J Ind Microbiol Biotechnol (2004) 31: 323–329
DOI 10.1007/s10295-004-0153-6

ORIGINAL PAPER

Svetoslav D. Todorov · Leon M. T. Dicks

Characterization of mesentericin ST99, a bacteriocin produced by *Leuconostoc mesenteroides* subsp. *dextranicum* ST99 isolated from boza

Received: 13 January 2004 / Accepted: 2 June 2004 / Published online: 14 July 2004

Abstract Lactic acid bacteria isolated from Boza, a cereal-fermented beverage from Belogratchik, Bulgaria, were screened for the production of bacteriocins. With the first screening, 13 of the 52 isolates inhibited the growth of *Listeria innocua* and *Lactobacillus plantarum*. The cell-free supernatant of one of these strains, classified as *Leuconostoc mesenteroides* subsp. *dextranicum* ST99, inhibited the growth of *Bacillus subtilis*, *Enterococcus faecalis*, several *Lactobacillus* spp., *Lactococcus lactis* subsp. *cremoris*, *Listeria innocua*, *Listeria monocytogenes*, *Pediococcus pentosaceus*, *Staphylococcus aureus* and *Streptococcus thermophilus*. *Clostridium* spp., *Carnobacterium* spp., *L. mesenteroides* and Gram-negative bacteria were not inhibited. Maximum antimicrobial activity, i.e. 6,400 arbitrary units (AU)/ml, was recorded in MRS broth after 24 h at 30°C. Incubation in the presence of protease IV and pronase E resulted in loss of antimicrobial activity, confirming that growth inhibition was caused by a bacteriocin, designated here as mesentericin ST99. No loss in activity was recorded after treatment with α-amylase, SDS, Tween 20, Tween 80, urea, Triton X-100, *N*-laurylsarcosin, EDTA and phenylmethylsulfonylfluoride. Mesentericin ST99 remained active after 30 min at 121°C and after 2 h of incubation at pH 2 to 12. Metabolically active cells of *L. innocua* treated with mesentericin ST99 did not undergo lysis. Mesentericin ST99 did not adhere to the cell surface of strain ST99. Precipitation with ammonium sulfate (70% saturation), followed by Sep-Pack C_{18} chromatography and reverse-phase HPLC on a C_{18} Nucleosil column yielded one antimicrobial peptide.

S. D. Todorov · L. M. T. Dicks (✉)
Department of Microbiology,
University of Stellenbosch,
7600 Stellenbosch, South Africa
E-mail: lmtd@sun.ac.za
Tel.: +27-21-8085849
Fax: +27-21-8085846

S. D. Todorov
Magura Winery, JSCo, 3938 Rabisha,
Vidin region, Bulgaria

Keywords Mesentericin ST99 · Boza · *Leuconostoc mesenteroides* subsp. *dextranicum*

Introduction

Lactic acid bacteria are widely used as starter cultures and play an important role in food preservation, microbiological stability, and production of aroma compounds in various food products [5, 6, 20, 27, 31, 35]. Many of these lactic acid bacteria produce bacteriocins [20, 31]. By definition, bacteriocins are small proteins with bactericidal or bacteriostatic activity against genetically closely related species [19, 37].

Countries of the Balkan region (North Turkey, North Greece, Yugoslavia, Albania, Bosnia and Herzegovina, and Bulgaria) are well known for the production of food and beverages fermented with lactic acid bacteria. Boza is one such traditional drink, produced by the fermentation of different cereals with yeast and lactic acid bacteria. Only a few papers have been published on the microbial composition of Boza [12, 13, 17, 41]. Most of the lactic acid bacteria that have been isolated belong to the genera *Lactobacillus*, *Lactococcus* and *Leuconostoc*. As many as 33 strains isolated from Boza showed antibacterial activity against various Gram-positive bacteria, including *Listeria innocua*, and Gram-negative bacteria such as *Escherichia coli* [17]. A bacteriocin produced by *Lactococcus lactis* subsp. *lactis* 14 has been partially characterised [17]. As far as we could determine, nothing has been reported on bacteriocins produced by the *Leuconostoc* spp. that have been isolated from Boza. *Leuconostoc* spp. are present in fairly high cell numbers in Boza (10^3 cfu/ml) and play an important role in the aroma and flavour development of the product [12, 13]. It is thus important to determine if they produce bacteriocins that may be active against other leuconostocs and lactic acid bacteria normally present in Boza. Unlike dairy products, from which many leuconostocs have been isolated, Boza does not contain

