Pediocin A, a bacteriocin produced by *Pediococcus pentosaceus* FBB61

Andrea Piva¹ and Denis R. Headon²

Author for correspondence: Andrea Piva. Tel: +39 51 792883. Fax: +39 51 792869.

¹ Istituto di Zootecnia e Nutrizione Animale, Facoltà di Medicina Veterinaria, Università di Bologna, 40064 Ozzano Emilia (BO), Italy

² Department of Biochemistry, Cell and Molecular Biology Group, University College Galway, Ireland

Pediocin A, a bacteriocin produced by *Pediococcus pentosaceus* FBB61, was purified and partially characterized. The purification was achieved by dialysis against PEG 20000, butanol extraction and electroendosmotic preparative electrophoresis. The protocol led to a 7843-fold increase in the specific activity, with 3.9% activity recovered. SDS-PAGE of pediocin A resulted in a single 80 kDa protein band. The antimicrobial compound was sensitive to proteolytic enzymes and heat (10 min at 100 °C). It exhibited inhibition against species of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Staphylococcus*, *Enterococcus*, *Listeria* and *Clostridium*.

Keywords: pediocin A, bacteriocin, *Pediococcus pentosaceus*, food microbiology

INTRODUCTION

An outstanding feature of lactic acid bacteria, accounting for their wide utilization in dairy, meat and vegetable fermentations, is their inhibitory activity against a variety of food-spoilage micro-organisms (Bhunia *et al.*, 1988; Pucci *et al.*, 1988; Spelhaug & Harlander, 1989). Among antimicrobial substances produced by lactic acid bacteria, bacteriocins have gained increasing interest as possible natural food preservatives. Bacteriocins are heterogeneous in terms of spectra of activity, optimal conditions for activity, molecular masses, biochemical characteristics and sequence homologies. Nisin, a bacteriocin produced by certain strains of *Lactococcus lactis* subsp. *lactis*, was approved as a GRAS human food ingredient by the FDA in 1988 (Food and Drug Administration, 1988).

Pediococcal strains are used for meat and vegetable fermentations. Pediocin AcH and pediocin PA-1, produced by different strains of *Pediococcus acidilactici* were purified (Bhunia *et al.*, 1988; Henderson *et al.*, 1992), and later demonstrated to be identical (Motlagh *et al.*, 1992). Pediocins have been successfully used to inhibit *Listeria monocytogenes* in several food systems (Pucci *et al.*, 1988; Yousef *et al.*, 1991). *Pediococcus pentosaceus* FBB61 was originally isolated in 1953 from a cucumber fermentation (Costilow *et al.*, 1956). The inhibitory activity of this strain was observed by Etchells *et al.* (1964) in pure culture fermentations of cucumbers, and further investigated by Fleming *et al.* (1975). Rueckert (1979)

characterized the chemical nature of the inhibitory material as proteinaceous and nondialysable across a semi-permeable membrane, and its action as bactericidal. Daeschel & Klaenhammer (1985) associated the production and immunity phenotype (Bac⁺ Imm⁺) to a 13.6 MDa plasmid. Spelhaug & Harlander (1989) demonstrated that *P. pentosaceus* FBB61 was inhibitory to several strains of *L. monocytogenes*, but had no effect against any Gram-negative bacteria tested.

No additional data are available about the characteristics of pediocin A, its genetic determinants, possible homologies with other bacteriocins and mode of action. This study deals with the purification and partial characterization of pediocin A, produced by *P. pentosaceus* FBB61, with a view to evaluating its possible utilization as a food preservative.

METHODS

Bacterial strains and media. *P. pentosaceus* FBB61 (ATCC 43200) and '*Lactococcus lactis* subsp. *diacetylactis*' (*L. lactis* subsp. *lactis*) CH001, used as an indicator strain, were propagated at 30 °C in M17 broth (Sambrook *et al.*, 1989). The medium used for bacteriocin production was M17 + 1% (w/v) glucose. Agar media were prepared by adding 1.5% (w/v) granulated agar to the broth media. Soft and overlay agars were prepared with 0.75% agar.

