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Purification and Characterization of Bacteriocin from *Pediococcus pentosaceus* ACCEL

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The *Pediococcus pentosaceus* ACCEL bacteriocin was purified to electrophoretical homogeneity by cell adsorption−desorption and Superose 12 fast performance liquid chromatography (FPLC). The purified bacteriocin, with a molecular mass of 17.5 kDa and an N-terminal sequence of −KYYGNGVTXGKHSXXVDXG−, belongs to class IIa and is designated pediocin ACCEL. It was inactivated by various proteases and stable at pH 2.0−6.0 and <100 °C. More than 80% activity was left even after 15 min of heating at 121 °C and pH 2.0−4.0. Gram-positive food-borne pathogens were inhibited by this bacteriocin, but Gram-negative ones were not. According to the storage stability study, the purified pediocin was stable at pH <6.0 and low temperature. No significant change in bactericidal activity was observed after freeze-drying and subsequent 1-month storage at room temperature.

KEYWORDS: Pediocin; *Pediococcus pentosaceus*; bacteriocin; lactic acid bacteria

INTRODUCTION

Lactic acid bacteria (LAB) have long been employed in fermentation as a food preservation technique due to the production of antimicrobial substances such as organic acids, diacetyl compounds, hydrogen peroxide, and bacteriocins (*1*−*4*). According to the definition by Tagg et al. (*5*), bacteriocins produced from GRAS (Generally Recognized as Safe) LAB are biologically active and usually ribosomally synthesized proteins or protein complexes. They reveal a bactericidal action mode against other bacterial species but not the producer strain. Bacteriocins from various strains can inhibit food-borne pathogens, including *Bacillus cereus*, *Clostridium perfringens*, *Listeria* species, and *Staphylococcus aureus* (*6*−*9*), suggesting their high potential for use as natural preservatives in the food industry.

Several strains of *Pediococcus acidilactici* and *Pediococcus pentosaceus* were found to produce pediocin (*10*−*15*). Among these, nucleotide and amino acid sequences were the same as those of pediocin PA-1/AcH (*16*). Most of them are small, heat-stable, and non-lanthionine-containing peptides, belonging to the class II that was proposed by Klaehammer (*17*).

$(NH_4)_2SO_4$ precipitation, gel filtration, ion-exchange chromatography, and reverse-phase high-performance liquid chromatography are frequently employed to purify the desired proteins including bacteriocins. However, these procedures seem to be complex and time-consuming (*18*). Some researchers used organic solvent to extract bacteriocins and obtained relatively high yield of the final products (*19*, *20*). Yang et al. (*21*) developed a cell adsorption−desorption method to partially purify large amounts of bacteriocins. This method has been successfully applied to purify several bacteriocins with some modifications (*14*, *22*, *23*).

This study aimed to purify and characterize the bacteriocin produced by *P. pentosaceus* ACCEL, which was provided by Kuo (*24*) using the cell adsorption−desorption method.

MATERIALS AND METHODS

Bacterial Strains and Growth Media. The stock culture of *P. pentosaceus* ACCEL, which was isolated from vacuum-packaged meat by Kuo (*24*), was stored at −70 °C in 50% glycerol. The *P. pentosaceus* ACCEL was activated on lactobacilli MRS supplemented with 1.5% Bacto-agar (Difco Laboratories, Detroit, MI). *Listeria monocytogenes* CCRC 14845 was used as indicator strain and also activated on Trypticase soy agar (BBL, Microbiology System, Cockeysville, MD) supplemented with 2.0% yeast extract (Difco Laboratories). Both cultures were transferred to the respective media and activated bimonthly.

Bacteriocin Production. About 0.1% fresh culture of *P. pentosaceus* ACCEL was inoculated into MRS broth and incubated at 37 °C for 16−18 h to obtain the maximum bacteriocin production. After 30 min of heating at 80 °C, the culture was centrifuged at 10500*g* and 4 °C for 20 min. The supernatant was filtered against a 0.45 μm filter (no. 4654, Gelman). The filtrate was used as crude bacteriocin.

Preparation of Cells for Bacteriocin Adsorption. The *P. pentosaceus* ACCEL and *L. monocytogenes* CCRC 14845 (indicator strain) were grown in 1 L of appropriate medium (MRS for *P. pentosaceus* ACCEL, TSB for *L. monocytogenes*) to ∼10^8 colony-forming units (CFU)/mL at 37 °C (∼24 h). After 30 min of heating at 80 °C, the cultures were then centrifuged at 10500*g* and 4 °C for 20 min. Both cells were washed twice using sterilized distilled water. The *P. pentosaceus* ACCEL cells were further resuspended in a 100 mM NaCl solution. After the pH had been adjusted to pH 2.0, the culture was incubated at 4 °C overnight, allowing the release of bacteriocin from cells. The cells were collected by centrifugation at 10500*g* for 20 min and stored at 4 °C until use.

