International Journal of Food Microbiology, 16 (1992) 141–151

FOOD 00510

Identification and characterization of two bacteriocin-producing strains of *Lactococcus lactis* isolated from vegetables *

L. Uhlman [1], U. Schillinger, J.R. Rupnow [1] and W.H. Holzapfel

Federal Research Centre for Nutrition, Institute of Hygiene and Toxicology, Karlsruhe, Germany

(Received 24 September 1991; accepted 17 April 1992)

Isolated from mixed salad and fermented carrots, 123 strains of lactic acid bacteria were screened for bacteriocin production. Two strains, D53 and 23, identified as *Lactococcus lactis* by DNA-DNA hybridizations, produced heat stable bacteriocins which were resistant to trypsin and pepsin, but were inactivated by α-chymotrypsin and proteinase K. The bacteriocins were active from pH 2 to 9 and inhibited species of *Listeria*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Bacillus* and *Staphylococcus*. Strain D53 produced bacteriocin at pH values of 4.5–8.0 and from 10 to 37°C.

Key words: Bacteriocin; *Lactococcus lactis*; Nisin-like; Vegetable

Introduction

A trend toward convenient yet healthy foods is being seen as part of a modern lifestyle. Typically, ready-to-eat vegetable salads are becoming more popular and can be found in almost every supermarket. Due to their origin, vegetables may contain heavy microbial loads which contribute to spoilage of the salads and may lead to foodborne illness.

Spoilage may be caused by lactic acid bacteria or yeasts. Carlin et al. (1989) observed that the growth of lactic acid bacteria, especially of *Leuconostoc mesenteroides* was responsible for a loss of firmness and the development of off flavours in ready-to-use grated carrots.

Other Gram-positive bacteria are known to cause food intoxications and infections, such as *Staphylococcus aureus* and *Bacillus cereus*, especially in heated,

Correspondence address: U. Schillinger, Federal Research Centre for Nutrition, Institute of Hygiene and Toxicology, Engesserstr. 20, W-7500 Karlsruhe 1, Germany. Tel.: 49-721-6625115; Fax: 49-721-6625111.

* Published as paper No 9693, J. Series, Nebraska Agricultural Research Division Lincoln, NE 68583-0919, USA.

[1] Present address: University of Nebraska Department of Food Science and Technology, Lincoln, NE 69583-0919, USA. Tel. (402) 472-2823.

cooked and processed vegetables (Zollinger, 1990). *Clostridium botulinum* types A and B were isolated from foods of plant origin, such as mushrooms, green peppers and cabbage and type A was able to grow and to produce toxin in shredded cabbage stored under modified atmosphere (Salomon et al., 1990). In recent years, *Listeria monocytogenes* has emerged as a food-borne pathogen of major concern and several studies have been conducted dealing with the occurrence of *Listeria monocytogenes* in different food environments. In one study (Sizmur and Walker, 1988), 60 prepared salads were purchased from local supermarkets and examined for the presence of *Listerias*. *L. monocytogenes* was isolated from four samples representing two salad varieties. One variety consisted of cabbage, celery, sultanas, onion and carrots, and the other consisted of lettuce, cucumbers, radishes, fennel, watercress and leeks. *Listeria innocua*, a non-pathogen, was isolated from 13 samples of five different varieties (Sizmur and Walker, 1988). *Listeria monocytogenes* has been isolated from lettuce heads purchased from retail outlets (Steinbruegge et al., 1988) and fresh cabbage, cucumbers, potatoes and radishes from Minneapolis area supermarkets (Heisick et al., 1989). The ability of *L. monocytogenes* to grow and survive in lettuce and cabbage juice was shown in two studies (Steinbruegge et al., 1988; Conner et al., 1986). The spoilage and health risks associated with contaminated vegetables processed for raw consumption are obvious, along with the particular susceptibility of cut products and pre-sliced salads (Zollinger, 1990).

In this study lactic acid bacteria strains isolated from vegetable sources were screened for antimicrobial activity against *Listeria innocua*, *Bacillus cereus* and *Staphylococcus aureus*. Two strains producing bactericidal proteins called bacteriocins, were identified and the substances excreted were characterized.

