci	Acid formation	
Cell size (μm)	0.5–1.5	Amygdalin	+
Cell arrangement	pairs, chains	Arabinose	+
Gram reaction	+	Arbutin	+
Motility	–	Cellobiose	+
Spore formation	–	Cellulose	–
Gas from glucose	+	Dextrin	+
Catalase	–	Esculin	–
Reaction in litmus milk:		Fructose	+
Reduction	–	Galactose	+
Peptonization	–	Glucose	+
Acid curd	–	Gluconate	+
Ammonia from arginine	–	Glycerol	+
Hydrolysis of esculin	–	Inulin	+
Hydrolysis of arginine	–	Lactose	+
Nitrate reduction	–	Maltose	+
Formation of indole	–	Maltotriose	+
Dextran formation	+	Mannitol	+
Growth at 10°C	+	Mannose	+
Growth at 15°C	+	Melezitose	+
Growth at 40°C	+	Melibiose	+
Growth at 45°C	–	Raffinose	+
Growth at 50°C	–	Rhamnose	+
Growth at pH 3.6	–	Ribose	+
Growth at pH 3.9	–	Salicin	+
Growth at pH 4.2	–	Sorbitol	+
Growth at pH 4.8	–	Sorbose	–
Growth at pH 8.6	+	Starch	+
Growth at pH 9.2	+	Sucrose	+
DAP in peptidoglycan	–	Trehalose	+
Isomer of lactic acid	D(–)	Xylose	+
G+C Content (mol%)	38.1		

Symbols: +, positive; -, negative.

Table 3. Cellular fatty acid composition of the bacteriocin-producing strain DU-0608 isolated from Kimchi and *Leuconostoc mesenteroides* subsp. *mesenteroides* JCM 6124[T].

Fatty acids	Strains	
	DU 0608	JCM 6124
Straight-chain acids		
$C_{12:0}$[a]	0.23[b]	0.67
$C_{14:0}$	7.75	6.76
$C_{16:0}$	33.35	32.73
$C_{16:1}$	4.85	7.01
$C_{17:0}$	2.04	1.63
$C_{18:0}$	3.13	4.99
$C_{18:1}$	20.72	23.18
$C_{20:1}$	7.11	56.72
Cyclopropane acid		
$C_{19:0}$	12.20	10.98
Unknown	8.19	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. [b]The number refers to the percentage of an acid to a total acid. [T]Type strain of the species.

(Table 3).

Therefore, the isolated strain was identified as *L. mesenteroides* subsp. *mesenteroides*.

Antimicrobial Spectrum and Characteristics of Bacteriocin

The spectrum of antimicrobial activity and physiochemical characteristics of the bacteriocin produced by the isolate are shown in Tables 4 and 5. The isolate was capable of inhibiting several strains of lactic acid bacteria, *Micrococcus luteus* and *Listeria monocytogenes*, but none of the Gram-negative bacteria tested were inhibited. The bacteriocin activity of the isolate was destroyed by treatment with α-chymotrypsin, trypsin and proteinase at pH 7.0 and with pepsin at pH 2.0. The activity was also reduced appreciably by treatment with α-amylase and lipase along with proteolytic enzymes at pH 7.0, and the residual activities were only 13% of starting activity. It seems probable that glyco and/or lipid moieties are associated with antagonistic activity. After treatment at 100°C for 30 min, bacteriocin activity was

Table 4. Antimicrobial spectrum of bacteriocin[a] produced by *Leuconostoc mesenteroides* subsp. *mesenteroides* DU-0608.

Indicator strain	Activity (AU/ml)[b]
Lactobacillus plantarum ATCC 14917	160
Lactobacillus plantarum DU 3003	0
Lactobacillus curvatus ATCC 25601	5,120
Lactobacillus sake JCM 1157	10
Lactobacillus sake DU 3011	160
Lactobacillus brevis JCM 1059	40
Lactobacillus brevis DU 3025	80
Enterococcus faecalis JCM 5803	20
Enterococcus faecalis DU 2103	320
Enterococcus faecium JCM 5804	40
Enterococcus faecium KCTC 3095	80
Enterococcus faecium DU 2102	80
Leuconostoc mesenteroides subsp. *mesenteroides* JCM 6124	40
Leuconostoc mesenteroides subsp. *mesenteroides* DU 1003	20
Leuconostoc mesenteroides subsp. *dextranicum* ATCC 19255	1,280
Pediococcus pentosaceus IAM 12300	40
Pediococcus pentosaceus DU 2203	160
Pediococcus acidilactici ATCC 8042	40
Aeromonas hydrophila ATCC 7966	0
Bacillus cereus ATCC 9634	0
Bacillus subtilis ATCC 21697	0
Clostridium perfringens ATCC 13124	0
Escherichia coli ATCC 9637	0
Listeria monocytogenes ATCC 19111	40
Micrococcus luteus NRRL B-1018	160
Salmonella typhimurium KCTC 1925	0
Staphylococcus aureus ATCC 25923	0

[a]Partially purified bacteriocin was used for assay. [b]Bacteriocin activity was measured by the agar well diffusion assay.

Table 5. Effects of various enzymes and heat treatments on the antimicrobial activity of partially purified bacteriocin from *Leuconostoc mesenteroides* subsp. *mesenteroides* DU-0608.

Enzymes		Heating at	
Catalase	100[a]	100°C, 10 min	100
Protease	13	100°C, 30 min	50
Pepsin	0	100°C, 60 min	0
Trypsin	0	121°C, 10 min	0
α-Chymotrypsin	0	121°C, 20 min	0
α-Amylase	13	121°C, 30 min	0
Lipase	13		

[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.

reduced to about 50% of starting activity. There have been many reports about the bacteriocin produced by *Leuconostoc* species, such as mesenterocin 5 (3), leucocin A (10), leuconocin S (2), carnocin LA54A (12), carnosin 44A (22), and the bacteriocin of *L. gelidum* IN139 (8) and *L. gelidum* UAL187 (10). Those bacteriocins inhibited *L. monocytogenes*. Among the bacteriocins of leuconostocs listed above, leuconocin S (2) and carnosin LA54A (12) are inactivated by α-amylase but not by lipase. We reported in the preceding paper (6) that the bacteriocin activity of *Leuconostoc mesenteroides* subsp. *mesenteroides* DU 0243 isolated from Kimchi, was also reduced by treatment with lipase and α-amylase as well as with proteolytic enzymes, but the bacteriocin did not inhibit *Micrococcus luteus* and *L. monocytogenes*. Despite the limitation of the data obtained in this study, it is considered that this isolate bacteriocin is different from other bacteriocins.

The Coomassie brilliant blue-stained gel showed a strong band, together with several broad bands within molecular weights ranging 5 to 40 kDa (Fig. 1A). In the MRS-agar plate used to examine antimicrobial activity of these bands, one clear inhibition zone of indicator was detected (Fig. 1B) and the zone corresponded to the strong band, whose molecular weight was estimated to be about 6 kDa, in the stained gel (Fig. 1).

Mode of Action

By the treatment of bacteriocin, changes in viable counts and the optical density (660 nm) of log- and stationary-phase cells of *L. sake* JCM 1157 are shown in Fig. 2. In both log- and stationary-phase cells, the optical density of the cell suspension gradually decreased and reached a quarter of the starting value, and the viable counts also decreased to below 10 cfu/ml after 3 h of incubation with the addition of bacteriocin solution (5,120 AU/ml). This indicates that the inhibitory effect was bactericidal, and that isolate bacteriocin could kill both log- and stationary-phase cells.

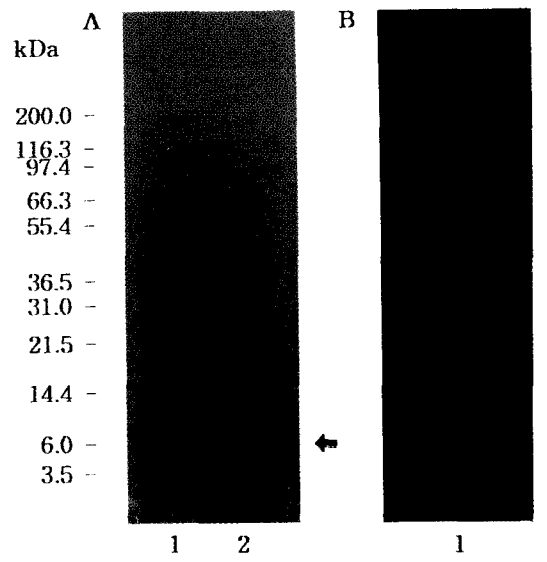

Fig. 1. Determination of the molecular weight of the bacteriocin produced by *Leuconostoc mesenteroides* subsp. *mesenteroides* DU 0608.