lactose and has an overall different population of lactic acid bacteria, which favours the possibility of finding a leuconostoc bacteriocin with a different spectrum of antibacterial activity.

The first bacteriocin described for *Leuconostoc* was from *Leuconostoc gelidium* UAL 187, a strain isolated from meat, and was named leucocin A [14, 15]. Subsequently, a number of bacteriocins have been described for *Leuconostoc mesenteroides* subsp. *mesenteroides* [4, 16, 23, 24, 28, 29, 32], *L. carnosum* [3, 9, 11, 18, 30, 38], *Leuconsotoc* sp. [2], and *L. paramesenteroides* (now *Weissella paramenteroides*) [21]. This is the first report of a bacteriocin produced by *L. mesenteroides* isolated from Boza.

Materials and methods

Bacterial strains and growth media

Samples of Boza from Belogratchik, Bulgaria, were serially diluted, plated onto MRS agar [7] and incubated at 30°C. Colonies of different morphology were selected from plates and cultured in MRS broth [7]. The growth media used for indicator strains included in this study are listed in Table 1. All strains were incubated at 30°C. Pure cultures were stored at −80°C in MRS broth supplemented with 15% (v/v) glycerol.

Screening for bacteriocin activity

Strains isolated from Boza were grown in MRS broth for 24 h at 30°C, harvested by centrifugation (8,000 *g*, 10 min, 4°C) and the cell-free supernatant adjusted to pH 6.0 with sterile 1 *N* NaOH. Screening for bacteriocin activity was according to the agar spot test and the well diffusion methods, described by Schillinger and Lücke [33] and Tagg and McGiven [36], respectively. The strains included in the test panel are listed in Table 1. Antimicrobial activity was expressed in arbitrary units (AU) per millilitre; 1 AU was defined as the reciprocal of the highest serial 2-fold dilution showing a clear zone of growth inhibition of the indicator strain [39]. Strain

Table 1 Indicator strains, growth media and sensitivity to *Leuconostoc mesenteroides* subsp. *dextranicum* ST99 cell-free supernatant. Incubation was at 30°C. *ENITIAA* Ecole Nationale des Ingenieurs des Techniques Agricoles et Alimentaires, Nantes, France; *ATCC* American Type Culture Collection, Rockville, Md.; *NCDO* National Collection of Dairy Organisms, Reading, UK; *IP* Institut Pasteur, Paris, France; *SD PC* sourdough private collection; *LdC* Levain de Cracker, USA (Boll); *INRA-CNRZ* Centre National de Recherche Zootechnique, INRA, Jouy en Josas, France; *Elliker* [8]; *NB* nutrient broth, Biokar, Beauvais, France; *MRS* [7]; *RCM* reinforced clostridial medium, Biokar, Beauvais, France

Strain	Origin	Media	Activity[a]
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 curvatus 1307 and 1371	SD PC	MRS	−
Lactobacillus delbrueckii subsp. *bulgaricus* C	ENITIAA	MRS	−
Lactobacillus fermentum 1386	IP	MRS	−
Lactobacillus helveticus 1 and 2	ENITIAA	MRS	+
Lactobacillus plantarum 1383, 1408, 1409, 14917 and 73	ENITIAA	MRS	+
L. plantarum 1390 and 1397	LdC Boll	MRS	+
L. plantarum subsp. *pseudoplantarum* 1128 and 1131	SD PC	MRS	+
Lactobacillus sakei 1	ENITIAA	MRS	−
L. lactis subsp. *cremoris* 117	INRA CNRZ	MRS	+
L. mesenteroides 1 and 2	ENITIAA	MRS	−
L. mesenteroides 1000, 1044, 1228 and 1324	SD PC	MRS	−
L. mesenteroides 8293	ATCC	MRS	−
L. mesenteroides subsp. *dextranicum* 1055 and 1185	SD PC	MRS	−
L. mesenteroides subsp. *dextranicum* 1414	LdC	MRS	−
L. innocua F and V1	ENITIAA	Elliker	+
L. monocytogenes R ser. 4b	ENITIAA	Elliker	+
Pediococcus damnosus 1	ENITIAA	Elliker	−
Pediococcus pentosaceus 1164 and 1272	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 6,400 arbitrary units (AU)/ml

