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Bacteriocin plasmids of *Pediococcus acidilactici*

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SUMMARY

Pediococcus acidilactici strains E, F and H isolated from fermented sausages produced bacteriocins which were protein in nature and inhibitory to a variety of spoilage and pathogenic microorganisms often encountered in foods. These strains harbored two to three plasmids ranging in size from 7.4 to 40.2 megadaltons. Curing experiments and plasmid profile analysis indicated the involvement of plasmid DNA with bacteriocin activity in all three strains. Carbohydrate fermentation and antibiotic resistance phenotypes did not appear to be associated with bacteriocin plasmids. Both bacteriocin activity and resistance determinants were linked in strain H and mediated by a 7.4-megadalton plasmid, whereas in strains E and F these two traits were not linked.

INTRODUCTION

Several species and strains of pediococci have been used as starter cultures in the fermentation of vegetables [22], meats [8, 24], sausage products [25] and Cheddar cheese [11]. Pediococci are lactic acid bacteria which help to preserve fermented foods and contribute in flavor development [11, 15]. Some pediococcal strains are specifically capable of inhibiting the growth of related lactic acid bacteria and some other bacteria, including some which are associated with food spoilage and food-borne illness [2, 6, 10, 14]. In these strains the antimicrobial agents were shown to be neither lactic acid nor hydrogen peroxide, but bacteriocin-like compounds [25]. An understanding of the genetic control for bacteriocin production in these strains will be beneficial to use them effectively. Pediococci, like other members of lactic acid bacteria, have not yet been extensively characterized with respect to involvement of plasmid DNA for important metabolic traits. However,

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recent studies have shown that bacteriocin production in several pediococci is under plasmid control [5, 12–14]. This communication presents a modified method for plasmid isolation from three *Pediococcus acidilactici* strains, curing studies in relation to plasmid linkage of bacteriocin activity and resistance in these strains, and several properties of their bacteriocins.

MATERIALS AND METHODS

Bacterial strains and media

Three *P. acidilactici* strains possessing bacteriocin activity were isolated from different fermented sausages. They were designated E, F and H, and maintained in our laboratory stock culture collection. The cultures were grown in MRS broth [7] at 30°C unless stated otherwise. As necessary, 1.5% agar was added to MRS broth to make a solid medium. The soft agar, used for overlaying MRS agar in some studies, was prepared by adding 0.75% agar to the broth. *Escherichia coli* V517 was propagated in tryptic soy broth and it provided standard molecular weight (MW) markers to estimate the MW of *P. Acidilactici* plasmids in megadaltons (MDa). In most studies, *Lactobacillus plantarum* WSO-39 was used as an indicator strain for bacteriocin assay. The inhibitory activity of bacteriocins was also tested against several food spoilage and pathogenic bacteria (Table 1).

Bacteriocin activity assay

Cell-free medium from cultures of *P. acidilactici* strains E, F and H was heated in boiling water for 1 h. An indicator lawn on MRS agar surface was prepared by adding 10 μl of an overnight culture of *L. plantarum* WSO-39 (about 1×10^8 cells/ml) to 5 ml of MRS soft agar. Sterile paper discs (6.25 mm diameter) were placed on the indicator lawn and 20 μl of heat-treated supernatant medium was spotted on the discs. The plate was incubated at 30°C for 18–24 h and examined for zones of growth inhibition. The retention or loss of bacteriocin activity in the supernatant medium was also tested after autoclaving, neutralizing to pH 7.0, and treating with catalase, DNase or protease. The media used for testing bacteriocin activity against food spoilage and pathogenic bacteria are listed in Table 1.

Curing methods

Elimination of bacteriocin activity was accomplished [19] by growing *P. acidilactici* strains in MRS broth containing acriflavine (5–25 μg/ml), ethidium bromide (3–18 μg/ml) or novobiocin (2–10 μg/ml) with three consecutive transfers in a 24-h period or by growing the cultures at 50°C for 24 h. After centrifugation and washing, the cells were diluted and cultured in pourplates. Plates with 10–25 isolated colonies were overlaid with MRS soft agar containing *L. plantarum* WSO-39 as before and incubated overnight. Colonies without a zone of growth inhibition were considered as bacteriocin-negative (Bac^-) mutants. The mutants were purified and tested again for inability to inhibit the growth of *L. plantarum* WSO-39.

