Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Isolation and partial characterization of a bacteriocin produced by *Pediococcus pentosaceus* K23-2 isolated from Kimchi

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Keywords
antimicrobial activity, bacteriocin, kimchi, pediocin, *Pediococcus pentosaceus*.

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2007/1184: received 25 July 2007, revised 21 December 2007 and accepted 2 January 2008

doi:10.1111/j.1365-2672.2008.03770.x

Abstract

Aims: Screening and partial characterization of a bacteriocin produced by *Pediococcus pentosaceus* K23-2 isolated from Kimchi, a traditional Korean fermented vegetable.

Methods and Results: A total of 1000 lactic acid bacteria were isolated from various Kimchi samples and screened for the production of bacteriocin. Pediocin K23-2, a bacteriocin produced by the *Pediococcus pentosaceus* K23-2 strain, showed strong inhibitory activity against *Listeria monocytogenes*. The bacteriocin activity remained unchanged after 15 min of heat treatment at 121°C or exposure to organic solvents; however, it diminished after treatment with proteolytic enzymes. The bacteriocin was maximally produced at 37°C, when the pH of the culture broth was maintained at 5·0 during the fermentation, although the optimum pH for growth was 7·0. The molecular weight of the bacteriocin was about 5 kDa according to a tricine SDS-PAGE analysis.

Conclusions: *Pediococcus pentosaceus* K23-2 isolated from Kimchi produces a bacteriocin, which shares similar characteristics to the Class IIa bacteriocins. The bacteriocin is heat stable and shows wide antimicrobial activity against Gram-positive bacteria, especially *L. monocytogenes*.

Significance and Impact of the Study: Pediocin K23-2 and pediocin K23-2-producing *P. pentosaceus* K23-2 could potentially be used in the food and feed industries as natural biopreservatives, and for probiotic application to humans or livestock.

Introduction

Lactic acid bacteria (LAB) are used to preserve fermented food products, including dairy products, vegetables, meats and cereals. LAB produce antimicrobial substances, such as organic acids, diacetyl, hydrogen peroxide and bacteriocins. Bacteriocins, which are ribosomally synthesized peptides or proteins with antimicrobial properties that often target closely related species to the producer strain, could potentially be used in the food and feed industries as natural preservatives and as probiotics for humans and livestock (Diez-Gonzalez 2007).

Pediococci are Gram-positive LAB commonly found on fermenting plant material and are commercially used as starters in meat, vegetable and silage fermentations (Fleming and McFeeters 1981; Smith and Palumbo 1983). Some strains of *Pediococcus* sp. produce pediocins, members of the Class IIa bacteriocins, which are small, heat-stable, nonmodified antilisterial peptides. The pediocins described to date include PA-1 (Gonzalez and Kunka 1987), AcH (Bhunia *et al.* 1988), JD (Richter *et al.* 1989), SJ-1 (Schved *et al.* 1993), 5 (Huang *et al.* 1996), AcM by *Pediococcus acidilactici* isolated from fermented meat (Elegado *et al.* 1997) and PD-1 by *Pediococcus damnosus* NCFB 1832 isolated from spoiled beer (Green *et al.* 1997). Bacteriocins produced by different strains of *Pediococcus pentosaceus* have also been reported, including pediocin A (Etchells *et al.* 1964; Fleming *et al.* 1975), pediocin N5p (Strasser de Saad and Manca de Nadra 1993), pediocin ACCEL (Wu *et al.* 2004 and pediocin P

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(Osmanağaoğlu *et al.* 2001). Pediocins are considered potential food biopreservatives owing to their antimicrobial activity against some food-spoilage and pathogenic bacteria, such as *Listeria monocytogenes* and *Staphylococcus aureus* (Cintas *et al.* 1998).

Kimchi is a traditional Korean vegetable dish that is fermented by various micro-organisms, especially LAB, such as those in the genera *Leuconostoc*, *Lactobacillus*, *Enterococcus*, *Lactococcus* and *Pediococcus* (Han *et al.* 1990; Park *et al.* 2003). Thus, Kimchi is a good source for screening novel bacteriocin-producing LAB. We describe the purification and partial characteristics of a bacteriocin produced by a *P. pentosaceus* K23-2 strain isolated from Kimchi that shows strong inhibitory activity against Gram-positive bacteria, especially *L. monocytogenes*.

