Plasmid-associated bacteriocin production by a *Lactobacillus sake* strain

CHRISTINA I. MØRTVEDT¹* and INGOLF F. NES²

¹Norwegian Food Research Institute, Osloveien 1, 1430 ÅS, Norway and ²Laboratory of Microbial Gene Technology, PO Box 51, 1432 ÅS-NLH, Norway

(Received 9 January 1990; revised 26 March 1990; accepted 11 April 1990)

A *Lactobacillus sake* strain L45 isolated from naturally fermented dry sausage, produced a bacteriocin designated lactocin S. The bacteriocin was moderately heat-stable and its activity was sensitive to proteases. Bacteriocin activity was found in the growth medium during the late exponential phase of growth and was directed against selected strains within the closely related genera *Lactobacillus*, *Leuconostoc* and *Pediococcus*. During propagation in liquid medium, non-bacteriocin-producing bacteria (Bac⁻) appeared with high frequency. Some isolates had also lost immunity to the bacteriocin (Imm⁻). Analyses of L45 Bac⁺Imm⁺ isolates revealed two plasmids of about 50 kb (pCIM1) and 34 kb (pCIM2). All of the L45 Bac⁻Imm⁻ variants had lost pCIM1. Three Bac⁻Imm⁺ isolates were found which still contained pCIM1. However, DNA restriction enzyme analyses disclosed differences between pCIM1 in the Bac⁺Imm⁺ and Bac⁻Imm⁺ isolates. These experiments strongly suggest that the pCIM1 plasmids are involved in production of the bacteriocin and in immunity to the bacteriocin.

Introduction

Lactic acid bacteria are of great significance in food fermentation. Strains of *Lactobacillus* are used as starter cultures for meat, vegetables, dairy and bakery products (Chassy, 1985).

Bacteriocins are proteins which show bactericidal activity towards closely related species (Tagg *et al.*, 1976). There are two main reasons for studying bacteriocins in lactobacilli. Firstly, bacteriocin-producing starter cultures may result in a more reliable fermentation process preventing growth of spoilage bacteria. Secondly, the genetic determinants for bacteriocin production and resistance to bacteriocins have great potential as genetic markers in recombinant DNA technology for application in the future production of food additives or supplements from micro-organisms.

Plasmid-encoded bacteriocins are commonly observed among both Gram-negative (Hardy, 1975; Cooper *et al.*, 1986) and Gram-positive bacteria (Tagg *et al.*, 1976; Scherwitz *et al.*, 1983; Daeschel & Klaenhammer, 1985; Kaletta & Entian, 1989). Bacteriocin production by strains of *Lactobacillus* has been reported by deKlerk & Smit, 1967; Upreti & Hinsdill, 1975; Barefoot & Klaenhammer, 1983; McCormick & Savage, 1983; Daeschel *et al.*, 1986; Joerger & Klaenhammer, 1986; Ferreira & Gilliland, 1988; West & Warner, 1988; Schillinger & Lücke, 1989. Most attempts to correlate

bacteriocin production with plasmids in *Lactobacillus* have not been successful (Upreti & Hinsdill, 1975; Barefoot & Klaenhammer, 1983; Joerger & Klaenhammer, 1986). There are only two reports on plasmid-linked bacteriocin activity in *Lactobacillus* species (Muriana & Klaenhammer, 1987; Schillinger & Lücke, 1989).

In this study, a *Lactobacillus sake* strain designated L45, which produces a bacteriocin-like compound termed lactocin S, has been isolated. Furthermore, different variants which have spontaneously lost both the ability to produce bacteriocin (Bac⁻) and host cell immunity (Imm⁻), or lost bacteriocin production ability only have been isolated, and the plasmid content of these isolates has been studied.

