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Review article

Bacteriocins: safe, natural antimicrobials for food preservation

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Abstract

Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria. Many lactic acid bacteria (LAB) produce a high diversity of different bacteriocins. Though these bacteriocins are produced by LAB found in numerous fermented and non-fermented foods, nisin is currently the only bacteriocin widely used as a food preservative. Many bacteriocins have been characterized biochemically and genetically, and though there is a basic understanding of their structure–function, biosynthesis, and mode of action, many aspects of these compounds are still unknown. This article gives an overview of bacteriocin applications, and differentiates bacteriocins from antibiotics. A comparison of the synthesis, mode of action, resistance and safety of the two types of molecules is covered. Toxicity data exist for only a few bacteriocins, but research and their long-time intentional use strongly suggest that bacteriocins can be safely used.

Keywords: Bacteriocin; Antimicrobial; Natural; Non-antibiotic; Food preservation

1. Introduction: the need for natural food preservation

Since food safety has become an increasingly important international concern, the application of antimicrobial peptides from lactic acid bacteria (LAB) that target food pathogens without toxic or other adverse effects has received great attention. Recent estimates from the Centers for Disease Control and Prevention in the United States suggest that there are 76 million cases of food-borne illness in the US each year, which result in about 5000 deaths (Mead et al., 1999). The US cost of foodborne illness associated with *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus* and *Toxoplasma gondii* is between $6.5 and $34.9 billion (Buzby and Roberts, 1997). Recent outbreaks of emerging pathogens such as *L. monocytogenes* have prompted the food industry, the public, and the government to question the adequacy of current methods of food preservation (http://aids.medscape.com/reuters/prof/1999/10/10.28/pb10289b.html, 2000). The consumption of more food that has been formulated with chemical preservatives has also increased consumer concern and created a demand for more "natural" and "minimally processed" food. As a result, there has been a great interest in naturally produced antimicrobial agents.

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Table 1
Antimicrobial peptides of eukaryotic origin

Antimicrobial Peptide	Source	Mode of Action	Antimicrobial Spectrum	Toxicity	References
Pardaxin	*Pardachiros maroratus* (Red Sea Moses Sole) and *Par. pavoninus* (peacock sole)	Forms barrel stave pores which induce release of neurotransmitters	Gram+, more effective against Gr−	Reduced hemolysis against human rbc	(Oren and Shai, 1996)
Melittin	Bee venom	α helix inserts in membrane	Gr+ and Gr−	Lyse mammalian and bacterial cells	(Oren and Shai, 1996)
Ceratotoxin	*Ceratitis capita*	unknown	Gr+ and Gr−	Lytic to *E. coli* K-12	(Marri et al., 1996)
Histatins	Human saliva	Form pores in membranes	Broad spectrum, bacteria and fungi	Little or none	(Helmerhorst et al., 1997)
Trichorzins	*Trichoderma* (soil fungi)	Forms voltage gated ion channels	*S. aureus* but not *E. coli*	Hemolytic	(Goulard et al., 1995)
Cecropins	Humoral immune system of some insects, i.e., *Hyalophora cecropia* (giant silk moth)	Disrupts lipid bilayer of membrane	Gr− more sensitive than Gr+	Lyse anionic liposomes and bacteria	(Moore et al., 1996; Hansen, 1993; Helmerhorst et al., 1997; Boman, 1991)
Magainins	Frogs and other amphibians, i.e., *Xenopus laevis*	Forms anion permeable channels in membrane	Bacteria and fungi	Lytic	(Higazi et al., 1996; Helmerhorst et al., 1997; Hansen, 1993)
Defensins	Mammalian neutrophils	Form voltage gated channels	Gr+, Gr−, fungi, enveloped viruses	Cytotoxic	(Higazi et al., 1996; Kagan et al., 1994)

2. Antimicrobial peptides from eukaryotes

To maintain their existence or ecological niche, many species have developed systems of antimicrobial defense against competitors or infections (Nissen-Meyer and Nes, 1997). The production of antimicrobial peptides is a first line of defense, and also part of the innate immunity, found in a variety of species. Table 1 provides examples of many antimicrobial peptides produced by eukaryotic organisms. Sometimes the peptides act against a specific group of competing organisms; sometimes their broad spectrum of activity serves as a more general defense mechanism. Antimicrobial peptides protect the host by different mechanisms, but most commonly by permeabilizing the target cell membrane, resulting in an irreversible leakage of cellular material and consequently cell death. Antimicrobial peptides from eukaryotes show varying degrees of toxicity. For example, defensins, produced by human neutrophils, are cytotoxic toward the producing cell at high concentrations (Higazi et al., 1996). Though many different antimicrobial peptides have been isolated from eukaryotes, their cytotoxicity makes them undesirable for use in foods.

3. Bacteriocins: antimicrobial peptides from bacteria

Bacteria are a source of antimicrobial peptides, which have been examined for applications in micro-

Table 2
Examples of bacteriocins isolated from foods

Source	Strain	Active against	References
Commercial probiotic product	*Streptococcus* sp. CNCM I-841	*Clostridium* sp., *L. monocytogenes*	(Gomez et al., 1997)
Bulgarian yellow cheese	*Lactob. delbrueckii* sp.	*L. monocytogenes*, *S. aureus*, *Ent. faecalis*, *E. coli*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*	(Miteva et al., 1998)
Vegetables	*Enterococcus mundtii*	*L. monocytogenes*, *C. botulinum*	(Bennik et al., 1998)
Radish	*Lac. lactis* supsp. *cremoris* R	*Clostridium*, *Staphylococcus*, *Listeria*, and *Leuconostoc* spp.	(Yildirim and Johnson, 1998)
"Waldorf" salad	*Lactob. plantarum* BFE905	*L. monocytogenes*	(Franz et al., 1998)
French mold-ripened soft cheese	*Carnobacterium piscicola* CP5	*Carnobacterium*, *Listeria*, and *Enterococcus* spp.	(Herbin et al., 1997)
Bean-sprouts	*Lac. lactis* subsp. *lactis* (NisZ)	*L. monocytogenes* Scott A	(Cai et al., 1997)
Munster cheese	*Lactob. plantarum* WHE92 (PedAcH)	*L. monocytogenes*	(Ennahar et al., 1996)
Spoiled ham	*C. piscicola* JG126	*L. monocytogenes*	(Jack et al., 1996)
Traditional French cheese	*Ent. faecalis* EFS2	*L. inocua*	(Maisnier-Patin et al., 1996)
Dry sausage	*Lactob. plantarum* UG1	*L. monocytogenes*, *Bacillus cereus*, *C. perfringens*, *C. sporogenes*	(Enan et al., 1996)
Irish kefir grain	*Lac. lactis* DPC3147	*Clostridium*, *Enterococcus*, *Listeria*, *Leuconostoc* spp.	(Ryan et al., 1996)
Dry fermented sausage	*Lac. lactis* (NisA)	*L. monocytogenes*	(Rodriguez et al., 1995)
Fermented sausage	*Lactob. plantarum* SA6	*Lactobacillus* spp.	(Rekhif et al., 1995)
Red smear cheese	*Brevibacterium lines* M18	*Listeria* and *Corinebacterium* spp.	(Valdes-Stauber and Scherer, 1994)
Meat	*Leuconostoc carnosum* Ta11A (LeuA)	*L. monocytogenes*	(Felix et al., 1994)
Sour doughs	*Lactob. bavaricus* (bavA)	*L. monocytogenes*	(Larsen et al., 1993)
Whey	*Ent. faecalis* 226	*L. monocytogenes*	(Villani et al., 1993)
Goat's milk	*Leu. mesenteroides* Y105	*L. monocytogenes*	(Hechard et al., 1992)
Sauerkraut	*Lac. lactis* subsp. lactis (Nis)	*L. monocytogenes*	(Hechard et al., 1992)

bial food safety. The bacteriocins were first characterized in Gram-negative bacteria. The colicins of *E. coli* are the most studied (Lazdunski, 1988). The colicins constitute a diverse group of antibacterial proteins, which kill closely related bacteria by various mechanisms such as inhibiting cell wall synthesis, permeabilizing the target cell membrane, or by inhibiting RNase or DNase activity. Among the Gram-positive bacteria, the lactic acid bacteria have been comprehensively exploited as a reservoir for antimicrobial peptides with food applications (Tables 2 and 3).

As previously mentioned, the antimicrobial proteins or peptides produced by bacteria are termed bacteriocins. They are ribosomally synthesized and kill closely related bacteria (Klaenhammer, 1993). This review will focus on LAB bacteriocins, which have been shown to be safe, and have potential as effective natural food preservatives. Bacteriocins have applications in hurdle technology, which utilizes synergies of combined treatments to more effectively preserve food (Table 4).

Since bacteriocins are isolated from foods such as meat and dairy products, which normally contain lactic acid bacteria (Table 3), they have unknowingly been consumed for centuries. A study of 40 wild-type strains of *Lactococcus lactis* showed that 35 produced nisin (Hurst, 1981). Nisin is approved for use in over 40 countries and has been in use as a food preservative for over 50 years. It is not, however, considered "natural" when it is applied in concentrations that exceed what is found in food naturally fermented with a nisin-producing starter culture. The term "natural" is also compromised when the bacte-

Table 3
Examples of patented food applications of bacteriocins

Author	US Patent	Patent Title	Use
Vandenbergh et al.	5,817,362 (10.06.98)	Method for inhibiting bacteria using a novel lactococcal bateriocin	A method for inhibiting Gram-positive bacteria in foods by using a novel bacteriocin produced by *Lac. lactis* NRRL-B-18535
Blackburn et al.	5,753,614 (05.19.98)	Nisin compositions for use as enhanced, broad range bactericides	Combination of nisin, a chelating agent and a surfactant to inhibit both Gram-positive and Gram-negative microorganisms in meat, eggs, cheese and fish, use as food preservative,
Wilhoit	5,573,801 (11.12.96)	Surface treatment of foodstuffs with antimicrobial compositions	Use of Streptococcus-derived or Pediococcus-derived bacteriocins in combination with a chelating agent to protect food against *Listeria*
Vedamuthu	5,445,835 (08.29.95)	Method of producing a yogurt product containing bacteriocin PA-1	A yogurt product with increased shelf life containing a bacteriocin derived from a *P. acidilactici*
Boudreaux et al.	5,219,603 (06.15.93)	Composition for extending the shelf life of processed meats	Use of a bacteriocin from *P. acidilactici* and a propionate salt to inhibit bacterial growth and to extend shelf life of raw and processed meat
Hutkins et al.	5,186,962 (02.16.93)	Composition and method for inhibiting pathogens and spoilage organisms in foods	Use of bacteriocin-producing lactic acid bacteria to inhibit growth of food-born pathogens
Collison et al.	5,015,487 (05.14.91)	Use of lanthionines for control of post-processing contamination in processed meat	Inhibiting the contamination of processed meat products by pathogenic or spoilage microorganisms by treating the surface of the meat product with a lantibiotic
Vandenbergh et al.	4,929,445 (05.29.90)	Method for inhibiting *L. monocytogenes* using a bacteriocin	Inhibition of *L. monocytogenes* by a bacteriocin produced by *P. acidilactici*
Gonzalez	4,883,673 (11.28.89)	Method for inhibiting bacterial spoilage and resulting compositions	Inhibition of food spoilage microorganisms in salads and salad dressings by a bacteriocin from *P. acidilactici*
Matrozza et al.	4,790,994 (12.13.88)	Method for inhibiting psychrotrophic bacteria in cream or milk based products using a pediococcus	Inhibition of bacterial growth in cottage cheese by a bacteriocin-producing *P. pentosaceus* cells

Table 4
Increased activity of bacteriocins when used as a part of hurdle technology

Bacteriocin	Other factors	Effect	References
Nisin A	N_2; CO_2; low temperature	Effect on *L. monocytogenes*: increase in the lag phase (400 IU/ml); inhibition of growth (1250 IU/ml)	(Szabo and Cahill, 1998)
Pediocin AcH	Hydrostatic pressure and high temperature	Combination of pressure (345 MPa), temperature (50 °C) and bacteriocin acts synergistically causing reduction of viability of *S. aureus*, *L. monocytogenes*, *E. coli* O157:H7, *Lactob. sake*, *Leu. mesenteroides*	(Kalchayanand et al., 1998)
Nisin A	Milk lactoperoxidase (LP) and low temperature	Nisin-producing *Lac. lactis* acts synergistically with LP in reduction of *L. monocytogenes*	(Rodriguez et al., 1997)
Nisin A	Calcium alginate gel	Gel-immobilized nisin is delivered more effectively than pure nisin and suppresses growth of *Bro. thermosphacta* on beed carcasses	(Cutter and Siragusa, 1998)
Pediocin AcH	Sodium diacetate	Combination of pediocin and sodium diacetate works synergistically against *L. monocytogenes* both at room and low temperature	(Schlyter et al., 1993)
Nisin	Sucrose fatty acid esters	Synergy against *L. monocytogenes*, *B. cereus*, *Lactob. plantarum* and *S. aureus*	(Thomas et al., 1998)
Nisin	Carbon Dioxide	Synergistic when used against wild-type and nisin-resistant *L. monocytogenes*	(Nilsson et al., 2000)
Nisin	Pulsed electric field	Synergistic activity against *B. cereus* (.06 μg/ml nisin and 16.7 kV/cm, 100 μs duration PEF)	(Pol et al., 2000)
Nisin	Modified atmosphere packaging (MAP)	Combination was more effective than either treatment alone at preventing growth of *L. monocytogenes*	(Fang and Lin, 1994)
Pediocin AcH	Emulsifier (Tween 80) or encapsulation of the pediocin within liposomes	Pediocin AcH possesses higher listericidal activity in slurries of nonfat milk, butterfat, or meat when present in encapsulated form or acts in the presence of Tween 80	(Degnan et al., 1993)

riocin is produced by genetically modified bacteria. Though nisin is currently the only bacteriocin approved for use in the United States, many bacteriocins produced by members of the LAB have potential application in food products.

4. Bacteriocins vs. antibiotics

Bacteriocins are often confused in the literature with antibiotics (Hansen, 1993; Hurst, 1981). This would limit their use in food applications from a legal standpoint. In some countries, it is critical to make the distinction between bacteriocins and antibiotics. The main differences between bacteriocins and antibiotics are summarized in Table 5. Bacteriocins, which are clearly distinguishable from clinical antibiotics, should be safely and effectively used to control the growth of target pathogens in foods. This review will differentiate bacteriocins from antibiotics on the basis of synthesis, mode of action, antimicrobial spectrum, toxicity and resistance mechanisms. Recognizing that bacteriocins are different from antibiotics, Hurst, 1981, in his review, proposed the term “biological food preservatives” since bacteriocins, unlike antibiotics, are not used for medicinal purposes.

5. Classification of bacteriocins

Bacteriocins are commonly divided into three or four groups (Klaenhammer, 1993; Nes et al., 1996) (Table 6). Nisin was discovered in 1928 (Hurst, 1967), and subtilin, a nisin analogue differing by 12 amino acid residues, was discovered in 1948 (Han-

Table 5
Bacteriocins vs. antibiotics

Characteristic	Bacteriocins	Antibiotics
Application	Food	Clinical
Synthesis	Ribosomal	Secondary metabolite
Activity	Narrow spectrum	Varying spectrum
Host cell immunity	Yes	No
Mechanism of target cell resistance or tolerance	Usually adaptation affecting cell membrane composition	Usually a genetically transferable determinant affecting different sites depending the mode of action
Interaction requirements	Sometimes docking molecules	Specific target
Mode of action	Mostly pore formation, but in a few cases possibly cell wall biosynthesis	Cell membrane or intracellular targets
Toxicity/side effects	None known	Yes

sen, 1993). Both belong to Class I, termed lantibiotics. The classification of bacteriocins is currently being revised to reflect similarities and differences observed in the discovery of new molecules. Class I is being further subdivided into Class Ia and Class Ib. In general, Class I peptides typically have from 19 to more than 50 amino acids. Class I bacteriocins are characterized by their unusual amino acids, such as lanthionine, methyl-lanthionine, dehydrobutyrine and dehydroalanine. Class Ia bacteriocins, which include nisin, consist of cationic and hydrophobic peptides that form pores in target membranes and have a flexible structure compared to the more rigid class Ib. Class Ib bacteriocins, which are globular peptides, have no net charge or a net negative charge (Altena et al., 2000). More detailed information on the structure and biosynthesis of lantibiotics is presented in a review by Sahl and Bierbaum (1998).

Class II contains small heat-stable, non-modified peptides, and can be further subdivided. According to conventional classification, Class IIa includes Pediocin-like *Listeria* active peptides with a conserved N-terminal sequence Tyr–Gly–Asn–Gly–Val and two cysteines forming a S–S bridge in the N-terminal half of the peptide. Bacteriocins composed of two different peptides comprise Class IIb. The two-peptide bacteriocins need both peptides to be fully active. The primary amino acid sequences of the peptides are different. Though each is encoded by its own adjacent genes, only one immunity gene is needed. Class IIc was originally proposed to contain the bacteriocins that are secreted by the general

Table 6
Classification of bacteriocins adapted from Klaenhammer (1993)

Group		Features		Bacteriocins (group representatives)
I	Ia	Lantibiotics, small (< 5 kDa) peptides containing lanthionine and β-methyl lanthionine	Flexible molecules comp to Ib	Nisin
	Ib		Globular peptides with no net charge or net negative charge	Mersacidin
II	IIa	Small heat-stable peptides, synthesized in a form of precursor which is processed after two glycine residues, active against *Listeria*, have a consensus sequence of YGNGV-C in the N-terminal		Pediocin PA-1, sakacins A and P, leucocin A, carnobacteriocins, etc.
	IIb	Two component systems: two different peptides required to form an active poration complex		Lactococcins G and F, lactacin F Plantaricin EF and JK
III		Large molecules sensitive to heat		Helveticins J and V-1829, acidophilucin A, lactacins A and B

sec-system (Nes et al., 1996). Since this proposal, it has been shown that Class IIa bacteriocins can use this secretory system and consequently the sub-class IIc should be eradicated (Cintas et al., 1997). The large and heat labile bacteriocins make up the Class III bacteriocins for which there is much less information available. A fourth class consists of bacteriocins that form large complexes with other macromolecules, has been proposed (Klaenhammer, 1993). However, presently, no such bacteriocins have been purified and there is good reason to believe that this type of bacteriocin is an artifact due to the cationic and hydrophobic properties of bacteriocins which result in complexing with other macromolecules in the crude extract. This phenomenon has been shown in the case of plantaricin S. First, it was claimed to be a large complex molecule, but later the activity was purified as a small peptide, and the complex disintegrated while the activity was maintained (Jimenez-Diaz et al., 1995). This paper will focus on the Class I and II bacteriocins since they are the best understood and most likely to be used in food applications due to their target specificity and robustness.

6. Effectiveness of bacteriocins in food systems

Though results obtained from broth systems show bacteriocins inhibit target organisms, applied studies must be done to confirm their effectiveness in food. The application of bacteriocins, particularly nisin, in food systems has been reviewed (Abee et al., 1995; Delves-Broughton et al., 1996; Wessels et al., 1998). The chemical composition and the physical conditions of food can have a significant influence on the activity of the bacteriocin. Nisin, for example, is 228 times more soluble at pH 2 than at pH 8 (Liu and Hansen, 1990).

Since lactic acid bacteria are commonly used as starter cultures in food fermentations, investigators have explored the use of bacteriocin producers as starter cultures. In some cases, natural bacteriocin producers, such as *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Enterococcus faecalis*, are and have been used in such studies (Campanini et al., 1993; Nunez et al., 1997). Nunez et al. (1997) found that counts of *L. monocytogenes* Ohio in Manchego cheese inoculated with a bacteriocin-producing *Ent. faecalis* strain decreased by 6 logs in 7 days, whereas the survival of the organism in cheese made with the commercial starter culture was not affected. Similarly, the surviving number of *L. monocytogenes* found in a naturally contaminated salami sausage decreased when the product was inoculated with the bacteriocin producer *Lactob. plantarum* MCS1 (Campanini et al., 1993). Most commercial starter cultures do not produce bacteriocins; however, a few bacteriocin-producing meat starter cultures are sold today.

Transposon-encoding nisin production and immunity was transformed into a commercial *Lac. lactis* starter culture for Gouda cheese (Abee et al., 1995). Because *Pediococcus* spp. do not have application as cheese starter cultures, the plasmid-encoding pediocin was expressed in *Lac. lactis* to aid in the preservation of cheddar cheese and to assure the microbial quality of the fermentation process (Buyong et al., 1998). This study concluded that control cheese made from milk spiked with 10^6 cfu/ml *L. monocytogenes* had 10^7 cfu/g after 2 weeks of ripening,

Table 7
Examples of effective use of nisin in food systems

Food product	Target organism	Effective nisin concentration (IU/ml)	References
Cottage cheese	*L. monocytogenes*	2000	(Ferreira and Lund, 1996)
Ricotta cheese	*L. monocytogenes*	100	(Davies et al., 1997)
Skim milk	*B. cereus* spores	4000	(Wandling et al., 1999)
Bologna-type sausage	*Lactob. sake* and *Lactob. curvatus*	1000	(Davies et al., 1999)
Lean beef	*Bro. thermosphacta*	400	(Cutter and Siragusa, 1998)
Kimchi	lactobacilli	100	(Choi and Park, 2000)

while cheese made with the pediocin-producing strain had only 10^2 cfu/g after 1 week. Pediocin PA-1 has also been expressed in *Streptococcus thermophilus*, an important organism in dairy fermentations (Coderre and Somkuti, 1999). In another study, pediocin PA-1 and nisin, bacteriocins of different classes that have both been shown to be safe and effective, were co-expressed in *Lac. lactis* (Horn et al., 1999). Though the transformed cells produced only 11.8%, the level of pediocin compared to the control pediocin producer, the co-production of bacteriocins may have major applications in improving food safety and minimizing the likelihood of resistant organisms. Pediocin PA-1 has also been expressed in the yeast *Saccharomyces cerevisiae* to improve preservation of wine, bread and other food products where yeast is used (Schoeman et al., 1999).

Bacteriocins have been directly added to foods such as cheese to prevent against *Clostridium* and *Listeria*. Nisin inhibits the outgrowth of *C. botulinum* spores in cheese spreads (Wessels et al., 1998) and it is approved as a food additive in the United States for this purpose (U.S. Food and Drug Administration 1988). Nisin has many applications in foods (Tables 7 and 8) and is approved for use in various foods throughout the world (Table 9). In long-life cottage cheese spiked with 10^4 cfu/g *L. monocytogenes*, the addition of 2000 IU/g nisin

Table 8
Bacteriocins as food preservatives: examples of suggested applications

Bacteriocin	Application	Conclusion	References
Nisin A	Incorporation of nisin into a meat binding system (Fibrimex)	Addition of nisin can reduce undesirable bacteria in restructured meat products	(Cutter and Siragusa, 1998)
Pediocin AcH	Use of a pediocin AcH producer *Lactob. plantarum* WHE 92 to spray on the Munster cheese surface at the beginning of the ripening period	Spray prevents outgrowth of *L. monocytogenes* and can be used as an antilisterial treatment	(Ennahar et al., 1996)
Enterocin 4	Use of an enterocin producer *Ent. faecalis* INIA4 as a starter culture for production of Manchego cheese	Use of an *Ent. faecails* INIA4 starter inhibits *L. monocytogenes* Ohio, but not *L. monocytogenes* Scott A	(Nunez et al., 1997)
Linocin M-18	Use of *Bre. lines* as a starter culture for production of red smear cheese	Causes 2 log reduction of *L. ivanovi* and *L. monocytogenes*	(Eppert et al., 1997)
Nisin A	Use of nisin to control *L. monocytogenes* in ricotta cheese	Nisin effectively inhibits *L. monocytogenes* for 8 weeks	(Davies et al., 1997)
Piscicolin 126	Use of piscicolin 126 to control *L. monocytogenes* in devilled ham paste	More effective than commercially available bacteriocins	(Jack et al., 1996)
Leucocin A	Use of a leucocine-producing *Leu. gelidum* UAL187 to control meat spoilage	Inoculation of a vacuum packed beef with the bacteriocin-producer delays the spoilage by *Lactob. sake* for up to 8 weeks	(Leisner et al., 1996)
Lactocin 705	Use of lactocin 705 to reduce growth of *L. monocytogenes* in ground beef	Lactocin 705 inhibits growth of *L. monocytogenes* in ground beef	(Vignolo et al., 1996)
Pediocin AcH	Use of the pediocin producer *P. acidilactici* to inhibit *L. monocytogenes*	*P. acidilactici* (Ped^+) starter culture contributes to effective reduction of *L. monocytogenes* during manufacture of chicken summer sausage	(Baccus-Taylor et al., 1993)
Pediocin	Expression of pediocin operon in *Sac. cerevisiae*	Potential application in preserving wine and baked products	(Schoeman et al., 1999)
Pediocin AcH	Add pediocin preparation to raw chicken	Controlled growth of *L. monocytogenes* at 5 °C for 28 days	(Goff et al., 1996)
Pediocin PA-1	Use of *P. acidilactici* (Ped^+) strain as a starter culture in sausage fermentation	Pediocin effectively contributes to inhibition of *L. monocytogenes*	(Foegeding et al., 1992)
Enterocin	Add enterocin to inoculated ham, pork, chicken breast, pate, sausage	Controlled growth of *L. monocytogenes* under several conditions	(Aymerich et al., 2000a,b)

Table 9
Examples of world-wide use of nisin (adapted from Aplin and Barrett)

Country	Food in which nisin is permitted	Maximum level (IU/g)
Argentina	Processed cheese	500
Australia	Cheese, processed cheese, canned tomatoes	No limit
Belgium	Cheese	100
Cyprus	Cheese, clotted cheese, canned vegetables	No limit
EU	E234, may also labeled as "natural preservative"	varies according to product and member state
France	Processed cheese	No limit
Italy	Cheese	500
Mexico	Nisin is a permitted additive	500
Netherlands	Factory cheese, processed cheese, cheese powder	800
Peru	Nisin is a permitted additive	No limit
Russia	Dietetic processed cheese, canned vegetables	8000
UK	Cheese, canned foods, clotted cream	No limit
US	Pasteurized processed cheese spreads	10,000

resulted in a 1000-fold decrease in *L. monocytogenes* after 7 day storage at 20 °C, compared to a 10-fold decrease in the control (Ferreira and Lund, 1996). Growth of a five strain cocktail of 10^2–10^3 cfu/ml *L. monocytogenes* in ricotta cheese was inhibited up to 55 days at 6–8 °C when 2.5 mg/l nisin was added. When the cheese was made with acetic acid, *L. monocytogenes* was completely inhibited for the duration of the study. The authors also found that after 10 weeks, there was only a 10–32% loss in nisin activity (Davies et al., 1997). The effect of pediocin PA-1 on the growth of *L. monocytogenes* has also been studied in cottage cheese, half-and-half cream and cheese sauce systems (Pucci et al., 1988). In that study, control counts of *L. monocytogenes* in the half-and-half and cheese sauce increased by almost 4 logs after 7 days at 4 °C (5.4×10^6 cfu/ml and 1.7×10^7 cfu/g, respectively). When 100 AU/ml pediocin was added, cell counts had just reached the detection limit for half-and-half (10^2 cfu/ml) and were 5 logs lower than the control in the cheese sauce.

7. Application of bacteriocins in meat

Though bacteriocins have applications in many food systems, foods should not be preserved by bacteriocins alone but rather as part of a system with multiple hurdles (Table 4). Since LAB are commonly found in meat, bacteriocins produced by these bacteria have been explored and isolated. Though most bacteriocins have been isolated from food-associated LAB, they are not necessarily effective in all food systems. However, several bacteriocins certainly do have potential in food applications when used under the proper conditions. One of the best-studied examples is the use of nisin in meat systems. Nitrates are commonly used to prevent clostridial growth in meat; however, safety concerns regarding the presence of nitrites have prompted the food industry to look for alternative methods of preservation. Nisin or its combination with lower levels of nitrate can prevent the growth of *Clostridium* (Rayman et al., 1981, 1983). Though some researchers concluded that nisin is not effective in meat applications due to high pH (Rayman et al., 1983), inability to uniformly distribute nisin, and interference by meat components such as phospholipids (de Vuyst and Vandamme, 1994), others find contradictory results (Chung et al., 1989). A presentation by Rose at the Workshop on the Bacteriocins of Lactic Acid Bacteria (Alberta, Canada 2000) showed that nisin is inactivated by glutathione in a reaction catalyzed by glutathione S-transferase. Glutathione is found in raw meat, and the reaction greatly diminishes the activity of nisin. Other work shows that nisin can be used in meat under certain conditions. A commonly examined system is sausage, since its spoilage is often attributable to lactic acid bacteria that can be inhibited by bacteriocins. Davies et al. (1999) examined the influence of fat content and phosphate emulsifier on the effectiveness of nisin in sausage and found that lower fat contents correlate with higher

nisin activity in the system. Other studies (Ariyapitipun et al., 1999, 2000) have used nisin in combination with lactic acid to show an increased effect when the preservatives are used together to inhibit gram negative organisms. No advantage to the combination is seen when used to inhibit *L. monocytogenes* Scott A or *Lactobacillus* spp. Nisin is also effective at inhibiting *Brochothrix thermosphacta* when incorporated in a cold meat-binding system (Cutter and Siragusa, 1998).

Since there are difficulties using nisin in raw meat applications, the use of other bacteriocins has been examined. Leucocin A, enterocins, sakacins and the carnobactericins A and B prolong the shelf life of fresh meat. The most promising results in meats were obtained using pediocin PA-1 (which has an amino acid sequence identical to AcH). Produced by *P. acidilactici*, pediocin PA-1 immediately reduces the number of target organisms (Nielsen et al., 1990) but is not yet an approved food additive in the United States. Used alone (Baccus-Taylor et al., 1993; Coventry et al., 1995; Nielsen et al., 1990) or in combination with diacetate (Schlyter et al., 1993), pediocin PA-1 is active against the foodborne pathogen *L. monocytogenes* and *Lactob. curvatus*, a spoilage organism. In the *Lactob. curvatus* study, however, pediocin PA-1 is less active than nisin in the model meat system, and neither preservative is effective when used in a commercially manufactured meat product (Coventry et al., 1995). Pediocin AcH (PA-1) successfully controlled the growth of *L. monocytogenes* in raw chicken in another study (Goff et al., 1996). The use of 2,400 AU/g pediocin resulted in 2.8 log cfu/g *L. monocytogenes* after 28 days of storage at 5 °C, whereas the control chicken had as high as 8.1 log cfu/g. Pediocin binds to raw chicken, but not to cooked. However, when raw chicken with applied pediocin was cooked, activity was retained. The authors suggest that pediocin should be applied to chicken before cooking for maximum effectiveness.

8. Synthesis of bacteriocins

The genetic determinants for bacteriocins are discussed in detailed reviews (Klaenhammer, 1993; Entian and de Vos, 1996; Nes et al., 1996; Sahl and Bierbaum, 1998). Genes for the production of active bacteriocins are usually in operon clusters. Operons containing the genes for lantibiotic production are well studied, and homologous genes are found among the many of the sequenced lantibiotic operons, as reviewed by Siezen et al. (1996). Most characterized lantibiotic operons belong to Class Ia. Complete gene clusters have recently been elucidated for the Class Ib lantibiotic mersadicin (Altena et al., 2000). Not surprisingly, many of the genes in the cluster transcribe similar proteins to those known for Class Ia. Genes encoding bacteriocin production can be located on the chromosome (Altena et al., 2000; Diep et al., 1996), or encoded in a plasmid or transposon (Engelke et al., 1992). Typically, organisms possess genes coding for the structural peptide (Rauch and de Vos, 1992), proteins that aid in processing to the active form (Engelke et al., 1992), proteins that aid in the transport of the bacteriocin across the membrane (Klein et al., 1992), regulatory proteins (Klein et al., 1993) and proteins that confer immunity to the host producer (Diep et al., 1996; Engelke et al., 1994; Klein and Entian, 1994; Qiao et al., 1996).

The genetics of many nonlantibiotics such as plantaricin, pediocin and sakacin have also been elucidated (Diep et al., 1994; Ehrmann et al., 2000; Marugg et al., 1992). While similarities exist with lantibiotic genes (structural, transport, regulatory genes, etc.), the genes for the plantaricin system also encode for multiple bacteriocins which share the transport and the regulatory systems. However, each bacteriocin has its own dedicated immune system (Diep et al., 1996).

While all classes of bacteriocins are ribosomally synthesized, only Class I is post-translationally modified to produce the active form (for more information on lantibiotic synthesis, see review by Kupke and Gotz, 1996). Different from bacteriocins, antibiotics are generally considered secondary metabolites. Antibiotics are not ribosomally synthesized. Although several antibiotics, such as vancomycin, are composed of amino acids, they are enzymaticly synthesized. In fact, several peptide antimicrobials are synthesized by a multiple-carrier thiotemplate mechanism, where peptide synthetases assemble amino acids to form the antibiotic molecule (Hancock and Chapple, 1999). Because bacteriocins are encoded by

one structural gene, active sites and structure–function relationships can be examined more simply by genetic manipulation. Molecular techniques also allow bacteriocin analogues with increased activity or with altered specificity to be constructed and evaluated, unlike antibiotics, which must be chemically synthesized or where the complexity in genetic manipulation results from the increased number of genes involved.

9. Bacteriocin immunity

The immunity of the cell synthesizing the bacteriocin to its product is a phenomenon that distinguishes bacteriocins from antibiotics. Genes coding for "immunity proteins" are in close genetic proximity to other bacteriocin structural and processing genes (Siegers and Entian, 1995). It is common for the structural bacteriocin gene and the immunity gene to be located on the same operon and often next to each other (Nes et al., 1996; Klein and Entian, 1994). The immunity of lantibiotics was initially thought to be due to an immunity gene, such as *nis*I for nisin and *spa*I for subtilin, which code for NisI and SpaI immunity proteins, respectively. It appears, however, that immunity to these bacteriocins is the result of the influence of several proteins, since the deletions of other genes result in altered host immunity (Klein and Entian, 1994). For example, non-nisin producing nisin-resistant strains of *Lac. lactis* do not have the genetic elements coding for NisI protein, but do have sequences similar to nisF, nisE and nisG (Duan et al., 1996). These are thought to render the strains resistant to nisin. Deletion of *nis*G makes the cells less resistant to nisin. The complexity of host immunity with respect to nisin is reviewed by Saris et al. (1996) but as of yet, there is not a clear understanding of how immunity proteins serve to protect the producing organism from its bacteriocin.

The phenomenon of immunity is simpler in the nonlantibiotics, Class II bacteriocins. One gene encodes for the immunity protein. Usually, it is a basic protein between 50 and 150 amino acid residues long that is loosely associated with the membrane. The lactococcin A immunity protein (LcnI) is by far the most studied one, yet the basic mechanism behind the immunity is still not understood (Nissen-Meyer et al., 1993; Venema et al., 1994, 1995).

10. Post-translational modifications resulting in active bacteriocin

Though bacteriocins are ribosomally synthesized, the resulting transcript must be modified before becoming active. Genes coding for the enzymes that facilitate the modifications are usually in close proximity to the structural gene. Lantibiotics experience the most extensive modification. LanB, a membrane-spanning protein, is transcribed by lantibiotic producers and enzymatically modifies the bacteriocin before transport out of the cell (Engelke et al., 1992). LanC also participates in the formation of thioether bonds in lantibiotics (Kupke and Gotz, 1996).

A characteristic of lantibiotic synthesis is the presence of an N-terminal leader peptide, followed by a C-terminal propeptide. The leader peptide was initially thought to serve as a recognition site for enzymes involved in the post-translational modification. Experiments using unmodified propeptide show that they are still able to undergo recognition and modification (Kupke and Gotz, 1996).

The extensive post-translational modification of lantibiotics includes the formation of several unusual amino acids. Ingram (1969, 1970) proposed that serine and threonine are modified to dehydroalanine and dehydrobutyrine, respectively, and that these amino acids serve as precursors to lanthionine and methyl-lanthionine, the formation of which occurs upon the addition of cysteine thiol groups. In all, over one dozen unusual amino acids are found in the lantibiotics, and are summarized in a review by Kupke and Gotz (1996).

The prepeptides of nonlantibiotics are also modified by cleavage of the leader sequence (Diep et al., 1996; Ehrmann et al., 2000). These modifications are necessary for secretion and transport across the cell membrane.

11. Transport across the cell membrane

Most bacteriocins in Class I and II are translocated to the outside of the cell by a dedicated ABC

transporter system. The only exceptions are the few (presently, 4–5) class II bacteriocins that are externalized by the sec-dependent system. The bacteriocins that are dependent on the ABC transporters can be divided into two major groups: bacteriocins with a double glycine-leader and bacteriocins with a different leader but not a sec-leader. The double-glycine leader bacteriocins are found mainly among the Class II bacteriocins but also include some lantibiotics (Havarstein et al., 1994; Nes et al., 1996). These bacteriocins are secreted by a unique form of ABC transporters, which possess an N-terminal leader of approximately 150 amino acid residues exerting a specific proteolytic activity that cleaves the double-glycine leader. Concomitant with the secretion, this specific ABC transporter cleaves the leader thereby activating the bacteriocin. An accessory protein is needed in this secretion process (Franke et al., 1996). The ABC transporters that secrete lantiobiotics with different type of leaders do not possess N-terminal proteolytic activity, and the removal of the leader is carried out by a dedicated protease such as NisP in the nisin system.

12. Mode of action

Bacteriocins, particularly lantibiotics, inhibit target cells by forming pores in the membrane, depleting the transmembrane potential ($\Delta\psi$) and/or the pH gradient, resulting in the leakage of cellular materials. Early studies suggest that in order for nisin to form pores, target cells require $\Delta\psi$ (inside negative) and ΔpH (inside alkaline) (Okereke and Montville, 1992).

Bacteriocins are positively charged molecules with hydrophobic patches. Electrostatic interactions with negatively charged phosphate groups on target cell membranes are thought to contribute to the initial binding with the target membrane (Chen et al., 1997a,b). The association of hydrophobic patches of bacteriocins with the hydrophobic membrane has also been modeled using computer simulation to predict the most favorable interaction (Lins et al., 1999). It is likely that the hydrophobic portion inserts into the membrane, forming pores. There is debate over the types of pores formed by nisin, with most groups favoring the "barrel-stave" or "wedge" models. In the "barrel-stave" model, each nisin molecule orients itself perpendicular to the membrane, forming an ion channel that spans the membrane (Ojcius and Young, 1991). According to the "wedge" model, after a critical number of nisin molecules associate with the membrane, they insert concurrently, forming a wedge (Driessen et al., 1995). More recent studies demonstrate the complexity of bacteriocin activity, where nisin must bind to lipid II on the susceptible cell membrane in order to kill (Breukink et al., 1999). For other cationic peptides, the peptide concentration required to cause membrane depolarization does not always correspond with the minimal inhibitory concentration (MIC) and does not necessarily cause cell death (Friedrich et al., 2000). One may speculate that entry into the cell may be required to access targets such as DNA, RNA, enzymes and other sites to kill the target cell. There is evidence that one Class II bacteriocin actually inhibits septum formation in susceptible bacteria (Martinez et al., 2000).

Because bacteriocins do not act equally against target species, researchers have examined the affinity of bacteriocins to specific species and strains. The phospholipid composition of the target strains and environmental pH influences the MIC values (Chen et al., 1997a,b). Instead of pore formation occurring indiscriminately, it appears that "docking molecules" on the target cell membrane facilitate the interaction with the bacteriocin, thereby increasing the effectiveness of the bacteriocin. This mechanism has been clearly demonstrated for nisin and mersacidin, which both use lipid II, a peptidoglycan precursor, as a docking molecule (Breukink et al., 1999; Brotz et al., 1998a,b). Mersacidin correspondingly inhibits peptidoglycan synthesis, whereas the primary mode of action of nisin is pore formation resulting in leakage of cellular materials. Interestingly, lipid II is also the recognition site for the antibiotic vancomycin. However, the specific interaction with lipid II is different for the different molecules. Vancomycin-resistant *Ent. faecium* is still sensitive to mersacidin (Brotz et al., 1998b). Additionally, lipid II can be considered a "target" for vancomycin whereas it is a "docking molecule" for nisin, since vancomycin acts by inhibiting peptidoglycan synthesis. They do not, however, bind to the same part of the lipid II molecule (Breukink et al., 1999). Now, it is believed that in

some cases cell wall biosynthesis may be a target for nisin action.

Other bacteriocins also interact with specific sites on target cell membranes which may be proteins (Chikindas et al., 1993; van Belkum et al., 1991). While this interaction may increase the effectiveness of the bacteriocin in vivo, it may not be a requirement for activity in artificial vesicles (Chen et al., 1997a,b).

Both pediocin PA1 and lactococcin A form voltage independent pores, (Chikindas et al., 1993; van Belkum et al., 1991). Lactococcin A permeabilize vesicles made from susceptible cells while liposomes made from same kind of cells are not affected. This suggests that a receptor-like entity on the cell surface is needed (van Belkum et al., 1991).

Some detailed mechanistic studies of two-peptide bacteriocins have been performed (Hauge et al., 1998; Moll et al., 1998, 1999). For example, while lactococcin G does not affect the pH gradient it leads to dissipation of monovalent cations.

13. Resistance mechanisms

Once a new preservative is found to be safe and effective, it is critical to ensure the longevity of its use by preventing the proliferation of resistant cells. Already, cells exhibit resistance to several antibiotics and the transferal of resistance between organisms has been documented. Although bacteriocins are not antibiotics, there is concern that exposure to bacteriocins will render cells more resistant to antibiotics. Since antibiotics and nisin have different modes of action, it has shown that exposure to nisin has no effect on the frequency of resistance of *L. monocytogenes* Scott A to ampicillin and chloramphenicol (Tchikindas et al., 2000). In another study, several multi-drug resistant bacteria were subjected to up to 400 IU/ml nisin, and these organisms remained sensitive to nisin (Severina et al., 1998). Cross-resistance between nisin and 33 other antimicrobials has also been studied, and penicillin resistant *S. aureus* was 50 times more sensitive to nisin (Szybalski, 1953). This was the only case of "collateral sensitivity" observed in the study. In addition to bacteriocins, other cationic peptides are active against antibiotic resistant organisms such as methicillin-resistant *S. aureus* and vancomycin-resistant *S. haemolyticus* (Friedrich et al., 2000).

Though bacteria exhibiting nisin resistance do not show cross-resistance with antibiotics, it is still important to understand the mechanism of resistance so that it can be avoided. Antibiotic resistance is usually associated with a genetic determinant, facilitating the transfer of resistance between cells, strains and species. Unlike most antibiotic resistance, bacteriocin resistance results from a physiological change in the target cell membrane (Crandall and Montville, 1998; Mazzotta et al., 1997; Ming and Daeschel, 1993). For *L. monocytogenes*, a more rigid membrane, usually having a lower C15:C17 ratio results in increased tolerance to nisin (Mazzotta et al., 1997). Ming and Daeschel (1993) also found that nisin resistant *L. monocytogenes* have reduced amounts of phosphatidylglycerol, diphosphatidylglycerol and bisphosphatidylglyceryl phosphate. Though most research shows that a change in cell membrane composition accounts for resistance, some mutants produce an enzyme, nisinase, which degrades nisin (Jarvis, 1967). Gravesen et al. (2000) reports that *L. monocytogenes* mutants resistant to pediocin PA-1 show increased expression of gene fragments that code for β-glucoside-specific phosphoenolpyruvate-dependent phosphotransferase systems (PTS). The mechanism by which β-glucoside-specific PTS interacts with pediocin to confer resistance must still be elucidated. In a study of the mode of action of mesentericin Y105, a bacteriocin bactericidal against *L. monocytogenes*, transposon mutants resistant to the bacteriocin resulted from the transposon insertion into a gene (rpoN) encoding a putative σ^{54} factors (Robichon et al., 1997).

Whether resistance is genetically encoded or the result of an adaptation, there is contradictory data regarding cross-resistance when bacteriocins from different classes are used (Crandall and Montville, 1998; Mazzotta et al., 1997; Rasch and Knochel, 1998; Song and Richard, 1997).

14. Use of bacteriocins in hurdle technology

Hurdle technology combines different preservation methods to inhibit microbial growth. The principles underlying hurdle technology, as well as poten-

tial hurdles in food systems, have been reviewed by Leistner (2000). Table 4 shows that bacteriocins often have synergies with other treatments, and can be used as a hurdle to improve the safety of food. An understanding of the mode of action of each individual hurdle allows the most effective combination of treatments. For example, the application of pulsed electric field (PEF), which increases the permeability of cell membranes, has been used together with nisin, which can also act at the level of the cell membrane (Terebiznik et al., 2000). The researchers found that some nisin was inactivated in the process, possibly due to the interaction between the hydrophobic portion of the peptide and the leakage of intracellular materials induced by PEF. The remaining, active nisin increased the lethality of PEF against *E. coli*. However, the effect was additive, not synergistic. The effectiveness of nisin against gram-negative cells is generally low. The growth of gram-negative pathogens such as *E. coli* O157:H7 and *Salmonella* (Stevens et al., 1991) can also be controlled when metal chelators such as EDTA are used in combination with nisin (Zhang and Mustapha, 1999). EDTA disrupts the outer membrane, allowing the penetration of nisin (Abee et al., 1995).

15. Regulatory considerations

From a regulatory standpoint, it is critical in some countries to distinguish bacteriocins from antibiotics, since the presence of antibiotics in food is often prohibited. Table 9 shows examples of the permitted use of nisin in various countries. For example, in Denmark, bacteria used to produce food additives must not produce toxins or antibiotics (Wessels et al., 1998). The use of bacteriocin-producing starter cultures as ingredients may not require special consideration in the United States if the culture (microorganism) is considered Generally Recognized as Safe (GRAS) because of its history of safe use by food industries prior to the 1958 Food Additives Amendment (Muriana, 1996). If a purified bacteriocin is used as a food preservative, the substance might be self-affirmed as GRAS by the company according to the Code of Federal Regulations (U.S. Government Printing Office, 1990), but the Food and Drug Administration (FDA) may require justification of the affirmation. With the formation of the European Union, food additives have been given "E" numbers. Nisin is listed as E234, and may also be labeled as "nisin preservative" or "natural preservative".

In the United States, where antibiotics are prohibited in foods, nisin was confirmed Generally Recognized as Safe (GRAS) in 1988 (U.S. Food and Drug Administration). Several authors have outlined issues involved in the approval of new bacteriocins for food use (Fields, 1996; Harlander, 1993; Post, 1996) and the USDA publishes guidelines for the safety assessment of a new preservative (U.S. Food and Drug Administration, 1993). For approval to be granted, the bacteriocin must be chemically identified and characterized, and its use and efficacy must be shown. The manufacturing process must be described and assays used for quantification and standardization of the peptide must be shown. Toxicological data and the fate of the molecule after ingestion are also needed.

16. Bacteriocin toxicity

Bacteriocins have been consumed for centuries as products of LAB. The approval of nisin was based on published and unpublished data regarding its safety, not on history of common use (U.S. Food and Drug Administration, 1988). Acute, subchronic, and chronic toxicity studies, as well as reproduction, sensitization, in vitro and cross-resistance studies showed that nisin is safe for human consumption at an Acceptable Daily Intake (ADI) of 2.9 mg/person/day (U.S. Food and Drug Administration, 1988). Frazer et al. (1962) performed many of the studies upon which the recommendation was formed, including examination of rats and guinea pigs. Since nisin is consumed orally, the effect of nisin on the oral microflora was also examined. It was found that 1 min after the consumption of chocolate milk containing nisin was assayed, only 1/40 of the activity of the original nisin concentration could be detected in the saliva. Control saliva showed 1/100 activity. In contrast, the same study found that, when the chocolate milk contained penicillin, the saliva showed antibacterial activity for a greater length of time

(Claypool et al., 1966). Another study showed the effect of gastric enzymes on nisin. Trypsin inactivated the peptide, and it was concluded that ingested nisin would not have an effect on beneficial organisms, such as the microflora of the gut (Hara et al., 1962).

A comprehensive literature search shows that most of the information regarding the safety of nisin was collected over 20 years ago (Claypool et al., 1966; Fowler, 1973; Frazer et al., 1962; Hara et al., 1962; Shtenberg, 1973). It is likely that more information regarding nisin safety exists, but is not available to the public. Patents claiming nisin as an antibacterial agent in food, personal care products or for medical applications do not provide new data, and instead rely on previously published information (Blackburn et al., 1998). When patents for new bacteriocins are submitted, often full toxicological data is not complete (Vedamuthu et al., 1992).

Though nisin is currently the most commercially used bacteriocin, the safety of other bacteriocins with potential applications in food has also been evaluated. Pediocin PA-1 (AcH) was injected into mice and rabbits, and immunoblotting showed that it was non-immunogenic in both animals (Bhunia et al., 1990). This peptide is also susceptible to proteolysis by trypsin and chymotrypsin (Bhunia et al., 1990).

17. Conclusion

The effectiveness of bacteriocins as food preservatives is well demonstrated. Though nisin is the only purified bacteriocin used commercially, others, such as pediocin, have application in food systems. Though bacteriocins are inhibitory against foodborne pathogens such as *L. monocytogenes*, they are not antibiotics. Their synthesis and mode of action distinguish them from clinical antibiotics. Additionally, organisms that show resistance to antibiotics are generally not cross-resistant with bacteriocins, and unlike antibiotic resistance, bacteriocin resistance is not usually genetically determined. This review has highlighted the key differences between the two types of molecules, summarized in Table 5, and shown that bacteriocins are not only effective, but are also safe for use in the food supply.

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References

Abee, T., Krockel, L., Hill, C., 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int. J. Food Microbiol. 28, 169–185.

Altena, K., Guder, A., Cramer, C., Bierbaum, G., 2000. Biosynthesis of the lantibiotic mersacidin: organization of a type B lantibiotic gene cluster. Appl. Environ. Microbiol. 66, 2565–2571.

Ariyapitipun, T., Mustapha, A., Clarke, A.D., 1999. Microbial shelf life determination of vacuum-packaged fresh beef treated with polylactic acid, lactic acid, and nisin solutions. J. Food Prot. 62, 913–920.

Ariyapitipun, T., Mustapha, A., Clarke, A.D., 2000. Survival of *Listeria monocytogenes* Scott A on vacuum-packaged raw beef treated with polylactic acid, lactic acid, and nisin. J. Food Prot. 63, 131–136.

Aymerich, T., Garriga, M., Ylla, J., Vallier, J., Monfort, J.M., Hugas, M., 2000a. Application of enterocins as biopreservatives against *Listeria innocua* in meat products. J. Food Prot. 63, 721–726.

Aymerich, T., Artigas, M.G., Garriga, M., Monfort, J.M., Hugas, M., 2000b. Effect of sausage ingredients and additives on the production of enterocin A and B by *Enterococcus faecium* CTC492. Optimization of in vitro production and anti-listerial effect in dry fermented sausages. J. Appl. Microbiol. 88, 686–694.

Baccus-Taylor, G., Glass, K.A., Luchansky, J.B., Maurer, A.J., 1993. Fate of *Listeria monocytogenes* and pediococcal starter cultures during the manufacture of chicken summer sausage. Poult. Sci. 72, 1772–1778.

Bennik, M.H., Vanloo, B., Brasseur, R., Gorris, L.G., Smid, E.J., 1998. A novel bacteriocin with a YGNGV motif from vegetable-associated *Enterococcus mundtii*: full characterization and interaction with target organisms. Biochim. Biophys. Acta 1373, 47–58.

Bhunia, A.K., Johnson, M.C., Ray, B., Belden, E.L., 1990. Antigenic property of pediocin AcH produced by *Pediococcus acidilactici* H. J. Appl. Bacteriol. 69, 211–215.

Blackburn, P., Polak, J., Gusik, S., Rubino, S., 1998. Nisin Compositions for Use as Enhanced, Broad Range Bactericides. AMBI, Tarrytown, NY, USA, 470929 5,753,614.

Boman, H.G., 1991. Antibacterial peptides: key components needed in immunity. Cell 65, 205–207.

Breukink, E., Wiedemann, I., van Kraaij, C., Kuipers, O.P., Sahl, H., de Kruijff, B., 1999. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. Science 286, 2361–2364.

Brotz, H., Josten, M., Wiedemann, I., Schneider, U., Gotz, F., Bierbaum, G., Sahl, H.G., 1998a. Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. Mol. Microbiol. 30, 317–327.

Brotz, H., Bierbaum, G., Leopold, K., Reynolds, P.E., Sahl, H.G., 1998b. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. Antimicrob. Agents Chemother. 42, 154–160.

Buyong, N., Kok, J., Luchansky, J.B., 1998. Use of a genetically enhanced, pediocin-producing starter culture, *Lactococcus lactis* subsp. lactis MM217, to control *Listeria monocytogenes* in cheddar cheese. Appl. Environ. Microbiol. 64, 4842–4845.

Buzby, J.C., Roberts, T., 1997. Economic costs and trade impacts of microbial foodborne illness. World Heath Stat. Q. 50 (1–2), 57–66.

Cai, Y., Ng, L.K., Farber, J.M., 1997. Isolation and characterization of nisin-producing *Lactococcus lactis* subsp. lactis from bean-sprouts. J. Appl. Microbiol. 83, 499–507.

Campanini, M., Pedrazzoni, I., Barbuti, S., Baldini, P., 1993. Behaviour of *Listeria monocytogenes* during the maturation of naturally and artificially contaminated salami: effect of lactic-acid bacteria starter cultures. Int. J. Food Microbiol. 20, 169–175.

Chen, Y., Ludescher, R.D., Montville, T.J., 1997a. Electrostatic interactions, but not the YGNGV consensus motif, govern the binding of pediocin PA-1 and its fragments to phospholipid vesicles. Appl. Environ. Microbiol. 63, 4770–4777.

Chen, Y., Shapira, R., Eisenstein, M., Montville, T.J., 1997b. Functional characterization of pediocin PA-1 binding to liposomes in the absence of a protein receptor and its relationship to a predicted tertiary structure. Appl. Environ. Microbiol. 63, 524–531.

Chikindas, M.L., Garcia-Garcera, M.J., Driessen, A.J., Ledeboer, A.M., Nissen-Meyer, J., Nes, I.F., Abee, T., Konings, W.N., Venema, G., 1993. Pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC1.0, forms hydrophilic pores in the cytoplasmic membrane of target cells. Appl. Environ. Microbiol. 59, 3577–3584.

Choi, M.H., Park, Y.H., 2000. Selective control of lactobacilli in kimchi with nisin. Lett. Appl. Microbiol. 30, 173–177.

Chung, K.T., Dickson, J.S., Crouse, J.D., 1989. Effects of nisin on growth of bacteria attached to meat. Appl. Environ. Microbiol. 55, 1329–1333.

Cintas, L.M., Casaus, P., Havarstein, L.S., Hernandez, P.E., Nes, I.F., 1997. Biochemical and genetic characterization of enterocin P, a novel sec-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. Appl. Environ. Microbiol. 63, 4321–4330.

Claypool, L., Heinemann, B., Voris, L., Stumbo, C.R., 1966. Residence time of nisin in the oral cavity following consumption of chocolate milk containing nisin. J. Dairy Sci. 49, 314–316.

Coderre, P.E., Somkuti, G.A., 1999. Cloning and expression of the pediocin operon in *Streptococcus thermophilus* and other lactic fermentation bacteria. Curr. Microbiol. 39, 295–301.

Coventry, M.J., Muirhead, K., Hickey, M.W., 1995. Partial characterisation of pediocin PO2 and comparison with nisin for biopreservation of meat products. Int. J. Food Microbiol. 26, 133–145.

Crandall, A.D., Montville, T.J., 1998. Nisin resistance in *Listeria monocytogenes* ATCC 700302 is a complex phenotype. Appl. Environ. Microbiol. 64, 231–237.

Cutter, C.N., Siragusa, G.R., 1998. Incorporation of nisin into a meat binding system to inhibit bacteria on beef surfaces. Lett. Appl. Microbiol. 27, 19–23.

Davies, E.A., Bevis, H.E., Delves-Broughton, J., 1997. The use of the bacteriocin, nisin, as a preservative in ricotta-type cheeses to control the food-borne pathogen *Listeria monocytogenes*. Lett. Appl. Microbiol. 24, 343–346.

Davies, E.A., Milne, C.F., Bevis, H.E., Potter, R.W., Harris, J.M., Williams, G.C., Thomas, L.V., Delves-Broughton, J., 1999. Effective use of nisin to control lactic acid bacterial spoilage in vacuum-packed bologna-type sausage. J. Food Prot. 62, 1004–1010.

Degnan, A.J., Buyong, N., Luchansky, J.B., 1993. Antilisterial activity of pediocin AcH in model food systems in the presence of an emulsifier or encapsulated within liposomes. Int. J. Food Microbiol. 18, 127–138.

Delves-Broughton, J., Blackburn, P., Evans, R.J., Hugenholtz, J., 1996. Applications of the bacteriocin, nisin. Antonie van Leeuwenhoek 69, 193–202.

de Vuyst, L., Vandamme, E., 1994. Nisin, a lantibiotic produced by *Lactococcus lactis* subsp. lactis: properties, biosynthesis and applications. In: de Vuyst, L., Vandamme, E. (Eds.), Bacteriocins of Lactic Acid Bacteria. Microbiology, Genetics and Applications, Blackie Academic and Professional, London, pp. 151–221.

Diep, D.B., Havarstein, L.S., Nissen-Meyer, J., Nes, I.F., 1994. The gene encoding plantaricin A, a bacteriocin from *Lactobacillus plantarum* C11, is located on the same transcription unit as an agr-like regulatory system. Appl. Environ. Microbiol. 60, 160–166.

Diep, D.B., Havarstein, L.S., Nes, I.F., 1996. Characterization of the locus responsible for the bacteriocin production in *Lactobacillus plantarum* C11. J. Bacteriol. 178, 4472–4483.

Driessen, A.J., van den Hooven, H.W., Kuiper, W., van de, K.M., Sahl, H.G., Konings, R.N., Konings, W.N., 1995. Mechanistic studies of lantibiotic-induced permeabilization of phospholipid vesicles. Biochemistry 34, 1606–1614.

Duan, K., Harvey, M.L., Liu, C.Q., Dunn, N.W., 1996. Identification and characterization of a mobilizing plasmid, pND300, in *Lactococcus lactis* M189 and its encoded nisin resistance determinant. J. Appl. Bacteriol. 81, 493–500.

Ehrmann, M.A., Remiger, A., Eijsink, V.G., Vogel, R.F., 2000. A gene cluster encoding plantaricin 1.25beta and other bacteriocin-like peptides in *Lactobacillus plantarum* TMW1.25. Biochim. Biophys. Acta 1490, 355–361.

Enan, G., el Essawy, A.A., Uyttendaele, M., Debevere, J., 1996. Antibacterial activity of *Lactobacillus plantarum* UG1 iso-

lated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. Int. J. Food Microbiol. 30, 189–215.

Engelke, G., Gutowski-Eckel, Z., Hammelmann, M., Entian, K.D., 1992. Biosynthesis of the lantibiotic nisin: genomic organization and membrane localization of the NisB protein. Appl. Environ. Microbiol. 58, 3730–3743.

Engelke, G., Gutowski-Eckel, Z., Kiesau, P., Siegers, K., Hammelmann, M., Entian, K.D., 1994. Regulation of nisin biosynthesis and immunity in *Lactococcus lactis* 6F3. Appl. Environ. Microbiol. 60, 814–825.

Ennahar, S., Aoude-Werner, D., Sorokine, O., Van Dorsselaer, A., Bringel, F., Hubert, J.C., Hasselmann, C., 1996. Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. Appl. Environ. Microbiol. 62, 4381–4387.

Entian, K.D., de Vos, W.M., 1996. Genetics of subtilin and nisin biosyntheses: biosynthesis of lantibiotics. Antonie van Leeuwenhoek 69, 109–117.

Eppert, I., Valdes-Stauber, N., Gotz, H., Busse, M., Scherer, S., 1997. Growth reduction of Listeria spp. caused by undefined industrial red smear cheese cultures and bacteriocin-producing *Brevibacterium lines* as evaluated in situ on soft cheese. Appl. Environ. Microbiol. 63, 4812–4817.

Fang, T.J., Lin, L.-W., 1994. Growth of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked pork in a modified atmosphere packaging/nisin combination. J. Food Prot. 57, 479–485.

Felix, J.V., Papathanasopoulos, M.A., Smith, A.A., von Holy, A., Hastings, J.W., 1994. Characterization of leucocin B-Ta11a: a bacteriocin from *Leuconostoc carnosum* Ta11a isolated from meat. Curr. Microbiol. 29, 207–212.

Ferreira, M.A., Lund, B.M., 1996. The effect of nisin on *Listeria monocytogenes* in culture medium and long-life cottage cheese. Lett. Appl. Microbiol. 22, 433–438.

Fields, F., 1996. Use of bacteriocins in food: regulatory considerations. J. Food Prot. 59, 72–77.

Foegeding, P.M., Thomas, A.B., Pilkington, D.H., Klaenhammer, T.R., 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production [published erratum appears in Appl. Environ. Microbiol. 1992, Jun; 58 (6): 2102]. Appl. Environ. Microbiol. 58, 884–890.

Fowler, G.G., 1973. Toxicology of nisin. Food Cosmet. Toxicol. 11, 351–352.

Franke, C.M., Leenhouts, K.J., Haandrikman, A.J., Kok, J., Venema, G., Venema, K., 1996. Topology of LcnD, a protein implicated in the transport of bacteriocins from *Lactococcus lactis*. J. Bacteriol. 178, 1766–1769.

Franz, C.M., Du, T.M., Olasupo, N.A., Schillinger, U., Holzapfel, W.H., 1998. Plantaricin D, a bacteriocin produced by *Lactobacillus plantarum* BFE 905 ready-to-eat salad. Lett. Appl. Microbiol. 26, 231–235.

Frazer, A., Sharratt, M., Hickman, J., 1962. Biological effects of food additives. J. Sci. Food Agric. 13, 32–42.

Friedrich, C.L., Moyles, D., Beveridge, T.J., Hancock, R.E., 2000. Antibacterial action of structurally diverse cationic peptides on Gram-positive bacteria. Antimicrob. Agents Chemother. 44, 2086–2092.

Goff, J.H., Bhunia, A.K., Johnson, M.G., 1996. Complete inhibition of low levels of *Listeria monocytogenes* on refrigerated chicken meat with Pediocin AcH bound to heat-killed *Pediococcus acidilactici* cells. J. Food Prot. 59, 1187–1192.

Gomez, S., Cosson, C., Deschamps, A.M., 1997. Evidence for a bacteriocin-like substance produced by a new strain of *Streptococcus* sp., inhibitory to gram-positive food-borne pathogens. Res. Microbiol. 148, 757–766.

Goulard, C., Hlimi, S., Rebuffat, S., Bodo, B., 1995. Trichorzins HA and MA, antibiotic peptides from *Trichoderma harzianum*: I. Fermentation, isolation and biological properties. J. Antibiot. (Tokyo) 48, 1248–1253.

Gravesen, A., Warthoe, P., Knochel, S., Thirstrup, K., 2000. Restriction fragment differential display of pediocin-resistant *Listeria monocytogenes* 412 mutants shows consistent overexpression of a putative beta-glucoside-specific PTS system. Microbiology 146 (Pt. 6), 1381–1389.

Hancock, R.E., Chapple, D.S., 1999. Peptide antibiotics. Antimicrob. Agents Chemother. 43, 1317–1323.

Hansen, J.N., 1993. Antibiotics synthesized by post translational modification. Annu. Rev. Microbiol. 47, 535–564.

Hara, S., Yakazo, K., Nakakawaji, K., Takeuchi, T., Kobayasi, T., Sata, M., Imai, Z., Shibuya, T., 1962. An investigation of toxicity of nisin with a particular reference to experimental studies of its oral administration and influences by digestive enzymes. J. Tokyo Med. Coll. 20, 176–207.

Harlander, S.K., 1993. Regulatory aspects of bacteriocin use. Bacteriocins of Lactic Acid Bacteria. Academic Press, San Diego, CA, pp. 233–247.

Hauge, H.H., Nissen-Meyer, J., Nes, I.F., Eijsink, V.G., 1998. Amphiphilic alpha-helices are important structural motifs in the alpha and beta peptides that constitute the bacteriocin lactococcin G-enhancement of helix formation upon alpha–beta interaction. Eur. J. Biochem. 251, 565–572.

Havarstein, L.S., Holo, H., Nes, I.F., 1994. The leader peptide of colicin V shares consensus sequences with leader peptides that are common among peptide bacteriocins produced by gram-positive bacteria. Microbiology 140 (Pt. 9), 2383–2389.

Hechard, Y., Derijard, B., Letellier, F., Cenatiempo, Y., 1992. Characterization and purification of mesentericin Y105, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*. J. Gen. Microbiol. 138 (Pt. 12), 2725–2731.

Helmerhorst, E.J., van't Hof, W., Veerman, E.C., Simoons-Smit, I., Nieuw Amerongen, A.V., 1997. Synthetic histatin analogues with broad-spectrum antimicrobial activity. Biochem. J. 326 (Pt. 1), 39–45.

Herbin, S., Mathieu, F., Brule, F., Branlant, C., Lefebvre, G., Lebrihi, A., 1997. Characteristics and genetic determinants of bacteriocin activities produced by *Carnobacterium piscicola* CP5 isolated from cheese. Curr. Microbiol. 35, 319–326.

Higazi, A.A.R., Ganz, T., Kariko, K., Cines, D.B., 1996. Defensin modulates tissue-type plasminogen activator and plasminogen binding to fibrin and endothelial cells. J. Biol. Chem. 271, 17650–17655.

Horn, N., Martinez, M.I., Martinez, J.M., Hernandez, P.E., Gasson, M.J., Rodriguez, J.M., Dodd, H.M., 1999. Enhanced production of pediocin PA-1 and coproduction of nisin and pediocin PA-1 by *Lactococcus lactis*. Appl. Environ. Microbiol. 65, 4443–4450.

http://aids.medscape.com/reuters/prof/1999/10/10.28/pb10289b.html, 2000. Marathon Enterprises Recalls Hotdogs Due to Possible Listeria Contamination.

Hurst, A., 1967. Function of nisin and nisin-like basic proteins in the growth cycle of *Streptococcus lactis*. Nature 214, 1232–1234.

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

Ingram, L.C., 1969. Synthesis of the antibiotic nisin: formation of lanthionine and beta-methyl-lanthionine. Biochim. Biophys. Acta 184, 216–219.

Ingram, L., 1970. A ribosomal mechanism for synthesis of peptides related to nisin. Biochim. Biophys. Acta 224, 263–265.

Jack, R.W., Wan, J., Gordon, J., Harmark, K., Davidson, B.E., Hillier, A.J., Wettenhall, R.E., Hickey, M.W., Coventry, M.J., 1996. Characterization of the chemical and antimicrobial properties of piscicolin 126, a bacteriocin produced by *Carnobacterium piscicola* JG126. Appl. Environ. Microbiol. 62, 2897–2903.

Jarvis, B., 1967. Resistance to nisin and production of nisin-inactivating enzymes by several *Bacillus* species. J. Gen. Microbiol. 47, 33–48.

Jimenez-Diaz, R., Ruiz-Barba, J.L., Cathcart, D.P., Holo, H., Nes, I.F., Sletten, K.H., Warner, P.J., 1995. Purification and partial amino acid sequence of plantaricin S, a bacteriocin produced by *Lactobacillus plantarum* LPCO10, the activity of which depends on the complementary action of two peptides. Appl. Environ. Microbiol. 61, 4459–4463.

Kagan, B.L., Ganz, T., Lehrer, R.I., 1994. Defensins: a family of antimicrobial and cytotoxic peptides. Toxicology 87, 131–149.

Kalchayanand, N., Sikes, A., Dunne, C.P., Ray, B., 1998. Interaction of hydrostatic pressure, time and temperature of pressurization and pediocin AcH on inactivation of foodborne bacteria. J. Food Prot. 61, 425–431.

Klaenhammer, T.R., 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. 12, 39–85.

Klein, C., Entian, K.D., 1994. Genes involved in self-protection against the lantibiotic subtilin produced by *Bacillus subtilis* ATCC 6633. Appl. Environ. Microbiol. 60, 2793–2801.

Klein, C., Kaletta, C., Schnell, N., Entian, K.D., 1992. Analysis of genes involved in biosynthesis of the lantibiotic subtilin [published erratum appears in Appl. Environ. Microbiol. 1992, May; 58 (5): 1795]. Appl. Environ. Microbiol. 58, 132–142.

Klein, C., Kaletta, C., Entian, K.D., 1993. Biosynthesis of the lantibiotic subtilin is regulated by a histidine kinase/response regulator system. Appl. Environ. Microbiol. 59, 296–303.

Kupke, T., Gotz, F., 1996. Post-translational modifications of lantibiotics. Antonie van Leeuwenhoek 69, 139–150.

Larsen, A.G., Vogensen, F.K., Josephsen, J., 1993. Antimicrobial activity of lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* MI401. J. Appl. Bacteriol. 75, 113–122.

Lazdunski, C.J., 1988. Pore-forming colicins: synthesis, extracellular release, mode of action, immunity. Biochimie 70, 1291–1296.

Leisner, J.J., Greer, G.G., Stiles, M.E., 1996. Control of beef spoilage by a sulfide-producing *Lactobacillus sake* strain with bacteriocinogenic *Leuconostoc gelidum* UAL187 during anaerobic storage at 2 °C. Appl. Environ. Microbiol. 62, 2610–2614.

Leistner, L., 2000. Basic aspects of food preservation by hurdle technology. Int. J. Food Microbiol. 55, 181–186.

Lins, L., Ducarme, P., Breukink, E., Brasseur, R., 1999. