ELSEVIER

Process Biochemistry 40 (2005) 365–370

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

Pediocin ST18, an anti-listerial bacteriocin produced by *Pediococcus pentosaceus* ST18 isolated from boza, a traditional cereal beverage from Bulgaria

Svetoslav D. Todorov [a], Leon M.T. Dicks [b,*]

[a] *Magura Winery, JSCo, 3938 Rabisha, Vidin Region, Bulgaria*
[b] *Department of Microbiology, University of Stellenbosch, 7600 Stellenbosch, South Africa*

Received 22 August 2003; accepted 3 January 2004

Abstract

Lactic acid bacteria isolated from boza, a cereal-fermented beverage from Belogratchik, Bulgaria, were screened for the production of bacteriocins. Thirteen of the 52 strains isolated inhibited the growth of *Pediococcus* spp., *Listeria innocua* and *Lactobacillus plantarum*. One of the strains, identified as *Pediococcus pentosaceus* ST18, produced pediocin ST18 at 3200 arbitrary units (AU) ml^{-1} in MRS broth at the end of logarithmic growth (i.e. after 24 h). Concentration by ammonium sulfate precipitation, followed by separation in a Sep-Pack C_{18} column and reverse-phase HPLC on a C18 Nucleosil column yielded two active antimicrobial peptides, which suggests that pediocin ST18 may be a two-peptide bacteriocin. The peptide had bacteriostatic action towards *L. innocua*, with no cell lyses. Pediocin ST18 remained active after 30 min at 121 °C and after 2 h of incubation at pH 2–12. No loss in activity was recorded after treatment with α-amylase, SDS, Tween 20, Tween 80, urea, Triton X-100, *N*-laurylsarcosin, EDTA and PMSF. Pediocin ST18 does not adhere to the cell surface of the producer strain.

Keywords: Boza; *Pediococcus pentosaceus*; Pediocin ST18

1. Introduction

Lactic acid bacteria play an important role in the preservation, microbiological stability and production of organoleptic compounds of fermented foods [1]. Boza, a traditional drink produced from fermented cereal, contains a large population of lactic acid bacteria and yeast. However, only a few papers have been published on the microbial composition of boza [2–5]. Most of the strains thus far isolated belonged to the genera *Lactobacillus*, *Lactococcus* and *Leuconostoc*.

In one study, reported by Kabadjova et al. [4], 33 strains isolated from boza showed antibacterial activity against various Gram-positive bacteria, including *Listeria innocua*, and Gram-negative bacteria such as *Escherichia coli*. The antimicrobial compound produced by one of these strains, *Lactococcus lactis* subsp. *lactis* 14, was reported to be a bacteriocin [4].

As far as we could determine, nothing has been reported on bacteriocins produced by *Pediococcus* spp. isolated from boza. The aim of this study was to determine the cell numbers of pediococci in boza, identify the species, and determine if bacteriocin-producing pediococci exist.

A number of bacteriocins have been described for pediococci, viz. pediocin A, produced by different strains of *Pediococcus pentosaceus* isolated from cucumber brine [6,7]; pediocin AcH (also referred to as pediocin H), pediocin PA-1, pediocin JD1 and pediocin SJ-1, produced by *Pediococcus acidilactici* isolated from fermented meat [8–11]; and pediocin PD-1, produced by *Pediococcus damnosus* NCFB 1832, originally isolated from spoiled beer [12]. Pediocins have also been reported for *P. pentosaceus* strains isolated from pepperoni [13,14] and strains of *P. acidilactici* isolated from human clinical sources [14]. More recently, strains of *Lactobacillus plantarum* and *L. lactis* with the

* Corresponding author. Tel.: +27-21-808-5849; fax: +27-21-808-5846.
E-mail address: lmtd@sun.ac.za (L.M.T. Dicks).

0032-9592/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2004.01.011

ability to produce pediocins AcH and PA-1, respectively, have also been reported [15,16].