The bacteriocin was purified by ammonium sulfate precipitation from culture supernatants of the strain, dialysis and lyophilization. The bacteriocin was electrophoresed on a 10-20% continuous gradient gel. After electrophoresis, the gel was divided into two parts. One part (A) was Coomassie blue stained. Lane 1 shows molecular weight standards (size indicated on the left); lane 2, the partially purified bacteriocin. The other part (B) was overlaied with MRS soft agar containing *L. sake* JCM 1157 as indicator. Lane 1 shows the inhibiting zone by electrophoresed partially purified bacteriocin against indicator cells. Arrow head indicates the bacteriocin band (MW 6 kDa) which correspond with the inhibiting zone.

Scanning Electron Microscopic Observation of Bacteriocin-induced Structural Changes in Indicator Strain

Intact cells of the indicator strain, *L. sake* JCM 1157, were typical short-rod (Fig. 3A). When treated with DU-0608 strain bacteriocin solution (5,120 AU/ml) and nisin (1,000 IU/ml), the structural appearance of these cells was different from each other. In the case of treatment with DU-0608 strain bacteriocin, the cells of indicator strain were damaged at the end regions of the cell wall (Fig. 3B), but after treatment with nisin, the cells were damaged at many places around the cell wall (Fig. 3C).

The mechanisms by which bacteriocins inhibit or kill sensitive organisms are not fully understood. The bacteriocin molecules bind to specific cell surface receptors of gram-positive bacteria. According to Ray (21), following binding on sensitive cells, a bacteriocin molecule disorganizes the barrier property of the cell wall, allowing the bacteriocin molecules to cross the wall, to come in contact with the cytoplasmic membrane, to destabilize its function, and to cause viability loss, but the binding of a bacteriocin on a resistant cell does not disorganize the

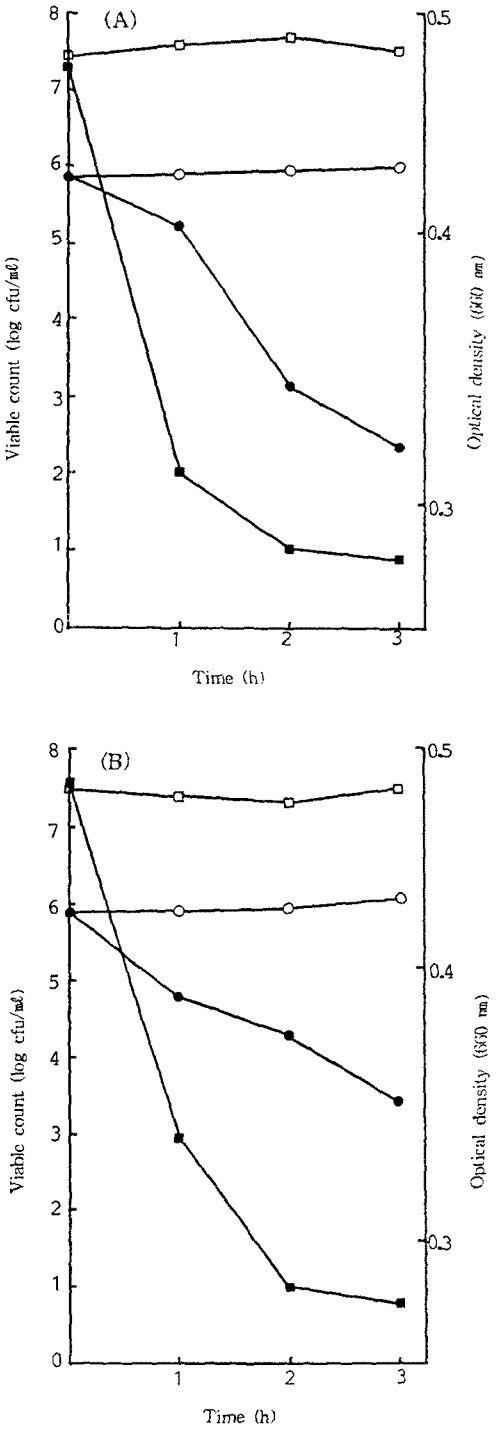

Fig. 2. Bactericidal effects of the partially purified bacteriocin of *Leuconostoc mesenteroides* subsp. *mesenteroides* DU-0608 against log-phase cells (A), and stationary-phase cells (B) of *Lactobacillus sake* JCM 1157.

○, Optical density in control (without bacteriocin); ●, optical density in test (with bacteriocin); □, viable count in control (without bacteriocin); ■, viable count in test (with bacteriocin).

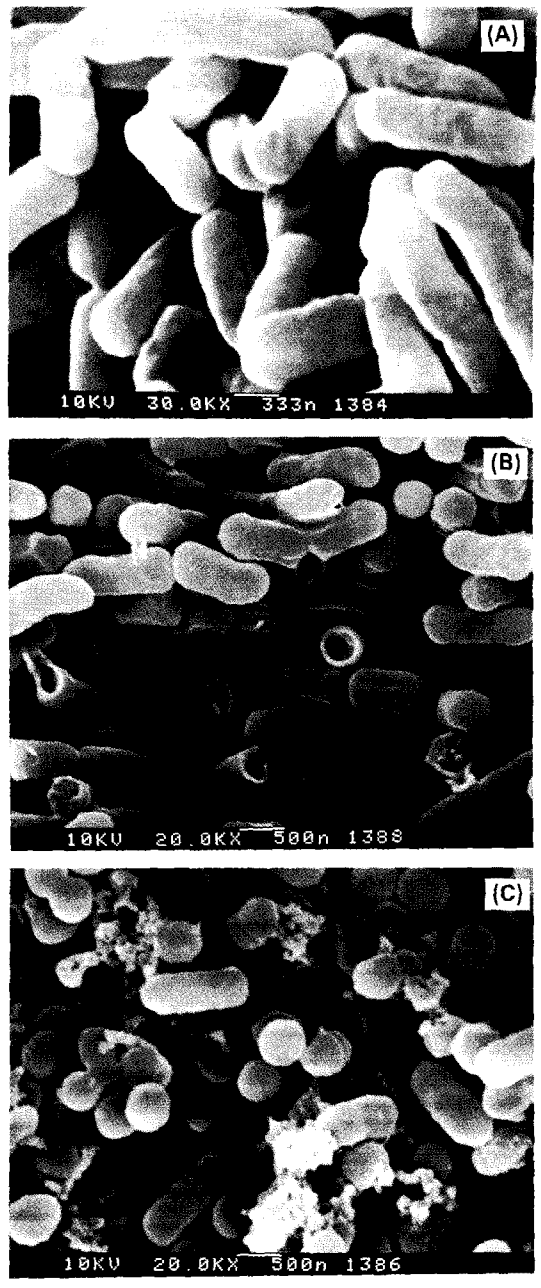

Fig. 3. Scanning electron micrographs of cells of *L. sake* JCM 1157 showing the structural changes of the cells after treatement with the bacteriocin of *L. mesenteroides* subsp. *mesenteroides* DU-0608 (isolate) and nisin.

(A), untreated cells; (B), treated cells with the bacteriocin of *L. mesenteroides* subsp. *mesenteroides* DU-0608; (C), treated cells with nisin.

cell wall. Accordingly, the difference in appearance between indicator cells treated with isolate bacteriocin and those treated with nisin indicates that the receptor sites on the cell wall of the indicator strain which are

disorganized by the isolate bacteriocin are different from those affected by nisin.

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REFERENCES

1. Bhunia, A. K., M. C. Johnson, and B. Ray. 1987. Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Ind. Microbiol.* **2**: 319-322.
2. Catherine, B. L., S. Sun, and T. J. Montville. 1992. Production of an amylase sensitive bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. *Appl. Environ. Microbiol.* **58**: 143-149.
3. Daba, H., S. Pandian, J. F. Gosselin, R. E. Simard, J. Huang, and C. Lacroix. 1991. Detection and activity of a bacteriocin produced by *Leuconostoc mesenteroides*. *Appl. Environ. Microbiol.* **57**: 3450-3455.
4. DeWald, D. B., L. D. Adams, and J. D. Pearson. 1986, A nonurea electrophoretic gel system for resolution of polypeptides of Mr 2,000 to Mr 200,000. *Anal. Biochem.* **154**: 502-508.
5. Elisabeth, M. and T. F. Fryer. 1966. Identification of the lactic acid bacteria. p. 65-79. *In* B. M. Gibbs and F. A. Skinner (eds.), *Identification Methods for Microbiologists*, Part A. Academic Press, New York.
6. Ha, D. M., D. S. Cha, and S. G. Han. 1994. Identification of bacteriocin-producing lactic acid bacteria from Kimchi and partial characterization of their bacteriocin. *J. Microbiol. Biotechnol.* **4**: 305-315.
7. Hames, B. D. and D. Rickwood. 1990. *Gel electrophoresis of proteins*. p. 53-58. Oxford University Press, New York.
8. Harding, C. D. and B. G. Shaw. 1990. Antimicrobial activity of *Leuconostoc gelidum* against closely related species and *Listeria monocytogenes*. *J. Appl. Bacteriol.* **69**: 648-654.
9. Harrigan, W. F. and E. M. Margaret. 1976. *Laboratory Method in Food and Dairy Microbiology*. p. 258-276. Academic Press, London.
10. Hastings, J. W. and M. E. Stiles. 1991. Antibiosis of *Leuconostoc gelidum* isolated from meat. *J. Appl. Bacteriol.* **70**: 127-134.
11. Jones, B. R. 1985. Preparation of specimens for scanning electron microscopy. p. 143-149. *In* B. R. Jones (ed.) *Electron Microscopy*. Library Reserch Associates Inc., New York.
12. Keppler, K., R. Geisen, and W. H. Holzafel. 1994. An α-amylase sensitive bacteriocin of *Leuconostoc carnosum*. *Food Microbiol.* **11**: 39-45.
13. Kim, J. H. 1995. Inhibition of *Listeria monocytogenes* by bacteriocin(s) from lactic acid bacteria isolated from Kimchi. *Agric. Chem. Biotechnol.* **38**: 302-307.
14. Komagata, K. and K. Suzuki. 1987. Lipid and cell-wall analysis in bacterial systematics, p. 161-207. *In* R. R. Cowell, and R. Grigorova (eds.), *Method in Microbiology*, vol. 19, Academic Press, London.
15. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.
16. Lee, C. W., C. Y. Ko, and D. M. Ha. 1992. Microfloral changes of the lactic acid bacteria during Kimchi fermentation and identification of the isolates. *Kor. J. Appl. Microbiol. Biotechnol.* **20**: 102-109.
17. Mathieu, F., I. S. Suwandhi, N. Rekhif, J. B. Milliere, and G. Lefebvre. 1993. Mesenterocin 52, a bacteriocin produced by *Leuconostoc mesenteroides* ssp. *mesenteroides* FR 52. *J. Appl. Bacteriol.* **74**: 372-379.
18. Mheen, T. I. and T. W. Kwon. 1984. Effect of temperature and salt concentration on Kimchi fermentation. *Korean J. Food Sci. Technol.* **16**: 443-450.
19. Okada, S., T. Toyoda, and M. Kozaki. 1978. An easy method for the determination of the optical types of lactic acid produced by lactic acid bacteria. *Agric. Biol. Chem.* **42**: 1781-1793.
20. Paulo. K. O. and W. E. Sandine. 1984. Common occurrence of plasmid DNA and vancomycin resistance in *Leuconostoc* spp. *Appl. Environ. Microbiol.* **48**: 1129-1133.
21. Ray. B. 1993. Sublethal injury, bacteriocins and food microbiology. *ASM News* **59**: 285-291.
22. Riette L. J. M. van Laack, U. Schillinger, and W. H. Holzapfel. 1992. Characterization and partial purification of a bacteriocin produced by *Leuconostoc carnosum* LA44A. *Int. J. Food Microbiol.* **16**: 183-195.
23. Saito, B. and K. Miura 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochemi. Biophys. Acta.* **72**: 619-629.
24. Schleifer, K. H. 1986. Gram-positive cocci, p. 993-1103. *In* N. R. Krieg, and J. G. Holt (eds.), *Bergey's Manual of Systematic Bacteriology*, vol. 2. Williams and Wilkins Co., Baltimore, MD.
25. Smibert, R. M. and R. K. Nobel. 1994. Phenotypic characterization, p. 607-654. *In* P. Gerhardt, R. G. E. Murray, W. A. Wood and N. R. Trige (eds.), *Methods for General and Molecular Bacteriology*, American society for Microbiology, Washington, D.C.
26. Stamer, J. R., B. O. Stoyla, and B. A. Dunckel. 1971. Growth rates and fermentation patterns of lactic acid bacteria associated with the sauerkraut fermentation. *J. Milk Food Technol.* **34**: 521-525.
27. Stiles, M. E. 1994. Bacteriocins produced by *Leuconostoc* species. *J. Dairy Sci.* **77**: 2718-2724.
28. Tagg, J. R. and A. R. McGiven. 1971. Assay system for bacteriocins. *Appl. Microbiol.* **21**: 943.
29. Tamaoka, J. and K. Komagata. 1984. Determination of DNA base composition by reversed phase high performance liquid chromatography. *FEMS Microbiol. Lett.* **25**: 125-128.

30. Uchinura, T. and S. Okada. 1992. Identification methods for lactic acid bacteria, p. 21-137. *In* M. Kozaki (ed.), *Nyusankin-jitken-manual*, Asakurashoten, Tokyo.
31. Walker, J. M. 1984. Gradient SDS polyacrylamide gel electrophoresis. p. 57-61. *In* Walker, J. M. (ed), *Methods in Molecular Biology*, vol. 1. Proteins. The Humana Press Inc., Clifton, NJ.

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