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Mode of action, purification and amino acid sequence of plantaricin C19, an anti-*Listeria* bacteriocin produced by *Lactobacillus plantarum* C19

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Abstract

Plantaricin C19, an anti-*Listeria* bacteriocin, was successfully purified by adsorption to and release from producing cells at low pH combined with reverse phase high-performance liquid chromatography (HPLC). The purification resulted in a 900-fold increase in specific activity with a yield of 15% of the original activity. Mass spectrometry analysis gave a molecular weight of 3845.3. Protein microsequencing identified 36 amino acids. Plantaricin C19 is rich in both hydrophobic and basic amino acids in good accordance with its basic and hydrophobic character. Comparison of the amino acid sequence of plantaricin C19, with the sequence of some other anti-*Listeria* bacteriocins produced with lactic acid bacteria, revealed that plantaricin C19 has in its N-terminal region the consensus sequence—YYGNGL—(uniquely with Valine instead of Leucine as found in all other bacteriocins), identifying plantaricin C19 as a pediocin-like bacteriocin. Plantaricin C19 exerted a bacteriostatic action on sensitive cells of *Listeria grayi* IP 6818 in BHI broth. No loss of intracellular K^+, Mg^{2+} or UV-absorbing materials was observed. Adsorption of plantaricin C19 on *L. grayi* CIP 6818 decreased in the presence of salts. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Lactobacillus plantarum*; Plantaricin C19; Purification; Amino acid sequence; Mode of action

1. Introduction

Lactic acid bacteria are widely distributed in nature. They are found commonly on vegetables and grains, in milk and on fresh meat. In several food fermentations, lactic acid bacteria are found as the dominating microflora, resulting in acidification and in eventual inhibition of spoilage and pathogenic bacteria. Although it is generally assumed that this antimicrobial activity is due to organic acids, diacetyl, hydrogen peroxide or other low molecular weight compounds such as reuterin (Piard and Desmazeaud, 1992a,b; Vandenberg, 1993), the inhibition could also be due to bacteriocins, a family of antimi-

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crobial peptides. These substances, which have gained increasing interest, exerted in some cases a narrow, or in other cases, a relatively broad spectrum of antimicrobial activity. Their proteinaceous nature implies their putative degradation in the gastro-intestinal tract of man and animals. This suggests that some bacteriocin-producing lactic acid bacteria or purified bacteriocin could be used as natural preservatives in the food industry (Daeschel, 1989; Eckner, 1992). The effective use of bacteriocins in food preservation (Ray, 1992; De Vuyst and Vandamme, 1994) requires the understanding of their mode of action and the effect on their inhibitory action of different biochemical conditions naturally occurring in food. *Lactobacillus plantarum* is commonly associated with plant material (Daeschel et al., 1987) and has industrial application as a starter culture for the fermentation of vegetables and sausage products (McKay and Baldwin, 1990). Several authors have reported the production of bacteriocins by *Lb. plantarum* strains (Atrih et al., 1993a; Daeschel et al., 1990; Enan et al., 1996; Fricourt et al., 1994; Garriga et al., 1993; Gonzalez et al., 1994; Jimenez-Diaz et al., 1993; Lewus et al., 1991; Kato et al., 1994; West and Warner, 1988; Moll et al., 1999). Some of these bacteriocins have been purified to homogeneity . It concerns plantaricin A (Daeschel et al., 1990; Nissen-Meyer et al., 1993, Andersen et al., 1998), plantaricin S (Jimenez-Diaz et al., 1995), plantaricin C (Gonzalez et al., 1994), plantaricin-149 (Kato et al., 1994), plantaricin 423 (van Reenen et al., 1998), plantaricin 1.25 (Remiger et al., 1999) and plantaricin ST31 (Todorov et al., 1999).

Other bacteriocins (plantaricins EF and JK) are two peptide bacteriocins produced by *L. plantarum* C11. This strain produce plantaricin A (plnA) with 26 amino acid residues and two N-terminally truncated shorter forms of plnA (22 and 23 amino acid residues). These three peptides induce the transcription of *pln* genes (Anderssen et al., 1998; Moll et al., 1999).

This paper describes a two-step purification procedure of plantaricin C19, exploiting adsorption and desorption properties of the bacteriocin in function of pH, combined with reverse phase high-performance liquid chromatography (HPLC). The amino acid sequence, the mass spectrum, the mode of action of plantaricin C19 and the effect of salts on both adsorption and inhibitory activity of the bacteriocin, are also reported.

2. Materials and methods

2.1. Bacterial strains, media and antimicrobial assay of plantaricin C19

The plantaricin C19 producer, *Lb. plantarum* C19, isolated from fermented cucumbers (Atrih et al., 1993a) was propagated at 30°C in de Man, Rogosa and Sharpe (MRS) broth (de Man et al., 1960) (Biokar Diagnostics, Beauvais, France). The indicator strain (*Listeria grayi* CIP 6818), obtained from Institut Pasteur Collection (CIP, Paris, France) was incubated at 30°C in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI). *Lactococcus lactis* subsp. *lactis* INRA 141 was cultivated in Elliker (Elliker et al., 1956) broth (Difco) and was used to assess plantaricin C19 adsorption as a function of pH. Antimicrobial activity was detected by the agar well diffusion method, and quantitative measurements of the activity were carried out by the critical dilution assay as previously described (Atrih et al., 1993a,b).