2. Materials and methods

2.1. Bacterial strains and growth media

Strain ST18 was isolated from boza Belogratchik (Belogratchik, Bulgaria) and identified as *P. pentosaceus* by using the physiological and biochemical tests described by Müller [17], Garver and Muriana [18] and Atrih et al. [19]. Sugar fermentation reactions were obtained by using the API 50 CHL system (BioMérieux, France). Final identification was made by comparing the characteristics with those listed for the species [20].

Strain ST18 was grown in MRS medium [21] and incubated at 30 °C. The growth media and origin of the other strains included in this study are listed in Table 1. The strains were stored at −80 °C in MRS broth containing 15% (v/v) glycerol.

2.2. Bacteriocin bioassay

Bacteriocin screening was performed by using the agar spot test and the well diffusion method, as described by Schillinger and Lücke [22] and Tagg and McGiven [23], respectively. To eliminate the antimicrobial effect of lactic acid, the pH of the supernatants was adjusted to 6.0 with sterile 1N NaOH and the activity expressed in arbitrary units (AU) ml^{-1}. One AU was defined as the reciprocal of the highest serial two-fold dilution showing a clear zone of growth inhibition of the indicator strain [24].

2.3. Production

Sterile MRS broth, without Tween 80, was inoculated with 2% (v/v) of an 8-h-old culture of *P. pentosaceus* ST18. Incubation was at 30 °C, without agitation. Samples were taken at different time intervals to determine the optical density (at 600 nm) of the culture and the antimicrobial activity (AU ml^{-1}) of the bacteriocin produced.

2.4. Effect of enzymes, pH, detergents, protease inhibitors and temperature

Strain ST18 was grown in MRS broth at 30 °C for 24 h, the cells harvested by centrifugation (8000 × *g*, 10 min, 4 °C), and the cell-free supernatant, adjusted to pH 6.0, conducted to the following tests. Samples of 100 μl were incubated for 2 h in the presence of 1 and 0.1 mg ml^{-1} (final concentration) protease IV (Sigma, France), Pronase (Sigma) and α-amylase (Sigma), respectively, and tested for antimicrobial activity by using the agar spot test method as described before.

In a separate experiment the effect of surfactants on the bacteriocins was tested by adding sodium dodecyl sulfate (SDS), Tween 20, Tween 80, urea, *N*-laurylsarcosin and Triton X-100 (1%, v/v, final concentration), respectively, to the cell-free supernatant. The protease inhibitors EDTA and PMSF were added to the cell-free supernatant to yield a final concentration of 0.1, 2.0 and 5.0 mM, respectively. Untreated cell-free supernatant and the detergents at these respective concentrations were used as controls. All samples were incubated at 37 °C for 5 h and then tested for antimicrobial activity by using the agar spot test method, as described before.

The effect of pH on the bacteriocins was tested by adjusting cell-free supernatants to pH 2.0–12.0 (at increments of 1 pH unit) with sterile 1N NaOH or 1N HCl. After 30 min and 2 h of incubation at room temperature (25 °C), each of the samples was tested for antimicrobial activity by using the agar spot test method, as described before.

The effect of temperature on the activity of the bacteriocins was tested by heating the cell-free supernatant from 30 to 100 °C (with 10 °C intervals) and at 121 °C, respectively. Residual bacteriocin activity was tested after 5, 10, 15, 20 and 30 min, respectively, at each of the latter temperatures. The agar spot test method was used as described before.

2.5. Cell lysis

A bacteriocin-containing cell-free supernatant (20 ml at pH 6.0) was added to a 100 ml culture of *L. innocua* F in early exponential phase. The OD (600 nm) of the culture was determined every hour for 9 h.

2.6. Adsorption studies

Adsorption of bacteriocins to the producer, strain ST18, was studied by using the method described by Yang et al. [25]. After 18 h of growth at 30 °C, the culture was adjusted to pH 6.0, the cells harvested by centrifugation (20,000 × *g*, 15 min) and washed with sterile 0.1 M phosphate buffer (pH 6.5). The cells were resuspended in 10 ml of 100 mM NaCl (pH 2.0) and stirred for 1 h at 4 °C. The cells were harvested by centrifugation (15 min at 20,000 × *g*), the cell-free supernatant neutralized to pH 7.0 with sterile 1N NaOH and tested for activity as described above.