Bacteriocin production. *P. pentosaceus* FBB61 was grown in M17 + 1% glucose at 30 °C for 24 h. The culture supernatant was then collected, adjusted to pH 6.5, filtered through 0.22 µm pore-size filters, concentrated to 0.1 volume by polyethylene glycol dialysis (Sigma, PEG 20000) and again filter-sterilized. This material was designated crude pediocin A and was frozen at –20 °C when not used immediately.

Abbreviations: AU, activity units; EPE, electroendosmotic preparative electrophoresis.

Bacteriocin titration. Serial twofold dilutions of the crude extract and purified protein were made in sterile distilled water. From each dilution 30 µl aliquots were delivered into wells in an M17 agar (1.5%) plate previously seeded with 10 µl of a fresh overnight culture (*Lactococcus lactis* subsp. *lactis* CH001) (approx. 10^7 c.f.u.) and incubated at 30 °C. The sample titre was defined as the reciprocal of the highest dilution showing definite inhibition of the indicator lawn and was expressed in activity units (AU) ml⁻¹.

Purification of pediocin A. (i) Butanol extraction. Crude pediocin A, prepared from M17 supernatant, was mixed with butanol (2:3 ratio). The mixture was vortexed for 30 s and left for 30 min. The butanol phase was removed and freeze-dried. All manipulations were at 0 °C. The powder was resuspended in ultrapure water in one-fifteenth of the original volume.

(ii) Non-denaturing gels and direct detection of antimicrobial activity. Non-denaturing polyacrylamide gels were prepared with a mixture of 15% (w/v) acrylamide and 0.4% (w/v) bisacrylamide, without SDS. Butanol extract was loaded in two parallel lanes (30 µg per lane). After the electrophoresis, one lane was stained with Coomassie blue R-250 and silver. To detect antimicrobial activity, the other lane was overlaid with 10 ml M17 soft agar seeded with 10 µl of a fresh overnight culture of *L. lactis* subsp. *lactis* CH001. Plates were incubated at 30 °C for 20 h. The antimicrobial activity was eluted from these gels as described for helveticin J by Joerger & Klaenhammer (1986).

(iii) Electroendosmotic preparative electrophoresis (EPE). Recovery of the purified protein from the butanol extract was achieved by EPE (Curioni *et al.*, 1988, 1989) using native polyacrylamide gels as described by Reniero *et al.* (1992). The stacking (T = 3%) and the running gel (T = 10%) were 2 cm and 3 cm in length, respectively. The fraction-collecting device was set to obtain one fraction every 20 drops (400 µl) at a flow rate of 4.8 ml h⁻¹.

SDS-PAGE. SDS-PAGE was carried out with a mixture of 15% (w/v) acrylamide and 0.4% (w/v) bisacrylamide according to Laemmli (1970). After the electrophoresis, the gels were stained with Coomassie blue R-250 or silver.

Sensitivity to heat and proteolytic enzymes. Crude and purified pediocin A was heated in a boiling water bath. Samples were removed at 0, 5, 10, 30 and 60 min, and titres were determined to detect activity. To determine the influence of proteolytic enzymes, the following enzymes (1 mg ml⁻¹) were used: trypsin (EC 3.4.21.4) (110 U mg⁻¹), pronase (7 U mg⁻¹), and proteinase K (EC 3.4.21.64) (20 U mg⁻¹). Pediocin A/enzyme mixtures were incubated for 30 min at 37 °C.

Determination of minimal inhibitory concentration (MIC). One-millilitre vials of M17 were inoculated with approximately 10^3 c.f.u. ml⁻¹ of a fresh overnight culture of the indicator

strain. Twofold dilutions of purified bacteriocin were added to each vial, ranging from 1.95 to 1000 AU ml⁻¹. The MIC was defined as the minimal bacteriocin concentration required to inhibit growth after 24 h incubation.

Antimicrobial spectrum. Bacterial strains and growth conditions for activity spectrum determination of purified pediocin A are listed in Table 2. Strain sensitivity to pediocin A was measured by serial twofold dilutions of purified pediocin A (133.3 AU), as described above (bacteriocin titration). Agar plates were seeded with the strains listed in Table 2 and incubated at the growth temperature of the strain. Strain sensitivities were expressed as a percentage of *L. lactis* subsp. *lactis* CH001 sensitivity to 133.3 AU of pediocin A.