Carbohydrate utilization test

A double-strength MRS basal broth, containing hydrolyzed casein instead of beef extract and without dextrose, was used. Individual solutions (10%, w/v) of 16 carbohydrates were sterilized and added to the basal broth at a final concentration of 1%. Bromo cresol purple (BCP) at a final concentration of 0.008% was used as pH indicator. Overnight cultures of *P. acidilactici* strains were washed and resuspended in the same volume of sterile 0.85% NaCl. Each tube containing 10 ml of carbohydrate broth was inoculated with 10 μl of cell suspension. Acid production (yellow color) after 24–48 h incubation at 30°C was considered as utilization of carbohydrate.

The carbohydrates utilized by bacteriocin-producing (Bac^+) wild strains were then employed in sugar indicator agar plates [19] with 0.005% (w/v) 2,3,5-triphenyl tetrazolium chloride to examine their utilization by Bac^- mutants. Strains capable of fermenting a sugar would produce white colonies, while the nonfermenters would produce red colonies.

Antibiotic sensitivity test

An overnight culture (10 μl) of Bac^+ strain or Bac^- mutant was mixed with 5 ml of MRS soft agar and poured onto MRS agar plates [27]. Commercially available sensi-discs (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, MD 21030, U.S.A.) of 31 different antibiotics were placed on the agar surface and diffusion was allowed to occur overnight at 4°C. The plates were incubated at 30°C for 24–48 h. Antibiotic sensitivity was defined as the presence of at least a 2 mm zone of growth inhibition around a disc.

Sensitivity or resistance of Bac^- mutant to bacteriocin

Small wells were made in an MRS agar plate with a cork-borer of diameter 4 mm. Freshly grown culture (20 μl) of Bac^+ strain was added to each well and sealed with soft agar. Then the MRS agar surface was overlaid with a lawn of the corresponding Bac^- mutant. The plates were incubated overnight and examined for growth inhibition of Bac^- lawn.

Cell lysis with lysozyme and mutanolysin

Each *P. acidilactici* strain (1% inoculum) was grown to mid-exponential phase in 100 ml MRS broth supplemented with DL-threonine [27]. Cells were washed with TES buffer [18] and resuspended in ST buffer [16] to a final optical density (O.D.) at 600 nm of 1.0. Six 900-μl aliquots were removed in Eppendorf tubes and held on ice for 10 min. Lysozyme or mutanolysin (100 μl of a 1-mg enzyme/ml ST buffer solution) was added to two aliquots. Only ST buffer (100 μl) was added to the control. For each strain, a set of three tubes (lysozyme, mutanolysin and control) was incubated at 0°C and another set at 37°C. A 250-μl sample was removed from each tube at 30 min and another at 60 min, and each sample was added to a cuvette containing 2 ml of 50 mM Tris-HCl, pH 7.0. After mixing the contents, the O.D. at 600 nm was measured in a Beckman DU-50 Spectrophotometer. The percent lysis by each enzyme at 30 or 60 min was determined considering the O.D. of control as 100%.