2.7. Bacteriocin purification

A 24-h-old culture of strain ST18 was centrifuged for 15 min at 20,000 × *g* and the cell-free supernatant treated for 10 min at 80 °C to prevent proteolytic degradation of the bacteriocin. Ammonium sulfate was gently added to the cell-free supernatant (80% saturation), stirred for 4 h at 4 °C and then centrifuged (20,000 × *g*, 1 h, 4 °C). The pellet was resuspended in 25 mM ammonium acetate (pH 6.5) and loaded on a Sep-Pack C_{18} column (Waters Millipore, MA, USA). The column was washed with 20% (v/v) *iso*-propanol

Table 1
Indicator strains, growth media and sensitivity to *P. pentosaceus* ST18 cell-free supernatant[a]

Strain	Origin	Media	Activity
Bacillus cereus 1 and 2	ENITIAA	NB	−
Bacillus stearothermophilus	ENITIAA	NB	−
Bacillus subtilis 6633	ATCC	NB	+
Carnobacterium divergens 2763	NCDO	Elliker	+
Carnobacterium piscicola 2762	NCDO	Elliker	−
Citrobacter freundii 2	ENITIAA	NB	−
Clostridium perfringens 2	ENITIAA	RCM	−
Clostridium sporogenes 2	ENITIAA	RCM	−
Clostridium tyrobutyricum 1	ENITIAA	RCM	−
Enterococcus faecalis 1	ENITIAA	Elliker	+
E. coli 1 and 2	ENITIAA	NB	−
Klebsiella pneumoniae 1	ENITIAA	NB	−
Lactobacillus amylophilus 1394	IP	MRS	+
Lactobacillus brevis 1104	SD PC	MRS	+
Lactobacillus casei subsp. *casei* 1038	SD PC	MRS	−
L. casei subsp. *casei* 1416	IP	MRS	−
Lactobacillus delbrueckii subsp. *bulgaricus* C	ENITIAA	MRS	+
Lactobacillus fermentum 1386	IP	MRS	+
Lactobacillus helveticus 1 and 2	ENITIAA	MRS	+
L. plantarum 1390 and 1397	LdC Boll	MRS	+
L. plantarum 1408 and 1409	ENITIAA	MRS	−
L. plantarum 73, 1383 and 14917	ENITIAA	MRS	+
L. plantarum subsp. *pseudoplantarum* 1128 and 1131	SD PC	MRS	+
L. lactis subsp. *cremoris* 117	INRA CNRZ	MRS	−
Leuconostoc mesenteroides 1 and 2	ENITIAA	MRS	−
L. mesenteroides 1000 and 1228	SD PC	MRS	−
L. mesenteroides 1044 and 1324	SD PC	MRS	+
L. mesenteroides 8293	ATCC	MRS	+
L. mesenteroides subsp. *dextranicum* 1055	SD PC	MRS	+
L. mesenteroides subsp. *dextranicum* 1185	SD PC	MRS	−
L. mesenteroides subsp. *dextranicum* 1414	LdC	MRS	+
L. innocua V1 and F	ENITIAA	Elliker	+
L. monocytogenes R ser. 4b	ENITIAA	Elliker	+
P. damnosus 1	ENITIAA	Elliker	+
P. pentosaceus 1272 and 1164	SD PC	Elliker	+
Proteus vulgarus 2	ENITIAA	NB	−
Salmonella heidelberg 1	ENITIAA	NB	−
Serratia marcescens 1	ENITIAA	NB	−
Staphylococcus aureus 1	ENITIAA	NB	+
Streptococcus thermophilus 1	ENITIAA	Elliker	+
Yersinia enterocolitica 3	ENITIAA	NB	−