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Effects of pH and Mass Ratio of Producer or Indicator Cells to Bacteriocin on the Adsorption. Both producer and indicator strains were employed to absorb the bacteriocin. Various amounts of adsorptive strains, *P. pentosaceus* ACCEL and *L. monocytogenes* cells, were added to the desired volumes of crude bacteriocin, which were produced by equal amounts of *P. pentosaceus* ACCEL cells. The mass ratios of adsorptive cells to the crude bacteriocin were 1, 5, 10, 20, 30, 40, 50, and 100. The pH was then adjusted to 2.0−7.0 by using 1.0 N NaOH or 1.0 N HCl and incubated at 4 °C for 24 h. After 20 min of centrifugation at 10500*g*, the pH of all supernatants was adjusted to 4.0 and the bacteriocin activity was measured. The bacteriocin activity was expressed as [(diameter of blank inhibition zone − diameter of unabsorbed bacteriocin inhibition zone)^2/(diameter of blank inhibition zone)^2] × 100%.

Purification of Bacteriocin. After 18 h of incubation at 37 °C, the MRS culture was heated at 80 °C for 30 min, adjusted to pH 6, and stirred at 4 °C for 4 h. Cells were collected by 20 min of centrifugation at 10500*g* and 4 °C and washed twice with 0.1 volume of 5 mM sodium phosphate buffer (pH 6.0). The cells were resuspended in a 0.25 volume of 100 mM NaCl (pH 2.0) and stirred at 4 °C for 4 h. The resultant samples were centrifuged at 10500*g* and 4 °C for 20 min. After the pH of the supernatants had been adjusted to 4.0, the resultant samples were filtered through a 0.45 μm filter and further chromatographed on Superose 12 fast performance liquid chromatography (FPLC) (Pharmacia LKB). The bacteriocin was eluted using sodium acetate buffer (pH 4.4) and monitored at 280 nm (flow rate = 0.5 mL/min). Fractions of 1 mL were collected and subjected to the antimicrobial activity assessment. Fractions with antimicrobial activity were collected and dialyzed against distilled water overnight using a Spectra/Por membrane with a molecular cutoff of 3500 Da (Spectrum Co.). The resulting sample was freeze-dried and stored at room temperature for the further measurements.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). The freeze-dried bacteriocin was dissolved in a dissociating buffer (0.5 M Tris-HCl buffer, pH 6.8, containing 10% SDS and 0.1% bromophenol blue) and heated at 100 °C for 5 min. The purity and molecular mass of purified bacteriocin were determined using 15% polyacrylamide SDS-PAGE according to the method of Laemmli (*25*). Low molecular weight protein kits (Pharmacia Chemical Co.) were used as markers.

Determination of the Amino Acid Sequence of Bacteriocin. The purified bacteriocin appeared on SDS-PAGE was transferred to Sequi-Blot PVDF membrane (Bio-Rad). Edman degradation sequencing of the protein was performed using an Applied Biosystems Procise sequencer.

Protein Concentration. Protein concentrations were determined by using the dye-binding method (*26*). Bovine serum albumin (Pharmacia LKB) was used as marker.

Inhibition Assay. The agar diffusion method was employed to determine the inhibition ability of bacteriocin according to the method of Piddock (*27*). The broth was inoculated with indicator organisms and incubated at 37 °C for 24 h. After the level of cells in the broth had been adjusted to ∼1.5 × 10^8 CFU/mL, 0.1 mL of indicator broth was mixed uniformly with 15 mL of warm agar (∼45 °C). The agar was poured into a Petri dish and allowed to stand at 4 °C for 1 h. After the agar was punched by using a stainless ring with a diameter of 6 mm, 25 μL of serial 2-fold diluted samples was added into the hole and incubated at 4 °C for 24 h to allow bacteriocin diffusion. The resulting samples were incubated at 37 °C for 6 h, and the size of the inhibition zone was measured to evaluate the inhibition ability. The percentage of inhibition activity was determined as

$$[(\text{diameter of inhibition zone} - 6\ \text{mm})^2/(\text{diameter of maximum inhibition zone} - 6\ \text{mm})^2] \times 100\%$$

Biochemical Properties. *Antimicrobial Spectrum of Purified Bacteriocin.* The agar diffusion method (*27*) was used to determine the antimicrobial spectrum of the purified bacteriocin against several spoilage bacteria, pathogens, and some LAB as indicated in **Table 1**.

