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Identification and Partial Characterization of Lacticin SA72, a Bacteriocin Produced by *Lactococcus lactis* SA72 Isolated from Jeot-gal

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Abstract Strain SA72 was isolated from Jeot-gal and identified as producer of a bacteriocin, which showed some bactericidal activity against *Lactobacillus delbrueckii* ATCC 4797. Strain SA72 was tentatively identified as *Lactococcus lactis* according to the API test. *Lactococcus lactis* SA72 showed a broad spectrum of activity against most of the non-pathogenic and pathogenic microorganisms, tested by the modified deferred method. The activity of lacticin SA72, named tentatively as a bacteriocin produced by *Lactococcus lactis* SA72, was detected during the mid-log growth phase, reached a maximum during the early stationary phase, and then declined after the late stationary phase. Lacticin SA72 also showed a relatively broad spectrum of activity against non-pathogenic and pathogenic microorganisms when assessed by the spot-on-lawn method. Its antimicrobial activity on sensitive indicator cells disappeared completely by protease XIV treatment. The inhibitory activity of lacticin SA72 remained after treatment for 15 min at 121°C, and was stable in a pH range of 2.0 to 9.0 and all organic solvents examined. It demonstrated a typical bactericidal mode of inhibition against *Lactobacillus delbrueckii* ATCC 4797. The apparent molecular mass of lacticin SA72 was in the region of 3-3.5 kDa, determined by SDS-PAGE.

Key words: Bacteriocin, Jeot-gal, *Lactococcus lactis*, identification, characterization, lacticin SA72

Lactic acid bacteria is a common microflora seen in various fermented foods such as dairy products and processed vegetables, and it plays an essential role in food fermentation processes. They have been used for centuries in preparing and preserving foods such as meat, milk, and vegetables, and are generally recognized to be safe (GRAS) [13]. Many lactic acid bacteria produce antimicrobial substances like organic acids, hydrogen peroxide, diacetyl, carbon dioxide, and bacteriocins [3, 5, 13, 33, 34].

Bacteriocins are defined as bactericidal proteins, which typically consist of a narrow spectrum of activity towards a species related to the producer culture [32]. Recently, bacteriocins have aroused great interest in the context of food preservation, and a possibility of genetically manipulating the genes which encode bacteriocins is considered to be one of the major reasons for undertaking bacteriocin research [14, 19]. Bacteriocin producers have developed protective systems against their bacteriocins, and this system is known as self-immunity [20, 28]. Bacteriocins are potentially useful for industrial applications because of their antibacterial characteristics. In addition, the bacteriocins can be used as biopreservatives and bioregulators of the microflora present in fermented-foods [26]. Nisin, a GRAS bacteriocin, is produced by certain strains of *L. lactis* subsp. *lactis*, and this is the only bacteriocin which has been approved for food use in many countries [6]. It is believed that further research will allow other bacteriocins to be successfully exploited as food preservatives.

Korea has inherited various fermented foods from its ancestors [15, 25]. Chang (fermented soybean sauce and pastes), Kimchi (fermented vegetables), and Jeot-gal (fermented fish foods) are the three major salt-fermented and preserved food categories in Korea. Kimchi is prepared with various vegetables, and it becomes palatable and preservable through a proper fermentation process caused by these lactic acid bacteria. Bacteriocin research conducted on Kimchi was carried out by screening bacteriocinogenic lactic acid bacteria to characterize and apply in various food systems [5, 16, 30]. Jeot-gal is a generic name for high-salt fermented fish foods, which is composed of partially hydrolyzed fish organs immersed in fish liquid exudates [34]. However, very little is known about the antibacterial properties of lactic acid bacteria and their bacteriocins in Jeot-gal [17, 21].

In this study, we report the identification and partial characterization of lacticin SA72, a Jeot-gal bacteriocin

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produced by *L. lactis* SA72. The characteristics of lacticin SA72 make this bacteriocin potentially interesting as an antimicrobial agent for controlling both spoilage and pathogenic organisms in foods.

MATERIALS AND METHODS

Bacterial Strains and Media

Producer strain *L. lactis* SA72 was isolated from Jeot-gal by using the general spreading method and incubating anaerobically at 37°C [17]. Stock cultures were maintained at -70°C in MRS broth (Difco Laboratories, Detroit, U.S.A.) to which 20% (v/v) glycerol was added. Working cultures were propagated in MRS broth at 30°C for 12 h before using them in experiments. *L. delbrueckii* ATCC 4797 was used as the indicator strain. The strains used for determining the antimicrobial spectrum of activity were obtained from different culture collections and indicator strains were grown in an appropriate media as indicated in Tables 1 and 2.

Detection of Antimicrobial Activity

L. lactis SA72 was examined for antimicrobial activity against indicator organisms on MRS agar plates by incorporating the modified deferred method [1]. After inoculating the selected producer with a toothpick, *L. lactis* SA72 was grown on a MRS agar at 30°C for 24 h. Five milliliters of soft MRS agar (0.75% agar), containing approximately 10^7 cells of the indicator strain per overlay, was overlaid on 1.5% MRS agar plates, and, after 24 h incubation period at the indicator strain's optimal growth temperature, an inhibition halo was clearly visible. The strength of the antimicrobial activity was expressed by the diameter (mm) of the halo, and results are presented as means of duplicate tests.

Lacticin SA72 Assay

The spot-on-lawn method was performed as follows. Soft MRS agar (0.75% agar), containing 10^7 cells of the indicator organism per overlay, was overlaid on 1.5% MRS agar plates. As the soft agar hardened, 5 µl of bacteriocin preparation was spotted on the plate and incubated for 12 h at 30°C, at which time an inhibition halo was clearly visible. Bacteriocin preparation was made by spotting serial two-fold dilutions of crude bacteriocin. The reciprocal of the greatest inhibitory dilution was used to calculate the activity units (AU) per milliliter of the original cultures. All experiments were performed in duplicate, and results are shown as means of duplicate trials.

Identification of Lacticin SA72 Producer

Bacteriocin-producing strain SA72 was identified by Gram staining, morphology by SEM (scanning electron microscopy), catalase test, and biochemical carbohydrates fermentation patterns using an API 50 CHL kit (BioMerieux, France) [12].

Production of Lacticin SA72

Lacticin SA72 production was performed in a 5-l jar fermenter (3-l working volume; Korea Fermenter Co., Inchon, Korea) in a fermentation medium of the MRS broth. *L. lactis* SA72 was inoculated (1%, v/v) into 250 ml of sterile MRS and the seed culture (1%, v/v) was transferred to the jar fermenter. Temperature was controlled at 30°C and the pH level was maintained at 6.0±0.1 by adding 3 N H_2SO_4 and 3 N NaOH. Agitation speed was 200 rpm in the jar fermenter and no aeration was provided. Samples were aseptically removed over 12 h periods to determine viable cells and bacteriocin activity at different time intervals. Cell growth was monitored spectrophotometerically and the bacteriocin activity of the culture broth was evaluated by the method previously described.

Preparation of Cell-Free Supernatant

Culture broth from the jar fermenter was centrifuged at 8.000 ×g for 20 min at 4°C and the supernatant was filter-sterilized by passing through 0.22 µm cellulose acetate [31].

Partial Purification of Lacticin SA72

Partially purified lacticin SA72 was obtained as follows [22, 23]. Cold ethanol was slowly added to the culture supernatant to reach a 60% saturation level at 4°C with constant stirring over a 5 h period. Slow stirring was continued for an additional 1 h at 4°C. Precipitated proteins were then pelletized by centrifugation at 12,000 ×g for 20 min at 4°C, and resuspended in 100 mM phosphate buffer (pH 7.0). Residual ethanol was evaporated at 30°C for 2 h. The samples were stored at -70°C.

Assay for Protein Concentration

Protein concentration was determined by the method described by Lowry *et al.* [18], using bovine serum albumin as the standard.

Antimicrobial Spectrum of Activity

The modified deferred and spot-on-lawn methods were used to assess the antimicrobial activity of the cell-free supernatant and the partially purified lacticin SA72 preparation against several gram-positive and -negative strains, which included food spoilage and pathogenic organisms, a yeast, and several molds. All strains were previously subcultured in an appropriate growth agar medium and were propagated in a liquid medium before being inoculated into a soft-agar medium (0.75% agar).

Effects of Enzymes, Heat, pH, and Organic Solvents

For enzyme stability, partially purified lacticin SA72 was treated at 30°C for 1 h with various enzymes at a final

Table 1. Antimicrobial spectrum of activity of *L. lactis* SA72 by the modified deferred method.

Organisms	Culture medium[b]	Incubation temp.	Inhibition zone diameter (mm)	
			Nisin producer (ATCC 40140)	*L. lactis* SA72
Gram-positive bacteria				
Bacillus cereus	NB	30°C	25	20
Bacillus cereus ATCC 11778	TSB	37°C	20	22
Bacillus pumilis	NB	30°C	41	39
Bacillus subtilis ATCC 6633	TSB	37°C	>30	>30
Clostridium perfringens ATCC 3624[a]	TSB	37°C	10	14
Enterococcus faecalis ATCC 19433	TSB	37°C	15	15
Lactobacillus delbrueckii ATCC 4797	MRS	37°C	32	28
Leuconostoc mesenteroides KCCM 11324	MRS	25°C	>30	25
Listeria innocua	TSB	37°C	23	28
Listeria monocytogenes ATCC 15313	TSB	30°C	22	30
Listeria monocytogenes	TSB	37°C	25	27
Micrococcus flavus ATCC 10240	NB	37°C	34	37
Propionibacterium acnes P3	NLB	32°C	11	7
Propionibacterium acidipropionici P9	NLB	32°C	>30	>30
Propionibacterium acidipropionici P200910	NLB	32°C	>30	>30
Propionibacterium thoenii P127	NLB	32°C	19	21
Rhodococcus equi	TSB	37°C	17	>30
Staphylococcus aureus ATCC 6538	TSB	37°C	23	27
Staphylococcus aureus ATCC 25923	TSB	37°C	27	22
Streptococcus bovis ATCC 9809	TSB	37°C	27	26
Gram-negative bacteria				
Escherichia coli ATCC 8739	TSB	37°C	>30	>30
Escherichia coli ATCC 25922	TSB	37°C	24	25
Escherichia coli O157:H7	TSB	37°C	20	26
Escherichia coli KCCM 32396	TSB	37°C	23	26
Escherichia coli JM109	TSB	37°C	9	10
Pseudomonas aeruginosa ATCC 15422	TSB	37°C	20	21
Pseudomonas syringae ATCC 12885	TSB	30°C	16	18
Salmonella enteritidis	TSB	37°C	21	33
Salmonella london E	TSB	37°C	18	27
Salmonella paratyphi	TSB	37°C	>30	>30
Salmonella typhi	TSB	37°C	27	>30
Salmonella typhimurium	TSB	37°C	24	27
Shigella flexneri	TSB	37°C	21	27
Shigella sonnei	TSB	37°C	25	20
Vibrio cholerae O139	TSB	37°C	17	25
Vibrio parahaemolyticus ATCC 17802	TSB	37°C	21	26
Vibrio parahaemolyticus	TSB	37°C	17	26
Vibrio vulnificus	TSB	37°C	>30	>30
Yersinia enterocoecus ATCC 27729	TSB	37°C	18	28
Molds				
Aspergillus niger KCCM 11239	PDA	25°C	-	-
Aspergillus oryzae KCCM 11371	PDA	25°C	-	-

[a]Incubated in anaerobic GasPak jar.
[b]NB, Nutrient broth; LB, Luria broth, TSB, Tryptic soy broth; MRS, Lactobacilli MRS broth; PDB, Potato dextrose broth; NLB, Sodium lactate broth.

concentration of 1 mg/ml. All enzymes were suspended in buffers as recommended by the supplier (Sigma Chemical Co., St. Louis, U.S.A.). Untreated bacteriocin plus buffers, buffers alone, and enzyme suspensions were used as controls. In order to determine the effect of heat on bacteriocin activity, aliquots (500 µl) of partially purified lacticin SA72 were incubated at various temperatures (40, 50, 60, 70, 80, 90, and 100°C) for 30 min or 121°C for

Table 2. Antimicrobial spectrum of activity of partially purified lacticin SA72 by the spot-on-lawn method.

Organisms	Culture medium[b]	Incubation temp.	Inhibition	
			Nisin	Partially purified lacticin SA72
Gram-positive bacteria				
Bacillus cereus	NB	30°C	+	+
Bacillus cereus ATCC 11778	TSB	37°C	+	+
Bacillus pumilis	NB	30°C	+	+
Bacillus subtilis ATCC 6633	TSB	37°C	+	+
Clostridium perfringens ATCC 3624[a]	TSB	37°C	+	+
Enterococcus faecalis ATCC 19433	TSB	37°C	+	+
Lactobacillus delbrueckii ATCC 4797	MRS	37°C	+	+
Leuconostoc mesenteroides KCCM 11324	MRS	25°C	+	+
Listeria innocua	TSB	37°C	+/-[a]	+
Listeria monocytogenes ATCC 15313	TSB	30°C	+/-	+
Listeria monocytogenes	TSB	37°C	+	+
Micrococcus flavus ATCC 10240	NB	37°C	+	+
Propionibacterium acnes P3	NLB	32°C	+	+
Propionibacterium acidipropionici P9	NLB	32°C	+	+
Propionibacterium acidipropionici P200910	NLB	32°C	+	+
Propionibacterium thoenii P127	NLB	32°C	+	+
Rhodococcus equi	TSB	37°C	+	+
Staphylococcus aureus ATCC 6538	TSB	37°C	+	+
Staphylococcus aureus ATCC 25923	TSB	37°C	+/-	+/-
Streptococcus bovis ATCC 9809	TSB	37°C	+	+
Gram-negative bacteria				
Escherichia coli ATCC 8739	TSB	37°C	-	-
Escherichia coli ATCC 25922	TSB	37°C	-	-
Escherichia coli O157:H7	TSB	37°C	-	-
Escherichia coli KCCM 32396	TSB	37°C	+	+
Escherichia coli JM109	TSB	37°C	-	-
Pseudomonas aeruginosa ATCC 15422	TSB	37°C	-	-
Pseudomonas syringae ATCC 12885	TSB	30°C	-	-
Salmonella enteritidis	TSB	37°C	-	-
Salmonella london E	TSB	37°C	-	-
Salmonella paratyphi	TSB	37°C	-	-
Salmonella typhi	TSB	37°C	-	-
Salmonella typhimurium	TSB	37°C	-	-
Shigella flexneri	TSB	37°C	-	-
Shigella sonnei	TSB	37°C	-	-
Vibrio cholerae O139	TSB	37°C	-	-
Vibrio parahaemolyticus ATCC 17802	TSB	37°C	-	-
Vibrio parahaemolyticus	TSB	37°C	-	-
Vibrio vulnificus	TSB	37°C	-	-
Yersinia enterococcus ATCC 27729	TSB	37°C	-	-
Molds				
Aspergillus niger KCCM 11239	PDA	25°C	-	-
Aspergillus oryzae KCCM 11371	PDA	25°C	-	-

[a]Incubated in anaerobic GasPak jar.
[b]NB, Nutrient broth; LB, Luria broth, TSB, Tryptic soy broth; MRS, Lactobacilli MRS broth, PDB, Potato dextrose broth; NLB, Sodium lactate broth

15 min [22, 23]. The pH stability of the partially purified lacticin SA72 was estimated after a 4 h storage at 4°C in the following buffers: 50 mM citrate buffer at pH 3-6; 50 mM phosphate buffer at pH 7.0; 50 mM Tris-HCl buffer at pH 8-9 [22, 23]. Partially purified bacteriocin was incubated at 30°C for 1 h with 50% organic solvents such as ethanol, methanol, acetone, toluene, chloroform, and isopropanol, and residual solvents were evaporated at 30°C for 2 h. In

this case, the bacteriocin in a buffer without solvents was used as the control. The residual bacteriocin activity was determined by the spot-on-lawn method. Results are shown as means of tests performed in duplicate.

Mode of Inhibition

Cells from the log-phase of *L. delbrueckii* ATCC 4797 were suspended in a sterile 100 mM phosphate buffer (pH 7.0), and the test was conducted at 30°C by adding 1,000 AU/ml of partially purified lacticin SA72. At specific times, viable cells (CFU/ml) were determined on MRS agar plates by the standard plate counting method [24].

Determination of Molecular Weight by SDS-PAGE

To estimate the molecular weight of partially purified lacticin SA72, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on 16% discontinuous gel. Twenty-microliters of the sample and molecular weight standards were applied to the gel. Protein standards and their molecular weights were as follows: ovalbumin, 43,000; carbonic anhydrase, 29,000; lactoglobulin, 18,400; lysozyme, 14,300; bovine trypsin inhibitor, 6,200; and insulin, 3,000. The sample was prepared by mixing the lacticin SA72 sample and buffer in a 1:1 ratio and boiling the mixture at 100°C for 5 min. Electrophoresis was then performed in a vertical slab gel apparatus (Protein Cell II: Bio-Rad, Richmond, U.S.A.) at a constant voltage (100 V) for 2 h. Half of the gel was stained with Coomassie brilliant blue R-250 as directed by the manufacturer, while the other half was assayed for bacteriocin activity by the direct method previously described [6]. Furthermore, the area of gel being assayed was fixed in 20% 2-propanol and 10% acetic acid for 2 h and soaked for at least 4 h in a sterile deionized water. It was then aseptically placed in a sterile petri dish and covered with 20 ml of soft agar containing 10^7 cells of *L. delbrueckii* ATCC 4797 as the indicator strain. The plate was then incubated at 37°C for 12 h and examined for zones of inhibition.

Results and Discussion

Identification of Lacticin SA72 Producer from Jeot-gal

It has been suggested for a long time that lactic acid bacteria would play an important role in antibiosis as well as in Jeot-gal fermentation. Strain SA72 was isolated from Jeot-gal and identified as a bacteriocin producer [17]. Upon dilution of lacticin SA72, zones of inhibition on lawns of the indicator strain diminished in size without any indication of appearance of plaque, suggesting that the inhibition was not caused by the replication of bacteriophages.

Bacteriocin-producing strain SA72 was identified by Gram staining, a catalase test, morphology by SEM, and by biochemical carbohydrates fermentation patterns using the API 50 CHL kit. Information on carbohydrate utilization patterns of strain SA72 coincided with those of *L. lactis* subsp. *lactis*. This isolate was Gram-positive, nonmotile and catalase-negative, and the cells were categorized as the coccus type. On the basis of these results, this strain was identified as *L. lactis*, and the isolate was tentatively named as *L. lactis* SA72 [17]. However, the complete identification of this strain has not yet been carried out and further experiments, including 16S rRNA sequence analysis, are needed. Lacticin SA72 was proposed as the tentative name of the bacteriocin produced by *L. lactis* SA72.

Production of Lacticin SA72

L. lactis SA72 in a MRS medium of pH 6.0 at 30°C produced extracellular inhibitory activity against *L. delbrueckii* ATCC 4797, (Fig. 1). Production of lacticin SA72 seems to follow a typical kinetic pattern of primary metabolite synthesis. Lacticin SA72 activity reached its maximum level of 2,400 AU/ml after incubation for 7 h, and it was maintained for 2 h in the early stationary phase, before dropping after the late stationary phase. Possible reasons for this rapid decrease in the bacteriocin activity include formation of an inhibitor, its degradation by extracellular proteolytic enzymes, binding of bacteriocin to cells, and formation of inactivating complex with other extracellular products [25]. Almost all the bacteriocins of lactic acid bacteria are produced during the exponential growth phase. Several lantibiotics are also synthesized during the exponential growth phase of the producer strain [8, 9, 29]. This comparatively early production of bacteriocin by *L. lactis* SA72 could be exploited as an industrially useful characteristic.

Partial Purification of Lacticin SA72

L. lactis SA72 was cultured in a jar fermenter at 30°C for 8 h and the culture broth was centrifuged. Partially purified lacticin SA72 was prepared by ethanol precipitation, and its activity was determined to be 1,331,200 AU/ml, with a yield of approximately 52% and the specific activity increased 13.9-fold (data not shown).

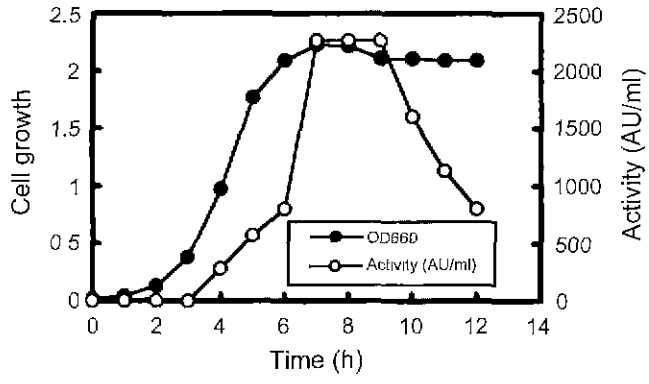

Fig. 1. Production of lacticin SA72 in the jar fermenter.

Antimicrobial Spectrum of Activity

To determine the spectrum of microbial activity, the cell-free supernatant and partially purified lacticin SA72 were tested against various non-pathogenic and pathogenic bacteria, a yeast, and molds by the modified deferred and spot-on-lawn methods (Tables 1 and 2).

L. lactis SA72 showed a broad spectrum of activity against all of the non-pathogenic and pathogenic bacteria tested by the modified deferred method, but it had no activity against a yeast and molds. When compared with other lactic acid bacteria bacteriocins, Lacticin SA72 also showed a relatively broad spectrum of activity against most lactic acid bacteria, including *Enterococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 25923, *Clostridium perfringens* ATCC 3624, some *Bacilli*, *Micrococcus flavus* ATCC 10240, *Listeria monocytogenes* ATCC 15313, *Yersinia enterocolitica* ATCC 27729, and *Escherichia coli* KCCM 32396, tested by the spot-on-lawn method. However, lacticin SA72 had no inhibitory activity on the yeast and molds. Antimicrobial activity of lacticin SA72 against *E. coli* KCCM 32396 makes this bacteriocin an interesting one. On the basis of its inhibitory spectrum, lacticin SA72 appeared to be similar to the lantibiotic nisin, which inhibits most Gram-positive bacteria [4]. In contrast, several other bacteriocins from *Lactobacillus* sp. have activity spectra that include only the same genus strains [2, 11, 27].

Effects of Various Enzymes, Heat, pH, and Organic Solvents

As shown in Table 3, treatment with protease XIV caused complete loss of bacteriocin activity. No activity was lost when lacticin SA72 was treated with other enzymes such as protease XIII, trypsin, α-chymotrypsin, and proteinase K. It is necessary to mention that buffers and enzyme solutions alone had no effect on the indicator strain.

Lacticin SA72 was relatively heat stable (Table 4); partially purified lacticin SA72 was stable to a heat treatment of 80°C for 30 min, and inhibitory activity was still detected during treatment for 15 min at 121°C. This heat stability could be due to formation of small globular structures and occurrence of strongly hydrophobic regions, stable cross-linkage, and high glycine content [7]. This heat stability also ruled out the possibility that the observed inhibitory action was due to bacteriophages.

Finally, the partially purified bacteriocin was stable in the pH range of 2.0 to 9.0, and it was not affected by any of the organic solvents shown in Table 4. This pH stability is certainly an important attribute to the food industry.

Table 3. Effect of various enzymes on partially purified lacticin SA72.

Enzyme treatment	Residual activity (AU/ml)
Control	12,800
Protease XIII	12,800
Protease XIV	0
Trypsin	12,800
α-Chymotrypsin	12,800
Proteinase K	12,800

Mode of Inhibition

To determine whether lacticin SA72 has a bactericidal or a bacteriostatic effect, the partially purified lacticin SA72 was added to indicator cells suspended in phosphate buffer (pH 7.0). Lacticin SA72 showed a bactericidal mode of action, since a decrease in CFU per milliliter was observed in indicator cells exposed to lacticin SA72 (Fig. 2). However, the intrinsic nature of this inhibition has not been identified and it requires further investigation.

Molecular Weight of Lacticin SA72

Several contaminating proteins were detected in the partially purified lacticin SA72 preparation (Fig. 3). Partially purified lacticin SA72 on 16% polyacrylamide gel was cut into two vertical parts. The portion containing the sample and the

Table 4. Effect of heat, pH, and organic solvent on partially purified lacticin SA72.

Treatment (pH)	Residual activity (AU/ml)	Treatment (Heat)	Residual activity (AU/ml)	Treatment (Organic solvent)	Residual activity (AU/ml)
Control	51,200	Control	25,600	Control	51,200
2	51,200	40°C	25,600	Ethanol	51,200
3	51,200	50°C	12,800	Methanol	51,200
4	51,200	60°C	12,800	Acetone	51,200
5	51,200	70°C	12,800	Toluene	51,200
6	51,200	80°C	12,800	Chloroform	51,200
7	51,200	90°C	9,051	Isopropyl alcohol	51,200
8	51,200	100°C*	4,525		
9	25,600	120°C**	400		

*Heat treatment for 30 min.

**Autoclave for 15 min.

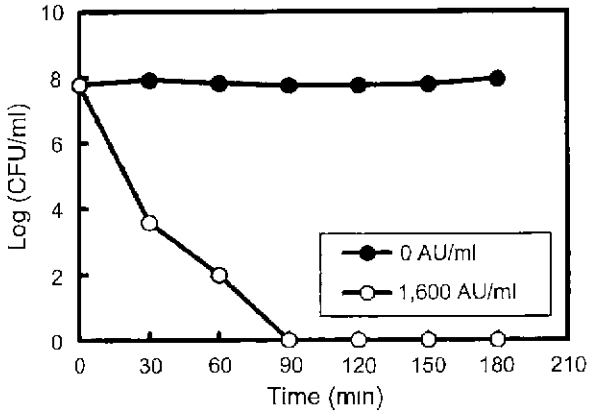

Fig. 2. Mode of inhibition of lacticin SA72 against *Lactobacillus delbrueckii* ATCC 4797 in phosphate buffer.

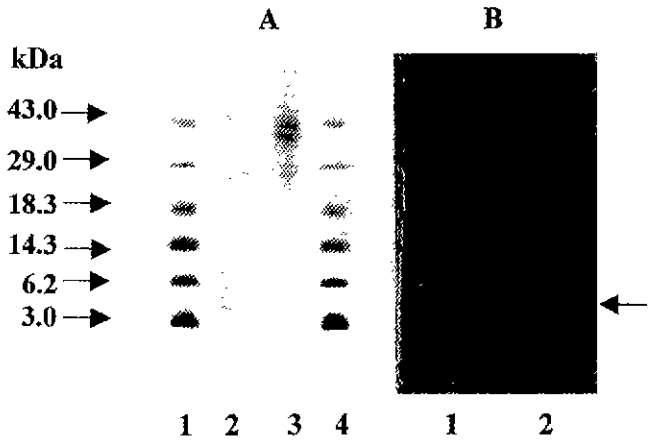

Fig. 3. SDS-PAGE of partially purified lacticin SA72 for determination of molecular weight.
(A) SDS-PAGE of partially purified lacticin SA72 after Coomassie brilliant blue stained. Lanes 1 and 4: size marker, 43 29, 18.3, 14.3, 6.2, 3 kDa, Lane 2: nisin (3.5 kDa); Lane 3: partially purified lacticin SA72. (B) The gel overlayed with indicator strain, *Lactobacillus delbrueckii* ATCC 4797. Lane 1: nisin; Lane 2: partially purified lacticin SA72

molecular weight markers was stained, while the remaining part, which contained only the sample, was fixed and used for direct detection of antimicrobial activity by the method of Daba *et al.* [6]. As shown in Fig. 3, the bactericidal activity of lacticin SA72 was found to be associated with a band with an apparent molecular mass of about 3-3.5 kDa. Thus, the apparent molecular mass of lacticin SA72 was determined to be about 3-3.5 kDa by directly detecting the bactericidal activity after SDS-PAGE.

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References

1. Ahn, C. and M. E. Stiles. 1990. Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. *Appl. Environ. Microbiol.* **56:** 2503-2510.
2. Barefoot, S. F. and T. R. Klaenhammer. 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lacticin B. *Antimicrob. Agents Chemother.* **26:** 328-334.
3. Bibek, R. and D. Mark. 1992. Food biopreservatives of microbial origin, pp. 323-342. *In* A. D. Mark (ed.), *Bacteriocins of Lactic Acid Bacteria*, CRC Press Inc., London. U.K.
4. Benkerroum, N. and W. E. Sandine. 1988. Inhibitory action of nisin against *Listeria monocytogenes*. *J. Dairy Sci.* **71:** 3237-3242.
5. Choi, M. H. and Y. H. Park. 1998. Inhibition of lactic acid bacteria in Kimchi fermentation by nisin. *J. Microbiol. Biotechnol.* **8:** 547-551.
6. 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.
7. De Vuyst, L. and E. J. Vandamme. 1994. Antimicrobial potential of lactic acid bacteria. pp. 91-142. *In* L. De Vuyst and E. J. Vandamme (eds.), *Bacteriocins of Lactic Acid Bacteria*, Blackie Academic and Professional, Glasgow, Scotland.
8. Höner, T., H. Zahner, R. Kellner, and G. Jung. 1989. Fermentation and isolation of epidermin, a lanthionine containing polypeptide antibiotic from *Staphylococcus epidermidis*. *Appl. Microbiol. Biotechnol.* **30:** 219-225.
9. Höner, T., V. Ungermann, H. Zahner, H. P. Fiedler, R. Utz, R. Kellner, and G. Jung. 1990. Comparative studies on the fermentative production of lantibiotics by *Staphylococcus*. *Appl. Microbiol. Biotechnol.* **32:** 511-517.
10. Joerger, M. C. and T. R. Klaenhammer. 1986. Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. *J. Bacteriol.* **167:** 439-446.
11. Kim, S.-K., E.-J. Lee, K.-Y. Park, and H.-K. Jun. 1998. Bacteriocin produced by *Lactobacillus curvatus* SE1 isolated from Kimchi. *J. Microbiol. Biotechnol.* **8:** 588-594.
12. Kim, W. J. 1996. Screening of bacteriocinogenic lactic acid bacteria and their antagonistic effects in sausage fermentation. *J. Microbiol. Biotechnol.* **6:** 461-467.
13. Klaenhammer, T. R. 1988. Bacteriocins of lactic acid bacteria. *Biochimie* **70:** 337-349.
14. Klaenhammer, T. R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.* **12:** 39-86.
15. Lee, C.-H. 1993. Fish fermentation technology. pp. 187-279. *In* C.-H. Lee, K. H. Steinkraus, and P. J. A. Reilly (eds.), *Fish Fermentation Technology in Korea*, United Nations University Press, Tokyo, Japan.

16. Lee, H.-J., C.-S. Park, Y.-J. Joo, S.-H. Kim, J.-H. Yoon, Y.-H. Park, I.-K. Hwang, J.-S. Ahn, and T.-I. Mheen. 1999. Identification and characterization of bacteriocin-producing lactic acid bacteria isolated from Kimchi. *J. Microbiol. Biotechnol.* **9:** 282-291.
17. Lee, N.-K., S.-A. Jun, J.-U. Ha, and H.-D. Paik. 2000. Screening and characterization of bacteriocinogenic lactic acid bacteria from Jeot-gal. *J. Microbiol. Biotechnol.* **10:** 423-428.
18. Lowry, O. H., N. J. Roseverough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **196:** 336-337.
19. Nes, I. F., D. B. Diep, L. S. Harvastein, M. B. Brurberg, V. Eijsink, and H. Holo. 1996. Biosynthesis of bacteriocins in lactic acid bacteria. *Antonie van Leeuwenhoek* **70:** 113-128.
20. Nissen-Meyer, J., A. G. Larsen, K. Sletten, and I. F. Nes. 1993. Purification and characterization of plantaricin A, a *Lactobacillus plantarum* bacteriocin whose activity depends on the action of two peptides. *J. Gen. Microbiol.* **139:** 1973-1978.
21. Østergaard, A., P. K. B. Embarek, C. Wedell-Neergaard, H. H. Huss, and L. Gram. 1998. Characterization of anti-listerial lactic acid bacteria isolated from Thai fermented fish products. *Food Microbiol.* **15:** 223-233.
22. Paik, H.-D. and B. A. Glatz. 1995. Purification and partial amino acid sequence of propionicin PLG-1, a bacteriocin produced *Propionibacterium thoenii* P127. *Lait* **75:** 367-377.
23. Paik, H.-D. and B. A. Glatz. 1997. Enhanced bacteriocin production by *Propionibacterium thoenii* in fed-batch fermentation. *J. Food Prot.* **60:** 1529-1533.
24. Paik, H.-D., S.-S. Bae, S.-H. Park, and J.-G. Pan. 1997. Identification and partial characterization of tochicin, a bacteriocin produced by *Bacillus thuringiensis* subsp. *tochigiensis*. *J. Ind. Microbiol. Biotechnol.* **19:** 294-298.
25. Park, S. K., K. T. Lee, and S. M. Kim. 1998. Changes in taste compound of low salt fermented pollack tripe during controlled freezing point aging. *Kor. J. Food Sci. Technol.* **30:** 49-53.
26. Ralph, W. J., J. R. Tagg, and B. Ray. 1995. Bacteriocins of Gram-positive bacteria. *Microbiol. Rev.* **59:** 171-200.
27. Rammelsberg, M., E. Muller, and F. Radler. 1990. Cascicin 80: Purification and characterization of a new bacteriocin from *Lactobacillus casei*. *Arch. Microbiol.* **154:** 249-252.
28. Reis, M., M. Eschbach-Bludau M. I Loglesias-Wind, T. Kupke, and H. G. Sahl. 1993 Producer immunity toward the lantibiotic Pep5: Identification of the immunity gene Pep I and localization and functional analysis of its gene product. *Appl. Environ. Microbiol.* **60:** 2876-2883.
29. Sahl, H. G. and H. Brandis. 1981. Production, purification and chemical properties of an antistaphylococcal agent produced by *Staphylococcus epidermidis*. *J. Gen. Microbiol.* **127:** 377-384.
30. Shin, J. Y. and C. Ahn. 1997. Characterization of bacteriocin production by *Lactococcus lactis* LAB 3113 isolated from Kimchi. *J. Food Sci. Nutr.* **2:** 101-108
31. Sudirman, I., F. Mathieu, M. Michel, and G. Lefebvre. 1993. Detection and properties of curvaticin 13, a bacteriocin-like substance produced by *Lactobacillus curvatus* SB13. *Curr. Microbiol.* **27:** 35-40.
32 Tagg, J. R., A. S. Dajani, and L. W. Wannamaker. 1976. Bacteriocins of Gram-positive bacteria. *Bacteriol. Rev.* **40:** 722-756.
33. Thomas, J. M. and A. L. Kaiser. 1993. Antimicrobial proteins: Classification, nomenclature, diversity, and relationship to bacteriocins, pp. 1-22. *In* D. G. Hoover and L. R. Steenson (eds.), *Bacteriocins of Lactic Acid Bacteria*, Academic Press, Inc., San Diego, U.S.A.
34. Um, M.-N. and C.-H. Lee. 1996. Isolation and identification of *Staphylococcus* sp. from Korean fermented fish products. *J. Microbiol. Biotechnol.* **6:** 340-346.