[a] Activity refers to inhibition with 3200 AU/ml. ATCC, American Type Culture Collection, Rockville, MD; ENITIAA, Ecole Nationale des Ingenieurs des Techniques Agricoles et Alimentaires, Nantes, France; INRA-CNRZ, Centre National de Recherche Zootechnique, INRA, Jouy en Josas, France; IP, Institut Pasteur, Paris, France; LdC, Levain de Cracker, USA (Boll); NCDO, National Collection of Dairy organisms, Reading, UK; SD PC, sourdough private collection. Elliker [26], NB (nutrient broth, Biokar, Beauvais, France), MRS [21], RCM (Biokar, Beauvais, France). Incubation was at 37 °C.

in 25 mM ammonium acetate (pH 6.5) and the bacteriocins eluted with 40% *iso*-propanol in 25 mM ammonium acetate (pH 6.5). After drying under vacuum (Speed-Vac; Savant, France), the fractions were pooled and dissolved in 0.1% (v/v) trifluoracetic acid (TFA). This fraction was subjected to reverse-phase HPLC on a C18 Nucleosil (Water, Millford, MA, USA) column (250 mm × 4.6 mm). Elution was performed by using a linear gradient from 100% of 0.1% TFA (solvent A) to 90% acetonitrile in 0.1% TFA (solvent B) over 65 min. Polypeptides were detected with an in-line optical density reader 220 nm. Fractions containing peptides were collected, dried under vacuum, dissolved in 1 ml sterile de-ionized water and stored at −20 °C. Activity tests were performed by using the agar spot test method as described before.

3. Results and discussion

The population of lactic acid bacteria recorded in boza was ca. 2×10^8 CFU ml^{-1}. Fifty-two isolates were selected based on differences in colony morphology on MRS agar. Only 13 isolates showed antibacterial activity against *L. innocua* F. From these strains one strain (ST18) was selected for further studies, based on its broad spectrum of antibacterial activity (Table 1).

Table 2
Identification of *P. pentosaceus* ST18 by carbohydrate fermentation reactions (API 50 CHL, Biomérieux, Marcy-l'Etoile, France)

Sugar	Reaction	Sugar	Reaction	Sugar	Reaction
Glycerol	−	Salicin	+	Esculin	+
Erythritol	−	Cellobiose	+	α-Methyl-D-mannoside	−
D-Arabinose	−	Maltose	+	α-Methyl-D-glucoside	−
L-Arabinose	+	Lactose	+	*N*-acetyl-glucosamine	+
Ribose	+	Melibiose	+	Amygdalin	+
D-Xylose	+	Sucrose	+	Arbutin	+
L-Xylose	−	Trehalose	+	D-Arabitol	−
Adonitol	−	Inulin	+	L-Arabitol	−
Inositol	−	Melezitose	−	Gluconate	−
Galactose	+	Raffinose	+	2-Keto-gluconate	−
Glucose	+	Starch	−	5-Keto-gluconate	−
Fructose	+	Glycogen	−	Mannitol	−
Mannose	+	Xylitol	−	Sorbitol	−
Sorbose	−	Gentiobiose	+	D-Fucose	−
Rhamnose	+	D-Turanose	−	L-Fucose	−
Dulcitol	−	D-Lyxose	−	b-Methyl-D-xyloside	−
		D-Tagatose	+		

Strain ST18 is a Gram-positive, catalase and oxidase negative coccus with a tetrad cell organization. Carbon dioxide is not produced from the fermentation of glucose and polysaccharides are not formed. Growth is optimal at 30 °C and slow at 16 °C (usually only after 48 h). No growth was observed at 45 °C. The latter characteristics, especially cell morphology, are typical for the genus *Pediococcus* [20]. Carbohydrate fermentation reactions recorded with the API 50CHL system (Table 2) classified strain ST18 as a member of *P. pentosaceus*.

Growth of *P. pentosaceus* ST18 under aerobic or anaerobic conditions resulted in more-or-less the same level of pediocin ST18 production (3200 AU ml^{-1}). Incubation temperature, however, had a significant effect on the growth of the strain and production of pediocin ST18. At 30 °C and the pH of the culture not regulated, pediocin ST18 was produced at 3200 AU ml^{-1} within 24 h (data not shown).