ST99, which had the broadest spectrum of antimicrobial activity, was selected for further studies.

Identification of strain ST99

Strain ST99 was subjected to physiological and biochemical tests, as described by Müller [25], Garver and Muriana [10] and Atrih et al. [1]. Sugar fermentation reactions were recorded using the API 50 CHL system (Biomérieux, Marcy-l'Etoile, France). Results obtained with the API identification system were compared to carbohydrate fermentation reactions listed in Bergey's manual of systematic bacteriology [34].

Bacteriocin production

The MRS broth, without Tween 80, was inoculated with an 8-h-old culture (2%, v/v) of strain ST99. Incubation was at 30°C, without agitation. Samples were taken at 1 h intervals to determine the optical density (at 600 nm) of the culture and the antimicrobial activity (AU/ml) of the bacteriocin produced.

Effect of enzymes, pH, detergents and temperature on bacteriocin activity

Strain ST99 was grown in MRS broth at 30°C for 24 h, the cells harvested by centrifugation (8,000 *g*, 10 min, 4°C), and the cell-free supernatant adjusted to pH 6.0. Samples of 500 µl were incubated for 2 h in the presence of 1 or 0.1 mg/ml (final concentration) protease IV (Sigma-Aldrich, France), pronase E (Sigma) and α-amylase (Sigma), and tested for antimicrobial activity using the agar spot test method.

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

The effect of pH on the bacteriocin was tested by adjusting cell-free supernatants to pH 2.0–12.0 (at increments of one pH unit) with sterile 1 *N* NaOH or 1 *N* HCl. After 30 min and 2 h of incubation at room temperature (25°C), the samples were re-adjusted to pH 6.5 with 1 *N* NaOH or 1 *N* HCl and tested for antimicrobial activity using the agar spot test method.

The effect of temperature on the activity of the bacteriocin was tested by heating the cell-free supernatant to 30, 40, 50, 60, 70, 80, 90, 100 and 121°C, respectively. Bacteriocin activity was tested after 5, 10, 15, 20 and 30 min at each of these temperatures. The agar spot test method was used.

Cell lysis

A 20 ml aliquot of bacteriocin-containing filter-sterilised and cell-free supernatant (pH 6.0) was added to a 100 ml-culture of *L. innocua* F in early exponential phase ($OD_{600} = 0.06$). The optical density of the culture was determined every hour for 9 h. The experiment was performed in triplicate.

Adsorption studies

Adsorption of the bacteriocin to the producer, strain ST99, was studied using the method described by Yang et al. [40]. 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, 4°C) and washed with sterile 0.1 M phosphate buffer (pH 6.5). The cells were resuspended in 10 ml 100 mM NaCl (pH 2.0), stirred for 1 h at 4°C and then harvested by centrifugation (20,000 *g*, 15 min, 4°C). The cell-free supernatant was neutralised to pH 7.0 with sterile 1 *N* NaOH and tested for activity as described above.