Isolation of plasmid DNA

A modification of two methods [1, 16] was developed that provided better isolation of plasmid DNA from *P. acidilactici* strains. The inoculum (2%) from an overnight culture was added to 50 ml of MRS broth containing 20 mM DL-threonine. Cells were grown at 37°C to an O.D. at 600 nm of 0.55. Pelleted cells were washed with TES buffer and resuspended in 5 ml of cold (4°C) sucrose buffer (5% w/v sucrose, 50 mM Tris-HCl, 5 mM EDTA, pH 8.0). The sample was cooled on ice for 10 min. A mixture [20] of 500 μl of lysozyme and mutanolysin (0.5 mg of each enzyme/ml in 50 mM Tris-HCl, 5 mM EDTA, pH 8.0) was added. The sample was incubated at 37°C for 1 h. Following this, 1 ml of SDS buffer (20% w/v SDS, 50 mM Tris, 20 mM EDTA, pH 8.0) was added and mixed on an orbital shaker at 200 rpm for 3 min. Freshly prepared 3 N NaOH (about 300 μl) was added dropwise to adjust the pH to 12.25 and the solution was mixed on the orbital shaker at 200 rpm for 10 min. The sample was heated in a water bath at 62°C for 30 min and cooled at room temperature for 15 min. Then, 2 M Tris-HCl, pH 7.0 (about 600 μl) was added rapidly to drop the pH of the sample to 8.75. After gentle mixing for 3 min, 0.12 volume of 5 M NaCl was added and incubated at 0°C for 30 min. The sample was treated twice with an equal volume of 3% NaCl-saturated phenol, vortexed slowly for 15 s and centrifuged at 5000 rpm in a swinging bucket rotor for 20 min. The aqueous phase (7 ml) was transferred to another tube, mixed with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged at 5000 rpm in a swinging bucket rotor for 10 min. The clear aqueous phase (6 ml) was removed to another tube and mixed with equal volume of isopropanol. The sample was held at −70°C for at least 1 h. DNA was pelleted by centrifugation at 16 000 rpm for 20 min at 4°C. The supernatant fluid was discarded, DNA was dried under vacuum and resuspended in 100 μl of TES buffer.

Purification of plasmid DNA

This was accomplished by cesium chloride-ethidi-

um bromide (CsCl-EtBr) density gradient ultracentrifugation at a final density of 1.55–1.57 g/ml [26]. The DNA isolated from 100 ml of culture was suspended in 2.5 ml of TE buffer (50 mM Tris, 20 mM EDTA, pH 7.5) and treated with 0.2 ml of RNase (100 μg/ml in TE buffer). To this was added 6.5 ml of CsCl (8.66 g/ml in TE buffer) and 0.8 ml of EtBr (10 mg/ml in TE buffer). The mixture was transferred to a Beckman polyallomer quick-seal centrifuge tube (16 × 76 mm). The remaining space in the tube was filled with sterile mineral oil. After sealing the tube, it was centrifuged at 45 000 rpm for 48 h in a Beckman 70.1 Ti rotor at 20°C. The plasmid DNA-EtBr complex, which banded below the chromosomal fraction, was removed with a syringe and No. 18 hypodermic needle. EtBr was extracted with (1:1) CsCl-saturated isopropanol/STE buffer (5 M NaCl, 10 mM Tris, 1 mM EDTA, pH 8.5), whereas CsCl was removed by filtration through an Amicon Centricon-30 microconcentrator according to the instructions of the manufacturer. The plasmid DNA was washed twice with TE buffer and resuspended in 100 μl of TES buffer.

Agarose gel electrophoresis

This was done after adding 25 μl of tracking dye [28] to 100 μl of plasmid DNA preparation. The sample was loaded on 0.7% agarose gel in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.2) and electrophoresis was conducted at 5 V/cm for 3–5 h. The gel was stained in EtBr (0.5 μg/ml) for 15 min, destained in 1 mM $MgSO_4$ for 30 min, viewed against UV light and photographed with a Polaroid camera.

RESULTS

Bacteriocin activity of cell-free media

The heated cell-free media from *P. acidilactici* strains E, F and H produced large zones of inhibition against *L. plantarum* WSO-39 (Fig. 1). The inhibitory activity of the supernatant medium was retained after autoclaving, neutralization to pH 7.0 and treatments with catalase and DNase, but disappeared after protease treatment (Table 1). The culture medium from all three strains also inhibited the growth of *L. viridiscens, Leuconostoc mesenteroides, Listeria monocytogenes* and *Clostridium perfringens*; however, the growth of *Staphylococcus aureus* was inhibited by the medium from H more effectively than that from strains E and F (Table 1).

Efficiency of lysozyme and mutanolysin in lysing the cells

The lysis of *P. acidilactici* E, F and H cells by either lysozyme or mutanolysin during incubation at 0–37°C for 30 or 60 min was examined (Table 2). At 37°C, mutanolysin was relatively more effective than lysozyme for lysis of all three strains.