2.2. Adsorption of plantaricin C19 onto producer or resistant bacterial cells

Adsorption of plantaricin C19 as a function of pH was determined as previously described for other bacteriocins (Yang et al., 1992) with minor modification. The producing cells (*Lb. plantarum* C19) and one type of resistant cells (*Lac. lactis* INRA 141) were grown at 30°C for 16 h, in 60 ml of MRS or Elliker broth, respectively, collected by centrifugation (10,000 × *g*, 10 min) and washed twice with 5 mmol l^{-1} phosphate buffer (pH 7). After stirring at 7°C for 30 min with sterile 1 mol l^{-1} NaCl solution at pH 2, cells were harvested by centrifugation and washed in sterile phosphate buffer (5 mmol l^{-1}, pH 6.5) until the pH was about 6, and finally suspended in 30 ml of phosphate buffer (5 mmol l^{-1}, pH 6.5). An aliquot of cell suspension (1.5 ml) was centrifuged and cells were resuspended in the same volume of phosphate buffer (5 mM) adjusted to a pH

range from 1.5 to 9 with 1 mol l^{-1} HCl or NaOH. Plantaricin C19 (320 AU ml^{-1}) was added and the mixtures were incubated at 30°C for 40 min. After centrifugation, the supernatants were adjusted to pH 7 and bacteriocin activity was assayed as previously described. The control was plantaricin C19 in phosphate buffer (5 mmol l^{-1}, pH 6.5).

2.3. Release of adsorbed bacteriocin from producing cells

For preparation of plantaricin C19, *Lb. plantarum* C19 was grown for 20 h at 30°C in 200 ml of MRS broth. The culture was heated for 10 min at 70°C, and the pH was adjusted to 6.8 using 1 mol l^{-1} NaOH after cooling to room temperature. Cells were then harvested by centrifugation (10,000 × *g*, 10 min, 7°C), washed twice with phosphate buffer (5 mmol l^{-1}, pH 6.8) and then suspended in 20 ml of the same buffer containing 100 mM NaCl. The pH was lowered to 2 with 1 mol l^{-1} HCl and cells were centrifuged within 1 min. The supernatant was stored at −20°C before high-performance liquid chromatography purification. Under these conditions of storage, plantaricin C19 showed no loss of activity for 6 months.

2.4. High-performance liquid chromatography (HPLC) analysis

The equipment used for plantaricin C19 purification consisted of 590 pump, U6K injector, 484 variable wavelength detector, 740 data module and C18 column (Delta pack). All equipment was obtained from Waters Scientific (Milford, MA, USA). Plantaricin C19 was eluted at room temperature, using the following conditions: 15% acetonitrile containing TFA for 15 min, followed by two-step linear gradient, 15–25% acetonitrile for 10 min, and finally, 25–45% for 60 min, at a constant flow rate of 1.5 ml/min. The eluted compounds were detected by monitoring absorbance at 220 nm, and fractions corresponding to peaks of OD were collected individually at the detector outlet. The activity was determined by directly placing 50-μl aliquots of individual peak fractions in wells cut in BHI agar plates containing indicator cells. The fractions containing inhibitory activity (purified plantaricin C19) from several runs were pooled and stored at −20°C. Protein concentration (mg/ml) was estimated as described by Scopes (1988).

2.5. Amino acid composition analysis

The pico-Tag amino acid analysis system (Waters) was used for amino acid composition. Prior to amino acid analysis, HPLC-purified plantaricin C19 was hydrolyzed in sealed evacuation vials in gaseous 6 mol l^{-1} HCl, 0.2 mmol l^{-1} phenol, for 20 h at 115°C. The hydrolysate was dried and derived with phenylisothiocynate as recommended by the manufacturers. The phenylthiocarbamyl amino acid derivatives were separated on a reverse phase HPLC pico-Tag column (3.9 × 150 mm, Waters), maintained at 38°C, and were detected at 254 nm.

2.6. N-terminal amino acid sequencing and mass spectrometry analysis

The amino-terminal sequence analysis of the plantaricin C19 was performed by Edman degradation using an Applied Biosystems (A.B.I) 476A gas-phase sequencer. Phenylthiohydantoin (PTH) amino acids were identified on line by reverse phase HPLC with Brownlee PTH-C18 column (A.B.I.). The sample was sequenced with polybrene-coated glass fiber as a support, and all the products and reagents used from A.B.I. Mass spectrometry analysis was performed by MALDI III (Katros Analytical, Manchester, UK), as previously reported (Atrih et al., 1996).

2.7. Effects of detergents or organic solvents on plantaricin C19 adsorption

Cells of *L. grayi* CIP 6818 were suspended in 1% sodium dodecyl sulfate (SDS), 2% Triton X-100, 2% isotridecylpoly(ethyleneglycolether)$_n$ or 4 mol l^{-1} guanidine–HCl, and heated at 60°C for 15 min, as reported by Bhunia et al. (1991). The suspensions were centrifuged; the pellets were washed three times with phosphate buffer (5 mmol l^{-1}, pH 7) and resuspended to the original volume in the same phosphate buffer. To test the effect of organic solvents on subsequent adsorption of plantaricin C19, cells were suspended in acetone, methanol, ethanol, butanol, hexan or chloroform, all used at 80% (v/v).

The mixtures were incubated at 30°C for 1 h and the solvents were removed by centrifugation and drying; the residual pellets were washed and thereafter resuspended in phosphate buffer (5 mmol l^{-1}, pH 7). Suspensions obtained after detergent or organic solvent treatments were mixed with plantaricin C19 (80 AU ml^{-1}) and incubated at 30°C for 30 min. After centrifugation, residual plantaricin C19 was assayed in supernatant fluids. Controls were cells without treatments in the presence of plantaricin C19 and plantaricin C19 in phosphate buffer only.

2.8. Effect of the presence of lipoteichoic acid on plantaricin C19 adsorption

Purified lipoteichoic acid (2 mg) prepared from *Staphylococcus aureus* (Sigma) was added to 2.6 ml of *L. grayi* CIP 6818 cell suspension (10^8 cfu ml^{-1}). Plantaricin C19 was added at a final concentration of 160 AU ml^{-1} and the mixture was incubated for 40 min at 30°C. After centrifugation ($15{,}000 \times g$, 10 min), the supernatant fluids were measured for residual non-adsorbed plantaricin C19. Controls were cells mixed with plantaricin C19 or lipoteichoic acid mixed with plantaricin C19.

2.9. Effect of different salts on bacteriocin adsorption and cell viability

To determine the effect of the presence of salts on plantaricin C19 adsorption, NaCl or KCl were prepared at 50, 100 or 200 mmol l^{-1}, while $MgSO_4$ or $CaCl_2$ were used at 10, 25 or 100 mmol l^{-1} in phosphate buffer (5 mmol l^{-1}, pH 7). Fractions (1 ml) of *L. grayi* CIP 6818 taken from an exponential preculture (10^8 cfu ml^{-1}), previously washed in phosphate buffer, were put in the same volume of each salt solution and plantaricin C19 was added at a final concentration of 80 AU ml^{-1}. Controls were: plantaricin C19 in phosphate buffer containing cells and without salt, salt solutions or plantaricin C19 without cells and salts. All samples were incubated at 30°C for 30 min and thereafter, remaining free bacteriocin was determined in fluid supernatants after centrifugation ($10{,}000 \times g$, 10 min).

Combined effect of salts (the same concentrations as indicated above) and plantaricin C19 on cell viability was examined in both phosphate buffer (5 mmol l^{-1}, pH 7.2) and BHI broth. *L. grayi* cells and plantaricin C19 were added, respectively, at 4×10^7 cfu ml^{-1} and 320 AU ml^{-1}, and incubation was performed at 15°C. After 24 h, equal portions of the cultures were then withdrawn for determination of viable cell counts, by plating dilutions on BHI agar. Controls were *L. grayi* CIP 6818 in phosphate buffer or BHI broth without salts and plantaricin C19 or *L. grayi* in phosphate buffer and BHI broth in the presence of plantaricin C19 only.

2.10. Effect of plantaricin C19 on viable cells at various temperatures and pH

To study the effect of plantaricin C19 on viable cells, plantaricin C19 obtained by precipitation with 60% ammonium sulphate (Atrih et al., 1993b) was used at 320 or 1280 AU ml^{-1}. Washed cells of *L. grayi* CIP 6818 were inoculated at 3×10^7 or 1×10^7 cfu ml^{-1}, and incubation was conducted at 7°C, 15°C, 30°C or 37°C. Samples were removed at different times and counts were performed in duplicate on agar BHI medium. The effect of pH was examined in BHI broth previously adjusted to pH 5.5, 6, 6.5, or 7 and inoculated with *L. grayi* CIP 6818. Plantaricin C19 was added at 320 AU ml^{-1} and incubation was carried out at 15°C. After 24 h, samples were removed and counts were performed in duplicate on agar BHI medium.

2.11. Effect of plantaricin C19 on cell membrane

Leakage of ultraviolet (UV) light-absorbing material and K^+ or Mg^{2+} ions were used as indicators of

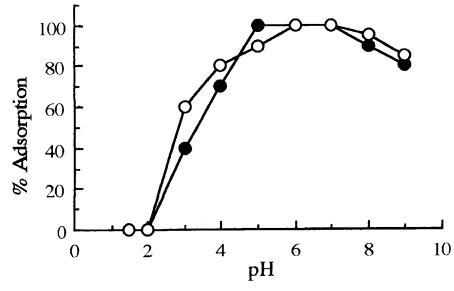

Fig. 1. Adsorption of plantaricin C19 onto producing (○) and resistant cells of *Lac. lactis* INRA 141 (●) at different pH (for conditions see text).

Table 1
Purification of plantaricin C19

Purification stage	Volume (ml)	Total activity (AU)	Total protein[a] (mg)	Specific activity (AU mg^{-1})	Recovery (%)	Purification fold
Culture supernatant	200	2048000	4500	455	100	1
Adsorption/release[b]	20	409600	23	17808	20	39
HPLC (C18)	15	307200	0.75	409600	15	900

[a]Protein concentration (mg/ml) was estimated as described by Scopes (1988).
[b]Adsorption at pH = 6.8 and release at pH = 2.

the loss of cell membrane integrity. *L. grayi* CIP 6818 cell suspension (10^8 cfu ml^{-1}) in 50 ml of phosphate buffer (5 mmol l^{-1}, pH 7.2) was mixed with plantaricin C19 (320 AU ml^{-1}) and incubated at 30°C. Samples were removed after 3 or 6 h and filtered through 0.22-mm pore size sterile mem-

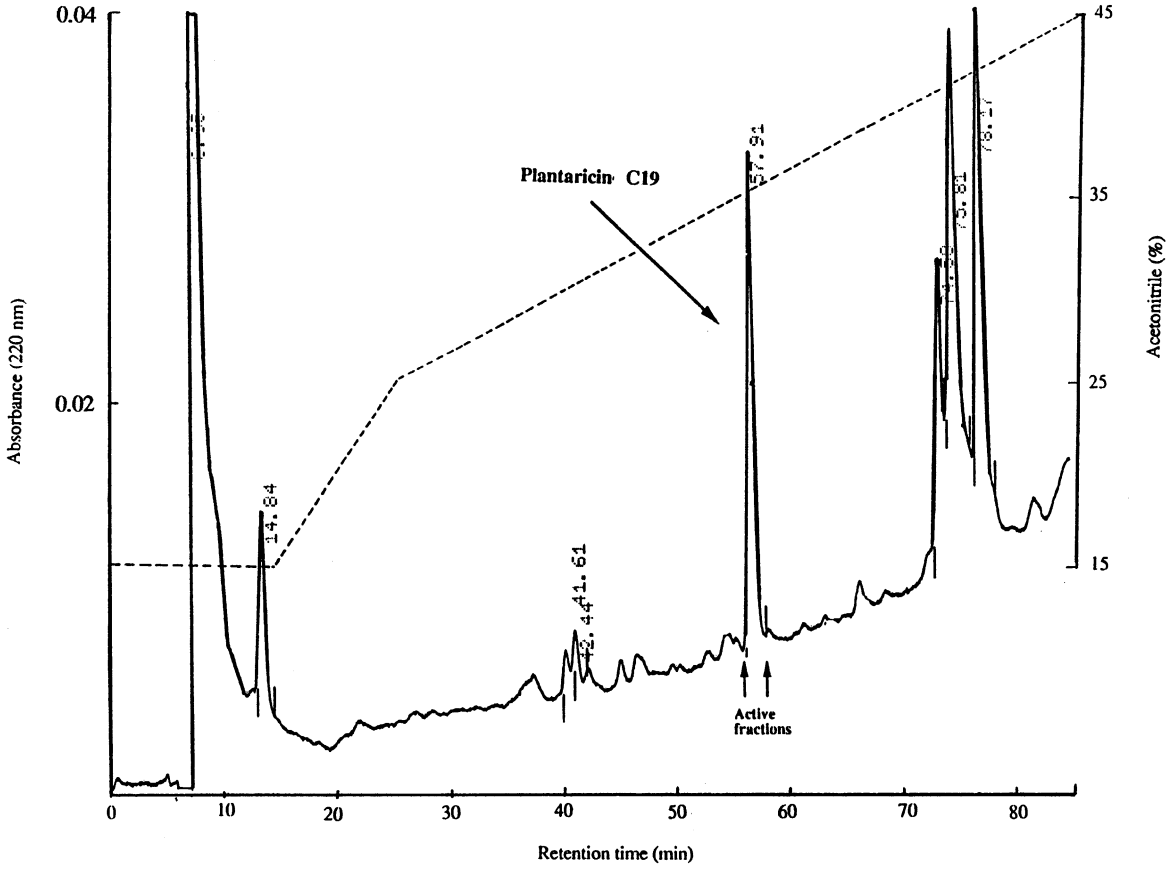

Fig. 2. Chromatogram of the final purification step. Semipreparative C_{18} reverse-phase column (Delta Pack, 300 A°, 30 cm × 7.8 mm) was used to isolate plantaricin C19 after adsorption/release from producer cells. The flow rate was 1.5 ml min^{-1} and 2-ml fractions were collected.

branes (Millipore). The absorbance of the filtrates was measured at 260 and 280 nm and the concentration of K^+ and Mg^{2+} ions was determined by atomic absorption spectroscopy (Perkin-Elmer 1100).

3. Results

3.1. Effect of pH on adsorption of bacteriocin onto producer or resistant cells

The influence of pH on the adsorption of plantaricin C19 onto producer (*Lb. plantarum* C19) or resistant cells (*Lac. lactis* INRA 141) is illustrated in Fig. 1. Adsorption on both producer and resistant strains was strongly influenced by the pH; maximum adsorption occurred between pH 5 and 7, and complete loss of adsorption was observed at pH 1.5 and 2.

3.2. Purification of plantaricin C19

The influence of pH on adsorption and release of plantaricin C19 from producing cells was exploited to purify plantaricin C19 from supernatant fluids. Since release of plantaricin C19 from producing cells at pH 2 occurred within a minute, the desorption of the bacteriocin was carried out without stirring the cells for a long time period (1 h), as reported earlier for other bacteriocins (Daba et al., 1994; Yang et al., 1992). Indeed, the same level of plantaricin C19 was released from the producing cells after stirring the cells at acidic condition for 1 h (data not shown), or centrifugation within 1 min after adjusting the pH to 2. The results of the purification process are summarized in Table 1. The crude concentrate obtained after release of the bacteriocin from the producer cells at pH 2, showed a high increase in specific activity, indicating that numerous proteins and peptides of MRS broth were eliminated by washing cells with phosphate buffer. The proteins present in this concentrate were probably extracted from cells along with plantaricin C19. At this stage, 20% of the original activity was recovered; the specific activity was 17,808 AU mg^{-1} proteins. When the preparation from the released concentrate was applied to C18 reverse phase HPLC column, plantaricin C19 was retained under initial conditions and then eluted in a single homogeneous peak with a proportion of 35–36% acetonitrile in the eluent (Fig. 2). After this step, the specific activity increased by 900-fold and 15% initial activity was recovered.

3.3. Amino acid composition, sequence and mass spectrometry of plantaricin C19

The amino acid composition of purified plantaricin C19 is presented in Table 2. Plantaricin C19 contains a high proportion of glycine, hydrophobic amino acids (mainly alanine and valine), and lysine. Cysteine was detected, but the instability of this amino acid under acid hydrolysis did not allow accurate quantification. The sequence of residues 1–36 of the bacteriocin as determined by automatic Edman degradation is: KYYGNGLSCSKKGCTVN-WGQAFSCGVNRVATAGHGK. No residue could be determined at position 37, presumably due to wash out of the sample from the sequencer. Mass spectrometry in the positive and the negative mode gave 3846.3 (Fig. 3) and 3844, respectively.

Table 2
Amino acid composition of plantaricin C19

Amino acid	Experimental value	Number of residues/molecule retained
Glycine	7.6	7–8
Alanine	4.2	3
Valine	3.3	3–4
Leucine	1.5	1
Ileucine	0	0
Proline	0	0
Methionine	0	0
Phenylalanine	1.3	1
Tryptophane	2.2	2
Glutamine/glutamate	1.2	1
Asparagine/aspartate	2.6	2–3
Serine	2.9	3
Theorine	2.2	2
Lysine	4.6	4
Arginine	1	1
Histidine	0.9	1
Cysteine	ND	–
Tryptophane	ND	–

Cystein and tryptophane were detected, however, their instability under acid hydrolysis did not allow accurate quantification.
ND: not determined.

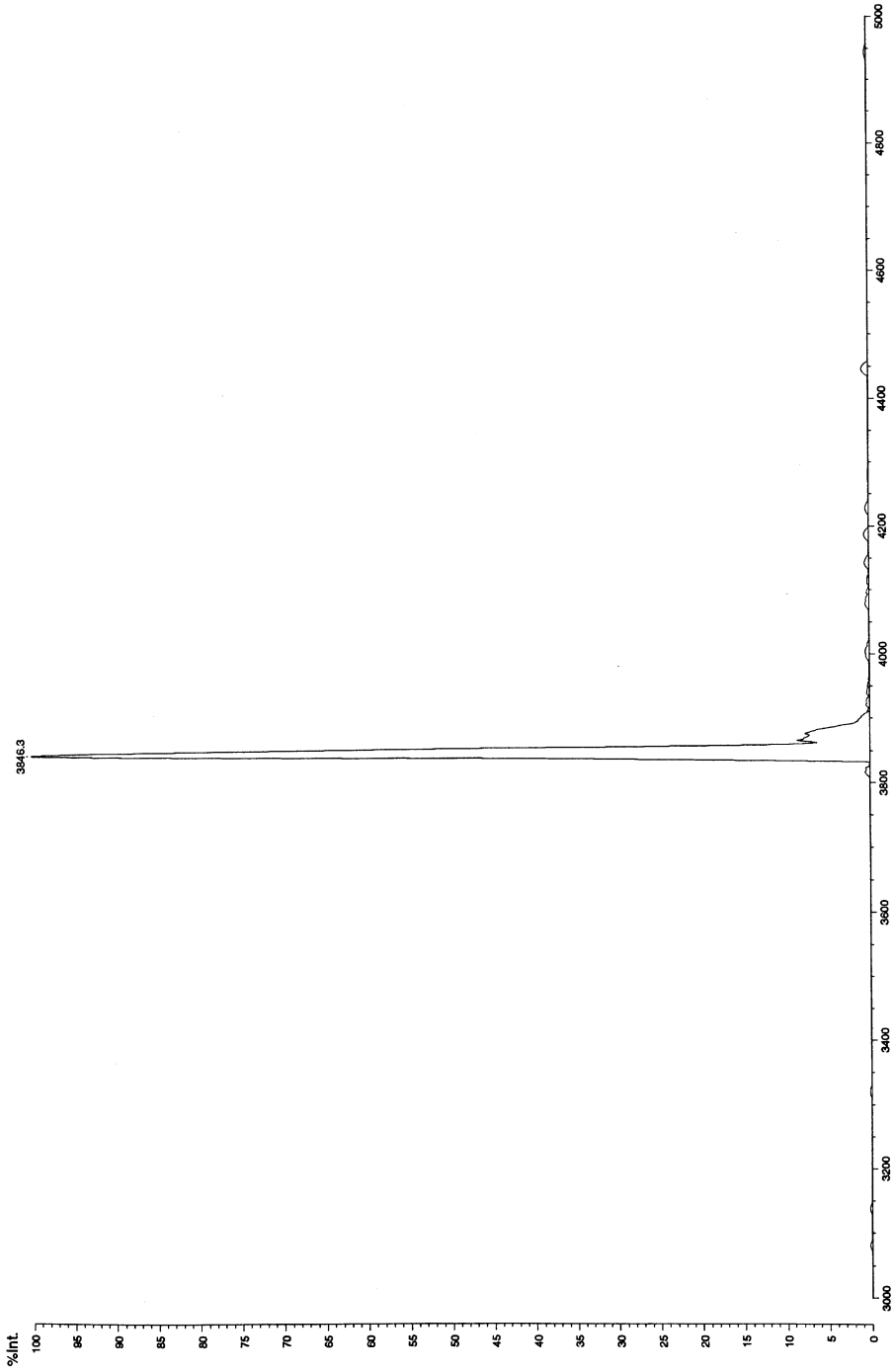

Fig. 3. Positive ion MALDI mass spectrum of pure plantaricin C19.

3.4. Effect of plantaricin C19, at different temperatures and pH, on target cells

With all the temperatures tested (7–37°C), plantaricin C19 exerted a bacteriostatic effect on growing *L. grayi* CIP 6818. At 7°C, a slight decrease in viability was noted (Fig. 4a); the inhibition was still observed until 192 h. However at 37°C, a partial regrowth was noted after 9 h (Fig. 4c). The different initial pH values (5.5–7) of BHI broth tested did not change the action of plantaricin C19 on *L. grayi* cells (results not shown).

3.5. Effect of detergents, organic solvents and lipoteichoic acid and salt on plantaricin C19 adsorption on target cells

Treatment of cells with 4 M guanidine HCl, 1% SDS, 2% isotridecylpoly(ethyleneglycolether)$_n$ or 2% Triton X-100, did not reduce the adsorption of plantaricin C19 onto *L. grayi* (results not shown). Cell treatment with methanol reduced plantaricin C19 adsorption to 90%; however, the other organic solvents tested had no effect (results not shown). The addition of lipoteichoic acid completely inhibited the adsorption of plantaricin C19 (results not shown).

The different tested salts ($MgSO_4$, KCl, $CaCl_2$ or NaCl) reduced the adsorption of plantaricin C19 (Table 3). This reduction was in terms of increased concentrations. The most important reduction was observed with $MgSO_4$. At 100 mmol l^{-1}, only 25% of plantaricin C19 was adsorbed. When the adsorption of plantaricin C19 was reduced, survival appeared not to be affected (Table 3).

3.6. Effect of plantaricin C19 on the release of cellular materials

The effect of plantaricin C19 on the integrity of cell membrane was measured using two parameters. Treatment of *L. grayi* CIP 6818 with plantaricin C19 did not cause leakage of UV-absorbing materials

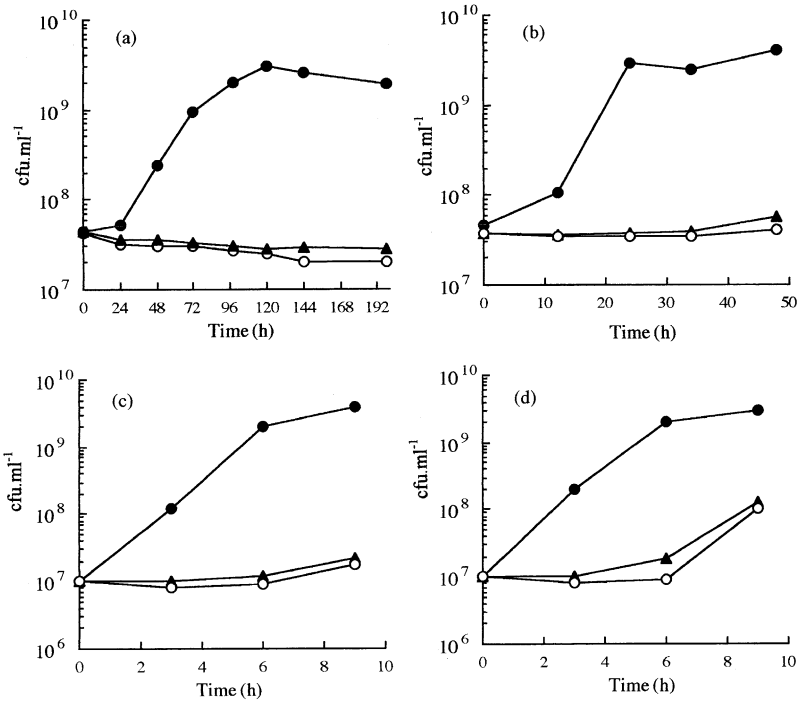

Fig. 4. Effect of an extract of plantaricin C19 obtained from precipitation with 60% ammonium on washed cells of *L. grayi* CIP 6818, incubated in BHI broth at 7°C (a), 15°C (b), 30°C (c) and 37°C (d). (●) Control : without bacteriocin; (○) 320 and (▲) 1280 AU ml^{-1}.

Table 3
Effect of lipoteichoic acid, detergents and organic solvents on plantaricin C19 adsorption on *L. grayi* CIP 6818

Salt (mM)		Adsorption (%) in phosphate buffer	Survival of *L. grayi* CIP 6818 (10^6 cfu ml^{-1})	
			BHI medium	Phosphate buffer
$MgSO_4$	0	100	34	12
	10	100	39	22
	25	70	55	28
	100	25	60	42
KCl	0	100	34	12
	50	85	38	14
	100	65	40	13
	200	25	54	10
NaCl	0	100	34	12
	50	90	39	13
	100	75	42	17
	200	50	45	14
$CaCl_2$	0	100	34	12
	10	100	42	nd
	25	75	46	nd
Control[a]	–	–	2200	40

nd: not determined.
[a] *L. grayi* CIP 6818 in the absence of plantaricin C19.

measured at 260 and 280 nm. Likewise, no leakage of K^+ or Mg^{2+} ions was observed after treatment of cells with this bacteriocin (result not shown).

4. Discussion

Purification of plantaricin C19 by ammonium sulphate precipitation, binding to cation exchanger, dialysis and reverse phase HPLC, resulted in a large loss of activity, especially after cation exchange chromatography and dialysis. Only 0.7% of initial activity was recovered after the final reverse phase HPLC step (result not shown). Adsorption to and release of bacteriocins from producer cells, as a function of pH and ionic strength, has been utilised as an alternative method of bacteriocin purification (Yang et al., 1992; Daba et al., 1994). A similar procedure combined with reverse phase HPLC allowed plantaricin C19 to be purified to apparent homogeneity, pure bacteriocin being obtained within 2 h. The extraction of plantaricin C19 from producer cells without heat treatment resulted in low recovery. Heat treatment could inactivate any heat-labile protease(s), which might be active at pH 6.8 (pH used to adsorb the plantaricin C19 on producing cells). Compared with the usual methods used to purify bacteriocins (Piard and Desmazeaud, 1992b), the method of adsorption to and release of bacteriocins from producing cells is easy to perform and more economical, as cells can be used for several extractions of bacteriocin from the supernatant. Assay of plantaricin C19 after each step indicated that the loss of bacteriocin into the supernatant, following harvesting of the cells, ranged from 40% to 50%, possibly due to saturation of receptors on cell surfaces.

The sequence of residues 1–36 of the purified preparation of plantaricin C19 were determined. The calculated molecular weight of this sequence is 3750. However, mass spectrometry analysis revealed a molecular weight of 3845.3, suggesting that plantaricin C19 contains 37 amino acids, the last amino acid corresponding probably to valine. Plantaricin C19 contains five basic amino acids (four Lys and one Arg); the presence of these amino acids explained its strong adsorption on cation exchange support (elution was obtained with 1.5 mol l^{-1} NaCl in phosphate buffer at pH 7.5). The molecular mass of plantaricin C19 (3845.3), determined by mass spectrometry, correlated well with that obtained with Sephadex G-50 gel filtration chromatography (Atrih et al., 1993b). It indicated that plantaricin C19 did not aggregate or associate with other substances under non-dissociating conditions. The amino acid sequence of plantaricin C19 indicates that it is a novel bacteriocin belonging to the pediocin-like family. It is different from plantaricin A (Nissen-Meyer et al., 1993), plantaricin-149, (Kato et al., 1994), plantaricin C (Gonzalez et al., 1994) and plantaricin S (Jimenez-Diaz et al., 1995). Plantaricin C19 amino acid sequence was compared with those of other bacteriocins present in the Swiss-prot protein sequence data-base (16,941) entries by using the Fast P program. The pediocin-like bacteriocins present in their N-terminal region the consensus sequence—YYGNGV; the same sequence was also present in the N-terminal region of plantaricin C19, but with Leu instead of Val (Fig. 5). Plantaricin C19, therefore, is a biochemical variant in the pediocin-like family bacteriocins; it shows 65%, 60% and 50%