Bacteriocin purification

A 24-h-old culture of strain ST99 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 gradually added to the cell-free supernatant (70% 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, Bedford, Mass.). The column was washed with 20% (v/v) iso-propanol 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 C_{18} Nucleosil (Waters) column (250×4.6 mm). Elution was performed using TFA (0.1%) in water (eluent A) and TFA (0.1%) in acetonitrile (eluent B). A linear gradient from 0 to 100% B was applied over 65 min and kept at 100% B for 10 min. Polypeptides were detected with an in-line optical density reader at 280 nm. Fractions were collected, dried under vacuum, dissolved in 1 ml sterile de-ionised water and stored at −20°C. Activity was tested by using the agar spot test method. Fractions with the highest activity from the first separation were pooled and again separated by HPLC, using the same

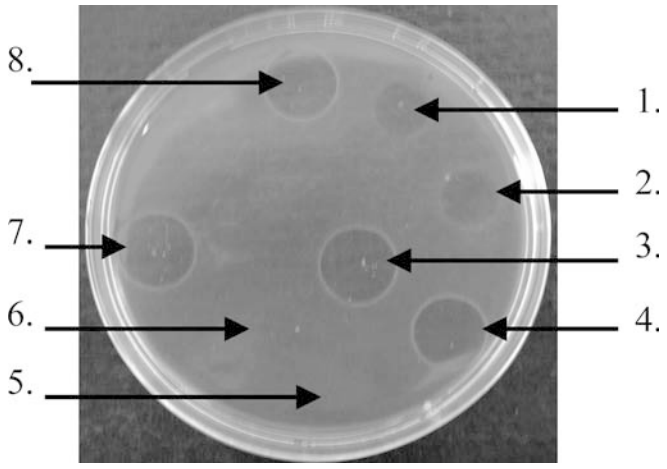

Fig. 1 Antimicrobial activity of mesentericin ST99. *1* Cell-free supernatant; *2* cell-free supernatant treated with α-amylase (0.1 mg/ml); *3*, *4* peak eluted at 46.96 min, separated by reverse phase HPLC; *5* cell-free supernatant treated with pronase E (0.1 mg/ml); *6* peak eluted at 46.96 min, treated with pronase E (0.1 mg/ml); *7*, *8* peak eluted at 46.96 min treated with α-amylase (0.1 mg/ml). *Lactobacillus plantarum* LAB 73 was used as the sensitive strain

conditions. The activity of the peak which eluted at 46.96 min is shown in Fig. 1.

Results and discussion

The population of lactic acid bacteria recorded in Boza was about 2×10^8 cfu/ml. A total of 52 colonies were selected from MRS agar plates based on differences in morphology. Only 13 isolates showed antibacterial activity against *L. innocua* F. No activity was recorded against the Gram-negative bacteria included in this study (Table 1). The bacteriocin produced by strain ST99 differs from mesentericin Y105, mesenterocin 52, and leucocins A, B, C and TA33a described for *L. mesenteroides* [16, 24, 29] in that it does not inhibit the growth of other *Leuconostoc* spp. Based on this characteristic, and its broad spectrum of activity (active against *Bacillus subtilis*, *Enterococcus faecalis*, several *Lactobacillus* spp., *Lactococcus lactis* subsp. *cremoris*, *Listeria innocua*, *Listeria monocytogenes*, *Pediococcus pentosaceus*, *Staphylococcus aureus* and *Streptococcus thermophilus*), the bacteriocin of strain ST99 was selected for further studies.

Strain ST99 is Gram-positive, catalase- and oxidase-negative. Cells in mid-log phase are coccoid, but somewhat elongated. Carbon dioxide is produced from the fermentation of glucose. Growth in MRS broth is viscous, which may be due to the formation of exopolysaccharides. Growth in MRS broth is optimal at 30°C, but slow at 16°C and usually only visible after 48 h. The final pH after 48 h at 30°C is about 4.6. No growth was observed at 45°C. Strain ST99 fermented L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, raffinose, gentiobiose, esculin, α-methyl-D-glucoside, *N*-acetyl-glucosamine, amygdalin, arbutin, gluconate and mannitol, but none of the other sugars in the API 50 CHL reaction test. Comparison of these carbohydrate fermentation reactions to the API 50 CHL databank revealed 99.9% homology to *L. mesenteroides* subsp. *dextranicum*, confirming its identification.