Materials and methods

Bacterial strains and culture conditions

Pediococcus pentosaceus K23-2 was isolated from Kimchi and maintained at −70°C in lactobacilli de Man Rogosa Sharpe (MRS) broth (Difco Laboratories, Detroit, USA) containing 50% (v/v) glycerol. Indicator organisms were obtained from the Korean Collection for Type Culture (KCTC), and propagated in appropriate media as indicated in Table 1.

Isolation of LAB from Kimchi

Various types of Kimchi, homemade or commercially sold, were collected. Chinese cabbage Kimchi, Baek Kimchi, Yeolmu Kimchi, Kkakdugi, Tongchimi and cucumber Kimchi were used for isolation of LAB. The samples of homogenized Kimchi were serially diluted tenfold with saline solution, plated on MRS agar, and incubated at 37°C for 2–3 days. Colonies on MRS agar were randomly selected with sterilized toothpicks and inoculated into 1 ml MRS broth in an Eppendorf tube. The isolates were grown in MRS broth for 2 days at 37°C, and then the cells were separated by centrifugation (10 000 g × 15 min). The supernatants, adjusted to pH 6·5 with 10 N NaOH, were used to detect the antagonistic activity against indicator organisms according to the

Table 1 Antimicrobial spectrum of the bacteriocin produced by *Pediococcus pentosaceus* K23-2

Indicator strains	Medium	Modified deferred method	Spot-on-lawn method (AU ml^{-1})
Bacillus cereus KCTC 1012	BHI	+	–
Escherichia coli KCTC 1467	BHI	+	–
E. coli KCTC 11682	BHI	+	–
Enterobacter aerogenes KCTC 2190	BHI	+	–
Enterococcus durans KCTC 3121	MRS	+	400
Enterococcus faecalis KCTC 2011	MRS	+	3200
E. faecalis KCTC 3206	MRS	+	800
Enterococcus faecium KCTC 3122	MRS	+	200
E. faecium KCTC 3513	MRS	+	800
Klebsiella pneumoniae KCTC 2208	BHI	+	–
Lactobacillus acidophilus KCTC 3111	MRS	+	–
Lactobacillus casei KCTC 3109	MRS	+	–
Lactobacillus delbrueckii KCTC 1047	MRS	+	–
Lactobacillus fermentum KCTC 3112	MRS	+	–
Lactobacillus plantarum KCTC 3108	MRS	+	–
Leuconostoc mesenteroides KCTC 3505	MRS	+	400
Listeria monocytogenes KCTC 3569	BHI	+	6400
L. monocytogenes KCTC 3710	BHI	+	6400
Listeria innocua KCTC 3586	BHI	+	3200
Pediococcus acidilactici KCTC 1626	MRS	+	200
Pediococcus dextrinicus KCTC 3506	MRS	+	6400
Proteus mirabillis KCTC 2566	BHI	+	–
Pseudomonas aeruginosa KCTC 1750	BHI	+	–
Staphylococcus aureus KCTC 1621	BHI	+	–
S. aureus KCTC 1916	BHI	+	–
Staphylococcus epidermidis KCTC 1917	BHI	+	–

BHI,; MRS, de Man Rogosa Sharpe.
–, no inhibition zone; +, clear inhibition zone.

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spot-on-lawn method (Mayr-Harting *et al.* 1972). The pellets were mixed with 10% skim milk and maintained as frozen stocks for master control.

Detection of bacteriocin-producing LAB

To detect antimicrobial activity, the spot-on-lawn method was used. The supernatants, adjusted to pH 6·5 and filtered through 0·2 μm pore size membrane filters, were serially diluted and 10 μl of samples were spotted onto the surface of a soft (0·7%) MRS agar seeded with an overnight culture of the indicator strain at a level of about $5{\cdot}0 \times 10^6$ CFU ml^{-1}. After incubation for 24 h at an appropriate temperature, the plates were checked for inhibition zones. Bacteriocin activity was expressed in terms of arbitrary units per ml (AU ml^{-1}), which was defined as the highest dilution showing definite inhibition of the indicator lawn.