Materials and Methods

Bacterial strains and media

The strains used in this study were isolated at the Federal Research Centre for Nutrition at Karlsruhe, Germany. The origins of other strains which were used to determine spectra of bacteriocin activities are listed in Tables I and III. The strains *Lactococcus lactis* ssp. *lactis* 20384 and *Enterococcus faecalis* 20478 were obtained from the Deutsche Sammlung von Mikroorganismen, Göttingen, Germany, to be used in DNA homology studies. Lactic acid bacteria were cultivated overnight at 25°C in MRS broth (E. Merck, Darmstadt, Germany) and non-lactic acid bacteria, Gram-negative bacteria and yeasts in Standard I broth (E. Merck), overnight at 25°C.

Identification of isolates

All growth tests were performed in MRS broth. Gas (CO_2)-production from glucose, fermentation of carbohydrates, the configuration of lactic acid enantiomers, and the presence of meso-diaminopimelic acid were determined with the methods used by Schillinger and Lücke (1987) in their identification of lactobacilli.

The pattern of fermented carbohydrates was determined in Minitek plates using MRS without glucose and meat extract as a basal medium. The test sugars were added as filter-sterilized solutions to a final concentration of 0.5%. The configuration of the lactic acid was determined enzymatically using the test kit from Boehringer (Mannheim, Germany). The presence or absence of mesodiaminopimelic acid in the cell wall was tested by the procedure decribed by Kandler and Weiss (1986).

DNA base composition and DNA-DNA hybridization

The DNA was extracted and purified according to the method of Marmur (1961) modified as described by Stackebrandt and Kandler (1979). The DNA base composition was estimated from the DNA melting temperature (T_M) according to Marmur and Doty (1962). The spectrophotometric determination of DNA-DNA hybridization from renaturation rates was performed using the modified (Huss et al., 1983) optical method of DeLey et al. (1970).

Detection of antibacterial activity

In the initial screening of isolates for antagonistic activity, the agar spot test method used by Schillinger and Lücke (1989) was performed under aerobic conditions. The isolates found positive for antagonistic activity were further tested after centrifuging (10000 × *g*, 15 min, 10°C) 20-h cultures of the isolates and adjusting (neutralizing) the pH to 6.5 with NaOH. The supernatant fluids were then heat treated for 3 min at 100°C. These fluids were spotted (10 μl) onto a fresh 7 ml soft agar lawn (0.7% agar) seeded with 0.1 ml of an overnight indicator culture and incubated overnight. In this way, the spectrum of activity was determined.

The action of *Lactococcus lactis* strains D53 and 23 neutralized supernatant fluids was compared with that of nisin against different *Listeria* serotypes. Nisin (Sigma, St. Louis, MO, USA) was suspended in 0.02 N HCl at a level of 10 mg/ml and adjusted to pH 3 with 1 N NaOH.

To quantitate bacteriocin activity, bacteriocin solutions were subjected to a series of two-fold dilutions in 0.9% NaCl and pipetted onto a soft agar bed containing the indicator strain (strain D17). Arbitrary activity units (AU) were assigned (Barefoot and Klaenhammer, 1983).

Influences of pH and temperature on growth and bacteriocin production

An overnight culture of D53 was used to inoculate MRS broth at a 1% level which was incubated at 15, 25, 30 and 37°C. Bacterial growth was monitored spectrophotometrically at 660 nm and bacteriocin activity was determined throughout growth. At 10 and 6°C, MRS broth was inoculated at a level of 3×10^5 cfu/ml, and growth evaluated daily by plate counts; bacteriocin activity was also determined. MRS, adjusted to various pH values between 4.5 and 8.0 with NaOH and HCl, was inoculated at a level of 1% with D53 and incubated at 25°C. Bacterial growth was monitored spectrophotometrically and bacteriocin activity was evaluated as previously described.

Bacteriocin properties

Neutralized supernatant fluids were treated 3, 10 and 20 min at 99°C, and autoclaved 20 min at 121°C. The treated and unheated control samples were evaluated for bacteriocin activity.