Effect of pH. Freeze-dried bacteriocin was dissolved in distilled water at a concentration of 1280 AU/mL. The pH of samples was adjusted to 2.0−13.0 using 1.0 N HCl or 1.0 N NaOH. After 3 h of incubation at 25 °C, the antimicrobial activities were assayed.

Table 1. Antibacterial Spectrum of Bacteriocin ACCEL

organism	ACCEL[a]
Gram-positive	
Bacillus cereus CCRC 10446	+
Bacillus cereus CCRC 10603	+
Bacillus subtilis CCRC 10255	+
Clostridium perfringens CCRC 10913	+
Lactobacillus helveticus CCRC 14092	+
Lactobacillus plantarum CCRC 10069	+
Lactococcus lactis subsp. *lactis* CCRC 10791	−
Lactococcus lactis subsp. *lactis* CCRC 12315	+
Listeria monocytogenes CCRC 14845	+
Listeria monocytogenes RamII	−
Pediococcus pentosaceus CCRC 14024	+
Pediococcus pentosaceus L	−
Pediococcus pentosaceus S	−
Staphylococcus aureus subsp. *aureus* ATCC 25923	−
Staphylococcus epidermidis ATCC 14990	+
Streptococcus faecalis DS-5	+
Gram-negative	
Alcaligenes faecalis subsp. *faecalis* ATCC 8750	−
Enterobacter aerogenes ATCC 13048	−
Escherichia coli ATCC 11229	−
Escherichia coli O:157 H:7	−
Klebsiella oxytoca ATCC 13182	−
Proteus vulgaris ATCC 13315	−
Pseudomonas fluorescens ATCC 13523	−
Salmonella enteritidis ATCC 13076	−
Salmonella typhimurium CCRC 10746	−
Salmonella typhimurium ATCC 14028	−
Vibrio cholerae	−
Vibrio damsela CCRC 15428	−
Vibrio parahaemolyticus	−
Vibriovulnificus CCRC 15430	−

[a] −, inhibition zone < 6 mm; +, inhibition zone > 6 mm.

Thermostability. The pH of purified bacteriocin (∼1280 AU/mL) was adjusted to 2.0, 4.0, 6.0, 8.0, and 10.0. The resulting samples were incubated at 80 or 100 °C for 15, 30, 45, and 60 min or at 121 °C for 15 min and then chilled to room temperature in ice−water. The inhibition ability was determined according to the method of Piddock (*27*).

Sensitivity to Proteases. After the pH of purified bacteriocin had been adjusted to 2.0, 4.0, 6.0, 8.0, or 10.0, appropriate amounts of proteases were added and incubated at 37 °C for 2 h. The reaction was stopped by heating in an 80 °C water bath for 15 min. After the mixture had cooled to room temperature, the inhibition ability was determined according to the method of Piddock (*27*). Proteases used in this study included pepsin (from porcine stomach mucosa, Sigma), α-chymotrypsin (from bovine pancreas, Sigma), Pronase (from *Streptomyces griseus*, Sigma), and bromelain (from pineapple stem, Sigma).

Storage Stability. The purified bacteriocin (1280 AU/mL; pH 2.0−11.0) was adjusted to pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 7.0, 9.0, 10.0, or 11.0 using 0.1 N HCl or 0.1 N NaOH solutions. One aliquot of these bacteriocins was kept in liquid, and the other one was freeze-dried. Both samples were stored at −20, 4, 15, 25, and 35 °C. The residual antimicrobial activity of the samples was determined at 1-day intervals during 1 month of storage. The liquid sample was directly subjected to the measurement of antimicrobial activity, whereas the freeze-dried sample was redissolved in sterilized distilled water to make the concentration the same as that before freeze-drying, and then residual inhibition activity was measured.

Bactericidal Action. The bactericidal action was determined according to the method of Enan et al. (*23*). *L. monocytogenes* CCRC 14845 was grown to log-phase (∼6 h) and stationary-phase (∼24 h) in TSB broth at 37 °C. Cells were harvested and suspended in a 4 mL TSB broth to make a 10^7 CFU/mL solution. Bacteriocin was then added. The final concentration of bacteriocin was 1280 AU/mL. Samples with and without bacteriocin were incubated at 37 °C for 10 h. The absorbance at 600 nm (OD_{600}) and viable count (CFU/mL) were

