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Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes

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Abstract

A review is presented on the present status of biological preservation of foods. Recent developments are discussed with respect to underlying mechanisms of inhibition by 'protective' cultures, and special reference is made to lactic acid bacteria (LAB) and their 'food-grade' safety. The role of bacteriocins, their limitations and potentiating role in biological systems, is also addressed. The use of enzymes (e.g. lysozyme) for food preservation is mainly restricted by economic factors, their inactivation by endogenous food components and their limited activity spectrum.

Practical applications of protective cultures refer to particular food commodities that either constitute novel systems with respect to packaging and/or composition, or represent special hygienic risks. It is concluded that biological preservation cannot substitute GMP; it, however, offers an additional (and acceptable) processing parameter for improving the safety and assuring the quality of a given food.

Keywords: Protective cultures; Lactic acid bacteria; Bacteriocins; Enzymes; Food safety; Biological preservation

Food biotechnology, as we know it today, is rooted in the development of fermented foods during 6000 to 12000 years of man's cultural history. More than anything else, man has, by trial and error, devised methods of 'controlled' fermentation in order to counteract undesired 'deterioration' of products of plant and animal origin. By these empirical approaches beneficial microorganisms were favoured by selection parameters whilst spoilage and other deleterious microbes

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Table 1
Commercial significance of metabolic products of lactic acid bacteria

Metabolite	Beneficial	Deleterious
Lactic acid	Preservation Sensory improvement Enhancement of: – digestion – nutrient uptake	Acidification
Acetic acid	Aroma	Off-taste
Diacetyl/acetoin	Aroma (dairy products)	Off-taste (beer)
CO_2	Preservation Taste enhancement	Gas production (blowing/bloaters)
H_2O_2	Preservation	Discolouration Greening
Biogenic amines	–	Health (allergies)
Slime	Stabilisation (e.g. yoghurt)	Sensory
Methane thiol. H_2S	Aroma	Sensory (off-taste and odour)
Growth factors	Aroma Nutritional value	Clostridia (gas) Yeasts
Bacteriocins	Preservation (inhibition of closely related bacteria)	Health? Inhibition of beneficial lactic acid bacteria
Wide-spectrum antimicrobials	Inhibition of pathogens and spoilage microorganisms	Allergies? Resistance of intestinal microorganisms

were inhibited. Following the earlier development of fermented beverages such as beer and wine, and also of fermented milk products (cheese, yoghurt, etc.), more 'recent' developments include the fermentation of meat (by the 9th century BC) and vegetables (3rd century BC) (Pederson, 1971, pp. 66–172). In most developing countries, fermented foods are still produced by traditional means, and constitute up to 60% and more of the daily diet. Lacking modern refrigeration facilities, inhabitants of many tropical and subtropical regions rely on fermentation as a means of preserving and safeguarding their food, at the same time appreciating the typical sensory characteristics and increased commercial value thus achieved. In Europe around 25% of our diet is contributed by fermented foods, some of which (e.g. sauerkraut, sour dough bread, etc.), although modern technologies are applied, are still produced without added commercial starter cultures.

Our present understanding of food fermentations gained during this century, recognises a number of physiological attributes of desired strains involved, notably lactic acid bacteria (LAB) used as starter cultures. As shown in Table 1, metabolism of starter cultures may contribute in a number of ways to the control of pathogens, shelf life and sensory qualities.

Looking back at recent progress in food biotechnology (cf. Holzapfel and Hammes, 1989), it is a fact that the problem of food safety and security still

remains to be solved. This will be the major challenge of the 90's to the food industry, food and biological scientists and legislating authorities alike. In spite of the introduction of modern technologies and safety concepts (e.g. HACCP) the reported number of food-borne illnesses and intoxications is still increasing. On the other hand, an increasing number of consumers prefer minimally processed foods, prepared without chemical preservatives, as well as 'mild' and 'light' products characterised by a low acid, sugar or fat content. Many of these 'ready-to-eat' and novel food types in fact represent new food systems with respect to health risks and spoilage association. Against this background, and relying on improved understanding and knowledge of the complexity of microbial interactions and combined preservation factors present in food systems, recent approaches are increasingly directed towards possibilities offered by biological or 'milder' preservation approaches. This implies so-called 'protective cultures' or their metabolites, notably enzymes and bacteriocins.

In general terms, starter cultures are applied to bring about beneficial metabolic and sensory changes of a food – generally accompanied by a preservation effect (Table 1). Modern approaches in biological preservation aim at the reduction of health risks without changing the sensory quality of the product.

A distinction is sometimes made between starter cultures and protective cultures; in reality it may be the same culture applied for different purposes under different conditions. For a starter culture metabolic activity (e.g. acid production) has technological importance whilst antimicrobial action may constitute a secondary-effect; for a protective culture the functional objectives are the inverse.

In this presentation biological preservation will be discussed relative to the potential of protective cultures, bacteriocins and food-grade enzymes.

1. Protective cultures

Protective cultures should in the first instance be considered as additional safety factor, with the potential of improving the microbiological safety of food. Their implementation should support good manufacturing practices, thereby reducing risks of growth and survival of pathogens and spoilage organisms. In addition, under abuse conditions of temperature, handling, etc., their metabolic activities (e.g. acid or gas production) may serve as an indicator of microbial risk.

The LAB, generally considered as 'food-grade' organisms, show special promise for selection and implementation as protective cultures. Involved in numerous food fermentations known to man for millennia, it is assumed that most representatives of this group do not pose any health risk to man, and some are designated as 'GRAS' ('*g*enerally *r*ecognised *a*s *s*afe') organisms. Reports on the involvement of LAB in human infections (Aguirre and Collins, 1993) indicate that some species may act as opportunistic pathogens in rare cases. However, there is no indication of a health risk of LAB involved in food fermentations; and any definite conclusions presently are speculative. On the other hand, in a number of product groups, especially dairy products, the use of biological preservation may also contribute to

Table 2
Desirable properties of protective cultures

1. No health risks
   - No production of toxins
   - No biogenic amines or other metabolites detrimental to health
   - Non pathogenic
2. Bring about beneficial effects in product
   - Adaptation to product/substrate
   - Reliability of consistent protective activity
   - Predictability of metabolic activity under given set of parameters (e.g. lactic acid production/ no gas)
   - Competitiveness against autochtonous organisms
   - Specific enzymatic acitivities, e.g. for meat:
     - nitrate reductase
     - catalase
3. No negative (sensory) effects on product under GMP (e.g. no production of acid, gas, slime, etc., depending on product type)
4. Function as 'indicator' under abuse conditions

the health benefits of a product, e.g. as for acidophilus milk. Such probiotic cultures are considered to provide substantial health benefits to man by means of stabilising or normalising the gastro-intestinal tract (Fernandes et al., 1992). Some LAB strains are even associated with anticarcinogenic action and tumour control (Adachi, 1992). These health traits may serve as important additional advantage for future selection and application of protective cultures.