RESULTS

Production of pediocin A in M17 medium

To maximize the recovery of antimicrobial activity, the relationship of culture growth and production was studied during a 24 h incubation. Titres of activity increased as the growth increased (Fig. 1). There was a linear relation between c.f.u. count and activity that was described by the following linear regression formula:

$$y = 0.475x - 3.105 \quad (r^2 = 0.91) \\ [x = \log(\text{c.f.u. ml}^{-1}); y = \log(\text{AU ml}^{-1})].$$

The antimicrobial protein was constitutively expressed, with no relation to the physiological status of the culture; and it appeared to be stable, with no loss of activity after the exponential phase as described for other bacteriocins

Fig. 1. Producer growth and pediocin A production. ●, Growth (c.f.u. ml⁻¹); ■, pediocin A activity. Means of five experiments are plotted.

Table 1. Purification of pediocin A

Purification stage	Vol (ml)	Total activity (AU)	Pediocin A activity (AU ml ⁻¹)	Total protein (mg)	Protein concn (mg ml ⁻¹)	Sp. act. (AU mg ⁻¹)	Activity recovered (%)	Purification (-fold)
Culture supernatant	1000	33000	33	17500	17.5	1.9	100	1
Dialysis against PEG	100	32000	320	13000	130	2.5	96.9	1.3
Butanol extraction	10	5333	533.3	6	0.6	889	16.2	468
EPE	0.4	1280	3200	0.086	0.215	14884	3.9	7834

Fig. 2. Non-denaturing PAGE of butanol extract (30 µg per lane). (a) Silver-stained gel; (b) detection of antimicrobial activity. The arrow indicates the band corresponding to the inhibitory halo. More than 20 non-denaturing PAGEs of butanol extract were run to detect antimicrobial activity, with different electrophoresis conditions. Inhibitory haloes always corresponded to the upper stained band.

Fig. 3. Coomassie-blue stained SDS-PAGE of pediocin A. Lane 1, molecular mass markers (rabbit muscle phosphorylase *b*, 97.4 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 42.7 kDa; bovine carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa). Lane 2, pediocin A (10 µg) eluted from EPE.

(Barefoot & Klaenhammer, 1984; Joerger & Klaenhammer, 1986).

Purification of pediocin A

Crude pediocin A preparation (100 ml, 3.2×10^4 AU) was subjected to butanol extraction. Titration of the butanol and aqueous phases for antimicrobial activity indicated that no activity remained in the aqueous phase, whereas the butanol phase, following freeze-drying, resuspension and dialysis against distilled water, was inhibitory against the indicator strain. This indicated the hydrophobic nature of pediocin A. Unfortunately, even if the extraction was carried out at low temperature, pediocin A activity was reduced to 20% by this extraction procedure; nevertheless the enrichment was highly significant (Table 1). Organic solvents can act as denaturing agents, causing considerable loss of activity (Wilson & Goulding, 1986).

Electrophoresis of the butanol extract on non-denaturing polyacrylamide gels resulted in two bands (Fig. 2); direct detection of antimicrobial activity by overlaying the gel with soft agar seeded with the indicator strain revealed that the upper band was active (Fig. 2). This band was eluted and analysed by SDS-PAGE, resulting in a single protein band of approximately 80 kDa, providing direct evidence that this band contained pediocin A.

To scale up the recovery of pure pediocin A, non-denaturing gel elution was replaced by nondenaturing EPE. Butanol extract (5333 AU) was applied to a native polyacrylamide column and the elution profile showed two peaks (data not shown) corresponding to the two bands seen on non-denaturing PAGE (Fig. 2). Each eluted fraction was assayed for antimicrobial activity and was analysed by SDS-PAGE. Only the fraction corresponding to the second peak was highly inhibitory to the indicator strain; on SDS-PAGE it showed only a single band, of approximately 80 kDa (Fig. 3). None of the buffers used in these experiments was inhibitory to *L. lactis* subsp. *lactis* CH001. Pediocin A was eluted following electrophoresis for 8.5 h. This protocol allowed 3.9% activity recovery (Table 1).

