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Purification and Characterization of an Antilisterial Bacteriocin Produced by *Leuconostoc* sp. W65

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Abstract This study was carried out to characterize the antilisterial substances produced by *Leuconostoc* sp. W65 and to evaluate the effects of pH, temperature, and time on inhibitory activity using response surface methodology. Leucocin W65, an antilisterial substance produced by *Leuconostoc* sp. W65, markedly inhibited the growth of *Listeria monocytogenes*, *L. innocua*, and *L. ivanovii*, whereas other pathogens including Gram-negative bacteria were not susceptible. The pH was the most effective factor with regard to bacteriocin activity, while temperature and time of heat treatment had no significant effect. Fifty percent of inhibitory activity remained after 22.8 min at pH 4.2 and 121°C. Leucocin W65 was purified by ammonium sulfate precipitation, hydrophobic interaction chromatography, and tricine-SDS-PAGE. Compositional analysis originally estimated the peptide to be 56 amino acids in length without asparagine, glutamine, and tryptophane. The sequence of partial N-terminal amino acid residues of purified bacteriocin was identified as follows: NH_2-XGXAGVXPXGGQQPXVPLXYP.

Key words: *Leuconostoc* sp. W65, bacteriocin, *Listeria*, inhibition, purification

Thirty years ago, *Listeria monocytogenes*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Vibrio parahaemolyticus*, and *Yersinia enterocolitica* were not known, however, these are now important foodborne pathogens that must be controlled in various foods to prevent foodborne illness [6]. *Listeria* species have been recognized as a foodborne pathogen since the 1970's and are among the most prevalent foodborne pathogens, since this genus has the ability to withstand various adverse environmental conditions such as refrigeration conditions, low pH, and high salt concentration. Therefore, *Listeria* can survive in various foods such as milk, meat products, and even fermented products [3].

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Bacteriocins are the product of the ribosomal translation from its own structural gene, whereas other antibiotics are secondary metabolites [9]. Lactic acid bacteria (LAB), producing bacteriocins inhibitory to *Listeria monocytogenes*, have been isolated from various food categories such as fermented foods and raw meat products [7, 16]. Nisin is the only bacteriocin currently accepted for use in food in the U.S. However, nisin resistance has been reported for *Listeria monocytogenes* [13]. This may indicate that addition of bacteriocins should be investigated, since it is possible that bacteriocin-resistant mutants may appear before commercial use.

Some bacteriocins and bacteriocin-like substances have been studied from *Leuconostoc* and *Pediococcus* species isolated from dairy, meat, and wine products [7, 4]. These have been identified as heat-stable with a bacteriocidal mode of action, effective mainly against Gram-positive bacteria [5, 15, 19]. Leucocin A, produced by *Leuconostoc gelidum* UAL187, was active against *Enterococcus faecalis*, *L. monocytogenes*, and LAB. Leucocin A is a heat-stable peptide of 3.9 kDa, as determined by mass spectrometry [8]. However, *Leuc. gelidum* also produces dextran from sucrose, which would restrict the direct use of these bacteria in meat preservation. *Leuc. mesentroides* UL5 isolated from Cheddar cheese produces a bacteriocin, Mesenterocin 5, with a molecular mass of 4.5 kDa, which is heat-stable and is bacteriostatic against *L. monocytogenes* strains [4].

The isolation and use of antilisterial bacteriocins from various LAB may contribute to a decrease in foodborne illness outbreaks due to *L. monocytogenes* [7]. The effectiveness of bacteriocins may also depend on the amount of bacteriocin inactivated by interaction with food processing conditions. During food processing conditions, several factors should be considered, such as heating temperature, time, pressure, and pH. Depending on the kinds of food, it is processed with a variety of physical and chemical treatments, since foods consist of biological materials [16, 17].

The purpose of this study was to characterize the antilisterial substances produced by *Leuconostoc* sp. W65 and to evaluate the effects of pH, temperature, and time on their inhibitory activity using response surface methodology (RSM).

MATERIALS AND METHODS

Bacterial Culture

Leuconostoc sp. W65 isolated from vacuum-packaged pepperoni was maintained as a frozen stock held at -80°C in Brain Heart Infusion broth (BHI; Difco, Detroit, U.S.A.) containing 20% glycerol. *Leuconostoc* sp. W65 was inoculated into BHI broth (1%) and propagated twice at 20°C before use. *Listeria* strains and other pathogens were propagated in Tryptic Soy (TS) broth (Difco) and incubated at 37°C.

Ammonium Sulfate Precipitation

Culture supernatant was prepared by growing *Leuconostoc* sp. W65 in BHI for 48 h, followed by centrifugation (8,000 ×g, 30 min, 4°C). The ammonium sulfate (Sigma, Chemical Co., St. Louis, MO, U.S.A.) was gradually added to the supernatant to a final concentration of 30% and stirred overnight at 4°C. The pellet was collected by centrifugation (8,000 ×g, 30 min, 4°C) and resuspended in 50 ml of 2-(4-morpholino)-ethane sulfonic acid (MES; Sigma) buffer (50 mM, pH 6.5). This preparation, designated Active Crude Bacteriocin (ACB), was stored at -20°C.

Chromatography, SDS-PAGE, and Western Blotting

ACB was applied to an octyl-Sepharose CL4B column, previously equilibrated with 3 bed volumes of 1.7 M ammonium sulfate. Proteins were eluted by a decreasing salt gradient. Strongly hydrophobic proteins were eluted with a linearly increasing ethanol gradient. After octyl-Sepharose CL4B elution, active fractions were pooled and concentrated by evaporation. The concentrated samples were electrophoresed on a tricine-SDS-PAGE and then blotted onto polyvinylidene difluoride (PVDF) membrane. A duplicate gel was washed 3 times with 500 ml Milli-Q water, and placed onto TS agar and overlayed with TS soft agar inoculated with 1% *L. ivanovii*. The band corresponding to inhibitory activity was excised from the membrane and used for amino acid analysis and N-terminal sequencing by Edman degradation [20].

Antimicrobial Assay

The spot-on-lawn method was used to determine the antimicrobial activity of the various bacteriocin fractions [1]. A 20 µl aliquot of ACB was spotted onto TS agar plates seeded with a fresh culture of indicator organisms. The plates were incubated at their optimum temperature, and examined for inhibition zones after 24 h of incubation.

Mode of Action

An overnight culture of *L. ivanovii* was centrifuged (3,000 ×g, 20 min, 4°C), and the pellet was resuspended in 0.1% peptone water to *ca.* 5×10^8 cells/ml. ACB was added at concentrations of 25 and 50 AU/ml (arbitrary units). Viable cell counts were determined by agar plate count methods.

Effect of pH, Heating Time, and Temperature

The pH, heating time, and temperature were selected for this study and the orthogonal rotatable central composite design was adapted for estimating the effect of bacteriocin activities (α=1.682) [14]. The experimental combination consisted of 22 runs for 3 variables including 8 center runs. The second-order polynomial model was assumed to describe relationships between the response, which was arbitrary units of bacteriocin activity against *L. ivanovii* (Y, AU/ml), and experimental factors (pH, time, and temperature; X_i):

$$Y=b+\sum_{i=1}^{3}b_iX_i+\sum_{i=1}^{2}\sum_{j=i+1}^{3}b_{ij}X_iX_j+\sum_{i=1}^{3}b_i^2iiX_i^2+e$$

where b is the intercept, b_i is the linear coefficient, b_i^2 is the quadratic coefficient, b_{ij} is the interaction coefficient, and e is random error.

Statistical analysis was carried out using RSREG procedures of the SAS-STAT software. Three-dimensional plots of the predicted values of the response variables were generated by the procedure G3D of SAS-GRAPH software [18].

Table 1. Inhibitory activity of bacteriocin against pathogenic bacteria.

Bacteria	Media	°C	Inhibition	Source
Acinetobacter baumanii	TSB*	37	-	Lab. isolate
Bacillus cereus ATCC 11778	TSB	37	-	ATCC‡
Enterobacter aerogenes	TSB	37	-	Lab. isolate
E. coli O157 ATCC 43889	LB†	37	-	ATCC
E. coli O157 ATCC 43893	LB	37	-	ATCC
E. coli O157 ATCC 43895	LB	37	-	ATCC
Klebsiella pneumoniae	TSB	37	-	Lab. isolate
Listeria innocua	TSB	25	+	Lab. isolate
Listeria ivanovii	TSB	25	+	Lab. isolate
Listeria monocytogenes	TSB	25	+	Lab. isolate
Pseudomonas fluorescens	TSB	25	-	Lab. isolate
Salmonella typhimurium	TSB	37	-	Lab. isolate
Shigella sonnei	TSB	37	-	Lab. isolate
Staphylococcus aureus	TSB	37	-	Lab. isolate
Yersinia enterocolitica	TSB	37	-	Lab. isolate

*TSB, Tryptic soy broth.
†LB, Luria Bertani broth.
‡ATCC, American Type Culture Collection.
+, Inhibited by crude bacteriocin; - not inhibited.