Complete inactivation or significant reduction in antimicrobial activity was observed after treatment of the cell-free supernatant with protease IV and pronase E, confirming its proteinaceous nature. Treatment of the bacteriocin with catalase and α-amylase did not change its activity (Table 3), indicating that the inhibition recorded was not hydrogen peroxide and that carbohydrate moieties were not required for antimicrobial activity, respectively. No decrease in activity was recorded after treatment at 121 °C for 30 min or when incubated in the presence of SDS, Tween 20, Tween 80, urea, *N*-laurylsarcosin and Triton X-100 (Table 3). The effect of detergents on different bacteriocins provided some information about the structure of the peptides. Anionic detergents often unfold proteins by complexion of the hydrophobic core of their native structure, which may affect their three-dimensional conformation. The bacteriocins remained stable after incubation for 2 h at pH values between 2.0 and 12.0 (Table 3). Similar results were recorded by Schved et al. [11], Bhunia et al. [8] and Green et al. [12].

The antimicrobial effect of pediocin ST18 was observed by recording the cell density ($OD_{600\,nm}$) of *L. innocua* F over 9 h (Fig. 1). Addition of the bacteriocin-containing cell-free supernatant (3200 AU ml^{-1}) to logarithmic-phase cells of *L. innocua* F (3 h old) resulted in growth inhibition after 1 h, followed by complete growth inhibition for 2 h. A very slow increase in optical density was recorded 3 h after the addition of pediocin ST18, suggesting that *L. innocua* F became resistant (Fig. 1). Addition of the same activity level of pediocin ST18 (3200 AU ml^{-1}) to stationary-phase cells of *L. innocua* F resulted in no growth inhibition (data not shown),

Table 3
Factors affecting the antimicrobial activity of pediocin ST18

Treatment	Pediocin ST18[a]
Enzymes (0.1 mg ml^{-1})	
Protease IV	−
Pronase E	−
α-Amylase	+
Surfactants (1% final concentration)	
SDS	+
Tween 20	+
Tween 80	+
Urea	+
N-laurylsarcosin	+
Triton X-100	+
Protease inhibitors (0.1, 2.0, 5.0 mM)	
EDTA	+, +, +
PMSF	+, +, +
pH	
2.0–7.0	+
8.0–12.0	+
Temperature (°C) (30 min)	
30–100	+
121	+

[a] +, activity (inhibition zone); −, no activity.

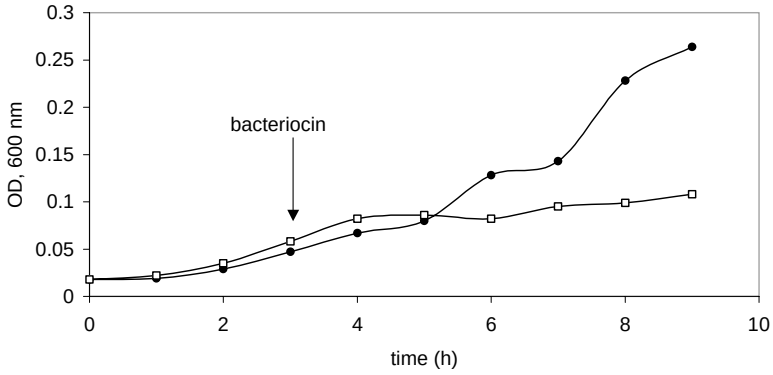

Fig. 1. Effect of pediocin ST18 on *L. innocua* F. *L. innocua* F was grown in Elliker broth at 30 °C without bacteriocin (●) and with 3200 AU/ml bacteriocin (□). The arrow indicates the point at which cell-free supernatant containing active pediocin ST18 was added to the culture.