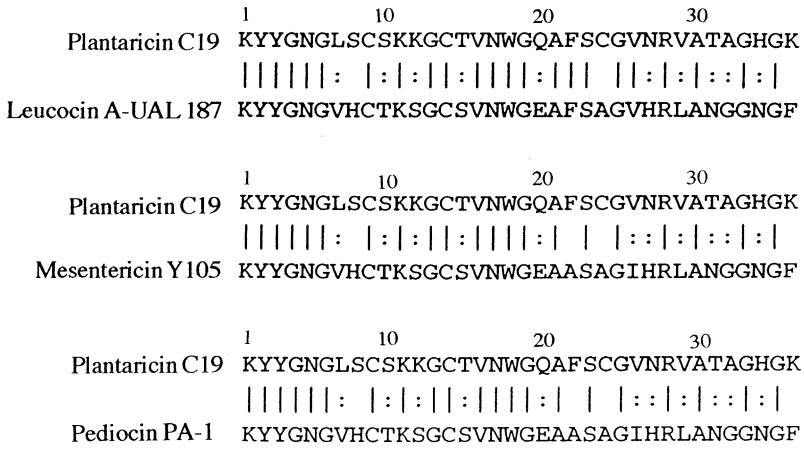

Fig. 5. Comparison of amino acid sequences of plantaricin C19 to leucocin A-UAL 187, mesentericin Y 105 and pediocin PA-1. Solid lines represent identical residues, double dots represent conservative substitutions.

identity, respectively, to leucocin A, mesentericin Y105 and pediocin PA-1 (Fig. 5).

The effect of plantaricin C19 on *L. grayi* CIP 6818 seemed to be bacteriostatic rather than bactericidal at the concentrations used in our study. The same effect observed with 320 or 1280 AU ml^{-1}, could be due to the saturation of the putative receptors. Several bacteriocins are bactericidal substances; however, a bacteriostatic effect was also reported for some other bacteriocins such as lactocin 27 (Upreti and Hinsdill, 1975) and leuconocin S (Lewus et al., 1992). The view that plantaricin C19 was bacteriostatic is consistent with the fact that it did not damage the cell membrane of *L. grayi* CIP 6818. Indeed, no release of UV-absorbing material was detected after treatment of cells with the bacteriocin. This effect was also observed with lactocin 27, although, in contrast to lactocin 27 (Upreti and Hinsdill, 1975), no release of K^+ ions was noted with plantaricin C19. The adsorption of plantaricin C19 to *L. grayii* was reduced in the presence of increased salts concentrations. In these conditions, the inhibitory effect of plantaricin C19 was slightly reduced in the presence of salts. This little protective effect of salt could be explained by the reduction of bacteriocin adsorption, which is suggested to be the first step in bacteriocin mode of action. Ray (1992) suggested that the loss of bacteriocin adsorption in the presence of salt is due to competitive ion adsorption on the bacterial cell surface, or to the blockage of the binding sites of the bacteriocin. Lipoteichoic acid inhibited adsorption of plantaricin C19 to the cells; earlier result also showed absence of plantaricin C19 adsorption on Gram-negative bacteria (Atrih et al., 1993b). These results support the hypothesis that lipoteichoic acid might represent the nonspecific receptor(s) of bacteriocins in Gram-positive bacteria (Bhunia et al.1991). The pediocin-like bacteriocins are widely distributed among several genera of lactic acid bacteria: *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Lactobacillus* (several *Lactobacillus* spp.: *Lb. sake*, *Lb. curvatus*, *Lb. bavaricus*, *Lb. bavaricus*, *Lb. plantarum*). In spite of peptide similarities and common anti-*Listeria* activity, a variety of spectrum activity and mode of action (bactericidal or bacteriostatic) activities have been reported. These features may suggest that different structural analogs with varied properties could be available naturally or constructed by site-directed mutagenesis. Comparison of plantaricin C19, which has a bacteriostatic effect in BHI broth, with other pediocin-like bacteriocins showing bactericidal action, may provide a consensus of structure–function relationships that defines particular peptide sequences or structures important to the inhibitory activity.

This manuscript is dedicated to the memory of Prof. G. Lefebvre, and in recognition of his major contribution to research on bacteriocins from lactic acid bacteria and physiology of *Streptomyces*.

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