RESULTS

Antimicrobial Spectrum

A bacteriocin produced by *Leuconostoc* sp. W65 showed inhibitory activity against a narrow spectrum of microorganisms (Table 1). It was only active against *L. monocytogenes*, *L. ivanovii*, and *L. innocua*.

Figure 1 shows the growth and production of the bacteriocin by *Leuconostoc* sp. W65 in BHI broth at various incubation temperatures. When *Leuconostoc* sp. W65 was incubated at 20°C, bacteriocin activity reached 800 AU/ml after 48 h. The maximum activity of the bacteriocin coincided with the maximum viable cell counts of *Leuconostoc* sp. W65. When *Leuconostoc* sp. W65 was incubated at 30°C, viable cell counts were the highest, as compared with 20°C and 7°C incubation. However, the production of bacteriocin reached a maximum of only 200 AU/ml. At 7°C incubation, *Leuconostoc* sp. W65 showed slow growth and low bacteriocin production.

Inhibitory Action

The inhibitory action of leucocin W65 is shown in Fig. 2. When 25 and 50 AU/ml of crude leucocin W65 were added to exponentially growing *Listeria ivanovii*, a dramatic decrease in the viable cells of *Listeria ivanovii* was observed. From

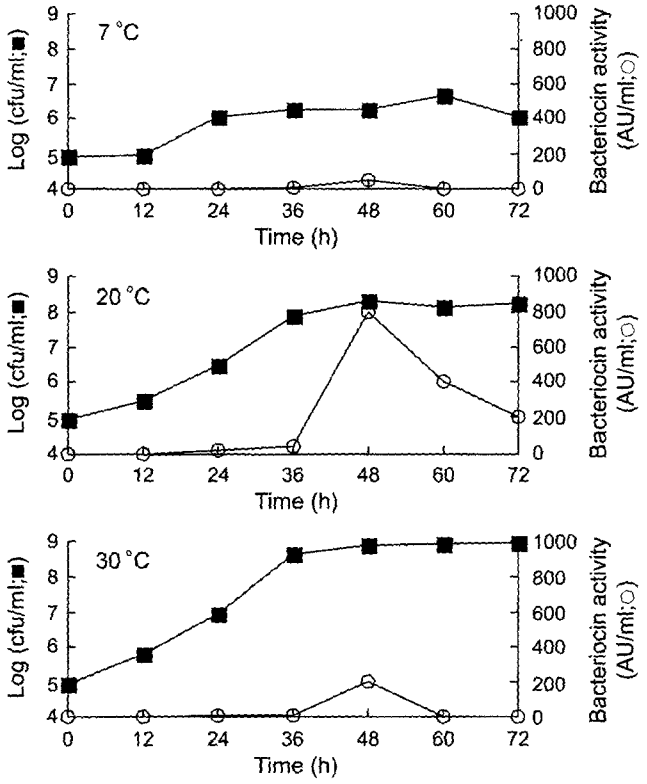

Fig. 1. Changes in viable cells and bacteriocin production during growth of *Leuconostoc* sp. W65 at 7, 20, and 30°C; (■), log numbers of viable cells; (○) bacteriocin activity.

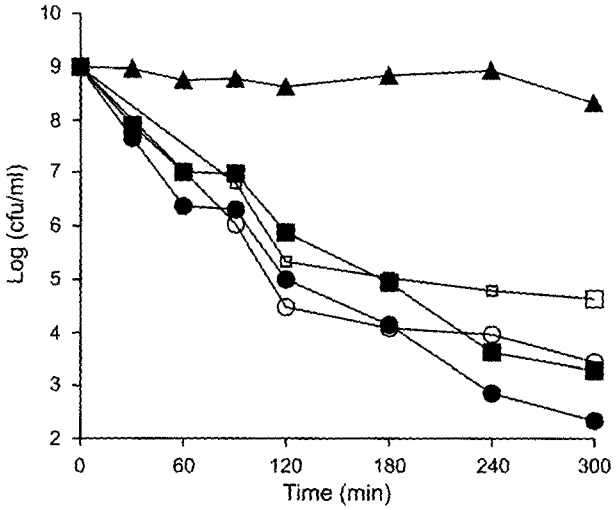

Fig. 2. Effect of crude bacteriocin of *Leuconostoc* sp. W65 on the growth of *Listeria ivanovii* at 25°C in 0.1% peptone water. (▲), Control; (■), 25 AU ml^{-1} bacteriocin; (□), addition of pronase-E (10 μg/ml) to peptone water containing 25 AU ml^{-1} bacteriocin after 60 min; (●), 50 AU ml^{-1} bacteriocin; (○), addition of pronase-E (10 μg/ml) to peptone water containing 50 AU ml^{-1} bacteriocin after 60 min.

an initial number of log 8.99 cfu/ml viable cells, the 25 and 50 unit/ml bacteriocin treatments dropped to log 3.48 and 2.32 cfu/ml after 5 h at 25°C, respectively. Pronase-E treated sample showed viable cells of log 4.61 and 3.49 cfu/ml,

Table 2. Treatment combination and bacteriocin activities.

Run	Temperature	pH	Time	Bacteriocin activity*
1	52.2	3.2	16.2	25,600
2	87.8	3.2	16.2	25,600
3	52.2	3.2	16.2	3,200
4	87.8	9.8	16.2	0
5	52.2	3.2	48.8	25,600
6	87.8	3.2	48.8	25,600
7	52.2	9.8	48.8	100
8	87.8	9.8	48.8	0
9	70	6.5	32.5	25,600
10	70	6.5	32.5	25,600
11	70	6.5	32.5	25,600
12	70	6.5	32.5	25,600
13	40	6.5	32.5	25,600
14	100	6.5	32.5	6,400
15	70	1	32.5	6,400
16	70	12	32.5	0
17	70	6.5	5	25,600
18	70	6.5	60	12,800
19	70	6.5	32.5	25,600
20	70	6.5	32.5	12,800
21	70	6.5	32.5	25,600
22	70	6.5	32.5	12,800
23	70	6.5	32.5	25,600

*AU/ml against *Listeria ivanovii*.

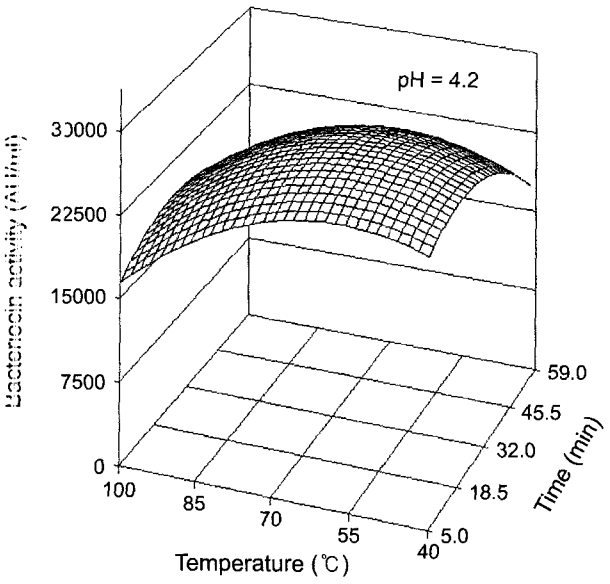

Fig. 3. Response surface for the effects of the heating temperature and holding time on the bacteriocin activity at pH=4.2.

