Journal of Industrial Microbiology & Biotechnology (1997) 19, 28–33

Detection and partial characterization of a bacteriocin produced by *Carnobacterium piscicola* 213

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BLIS 213, is a bacteriocin-like inhibitory substance produced by *Carnobacterium piscicola* 213. It is active against *Carnobacterium, Enterococcus* and *Listeria* spp. No activity was observed against tested *Lactobacillus, Lactococcus, Leuconostoc* and *Pediococcus* strains, nor against Gram-negative bacteria. The BLIS 213 activity was inactivated by several proteolytic enzymes. It was heat resistant (121°C for 20 min), and stable over a pH range of 2–8. Activity was determined by a dilution micromethod; it was increased after SDS treatment. A mutant strain which lacks bacteriocin production was isolated and designated as *Carnobacterium piscicola* 213a. It had the same phenotypic and biochemical properties as the parent strain, and was not sensitive to bacteriocin activity. The apparent molecular weight of the bacteriocin in the crude extract was greater than 10 kDa. It was about 6 kDa after SDS-PAGE of a partially purified bacteriocin by adsorption on producer cells. The isoelectric point of the BLIS 213 was around 9.3.

Keywords: lactic acid bacteria; Carnobacteria; bacteriocins

Introduction

Lactic acid bacteria (LAB) are known to produce several antibacterial compounds including organic acids, hydrogen peroxide, and bacteriocins [18]. LAB are predominant in fermented foods and in vacuum-packed meat [1]. A new genus, *Carnobacterium* [8], contains some species of non aciduric lactobacilli which are unable to grow on acetate agar. This genus includes *Carnobacterium piscicola*, *C. divergens*, *C. mobile* and *C. gallinarum*, especially isolated from poultry, meat and fish. Some species in the genus *Carnobacterium* have been shown to produce bacteriocins [2,12,16,22]. Bacteriocins are proteinaceous compounds, active generally against closely related species [24], including some pathogenic and food spoilage bacteria. These bacteriocins may be of interest in food preservation. In the last few years a number of new bacteriocins have been identified and characterized from the lactic acid bacteria. They are classified into four classes [15]: class I includes small ribosomally synthesized polypeptides, containing unusual amino acids (lanthionine and 3-methyl-lanthionine), which are called lantibiotics. The most well known is nisin which is produced by *Lactococcus lactis* subsp *lactis* strains. Carnocin U149 is a lantibiotic from *C. piscicola* U149 [23]. Class II is composed of small heat-stable, hydrophobic peptides with a high isoelectric pH. Carnobacteriocins BM1 and B2 are class II bacteriocins from *C. piscicola* LV17B strain [19]. Class II contains large (>30 kDa), heat-labile proteins and class IV includes complex bacteriocins.

In this study, many Carnobacteria strains have been screened for bacteriocin production (unpublished results), and we report in this paper the detection and properties of a bacteriocin-like inhibitory substance produced by *Carnobacterium piscicola* 213 (or BLIS 213).

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Received 21 January 1997; accepted 25 April 1997

Materials and methods

Bacterial strains and media

The producer strain *Carnobacterium piscicola* 213 was isolated from meat by Montel *et al* [17]. *C. piscicola* LMG 9839 was the indicator strain. Origins of all strains used in this study are listed in Table 1. Production of BLIS 213 by *C. piscicola* 213 was carried out on MRS* medium (modified MRS broth prepared without ammonium citrate or sodium acetate) containing 5 g L^{-1} glucose, at 25°C. Carnobacteria strains used as indicators were propagated in APT broth (Difco, Detroit, MI, USA) at 30°C. *Lactobacillus, Lactococcus, Leuconostoc* and *Pediococcus* strains were propagated in MRS broth (Merk Belgolabo, Overijse, Belgium) at 30°C. Strains of *Listeria, Enterococcus, Streptococcus, Staphylococcus*, and *Pseudomonas* were grown in TSB (Difco), and *Escherichia coli* in LB medium (Difco). All strains were maintained as frozen stocks at −80°C in 40% glycerol. Agar media were prepared by adding 1.5% agar (w/v) to broth media. Soft agar was prepared with 0.75% of agar.