Enzyme and heat inactivation

Pediocin A activity was completely eliminated upon treatment with trypsin, pronase or proteinase K (data not shown). After incubation of crude or purified pediocin A at 100 °C for 5 min the bacteriocin activity was reduced to 20%, and after 10 min only 5% of the original activity remained. No appreciable difference in inactivation was observed between crude and purified pediocin A. These results do not agree with those of Rueckert (1979): every attempt to detect antimicrobial activity from either crude or purified pediocin A heated at 100 °C for 60 min failed, confirming its heat sensitivity.

MIC

The MIC for *L. lactis* subsp. *lactis* CH001 was 31.25 AU ml⁻¹.

Table 2. Activity spectrum of pediocin A

Sensitivity of strains to pediocin A was measured with serial twofold dilutions of purified bacteriocin (133·3 AU). See Methods for details.

Target strain	Temp. (°C)	Medium*	Pediocin A sensitivity†
<i>Lactobacillus acidophilus</i> ATCC 4356	37	MRS	50
<i>Lactobacillus bulgaricus</i> ATCC 11842	42	MRS	25
<i>Lactobacillus casei</i> ATCC 334	37	MRS	5
<i>Lactobacillus curvatus</i> NCFB 2739	30	MRS	5
<i>Lactobacillus fermentum</i> ATCC 9338	37	MRS	65
<i>Lactobacillus helveticus</i> ATCC 15009	42	MRS	100
<i>Lactobacillus plantarum</i> NCDO 1193	37	MRS	5
<i>Lactobacillus reuteri</i> DMS 20016	37	MRS	5
<i>Lactobacillus sake</i> NCFB 2714	30	MRS	88
<i>Lactobacillus salivarius</i> NCFB 2747	37	MRS	10
<i>Pediococcus pentosaceus</i> FBB 63	30	M17	0
<i>Pediococcus pentosaceus</i> PC 1	30	M17	38
<i>Propionibacterium acidipropionici</i> NCDO 563	30	M17	50
<i>Lactococcus cremoris</i> CNRZ 177	25	M17	50
<i>Leuconostoc cremoris</i> DB 1275	25	M17	38
<i>Staphylococcus thermophilus</i> ST 20	42	M17	75
<i>Staphylococcus thermophilus</i> ST 112	42	M17	75
<i>Staphylococcus carnosus</i> MC 1	37	BHI	75
<i>Bacillus cereus</i> ATCC 9139	37	BHI	10
<i>Listeria innocua</i> BL 86/26	30	BHI	10
<i>Enterococcus faecalis</i> EF 1	37	BHI	65
<i>Clostridium sporogenes</i> C 22/10	37	RCM	65
<i>Clostridium tyrobutyricum</i> 3.5	30	RCM	50
<i>Clostridium tyrobutyricum</i> NCDO 1754	30	RCM	75
<i>Lactococcus lactis</i> CH001	30	M17	100
<i>Pediococcus pentosaceus</i> FBB61	30	M17	0
<i>Pediococcus pentosaceus</i> FBB61-2‡	30	M17	100

* MRS medium (De Man *et al.*, 1960) was used without Tween 80. BHI, brain-heart infusion (Difco); RCM, reinforced clostridial medium (Oxoid).

† Mean values of at least three experiments, expressed as a percentage of the sensitivity of the standard test strain *L. lactis* subsp. *lactis* CH001.

‡ Isogenic mutant that does not produce pediocin A (Daeschel & Klaenhammer, 1985).

Antimicrobial spectrum

P. pentosaceus FBB61 exhibited antimicrobial activity against almost all Gram-positive micro-organisms tested by the agar-spot method (data not shown). The utilization of serial dilutions of purified pediocin A against each strain tested allowed us to measure their relative sensitivities and to verify the broad spectrum of activity of this bacteriocin (Table 2).

DISCUSSION

We have confirmed that the antimicrobial agent produced by *P. pentosaceus* FBB61, pediocin A, is a bacteriocin. The sensitivity of pediocin A to heat and proteolytic enzymes demonstrated its proteinaceous nature. *P. pentosaceus* FBB61 and pure pediocin A had an identical broad spectrum of bactericidal activity against almost all Gram-

positive strains tested. Purification of pediocin A was achieved by PEG 20000 dialysis, butanol extraction and EPE. The purification resulted in an approximately 7834-fold increase in the specific activity of pediocin A and 3·9% recovery. Electrophoresis experiments showed that a 80 kDa protein could be identified as pediocin A.