LAB have been studied intensively for their physiology and interactions in food during this century, and more recently significant progress in research into their molecular biology has been made. Present knowledge enables us to distinguish between their beneficial and deleterious activities in food – often related to product type, time and consumers' expectations – and to understand the underlying mechanisms. This relationship is presented in Table 1, also suggesting the complexity of factors involved in preservative effects. 'Ideal' properties of a protective culture are summarised in Table 2.

2.1. Mechanisms of antagonism of LAB

Growth rate and competitiveness of a culture are determined by its adaptation to a substrate and by a number of intrinsic and extrinsic factors including redox potential (E_h), water activity (a_w), pH and temperature. Antagonism refers to the inhibition of other (e.g. undesired or pathogenic) microorganisms, caused by competition for nutrients, and by the production of antimicrobial metabolites. The use of large cell numbers as inoculum enables successful competition of a starter culture during fermentation of, e.g., milk or meat. Apart from its metabolic activity, the starter culture occupies vital nishes, thereby discouraging colonisation of undesired microorganisms.

Table 3
Metabolic products of lactic acid bacteria with antimicrobial properties

Product	Main target organisms
Organic acids	
– lactic acid	Putrefactive and Gram-negative bacteria, some fungi
– acetic acid	Putrefactive bacteria, clostridia, some yeasts and fungi
Hydrogen peroxide	Pathogens and spoilage organisms, especially in protein-rich foods
Enzymes	
– lactoperoxidase system with H_2O_2	Pathogens and spoilage bacteria (milk and dairy products)
– lysozyme (by recomb. DNA-technology)	Undesired Gram-positive bacteria
Low-molecular metabolites	
– reuterin (3-OH-propionaldehyde)	Wide spectrum of bacteria, moulds and yeasts
– diacetyl	Gram-negative bacteria
– fatty acids	Different bacteria
Bacteriocins	
– nisin	Some LAB and Gram-positive bacteria, notably endospore-formers
– other	Gram-positive bacteria, inhibitory spectrum according to producer strain and bacteriocin type

The production of one or more antimicrobially active metabolites is part of the complex mechanism by which a culture becomes established in the presence of other competing organisms. Understanding these mechanisms provides a valuable key towards 'biological' approaches in food preservation.

In Table 3 the antimicrobial properties of a number of metabolites from LAB are summarised. Depending on the product and processing situation, one or more of these metabolites may constitute a basis for the selection of a protective culture.

Organic acids. Lactic acid, the characteristic fermentation product of LAB may reduce pH to a level where putrefactive (e.g. clostridia and pseudomonads), pathogenic (e.g. salmonellae and *Listeria* spp.) and toxinogenic bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Clostridium botulinum*) will either be inhibited or destroyed. Moreover the undissociated acid, on account of its fat solubility (Brown and Booth, 1991), will diffuse into the bacterial cell, thereby reducing the intracellular pH and slowing down metabolic activities. Growth of *Escherichia coli* (e.g.) is inhibited at pH 5.1 by lactic acid, as compared to pH 4.5 in presence of hydrochloric acid (Gudkow, 1987). The rapid reduction of pH below 5.3 during raw sausage fermentation is sufficient to inhibit growth of salmonellae (Schillinger and Lücke, 1988) and *Staphylococcus aureus* (Hechelmann et al., 1988).

Because of its higher dissociation constant, *acetic acid* (pK_a 4.75) shows stronger inhibition than lactic acid (pK_a 3.1) at a given molar concentration and

pH. Produced by heterofermentative LAB (*Leuconostoc* spp. and some lactobacilli) in equimolar amounts with lactic acid from hexoses, acetic acid is usually present in small concentrations as result of LAB metabolism; it may, however, constitute a vital factor for the establishment of the initial LAB population (notably *Leuconostoc* spp.) during 'spontaneous' fermentation of sauerkraut, silage, etc. (Buckenhüskes et al., 1990). Under specific conditions of hexose limitation and/or availability of oxygen, homofermentative LAB (e.g. pediococci, lactococci and most *Lactobacillus* spp.) may dissimilate lactic acid to acetic acid, formic acid and/or CO_2 (Kandler, 1983).

In a number of fermented dairy products, the *benzoic acid* concentration significantly exceeds the naturally expected value, and ranges from ca. 7–11 mg/kg for Gouda cheese and up to 13–56 mg/kg for low fat yoghurt (Daeschel, 1989; Sieber et al., 1989). This is explained by the metabolism of hypuric acid by some LAB, e.g. *L.casei*, and constitutes an additional preservation factor in these dairy foods.

Hydrogen peroxide. Hydrogen peroxide is produced by a number of LAB in the presence of molecular oxygen together with lactate, pyruvate and NADH by flavin enzymes (Kandler, 1983). Undesired bacteria such as *Pseudomonas* spp. (Price and Lee, 1970) and *Staphylococcus aureus* are 2 to 10 times more sensitive to H_2O_2 than most LAB. The bacteriostatic concentration for staphylococci is around 6 μg/ml as compared to 23–35 μg/ml for pseudomonads (Gudkow, 1987). In addition, H_2O_2 may activate the lactoperoxidase system which is indigenous to fresh milk with the formation of hypothiocyanite and other antimicrobial products (Reiter and Härnulv, 1984).

As for other metabolic products, the tolerable amount of H_2O_2 is dependent on the product type and situation, and may be detrimental to sensory quality of e.g. processed meats.

Enzymes. Relative to other metabolites, enzymes produced by food-grade bacteria and especially LAB, are of little direct consequence to food preservation. Lipolytic activity, important in ripening of cheese, may, however, affect the production of fatty acids with antimicrobial properties.

Antimicrobial enzymes will be discussed below. Future prospects include using recombinant DNA technology for strain improvement, e.g. with respect to lysozyme production (Van de Guchte et al., 1992).

Low-molecular weight metabolites. A number of primary metabolites of low molecular weight is known for their relatively potent antimicrobial activities.