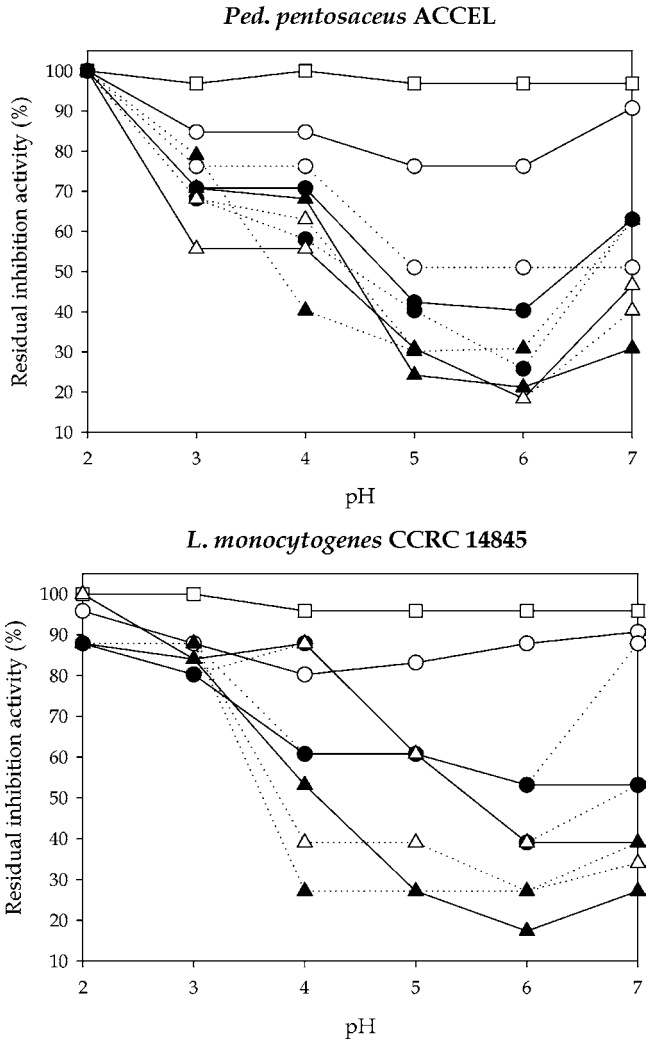

Figure 1. Effects of pH and mass ratio on the adsorption of bacteriocin ACCEL to the producer strain *Ped. pentosaceus* ACCEL and indicator strain *L. monocytogenes* CCRC 14845: –□–, control; –○–, 1; ···○···, 5; –●–, 10; ···●···, 20; –△–, 30; ···△···, 40; –▲–, 50; ···▲···, 100.

determined after 1 h of treatment with α-chymotrypsin (0.1 mg/mL) at 37 °C to inactivate the residual bacteriocin.

RESULTS

Influence of pH and Mass Ratios of Producer and Indicator to the Bacteriocin on the Efficiency of Adsorption. Adsorption of the bacteriocin was strongly influenced by pH. No bacteriocin was adsorbed by cells at pH 2.0 (**Figure 1**). The adsorption ability increased with the increase of pH, and maximal adsorption was found at pH 6.0. The adsorption ability decreased at pH 7.0. As indicated in **Figure 1**, at pH 6.0, only 20% of the bacteriocin was adsorbed at a mass ratio of 1, whereas more than 80% (by producer strain) or 70% (by indicator strain) of bacteriocin was absorbed at mass ratios of 30, 40, and 50. The optimal mass ratios for the adsorption of this bacteriocin were 30 or 40 for producer or 50 for the indicator strains, respectively (**Figure 1**).

Purification of Bacteriocin. Most of the antimicrobial substance was isolated after cell adsorption−desorption. However, some minor contaminations were still observed (**Figure 2**, lane 2); further purification was, therefore, performed using Superose 12 FPLC. The bacteriocin was purified to electrophoretical homogeneity after Superose 12 FPLC (**Figure 2**, lane 3). The molecular mass (*M*) was estimated to be 17.5 kDa by

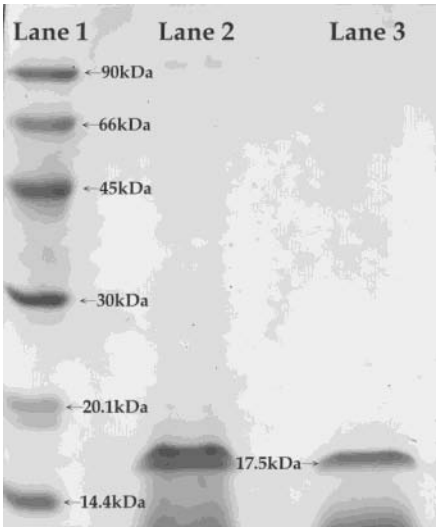

Figure 2. SDS-PAGE analysis of bacteriocin ACCEL purified from various stages: lane 1, protein marker; lane 2, from cell adsorption–desorption; lane 3, from Superose 12 FPLC.