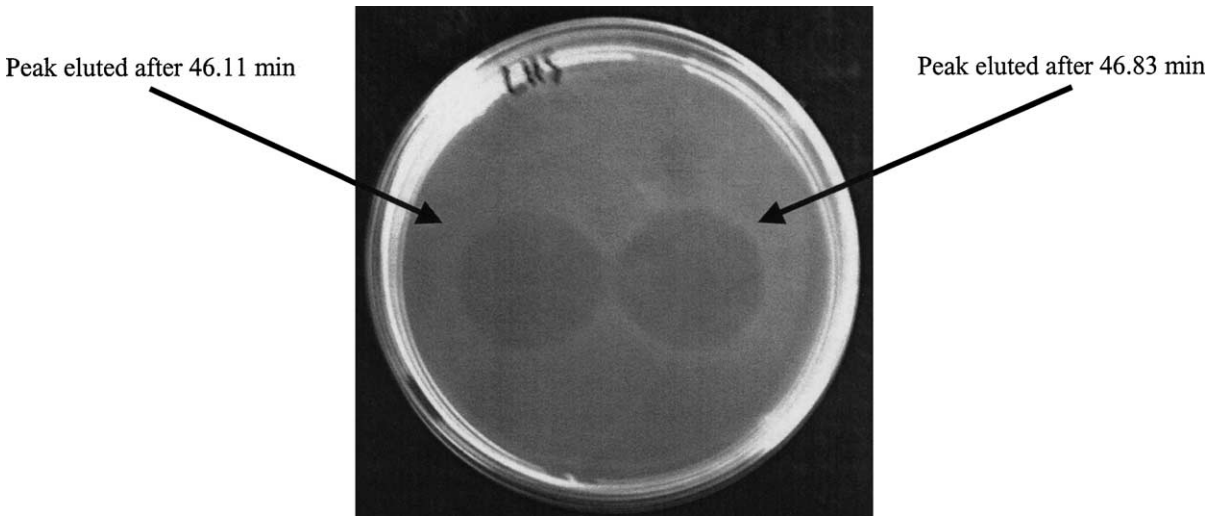

Fig. 2. Antimicrobial activity of two peptide peaks separated by reverse-phase HPLC. *L. plantarum* LAB 73 was used as sensitive strain.

suggesting that the peptide only affects metabolically active cells, or that the cell numbers were too high. Results obtained from the adsorption studies suggested that the bacteriocins did not adhere to the cell surface of the producer strain (data not shown).

Optimal production of pediocin ST18 was recorded after 24 h in MRS broth. Precipitation with ammonium sulfate resulted in an 80% recovery of pediocin ST18. Separation by Sep-Pack 18 and HPLC yielded two peaks with antimicrobial activity at 46.11 and 46.83 min (Fig. 2), suggesting that pediocin ST18 may be a two-peptide bacteriocin.

4. Conclusions

Pediocin ST18 is active against all strains of *Pediococcus* spp. included in this study and revealed good anti-listerial activity, including *Listeria monocytogenes*. From the 54 bacterial strains tested, 29 were sensitive to pediocin ST18. No activity was recorded against Gram-negative bacteria included in this study. This is the first report of a pediocin produced by *P. pentosaceus* isolated from boza. The amino acid sequence of pediocin ST18 is being determined.

Acknowledgements

This research was funded by the French Embassy (Service Culturel), in Sofia, Bulgaria.

References

[1] De Vuyst L, Vandamme EJ. Lactic acid bacteria and bacteriocins: their practical importance. In: De Vuyst L, Vandamme EJ, editors. Bacteriocins of lactic acid bacteria, microbiology, genetics and applications. London: Blackie, 1994. p. 1–12.
[2] Hancioglu O, Karapinar M. Microflora of boza, a traditional fermented Turkish beverage. Int J Food Microbiol 1997;35:271–4.
[3] Gotcheva V, Pandiella SS, Angelov A, Roshkova ZG, Webb C. Microflora identification of the Bulgarian cereal-based fermented beverage boza. Process Biochem 2000;36:127–30.
[4] Kabadjova P, Gotcheva I, Ivanova I, Dousset X. Investigation of bacteriocin activity of lactic acid bacteria isolated from boza. Biotechnol Biotech Equip 2000;14:56–9.