Preparation of the crude extract for antagonism assay and bacteriocin characterization

C. piscicola 213 was inoculated at 1% (v/v) into sterile MRS* broth at 25°C. After 24 h of growth, 500 ml of cell-free supernatant was adjusted to pH 6.5 with 2 N NaOH, filtred and concentrated 10-fold through a Diaflo hollow fibre cartridge with a 10-kDa cut-off (Amicon, Beverly, USA). The concentrated supernatant fluid was filter sterilized and stored at −20°C for further utilisation.

Table 1 Inhibitory spectrum of the bacteriocin produced by *C. piscicola* 213

Indicator strains	Source[a]	Sensitivity to the supernatant from *C. piscicola* 213[b]
Gram-positive strains		
Lactobacillus plantarum 6907	LMG	–
L. acidophilus 9433	LMG	–
L. curvatus 9188	LMG	–
L. brevis 7944	LMG	–
L. fermentum 6902	LMG	–
L. sake 9468	LMG	–
L. lactis ssp *cremoris* 6897	LMG	–
L. lactis ssp *lactis* 6890	LMG	–
Leuconostoc lactis 8894	LMG	–
Pediococcus acidilactici 11384	LMG	–
Carnobacterium piscicola 15	INRA	–
C. piscicola 213	INRA	–
C. piscicola 9839[C]	LMG	+
C. divergens 210	INRA	–
C. divergens 211	INRA	+
C. divergens 327	INRA	+
C. divergens 329	INRA	–
C. divergens 9129	LMG	+
C. gallinarum 9841	LMG	–
Enterococcus faecalis 29212	ATCC	+
Streptococcus pneumoniae 49619	ATCC	–
Staphylococcus aureus 6538	ATCC	–
S. aureus 25923	ATCC	–
Listeria monocytogenes 10357	Colindale (NCTC)	+
L. monocytogenes 5348	Colindale (NCTC)	+
L. monocytogenes 5105	Colindale (NCTC)	+
L. monocytogenes 5214	Colindale (NCTC)	+
L. monocytogenes 10225	Colindale	±
L. innocua 33090	ATCC	±
Gram-negative strains		
Pseudomonas aeruginosa 9027	ATCC	–
P. aeruginosa 27853	ATCC	–
E. coli 25923	ATCC	–

[a]LMG, Laboratory of Microbiology, University Gent, Belgium; INRA, Institut National de la Recherche Agronomique, France; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures, UK.
[b]Inhibition zones by an agar well diffusion assay. Symbols: –, non sensitive; ±, weakly sensitive; +, strongly sensitive.
[c] = ATCC 35586.

Detection of the bacteriocin activity

Agar well diffusion assay, and 'spot-on-lawn' antagonism were used for detection of the bacteriocin activity [24]. Plates were seeded with soft agar containing 0.1 ml of an overnight culture of the indicator strain. A 10-μl aliquot of the checked extract was added to wells previously cut in the agar plates, or directly on the lawn. The plates were then allowed to diffuse at 4°C for 2 h and incubated overnight at 30°C. The plates were inspected for inhibition zones.

Bacteriocin activity assay

Activity of the bacteriocin was assayed by a modified dilution micromethod in microtitre plates [9,11]. Two-fold dilutions of the bacteriocin samples were prepared in 160-μl volumes of APT broth in 96-well plates (Becton Dickinson Benelux SA, Aalst, Belgium). Wells were inoculated with 50 μl of 10^4-fold-diluted overnight culture of the sensitive strain *C. piscicola* LMG 9839 (10^4 cells). Microplates were incubated at 30°C for 16 h. The bacteriocin titre was defined as the reciprocal of the highest dilution which did not give a visible growth of the test organism in 160 μl and was expressed in activity units per ml (AU ml^{-1}).