Table 2. Summary of the Purification of Bacteriocin ACCEL

step	total protein (mg)	total activity (AU)	specific activity (AU/mg)	yield (%)	purification (fold)
culture	1192	1024000	859.1	100	1
cell extract	24	256000	106666.7	25	12
Superose 12	1.86	204800	82580.7	15	96

SDS-PAGE (**Figure 2**, lane 3). About 15% of the yield and 96-fold of purification were achieved after Superose 12 FPLC (**Table 2**).

Analysis of N-Terminal Sequence. The bacteriocin had 19 amino acid residues in the order KYYGNGVTXGKHSXX-VDXG. However, there were four unidentified residues designated X at positions 9, 14, 15, and 18 from the N-terminal. The sequence of purified bacteriocin is similar to that of pediocin PA-1/AcH. It belongs to the class IIa bacteriocins and is thus designated pediocin ACCEL.

Antimicrobial Spectrum. The pediocin ACCEL inhibited several food-borne pathogenic Gram-positive strains including *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens*, *Listeria monocytogenes* CCRC14845, *Staphylococcus epidermidis*, *Streptococcus faecalis*, and some LAB. It did not inhibit *Staphylococcus aureus*, some LAB, and all of the Gram-negative bacteria tested in this study (**Table 1**).

Effects of pH, Heat, and Proteolytic Enzymes. The antimicrobial activity of pediocin ACCEL was stable at pH between 2.0 and 10.0. Compared with that at pH 6.0, there was ∼59% activity left at pH 12.0. However, no activity was detected at pH 13.0 (data not shown).

No significant change in inhibitory activity of the purified pediocin ACCEL was observed after 15, 30, and 60 min of heating at 80 and 100 °C and pH 2.0 and 4.0 (**Table 3**). There were 76.6 and 69.4% activity left even after 15 min of heating at 121 °C and pH 2.0 and 4.0, respectively. More than 69 and 56% activity remained after 30 and 45 min of heating at 100 °C and pH 6.0. These data suggested that the purified pediocin ACCEL was stable at pH 2.0−6.0. The thermostability declined when the pH was >6.0 (**Table 3**), suggesting the thermostability is pH-dependent.

Proteolytic treatment, including pepsin, α-chymotrypsin, Pronase, and bromelain, completely inactivated the purified

Table 3. Effect of Heat Treatment on the Inhibition Activity of Pediocin ACCEL at Different pH Values against *L. monocytogenes* CCRC 14845

heat treatment	inhibition zone (mm)/inhibition activity (%)				
	pH 2.0	pH 4.0	pH 6.0	pH 8.0	pH 10
80 °C, 15 min	18.0/(100)	18.0/(100)	18.0/(100)	17.0/(84.0)	16.0/(69.4)
80 °C, 30 min	18.0/(100)	18.0/(100)	18.0/(100)	16.5/(76.6)	11.0/(17.4)
80 °C, 45 min	18.0/(100)	18.0/(100)	17.0/(84.4)	15.0/(56.3)	0
80 °C, 60 min	18.0/(100)	18.0/(100)	16.5/(76.6)	14.0/(44.4)	0
100 °C, 15 min	18.0/(100)	18.0/(100)	16.5/(76.6)	14.5/(50.2)	0
100 °C, 30 min	18.0/(100)	18.0/(100)	16.0/(69.4)	13.0/(34.0)	0
100 °C, 45 min	18.0/(100)	18.0/(100)	15.0/(56.3)	0	0
100 °C, 60 min	18.0/(100)	18.0/(100)	13.0/(34.0)	0	0
121 °C, 15 min	16.5/(76.6)	16.0/(69.4)	0	0	0