Growth of *L. mesenteroides* subsp. *dextranicum* ST99 under aerobic or anaerobic conditions resulted in production of more-or-less the same activity level of mesentericin ST99. Incubation temperature, however, had a significant effect on the growth of strain ST99 and production of mesentericin ST99. At 30°C, with the pH of the culture not regulated, mesentericin ST99 was produced at 6,400 AU/ml 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 (Fig. 1), confirming its proteinaceous nature. Treatment of the bacteriocin with catalase did not change its activity, indicating that the inhibition recorded was not hydrogen peroxide (Fig. 1). Incubation of mesentericin ST99 in the presence of α-amylase had no effect on the antibacterial activity recorded, suggesting that carbohydrates are not bound to the peptide (Fig. 1). In the

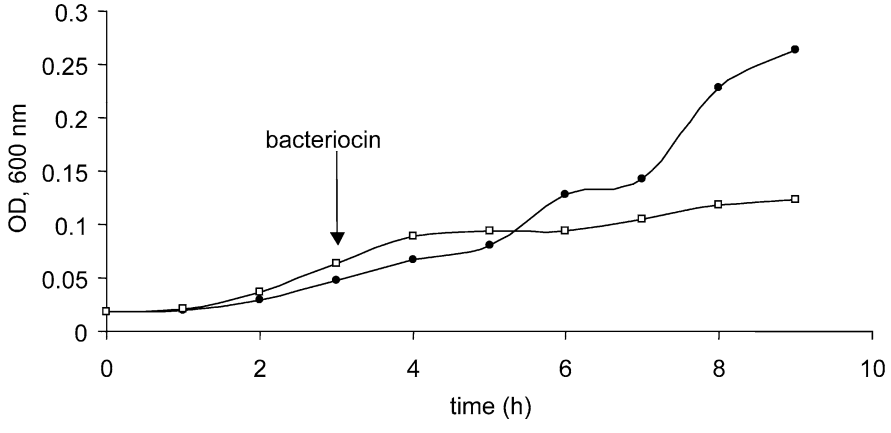

Fig. 2 Growth of *Listeria innocua* F in Elliker broth at 30°C in the absence of mesentericin ST99 (*filled circles*) and in the presence of 6,400 AU/ml mesentericin ST99 (*open squares*). *Arrow* Addition of cell-free supernatant containing the active bacteriocin

