REVIEW

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Bacteriocins of *Lactobacillus plantarum* Strains from Fermented Foods

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ABSTRACT. Bacteriocin-producing strains may be used as protective cultures to improve the microbial safety of foods. The crude or purified form of these antimicrobial agents may also be applied directly as food preservative. This review gives survey of the different bacteriocins produced by *Lactobacillus plantarum* isolated from fermented food products with particular emphasis on their genetic and biochemical properties. A number of bacteriocins are produced by *L. plantarum*. These include plantaricin B, plantaricin BN, plantaricin A, plantaricin C, plantaricin S and T, plantaricin F, plantaricin C19 and SA6 and other unnamed bacteriocins. However, with the exception of plantaricin A, information on the genetic and biochemical characteristics of *L. plantarum* bacteriocins is still scant.

CONTENTS

1 INTRODUCTION

Lactic acid bacteria (LAB) are used in food fermentations because they convert sugars into organic acids, thus improving the organoleptic and rheological properties of the product and also because they inhibit food spoilage by microorganisms through the production of inhibitors, such as biacetyl, hydrogen peroxide and bacteriocins. Of the inhibitors produced by LAB, bacteriocins have attracted much interest because they are usually safe for consumption and thus can be added to food as preservatives without any negative health implication. Bacteriocins have been defined by numerous authors (for instance Tagg *et al.* 1976; Klaenhammer 1988; Gonzalez *et al.* 1994) as antagonistic proteins or peptides that show bactericidal activity against related species.

One of the species of LAB, *Lactobacillus plantarum*, is a saprophyte often associated with plant and fermenting materials (Daeschel and Fleming 1984). In food and feed fermentations, *e.g.* of vegetables (Fleming *et al.* 1985), sausages (Bacus and Brown 1985) and silage (Beck 1978), it plays a major role in the preservation of the fermented products. In some cases *L. plantarum* has been used as a starter culture during food preparation.

Bacteriocins produced by different strains of *L. plantarum* have been identified and characterized in the literature (Table I), but it appears that information about bacteriocins of *L. plantarum* is not abundant or detailed. This paper intends to survey the present knowledge of different bacteriocins produced by *L. plantarum* strains with emphasis on their genetics and biochemistry.

2 PLANTARICINS

2.1 Plantaricin K

This was obtained from *Lactobacillus plantarum* strain DK 9 isolated from 'fufu', an African fermented maize product. The bacteriocin inhibited the growth of strains of *L. plantarum*, *L. brevis*, *L. sake* and *Enterococcus fæcalis*. Its activity against Gram-positive and -negative pathogens was not determined. The protein nature of plantaricin K was confirmed by its sensitivity to trypsin. It was stable

at 80 °C for 30 min after which the activity was significantly reduced (Olukoya *et al.* 1993). No information is available at the present time on the genetics of plantaricin K while information on the biochemical characterization appeared scanty.

Table I. Bacteriocins produced by *L. plantarum* strains

Bacteriocin	Strain	Origin	Reference
Plantaricin K	DK9	fufu (African fermented cassava product)	Olukoya *et al.* 1993
Unnamed	CTC305/306	fermented sausage	Garriga *et al.* 1993
	K12	kenkey (African fermented maize product)	Olasupo *et al.* 1994
Plantaricin A	C-11	fermented cucumber	Daeschel *et al.* 1990
Plantaricin C19	C19	fermented cucumber	Atrih *et al.* 1993
Plantaricin SA6	SA6	sausage	Atrih *et al.* 1993
Plantaricin S and T	LPC010	fermented Spanish-style green olive	Jimenez-Diaz *et al.* 1993
Plantaricin C	LL441	Cabrales cheese	Gonzalez *et al.* 1994
Plantaricin F	BF001	chilled processed channel catfish	Fricourt *et al.* 1994
Plantaricin B	NCDO 1193	National Collection of Dairy Organisms (NCDO)	West and Warner 1988
Plantaricin BN	BN	beef	Lewus and Montville 1992

2.2 Plantaricin A

It is produced by *L. plantarum* strain C-11 isolated from a cucumber fermentation. The inhibitory spectrum of the bacteriocin indicated bactericidal activity against some species of lactic acid bacteria: *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Streptococcus*. Plantaricin A was not effective against other Gram-positive or -negative bacteria. It was sensitive to proteinases, has a molar mass greater than 6 kDa, is heat-stable at 100 °C for 30 min and active over the pH range of 4.0–6.5 (Daeschel *et al.* 1990). Genetic analysis showed that *L. plantarum* C-11 contained two cryptic native plasmids of approximately 4.3 and 6.5 MDa. No difference was observed in the plasmid profiles of the mutant and wild-type strains, as both contained similar plasmid content and size. The bacteriocin-negative mutant (Bac^-) was immune to the bacteriocin produced by the parent (wild-type – Bac^+) strain, suggesting that the mutation responsible for the bacteriocin phenotype affected only the gene for bacteriocin production but did not affect the immunity to the bacteriocin.

Plantaricin A has been purified to homogeneity by ammonium sulfate precipitation, binding to cation exchanger and Octyl-Sepharose and reverse-phase chromatography (Nissen-Meyer *et al.* 1993). The bacteriocin activity of plantaricin A has been found to be associated with two peptides termed α and β, containing 21 and 22 amino acids, respectively, from the N-terminal end. Bacteriocin activity required the complementary action of both the α and β peptides. The molar mass of α and β as determined by mass spectroscopy was 2687 ± 30 and 2758 ± 30 Da, respectively (Nissen-Meyer *et al.* 1993), indicating that (*i*) the only difference between the α and β peptides was the presence of the N-terminal alanine residue in β, and that (*ii*) in addition to the sequence residues, two to three unidentified amino acid residues are present at the C-terminal ends of the α and β peptides. According to Ojcius and Young (1991) both the α and β peptide may form amphiphilic α-helices (Fig. 1), suggesting that they are pore-forming peptides that create cell membrane channels through a "barrel-stave" mechanism.

The gene encoding plantaricin A has been studied by Diep *et al.* (1994). They demonstrated that a single gene termed *plnA* encodes a plantaricin A precursor protein of 48 amino acids. The α (22-amino acid) and β (23-amino acid) peptides are most likely derived from this precursor protein by proteolytic cleavage.

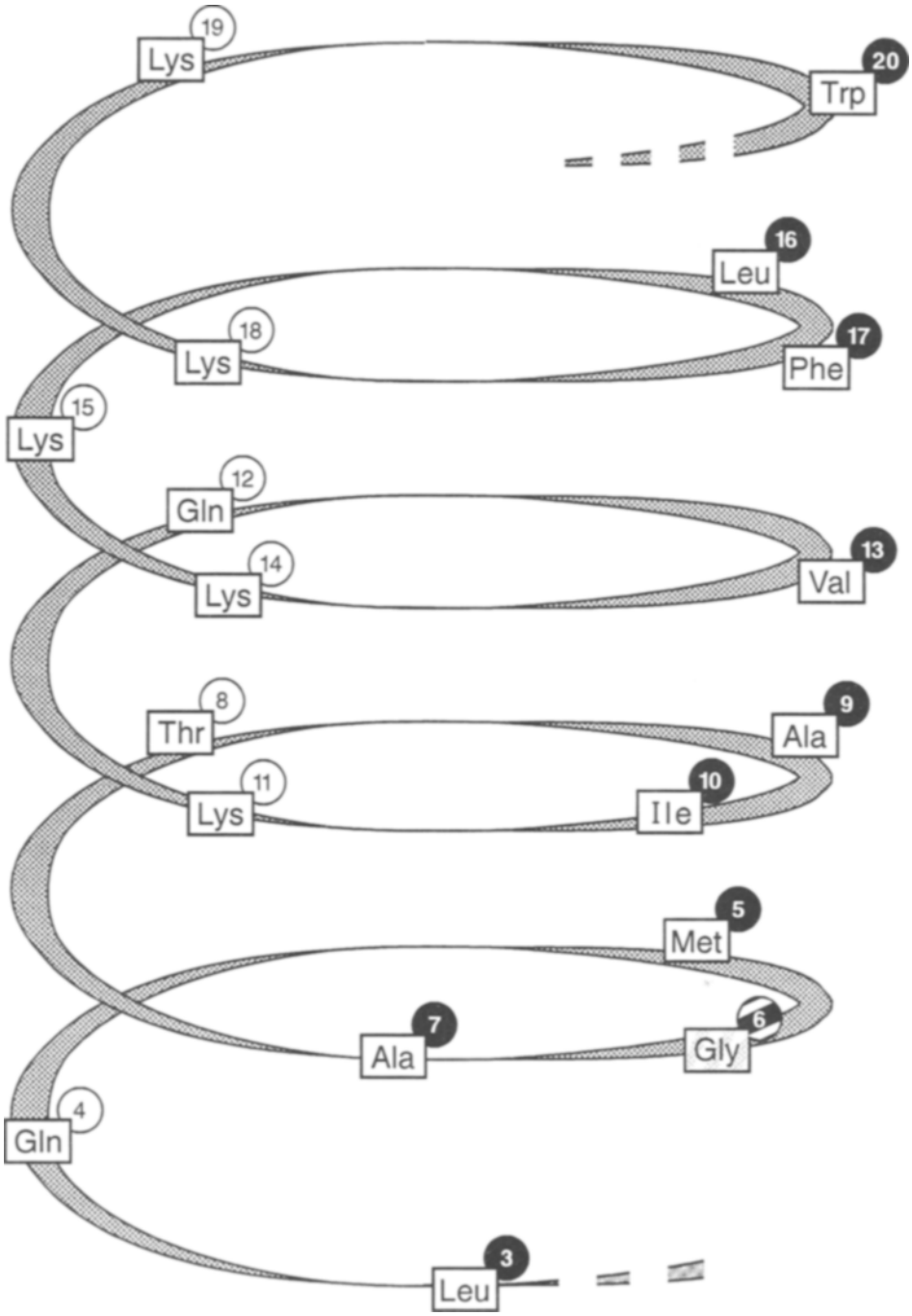

Fig. 1. α-Helical representation of the amphiphilic region in the α-peptide of plantaricin A. The amphiphilic region starts with residue 3 and ends with residue 20. Blank numbers on black background indicate nonpolar residues. An identical α-helical wheel may be constructed for the β-peptide, starting with amino acid residue 4 and ending with residue 21. (Source: Nissen-Meyer *et al.* 1993).

2.3 Plantaricin S and T

Plantaricin S is produced by *L. plantarum* strain LPC010 isolated from fermented green olives. It inhibits a wide range of lactic acid bacteria as well as some Gram-positive pathogens, including clostridia and propionibacteria. It also inhibits natural competitors of *L. plantarum* in olive fermentation brines. The bacteriocin provided no protection against Gram-negative bacteria. In addition to its sensitivity to proteolytic enzymes, the bacteriocin is also sensitive to glycolytic (α-amylase and dextranase) and lipolytic (lipase A and phospholipase C) enzymes, suggesting that this inhibitory substance is a glycolipoprotein. Plantaricin S is stable at 100 °C for 1 h and over the pH range of 3.0–7.0.

The molar mass of plantaricin S has been characterized using various approaches (Jimenez-Diaz *et al.* 1993). The ultrafiltration method of molar mass determination indicated that plantaricin occurred as multimolecular aggregates with the size of the smallest active form being between 3 and 10 kDa. However, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) revealed that plantaricin S migrated as a peptide of approximately 2 kDa.

Plantaricin T is also produced by *L. plantarum* strain LPC010 isolated from fermented green olives. Unlike plantaricin S which was produced during the exponential phase, plantaricin T was produced during the late stationary phase of *L. plantarum* LPC010. Plantaricin T is different from plantaricin S in its biological activity, its sensitivity to various enzymes and its molar mass which is lower than that of plantaricin S (Jimenez-Diaz *et al.* 1993). It has a similar inhibitory spectrum but with a lower level of activity. However, only plantaricin T inhibited the growth of *L. curvatus*. It exhibited the same heat resistance and sensitivity to enzymes as plantaricin S. However, in contrast to the latter, plantaricin T was not affected by treatment with α-amylase or lipase. The actual molar mass of plantaricin T is not known. SDS–PAGE characterization (Jimenez-Diaz *et al.* 1993) only indicated that plantaricin T migrated to a slightly lower position than the 2.5 kDa position of plantaricin S.

A plasmid profile study of strain LPC010 showed the presence of nine plasmids of sizes 49, 35, 27, 18, 16.5, 12.0, 8.4, 4.8 and 2.4 kb. Curing assay after novobiocin treatment resulted in four variants that have lost the ability to produce plantaricin S and T. Plasmid analysis of the four bacteriocin-deficient variants indicated that two of them lacked the 27- and 18-kb plasmids and the other two had lost the 49-, 27- and 18-kb plasmids. However, these plasmids were also lost in some other analyzed colonies that had retained the plantaricin S and T production phenotype. Hence, according to Jimenez-Diaz *et al.* (1993), the determinant for plantaricin S and T production does not appear to be plasmid-encoded.

2.4 Plantaricin C

Plantaricin C is produced by a strain of *L. plantarum* LL 441 of dairy origin from cabrales cheese. It inhibits a wide range of Gram-positive bacteria, including members of the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Propionibacterium*, *Bacillus*, *Streptococcus*, *Staphylococcus* and *Clostridium*. No inhibition was found toward the Gram-negative bacteria. Its activity was completely lost upon treatment with pronase, trypsin and α-chymotrypsin but was not affected by other proteolytic enzymes, such as pepsin and proteinase K, or by α-amylase or lipase. The bacteriocin remained stable under storage at 4 °C and −20 °C and when heated to 100 °C for 1 h or 121 °C for 10 min. Plantaricin C activity was found optimal under acidic and neutral pH while at alkaline pH, it lost its activity.

Studies of the mode of action of plantaricin C showed that it is bactericidal and in some cases, bacteriolytic (Gonzalez *et al.* 1994). Purification and amino acid sequencing of plantaricin C (Gonzalez *et al.* 1994) revealed that it is a peptide of 3.5 kDa whose aminoterminal sequence is H_2N-K–K–T–K–K–N–X–S–G–D–I.

2.5 Plantaricin F

Plantaricin F is a bacteriocin obtained from *L. plantarum* strain BF 001 isolated from chilled processed catfish. It shows a wider inhibitory spectrum; it inhibits both Gram-positive and Gram-negative bacteria including selected bacteria of the genera *Lactobacillus*, *Lactococcus*, *Listeria*, *Micrococcus*, *Leuconostoc*, *Pediococcus*, *Staphylococcus*, *Streptococcus*, *Salmonella* and *Pseudomonas*. The bacteriocin exhibited a bactericidal mode of action, active only at acidic pH of 3.0 to 4.5, displaying optimum activity at pH 3.5 and retaining full activity after 30 min at 100 °C (pH 3.5).

The effect of proteolytic enzymes on the bacteriocin activity was not clear. This is because the enzymes have their pH optimum around 7.5 to 8.0: trypsin (optimum pH 8.0, 25 °C), chymotrypsin (optimum pH 7.8, 25 °C), pronase E (optimum pH 7.5, 37 °C) and proteinase K (optimum pH 7.5,

37 °C) and these enzymes are not active within the pH range (pH 3.0–4.5) of plantaricin F (*Sigma Chemical Company* 1993). Hence it was not possible to prove exclusively that plantaricin C is a protein.

The molar mass of plantaricin C has been estimated by ultrafiltration (Fricourt *et al.* 1994) to be below 10 kDa. No information appeared to be available at the present time on the genetic determinant and amino acid sequences of plantaricin C.

2.6 Plantaricin B

It is produced by *L. plantarum* strain NCDO 1193 of dairy origin from the *National Collection of Dairy Organisms.* Plantaricin B inhibits only strains of closely related species including *L. plantarum*, *Leuconostoc mesenteroides* and *Pediococcus damnosus*. It was sensitive to proteolytic enzymes, such as pronase, pepsin, trypsin and α-chymotrypsin. Nonproteolytic enzymes, such as lipase and α-amylase, were found to substantially reduce the inhibitory capacity of *L. plantarum* NCDO 1193. At the present time, the mode of action of plantaricin B cannot be determined because the inhibitor could not be isolated in liquid culture (West and Warner 1988). While information on the biochemical properties of plantaricin B remained scanty, no information is available on its genetics.

2.7 Plantaricin C19 and SA6

Plantaricin C19 is produced by *L. plantarum* strain C19 isolated from fermented cucumber while plantaricin SA6 is produced by *L. plantarum* SA6 isolated from sausage. While plantaricin SA6 displayed a narrow inhibitory spectrum only against lactic acid bacteria of the genera *Lactococcus*, *Pediococcus*, *Leuconostoc* and *Enterococcus*; plantaricin C19 inhibited not only lactic acid bacteria but also several Gram-positive bacteria including *Staphylococcus aureus*, *Enterococci* (*E. fæcalis*, *E. liquefaciens*, *E. fæcium* and *E. durans*), *Listeria grayi* and *Listeria monocytogenes*. The effect of proteolytic, glycolytic and lipolytic enzymes on both bacteriocins indicated that activities in both were prevented by proteinase K, pronase E, pepsin, trypsin, α-chymotrypsin, ficin and thermolysin. However, the activity of plantaricin SA6 was partially destroyed by α-amylase, dextranase and phospholipase C. Both bacteriocins were heat-stable at 121 °C for 15 min and over a pH range from 4.0 to 8.0.

Studies on the purification and characterization of biochemical properties, the search for genetic determinants and practical application of plantaricin C19 and SA6 are currently in progress (Atrih *et al.* 1993).

2.8 Plantaricin BN

It is produced by *L. plantarum* strain BN isolated from beef. Plantaricin BN inhibits *Listeria monocytogenes*, *Aeromonas hydrophilia* (Lewus *et al.* 1991*a*); *Clostridium botulinum* (Okereke and Montville 1991) and *Lactobacillus sake* (Lewus and Montville 1991*b*). The bacteriocin is sensitive to proteinase K while its activity is not affected by other proteolytic enzymes, such as trypsin, pepsin and pronase E. Plantaricin BN exhibited a bactericidal mode of action and retained some activity after heating to 60 °C for 10 min or 100 °C for 5 min. Studies of the optimum pH and temperature for the production of plantaricin BN on solid media (Lewus and Montville 1992), indicated an optimum pH of 7.9 and temperature of 15 °C.

Preliminary molar mass estimation of crude plantaricin BN by SDS–PAGE and centricon 10 ultrafiltration suggested that the bacteriocin has a molar mass greater than 10 kDa. However, further purification and characterization of plantaricin BN requires further research (Lewus and Montville 1992).

Plasmid analysis of *L. plantarum* strain BN showed that plasmid DNA was not detected in the producer's strain but a chromosomal-DNA band was observed, suggesting possible linkage of bacteriocin production to the chromosome.

2.9 Unnamed bacteriocins

The detection of bacteriocin-producing *L. plantarum* strain K12 isolated from 'kenkey', an African fermented maize product was reported by Olasupo *et al.* (1994), but the bacteriocin was not assigned any name. The bacteriocin inhibited both Gram-positive and Gram-negative bacteria such as *L. plantarum*, *Pseudomonas* sp., *Aeromonas cavice* and *Aeromonas sobria*. Its activity is sensitive to proteolytic enzymes, such as pepsin and trypsin, and is moderately heat-stable (80 °C for 30 min). Genetic study of the plasmid profile of the bacteriocin-producing strain indicated the absence of plasmid DNA and the possibility of a chromosomally encoded trait. The high-molar-mass, chromosome-

coded bacteriocin of *L. plantarum* K12 with a wide-spectrum effect including Gram-negative species appeared to be similar to lactacin F (Muriana and Klaenhammer 1987). No extensive biochemical characterization of the bacteriocin has been conducted.

Garriga *et al.* (1993) also reported the detection of two bacteriocin-producing *L. plantarum* strains CTC 305 and CTC 306 isolated from fermented sausages. The bacteriocin from *L. plantarum* CTC 305 inhibited *L. plantarum*, *Enterococcus fœcalis* and *Listeria monocytogenes*, while bacteriocin from *L. plantarum* CTC 306 inhibited *L. plantarum*, *L. curvatus*, *Enterococcus fœcalis* and *Listeria monocytogenes*. The two bacteriocins were stable at 100 °C for 20 min and sensitive to proteolytic enzymes such as trypsin, pepsin, proteinase K and nagarase. Both bacteriocins are known to have a bactericidal mode of action with molar mass estimates greater than 10 kDa (Garriga *et al.* 1993) using ultrafiltration method.

Plasmid profile analysis of both strains indicated that they harbor several plasmids ranging from 2 kbp to 55 and from 3.6 kbp to 55, respectively. Curing experiments with the bacteriocin-producing strains CTC 305 and 306 resulted in mutants that have lost the ability to produce bacteriocin but with the same plasmid profile to the parent strains.

3 CONCLUSION

In general, bacteriocins from *L. plantarum* are small in number and are relatively heat-stable with promising inhibitory spectra of antimicrobial activities. Their general heat stability is an advantage, temperature stability being a very important parameter if a bacteriocin is to be used as a food preservative because many processing procedures involve a heating step (Hurst 1981). Further studies are still required on the genetic and biochemical characterization of *L. plantarum* bacteriocins to facilitate their application in practical food models. However, the bacteriocins from *L. plantarum* described in this paper appear quite promising as potential biopreservatives for fermented foods.

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REFERENCES

ATRIH A., MICHEL R.M., LEFEBVRE G.: Detection of bacteriocins produced by *Lactobacillus plantarum* strains isolated from different foods. *Microbios* **75**, 117–123 (1993).

BACUS J., BROWN W.: The lactobacilli: meat products, pp. 47–72 in S.E. Gilliand (Ed.): *Bacterial Starter Cultures of Foods.* CRC Press, Boca Raton (Florida) 1985.

BECK T.: The microbiology of silage fermentation, pp. 61–116 in M.E. McCullough (Ed.): *Fermentation of Silage: A Review.* National Food Ingredients Association, Des Moines (Iowa) 1978.

DAESCHEL M.A., FLEMING H.P.: Selection of lactic acid bacteria for use in vegetable fermentations. *Food Microbiol.* **1**, 303–313 (1984).

DAESCHEL M.A., MCKENNEY M.C., MCDONALD L.C.: Bacteriocidal activity of *Lactobacillus plantarum* C-11. *Food Microbiol.* 7, 91–98 (1990).

DIEP D.B., HAVARSTEIN L.S., NISSEN-MEYER J., NES I.F.: The gene encoding plantaricin A, a bacteriocin from *Lactobacillus plantarum* C-11, is located on the same transcription unit as an agar-like regulatory system. *Appl.Environ.Microbiol.* **60**, 160–166 (1994).

FRICOURT B.V., BAREFOOT S.F., TESTIN R.F., HAYASAKA S.S.: Detection and activity of plantaricin F, an antibacterial substance from *Lactobacillus plantarum* BF001 isolated from processed channel catfish. *J.Food Protect.* **57**, 698–702 (1994).

FLEMING H.P., MCFEETERS R.F., DAESCHEL M.A.: The lactobacilli, pediococcus and leuconostoc: vegetable products, pp. 97–118 in S.E. Gilliand (Ed.): *Bacterial Starter Cultures of Foods.* CRC Press, Inc., Boca Raton (Florida) 1985.

GARRIGA M., HUGAS M., AYMERICH T., MONFORT J.M.: Bacteriocinogenic activity of lactobacilli from fermented sausages. *J.Appl.Bacteriol.* **75**, 142–148 (1993).

GONZALEZ B., ARCA P., MAYO B., SUAREZ J.E.: Detection, purification and partial characterization of plantaricin C, a bacteriocin produced by a *Lactobacillus plantarum* strain of dairy origin. *Appl.Environ.Microbiol.* **60**, 2158–2163 (1994).

HURST A.: Nisin, pp. 85–123 in D. Perlman, A.I. Laskin (Eds): *Advances in Applied Microbiology.* Academic Press, New York 1981.

JIMENEZ-DIAZ R., RIOS-SANCHEZ R.M., DESMAZEAUD M., RUIZ-BARBA J.L., PIRARD J.C.: Plantaricins S and I, two new bacteriocins produced by *Lactobacillus plantarum* LPO10 isolated from a green olive fermentation. *Appl.Environ. Microbiol.* **59**, 1416–1424 (1993).

KLAENHAMMER T.R.: Bacteriocins of lactic acid bacteria. *Biochimie* **70**, 337–349 (1988).

LEWUS C.B., KAISER A., MONTVILLE T.J.: Inhibition of foodborne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. *Appl.Environ.Microbiol.* **57**, 1683–1688 (1991*a*).

LEWUS C.B., MONTVILLE T.J.: Detection of bacteriocins produced by lactic acid bacteria. *J.Microbiol.Methods* **13**, 145–150 (1991*b*).

LEWUS C.B., MONTVILLE T.J.: Further characterization of bacteriocin Plantaricin BN, Bavaricin MN and Pediocin A. *Food Biotechnol.* **6**, 153–174 (1992).

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

NISSEN-MAYER J.M., LARSEN A.G., SLETTEN K., DAESCHEL M., NES I.F.: Purification and characterization of Plantaricin A, a *Lactobacillus plantarum* bacteriocin whose activity depends on the action of two peptides. *J.Gen.Microbiol.* **139**, 1973–1978 (1993).

OJCIUS D.M., YOUNG J.D.E.: Cytolytic pore-forming proteins and peptides: Is there a common structural motif? *Trends Biochem.Sci.* **16**, 225–229 (1991).

OKEREKE A., MONTVILLE T.J.: Bacteriocin inhibition of *Clostridium botulinum* spores by lactic and bacteria. *J.Food Protect.* **54**, 349–353 (1991).

OLASUPO N.A., OLUKOYA D.K., ODUNFA S.A.: Plasmid profiles of bacteriocin-producing *Lactobacillus* species from African fermented foods. *Folia Microbiol.* **39**, 181–186 (1994).

OLUKOYA D.K., TICHACZEK P.S., BUTSCH A., VOGEL R.F., HAMMES W.P.: Characterization of the bacteriocins produced by *Lactobacillus pentosus* DK7 isolated from ogi and *Lactobacillus plantarum* DK9 from fufu. *Chem.Microbiol.Technol. Lebensm.* **15**, 65–68 (1993).

Sigma Chemical Company: Biochemicals organic compounds for research and diagnostic reagents. Sigma Chemical Co., St. Louis (USA) 1993.

TAGG J.R., DAJANI A.S., WANNAMAKER L.W.: Bacteriocins of Gram-positive bacteria. *Bacteriol.Rev.* **40**, 722–756 (1976).

WEST C.A., WARNER P.J.: Plantaricin B, a bacteriocin produced by *Lactobacillus plantarum* NCDO1193. *FEMS Microbiol.Lett.* **49**, 163–165 (1988).