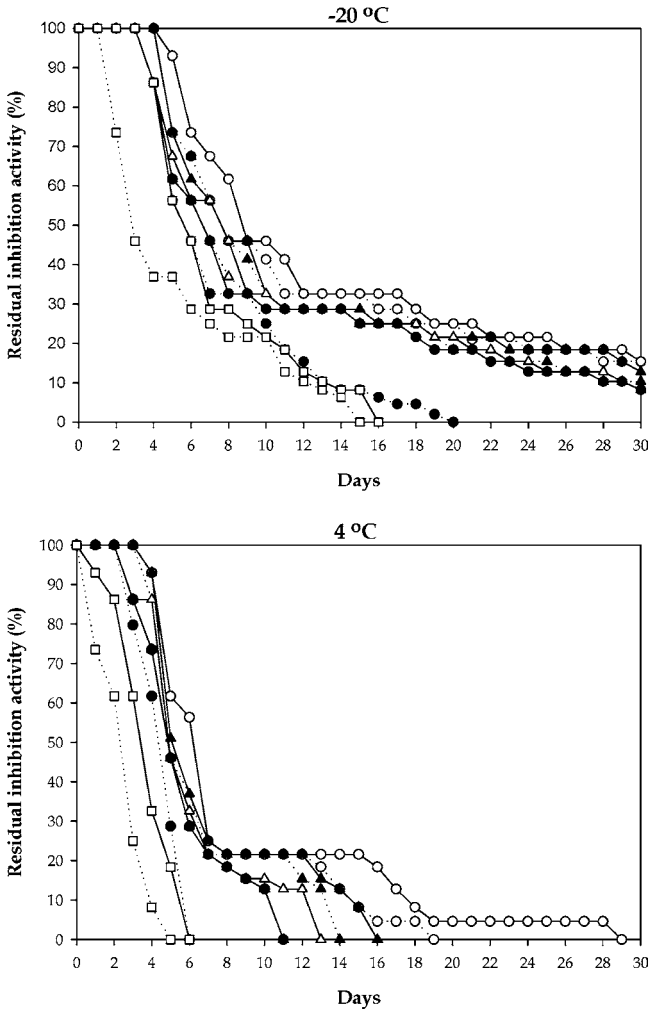

Figure 3. Stability of liquid states of pediocin ACCEL during storage at –20 and 4 °C at different pH values: –○–, pH 2; ···○···, pH 3; –▲–, pH 4; ···▲···, pH 5; –△–, pH 6; ···△···, pH 7; –●–, pH 8; ···●···, pH 9; –□–, pH 10; ···□···, pH 11.

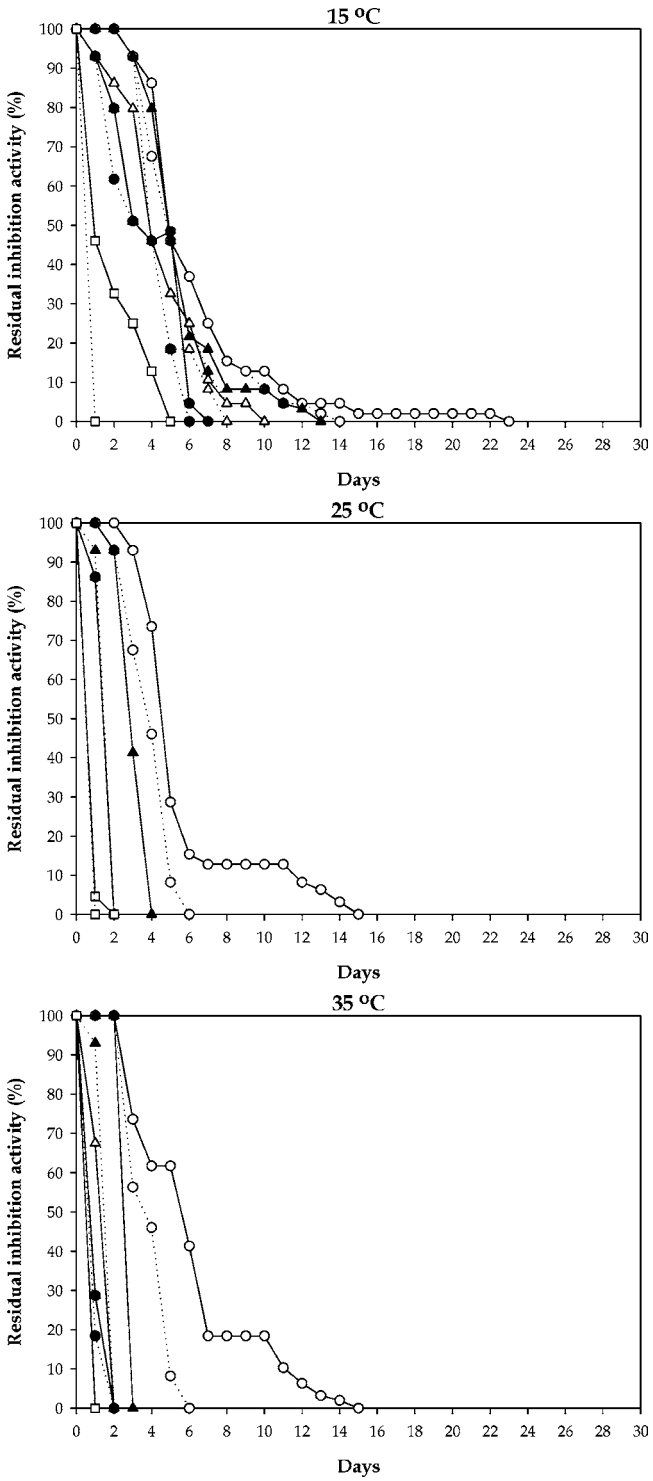

Figure 4. Stability of liquid states of pediocin ACCEL during storage at 15, 25, and 35 °C at different pH values: –○–, pH 2; ···○···, pH 3; –▲–, pH 4; ···▲···, pH 5; –△–, pH 6; ···△···, pH 7; –●–, pH 8; ···●···, pH 9; –□–, pH 10; ···□···, pH 11.