Effect of enzymes, heat treatment, pH, and dissociating agents on bacteriocin activity

Enzymes used in our tests and their sources are listed in Table 2. Proteolytic or non proteolytic enzymes were added to the concentrated supernatant at a final concentration of 1 mg ml^{-1}. To avoid possible inhibition by hydrogen peroxide, catalase was used at a final concentration of 68 U ml^{-1}. Controls consisted of samples of enzymes in sterile medium, and untreated bacteriocin solution. All preparations were incubated at 37°C for 90 min, except for catalase and trypsin for which the incubation temperature was 25°C. Enzymes were heat-inactivated (100°C for 10 min) and samples were tested on a lawn of *Carnobacterium piscicola* LMG 9839. After overnight incubation at 30°C, plates were checked for results. Stability of the BLIS 213 to heat was checked by heating the concentrated preparation at 70°C for 30 min, 100°C for 10 and 30 min, and 121°C for 20 min. Effect of pH on bacteriocin activity was determined by mixing a bacteriocin sample (800 AU ml^{-1}) with 50 mM sodium citrate (pH 2–5.5) or 50 mM sodium phosphate (pH 6–11). Bacteriocin assays were compared to the blanks done in the same conditions without addition of the bacteriocin. Activity was determined in different samples after storage for 30 and 60 min at 4°C. Sensitivity of the bacteriocin activity to detergents was determined as follows: 1% of Tween 80, Tween 20, Triton X-100, β-mercaptoethanol

Table 2 Effect of enzymes and heat treatment on the activity of cell supernatant from *C. piscicola* 213

Treatment	Activity[a]
Not treated	+
Catalase (Sigma)	+
Pronase (Boehringer)	–
Pepsin (Boehringer)	–
Papain (Sigma)	–
Subtilisin (Sigma)	–
Lipase (Sigma)	+
Lyzozyme (Sigma)	+
Trypsine (Boehringer)	–
70°C for 30 min	+
100°C for 10 min	+
100°C for 30 min	+
121°C for 20 min	+

[a]Activity was determined against *C. piscicola* LMG 9839 by the spot-on-lawn assay. Symbols: –, no inhibition zone; +, inhibition zone. Final concentration of enzymes was 1 mg ml^{-1} for proteases and 68 U ml^{-1} for catalase.

and SDS (sodium dodecyl sulfate) were added to crude bacteriocin solution (1600 AU ml^{-1}). The action of 1 mM DTT (dithiothreitol) and 8 M urea was also tested. Bacteriocin or detergents in sterile MRS* broth were used as controls. All samples and controls were incubated at 30°C for 4 h and titrated for the BLIS 213 activity.

Selection of mutant strains lacking bacteriocin production by plasmid-curing experiments

The deferred antagonism procedure was used for mutant detection [3]. *C. piscicola* 213 cells were grown to exponential phase in APT broth at 30°C. This served as inoculum for several tubes of APT broth containing different concentrations of acriflavine (10, 15, 20, 25, and 30 μg ml^{-1}). After three consecutive transfers every 24 h, cultures were serially diluted and surface plated on MRS* agar to obtain uncrowded colonies. Plates were incubated at 30°C for 24 h in anaerobic conditions using a Gazpak 150 System (BBL, Becton Dickinson). A lawn of the indicator bacteria *C. piscicola* LMG 9839 was poured on the survivor colonies. After overnight development of the lawn at 30°C, mutant strains were detected by the absence of the surrounding inhibition zone.

Carbohydrate fermentations of the selected mutant strain were compared to the parent strain by using the API 50 CH gallery and CHL medium designated for lactic acid bacteria according to the manufacturer's manual (bioMérieux, Marcy-l'Etoile, France). In this experiment *C. piscicola* LMG 9839 was used as reference.

Molecular weight and pI determination of the BLIS 213

Recovery of the bacteriocin adsorbed to bacterial cells: The method of Yang *et al* [25] was followed to yield a partially purified bacteriocin. Cells from an overnight culture (12–14 h) of *C. piscicola* 213 and its derivative bacteriocin-deficient strain grown in 1 L of MRS* at 25°C, were heated at 70°C for 30 min to kill cells and inactivate proteases. The culture was adjusted to pH 7 with 4 M NaOH. After 30 min of gentle stirring at room temperature, cells were harvested by centrifugation (9000 × *g* for 25 min). A sample of the supernatant fluid was taken to estimate the quantity of the bacteriocin not adsorbed to the cells. The cell pellets were washed twice in 5 mM sodium phosphate buffer (pH 7), and resuspended in 100 mM NaCl adjusted to pH 2.5 with 5% phosphoric acid. After mixing with a magnetic stirrer for 2 h at 4°C, cell suspension was centrifuged at 4°C (15 000 × *g* for 30 min), and the supernatant was dialysed overnight at 4°C in dialysis bags of 2-kDa cut-off (Spectra/Por CE) against deionized water (dH_2O). Five hundred microlitres of cell pellets were resuspended in 10 ml of 5 mM sodium phosphate buffer (pH 7) to estimate the quantity of bacteriocin still associated to the cells. Dialysed samples collected in each stage of the experiment were assayed for bacteriocin activity. Proteins desorbed from the producer and the mutant strain were freeze-dried in fractions and stored at –20°C for electrophoresis studies.

SDS-PAGE and isoelectric focusing (IEF): Samples of the freeze-dried preparations from the producer and mutant cells were dissolved in dH_2O, and analyzed by SDS-PAGE and IEF. Tricine-SDS-polyacrylamide gel electrophoresis (PAGE) as described by Schägger and Jagow [21] was used to determine the molecular weight of the bacteriocin on vertical slab gels (Bio-Rad Laboratories, Nazareth Eke, Belgium). Gels consisted of a 16.5% separating gel and a 4% stacking gel. Molecular size standards were MW-SDS-17S (Sigma-Aldrich, Bornem, Belgium). The bacteriocin band was detected directly on the SDS-PAGE [7]. Isoelectric focusing (IEF) was conducted on Phast system as recommended by the manufacturer (Phamacia LKB, Uppsala, Sweden). Phast gel IEF 3–9 and a calibration kit of pH range 3.5–9.3 were used. For detection of the bacteriocin band in the two electrophoresis systems, samples were applied onto gels in duplicate, and one half of each gel was stained with Coomassie brilliant blue, the other half was slightly coloured (in the case of SDS-PAGE only) and used to detect the bacteriocin band by growth inhibition of *C. piscicola* LMG 9839.

Results

Spectrum of the inhibitory activity

In order to test bacteriocin activity, concentrated cell-free supernatant from *C. piscicola* 213 was spotted on lawns containing different Gram-positive and Gram-negative bacteria (Table 1). The producer strain was not sensitive to the concentrated supernatant fluid. The antibacterial activity was effective against different Carnobacteria strains and *Listeria monocytogenes* strains. *Listeria monocytogenes* 10225 and the tested *Listeria innocua* strains were less sensitive. Other Gram-positive bacteria including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus* were not inhibited under the same conditions. No inhibition was observed when Gram-negative strains *Pseudomonas aeroginosa* and *E. coli* were tested. The inhibition was not due to the presence of the hydrogen peroxide nor to the low pH because the supernatant fluid was adjusted to pH 6.5 and concentrated by ultrafiltration.

Effect of enzymes, heat treatment, and pH on the BLIS 213 activity

Analysis for bacteriocin sensitivity to different enzymes and to heat was tested by the agar well diffusion or spot-on-lawn assays (Table 2). BLIS 213 activity was completely abolished by protease treatment, (pronase, pepsin, subtilisin (protease type VIII), trypsine and papaine). The activity was not affected by enzymes such as catalase, lipase and lyzozyme. Activity of the bacteriocin solution was maintained after heat treatment at 70°C for 30 min, 100°C for 10 min and 30 min and after sterilization (121°C for 20 min). These results demonstrated that the inhibitory compound produced by *C. piscicola* 213 is a heat-stable proteinaceous compound. The BLIS 213 activity was determined at different pHs at 4°C after 30 and 60 min incubating times. Activity seemed to be stable over a pH range of 2–8. Fifty percent of the activity was lost at a pH higher than 8. At pH 11, activity was completely lost.

Effect of dissociating agents on the bacteriocin activity

Testing the effect of dissociating agents on the activity of the BLIS 213 could inform us about the presence of bacteriocin aggregates in the active solution. As shown in Table 3, BLIS 213 activity was increased after treatment with 1% of anionic detergent SDS. SDS agent control had an inhibitory effect value of 400 AU ml^{-1} on the sensitive microorganism, so SDS resulted in a 75% increase of the initial bacteriocin activity. None of the other reagents resulted in an increase of the bacteriocin activity. In the case of the 8 M urea treatment, the bacteriocin titre remained constant. On the other hand, 8 M urea alone had a negligible inhibitory effect on the indicator, which was equivalent to 12.5 AU ml^{-1}.

Selection of a mutant lacking bacteriocin production

After treatment with acriflavine from 10 to 30 μg ml^{-1}, 243 colonies of *C. piscicola* 213 were screened for deficiency in bacteriocin production. One clone, from the culture treated with 10 μg ml^{-1} acriflavine, was unable to form an inhibition zone on *C. piscicola* LMG 9839 lawn. The Bac^- isolate was named *Carnobacterium piscicola* 213a. The same growth characteristics were observed for the producer parent strain and its mutant derivative at 25°C on an MRS* medium of pH 6.5. *C. piscicola* 213a was still immune towards the BLIS 213. As shown in Table 4, *C. piscicola* 213 and *C. piscicola* 213a have the same carbohydrate fermentation pattern. Results showed that *C. piscicola* LMG 9839 had small differences with the two compared strains, *C. piscicola* 213 and 213a. The latter produced acids from glycerol but not from galactose, while the reference strain had a weak acidification on both glycerol- and galactose-containing medium. The three strains are mannitol (+) and inulin (–).

Bacteriocin molecular weight estimation by SDS-PAGE and IEF analysis

Partial purification of the bacteriocin from cells:

Amounts of the bacteriocin prepared during the adsorbtion experience were calculated. After adsorbtion on *C. piscicola* 213 cells, 2% of bacteriocin activity was still present in the free supernatant. Desorbtion from cells yielded 1.6% of bacteriocin activity while 96.4% was still associated to the cells.

Table 3 Effect of dissociating agents on the BLIS 213 activity and on the sensitive microorganism

Dissociating agent	Activity (AU ml^{-1})	
	Treated BLIS 213	Dissociating agent control[a]
None	1600	–
Non ionic detergent[b]		
Tween 20	1600	0
Tween 80	1600	0
Triton X-100	1600	0
Anionic detergent		
SDS	3200	400
1 mM DTT	1600	0
8 M Urea	1600	12.5
1% β-mercaptoethanol	1600	0

[a]Inhibitory effect was measured as equivalent to AU ml^{-1}.
[b]All detergents were tested at a final concentration of 1%.

Table 4 Comparison of carbohydrate fermentation of the mutant strain, *C. piscicola* 213a, with those of the parent strain, *C. piscicola* 213, and *C. piscicola* LMG 9839 (ATCC 35586)

Strain	*C. piscicola* 213a	*C. piscicola* 213	*C. piscicola* LMG 9839
Acid produced from[a]			
Galactose	–	–	(+)
Glycerol	+	+	(+)
Lactose	(+)	(+)	(+)
Mannitol	+	+	+
Inulin	–	–	–

[a]Acid from carbohydrate was determined by using the API 50CH system. Results were recorded after 72 h of incubation at 30°C. +, Positive reaction; –, negative reaction; (+), weak positive reaction. All strains produced acid from D-glucose, D-fructose, D-mannose, ribose, maltose, sucrose, trehalose, β-gentiobiose, amygdalin, arbutin, esculin, salicin, cellobiose and *N*-acetylglucosamine. No strain fermented D-arabinose, erythritol, D- or L-xylose, β-methylxyloside, melibiose, melizitose, sorbose, rhamnose, inositol, dulcitol, μ-methyl-D-mannoside, μ-methyl-D-glucoside, D-raffinose, starch, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate or 2- or 5-ketogluconate.

Protein analysis by electrophoresis:

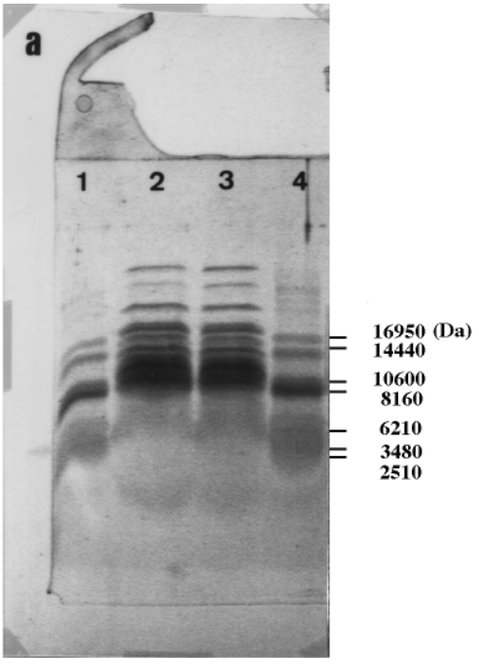
Electrophoresis of the desorbed solutions showed the same bands for the two preparations from the parent and mutant strains (Figure 1a). A growth inhibition zone was observed only in the case of proteins from producer cells which corresponded to a low molecular weight band estimated at 6 kDa (Figure 1b). In IEF analysis the active band was localized in a high pH range. The isoelectric point was around 9.3.

Discussion

Carnobacterium piscicola 213 produces an antagonistic substance. Loss of activity after protease treatment demonstrated its proteinaceous nature. The inhibitory spectrum included closely related species (Carnobacteria), some *Listeria* spp strains and an *Enterococcus* strain as observed previously for many bacteriocins from Carnobacteria strains [2,16]. Unlike carnocin U149 [22] and bacteriocins from *C. piscicola* LV17 [2], the BLIS 213 did not inhibit any strains of *Lactobacillus* spp. The activity was not affected by lipase, so lipidic moieties were not implicated in biological activity.

The BLIS 213 was heat resistant (121°C for 20 min) contrary to the carnocin CP5 [16]. Also activity was stable over a wide pH range (2–8); at pH >8 activity was decreased and totally lost at pH 11.

After concentration by ultrafiltration of the supernatant fluid, the molecular weight of the BLIS 213 was estimated to be greater than 10 kDa. After SDS-PAGE of the bacteriocin purified from producer cells [25] and direct detection of the inhibitory zone on the gel electrophoresis [7], the apparent molecular weight of the BLIS 213 was estimated to be 6 kDa.



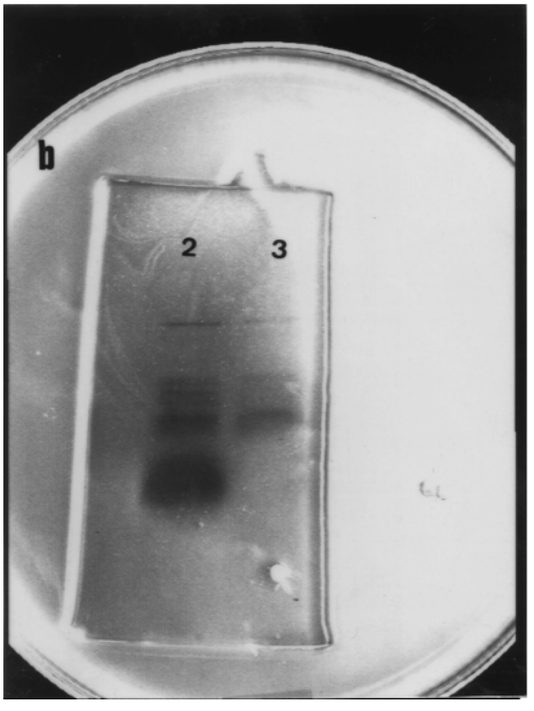


Figure 1 Detection of the BLIS 213 activity on a tricine-SDS-polyacrylamide gel by overlying the test indicator strain, *C. piscicola LMG* 9839. (a) Stained half used for protein band detection. Lanes 1 and 4 contained molecular weight standards (MW-SDS-17S; Sigma). Lanes 2 and 3 show the protein patterns of the desorbed preparations from *C. piscicola* 213 and *C. piscicola* 213a cells respectively. (b) The slightly stained half overlaid with the indicator strain showing one growth inhibition zone corresponding to the BLIS 213 activity in the case of *C. piscicola* 213 proteins (lane 2). No antibacterial activity was observed for proteins from the non producing strain *C. piscicola* 213a (lane 3). The bacteriocin activity was located at 6000 Da.

SDS treatment leads to bacteriocin aggregate dissociation and results in low molecular-weight, more active forms. SDS effect was observed for the bacteriocins, lactacin B [4] and helviticin J [14]. Also, SDS may increase the sensitivity of the indicator strain to the BLIS 213 activity. The non ionique detergent Tween 80 had no effect on the BLIS 213 activity. Tween 80 (0.1%) is added to the MRS* medium to moderate the cell permeability of the producer strain and to prevent the cell aggregation, therefore it facilitates diffusion of compounds through the cell membrane. In a previous experiment, the addition of the Tween 80 to the culture supernatant of cells grown without Tween 80 had no effect on the bacteriocin titre [13].

The recovery of the bacteriocin from cells was very helpful in eliminating the excess of proteins contained in fermentation medium which can form aggregates with the bacteriocin. On the other hand the quantity recovered was very low compared to the amount of bacteriocin still associated with the cells. In results presented by Yang *et al* [25], the recovery was over 90% for pediocin AcH, nisin and leuconocin Lcm1, and 44% for sakacin A. The quantity recovered for pediocin PA-1/AcH in Daba *et al* [10] did not exceed 10% of total activity. These discrepancies may be due either to the differences in bacteriocin activity assays, or to the extraction efficiency depending on the surface proteins in *Lactobacillus* strains [6,20].

The same protein patterns were observed for the two strains *C. piscicola* 213 and 213a but we could not conclude that the absence of bacteriocin activity in the case of proteins from *C. piscicola* 213a was related to the bacteriocin band deficiency.

The high isoelectric point of the bacteriocin (around 9.3) indicated that it contained a high content of basic amino acids residues. High pI was observed for many class II bacteriocins. The mutant strain which lacks bacteriocin production was identified as a derivative of the parent strain *C. piscicola* 213. The bacteriocin genetic information could be plasmid-mediated.

C. piscicola strains were characterized previously on the basis of their carbohydrate patterns. *C. piscicola* 213 [17] and *C. piscicola* ATCC 35586 [5] fermented both mannitol and inulin. In our results (Table 4), the two strains are mannitol (+) and inulin (–). Similar results were observed for *C. piscicola* U149 [22], and also a weakly positive reaction for inulin was observed for some strains identified as *C. piscicola* [5]. This was attributed to the sensivity of the API 50CHL test system, to the differences in the basal medium and to the time of reading.

Studies are now conducted to further characterize the BLIS 213 at the molecular level, to determine the bacteriocin genetic information, and also to better understand the regulation of the production/secretion systems.

Acknowledgements

We thank Dr Ingolf F Nes for critical reading of the manuscript.

References

1 Ahn C and ME Stiles. 1990. Antibacterial activity of lactic acid bacteria isolated from vacuum packaged meats. J Appl Bacteriol 69: 302–310.

2 Ahn C and ME Stiles. 1990. Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. Appl Environ Microbiol 56: 2503–2510.
3 Barefoot SF and TR Klaenhammer. 1983. Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl Environ Microbiol 45: 1808–1815.
4 Barefoot SF and TR Klaenhammer. 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lacticin B. Antimicrob Agents Chemother 26: 328–334.
5 Baya AM, AE Toranzo, B Lupiani, T Li, BS Roberson and FM Hetrick. 1991. Biochemical and serological characterization of *Carnobacterium* spp isolated from farmed and natural populations of striped bass and catfish. Appl Environ Microbiol 57: 3114–3120.
6 Bhowmik T, MC Johnson and B Ray. 1985. Isolation and partial characterization of the surface layer proteins of *Lactobacillus acidophillus*. Int J Food Microbiol 2: 311–321.
7 Bhunia AK and MG Johnson. 1992. A modified method to directly detect in SDS-PAGE the bacteriocin of *Pediococcus acidilactici*. Lett Appl Microbiol 15: 5–7.
8 Collins MD, JAE Farrow, BA Phillips, S Ferusu and D Johns. 1987. Classification of *Lactobacillus divergens, Lactobacillus piscicola*, and some catalase-negative, asporogenous, rod-shaped bacteria from poultry in a new genus, *Carnobacterium*. Int J Syst Bacteriol 37: 310–316.
9 Daba H, S Pandian, JF Gosselin, RE Simard, J Huang and C Lacroix. 1991. Detection and activity of a bacteriocin produced by *Leuconostoc mesenteroides*. Appl Environ Microbiol 57: 3450–3455.
10 Daba H, C Lacroix, J Huang, RE Simard and L. Lemieux. 1994. Simple method of purification and sequencing of a bacteriocin produced by *Pediococcus acidilactici* UL5. J Appl Bacteriol 77: 682–688.
11 Dupas H, PH Nougayred and G Perrin. 1981. Contrôle de l'activité bactericide des désinfectants. Présentation d'une microméthode destinée à determiner la 'concentration minimale bactericide'. Rev Med Vet 132: 209–215.
12 Bolck AL, L Axelsson and U Shillinger. 1994. Purification and cloning of piscicolin 61, a bacteriocin from *Carnobacterium piscicola* LV61. Curr Microbiol 29: 63–68.
13 Huot E, G Barrena-Gonzalez and H Petitdemange. 1996. Tween 80 effect on bacteriocin synthesis by *Lactococcus lactis* subsp *cremoris* J46. Lett Appl Microbiol 22: 307–310.
14 Joerger MC and TR Klaenhammer. 1986. Characterization and purification of helviticin J and evidence for chromosomally determined bacteriocin produced by *Lactobacillus helviticus* 481. J Bacteriol 167: 439–446.
15 Klaenhammer TR. 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol Rev 12: 39–86.
16 Mathieu F, M Michel and G Lefebvre. 1993. Properties of a bacteriocin produced by *Carnobacterium piscicola* CP5. Biotech Lett 15: 587–590.
17 Montel MC, R Talon, J Fournaud and MC Champomier. 1991. A simplified key for identifying homofermentative *Lactobacillus* and *Carnobacterium* spp from meat. J Appl Bacteriol 70: 469–472.
18 Piard JC and M Desmazeaud. 1992. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. Lait 72: 113–142.
19 Quadri LEN, M Sailer, KL Roy, JC Vederas and ME Stiles. 1994. Chemical and genetic characterization of bacteriocin produced by *Carnobacterium piscicola* LV17B. J Biol Chem 269: 12204–12211.
20 Ray B and MC Johnson. 1986. Freeze-drying injury of surface layer protein and its protection in *Lactobacillus acidophilus*. Cryo Lett 7: 210–217.
21 Schägger H and GV Jagow. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166: 368–379.
22 Stoffels G, IF Nes and A Gudmundsdottir. 1992. Isolation and properties of a bacteriocin-producing *Carnobacterium piscicola* isolated from fish. J Appl Bacteriol 73: 309–316.
23 Stoffels G, J Nissen-Meyer, A Gudmondsdottir, K Sletten, H Holo and IF Nes. 1992. Purification and characterization of a new bacteriocin isolated from a *Carnobacterium* sp. Appl Environ Microbiol 58: 1417–1422.
24 Tagg JR, AS Dajani and LW Wannamaker. 1976. Bacteriocins of gram-positive bacteria. Bacteriol Rev 40: 722–756.
25 Yang R, MC Johnson and B Ray. 1992. Novel method to extract large amounts of bacteriocins from lactic acid bacteria. Appl Environ Microbiol 58: 3355–3359.