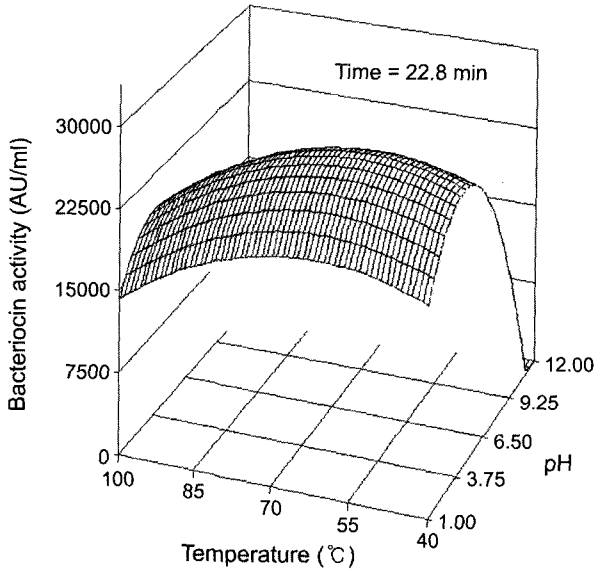

Fig. 5. Response surface for the effects of the heating temperature and pH on the bacteriocin activity at holding time=22.8 min.

respectively. The addition of pronase-E to suspended cells after 60 min in the presence of bacteriocin may affect the bacteriocin activity. During the experiments, optical density at 600 nm was decreased, indicating that cell lysis occurred (data not shown).

Effect of pH, Heating Time, and Temperature on Bacteriocin Activity

The experimental combination and their results are shown in Table 2. Optimum conditions for maximum bacteriocin activity were predicted to be 22.8 min, 59.4°C, and pH 4.2, corresponding to bacteriocin activity=27,100 AU/ml. Response surface plots were developed to determine the effects of pH, heating time, and temperature on the bacteriocin activity (Figs. 3, 4, and 5). The pH was shown to be the critical factor for bacteriocin activity. Bacteriocin activity dramatically increased with a reduction in the pH from 12 to 4.2, but it tended to decrease below pH 4.2. Greater losses of activity were predicted at above pH 10. RSM also predicted the heating time and temperature for bacteriocin activity at pH 4.2. Bacteriocin activity was maintained at 15,000 AU/ml over the range of temperature from 40 to 100°C and holding time from 5 to 60 min (Figs. 3, 4, and 5). This result indicates that the bacteriocin may have activity under normal food processing conditions in the pH range of 2 to 7.

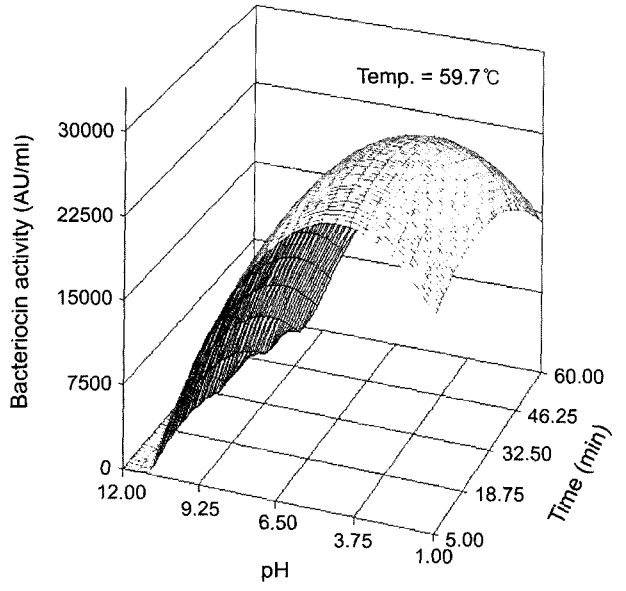

Fig. 4. Response surface for the effects of the pH and holding time on the bacteriocin activity at heating temperature=59.7°C.

Predicted activities were compared to experimental activities using the response surface model (Table 3). In the validation test, the pH and heating time were constant at 4.2 and 22.8 min, respectively, and heating temperatures varied from 40 to 121°C. At temperatures between 40 to 80°C, bacteriocin activities showed

Table 3. Comparison of bacteriocin activity with predicted activity at the pH 4.2 and heating time of 22.8 min.

Temperature (°C)	Activity	Predicted activity
40	25,600	25,119.4
60	25,600	26,899.3
80	25,600	24,920.5
100	12,800	19,182.9
121	12,800	9,113.9

Table 4. Purification of bacteriocin produced by *Leuconostoc* sp. W65.