pediocin ACCEL at pH 2.0−10.0 (data not shown), suggesting the purified bacteriocin is a protein type antimicrobial substance.

Storage Stability. As indicated in **Figures 3** and **4**, the stability of the pediocin ACCEL decreased with the increases of storage temperature, time, and pH. During 1 month of storage at −20 °C, >85% inhibitory activity of the pediocin ACCEL (1280 AU/mL) was lost at pH 2.0−8.0, and the activity was almost completely lost after 20, 16, and 15 days of storage at pH 9.0, 10.0, and 11.0, respectively. However, no decrease in inhibitory activity of freeze-dried samples was observed at tested pH values and temperatures during 1 month of storage (data not shown).

Bactericidal Action. The viability of log- and stationary-phase cells of *L. monocytogenes* CCRC 14845 with and without pediocin ACCEL was investigated. Both log- and stationary-phase cells were sensitive to pediocin ACCEL (**Figure 5**). Obviously, the effect of pediocin ACCEL on *L. monocytogenes* was not dependent on the physiological state. After 1 h of

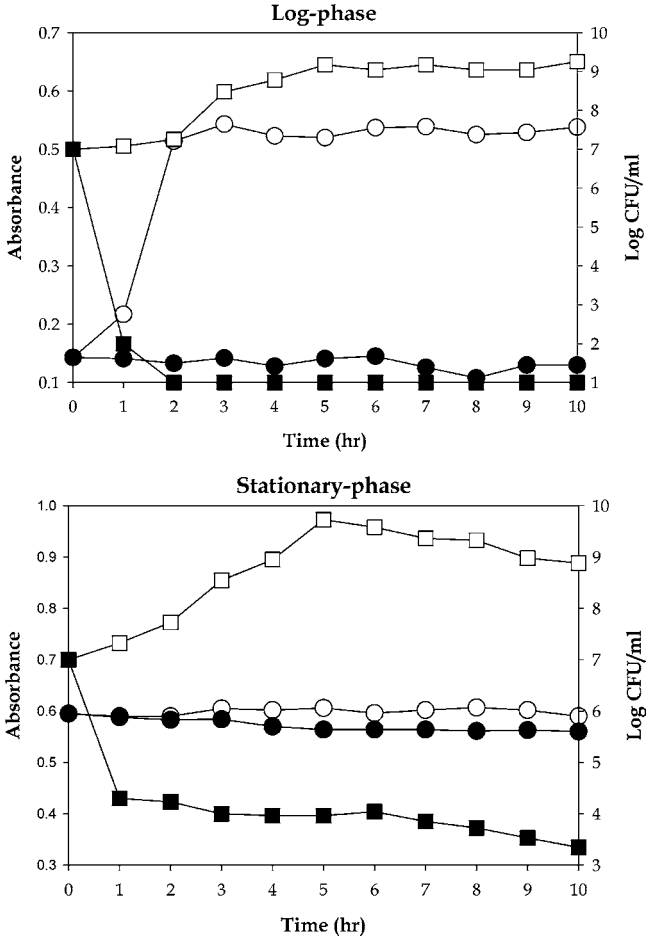

Figure 5. Antimicrobial action of pediocin ACCEL on the growth of *L. monocytogenes* CCRC 14845: ○, OD_{600} without bacteriocin; ●, OD_{600} with bacteriocin; □, viable count without bacteriocin; ■, viable count with bacteriocin.

incubation with pediocin ACCEL at 37 °C, the log-phase cells reduced from 10^7 to 10^2 CFU/mL and were killed completely after 2 h of incubation. On the other hand, the stationary-phase cells reduced from 10^7 to 10^4 CFU/mL after 1 h of incubation with pediocin ACCEL at 37 °C, and the colony counts also deceased during the further incubation. This phenomenon suggested that the log-phase cells were more sensitive to pediocin ACCEL than the stationary-phase cells.

No significant difference in OD_{600} between the stationary-phase cells treated with and without pediocin ACCEL was obtained during 10 h of incubation (**Figure 5**), indicating that no lysis of cells occurred in the stationary-phase cells treated with pediocin ACCEL.

DISCUSSION

According to the early studies on the action mode of bacteriocin bound to cell surface (*21*, *28*), a cell adsorption−desorption purification method was built up. A similar approach has been performed with the combination of RP-HPLC (*14*, *22*). In this study, the cell adsorption−desorption was employed with some modifications. However, the adsorptive amount of pediocin ACCEL to the producer or indicator strains was low when the modified cell adsorption−desorption methods were used. This is similar to that used in sakacin A. Although decreases in sakacin A and cells in supernatant were observed, the recovery of sakacin A was still low (*21*). They were not as high yield as those used in pediocin AcH, nisin, and leuconocin Lcm1 (in general, between 93 and 100%). However, in this study, the adsorptive ability of the producer or indicator strains is cell mass limiting (**Figure 1**). The adsorptive amount increased with increase of cell mass. Even though the mass ratio was raised to 30 in the preparation of 2 L of crude bacteriocin using the producer strain, almost 100% of the pediocin ACCEL in supernatant was absorbed (data not shown). In the study by Manca et al. (*15*), the adsorption abilities of pediocin N5p to the strains they used were different. It was adsorbed by both sensitive and resistant strains in nonspecific sites. High adsorption abilities were observed in sensitive strains, suggesting the presence of particular receptors. The adsorptive abilities of pediocin ACCEL to the strains used in this study need further studies to investigate whether the absorptive receptors interact with bacteriocin or not. Taking the results of this purification method and SDS-PAGE of the experiments described above into consideration, cell adsorption−desorption is a simple and convenient method in the purification of pediocin ACCEL.

Class IIa bacteriocins have a YGNGV consensus sequence near their N termini (*29*), and several of these consensus peptides have been fully characterized. These peptides have molecular masses between 3 and 6 kDa (*30*). In this study, pediocin ACCEL also has this consensus YGNGV sequence near its N terminus. Although several pediocin-producing strains have been isolated and identified, most of them were found to have consensus sequences identical to that of pediocin PA-1/AcH (*16*). As reported by Piva and Headoson (*13*), pediocin A produced from *P. pentosaceus* FBB61 had an *M* of 80 kDa but did not have the same consensus sequence as pediocin PA-1/AcH, which had an *M* of 4.6 kDa. No bacteriocins produced from the genus *Pediococcus* with a similar consensus sequence at their N termini and with a molecular mass of 17.5 kDa thus far have been reported.

The purified pediocin ACCEL almost completely lost its antimicrobial activity after proteolytic treatment, but it was still active even after high-temperature treatment at low pH (**Table 3**). This phenomenon was similar to those of bacteriocins produced by some LAB such as lactacin B (*31*), lactocin 27 (*32*, *33*), pediocin PA-1/AcH (*11*, *12*), and pediocin A (*10*).

Ray (*34*) found the antibacterial activity of pediocin AcH was retained after 15 min of heating at 70−80 °C under a pH of 2.5−9; ∼60% activity remained after 15 min of heating at 121 °C, and the activity was completely lost within 24 h at pH 10 and 25 °C. During storage at 25 °C, >50% of the activity of pediocin AcH was lost within 12 weeks. Physicochemical properties similar to those of pediocin AcH exist in pediocin ACCEL, although the discrepancy of molecular mass between these two bacteriocins was observed. Compared to the other bacteriocins, pediocin ACCEL is a rather large molecule, but, interestingly, it still shares similar biochemical properties. Therefore, further studies on the molecular genetics of pediocin ACCEL would be necessary.

Generally, class II bacteriocins strongly inhibit the strains of genus *Listeria* (*30*). According to Bhunia et al. (*28*), the adsorption of pediocin AcH resulting in cell death was considered to be due to the presence of nonspecific receptors. Recent studies on the effect of pediocin PA-1/AcH on lipid vesicles indicated that this phenomenon was not absolutely due to the presence of nonspecific receptors (*29*, *35*, *36*). As indicated in **Table 1**, the pediocin ACCEL inhibited *L. monocytogenes* CCRC 14845 but it did not inhibit *L. monocytogenes* Ram II. Perhaps there are some immunity mechanisms in the pediocin ACCEL resistant Gram-positive strains, which is mentioned in some reviews (*16*, *30*). The bactericidal effect

to the sensitive strains of pediocin ACCEL was revealed to be not due to cell lysis, but might be because of the common action of many bacteriocins produced by LAB, which have been well characterized (*37*). However, this kind of bactericidal action still needs further investigation.

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