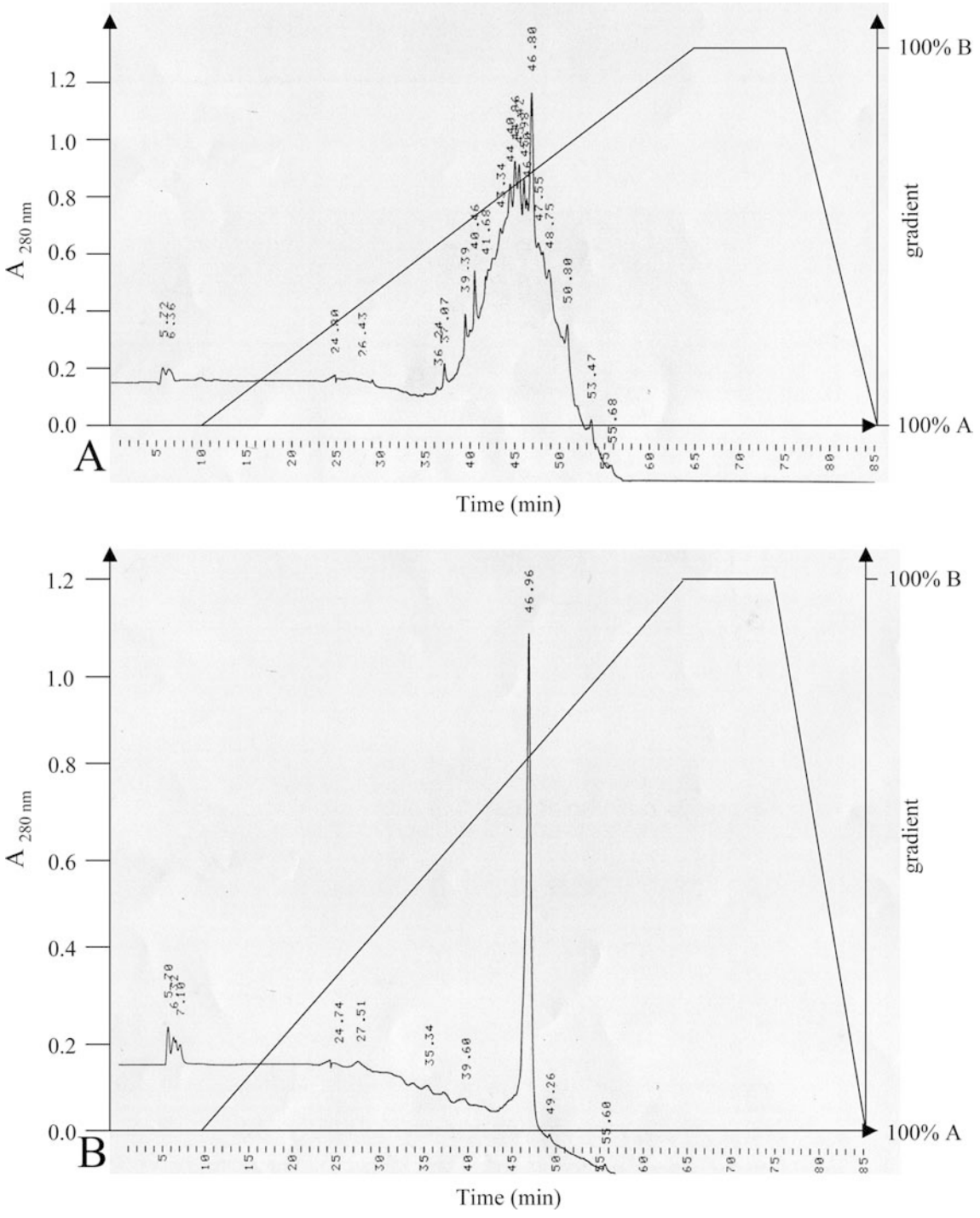

Fig. 3a, b Separation of mesentericin ST99 by reverse-phase HPLC on a C18 Nucleosil column (250×4.6 mm). The eluents used were trifluoroacetic acid (TFA; 0.1%) in water (eluent A) and TFA (0.1%) in acetonitrile (eluent B). A gradient from 0 to 100% B was applied over 65 min and kept at 100% B for 10 min. Active fractions from the first separation (**a**) were pooled and re-injected, which produced a single active peak at a retention time of 46.96 min (**b**)

case of leuconocin S [22] and carnocin 54 [18], treatment with α-amylase results in lower activity levels, suggesting that these peptides are linked to essential carbohydrates. No decrease in mesentericin ST99 activity was recorded when incubated in the presence of SDS, Tween 20, Tween 80, urea, *N*-laurylsarcosin, Triton X-100, EDTA or PMSF. Mesentericin ST99 remained stable after incubation for 2 h at pH values between 2.0 and 12.0. Similar results were recorded for leucocin F10 by Parente et al. [30].

Like most bacteriocins, including those produced by *Leuconostoc* strains [18, 24, 26], mesentericin ST99 is extremely heat tolerant (remained active after 30 min at 121°C, at pH 4.6).

Addition of mesentericin ST99 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 (Fig. 2). A slow increase in optical density was recorded 3 h after the addition of mesentericin ST99, suggesting that *L. innocua* F became resistant to the bacteriocin (Fig. 2). Addition of 6,400 AU/ml of mesentericin ST99 to stationary-phase cells of *L. innocua* F resulted in no inhibition (data not shown), suggesting that the cells became resistant to the bacteriocin. The data recorded for the inhibition of *L. innocua* by mesentericin ST99 represents an average of three repeats and did not vary by more than 5%. Single data points are, therefore presented in Fig. 2 without standard deviation bars.