Methods

Bacterial cultures and media. The lactic acid bacteria used are described in Table 1. These were propagated in MRS broth (Difco). Strain L45 was identified as *Lactobacillus* by Gram-staining, growth at 8, 37 and 42 °C, and by studying the carbohydrate fermentation pattern (Api 50ch, Api system). Organic acids produced by L45 in MRS broth were analysed by HPLC using a Bio-Rad HPX 87H column according to a previously published method (Nes & Sørheim, 1984). Enzymic determination of D- and L-lactic acid was accomplished by using the L-lactic acid assay kit (Boehringer Mannheim) for determination of L-lactic acid in food (Nordal & Slinde, 1980).

Bacteriocin assay. The facultative heterofermentative lactobacilli isolated from naturally fermented sausages, listed in Table 1, were

screened for production of bacteriocin. Modifications of the deferred method (Mayr-Harting *et al.*, 1972) and a microtitre assay were as follows. In the deferred assay, overnight cultures were diluted in sterile saline (0.9% NaCl) and plated on MRS agar (1.5%, w/v). After 48 h of incubation at 30 °C, the plates were overlaid with soft agar (0.8%) seeded with an overnight culture of the indicator organism and incubated for an additional 12 h. Colonies surrounded by clear zones of inhibition indicated bacteriocin activity. The largest zones of inhibition were observed when *Pediococcus acidilactici* Pac 1.0 was used as indicator organism. Accordingly this strain was used as indicator strain in the routine test.

To quantify bacteriocin activity, a microtitre plate assay system was developed. Twofold dilutions of bacteriocin extracts in MRS were prepared in microtitre plates (Costar). Fresh indicator organism (20 µl; OD₆₀₀ = 0.1–0.6) was added and the plates were incubated overnight at 30 °C. Growth inhibition of the indicator organism was attributed to the bacteriocin activity. One bacteriocin unit (BU ml⁻¹) was defined as the reciprocal of the highest dilution exhibiting 50% growth inhibition of the indicator organism measured as 50% of maximum turbidity in the microtitre plate assay system.

Preparation of bacteriocin extracts. L45 bacteria grown overnight in MRS broth, were removed by centrifugation (10000 r.p.m. for 10 min, Sorvall centrifuge, SS-34 rotor). The supernatant fraction was adjusted to pH 6.5 with 5 M-NaOH, dialysed (cut off 12000, Spectrapor, Spectrum Medical Industries Inc.) against 10 mM-KH₂PO₄ containing 0.1 mM-EDTA (pH 7.0) and sterilized by filtration (Millex-GS, 0.22 µm, Millipore).

Sensitivity to proteolytic enzymes. Bacteriocin extracts were tested for sensitivity to trypsin (bovine pancreas type III-S, Sigma) and protease (*Streptomyces griseus* type XIV, Sigma). Trypsin was inactivated by adding trypsin inhibitor (Sigma).

DNA isolation and analyses. Plasmid DNA from *Lactobacillus* L45 was isolated by the alkaline lysis procedure (Maniatis *et al.*, 1982) using 20 mg lysozyme ml⁻¹ in the lysis buffer. Chromosomal DNA from L45 was isolated as described by Marmur (1961). DNA melting analysis was done (Marmur & Mandel, 1968) using a Beckman model 25 spectrophotometer equipped with a water-circulated, heated cuvette holder.

Plasmid DNA was treated with restriction enzymes obtained from Bethesda Research Laboratories and New England Biolabs. DNA was analysed by agarose gel electrophoresis (0.8% or 1.0%). The gel was stained with ethidium bromide and destained in distilled water.

Plasmid DNA molecular size markers were prepared from *Escherichia coli* V517 (Macrina *et al.*, 1978). Linear DNA molecular size markers were obtained by HindIII-digestion of λ DNA.

Restriction fragments of plasmid DNA were purified from agarose gels by using Gene Clean (Bio 101) and stored in TE buffer (10 mM-Tris/HCl, 1 mM-EDTA), pH 8.0.

DNA was transferred to nylon membranes (Hybond-N, Amersham) by electroblotting at 200 mA overnight at 4 °C. DNA was fixed to the membranes by illumination with UV light. The hybridization reaction and autoradiography were done as described by Southern (1975). DNA was labelled by nick translation with [α -³²P]dCTP according to the instructions of the enzyme supplier.

Isolation of Bac⁻ variants. For selection of non-bacteriocin variants, L45 was grown to the stationary phase of growth. The stationary culture was diluted and plated on MRS agar to obtain 20–30 colonies per plate. After 1–2 d of incubation replica-plating was done. The master agar plate was overlaid with soft agar seeded with indicator organisms. The next day, colonies that did not produce bacteriocin were isolated.

Results

Isolation and identification of the bacteriocin-producing Lactobacillus L45

The 39 facultative heterofermentative lactobacilli listed in Table 1 were isolated from Norwegian fermented dry sausages by standard procedures (Nordal & Slinde, 1984). Among the isolates one strain, designated L45, was found to produce a substance, termed lactocin S, which was inhibitory to growth of a variety of closely related bacteria within the genera *Lactobacillus*, *Pediococcus* and *Leuconostoc* (Table 1). Antagonistic activity (bacteriocin) was measured by the deferred assay (Fig. 1). Dairy lactococcus species were not inhibited by the substance produced by strain L45 (Table 1). Several Gram-negative bacteria (*E. coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Yersinia enterocolitica*) and Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Listeria seeligeri*) were also tested but no effect was observed (data not shown).

Strain L45 was a rod-shaped, facultatively heterofermentative, Gram-positive, catalase-negative, lactic-acid-producing bacterium. From glucose, L45 produced only lactic acid as a fermentation product, as demonstrated by HPLC analysis. Enzymic determination showed that about 60% and 40% L- and D-lactic acid, respectively, were formed in MRS broth. The bacterium was capable of growing in MRS broth at 8 °C and 45 °C and at high concentrations of NaCl (7.5%). DNA melting analysis revealed a guanine-plus-cytosine content of 41 ± 1%. For further characterization of L45, analyses of fermentable carbohydrates were done. L45 did not grow on mannitol, maltose, but grew well on ribose, mellibiose, sucrose and trehalose. Taken together these data indicate that L45 is a *Lactobacillus sake* strain (Kandler & Weiss, 1986; Schillinger & Lücke, 1987).

Properties of the bacteriocin activity

Antagonistic activity was found in the medium during the late exponential phase of growth. Usually 320 BU ml⁻¹ were obtained in the supernatant of a fresh overnight culture of L45. Activity was retained in the dialysis membrane tube after extensive dialysis to remove low-M_r compounds which may have an inhibitory effect on other bacteria.

Heat treatment for 1 h at 100 °C reduced the antagonistic activity by 50%. The activity appeared to be sensitive to proteases (80% inactivation in 1 h with 0.3 mg protease ml⁻¹). These experiments suggested that lactocin S is moderately heat-stable and contains a proteinaceous moiety.

Table 1. *Strains of lactic acid bacteria tested for sensitivity to L45*

Strains	Sensitivity	Source
<i>Lactobacillus</i> isolated from natural fermented sausages:		Our collection*
L48, 49, 50, 52, 53, 54,	+	
55, 60, 61, 62, 63, 64,	+	
65, 66, 69, 74, 78,	+	
44, 45, 46, 47, 51,	—	
56, 57, 58, 59, 68,	—	
70, 71, 72, 73, 75,	—	
76, 77, 79, 80, 81,	—	
83, 84	—	
<i>Lactobacillus plantarum</i> ATCC 14917	+	
WSO	+	M. Daeschel†
C11	+	M. Daeschel†
<i>Lactobacillus reuteri</i> DSM 2006	+	
<i>Lactobacillus acidophilus</i>	+	D. Lillehaug‡
<i>Lactobacillus lactis</i> Plom	+	Our collection
<i>Lactobacillus pentosaceus</i> ATCC 8041	+	
<i>Lactobacillus casei</i>		
ATCC 393	—	
NCDO 151	—	
<i>Lactococcus lactis</i> subsp. <i>cremoris</i> BC101	—	H. Holo‡
<i>Lactococcus lactis</i> subsp. <i>lactis</i>		
var. <i>diacetylactis</i>		
E2-6	—	Chr. Hansen,
184-2	—	Laboratory A/S,
		Denmark
<i>Lactococcus lactis</i> subsp. <i>lactis</i>		D. Lillehaug‡
L5	—	
L2	—	
<i>Leuconostoc mesenteroides</i> ATCC 8293	+	
<i>Pediococcus acidilactici</i> Pac 1.0	+	M. Daeschel†
<i>P. pentosaceus</i> 61.1	+	M. Daeschel†

* Norwegian Food Research Institute, Norway.

† Department of Food Science, North Carolina State University, USA.

‡ Laboratory of Microbial Gene Technology, Norway.

Instability of bacteriocin production

A decreased amount of bacteriocin activity was occasionally found in the culture medium. This observation led us to search for spontaneous loss of bacteriocin-producing bacteria within a population of L45. Starting from a single colony, L45 was grown to the stationary phase of growth. From approximately 3000 colonies tested for bacteriocin production, nine colonies were found not to produce bacteriocin. One of these isolates is shown in Fig. 1. These non-producers were stable. Several thousand colonies of the isolates that did not produce the bacteriocin were further cultivated but it appeared impossible to re-establish bacteriocin production by this procedure.

Plasmid DNA analyses

Frequent loss of biological activity can be attributed to plasmid instability. Consequently, plasmid analyses of

Table 2. *Characteristics of Lactobacillus sake* L45 variants

	Group				
	1	2	3	4	5
Bacteriocin production	+	+	—	—	—
Immunity to lactocin S	+	+	—	+	+
Plasmid pCIM1	+	+	—	+	+
pCIM2	+	—	+	+	—

the non-producing isolates and several bacteriocin producing colonies were done (Fig. 2). In conjunction with plasmid analyses, immunity to the bacteriocin was tested by the deferred assay. The isolates could be separated into five different groups according to their plasmid content and phenotype (Table 2).

Two groups of isolates exhibited the phenotype Bac⁺Imm⁺. The original bacteriocin-producing strain L45, belonging to group 1 (Fig. 2, lane 2), contained two plasmids of 50 kb (pCIM1) and 34 kb (pCIM2), respec-

Fig. 1. Inhibition of *Pediococcus acidilactici* Pac 1.0 by lactocin S demonstrated by clear zones. The arrow shows a non-producing colony (Bac⁻).

tively. The size of the plasmids was confirmed by restriction enzyme analyses using *Eco*RI. From approximately 100 Bac⁺Imm⁺ colonies tested, one isolate was found to contain only pCIM1 (Fig. 2, lane 3) and was designated 'group' 2.

Of the nine isolates that did not produce the bacteriocin, three groups termed 3, 4 and 5, consisting of 6, 2 and 1 isolates, respectively, were identified (Table 2). The six isolates of group 3 were Bac⁻Imm⁻. These strains had lost pCIM1 (Fig. 2, lane 4), suggesting a correlation between this plasmid and the genetic determinants for bacteriocin production and immunity.

The two variants of group 4 were isolated from a culture of group 1, whereas the only variant of 'group' 5 was isolated from a culture of 'group' 2. The isolates from groups 4 (Fig. 2, lane 5) and 5 (Fig. 2, lane 6) which harboured pCIM1 did not produce bacteriocin (Bac⁻), but were still immune to the bacteriocin (Imm⁺).

Restriction enzyme and DNA hybridization analyses of plasmids

To investigate further whether bacteriocin activity was associated with pCIM1, DNA restriction analysis of the plasmids taken from the various isolates was done. The results from *Eco*RI and *Hind*III digests are presented in Figs 3 and 4(a), respectively.

The Bac⁻Imm⁺ isolates of group 4 and the isolate from 'group' 5 showed different digestion patterns compared with Bac⁺Imm⁺ isolates (group 1 and 2). *Eco*RI-digested

Fig. 2. Agarose gel electrophoresis of plasmids isolated from *L. sake* variants. Lane 1; molecular size markers of plasmid DNAs from *E. coli* V517; 2, Bac⁺Imm⁺ isolate of group 1; 3, Bac⁺Imm⁺ isolate of 'group' 2; 4, Bac⁻Imm⁻ isolate of group 3; 5, Bac⁻Imm⁺ isolate of group 4; 6, Bac⁻Imm⁺ isolate of 'group' 5.

Fig. 3. Agarose gel electrophoresis of *Eco*RI-digested plasmids from various isolates. Lane 1, *Hind*III digestion of λ DNA (molecular size markers); 2, Bac⁺Imm⁺ isolate of group 1; 3, Bac⁻Imm⁺ isolate of group 4. The arrow indicates a DNA fragment of 1.5 kb. 4, Bac⁻Imm⁻ isolate of group 3; 5, Bac⁺Imm⁺ isolate of 'group' 2; 6, Bac⁻Imm⁺ isolate of 'group' 5. The arrowheads indicate bands of 6.5 kb and 2.4 kb in lane 6.

Fig. 4. (a) Agarose gel electrophoresis of *Hind*III-digested plasmids. (b) Autoradiogram prepared after hybridization with the [α - 32 P]dCTP-labelled 6.5 kb fragment of isolate of group 4. Lane 1, *Hind*III digestion of λ DNA (molecular size markers); 2, Bac⁺Imm⁺ isolate of group 1; 3, Bac⁻Imm⁺ isolate of group 4; 4, Bac⁻Imm⁻ isolate of group 3; 5, Bac⁺Imm⁺ isolate of 'group' 2; 6, Bac⁻Imm⁺ isolate of 'group' 5.

plasmids of group 4 (Bac⁻Imm⁺) revealed at least one new DNA fragment of approximately 1.5 kb (Fig. 3, lane 3) which was not found in the Bac⁺Imm⁺, wild-type strain (Fig. 3, lane 2). The plasmid from the isolate of 'group' 5 contained two new *Eco*RI fragments of approximately 6.5 kb and 2.4 kb, respectively (Fig. 3, lane 6). These *Eco*RI fragments may originate from the 8.4 kb fragment present in the parental group 2 (Fig. 3, lane 5).

When the two isolates of group 4 were digested with *Hind*III, a 6.5 kb DNA fragment was obtained (Fig. 4a, lane 3). The wild-type isolates of group 1 did not show this fragment (Fig. 4a, lane 2). This 6.5 kb *Hind*III fragment seemed to originate from a 7.5 kb *Hind*III fragment which is within a double DNA band in the wild-type plasmid isolates in group 1 (Fig. 4a, lane 2). *Hind*III-digests of pCIM1 isolated from 'group' 2 or from 'group' 5 bacteria gave similar results. The double 7.5 kb *Hind*III fragment (Fig. 4a, lane 5) was only found in the plasmid of 'group' 2. Change in the *Hind*III profile of pCIM1 in 'group' 5 was observed by increased staining of the 6 kb fragment (Fig. 4a, lane 6).

To investigate the relationship between the 6.5 kb DNA fragment and the other DNA fragments, hybridization experiments using the 6.5 kb fragment as a probe were done. The 6.5 kb fragment hybridized with a 7.5 kb fragment in strains of group 1 (Fig. 4b, lane 2) and of 'group' 2 (Fig. 4b, lane 5), which is in agreement with the suggestion mentioned above. The probe also hybridized

to a 6 kb fragment and a 3 kb fragment in the isolate of 'group' 5 (Fig. 4b, lane 6). There was no hybridization with plasmid DNA isolated from group 3. The hybridization experiment together with DNA restriction analyses strongly suggested that pCIM1 (Bac⁺Imm⁺) isolates from group 1 and 2 had some kind of alteration in a 7.5 kb *Hind*III fragment leading to a new 6.5 kb *Hind*III fragment in group 4 and to two new fragments in 'group' 5 of 6 kb and 3 kb, respectively. These changes in pCIM1 were concomitant with loss of bacteriocin production, but immunity to the bacteriocin was retained.

The restriction patterns of the *Hind*III digest of pCIM1 and pCIM2 were very similar except for the unique molecular size fragments of 7.5, 6.0 and 4.4 kb found in pCIM1 (Fig. 4a). Southern blot analysis showed that nick-translated pCIM2 hybridized quantitatively to the common *Hind*III fragments of pCIM1 (data not shown). However, when pCIM1 was used as a radioactive probe, quantitative hybridization to all DNA fragments of pCIM2 was observed (data not shown). These data suggested that the 34 kb pCIM2 plasmid constitutes a part of the 50 kb pCIM1 plasmid. pCIM2 appears to be a modified form of pCIM1.

Discussion

In general, bacteriocins found in lactobacilli have been characterized as proteinaceous antagonists, displaying a

narrow range of inhibitory activity towards closely related species within *Lactobacillaceae* (Klaenhammer, 1988). The bacteriocin described in this study inhibited selected members of *Pediococcus* and *Leuconostoc* as well as *Lactobacillus*, but not strains of *Lactococcus*. It has previously been shown that among lactic acid bacteria a *Lactococcus* subcluster can clearly be distinguished, whereas the genus *Lactobacillus* is not as clearly separated from the genera *Leuconostoc* and *Pediococcus* as *Lactococcus* (Kandler & Weiss, 1986).

The antimicrobial activity was tested under conditions which eliminate the effects of low- M_r compounds, indicating that inhibition was not due to organic acids or hydrogen peroxide. The antagonistic effect was reduced upon heat treatment and was lost upon incubation of the antagonistic substances with proteases, implying the presence of an essential protein or peptide moiety in the antagonistic compound. Consequently, the antimicrobial compound produced by L45 should be defined as a bacteriocin. This bacteriocin was termed lactocin S.

Among the very few bacteriocins studied in *Lactobacillus* there are only two reports that demonstrate a linkage between plasmid genes and bacteriocin production and immunity (Muriana & Klaenhammer, 1987; Schillinger & Lücke, 1989).

The bacteriocin produced by *Lactobacillus sake*, sakacin A, identified by Schillinger & Lücke (1989), possesses some of the characteristics of lactocin S. Both exhibit inhibitory activity against closely related bacteria, although not to identical strains. It was suggested that sakacin A and its immunity gene were associated with a 18 MDa plasmid (28 kb) which, in contrast to pCIM1 is rather stable.

Evidence for plasmid-encoded bacteriocin production is normally obtained by plasmid curing (Upreti & Hinsdill, 1975; Barefoot & Klaenhammer, 1983; Daeschel *et al.*, 1986; Gonzales & Kunka, 1987; Hoover *et al.*, 1988; Schillinger & Lücke, 1989). In this study, spontaneous loss of bacteriocin activity as well as immunity was observed with simultaneous loss or modification of pCIM1, whereas loss of pCIM2 did not provoke any changes with respect to these two properties. The fact that the Bac⁻ isolates sometimes contained the pCIM1 plasmid does not disprove our interpretation. These isolates (Bac⁻) had an altered DNA restriction fragment pattern compared to the Bac⁺ plasmids. These changes could affect the bacteriocin-producing ability without affecting host immunity to the bacteriocin.

The changes observed in the Bac⁻Imm⁺ isolates retaining pCIM1 could be due to deletions or rearrangements caused by transposition. Gasson (1983) reported that the 52 kb plasmid pLP712 in *Lactococcus lactis* NCDO 712, which encodes enzymes involved in lactose and protein utilization, was unusually prone to deletion.

Loss of these activities could be explained by this phenomenon. Plasmid rearrangements have also been observed in *Lactobacillus plantarum* (Husby & Nes, 1986).

There is only one report on a transposition-like phenomenon in *Lactobacillus*. Shimizu-Kadota *et al.* (1988) identified insertion sequences (ISL1) from *Lactobacillus casei*. In two of the *Lactobacillus casei* strains, the ISL1-like sequences were found on plasmids associated with lactose metabolism. It has also been shown by Aukrust & Nes (1988) that pTV1, a Tn917-carrying plasmid, transposes at high frequency into plasmids of *Lactobacillus plantarum*.

Hybridization experiments showed that all fragments in pCIM2 were found in pCIM1. pCIM2 could therefore be a deleted form of pCIM1. Because of the extensive homology between pCIM1 and pCIM2 one would expect the plasmids to be incompatible (Novick, 1987). However, pCIM1 may carry a second and dominant compatible replicon.

On the basis of the observations presented in this study it is proposed that the 50 kb pCIM1 plasmid in *Lactobacillus sake* L45 is involved in coding both a bacteriocin and host immunity. This plasmid is prone to instability and to changes affecting bacteriocin production. The strongest evidence for plasmid-encoded bacteriocin production and host cell immunity to the bacteriocin would be obtained by re-introducing pCIM1 into a cured strain to re-establish these properties. However, a functional DNA transformation system for *Lactobacillus sake* is not yet available.

This study was supported by both a fellowship to C.I.M. and a research grant from the Agricultural Research Council of Norway.

References

- AUKRUST, T. & NES, I. F. (1988). Transformation of *Lactobacillus plantarum* with the plasmid pTV1 by electroporation. *FEMS Microbiology Letters* **52**, 127–132.
- BAREFOOT, S. F. & KLAENHAMMER, T. R. (1983). Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology* **45**, 1808–1815.
- CHASSY, B. M. (1985). Prospects for improving economically significant *Lactobacillus* strains by 'genetic technology'. *Trends in Biotechnology* **3**, 273–275.
- COOPER, P. C., HAWKINS, F. K. L. & JAMES, R. (1986). Incompatibility between E colicin plasmids. *Journal of General Microbiology* **132**, 1859–1862.
- DAESCHEL, M. A., MCKENNY, M. C. & McDONALD, L. C. (1986). Characterization of a bacteriocin from *Lactobacillus plantarum*. *Abstracts of the Annual Meeting of the American Society for Microbiology* **86**, 277.
- DAESCHEL, M. A. & KLAENHAMMER, T. R. (1985). Association of a 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. *Applied and Environmental Microbiology* **50**, 1538–1541.
- DEKLERK, H. C. & SMIT, J. A. (1967). Properties of a *Lactobacillus fermenti* bacteriocin. *Journal of General Microbiology* **48**, 309–316.

- FERREIRA, C. L. & GILLILAND, S. E. (1988). Bacteriocin involved in premature death of *Lactobacillus acidophilus* NCFM during growth at pH 6. *Journal of Dairy Science* **71**, 306–315.
- GASSON, M. J. (1983). Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. *Journal of Bacteriology* **154**, 1–9.
- GONZALES, C. F. & KUNKA, B. S. (1987). Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. *Applied and Environmental Microbiology* **53**, 2534–2538.
- HARDY, K. G. (1975). Colicinogeny and related phenomena. *Bacteriological Reviews* **39**, 464–515.
- HOOVER, D. G., WALSH, P. M., KOLAETIS, K. M. & DALY, M. M. (1988). A bacteriocin produced by *Pediococcus* species associated with a 5.5-megadalton plasmid. *Journal of Food Protection* **51**, 29–31.
- HUSBY, K. O. v. & NES, I. F. (1986). Changes in the plasmid profile of *Lactobacillus plantarum* obtained from commercial meat starter cultures. *Journal of Applied Bacteriology* **60**, 413–417.
- 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. *Journal of Bacteriology* **167**, 439–446.
- KALETTA, C. & ENTIAN, K. D. (1989). Nisin, a peptide antibiotic: cloning and sequencing of the *nisA* gene and posttranslational processing of its peptide product. *Journal of Bacteriology* **171**, 1597–1601.
- KANDLER, O. & WEISS, N. (1986). *Lactobacillus sake*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, p. 1229. Edited by P. H. A. Sneath. Baltimore: Williams & Wilkins.
- KLAENHAMMER, T. R. (1988). Bacteriocins of lactic acid bacteria. *Biochimie (Paris)* **70**, 337–349.
- MACRINA, F. L., KOPEICO, D. J., JONES, K. R., AYERS, D. J. & MCCOWEN, S. M. (1978). A multiple plasmid-containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. *Plasmid* **1**, 417–420.
- MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: a Laboratory Manual*, pp. 368–369. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- MARMUR, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *Journal of Molecular Biology* **3**, 208–218.
- MARMUR, J. & MANDEL, M. (1968). Use of ultraviolet absorbance – temperature profile for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **12**, 195–206.
- MAYR-HARTING, A., HEDGES, A. J. & BERKELEY, R. C. W. (1972). Methods for studying bacteriocins. *Methods in Microbiology* **7**, 315–422.
- MCCORMICK, E. L. & SAVAGE, D. C. (1983). Characterization of *Lactobacillus* sp. strain 100-37 from the murine gastrointestinal tract: ecology, plasmid content and antagonistic activity toward *Clostridium ramosum* H1. *Applied and Environmental Microbiology* **46**, 1103–1112.
- MURIANA, P. M. & KLAENHAMMER, T. R. (1987). Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. *Applied and Environmental Microbiology* **53**, 553–560.
- NES, I. F. & SØRHEIM, O. (1984). Effect of infection of a bacteriophage in a starter culture during the production of salami dry sausage: a model study. *Journal of Food Science* **49**, 337–340.
- NORDAL, J. & SLINDE, E. (1980). Characteristics of some lactic acid bacteria used as starter cultures in dry sausage production. *Applied and Environmental Microbiology* **40**, 472–475.
- NOVICK, R. P. (1987). Plasmid incompatibility. *Microbiological Reviews* **51**, 381–395.
- SCHERWITZ, K. M., BALDWIN, K. A. & MCKAY, L. L. (1983). Plasmid linkage of a bacteriocin-like substance in *Streptococcus lactis* subsp. *diacetylactis* strain WM₄: transferability to *Streptococcus lactis*. *Applied and Environmental Microbiology* **45**, 1506–1512.
- SCHILLINGER, U. & LÜCKE, F.-K. (1987). Identification of lactobacilli from meat and meat products. *Food Microbiology (London)* **4**, 199–208.
- SCHILLINGER, U. & LÜCKE, F.-K. (1989). Antibacterial activity of *Lactobacillus sake* isolated from meat. *Applied and Environmental Microbiology* **55**, 1901–1906.
- SHIMIZU-KADOTA, M., FLICKINGER, J. L. & CHASSY, B. M. (1988). Evidence that *Lactobacillus casei* insertion element ISL1 has a narrow host range. *Journal of Bacteriology* **170**, 4976–4978.
- SOUTHERN, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503–517.
- TAGG, J. R., DAJANI, A. S. & WANNAMAKER, L. W. (1976). Bacteriocins of Gram-positive bacteria. *Bacteriological Reviews* **40**, 722–756.
- UPRETI, G. C. & HINS DILL, R. D. (1975). Production and mode of action of lactocin 27: bacteriocin from a homofermentative *Lactobacillus*. *Antimicrobial Agents and Chemotherapy* **7**, 139–145.
- WEST, C. A. & WARNER, P. J. (1988). Plantacin B, a bacteriocin produced by *Lactobacillus plantarum* NCDO 1193. *FEMS Microbiology Letters* **49**, 163–165.