Identification of bacterial strain

The morphological and biochemical properties of the samples were examined to identify a bacteriocin-producing strain K23-2, using Bergey's manual (Holt *et al.* 1994). We assessed Gram staining, morphology, catalase activity, salt tolerance, gas production, growth temperature range and biochemical carbohydrate fermentation patterns using an API 50 CHL kit (Biomérieux, Lyan, France). We sequenced 16S rDNA using the Big Dye terminator cycle sequencing kit (Applied BioSystems, Foster City, USA), and sequences were resolved on an automated DNA sequencing system (Applied Biosystems model 3730XL; Applied BioSystems). The 16S rDNA sequence of the strain was aligned to the 16S rRNA gene sequence of the LAB and other related taxa to compare the levels of similarity.

Preparation of crude bacteriocin

Bacteriocin-producing strains were grown at 37°C in MRS broth for 30 h. The bacterial cells were removed by centrifugation (8000 rev min^{-1} for 15 min at 4°C) and the supernatant was passed through a filter membrane (0·45 μm pore size) and then boiled for 10 min to inactivate the proteases. Ammonium sulfate was added to achieve 50% saturation. After precipitation for 24 h at 4°C, the saturated solution was centrifuged (8000 rev min^{-1} for 20 min at 4°C). The pellet was resuspended in distilled water and dialysed with a membrane with a 1 kDa cutoff (Spectrum Medical Inc., LA, USA) for 24 h at 4°C. The dialysed material (crude bacteriocin) was stored at −20°C until use.

Partial purification of the bacteriocin produced by the K23-2 strain

The crude bacteriocin solution was subjected to hydrophobic interaction column chromatography (HIC) using octyl-sepharose 4 fast flow (Sigma) linked to an FPLC system (Biologic system; Bio-Rad, CA, USA). The column was equilibrated with 1·7 mol l^{-1} ammonium sulfate and then eluted with a linear increasing gradient using water and ethanol (0–75%) at a flow rate of 1 ml min^{-1}. After HIC, active fractions (2 ml) were pooled and concentrated by a vaccum concentrator. The bacteriocin activity of each fraction was determined by the spot-on-lawn assay.

Antimicrobial spectrum of activity

The antimicrobial activity of *P. pentosaceus* K23-2 and crude bacteriocin against several Gram-positive and Gram-negative strains was tested using spot-on-lawn method and the modified deferred antagonism method (Kwon *et al.* 2002) as follows: an overnight culture of the isolated strain was stabbed onto the surface of an MRS agar plate, and then incubated at 37°C for 24 h to allow for colony development. Approximately 5×10^6 CFU ml^{-1} of the indicator strain was inoculated into 7 ml of a soft (0·7%) MRS agar and poured over the plate in which the bacteriocin-producing bacteria was grown. After 24 h of incubation, the plates were checked for the appearance of an inhibition zone. Indicator strains were subcultured in the appropriate medium and then inoculated into soft agar medium of the same composition.

Culture conditions for bacteriocin production by K23-2 strain

The effect of growth temperature and pH on bacteriocin production was investigated. The K23-2 strain was incubated in MRS broth in a 5 l jar fermenter (Fermentec Co., Cheongju, Korea). Temperature was maintained at 30 and 37°C, and pH was maintained at 5·0, 6·0 and 7·0 by adding 10 N NaOH. The agitation speed was 50 rev min^{-1}. Samples were taken at 3 h intervals to measure cell counts and bacteriocin activity. The viable cell counts of K23-2 strain were determined by the pour plate method on MRS agar and bacteriocin activity was tested by the spot-on-lawn assy.

Effects of heat, pH, enzymes and organic solvents

The crude bacteriocin was heated for 30 min at 60 or 90°C, or at 121°C for 15 min, and then the residual bacteriocin activity was determined by the spot-on-lawn

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assay. To investigate the effects of pH on antimicrobial stability, the pH of the crude bacteriocin was adjusted to 2–10 with either 1 N HCl or 1 N NaOH and incubated at 30°C for 1 h. The crude bacteriocin was treated with various enzymes in a final concentration of 1 mg ml^{-1}. All enzymes (proteinase K, protease type XIV, pepsin, trypsin, α-amylase, β-amylase and catalase) were dissolved in buffers recommended by the supplier (Sigma Chemical Co., St Louis, USA). The mixture was incubated at 30°C for 1 h and heated at 80°C for 10 min to inactivate the enzymes. The crude bacteriocin was also treated with 50% organic solvents, including ethanol, methanol, chloroform, acetone, acetonitrile, hexane and cyclohexane. The solvent-treated samples were incubated at 37°C for 1 h.