Diacetyl is produced by some *Lactococcus*, *Leuconostoc* and *Pediococcus* spp., e.g. during degradation of citric acid. Due to its intensive aroma, diacetyl has little direct potential for the preservation of food.

Carbon dioxide produced by heterofermenters from hexoses, contributes to a reduced E_h and is directly toxic to a number of aerobic putrefactive bacteria but may promote the growth of others.

Reuterin, or 3-hydroxypropionaldehyde, is produced from glycerol, dependent of coenzyme B_{12} by *Lactobacillus reuteri*. It shows broad-spectrum antimicrobial activity, probably by inhibition of ribonucleotide reductase, and has been suggested for biopreservation of fish and meat, using *L. reuteri* (Daeschel, 1989; Lindgren and Dobrogosz, 1990).

Bacteriocins. Bacteriocins can be defined as a group of potent antimicrobial peptides or proteins primarily active against closely related organisms. They are ribosomally produced as 'secondary' metabolites, and are probably inactivated by proteases in the gastro-intestinal tract. Most of them are small cationic molecules (30 – 60 amino acid residues) forming amphiphilic helices and being thermostable (100°C/10 min).

Further aspects of bacteriocins will be discussed below.

2.2. Applications

Application of a protective culture for antimicrobial protection of food should be considered only as an additional measure to good manufacturing, processing, storage and distribution practices. Its eventual use will be determined by a number of factors, amongst which its ('food-grade') safety, and adaptation and suitability for a specific food system are the most important ones.

The effectiveness of some bacteriocinogenic protective cultures has been studied in several food systems (Table 4).

Table 4
Food systems in which effectiveness of bacteriocinogenic protective cultures against *Listeria monocytogenes* was tested

Food system	Protective culture	Reference
1. *Meats*		
– fresh meat	*Pediococcus acidilactici* PAC 1.0	Nielsen *et al.* (1990)
– comminuted, cured raw pork	*Lactobacillus sake* Lb 706	Schillinger *et al.* (1991)
– minced meat	*Lactobacillus sake* Lb 706	Schillinger *et al.* (1991)
	Pediococcus damnosus VTT-E-760653	Skyttä *et al.* (1991)
– fermented sausages	*Pediococcus acidilactici* JC 1–23	Berry *et al.* (1990)
	Pediococcus acidilactici PAC 1.0	Foegeding *et al.* (1992)
	Pediococcus acidilactici H, PAC 1.0, P 02, JBL 1350	Luchansky *et al.* (1992)
vacuumpacked:		
– minimally heat-treated beef	*Lactobacillus bavaricus* MN	Winkowski *et al.* (1993)
– frankfurters	*Pediococcus acidilactici* JD 1–23	Berry *et al.* (1991)
– wieners	*Pediococcus acidilactici* JBL 1095	Degnan *et al.* (1992)
2. *Vegetables*		
– ready-to-use salads	*Lactococcus lactis* D 53	Lichter(1993) M.Sc. dissertation (unpublished results)

In most cases a strain of *Pediococcus acidilactici* was used. An inhibitory activity against *Listeria monocytogenes* could be demonstrated in various meats such as fermented sausages and vacuum-packaged products as well as in vegetable-type

Table 5
Potential application of protective cultures in different food systems

(A) Milk and dairy products

Product	Target organism(s)
Mould-ripened cheese	*Listeria monocytogenes*
Hard and semi-hard cheeses	Clostridia causing late-blowing
Fresh cheese types (quarg, etc.)	Moulds and yeasts
Yoghurt (especially with added fruit, nuts and cereals)	Yeasts and moulds

(B) Meat, fish and poultry

Product	Target organism(s)
Soft-type raw sausage (e.g. Mettwurst)	*Staphylococcus aureus*
	Listeria monocytogenes
Mould-ripened fermented sausage	*Staphylococcus aureus*
Prepackaged fish and lightly preserved foods	*Clostridium botulinum* types E. B and F
such as cold-smoked fish, brined shrimps	*Listeria monocytogenes*
Fresh meat	Pseudomonads
	Salmonellae
	Listeria monocytogenes
	Enteropathogenic *E. coli*
Self service packages of processed meat products	*Listeria monocytogenes*
Poultry	*Salmonella* spp.
	Campylobacter spp.

(C) Vegetable type products

Product	Target Organism(s)
Fermented products (general)	Yeasts, moulds
Fermented products:	
– cucumbers	Gas producing LAB
– sauerkraut	Slime producers (e.g. leuconostocs)
	Producers of off-flavours and of biogenic amines
Prepacked mixed salads	Enterobacteriaceae
	Salmonella spp.
	Other Gram-negative pathogens
	Listeria monocytogenes

(D) Delicatessen and novel type foods

Product	Target organisms
Different delicatessen type foods (refrigerated)	Heterofermentative LAB
	Staphylococcus aureus
	Yeasts
Novel type foods	Psychrotolerant Clostridia
– 'sous vides'	*Listeria, Bacillus*
– other cooked-chilled foods and ready-to-eat products	determined by (novel) ecological parameters

Table 6
Effect of a protective culture, *Lactobacillus sake* Lb 706, on *Listeria monocytogenes* in vacuum-packaged sliced bologna (according to M. Kaya, U. Schmidt and L. Leistner, unpublished results/personal communication, 1990)

Inoculum (10^3/g)	Days ('lag time') to increase of *Listeria* cfu from 10^3 to 10^4/g at			
	2°C	4°C	7°C	10°C
Lb 706 (bacteriocin-positive)	> 49	> 42	> 21	7
Lb 706-B (bacteriocin-negative)	> 49	28	7	3

foods. Table 5 gives an overview on food products in which protective cultures may be applied in the future. However, at present protective cultures are not available for all those purposes.

The modern trend towards 'healthy' and fresh food can only be satisfied when additional, acceptable safety factors are applied, e.g. for transport, presentation in retail outlets and storage. Refrigeration is often marred by negligence and abuse; protective cultures may reduce the hygienic risk during e.g. interruption of the cold chain. An experimental example of successful application of a protective cultures against *Listeria* is given in Table 6 for vacuum-packaged sliced bologna (M. Kaya, U. Schmidt and L. Leistner, unpublished results/personal communication, 1990).

In this experiment, an antilisterial activity of *Lactobacillus sake* Lb 706 was observed in sliced bologna type sausages inoculated with *Listeria monocytogenes*. When such sausages were stored under vacuum-packaging at 7°C, the protective culture was able to prevent *Listeria* multiplication for more than 3 weeks, whereas viable numbers of *Listeria* increased from 10^3 to 10^4/g after only one week in sausages inoculated with a non-bacteriocin producing variant of *Lactobacillus sake* Lb 706 (Table 6).

2. Bacteriocins

Based on present knowledge, bacteriocins of LAB may be classified into four groups:

- the lantibiotics, which are characterised by some unusual amino acids such as lanthionine or β-methyllanthionine;
- peptide bacteriocins, which are small hydrophobic membrane-active peptides;
- protein bacteriocins which are of a higher molecular mass; and
- complex bacteriocins containing a glyco and/or lipid moiety essential for their activity (see Table 7).

Mode of action. For most bacteriocins, the antimicrobial effect seems to be bactericidal (Schillinger, 1990, Schillinger and Lücke, 1989), with some exceptions, e.g. leuconocin S (Lewus et al., 1992) and leucocin A-UAL 187 (Hastings et al., 1991) being bacteriostatic.

Table 7
Classification of bacteriocins from lactic acid bacteria

Type	Structure	Heat stability	Antibacterial spectrum	Example	References
Lantibiotics	small (< 5 kDa) unusual amino acids (e.g. lanthionine)	+	medium to broad	nisin lacticin 481	Hurst, 1981 Piard et al., 1990
Peptide bacteriocins	small (< 10 kDa) no lanthionine	+	medium to broad	pediocin AcH sakacin A leucocin UAL 187	Bhunia et al., 1988 Holck et al., 1992 Hastings et al., 1991
Protein bacteriocins	large (> 10 kDa) no lanthionine	−	narrow	helveticin J cascicin 80	Joerger and Klaenhammer, 1986 Rammelsberg et al., 1990
Complex bacteriocins	glyco- and/or lipid moiety	+	medium	leuconocin S pediocin SJ-1	Lewus et al., 1992 Schved et al., 1993

Table 8
Bacteriocins from LAB with activity against Gram-positive foodborne pathogens

Genus	Bacteriocin	Activity against					References
		Listeria monocytogenes	*Staphylococcus aureus*	*Bacillus cereus*	*Clostridium botulinum*	*Clostridium perfringens*	
Carnobacterium	Unnamed from *C. piscicola* LK5	+	n.d.	n.d.	n.d.	n.d.	Buchanan and Klawitter, 1992
	Carnobacteriocins A and B	+	n.d.	n.d.	n.d.	n.d.	Ahn and Stiles, 1990
	Piscicolin 61	+	−	−	n.d.	n.d.	Schillinger and Holzapfel, 1990
Lactobacillus	Bavaricin A	+	−	−	n.d.	n.d.	Larsen et al., 1993
	Bavaricin MN	+	−	n.d.	−	n.d.	Lewus et al., 1991
	Curvacin A	+	(+)	−	n.d.	n.d.	Tichaczek at al., 1992
	Curvaticin 13	+	+	+	n.d.	n.d.	Sudirman et al., 1993
	Plantaricin BN	+	−	n.d.	+	n.d.	Okereke and Montville, 1991
	Sakacin A	+	−	n.d.	−	n.d.	Schillinger and Lücke, 1989
	Sakacin M	+	(+)	−	(+)	(+)	Sobrino et al., 1991
	Sakacin P	+	−	−	n.d.	n.d.	Tichaczek et al., 1992
Leuconostoc	Carnocin 44	+	−	−	n.d.	n.d.	Van Laack et al., 1992
	Leucocin UAL 187	+	−	n.d.	n.d.	n.d.	Hastings and Stiles, 1991
	Leuconocin S	+	+	n.d.	+	n.d.	Lewus et al., 1992
	Mesenterocin 5	+	−	n.d.	n.d.	n.d.	Daba et al., 1991
	Mesenterocin 52	+	−	−	n.d.	n.d.	Mathieu et al., 1993
	Mesentericin Y 105	+	−	−	n.d.	n.d.	Hechard et al., 1992
	Unnamed from *Leuconostoc gelidum* IN 139	+	−	−	−	−	Harding and Shaw, 1990
Pediococcus	Pediocin A	+	+	n.d.	+	+	Daeschel and Klaenhammer, 1985
	Pediocin AcH	+	+	+	− (spores)	+	Bhunia et al., 1988
	Pediocin PA-1	+	−	n.d.	n.d.	n.d.	Gonzalez and Kunka, 1987
	Pediocin PC	+	−	−	n.d.	+	Jager and Harlander, 1992
	Pediocin SJ1	+	−	n.d.	n.d.	+	Schved et al., 1993

n.d., not determined.

The site of bacteriocin action is the cytoplasmic membrane. Bacteriocins such as nisin (Ruhr and Sahl, 1985) and pediocin JD (Christensen and Hutkins, 1992) dissipate the membrane potential and cause a collapse of proton motive force (Bruno and Montville, 1993).

Inhibitory spectrum. The activity spectrum of bacteriocins is, per definition, restricted to closely related organisms (Tagg et al., 1976). For LAB this fact implies two main disadvantages: (a) bacteriocins produced by protective cultures may inhibit other desired starter cultures and (b) are not active against Gram-negative pathogens and spoilage bacteria.

A number of Gram-positive toxinogenic and pathogenic bacteria have, however, been found sensitive to bacteriocins of certain LAB (see Table 8).

Gram-negative bacteria such as salmonellae are not sensitive to bacteriocins from lactic acid bacteria obviously because of their outer membrane protecting them by excluding the bacteriocins. However, chelating agents such as EDTA or citrate can be used to bind magnesium ions in the lipopolysaccharide layer of the outer membrane of Gram-negative bacteria and rendering these organisms susceptible to nisin and other bacteriocins (Stevens et al., 1992).

Molecular biological attributes. Despite increasing research activities, information on the genes involved in bacteriocin synthesis, processing, secretion and regulation, is still limited. Bacteriocin production can be either chromosomally or plasmid mediated, and, for the few cases so far investigated, is encoded by multiple genes organized in operon-like structures. Genetic investigations of peptide bacteriocins (group 2, Table 7) have shown that the genes encoding transport, maturation and procession, and immunity are closely linked with the structural gene (Marugg et al., 1992). These bacteriocins are transcribed as precursor peptides, typically with an *N*-terminal leader peptide of about 18 to 20 amino acids. The leader sequences are apparently not typical Gram-positive secretion signals (Tichaczek et al., 1993).

Nisin. The bacteriocins of LAB are of special interest with regard to the health acceptability and potential use of this group in biopreservation. Nisin is the best studied factor; it is produced by strains of *Lactococcus lactis* (Jarvis and Farr, 1971; Hurst, 1981) and is the best studied representative of the lantibiotics (Klaenhammer, 1988). It has found special application in the prevention of late-blowing of cheese by inhibiting the outgrowth of *Clostridium* spores (Daeschel, 1989) and is used in selected pasteurized cheese spreads. Although not considered by all workers as a typical bacteriocin, but rather as a peptide antibiotic (Schüller et al., 1989), its use as a food additive has been accepted by the Joint FAO/WHO Expert Committee in 1969 (Falbe and Regitz, 1991). Its antibacterial activity and possible use as biopreservative has been studied in a large number of food systems; application for the control of some pathogens and food spoilage organisms is approved in a number of countries, and is reviewed by Delves-Broughton (1990). In Europe, eight EC-countries have approved the use of nisin for preservation of processed cheese (Belgium, France, Ireland, Portugal and Spain), fresh cheese,

quarg, etc. (The Netherlands), processed vegetables (Italy), canned foods (United Kingdom), generally as part of 'botulinum-safe' thermal processing ($F_0 > 3.0$).

Considering the non-specific antagonistic effects resulting from other metabolic activities of LAB (see Table 3), bacteriocins may provide a valuable additional, controllable and specific tool for the inhibition of some deleterious food-associated microorganisms. The use of the pure substance as food additive may still be controversial; in addition, considerable consumer resistance can be expected. As for nisin and the dairy starter culture *Lactococcus lactis*, the benefits of bacteriocins may, however, be utilised by means of a 'food-grade' producer strain, for which special labelling is not necessary. Bacteriocinogeny may thus be considered as a most desirable and potentiating trait of a protective culture. Under special conditions, e.g. during fermentation, it may also increase the competitiveness of a starter culture.

Limitations and future prospects of protective cultures and bacteriocins

Present knowledge and experience indicate that protective cultures may be applied within certain limits, indicated above with relation to food systems, as additional safety factor. These limitations concern three main features of LAB and other bacterial cultures, *viz.*:

- Adaptation
  - relative to product group
  - persistance and competition
  - sensitivity to processing parameters
- Metabolic activity
  - essential in a food system (risk of inactivation)
  - possible deleterious sensory effects
- Specific antibacterial factors such as bacteriocins
  - activity spectrum
  - inactivation (e.g. by product specific proteases)
  - limited diffusion in solid matrix
  - no influence on Gram-negative bacteria
  - inducible resistance
  - unspecific binding to food ingredients (e.g. inactivation by lipids).

Recent advances and increased knowledge on the physiology and molecular biology of the LAB, in addition to progresses in selection and culture techniques, give reason for optimism towards the development of improved, tailor-made protective cultures. The main research activities will probably be directed towards the following achievements:

- Improved and targeted selection and screening methods
- Optimisation ('tailoring') by recombinant DNA technology
  - transfer of bacteriocin genes within the LAB; construction of multibacteriocinogenic strains
  - transfer of resistance genes
  - development of high potential multiple strain cultures

Table 9
Examples of the genetic optimisation of protective cultures

Desired trait	Result	Reference
Expression of lysozyme gene in lactococci	Amplification of antagonism against clostridia and Gram-positive bacteria	Van de Guchte et al. (1992)
Expression of lysostaphin gene in *Lactobacillus casei*	Increased inhibition of staphylococci	Gaier et al. (1992)
Expression of phage resistance genes	Increased phage resistance/process safety	Harrington and Hill (1991)

- transfer of genes encoding for wide-spectrum antibacterial proteins (activity e.g. against Gram-negative pathogens) from other 'GRAS' organisms including yeasts and moulds.

- Extending of activity spectrum – in combination with food-grade additives or natural ingredients.

Genetic optimisation of protective cultures is a valuable tool towards improving process security (e.g. phage resistance), improving efficiency (e.g. increased proteolytic activity) and reducing health risks (increased antagonism). The genes of encoding for some antagonistic properties have already been successfully expressed in lactic acid bacteria (Table 9).

3. Enzymes

The excretion of an antibacterial enzyme, such as lysozyme or lysostaphin, may improve the activity spectrum of protective cultures, and may render such a strain more suitable for application under versatile conditions.

As for antimicrobial metabolites, a number of enzymes serve in nature to protect a biological system against invasion of certain microorganisms. Typical examples are lysozyme in egg albumen and lactoperoxidase in milk. Food systems which contain such antagonistic enzymes possess an intrinsic stability against microorganisms. On the other hand purified antagonistic enzymes can be used as biopreservatives for foods which do not contain such antagonistic systems, as for example the addition of lysozyme to cheese to prevent late blowing due to *Clostridium tyrobutyricum* (Bester and Lombard, 1990).

Legally, enzymes are considered as food additives and require special approval of 'food grade' quality or 'GRAS' status.

Enzymes with antagonistic activity are listed in Table 10.

- One of the most important enzymes is *lysozyme*, which can be found in milk and eggs. It is a muraminidase that hydrolyses β-1,4 linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan, leading to lysis of bacterial cells. Gram-negative bacteria posses an outer membrane, which may protect the cell wall against the action of lysozyme. After disruption (weakening) of the outer membrane by chelating membrane-stabilizing divalent cations with

Table 10
Naturally occurring antagonistic enzyme systems in foods

Enzyme	Inhibited microorganisms	Mechanisms of action
Lysozyme	Mainly Gram-positive	Lysis
Glucose oxidase	Gram-positive Gram-negative	H_2O_2?, pH?
Lactoperoxidase	Gram-positive Gram-negative	Oxidation
Lactoferrin	Gram-positive Gram-negative	Iron binding

EDTA, also Gram-negative microorganisms become susceptible to the action of lysozyme.

Lysozyme is especially active against the outgrowth of clostridial spores but it also shows activity against other pathogenic or toxinogenic bacteria such as *Bacillus* and *Listeria*.

In recent years investigations have especially concerned with control of the growth of *Listeria monocytogenes* by the action of lysozyme (Hughey and Johnson, 1987; Carminati and Carini, 1989; Bester and Lombard, 1990). Lysozyme may control the growth of *Listeria* in culture broth systems (Hughey and Johnson, 1987) and in real food systems like milk (Carminati and Carini, 1989) and cheese (Bester and Lombard, 1990). In some food products lysozyme shows a remarkable stability. It is still active in Camembert cheese after 3–4 weeks (Hughey et al. 1989).

- *Lactoperoxidase* (LPS) is an enzyme system occurring naturally in milk. It represses the growth of *Staphylococcus aureus* and *Listeria monocytogenes* if activated in milk. The LPS system is activated by thiocyanate ions (SCN^-) and H_2O_2. In the presence of both substrates the LPS system oxidizes thiocyanate to hypothiocyanite ($OSCN^-$). This is a strong oxidizing agent which in turn can oxidize essential sulfhydryl groups in bacterial proteins but is harmless to host cells (Kamau et al., 1990).

  Thiocyanate ions are present in milk (Siragusa and Johnson, 1989) and H_2O_2 can be produced by accompanying lactic acid bacteria.
- *Glucose oxidase* oxidizes glucose to glucuronic acid in the presence of O_2. In addition hydrogen peroxide is produced. This system is already used in the food industry to remove O_2 from various packaged or canned food products. It is in addition able to repress the growth of certain Gram-positive as well as Gram-negative microorganisms. The inhibitory effect depends on the concentration of the enzyme in the medium and on the concentration of glucose as substrate. Higher inhibitory activity can be observed at higher enzyme concentrations (Tiina and Sandholm, 1989). These experiments were done in broth culture. Jeong et al. (1992) demonstrated that glucose oxidase was not useful to inhibit

the growth of *Pseudomonas* or *Salmonella* on chicken breast skin. Glucose oxidase might be more active in liquid food systems.

- *Lactoferrin* is an iron binding protein in milk, with activity against *Bacillus subtilis*, *B. stearothermophilus* and *Escherichia coli*. The microorganisms are apparently inhibited due to the iron chelating capacity of this protein. Lactoferricin B is a proteolytic cleavage product of lactoferrin. It is a peptide of 25 amino acids and has a much higher antagonistic activity than lactoferrin. 0.3 to 150 μg/ml of that peptide is enough to inhibit the following microorganisms completely: *E. coli*, *Salmonella enteritidis*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Clostridium perfringens* (Bellamy et al., 1992)

4. Conclusions

Biological preservation can only be considered as an additional processing parameter for improved safety and quality assurance of a food. It can never substitute GMP, and its implementation and acceptance will (amongst others) depend on careful selection and application of suitable protective cultures and probably certain enzymes for particular food systems.

Literature

Adachi, S. (1992) Lactic acid bacteria and the control of tumours. In: B.J.B. Wood (editor) The Lactic Acid Bacteria in Health and Disease, I. Elsevier Applied Science, London/New York.

Aguirre, M. and Collins, M.D. (1993) Lactic acid bacteria and human clinical infection. J. Appl. Bacteriol. 75, 95–107.

Ahn, C. and Stiles, M.E. (1990) Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. Appl. Environ. Microbiol. 56, 2503–2510.

Bellamy, W., Takase, M., Wakabayashi, H., Kawase, K. and Tomita, M. (1992) Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the *n*-terminal region of bovin lactoferrin. J. Appl. Bact. 73, 472–479.

Berry, E.D., Liewen, M.B., Mandigo, R.M. and Hutkins, R.W. (1990) Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus* during manufacture of fermented semi-dry sausage J. Food Prot. 53, 194–197.

Berry, E.D., Hutkins, R.W. and Mandigo, R.W. (1991) The use of bacteriocin-producing *Pediococcus acidilactici* to control postprocessing *Listeria monocytogenes* contamination of Frankfurters. J. Food Prot. 54, 681–686.

Bester, B.H. and Lombard, S.H. (1990) Influence of lysozyme on selected bacteria associated with gouda cheese. J. Food Prot. 53, 306–311.

Bhunia, A.K., Johnson, M.C. and Ray, B. (1988) Purification, characterisation and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. J. Appl. Bacteriol. 65, 261–268.

Brown, M.H. and Booth, J.R. (1991) Acidulants and low pH. In: N.J. Russell and G.W. Gould (editors), Food Preservatives. Blackie, Glasgow, pp. 22–43.

Bruno, M.E. and Montville, T.J. (1993) Common mechanistic action of bacteriocins from lactic acid bacteria. Appl. Environ. Microbiol. 59, 3003–3010.

Buchanan, R.L. and Klawitter, L.A. (1992) Characterization of a lactic acid bacterium, *Carnobacterium piscicola* LK5, with activity against *Listeria monocytogenes* at refrigeration temperatures. J. Food Safety 12, 199–217.

Buckenhüskes, H., Aabye Jensen, H., Andersson, R., Garrido Fernandez, A. and Rodrigo, M. (1990) Fermented vegetables. In: P. Zeuthen, J.C. Chefiel, C. Eriksson, T.R. Cormley, P. Linko and K. Paulus (editors) Processing and Quality of Foods, Vol. 2: Food Biotechnology. Elsevier Applied Science, London, pp. 2162–2187.

Carminati, D. and Carini, S. (1989) Antimicrobial activity of lysozyme against *Listeria monocytogenes* in milk. Microbiologie-Aliments-Nutrition 7, 49–56.

Christensen, D.P. and Hutkins, R.W. (1992) Collapse of the proton motive forcein *Listeria monocytogenes* caused by a bacteriocin produced by *Pediococcus acidilactici*. Appl. Environ. Microbiol. 58, 3312–3315.

Daba, H., Pandian, S., Gosselin, J.F., Simard, R.E., Huang, J. and Lacroix, C. (1991) Detection and activity of a bacteriocin produced by *Leuconostoc mesenteroides*. Appl. Environ. Microbiol. 57, 3450–3455.

Daeschel, M.A. (1989) Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol. 43., 164–166.

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.

Degnan, A.J., Yousef, A.E. and Luchansky, J.B. (1992) Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. J. Food Prot. 55, 98–103.

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

Falbe, J. and Regitz, M., editors (1991) Römpp Chemielexikon (9th edition) Vol. 4. Thieme, Stuttgart.

Fernandes, C.F., Chandan, R.C. and Shahani, K.M. (1992) Fermented Dairy Products and Health. In: B.J.B. Wood (editor) The Lactic Acid Bacteria in Health and Disease, I. Elsevier Applied Science, London/New York.

Foegeding, P.M., Thomas, A.B., Pilkington, D.H. and Klaenhammer, T.R. (1992) Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. 58, 884–890.

Gaier, W., Vogel, R.F. and Hammes, W.P. (1992) Cloning and expression of the lysostaphin gene in *Bacillus subtilis* and *Lactobacillus casei*. Lett. Appl. Microbiol. 14, 72–76.

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

Gudkow, A.V. (1987) Starters: as a means of controlling contaminating organisms. Milk – The Vital Force, pp. 83–93.

Hammes, W.P. and Vogel, R.F. (1990) Gentechnik zur Modifizierung von Starterorganismen. Lebensmitteltechnik 1–2, 24–32.

Harding, C.D. and Shaw, B.G. (1990) Antimicrobial activity of *Leuconostoc gelidum* against closely related species and *Listeria monocytogenes*. J. Appl. Bacteriol. 69, 648–654.

Harrington, A. and Hill, C. (1991) Confirmation of a bacteriophage-resistant derivative of *Lactococcus lactis* subsp. *lactis* 425A by lysing the conjugal plasmid pNP 40. Appl. Environ. Microbiol. 57, 3405 – 3409.

Hastings, J.W. and Stiles, M.E. (1991) Antibiosis of *Leuconostoc gelidum* isolated from meat. J. Appl. Bacteriol. 70, 127 – 134.

Hastings, J.W., Sailer, M., Johnson, K., Roy, K.L., Vederas, J.C. and Stiles, M.E. (1991) Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. J. Bacteriol. 173, 7491–7500.

Hechard, Y., Derijard, B., Letellier, F. and Cenatiempo, Y. (1992) Characterization and purification of mesentericin Y150, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*. J. Gen. Microbiol. 138, 2725–2731.

Hechelmann, H., Lücke, F.-K. and Schillinger, U. (1988) Ursachen und Vermeidung von *Staphylococcus aureus*-Intoxikationen nach Verzehr von Rohwurst und Rohschinken. Mittbl. Bundesanstalt Fleischforsch. Kulmbach 100, 7956–7964.

Holck, A., Axelsson, L., Birkeland, S.E., Aukrust, T. and Blom, H. (1992) Purification and amino acid sequence of sakacin A, a bacteriocin from *Lactobacillus sake* Lb706. J. Gen. Microbiol. 138, 2715–2720.

Holzapfel, W.H. and Botha, S.F. (1988) Physiology of *Sporolactobacillus* strains isolated from different

habitats and the indication of in vitro antagonism against *Bacillus* species. Int. J. Food Microbiol. 7, 161–168.

Holzapfel, W.H. und Hammes, W.P. (1989) Die Bedeutung moderner biotechnischer Methoden für die Lebensmittelherstellung. In: Biotechnologie in der Agrar- und Ernährungswirtschaft. Berichte über Landwirtschaft, Neue Folge, 201. Sonderheft, Parey, Hamburg/Berlin pp. 47–65.

Hughey, V.L. and Johnson, E.A. (1987) Antimicrobial activity of lysozyme against bacteria involved in food spoilage and food-borne disease. Appl. Environ. Microbiol. 53, 2165–2170.

Hughey, V.L., Wilder, P.A. and Johnson, E.A. (1989) Antibacterial activity of hen egg white lysozyme against *Listeria monocytogenes* Scott A in foods. Appl. Environ. Microbiol. 55, 631–638.

Hurst, A. (1981) Nisin. Adv. Appl. Microbiol. 27, 85–123.

Jager, K. and Harlander, S. (1992) Characterization of a bacteriocin from *Pediococcus acidilactici* PC and comparison of bacteriocin-producing strains using molecular typing procedures. Appl. Microbiol. 37, 631–637.

Jarvis, B. and Farr, J. (1971) Partial purification, specificity and mechanism of action of the nisin-inactivating enzyme from *Bacillus cereus*. Biochim. Biophys. Acta 227, 232–240.

Jeong, D.K. and Harrison, M.A., Frank, J.F. and Wicker, L. (1992) Trials on the antibacterial effect of glucose oxidase on chicken breast skin and muscle. J. Food Saf. 13, 43–49.

Joerger, M.C. and Klaenhammer, T.R. (1986) Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. J. Bacteriol. 167, 439–446.

Kamau, D.N., Doores, S. and Pruitt, K.M. (1990) Antibacterial activity of the lactoperoxidase system against *Listeria monocytogenes* and *Staphylococcus aureus* in milk. J. Food Prot. 53, 1010–1014.

Kandler, O. (1983) Carbohydrate metabolism in lactic acid bacteria. Antonie van Leuwenhoek 49, 202–224.

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

Larsen, A.G., Vogensen, F.-K. and Josephsen (1993) Antimicrobiol activity of lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavarius* MI 401. J. Appl. Bacteriol. 75, 113–122.

Lewus, C.B., Kaiser, A. and Montville, T.J. (1991) Inhibition for food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. 57, 1683–1688.

Lewus, C.B., Sun, S. and Montville, T.J. (1992) Production of an amylase-sensitive bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. Appl. Environ. Microbiol. 58, 143–149.

Lichter, G. (1993) Anwendung von Schutzkulturen zur mikrobiologischen Sicherung von vorverpacktem Mischsalat und Eisbergsalat. M.Sc. Dissertation, Univ. Karlsruhe.

Lindgren, S.E. and Dobrogosz, W.J. (1990) Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. 87, 149–163.

Luchansky, J.B., Glass, K.A., Harsono, K.D., Degnan, A.J., Faith, N.G., Cauvin, B., Baccus-Taylor, G., Arinara, K., Bater, B., Maurer, A.J. and Cassens, R.G. (1992) Genomic analysis of *Pediococcus* starter cultures used to control *Listeria monocytogenes* in Turkey summer sausage. Appl. Environ. Microbiol. 58, 3053–3059.

Marugg, J.D., Gonzalez, C.F., Kunka, B.S., Ledeboer, A.M., Pucci, M.J., Tonen, M.Y., Walker, S.A., Zoetmulder, L.C.M. and Vandenbergh, P.A. (1992) Cloning, expression, and nucleotide sequence of genes involved in production of pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC 1.0. Appl. Environ. Microbiol. 58, 2360–2367.

Mathieu, F., Sudirman Suwandhi, I., Rekhif, N., Milliere, J.B. and Lefebvre, G. (1993) Mesenterocin 52, a bacteriocin produced by *Leuconostoc mesenteroides* ssp. *mesenteroides* FR 52. J. Appl. Bacteriol. 74, 372–379.

Motlagh, A.M., Holla, S., Johnson, M.C., Ray, B. and Field, R.A. (1992) Inhibition of *Listeria* spp. in sterile food systems by pediocin AcH, a bacteriocin produced by *Pediococcus acidilactici* H.J. Food Prot. 55, 337–343.

Nielsen, J.W., Dickson, J.S. and Crouse, J.D. (1990) Use of a bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. Appl. Environ. Microbiol. 56, 2142–2145.

Okereke, A. and Montville, T.J. (1991) Bacteriocin-mediated inhibition of *Clostridium botulinum* spores

by lactic acid bacteria at refrigeration and abuse temperatures. Appl. Environ. Microbiol. 57, 3423–3428.

Pederson, C.S. (1971) Microbiology of Food Fermentations. AVI, Westport, CT.

Piard, J.-C., Muriana, P.M., Deszmazeaud, M.J. and Klaenhammer, T.R. (1992) Purification and partial characterization of lacticin 481, a lanthionine-containing bacteriocin produced by *Lactococcus lactis* subsp. *lactis* CNRZ 481. Appl. Environ. Microbiol. 58, 279–284.

Price, R.J. and Lee, J.S. (1970) Inhibition of *Pseudomonas* species by hydrogen peroxide producing lactobacilli. J. Milk Food Technol. 33, 13–18.

Rammelsberg, M., Müller, E. and Radler, F. (1990) Caseicin 80: purification and characterization of a new bacteriocin from *Lactobacillus casei*. Arch. Microbiol. 154, 249–252.

Ray, B. (1992) Bacteriocins of starter culture bacteria as food biopreservatives: an overview. In: B. Ray and M. Daeschel (editors) Food Biopreservatives of Microbial Origin. CRC, Boca Raton/Ann Arbor.

Reiter, B. and Härnulv, B.G. (1984) Lactoperoxidase antibacterial system: natural occurrence, biological functions and practical application. J. Food Prot. 47, 724–732.

Ruhr, E. and Sahl, H.-G. (1985) Mode of action of the peptide antibiotic nisin and influence on the membrane potential of whole cells and on cytoplasmic and artificial membrane vesicles. Antimicrob. Agents Chemother. 27, 841–845.

Schillinger, U. (1990) Bacteriocins of lactic acid bacteria. In: D.D. Bills and Shain-dow Kung (editors) Biotechnology and Food Safety. Butterworth-Heinemann, Boston, pp. 55–74.

Schillinger, U. and Lücke, F.-K. (1988) Hemmung des Salmonellenwachstums in frischer, streichfähiger Mettwurst ohne Zuckerstoffe. Fleischwirtschaft 68, 1056–1067.

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

Schillinger, U. and Holzapfel, W.H. (1990) Antibacterial activity of carnobacteria. Food Microbiol. 7, 305–310.

Schillinger, U., Kaya, M. and Lücke, F.-K. (1991) Behavior of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. 70, 473–478.

Schüller, F., Benz, R. and Sahl, H.-G. (1989) The peptide antibiotic subtilin acts by formation of voltage-dependent multi-state pores in bacterial and artificial membranes. Eur. J. Biochem. 182, 181–186.

Schved, F., Lalazar, A., Henis, Y. and Juven, B.J. (1993) Purification, partial characterization and plasmid-linkage of pediocin SJ-1, a bacteriocin produced by *Pediococcus acidilactici*. J. Appl. Bacteriol. 74, 67–77.

Sieber, R., Bütikofer, U., Bosset, J.O. and Rüegg, M. (1989) Benzoesäure als natürlicher Bestandteil von Lebensmitteln – eine Übersicht. Mitt. Gebiete Lebensmittelhyg. 80, 345–362.

Siragusa, G.R. and Johnson, M.G. (1989) Inhibition of *Listeria monocytogenes* growth by the lactoperoxidase-thiocyanat-H_2O_2 antimicrobial system. Appl. Environ. Microbiol. 55, 2802–2805.

Skyttä, E., Mereijgers, W. and Mattila-Sandholm, T. (1991) Broad spectrum antibacterial activity of *Pediococcus damnosus* and *Pediococcus pentosaceus* in minced meat. Food Microbiol. 8, 231–237.

Sobrino, O.J., Rodriguez, J.M., Moreira, W.L., Fernandez, M.F., Sanz, B. and Hernandez, P.E. (1991) Antibacterial activity of *Lactobacillus sake* isolated from dry fermented sausages. Int. J. Food Microbiol. 13, 1–10.

Stevens, K.A., Sheldon, B.W., Klapes, N.A. and Klaenhammer, T.R. (1992) Nisin treatment for inactivation of *Salmonella* species and other Gram-negative bacteria. Appl. Environ Microbiol. 57, 3613–3615.

Sudirman, I., Mathieu, F., Michel, M. and Lefebvre, G. (1993) Detection and properties of curvaticin 13, a bacteriocin-like substance produced by *Lactobacillus curvatus* SB 13. Current Microbiol. 27, 35–40.

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

Tichaczek, P.S., Nissen-Meyer, J., Nes, I.F., Vogel, R.F. and Hammes, W.P. (1992) Characterization of the bacteriocins curvacin A from *Lactobacillus curvatus* LTH 1174 and sakacin P from *Lactobacillus sake* LTH 673. System. Appl. Microbiol. 15, 460–468.

Tichaczek, P.S., Vogel, R.F. and Hammes, W.P. (1993) Cloning and sequencing of cur A encoding curvacin A, the bacteriocin produced by *Lactobacillus curvatus* LTH 1174. Arch. Microbiol. 160, 279–283.

Tiina, M. and Sandholm, M. (1989) Antibacterial effect of the glucose oxidase-glucose system on food-poisoning organisms. Int. J. Food Microbiol. 8, 165–174.

Van de Guchte, M., Van der Wal, F.M., Kok, J. and Venema, G. (1992) Lysozyme expression in *Lactococcus lactis*. Appl. Microbiol. Biotechnol. 37, 216–224

Van Laack, R.L.J.M., Schillinger, U. and Holzapfel, W.H. (1992) Characterization and partial purification of bacteriocin produced by *Leuconostoc carnosum* LA44A. Int. J. Food Microbiol. 16, 183–195.

Winkowski, K., Crandall, A.D. and Montville, T.J. (1993) Inhibition of *Listeria monocytogenes* by *Lactobacillus bavaricus* MN in beef systems at refrigeration temperatures. Appl. Environ. Microbiol. 59, 2552–2557.