No mesentericin ST99 activity was recorded after treating the cells with NaCl at low pH (data not shown), suggesting that the bacteriocin did not adhere to the cell surface.

Precipitation with ammonium sulfate resulted in a 70% recovery of mesentericin ST99. The first separation by HPLC yielded active fractions with a retention time of between 45 and 50 min (Fig. 3a). When these fractions were pooled and re-injected under the same conditions, a single active peak with a retention time of 46.96 min was produced (Fig. 3b). This suggests that mesentericin ST99 may be a single-peptide bacteriocin.

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

References

1. Atrih A, Rekhif N, Milliere JB, Lefebvre G (1993) Detection and characterization of a bacteriocin produced by *Lactobacillus plantarum* C19. Can J Microbiol 39:1173–1179
2. Blom H, Katla T, Holck A, Sletten K, Axelsson L, Holo H (1999) Characterization, production and purification of leucocin H, a two-peptide bacteriocin from *Leuconostoc* MF215B. Curr Microbiol 39:43–48
3. Budde BB, Horubaek T, Jacobsen T, Barkholt V, Koch AG (2003) *Leuconostoc carnosum* 4010 has the potential for use as a protective culture for vacuum-packed meats: culture isolation, bacteriocin identification, and meat application experiments. Int J Food Microbiol 83:171–184
4. Daba H, Lacroix C, Huang J, Simard RE (1993) Influence of growth conditions on production and activity of mesenterocin 5 by a strain of *Leuconostoc mesenteroides*. Appl Microbiol Biotechnol 39:166–173
5. Degnan AJ, Yousef AE, Luchansky JB (1992) Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged Wieners. J Food Protect 55:98–103
6. Delves-Broughton J (1990) Nisin and its uses of the DGHM preservative. Food Technol 44:100–117
7. De Man JC, Rogosa M, Sharpe E (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23:130–135
8. Elliker PR, Anderson AW, Hannesson G (1956) An agar culture medium for lactic acid streptococci and lactobacilli. J Dairy Sci 39:1611
9. Felix JV, Papathanasopoulos MA, Smith AA, Von Holy A, Hastings JW (1994) Characterization of leucocin B-Ta11a: a bacteriocin from *Leuconostoc carnosum* Ta11a isolated from meat. Curr Microbiol 29:207–212
10. Garver KI, Muriana M (1993) Detection and characterization of bacteriocin producing lactic acid bacteria from retail food products. Int J Food Microbiol 19:241–258
11. Geisen R, Becker B, Holzapfel WH (1993) Bacteriocin production of *Leuconostoc carnosum* LA54A at different combinations of pH and temperature. J Ind Microbiol 12:337–340
12. Gotcheva V, Pandiella SS, Angelov A, Roshkova ZG, Webb C (2000) Microflora identification of the Bulgarian cereal-based fermented beverage boza. Process Biochem 36:127–130
13. Hancioglu O, Karapinar M (1997) Microflora of Boza, a traditional fermented Turkish beverage. Int J Food Microbiol 35:271–274
14. Harding CD, Shaw BG (1990) Antimicrobial activity of *Leuconostoc gelidium* against related species and *Listeria monocytogenes*. J Appl Bacteriol 69:648–654
15. Hasting JW, Sailer M, Johnson K, Roy KL, Vederas JC, Stiles ME (1991) Characterization of leucocin AUAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidium*. J Bacteriol 173:7491–7500
16. Hechard Y, Derijard B, Letellier F, Cenatiempo Y (1992) Characterization and purification of mesenterocin Y105, an anti-Listeria bacteriocin from *Leuconostoc mesenteroides*. J Gen Microbiol 138:2725–2731
17. Kabadjova P, Gotcheva I, Ivanova I, Dousset X (2000) Investigation of bacteriocin activity of lactic acid bacteria isolated from boza. Biotechnol Biotechnol Eq 14:56–59
18. Keppler K, Geisen R, Holzapfel WH (1994) An α-amylase sensitive bacteriocin of *Leuconostoc carnosum*. Food Microbiol 11:39–45
19. Klaenhammer TR (1988) Bacteriocins of lactic acid bacteria. Biochimie 70:337–349
20. Klaenhammer TR (1993) Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev 12:39–86
21. Lewus CB, Kaiser A, Montville TJ (1991) Inhibition of foodborne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl Environ Microbiol 57:1683–1688
22. Lewus CB, Sun S, Montville TJ (1992) Production of an α-amylase bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. Appl Environ Microbiol 58:143–149
23. Mataragas M, Metaxopoulos J, Galiotou M, Drosinos EH (2003) Influence of pH and temperature on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442. Meat Sci 64:265–271
24. Mathieu F, Sudirman Suwandhi I, Rekhif N, Milliere JB, Lefebvre G (1992) Mesenterocin 52, a bacteriocin produced by *Leuconostoc mesenteroides* ssp. *mesenteroides* FR52. J Appl Bacteriol 74:372–349
25. Müller T (1990) Comparison of methods for differentiation between homofermentative and heterofermentative lactic acid bacteria. Zentralbl Microbiol 145:363–366
26. Nettles CG, Barefoot SF (1993) Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. J Food Prot 56:338–356
27. Nielsen JW, Dickson JS, Crouse JD (1990) Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. Appl Environ Microbiol 56:2142–2145
28. Papathanasopoulos MA, Krier F, Revol Junelles AM, Lefebvre G, Caer JP, Von Holy A, Hastings JW (1997) Multiple bacteriocin production by *Leuconostoc mesenteroides* TA33a and other *Leuconostoc*/*Weissella* strains. Curr Microbiol 35:331–335
29. Papathanasopoulos MA, Dykes GA, Revol Junelles AM, Delfour A, Von Holy A, Hastings JW (1998) Sequence and structural relationships of leucocins A-, B- and C-TA33a from *Leuconostoc mesenteroides* TA33a. Microbiology 144:1343–1348
30. Parente E, Moles M, Ricciardi A (1996) Leucocin F10, a bacteriocin from *Leuconostoc carnosum*. Int J Food Microbiol 33:231–243