Determination of molecular weight of the antimicrobial substance by SDS-PAGE

The concentrated sample from the column chromatography was electophoresed on a 16·5% tricine-SDS polyacrylamide gel. Then, part of the gel was stained with 0·1% Coomassie Brilliant Blue G250 (Bio-Rad) and destained using a methanol–acetic acid–water (3 : 1 : 6) solution. The molecular weight of the bacteriocin was estimated by comparing molecular mass markers. The other part of the gel was washed three times in sterile water for 30 min and overlaid on a BHI soft agar (0·8%) inoculated with fresh cultures of *L. monocytogenes*.

Activity against Listeria monocytogenes

Cells of *L. monocytogenes* in log-phase growth were centrifuged, washed and resuspended in 10 ml of 50 mmol l^{-1} phosphate buffer (pH 6·8) to a final concentration of $2{\cdot}0 \times 10^8$ CFU ml^{-1}. The crude bacteriocin was added at concentrations of 200, 400 and 800 AU ml^{-1}. Aliquots were taken at predetermined time intervals to count viable cells.

Results

Screening and identification of bacteriocin-producing LAB

About 1000 isolates were taken from the Kimchi samples. More than 90% of them showed inhibitory activity against at least one of the 24 indicator strains tested using the deferred antagonism method. However, only one strain, K23-2, produced an inhibition zone when the cell-free supernatants were adjusted to pH 6·5 and checked using the spot-on-lawn method. The K23-2 strain showed the largest zone of inhibition against *L. monocytogenes*; therefore, it was selected for further characterization. The strain was Gram-positive, catalase-negative, facultatively anaerobic cocci with tetrad cell organization (Fig. 1a). The result of the API test (carbohydrate fermentation test) showed the characteristics of *P. pentosaceus* (data not shown). Therefore, the isolated strain was named *P. pentosaceus* K23-2. The 16S rDNA sequence of K23-2 strain showed 98·6% similarity with *P. pentosaceus* ATCC 25745.

Spectrum of antimicrobial activity

The culture broth and partially purified bacteriocin were tested for their antimicrobial activities against various Gram-positive and Gram-negative bacteria using the modified deferred and spot-on-lawn methods (Table 1). The K23-2 strain showed a broad spectrum of activity against all of the pathogenic and nonpathogenic bacteria tested by the modified deferred method, and a relatively broad spectrum of activity against all *Enterococcus* strains, *Leuconostoc*, *Listeria* and *Pediococcus* strains tested by the spot-on-lawn method. The bacteriocin was not active against Gram-negative bacteria, *Escherichia coli* or *Salmonella typhimurium*. However, the bacteriocin of K23-2 strain showed a high antilisterial activity compared with other sensitive strains.

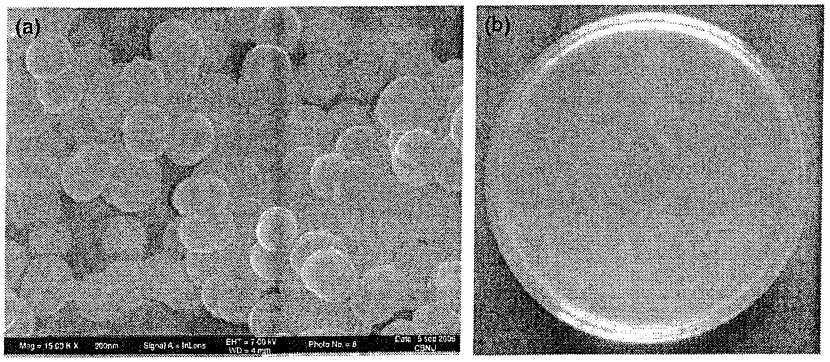

Figure 1 Scanning electron micrograph of the *Pediococcus pentosaceus* K23-2 strain isolated from Kimchi (a) and growth inhibition of *Listeria monocytogenes* by the bacteriocin produced by K23-2 (b).

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Influence of pH on cell growth and bacteriocin production

When the pH of culture broth was not controlled, the production of bacteriocin started in the middle of the exponential growth phase, reached maximum levels (6400 AU ml^{-1}) in the stationary phase, and then declined (Fig. 2a). The pH of the medium dropped from 6·5 to 3·8 during the 75 h incubation period. The optimal pH value for bacteriocin production did not coincide with the optimum pH for growth of the K23-2 strain (Fig. 2). The bacteriocin was maximally produced at 37°C, when the pH of the culture broth was 5·0 or less during fermentation, whereas the optimum pH for growth was 6·0–7·0 (Fig. 2).

Effects of enzymes, heat treatment, pH and organic solvents on bacteriocin activity

Bacteriocin activity was completely inactivated by treatment with proteinase K and pepsin but not protease type XIV, α-amylase, β-amylase and catalase (Table 2). The bacteriocin of the K23-2 strain was highly thermostable, maintaining antimicrobial activity even after treatment at 121°C for 15 min. Bacteriocin activity was stable from pH 2 to 8 for 1 h, whereas most activity was lost at pH 9·0 and higher. It was not affected by exposure to organic solvents in 50% concentrations.

Purification of bacteriocin

Two peaks of bacteriocin activity were detected in distilled water and 50% ethanol in the HIC (Fig. 3). Brown-coloured contaminants were contained in the first pooled active fraction of distilled water but not in the second one. To estimate the molecular weight of the bacteriocin, the second pooled active fraction was subjected to tricine SDS-PAGE. A single band appeared at approximately 5 kDa molecular weight. When the duplicate gel was tested for antimicrobial activity against *L. monocytogenes* as an indicator, a clear inhibition zone was detected at the same position (Fig. 4).

Activity against *Listeria monocytogenes*

To determine whether the bacteriocin produced by *P. pentosaceus* K23-2 was bactericidal or bacteriostatic against sensitive strains, crude bacteriocin was added at different concentrations to *L. monocytogenes* suspended in 50 mmol l^{-1} phosphate buffer. The number of viable bacterial cells decreased rapidly after the first 30 min and then slowly to 3 h (Fig. 5). Over the 3 h period, the reduction in viable cell number was approx. 5·4 log scale

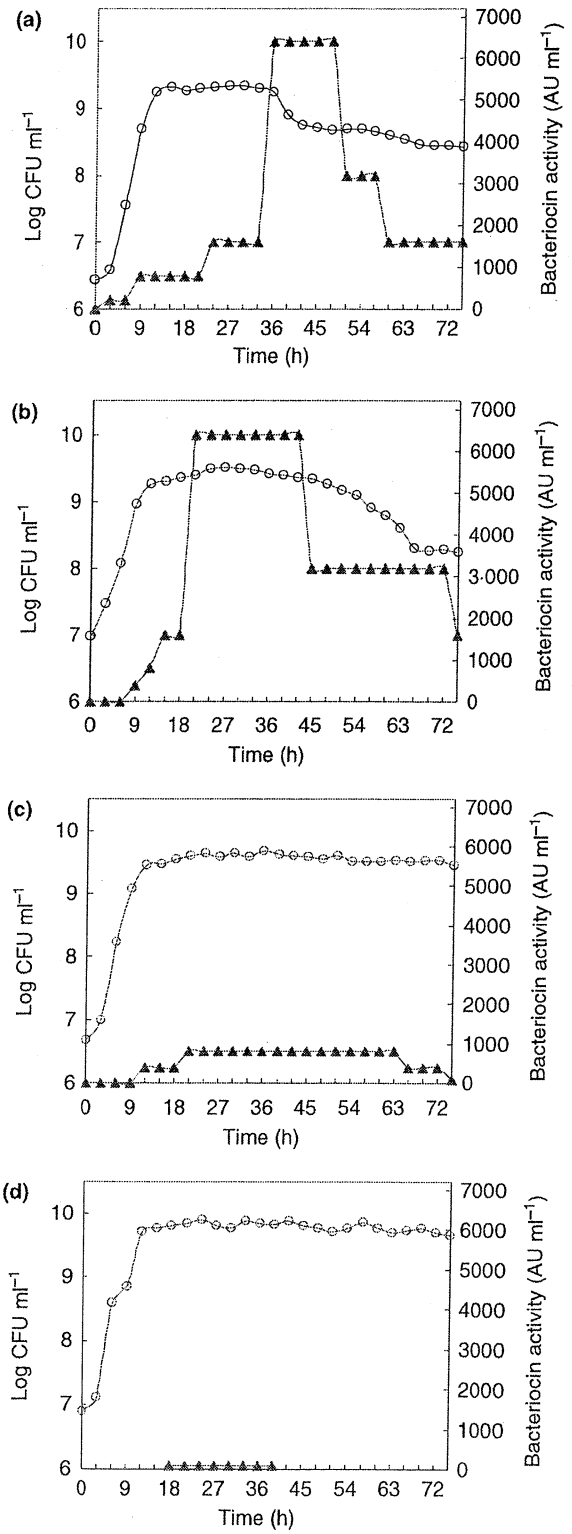

Figure 2 Cell growth and bacteriocin production of *Pediococcus pentosaceus* K23-2 at different pH in de Man Rogosa Sharpe broth in a 5 l jar fermenter. (○) viable cell count; (▲) bacteriocin activity; (a) uncontrolled pH; (b) pH 5·0; (c) pH 6·0; (d) pH 7·0.

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Table 2 Effects of enzymes, heat, pH and organic solvents on the activity of the bacteriocin partially purified from the *Pediococcus pentosaceus* K23-2 strain

	Treatment	Relative bacteriocin activity (%)
Enzymes	Proteinase K	0
	Protease	100
	Pepsin	0
	Trypsin	100
	α-Amylase	100
	β-Amylase	100
	Catalase	100
Heating	60°C, 30 min	100
	95°C, 30 min	100
	121°C, 15 min	100
PH	pH 2·0	100
	pH 3·0	100
	pH 4·0	100
	pH 5·0	100
	pH 6·0	100
	pH 7·0	100
	pH 8·0	50
	pH 9·0	0
	PH 10·0	0
Organic Solvents	Ethanol	100
	Methanol	100
	Chloroform	100
	Acetone	100
	Acetonitrile	100
	Hexane	100
	Cyclohexane	100

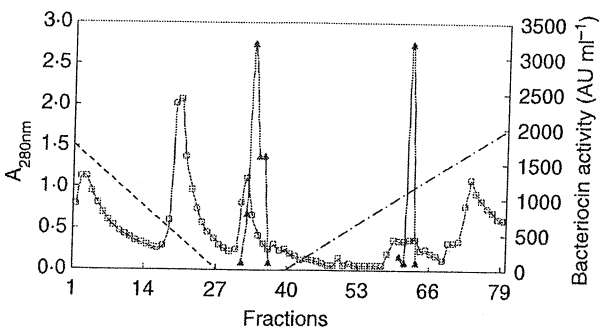

Figure 3 Separation of pediocin K23-2 by octyl-sepharose 4 fast-flow column chromatography. (□) absorbance at 280 nm; (▲) bacteriocin activity (AU ml^{-1}); (- - - - -) linear gradient of 20–0% $(NH_4)_2SO_4$; (——) linear gradient of 0–75% ethanol.

at 800 AU ml^{-1}, 4·9 log scale at 400 AU ml^{-1} and 4·5 log scale at 200 AU ml^{-1}.

Discussion

This study describes the partial characterization of an antilisterial bacteriocin, pediocin K23-2, produced by *P. pentosaceus* K23-2, which was isolated from cabbage Kimchi, a traditional Korean fermented vegetable. Several bacteriocin-producing bacteria have been isolated from different varieties of Kimchi, including *Lactococcus lactis* (Kwak *et al.* 2001; Lee *et al.* 2002), *Enterococcus faecium* (Ha *et al.* 1994; Moon *et al.* 2004), *Lactobacillus plantarum* (Kim *et al.* 2003; Lee *et al.* 2005), *Leuconostoc mesenteroides* (Cha and Ha 1996; Yang *et al.* 2002), and *P. acidilactici* (Kwon *et al.* 2002; Moon *et al.* 2005). However, there are strain differences in the production yield of bacteriocin and inhibition spectra against pathogens (Strasser de Saad and Manca de Nadra 1993; Green *et al.* 1997; Rodríguez *et al.* 2002). Moreover, the use of more than one bacteriocin as a combination biopreservative can be advantageous over a single bacteriocin (Hanlin *et al.* 1993). A new bacteriocin isolated from different sources or other species will be useful to develop upgraded natural biopreservatives.

The spectrum of antimicrobial activity of pediocin K23-2 is similar to that reported for other Class IIa bacteriocins, especially pediocin PA-1 (Gonzalez and Kunka 1987), pediocin SJ-1 (Schved *et al.* 1993) and N5p (Strasser de Saad and Manca de Nadra 1993). LAB produce inhibitory substances, such as organic acids, diacetyl, hydrogen peroxide and bacteriocins. The inhibitory activity of the *P. pentosaceus* K23-2 strain against Gram-negative strains in the modified deferred analysis could be explained primarily by the accumulation of lactic acid.

The antimicrobial activity of pediocin K23-2 in prolonged fermentation dramatically decreased peaking at 36–48 h (Fig. 2a). This pattern has been observed for other LAB bacteriocins (Barefoot and Klaenhammer 1984; Daba *et al.* 1991; Parente *et al.* 1994; Matsusaki *et al.* 1996; Aasen *et al.* 2000; Mataragas *et al.* 2003). Bacteriocins are often produced during the growth phase and then decrease owing to proteolytic degradation, protein aggregation and adsorption by the cells (Parente and Ricciardi 1994; Parente *et al.* 1994; de Vuyst *et al.* 1996; Aasen *et al.* 2000). Pediocin K23-2 production was dependent on the pH of the culture broth. The optimum pH for growth was 7·0, and for pediocin K23-2 production, it was 5·0 or not controlled. The optimal pH for the production of lactobacilli or lactococci bacteriocins is usually between 5 and 7 (Parente and Ricciardi 1994; Parente *et al.* 1994; Mortvedt-Abildgaard *et al.* 1995; Matsusaki *et al.* 1996; de Vuyst *et al.* 1996; Krier *et al.* 1998). However, Biswas *et al.* (1991) reported that pediocin AcH production was negligible when the pH of the medium was maintained at 5·0 or higher, even in the presence of high cell mass, because of the need for a low pH to convert prepediocin into active pediocins. Yang *et al.* (1992) found that almost all molecules of pediocin AcH, leuconocin Lcm1, sakacin A and nisin were adsorbed into cell surfaces at pH near

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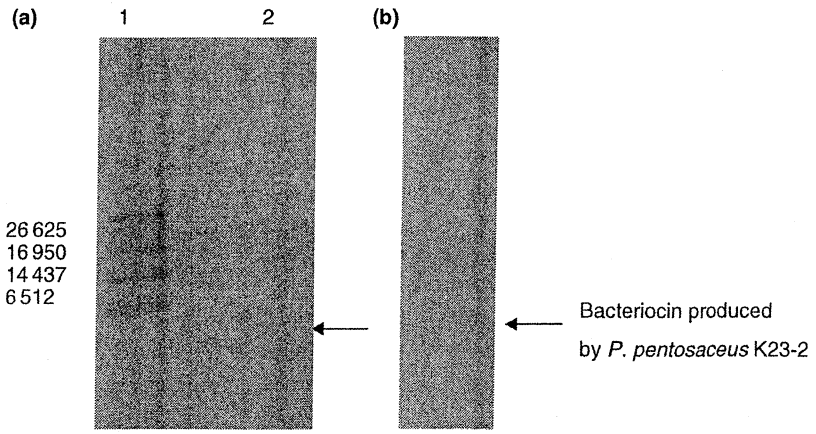

Figure 4 SDS-PAGE and detection of antimicrobial activity of the purified bacteriocin. (a) Gel stained with Coomassie Brilliant Blue G2-50; lane 1, molecular weight standards; lane 2, partially purified bacteriocin by hydrophobic interaction column chromatography. (b) Gel overlaid with *Listeria monocytogenes* inoculated into a de Man Rogosa Sharpe soft agar.

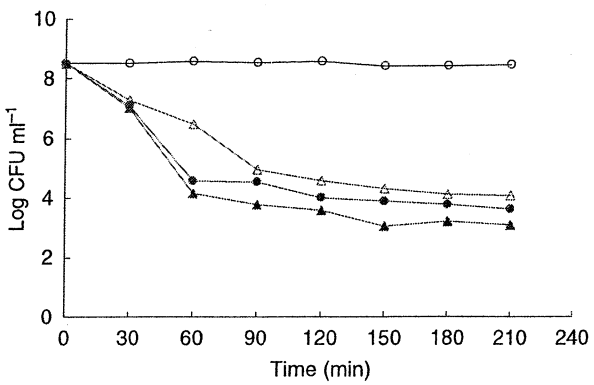

Figure 5 Type of inhibitory action of K23-2 bacteriocin. *Listeria monocytogenes* was incubated at 37°C in 10 mmol l^{-1} phosphate buffer containing bacteriocin. Control (○) had no bacteriocin, (△), 200 AU; (●), 400 AU; (▲), 800 AU.

6·0, and that the lowest adsorption took place at pH 1·5–2·0. The *P. pentosaceus* K23-2 strain cells grown in a fermenter at pH 7·0 were collected, and the bacteriocin was extracted using the adsorption–desorption method described by Yang *et al.* (1992). No bacteriocin activity was observed from these cells (data not shown), suggesting that the low bacteriocin production at pH 7·0 was not the result of the binding of bacteriocin to the cells.

The antimicrobial activity was sensitive to two out of three proteolytic enzymes tested, indicating that the inhibitory substance produced by *P. pentosaceus* K23-2 was proteinaceous. There was no loss of antimicrobial activity following treatment with amylase, organic solvents and catalase, probably owing to the absence of carbohydrate moieties in the molecule and hydrogen peroxide. These results suggest that the inhibitory compound is a bacteriocin; it was designated pediocin K23-2. The bacteriocin was stable over a wide range of pH (2–8), especially in more acidic conditions. Similar results have been described by previous authors (Daeschel and Klaenhammer 1985; Gonzalez and Kunka 1987; Bhunia *et al.* 1988; Osmanağaoğlu *et al.* 2001; Jamuna and Jeevaratnam 2004; Todorov and Dicks 2005). However, Elegado *et al.* (1997) found that the bacteriocin activity of pure pediocin at high concentrations remained even after incubation at pH 1·0–12, although antimicrobial activity at a low bacteriocin concentration was detected at pH 1·0–9·0.

The molecular weight of pediocin K23-2 was about 5 kDa, according to a tricine SDS-PAGE analysis, which corresponds to previous results obtained for other pediocins, including PA-1 (Rodríguez *et al.* 2002), AcH (Bhunia *et al.* 1988), SJ-1 (Schved *et al.* 1993) and AcM (Kim *et al.* 1991). The rapid decrease in the number of viable cells of *L. monocytogenes* treated with pediocin K23-2 suggests that the mechanism of activity of pediocin K23-2 is bactericidal for sensitive bacterial cells. The effectiveness of inhibition was in proportion to the concentration of bacteriocin. A similar mode of action has been observed in many other bacteriocins from LAB (Bhunia *et al.* 1991; Nettles and Barefoot 1993; de Vuyst and Vandamme 1994; Jack *et al.* 1995; Elegado *et al.* 1997).

In conclusion, pediocin K23-2 produced by *P. pentosaceus* isolated from Kimchi showed a wide spectrum of inhibitory activity against Gram-positive food-spoilage bacteria and pathogens. Pediocin K23-2 and pediocin K23-2-producing *P. pentosaceus* K23-2 could potentially be used in the food and feed industries as natural biopreservatives, and for probiotic application to humans or livestock. Further studies are necessary to determine whether the mode of pediocin K23-2 activity relates to that described for other pediocins. Research on the optimum conditions for industrial-scale production and purification of the bacteriocin is currently in progress.

Acknowledgements

This study was supported by the Technology Development program of the Ministry of Agriculture and Forestry, Republic of Korea.

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