[5] Zorba M, Hancioglu O, Genc M, Karapinar M, Ova G. The use of starter culture in the fermentation of boza, a traditional Turkish beverage. Process Biochem 2003;38:1405–11.
[6] Etchells JL, Costilow RN, Anderson TE, Bell TA. Pure culture fermentation of brine cucumbers. Appl Microbiol 1964;12:523–35.
[7] Daeschel MA, Klaenhammer TR. Association of a 13.6 megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl Environ Microbiol 1985;50:1538–41.
[8] Bhunia AK, Kim WJ, Johnson MC, Ray B. Partial purification and characterization of antibacterial substances form *Pediococcus acidilactici* and *Lactobacillus plantarum*. In: Proceedings of the Annual Meeting, Program Abstract, Institute of Food Technologists, Las Vegas, 16–19 June 1987 (Abstract No. 143).
[9] Gonzalez CF, Kunka BS. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. Appl Environ Microbiol 1987;53:2534–8.
[10] Richter KS, Mutsapa A, Liewen MB, Hutkins RW. Properties of a bacteriocin produced by a *Pediococcus* sp. active against *Listeria monocytogenes*. In: Proceedings of the 89th Annual Meeting of the American Society for Microbiology, New Orleans, LA, 1989 (Abstract Book).
[11] Schved F, Lalazar Y, Henis Y, Juven BJ. Purification, partial characterization and plasmid-linkage of pediocin SJ-1, a bacteriocin produced by *Pediococcus acidilactici*. J Appl Bacteriol 1993;74:67–77.
[12] Green G, Dicks LMT, Bruggeman G, Vandamme EJ, Chikindas ML. Pediocin PD-1, a bactericidal antimicrobial peptide from *Pediococcus damnosus* NCFB 1832. J Appl Microbiol 1997;83:127–32.
[13] Hoover DG, Walsh PM, Kolaetis KM, Daly MM. A bacteriocin produced by *Pediococcus* species associated with a 5.5 Megadalton plasmid. J Food Prot 1988;51:29–31.
[14] Hoover DG, Dishert KJ, Hermes MA. Antagonistic effect of *Pediococcus* species against *Listeria monocytogenes*. Food Biotechnol 1989;3:183–9.
[15] Ennahar S, Aoude-Werner D, Sorokine O, Dorsselaer A, Bringel F, Hubert J-C, et al. Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. Appl Environ Microbiol 1996;62:4381–7.
[16] Horn N, Martinez MI, Martinez JM, Hernandez PE, Gasson MJ, Rodriguez JM, et al. Production of pediocin PA-1 by *Lactococcus lactis* using the lactococcin A secretory apparatus. Appl Environ Microbiol 1998;64:818–23.
[17] Müller T. Comparison of methods for differentiation between homofermentative and heterofermentative lactic acid bacteria. Zentralbl Microbiol 1990;145:363–6.
[18] Garver KI, Muriana M. Detection and characterization of bacteriocin producing lactic acid bacteria from retail food products. Int J Food Microbiol 1993;19:241–58.
[19] Atrih A, Rekhif N, Milliere JB, Lefebvre G. Detection and characterization of a bacteriocin produced by *Lactobacillus plantarum* C19. Can J Microbiol 1993;39:1173–9.
[20] Garvie EJ. Genus *Pediococcus*. In: Sneath PHA, Hold JG, editors. Bergeys's manual of systematic bacteriology. Baltimore: Williams and Wilkins Co., 1986. p. 1075–79.
[21] De Man JC, Rogosa M, Sharpe E. A medium for the cultivation of *Lactobacilli*. J Appl Bacteriol 1960;23:130–5.
[22] Schillinger U, Lücke FK. Antibacterial activity of *Lactobacillus sake* isolated from meat. J Appl Bacteriol 1989;70:473–8.
[23] Tagg JR, McGiven AR. Assay system for bacteriocins. Appl Microbiol 1971;21:943.
[24] Van Reenen CA, Dicks LMT, Chikindas ML. Isolation 423, a bacteriocin produced by *Lactobacillus plantarum*. J Appl Microbiol 1998;84:1131–7.
[25] Yang R, Johnson M, Ray B. A novel method to extract large amounts of bacteriocins from lactic acid bacteria. Appl Environ Microbiol 1992;58:3355–9.
[26] Elliker PR, Anderson AW, Hannesson G. An agar culture medium for lactic acid streptococci and lactobacilli. J Dairy Sci 1956;39:1611.