31. Requena T, Pelàez C (1995) Actividad antimicrobiana de bacterias lacticas. Produccion de bacteriocinas. Rev Esp Cienc Tecnol Aliment 35:19–44
32. Revol Junelles AM, Mathis R, Krier F, Fleury Y, Delfour A, Lefebvre G (1996) *Leuconostoc mesenteroides* subsp. *mesenteroides* FR52 synthesizes two distinct bacteriocins. Lett Appl Microbiol 23:120–124
33. Schillinger U, Lücke FK (1989) Antibacterial activity of *Lactobacillus sake* isolated from meat. J Appl Bacteriol 70:473–478
34. Sneath PHA, Mair NS, Sharpe ME, Holt JG (1986) In: Sneath PHA, Hold JG (eds) Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore, pp 1071–1075
35. Stiles ME, Hastings JW (1991) Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. Trends Food Sci Technol 2:247–251
36. Tagg JR, McGiven AR (1971) Assay system for bacteriocins. Appl Microbiol 21:943
37. Tagg JR, Dajani AS, Wannamaker LW (1976) Bacteriocins of Gram-positive bacteria. Bacteriol Rev 40:722–756
38. Van Laack RL, Schillinger U, Holzapfel WH (1992) Characterization and partial purification of a bacteriocin produced by *Leuconostoc carnosum* LA44A. Int J Food Microbiol 16:183–195
39. Van Reenen CA, Dicks LMT, Chikindas ML (1998) Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum*. J Appl Microbiol 84:1131–1137
40. Yang R, Johnson M, Ray B (1992) A novel method to extract large amounts of bacteriocins from lactic acid bacteria. Appl Environ Microbiol 58:3355–3359
41. Zorba M, Hancioglu O, Genc M, Karapinar M, Ova G (2003) The use of starter culture in the fermentation of boza, a traditional Turkish beverage. Process Biochem 38:1405–1411