Purification step	Total protein (mg)	Total activity (AU/ml)	Specific activity	Purification fold	Recovery (%)
Culture supernatant (900 ml)	2798.1	720,000	241.77	1	100
Ammonium sulfate Precipitate					
30%	53.61	512,000	9,549.74	39.5	71.1
30%-35%	34.96	64,000	1,830.66	7.6	8.9
35%-40%	55.46	32,000	576.99	2.4	4.4
40%-50%	86.46	16,000	185.06	0.8	2.2
Octyl-sepharose chromatography	0.644	36,800	57,142.86	236.4	5.1

almost the same values as compared with the predicted activities.

Purification and SDS-PAGE of Bacteriocin

The results from the purification procedure are summarized in Table 4. The elution profile of bacteriocin on the octyl-Sepharose column is shown in Fig. 6. Seventy-one percent of total bacteriocin was recovered, following 30% saturation of the culture broth with ammonium sulfate. Upon hydrophobic interaction chromatography on octyl-Sepharose, the bacteriocin could be eluted in 8 fractions (fraction nos. 251-257), using a linear ethanol gradient. There was a 236.4-fold increase in the purification, but the total recovery of bacteriocin was only 5.1%. In order to examine the size of the bacteriocin, fractionated bacteriocin was subjected to tricine-SDS-PAGE. After electrophoresis, part of the gel was stained by silver staining and the other part of the gel was tested for inhibitory activity using *L. ivanovii* as the indicator organism. Bacteriocin was observed at a migration distance slightly above the 3.5 kDa molecular size marker and the inhibition zone was detected at the same position (Fig. 7). The molecular mass of bacteriocin was estimated to be between 3.5 kDa and 5 kDa by SDS-PAGE.

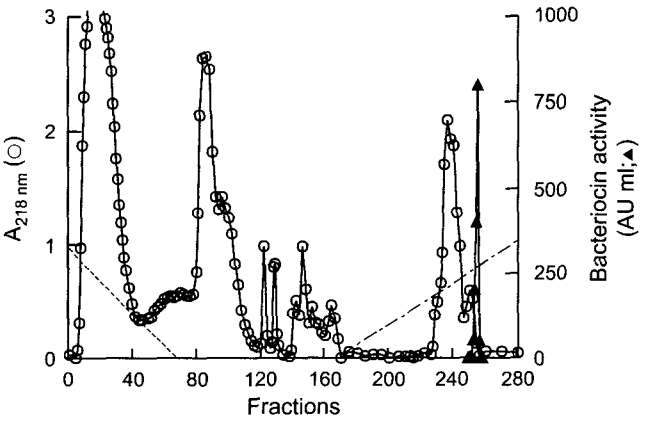

Fig. 6. Separation of bacteriocin by octyl-Sepharose CL4B column chromatography.
-----, linear gradient of 1.7 M $(NH_4)_2SO_4$; ----, linear gradient of 0 to 75% ethanol.

Amino Acid Composition and N-Terminal Amino Acid Sequencing

The amino acid composition of the purified bacteriocin is shown as Table 5. Calculation of the number of amino acid residues in the bacteriocin revealed that it consists of 56 residues, assuming the molecular weight of bacteriocin to be about 5.7 kDa. Cysteine, tryptophan, and modified amino acid such as lanthionine were not detected in the bacteriocin. The sequence of N-terminal amino acid residues of bacteriocin was identified as follows; NH_2-XGXAGVXPXGGQQPXVPLXYP.

Discussion

Listeria has caused major foodborne outbreaks worldwide. It has been isolated from soil, dust, animal, feed, water, and sewage. Therefore, humans can be exposed to listeria in various ways. Five species of *Listeria* are currently recognized; *L. innocua*, *L. welshimeri*, and *L. seeligeri* are

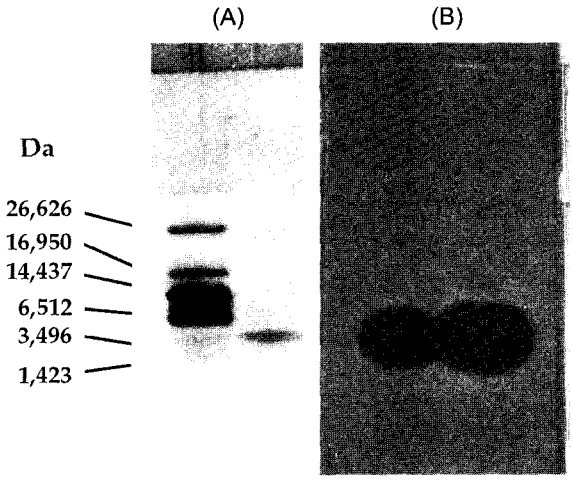

Fig. 7. Silver-stained and inhibition on tricine-SDS-PAGE of purified bacteriocin by *Leuconostoc* sp. W65.
(A) Gel stained with silver stain; lane 1, low molecular mass protein standards (Bio-Rad, U.S.A.); lane 2, purified bacteriocin. (B) Gel overlayed with cells of *Listeria ivanovii* inoculated tryptic soy agar.

Table 5. Amino acid composition of leucocin W65.

Amino acid	pmole	No. of residues*
Asp	129.8	4
Glu	182.4	6
Ser	164.6	5
Gly	275.9	8
His	33.1	1
Arg	59.1	2
Thr	90.5	3
Ala	223.9	7
Pro	127.5	4
Tyr	48.1	1
Val	106.2	3
Met	22.3	1
Ile	63.8	2
Leu	115.6	4
Phe	69.5	2
Lys	90.7	3
Total	1803	56

*Calculated number of residue per molecule.

considered to be avirulent. *L. ivanovii* may produce disease under certain conditions [2]. Only *L. monocytogenes* is believed to be pathogenic for humans, although a few reports suggest that some of the other species have caused human illness [3].

Typically, LAB bacteriocins are active against closely related microorganisms, therefore, many LAB bacteriocins are capable of inhibiting Gram-positive pathogens such as *Listeria* [10]. Some bacteriocin-producing LAB strains have been investigated as potential starter cultures for manufacturing fermented foods [12]. The ability of bacteriocin-producing LAB to inhibit *L. monocytogenes* as well as other Gram-positive foodborne pathogens provides an approach for the control of pathogens in fermented foods [15].

Three general classes of bacteriocins from LAB have been characterized, such as lantibiotics (class I), small hydrophobic heat-stable peptides (class II), and larger (>30 kDa) heat-labile proteins (class III) [10]. Nisin, a lantibiotic produced by *Lact. lactis*, is the first antilisterial bacteriocin to have been studied as a food preservative. Recently, several studies reported that bacteriocins belonging to class II are more effective at killing *Listeria* strains even though they have a narrower inhibitory spectrum [8, 9]. The bacteriocins are identified on the basis of their high amino acid sequence similarity, particularly their N-terminal region (YYGNGV) [10].

The inhibitory activity of bacteriocin employed in this study was restricted to pathogenic bacteria. Leucocin W65, a bacteriocin produced by *Leuconostoc* sp. W65, was able to inhibit different *Listeria* species tested in the same way as other bacteriocins. The leucocin W65 was detected during the early stationary phase of growth. Heat treatment (60-100°C, 60 min) had little effect on the bacteriocin activity. pH was found to be the most important factor in bacteriocin activity and this activity was more stable at acidic pHs than basic pHs. At pH 10 and higher, bacteriocin activity was completely lost, but over 1,000 AU/ml of bacteriocin activity was retained at pH 1. The 30% ammonium sulfate precipitation for the bacteriocin resulted in successful recovery rates of 71.1% and 5.1% after octyl-Sepharose column chromatography. In tricine-SDS-PAGE, a single peptide band was detected, indicating that leucocin W65 had been purified to apparent homogeneity. The leucocin W65 presumably consists of 65 amino acids and was partially sequenced.

Several antilisterial bacteriocins, belonging to class II, have a YYGNGV consensus of the N-terminal region, including bavaricin A, carnobacteriocin B2, leucocin A, pediocin AcH, mesentericin Y105, and piscicocin V1a and V1b [11, 17]. However, leucocin W65 did not contain such region.

Leucocin W65 has a broad pH range activity and heat-resistance, and is active against *Listeria* spp. These characteristics may make bacteriocin effective as a food preservative. We suggest that the bacteriocin (leucocin W65) produced by *Leuconostoc* sp. W65 is potentially useful in controlling species of *Listeria*, which is one of the most prevalent pathogens in food products.

REFERENCES

1. Ahn, C. and M. E. Stiles. 1990. Antibacterial activity of lactic acid bacteria isolated from vacuum-packaged meats. *J. Appl. Bacteriol.* **69:** 302-310.
2. Bahk, J. and E. H. Marth. 1990. Listeriolosis and *Listeria monocytogenes*, pp. 247-257. *In* D. O. Cliver (ed.), *Foodborne Diseases*. Academic Press, Inc., New York, U.S.A.
3. Brackett, R. E., E. H. Marth, M. P. Doyle, J. Lovett, and W. F. Schlech. 1988. *Listeria monocytogenes*: A foodborne pathogen (symposium). *Food Technology* **42:** 161-178.
4. Daba, H., S. Pandian, J. F. Gosselin, R. E. Simard, J. Huang, and C. Lacroix. 1991. Detection and activity of bacteriocin produced by *Leuconostoc mesenteroides*. *Appl. Environ. Microbiol.* **57:** 3450-3455.
5. Degnan, A. J., A. E. Yousef, and J. B. Luchansky. 1992. Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. *J. Food Prot.* **55:** 98-103.
6. Foster, E. M. 1997. Historical overview of key issues in food safety. *Emerg. Infect. Dis.* **3:** 481-482.
7. Harris, L. J., M. A. Saeschel, M. E. Stiles, and T. R. Klaenhammer. 1989. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes*. *J. Food Protect.* **52:** 384-387.
8. Hastings, J. W., M. Sailer, K. Johnson, K. L. Roy, J. C. Venderas, and M. E. Stiles. 1991. Characterization of leucocin A-UAL187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. *J. Bacteriol.* **173:** 7491-7500.

9. Jack, R. W., J. R. Tagg, and B. Ray. 1995. Bacteriocins of Gram-positive bacteria. *Microbiol. Rev.* **59:** 171-200.
10. Klaenhammer, T. R., C. Fremaux, C. Ahn, and K. Milton. 1993. Molecular biology of bacteriocins produced by *Lactobacillus*, pp. 151-180. *In* D. G. Hoover and L. R. Steenson (eds.), *Bacteriocins of Lactic Acid Bacteria.* Academic Press, New York, U.S.A.
11. Klaenhammer, T. R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.* **12:** 39-86.
12. McAuliffe, O., C. Hill, and R. P. Ross. 1999. Inhibition of *Listeria monocytogenes* in cottage cheese manufactured with a lacticin 3147-producing starter culture. *J. Appl. Microbiol.* **86:** 251-256.
13. Ming, X. and M. A. Daeschel. 1993. Nisin resistance of foodborne bacteria and the specific resistance responses of *Listeria monocytogenes* Scott A. *J. Food Prot.* **56:** 944-948.
14. Montgomery, D. C. 1991. Response surface methods and designs. *In: Design and Analysis of Experiments* 3rd ed. John Wiley & Sons, Inc. New York, U.S.A.
15. Moon, G. S., W. J. Kim, and M. Kim. 2002. Synergistic effects of bacteriocin-producing *Pediococcus acidilactici* K10 and organic acids on inhibiting *Escherichia coli* O157:H7 and applications in ground beef. *J. Microbiol. Biotechnol.* **12:** 936-942.
16. Muriana, P. M. 1996. Bacteriocins for control of *Listeria* spp. in food. *J. Food Prot.* **Supplement**: 54-63.
17. Rheem, S., S. Oh, K. S. Han, J. Y. Imm, and S. H. Kim. 2002. New response surface approach to optimize medium composition for production of bacteriocin by *Lactobacillus acidophilus* ATCC 4356. *J. Microbiol. Biotechnol.* **12:** 449-456.
18. SAS Institute Inc. 1985. SAS® User's guide: Statistics, Version 5 Edition. Cary, NC: SAS Institute Inc.
19. Stiles, M. E. 1993. Bacteriocins from *Carnobacterium* and *Leuconostoc*, pp. 211-218. *In* D. G. Hoover and L. R. Steenson (eds.), *Bacteriocins of Lactic Acid Bacteria.* Academic Press, New York, U.S.A.
20. Worobo, R. W., M. J. van Belkum, M. Sailer, K. L. Roy, J. C. Vederas, and M. E. Stiles. 1995. A signal peptide secretion-dependent bacteriocin from *Carnobacterium divergens*. *J. Bacteriol.* **177:** 3143-3149.