Plasmid profile of wild strains

The *P. acidilactici* strains E, F and H were lysed with a mixture of lysozyme and mutanolysin to examine the presence of plasmid DNA. Strains E and H harbored three plasmids, whereas strain F harbored only two plasmids (Fig. 2). The plasmid sizes (Table 3) were calculated from a calibration curve of log MW (in MDa) of the standard plasmids of *E. coli* V517 versus their respective mobility (in mm) in a 0.7% agarose gel. In the three strains the size of

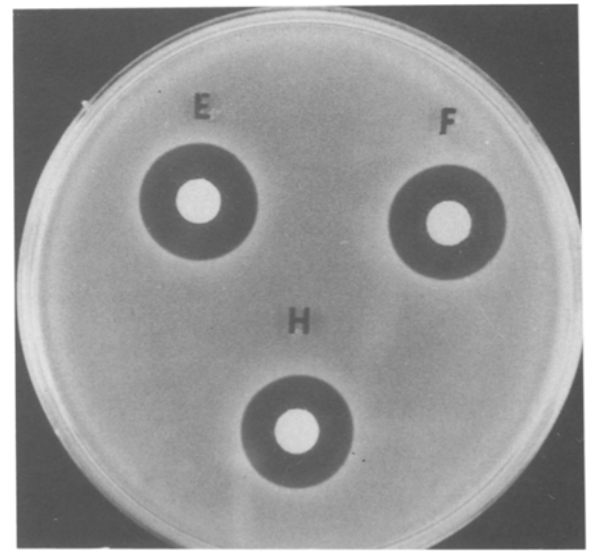

Fig. 1. Agar-disc assay showing the growth inhibition of indicator *L. plantarum* WSO-39 by heated cell-free medium from strain E (top left), F (top right) and H (bottom).

Table 1

Properties of bacteriocins from *P. acidilactici* strains

Bacteriocin activity tested against[a]	Source	Inhibition[b] by bacteriocin from *P. acidilactici* strain		
		E	F	H
L. plantarum WSO-39 after:	North Carolina State University			
autoclaving		+	+	+
neutralizing to pH 7.0		+	+	+
catalase treatment		+	+	+
DNase treatment		+	+	+
protease treatment		−	−	−
L. viridiscens	ATCC	+	+	+
Leuc. mesenteriodes	Oregon State University	+	+	+
List. monocytogenes	Silliker Lab, Chicago, IL	+	+	+
C. perfringens	FDA, Washington, DC.	+	+	+
S. aureus	FDA, Washington, DC.	±	±	+

[a] Cell-free supernatants were heated in a boiling water bath for 1 h and then used to test activity against these strains. About 1×10^6 cells of each strain were used for making indicator lawn on the agar plate. Autoclaving was done at 121°C for 15 min. The supernatant was neutralized to pH 7.0 with sterile 0.1 N NaOH. Catalase (200 IU/ml), DNase (100 μg/ml) and protease (200 μg/ml) treatments were done at 37°C for 30 min. The media used were MRS agar for *L. viridiscens* and *Leuc. mesenteroides*, tryptic soy agar for *List. monocytogenes* and *S. aureus* and thioglycollate agar for *C. perfringens*.

[b] Inhibition was determined by the presence (+) or absence (−) of a 2 mm or larger clear zone around the disc containing 20 μl of treated cell-free medium.

small plasmids ranged from 7.4 to 8.0 MDa and the medium plasmids ranged from 23.0 to 24.5 MDa; however, the size of large plasmids in strains E and H were about 40.0 MDa.

Table 2

Efficiency of lysozyme and mutanolysin in lysing three *P. acidilactici* strains

Strain	Enzyme (100 μg/ml)	% Lysis[a]			
		0°C		37°C	
		30 min	60 min	30 min	60 min
P. acidilactici E	lysozyme	1.7	4.3	83.7	91.4
	mutanolysin	8.4	9.8	97.5	98.3
P. acidilactici F	lysozyme	1.1	6.0	88.9	95.0
	mutanolysin	7.7	14.1	98.6	98.6
P. acidilactici H	lysozyme	5.3	12.7	85.0	96.2
	mutanolysin	23.8	56.6	99.5	100.0

[a] Calculated as percent loss in O.D. at 600 nm as compared to control sample containing cells without enzyme.

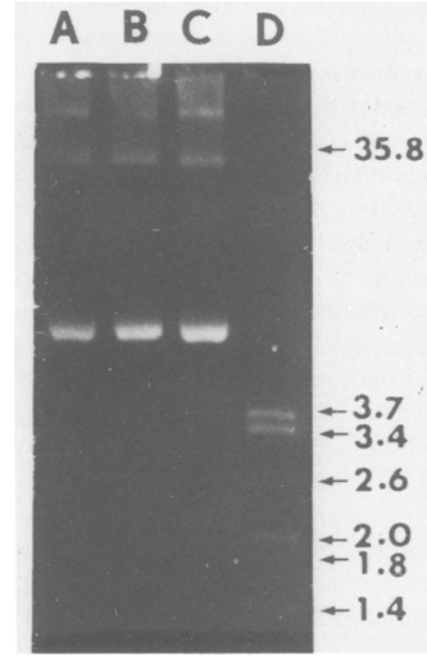

Fig. 2. Agarose gel electrophoresis of plasmid DNA isolated from Bac$^+$ strains used in this study. Lane A, *P. acidilactici* E; lane B, *P. acidilactici* F; lane C, *P. acidilactici* H; and lane D, *E. coli* V517 standard. The numbers on the right indicate molecular mass of standard plasmids in MDa.

Table 3

Plasmid profiles of *P. acidilactici* strains and mutants derived therefrom

Strains[a]	Description[b]	Plasmid contents (MDa)	Source
P. acidilactici E	wild, Bac^+Bac^r	40.2, 23.0, 8.0	this study
NB1	mutant, Bac^-Bac^r	40.2, 23.0	this study
P. acidilactici F	wild, Bac^+Bac^r	24.5, 7.8	this study
AF1	mutant, Bac^-Bac^r	24.5	this study
P. acidilactici H	wild, Bac^+Bac^r	40.0, 23.0, 7.4	this study
AF8	mutant, Bac^-Bac^s	40.0, 23.0	this study
E. coli V517	source of reference plasmids	35.8, 3.7, 3.4, 2.6 2.0, 1.8, 1.4	E.M. Lederberg[c]

[a] Bacteriocin-non-producing mutants derived from each strain after treatment with various curing agents had the same plasmid profiles. NB, novobiocin and AF, acriflavine induced mutants.

[b] Bac^+, bacteriocin-producing; Bac^-, bacteriocin-non-producing; Bac^r, bacteriocin-resistant; Bac^s, bacteriocin-sensitive.

[c] Plasmid Reference Center, Stanford University School of Medicine, CA 94305, U.S.A.

Plasmid curing for phenotypic assignment of bacteriocin activity and immunity

There were some spontaneously generated bacteriocin-negative (Bac^-) mutants from each bacteriocin-producing (Bac^+) strain. The occurrence of Bac^- mutants increased in each strain by growing the cells either with chemical curing agents or at elevated temperature (data not shown). Comparison of plasmid contents (Fig. 3) of Bac^+ strains and Bac^- mutants indicated that bacteriocin activity was encoded by 8.0, 7.8 and 7.4 MDa plasmids in strains E, F and H, respectively (Table 3).

The possibility of the linkage of two traits, bacteriocin production (Bac^+) and host cell resistance to bacteriocin (Bac^r), was also studied (Fig. 4). The growth of Bac^- mutant derived from strain H was inhibited by its parent strain, whereas the growth of Bac^- mutants derived from strains E and F was not inhibited by their respective parent strains.

Carbohydrate utilization and antibiotic resistance patterns

The relationship between bacteriocin production and carbohydrate metabolism in *P. acidilactici* strains E, F and H was studied. The fermentable sugars (Table 4) such as glucose, sucrose, fructose, xylose, cellobiose, trehalose and mannose were used in Miller's indicator agar plates to examine their utilization by the mutants lacking bacteriocin plasmid. However, the Bac^- mutants and parental strains had similar carbohydrate utilization patterns. Also, antibiotic resistance patterns in Bac^-

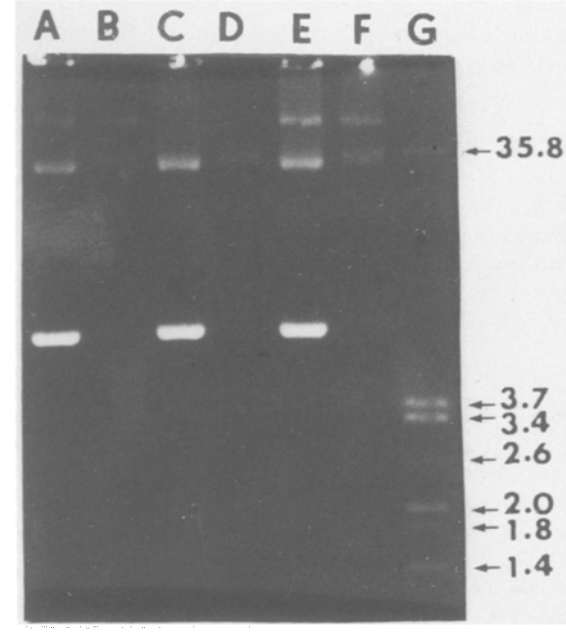

Fig. 3. Agarose gel electrophoresis of plasmid DNA isolated from Bac^+ strains and their Bac^- mutants. Lane A, E (Bac^+); lane B, E (Bac^-); lane C, F (Bac^+); lane D, F (Bac^-); lane E, H (Bac^+); lane F, H (Bac^-); and lane G, *E. coli* V517 standard. The numbers on the right indicate molecular mass of standard plasmids in MDa.

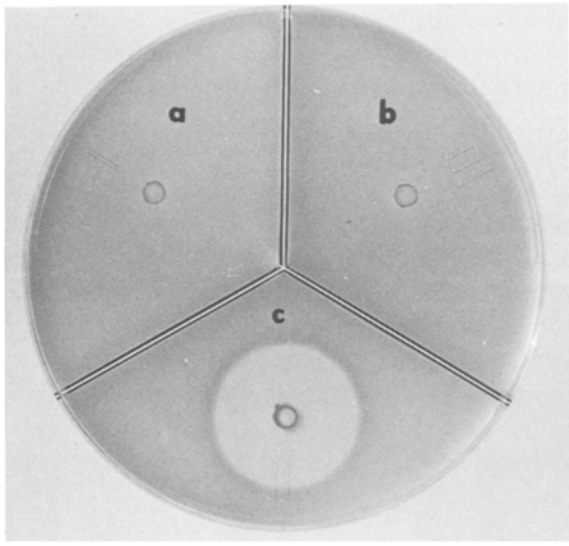

Fig. 4. Sensitivity of Bac$^-$ mutants to their respective parent strains. a, E (Bac$^-$) lawn is not inhibited by E (Bac$^+$); b, F (Bac$^-$) lawn is not inhibited by F (Bac$^+$); and c, H (Bac$^-$) lawn is inhibited around the well containing H (Bac$^+$).

mutants showed no change from the parental strains (Table 4).

DISCUSSION

The antimicrobial activity of *P. acidilactici* strains E, F and H reported here was not due to organic acid, hydrogen peroxide or bacteriophage as the cell-free media remained active following neutralization to pH 7.0, treatments with catalase and DNase or autoclaving. Treatment with protease destroyed activity indicating that the antimicrobial compounds could be heat-stable proteins and most probably bacteriocins [25]. There are reports [2, 12] that bacteriocins produced by *P. acidilactici* strains H and PAC1.0 remained active after heat treatment but became inactivated by proteolytic enzymes. The MW of these proteins differed greatly, about 2700 Da in strain H [2] and about 16

Table 4

Carbohydrate fermentation and antibiotic sensitivity patterns of *P. acidilactici* strains E, F and H

Carbohydrate fermentation[a]		Antibiotic sensitivity[b]	
+	−	R	S
Glucose	Lactose	Vancomycin (30 μg)	Erythromycin (15 μg)
Sucrose	Maltose	Polymyxin B (50 units)	Bacitracin (10 units)
Fructose	Glycerol	Streptomycin (10 μg)	Neomycin (30 μg)
Xylose	Sorbitol	Kanamycin (30 μg)	Penicillin (10 units)
Cellobiose	Mannitol	Cycloserine (20 μg)	Tetracycline (30 μg)
Trehalose	Melibiose	Amphotericin B (20 μg)	Chloramphenicol (30 μg)
Mannose	Inositol	Nalidixic acid (30 μg)	Rifampin (15 μg)
	Arabinose	Colistin (10 μg)	Chlortetracycline (30 μg)
	Starch	Griseofulvin (20 μg)	Ampicillin (10 μg)
			Novobiocin (5 μg)
			Actinomycin D (2 μg)
			Carbenicillin (20 μg)
			Oleandomycin (20 μg)
			Rifamycin SV (20 μg)
			Cephalothin (30 μg)
			Mytomycin C (5 μg)
			Cloxacellin (20 μg)
			Gentamycin (10 μg)
			Lincomycin (2 μg)
			Nitrofurantoin (300 μg)

[a] +, fermented; −, non-fermented at 30°C within 48 h.

[b] R, resistant; S, sensitive to antibiotic (concentration shown in bracket).

All three strains showed similar phenotypic patterns for these traits.

500 Da in PAC1.0 [12]. This difference could be due to differences in the techniques used. Our preliminary studies by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) indicated that the MW of bacteriocins produced by strains E and F were approximately 2700 Da (data not presented).

The bacteriocins from strains E, F and H inhibited the growth of food spoilage bacteria such as *L. viridiscens* and *Leuc. mesenteroides*, and food-borne pathogenic bacteria such as *List. monocytogenes, C. perfringens* and *S. aureus*. The bacteriocin from strain H, designated pediocin AcH, also inhibited the growth of other bacteria [3]. Other workers also reported similar results on bacteriocins from *P. acidilactici* and *P. pentosaceus* strains that were quite active against many taxonomically related and unrelated bacteria [5, 12–14].

The results of our study of lysis of *P. acidilactici* cells by lysozyme and mutanolysin (Table 2) are in good agreement with published reports [17, 20] that gram-positive lactic acid bacteria are more sensitive to mutanolysin than lysozyme. However, we used both enzymes to get better lysis.

The bacteriocin production traits in strains E, F and H are encoded by plasmids which are quite similar in MW, ranging from 7.4 to 8.0 MDa. In other reports on plasmid-associated bacteriocin production by strains of *P acidilactici* and *P. pentosaceus*, the MW of plasmids ranged from 5.5 to 13.5 MDa [5, 12–14].

In our study it was evident that the bacteriocin plasmid in strain H encoded for both the traits bacteriocin production (Bac^+) and resistance to bacteriocin (Bac^r), since its Bac^- mutants are sensitive to bacteriocin (Bac^s). However, in strains E and F the Bac^r is not linked to bacteriocin plasmid as their Bac^- mutants are also Bac^r. The linkage of the traits Bac^+ and Bac^r was also observed [5] in *P. pentosaceus* FBB61, and the absence of the linkage of these two traits was reported [12] in *P. acidilactici* PAC1.0. Moreover, our studies demonstrated that bacteriocin plasmids of strains E, F and H do not control either fermentation of carbohydrates or antibiotic resistance (Table 4). Although strains E, F and H could not be differentiated on the basis of these phenotypic characteristics, their plasmid profiles as well as the linkage of Bac^+ and Bac^r traits in strain H indicated a distinct identity of each of the three strains.

Strains E, F and H of *P. acidilactici* possessing Bac^+ phenotype may effectively be employed in meat fermentation. Besides their ability to produce lactic acid, they are effective in controlling the growth of many spoilage and pathogenic bacteria during fermentation. Their active proteins may also have potential as biopreservatives in a variety of perishable foods. Future studies may prove them useful in constructing ideal plasmid cloning vectors to design new starter strains with desirable properties.

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REFERENCES

1 Anderson, D.G. and L.L. McKay. 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Microbiol. 46: 549–552.

2 Bhunia, A., M.C. Johnson and B. Ray. 1987. Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulphate-polyacrylamide gel electrophoresis. J. Ind. Microbiol. 2: 319–322.

3 Bhunia, A.K., M.C. Johnson and B. Ray. 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. J. Appl. Bacteriol. (in press).

4 Clewell, D.B. and D.R. Helinski. 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry 9: 4428–4440.

5 Daeschel, M.A. and T.R. Klaenhammer. 1985. Association of a 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl. Environ. Microbiol. 50: 1538-1541.

6 Daly, C., M. LaChance, W.E. Sandine and P.R. Elliker. 1973. Control of *Staphylococcus aureus* in sausage by starter cultures and chemical acidification. J. Food Sci. 38: 426–430.

7 De Man, J.C., M. Rogosa and M.E. Sharpe. 1960. A medium for the cultivation of lactobacilli, J. Appl. Bacteriol. 23: 130–135.

8 Diebel, R.H., G.D. Wilson and C.F. Niven, Jr. 1961. Microbiology of meat curing. IV. A lyophilized *Pediococcus cerevisiae* starter culture for fermented sausage. Appl. Microbiol. 9: 239–243.

9 Etchells, J.L., R.N. Costilow, T.E. Anderson and T.A. Bell. 1964. Pure culture fermentation of brined cucumbers. Appl. Microbiol. 12: 523–535.

10 Fleming, H.P., J.L. Etchells and R.N. Costilow. 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. Appl. Microbiol. 30: 1040–1042.

11 Franklin, J.G. and M.E. Sharpe. 1963. The incidence of bacteria in cheese milk and Cheddar cheese and their association with flavor. J. Dairy Res. 30: 87–99.

12 Gonzalez, C.F. and B.S. Kunka. 1987. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. Appl. Environ. Microbiol. 53: 2534–2538.

13 Graham, D.C. and L. L. McKay. 1985. Plasmid DNA in strains of *Pediococcus cerevisiae* and *Pediococcus pentosaceus*. Appl. Environ. Microbiol. 50: 532–534.

14 Hoover, D.G., P.M. Walsh, K.M. Kolaetis and M.M. Daly. 1988. A bacteriocin produced by *Pediococcus* species associated with a 5.5-megadalton plasmid. J. Food Prot. 51: 29–31.

15 Keen, A.R., N.J. Walker and M.F. Peberdy. 1974. The formation of 2-butanone and 2-butanol in Cheddar cheese. J. Dairy Res. 41: 249–257.

16 Klaenhammer, T.R. 1984. A general method for plasmid isolation in lactobacilli. Curr. Microbiol. 10: 23–28.

17 Kondo, J.K. and L.L. McKay. 1982. Mutanolysin for improved lysis and rapid protoplast formation in dairy streptococci. J. Dairy Sci. 65: 1428–1431.

18 Meyers, J.A., D. Sanchez, L.P. Elwell and S. Falkow. 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. 127: 1529–1537.

19 Miller, J.H. 1972. Experiments in Molecular Genetics, p. 54, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

20 Monsen, T.J., S.E. Holm and L.G Burman. 1983. A general method for cell lysis and preparation of deoxyribonucleic acid from streptococci. FEMS Microbiol. Lett. 16: 19–24.

21 Muriana, P.M. and T.R. Klaenhammer. 1987. Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. Appl. Environ. Microbiol. 53: 553–560.

22 Pederson, C.S. 1949. The genus *Pediococcus*. Bacteriol. Rev. 13: 225–232.

23 Smith, J.L. and S.A. Palumbo. 1981. Microoorganisms as food additives. J. Food Prot. 44: 936–955.

24 Smith, J.L. and S.A. Palumbo. 1983. Use of starter cultures in meats. J. Food Prot. 46: 997–1006.

25 Tagg, J.R., A.S. Dajani and L.W. Wannamaker. 1976. Bacteriocins of gram-positive bacteria. Bacteriol. Rev. 40: 722–756.

26 Tsai, H.J. and W.E. Sandine. 1987. Conjugal transfer of nisin plasmid genes from *Streptococcus lactis* 7962 to *Leuconostoc dextranicum* 181. Appl. Environ. Microbiol. 53: 352–357.

27 Vescovo, M., L. Morelli and V. Bottazzi. 1982. Drug resistance plasmids in *Lactobacillus acidophilus* and *Lactobacillus reuteri*. Appl. Environ. Microbiol. 43: 50–56.

28 Walsh, P.M. and L.L. McKay. 1980. Restriction endonuclease analysis of the lactose plasmid in *Streptococcus lactis* ML3 and two recombinant lactose plasmids. Appl. Environ. Microbiol. 43: 1006–1010.