Among bacteriocins of the genus *Pediococcus* that have been purified to date, pediocin A is the largest, at 80 kDa. A wide range of different molecular masses was also observed for *Lactobacillus* bacteriocins (Joerger & Klaenhammer, 1986; Rammelsberg & Radler, 1990). The heat sensitivity of pediocin A appeared to be consistent with the size and the apparent complexity of this protein compared with other bacteriocins.

Every attempt to detect the antimicrobial activity directly in SDS-PAGE failed, whereas it was successful for pediocin AcH (Bhunia *et al.*, 1987). However, SDS was

highly inhibitory to the indicator strain; it is also possible that SDS inactivated pediocin A by altering its native state.

Pediocin A was also purified by ion-exchange and hydrophobic interaction chromatography (HIC) from a semi-synthetic medium prepared as described by Reniero *et al.* (1993) (data not shown). The hydrophobic nature of pediocin A was evidenced by the butanol extraction and the HIC elution profile. Chromatography of pediocin A on a C₁₈ reversed-phase column revealed that the bacteriocin was eluted with 32% acetonitrile (data not shown). Hydrophobicity is a common feature of several bacteriocins (Joerger & Klaenhammer, 1986; Muriana & Klaenhammer, 1991; Mortvedt *et al.*, 1991; Piard *et al.*, 1992). This suggests that the cell membrane is a possible target, as demonstrated for lactococcin A (van Belkum *et al.*, 1991).

Because of the potential use of bacteriocins of lactic acid bacteria as food preservatives, it is of great importance to gain insight into their chemical structure and their interaction with different food components that may affect their antimicrobial activity. The choice of M17 medium for our experiments was essentially on the basis of the absence of the non-ionic detergent Tween 80. If 0.01% Tween 80 was included in this medium, no antimicrobial activity was detectable. As previously reported for lactocin S (Mortvedt *et al.*, 1991), the exclusion of Tween 80 from MRS medium resulted in a high recovery of antimicrobial activity. Common food additives like soybean and egg yolk phosphatidylcholine showed the same effects as Tween 80 in inhibiting the antimicrobial activity of pediocin A (data not shown). Clearly, further studies of pediocin A are required to elucidate its possible role as a food preservative.

ACKNOWLEDGEMENTS

We gratefully acknowledge Dr M. C. Callegari, Dr R. Reniero and Dr D. McDonagh for their efficient help.