Supernatant fluids of D53 and 23 were neutralized and treated with the following enzymes: catalase from bovine liver (2600 U/mg, Sigma) 5 mg/ml, pH 7; trypsin from bovine pancreas (40 U/mg, Merck) 1 mg/ml, pH 7; proteinase K from *Tritirachium album* (11.5 U/mg, Sigma) 0.5 mg/ml, pH 7; α-chymotrypsin from bovine pancreas (45 U/mg, Serva) 1 mg/ml, ph 7; pepsin (35000 U/g, Merck) 1 mg/ml, pH 3; α-amylase from *Bacillus subtilis* (50 U/mg, Fluka) 1 mg/ml, pH 6; lipase from *Rhizopus* sp. (68 U/mg, Serva) 1 mg/ml, pH 7; phospholipase C from *Clostridium perfringens* (3.8 U/mg, Sigma) 0.1 mg/ml, pH 7; DNase I from bovine pancreas (3050 U/mg, Fluka) 1 mg/ml, pH 7; and lysozyme from chicken egg white (206000 U/mg, Serva) 1 mg/ml, pH 7. The supernatant fluids containing enzymes and the controls were incubated for 2 h at 37°C, then heated for 3 min at 100°C to denature the enzymes. All solutions were analysed for bacteriocin activity.

To investigate the pH stability of the bacteriocins, supernatant fluids were adjusted with NaOH and HCl to various pH values from 2 to 9 and allowed to stand at room temperature for 2 h. Supernatants treated with α-chymotrypsin to inactivate the bacteriocins present were pH adjusted and served as controls.

Results

Screening of isolates for antibacterial activity

Using the agar spot test, 123 strains of lactic acid bacteria isolated from foods were screened for antimicrobial activity. Two strains, D53 and 23, showed inhibition against *Listeria innocua, Bacillus cereus* and *Staphylococcus aureus*. Strain D53 had been isolated from a commercial salad mix consisting of endives, lettuce, and radishes. Strain 23 was isolated from fermented carrots. Antimicrobial activity was not observed in any of the other strains investigated.

Identification of isolates

In liquid media, the cells of D53 and 23 were coccoid in shape and existed in short chains. Both strains were Gram-positive, catalase-negative, non-motile and did not produce gas from glucose. The coccoid appearance, the formation of L(+)-lactic acid only, and the ability to grow at 10°C but not at 45°C indicated that 23 and D53 belonged to the genus *Lactococcus* (Schleifer and Kilpper-Bälz, 1987). Both strains fermented a similar range of carbohydrates and had similar G + C contents (Table II). To identify the strains to species level, DNA-DNA hybridizations were performed where strain D53 showed a 73% homology with strain 23, a 76% homology with *Lactococcus lactis* ssp. *lactis* DSM 20384, and a 29% homology with *Enterococcus faecalis* DSM 20478. Strain 23 showed an 80% homology to the *L. lactis* DSM 20384 strain. As organisms showing homology values of more

TABLE I

Spectrum of bacteriocin activity of *Lactococcus lactis* strains D53 and 23

Indicator organism	Origin	Inhibition by	
		D53	23
Lactococcus lactis ssp. *lactis* 20384	DSM	+	+
Enterococcus faecalis 20478	DSM	+	+
Enterococcus faecium SF68	BFE	+	+
Lactobacillus sp. D17	BFE	+	+
Lactobacillus sake 20017	DSM	+	+
Lactobacillus viridescens 20410	DSM	+	+
Pediococcus acidilactici 20333	DSM	+	+
Pediococcus sp. 8459	ATCC	+	+
Lactobacillus plantarum 20174	DSM	+	+
Leuconostoc mesenteroides 20343	DSM	+	+
Leuconostoc paramesenteroides 20288	DSM	+	+
Carnobacterium piscicola 20730	DSM	+	+
Pichia membranaefaciens 2	Fraunhofer Inst.	−	−
Rhodotorula mucilaginosa 20-1-1	CCY	−	−
Saccharomyces cerevisiae 5926	CBS	−	−
Bacillus cereus 2010	CCM	−	+
Staphylococcus aureus 14458	ATCC	−	+
Pseudomonas fluorescens 50091	DSM	−	−
Proteus vulgaris 5	Hohenheim	−	−
Escherichia coli 30083	DSM	−	−

DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Germany; BFE, Federal Research Centre for Nutrition, Karlsruhe, Germany; ATCC, American Type Culture Collection, USA; CCY, Czechoslovak Collection of Yeasts, Bratislava, Czechoslovakia; CBS, Centralbureau voor Schimmelcultures, Baarn, The Netherlands; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia.

than 65% are considered members of one species (Schleifer and Stackebrandt, 1983), these results clearly indicated that both strains belonged to the species *Lactococcus lactis*. Additionally the allocation of strain D53 to *Lactococcus lactis* was confirmed by Schleifer et al. (unpublished results) by hybridization with a 23s rRNA-targeted oligonucleotide probe specific for *Lactococcus lactis* (Betzl et al., 1990).

Spectrum of antibacterial activity

The strains 23 and D53 identified at *Lactococcus lactis* showed inhibitory activity against a broad range of lactic acid bacteria including enterococci, lactobacilli, leuconostocs, pediococci, and carnobacteria (Table I). In addition, neutralized supernatant fluid from *L. lactis* 23 was active against the food pathogens *Staphylococcus aureus* and *Bacillus cereus* and inhibited 11 of 15 strains of *Listeria* (Table III). Strain D53 did not produce inhibition zones against *S. aureus* and *B. cereus*, but was active against nearly the same spectrum of *Listeria* strains. Several Gram-negative bacteria (*Pseudomonas fluorescens*, *Proteus vulgaris*, *Escherichia*

TABLE II

Physiological and biochemical characteristics of *Lactococcus lactis* D53 and 23

Characteristics	D53	23
Growth at		
4°C	weak	weak
45°C	–	–
pH 9.6	+	+
Growth in		
4% NaCl	+	+
6.5% NaCl	+	+
10% NaCl	–	–
Gas production	–	–
mDAP in cell wall	–	–
Lactic acid configuration	L(+)	L(+)
mol% G+C in the DNA	36.8	34.4
Acid from		
L-Arabinose	+	+
Cellobiose	+	+
Galactose	+	+
Gluconate	–	+
Lactose	+	–
Maltose	+	+
Mannitol	+	+
Melezitose	–	–
Melibiose	–	–
Raffinose	–	–
Rhamnose	–	–
Ribose	+	+
Salicin	+	+
Sorbitol	–	–
Trehalose	+	+

coli) and yeasts (*Pichia membranaefaciens*, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae*) were not inhibited.

Influences of pH and temperature on growth and bacteriocin production

Lactococcus lactis D53 exhibited growth and produced bacteriocin at starting pH values from 4.5 to 8.0 (Table IV). Over a pH range of 6.0–8.0, D53 grew and exhibited antimicrobial activity of 128 AU/ml, which was reached after 28 h of growth and O.D. = 0.9. At pH 5.5, bacteriocin activity reached 32 AU/ml after 28 h and O.D. = 0.6. Growth was slower at pH 5.0 and 4.5. After 28 h at pH 5.0 (O.D. = 0.4), an activity of 8 AU/ml was determined, and then activity decreased in the following h. Activity was detected only after 76 h at pH 4.5 (O.D. = 0.19) at 1 AU/ml.

Bacteriocin was detected at temperatures from 10 to 37°C (Table V). No bacteriocin was detected at 6°C, although growth was evident. Growth and bacteri-

TABLE III

Inhibition of *Listeria* spp. by bacteriocinogenic *Lactococcus lactis* strains D53 and 23 in comparison to nisin

Target species	Origin	Serotype	Inhibition by		
			D53	23	Nisin
Listeria innocua 2258	WS	6b	+	+	+
Listeria innocua 2257	WS	6a	−	−	+
Listeria ivanovii 2255	WS	5	+	+	+
Listeria monocytogenes 2251	WS		+	+	+
Listeria monocytogenes 2249	WS	4b	−	−	+
Listeria monocytogenes 2250	WS	4b	+	+	+
Listeria monocytogenes 2247	WS	1/2c	−	+	+
Listeria seeligeri 2253	WS	1/2b	+	+	+
Listeria welshimeri 2254	WS	6a	−	−	+
Listeria sp. 125	BAFF		+	+	+
Listeria sp. 127	BAFF		+	+	+
Listeria sp. 1281	BFE		+	+	+
Listeria sp. 1283	BFE		−	−	+
Listeria sp. 2274	BFE		+	+	+
Listeria sp. 2279	BFE		+	+	+
Lactococcus lactis D53	BFE		−	−	+
Lactococcus lactis 23	BFE		−	−	−
Lactobacillus sp. D17	BFE		+	+	+

BAFF, Federal Meat Research Institute, Kulmbach, Germany; WS, Technical University, München-Weihenstephan, Germany. For other details, see legend to Table I.

ocin production was optimal at 25 and 30°C, although when the cells reached stationary phase at 30°C, activity declined more rapidly than at 25°C. At 37°C, the cells reached O.D. = 0.98 after 12 h and had an activity of 32 AU/ml. Further incubation resulted in an increase in optical density but a rapid reduction in activity. At 15°C, 52 h (O.D. = 0.61) were needed to reach an activity of 64

TABLE IV

Influences of pH on growth and bacteriocin production by *Lactococcus lactis* D53 at 25°C

Initial pH	Maximum bacteriocin activity (AU/ml) [a]	Optical density (660 nm)	Time (h)
4.5	1	0.19	76
5.0	8	0.44	28
5.5	32	0.68	28
6.0	128	0.88	28
6.5	128	1.00	28
7.0	128	1.14	28
8.0	128	1.20	28

[a] *Lactobacillus* sp. D17 served as indicator strain.

TABLE V

Influence of temperature on growth and bacteriocin production of *Lactococcus lactis* D53 at pH 6.5

Incubation temperature (°C)	Maximum bacteriocin activity [a] (AU/ml)	Optical density (660 nm)	Colony forming Units	Time (h)
6	0		6.2×10^{7}	312
10	8		7.8×10^{8}	72
15	64	0.61		52
25	128	0.81		10
30	128	0.90		10
37	32	0.98		12

[a] *Lactobacillus* sp. D17 served as indicator strain.

AU/ml. After 72 h at 10°C and at 7.8×10^{8} cfu/ml, an activity of 8 AU/ml was found.

Bacteriocin properties

The *L. lactis* D53 and 23 bacteriocins responded in the same way to heat and enzyme treatments. Bacteriocin activity remained strong after autoclaving with activities of 16 AU/ml or greater. No loss in activity occurred by heating 10 min at 99°C.

Bacteriocin activity was not affected by treatment with catalase, pepsin, α-amylase, lipase, phospholipase C, DNase I, lysozyme and trypsin. Activity was lost with the addition of proteinase K and α-chymotrypsin. Some activity remained after an incubation of only 2 h with α-chymotrypsin and 24 h were required to eliminate all activity.

The bacteriocins were active over a wide pH range (Table VI), with the highest activity at low pH (pH 3–5).

TABLE VI

pH stability of the bacteriocins of *Lactococcus lactis* strains D53 and 23

pH	Activity units [a] (AU/ml)	
	D53	23
2	32	64
3	64	128
4	128	64
5	128	64
6	32	64
7	32	32
8	32	32
9	16	32

[a] *Lactobacillus* sp. D17 served as indicator strain.

Discussion

Results of this investigation reveal that bacteriocin-producing strains naturally occur and survive on fresh and fermented vegetable products. Further cases to support this statement include the isolation of a *Lactobacillus plantarum* strain from fermented carrots, which produced a bacteriocin (Andersson, 1986). However, it was discovered later that this strain *L. plantarum* SIK-83 was most likely a *Lactococcus lactis*, producing a nisin-like compound (Andersson et al., 1988). Another bacteriocin, plantaricin A, is produced by a strain of *L. plantarum* isolated from a cucumber fermentation (Daeschel et al., 1990). A cucumber fermentation was also the source of a *Pediococcus pentosaceus* strain which produced a bacteriocin (Fleming et al., 1975) designated pediocin A (Daeschel and Klaenhammer, 1985). The bacteriocin-producing strains D53 and 23, identified as *Lactococcus lactis* strains, were isolated from a salad mix of endives, lettuce and radishes, and from fermented carrots, respectively. This suggests that bacteriocin-producing strains of *Lactococcus lactis* are associated not only with dairy products.

Nisin is an extensively characterized bacteriocin produced by some strains of *Lactococcus lactis* isolated from dairy products and applied internationally in many food products as a preservative (Delves-Broughton, 1990). In a comparison of nisin to the bacteriocins of *Lactococcus lactis* D53 and 23, it is important to note that strain D53 was inhibited by nisin, and strain 23 was not. The resistance of strain 23 to nisin suggests a possible relationship of the bacteriocin produced by strain 23 to nisin.

Strains 23 and D53 showed similar spectra of activity. However, *S. aureus, B. cereus* and one serotype of *L. monocytogenes* were inhibited by 23, but not by D53. Although nisin inhibited all strains of *Listeria* tested, *Lactococcus lactis* 23 and D53 exhibited selective antagonistic activity (Table III).

In the investigation of the influence of pH and temperature on growth, *Lactococcus lactis* D53 demonstrated the ability to produce bacteriocin over a wide pH range at 25°C. *L. lactis* D53 produced bacteriocin over a temperature range of 10–37°C, which in part represents ambient salad bar temperatures.

The bacteriocins of *L. lactis* D53 and 23 were quite heat stable and resistant to many enzymes. In contrast to nisin, the bacteriocins still retained activity after autoclaving at a neutral pH. After autoclaving at pH 6.8, nisin loses over 90% of its activity (Tramer, 1964). The bacteriocins were resistant to many of the enzymes tested, including pepsin and trypsin, which is also true for nisin (Jarvis and Mahoney, 1969). Like nisin, the bacteriocins were inactivated by α-chymotrypsin (Jarvis and Mahoney, 1969). Both bacteriocins were inactivated by proteinase K, although this enzyme has no effect on nisin (Kojic et al., 1991). These results could be compared with another study of 280 strains of lactococci, where bacteriocin-producing strains were arranged into eight categories (Geis et al., 1983). However, no category contained bacteriocins resistant to both heat and trypsin digestion.

From pH 2 to 9, both bacteriocins exhibited high activities, although activity was higher in the acidic range. Nisin is most stable and soluble at pH 2 and its activity decreases drastically or is lost at basic pH values between 8 and 12 (Liu and

Hansen, 1990). In a study of 67 lactostrepcins produced by lactococci, the majority of bacteriocins were active only at acid pH values (Kozak et al., 1978).

Our results suggest the production of two nisin-like bacteriocins by *Lactococcus lactis* strains associated with vegetable-type products. These however, showed activity over a wider pH range than nisin, were not inactivated by a number of proteolytic enzymes, and did not lose activity even after autoclaving at pH 6.8. Today, potential advantages of the use of bacteriocin-producing strains in fermentation starter cultures and even the use of bacteriocins directly as preservatives are being realized. Characterizations of bacteriocins and bacteriocin-producers and experimental applications to model food systems are necessary to determine the suitability and possible applications in the safeguarding and quality assurance of a range of traditional and novel food commodities.

Acknowledgements

The authors would like to express their appreciation to the Institute of Food Technologists and to the Fulbright Commission for their generous support of this research project.

References

Andersson, R. (1986) Inhibition of *Staphylococcus aureus* and spheroplasts of gramnegative bacteria by an antagonistic compound produced by a strain of *Lactobacillus plantarum*. Int. J. Food Microbiol. 3, 149–160.

Andersson, R., Daeschel, M.A. and Hassan, H.M. (1988) Antibacterial activity of plantaricin SIK-83, a bacteriocin produced by *Lactobacillus plantarum*. Biochimie 70, 381–390.

Barefoot, S.F. and Klaenhammer, T.R. (1983) Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 45, 1808–1815.

Betzl, D., Ludwig, W. and Schleifer, K.H. (1990) Identification of lactococci and enterococci by colony hybridization with 23s rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 56, 2927–2929.

Carlin, F., Nguyen-The, C., Cudennec, P. and Reich, M. (1989) Microbiological spoilage of fresh "ready-to-use" grated carrots. Sci. Alim. 9, 371–386.

Conner, D.E., Brackett, R.E. and Beuchat, L.R. (1986) Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. 52, 59–63.

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

Daeschel, M.A., McKenney, M.C. and McDonald, L.C. (1990) Bacteriocidal activity of *Lactobacillus plantarum* C-11. Food Microbiol. 7, 91–98.

DeLey, J., Cattoir, H. and Reynaerts, A. (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur. J. Biochem. 12, 133–142.

Delves-Broughton, J. (1990) Nisin and its uses as a food preservative. Food Technol. 44, 100–117.

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

Geis, A., Singh, J. and Teuber, M. (1983) Potential of lactic streptococci to produce bacteriocin. Appl. Environ. Microbiol. 45, 205–211.

Heisick, J.E., Wagner, D.E., Nierman, M.L. and Peeler, J.T. (1989) *Listeria* spp. found on fresh market produce. Appl. Environ. Microbiol. 55, 1925–1927.

Hass, V., Festl, H. and Schleifer, K.H. (1983) Studies on the spectrophotometric determination of the DNA hybridization from renaturation rates. System. Appl. Microbiol. 4, 184–192.

Jarvis, B. and Mahoney, R.R. (1969) Inactivation of nisin by alpha chymotrypsin. J. Dairy Sci. 52, 1448–1450.

Kandler, O. and Weiss, N. (1986) Genus Lactobacillus. In: P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.) Bergey's Manual of Systematic Bacteriology, Vol. 2, Williams and Wilkins, Baltimore, pp. 1209–1234.

Kojic, M., Svircevic, J., Banina, A. and Topisirovic, L. (1991) Bacteriocin-producing strain of *Lactococcus lactis* subsp. *diacitilactis* S50. Appl. Environ. Microbiol. 57, 1835–1837.

Kozak, W., Bardowski, J. and Dobrzanski, W.T. (1978) Lactostrepcins-acid bacteriocins produced by lactic streptococci. J. Dairy Research 45, 247–257.

Liu, W. and Hansen, J.N. (1990) Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. Appl. Environ. Microbiol. 56, 2551–2558.

Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3, 208–218.

Marmur, J. and Doty, P. (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. 5, 109–118.

McKay, L.L. (1983) Functional properties of plasmids in lactic streptococci. Antonie van Leeuwenhoek. 49, 259–274.

Schillinger, U. and Lücke, F. (1987) Identification of lactobacilli from meat and meat products. Food Microbiol. 4, 199–208.

Schillinger, U. and Lücke, F. (1989) Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 55, 1901–1906.

Schleifer, K.H. and Kilpper-Bälz, R. (1987) Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. System. Appl. Microbiol. 10, 1–19.

Schleifer, K.H. and Stackebrandt, E. (1983) Molecular systematics of prokaryotes. Ann. Rev. Microbiol. 37, 143–187.

Sizmur, K. and Walker, C.W. (1988) *Listeria* in prepacked salads. Lancet. ii, 1167.

Solomon, H.M., Kautter, D.A., Lilly, T. and Rhodehamel, E.J. (1990) Outgrowth of *Clostridium botulinum* in shredded cabbage at room temperature under a modified atmosphere. J. Food Prot. 53, 831–833.

Stackebrandt, E. and Kandler, O. (1979) Taxonomy of the genus *Cellulomonas*, based on phenotypic characters and deoxyribonucleic acid-deoxyribonucleic acid homology and proposal of seven neotype strains. Int. J. System. Bacteriol. 29, 273–282.

Steinbruegge, E.G., Maxcy, R.B., and Liewen, M.B. (1988) Fate of *Listeria monocytogenes* on ready to serve lettuce. J. Food Prot. 51, 596–599.

Tramer, J. (1964) The inhibitory action of nisin on *Bacillus stearothermophilus*. In: N. Molin (Ed.), Microbial Inhibitors in Foods. Almqvist and Wiksell, Stockholm, p. 26.

Zollinger, W. (1990) Mikrobiologie - spezielle Aspekte bei Früchten und Gemüse. Lebensmittel-Technologie. 23, 262–264.