REFERENCES

- Barefoot, S. F. & Klaenhammer, T. R. (1984). Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. *Antimicrob Agents Chemother* **26**, 328–334.
- van Belkum, M. J., Kok, J., Venema, G., Holo, H., Nes, I. F., Konings, W. N. & Abee, T. (1991). The bacteriocin lactococcin A specifically increases permeability of lactococcal cytoplasmic membranes in a voltage-independent, protein-mediated manner. *J Bacteriol* **173**, 7934–7941.
- Bhunia, A. K., Johnson, M. C. & Ray, B. (1987). Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J Ind Microbiol* **2**, 319–322.
- Bhunia, A. K., Johnson, M. C. & Ray, B. (1988). Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. *J Appl Bacteriol* **65**, 261–268.
- Costilow, R. N., Coughlin, F. M., Robach, D. L. & Ragheb, H. S. (1956). A study of the acid-forming bacteria from cucumber fermentations in Michigan. *Food Res* **21**, 27–33.
- Curioni, A., dal Belin Peruffo, A. & Nuti, M. P. (1988). Purification of cellulases from *Streptomyces* strain A20 by electroendosmotic preparative electrophoresis. *Electrophoresis* **9**, 327–330.
- Curioni, A., dal Belin Peruffo, A. & Pogna, N. E. (1989). Electroendosmotic preparative electrophoresis as a one-step method for purification of high molecular weight subunits of wheat glutenin. *Cereal Chem* **66**, 133–135.
- Daeschel, M. A. & Klaenhammer, T. R. (1985). Association of a 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. *Appl Environ Microbiol* **50**, 1538–1541.
- De Man, J. C., Rogosa, M. & Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *J Appl Bacteriol* **23**, 130–135.
- Etchells, J. L., Costilow, R. N., Anderson, T. E. & Bell, T. A. (1964). Pure culture fermentation of brined cucumbers. *Appl Microbiol* **12**, 523–535.
- Food and Drug Administration (1988). Nisin preparation: affirmation of GRAS status as a direct human food ingredient. *Fed Reg* April 6, 1988, **53**, 11247–11250.
- Fleming, H. P., Etchells, J. L. & Costilow, R. N. (1975). Microbial inhibition by an isolate of *Pedococcus* from cucumber brines. *Appl Microbiol* **30**, 1040–1042.
- Henderson, J. T., Chopko, A. L. & van Wassenaar, P. D. (1992). Purification and primary structure of pediocin PA-1 produced by *Pediococcus pentosaceus* PAC 1.0. *Arch Biochem Biophys* **295**, 5–12.
- Joerger, M. C. & Klaenhammer, T. R. (1986). Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. *J Bacteriol* **167**, 439–446.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Motlagh, A. M., Bhunia, A. K., Szostek, F., Hansen, T. R., Johnson, M. C. & Ray, B. (1992). Nucleotide and amino acid sequence of PAP-gene pediocin AcH production in *Pediococcus acidilactici* H. *Lett Appl Microbiol* **15** (2), 45–48.
- Mortvedt, C. I., Nissen-Meyer, J., Sletten, K. & Nes, I. F. (1991). Purification and amino acid sequence of lactocin S, a bacteriocin produced by *Lactobacillus sake* L45. *Appl Environ Microbiol* **57**, 1829–1834.
- Muriana, P. M. & Klaenhammer, T. R. (1991). Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. *Appl Environ Microbiol* **57**, 114–121.
- Piard, J. C., Muriana, P. M., Desmazeaud, M. J. & Klaenhammer, T. R. (1992). Purification and partial characterization of lactacin 481, a lanthionine-containing bacteriocin produced by *Lactococcus lactis* subs. *lactis* CNRZ 481. *Appl Environ Microbiol* **58**, 279–284.
- Pucci, M. J., Vedamuthu, E. R., Kunka, B. S. & Vandenberg, P. A. (1988). Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. *Appl Environ Microbiol* **54**, 2349–2353.
- Rammelsberg, M. & Radler, F. (1990). Antibacterial polypeptides of *Lactobacillus* species. *J Appl Bacteriol* **69**, 177–184.
- Reniero, R., Cocconcilli, P. S., Bottazzi, V. & Morelli, L. (1992). High frequency of conjugation in *Lactobacillus* mediated by an aggregation-promoting factor. *J Gen Microbiol* **138**, 763–768.
- Reniero, R., Piva, A., Morelli, L., Bottazzi, V. & Cocconcilli, P. S. (1993). Purification of *Lactobacillus* secreted proteins. *Biotechnol Tech* **7**, 401–406.
- Rueckert, P. W. (1979). *Studies on a bacteriocin-like activity produced by Pediococcus pentosaceus effective against gram-positive organisms*. MS thesis, Michigan State University, East Lansing.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning*:

a *Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Spelhaug, S. R. & Harlander, S. K. (1989). Inhibition of foodborne bacterial pathogens by bacteriocins from *Lactococcus lactis* and *Pediococcus pentosaceus*. *J Food Prot* **52**, 856–862.

Wilson, K. & Goulding, K. H. (1986). Purificazione degli enzimi. In *Biochimica Applicata*, pp. 90–93. Edited by M. Pilone Simonetta & G. Hanozet. Milan: R. Cortina.

Yousef, A. E., Luchansky, J. B., Degnan, A. J. & Doyle, M. P. (1991). Behavior of *Listeria monocytogenes* in weiner exudates in the presence of *Pediococcus acidilactici* H or pediocin AcH during storage at 4 or 25 °C. *Appl Environ Microbiol* **57**, 1461–1467.

Received 3 September 1993; revised 14 October 1993; accepted 22 October 1993.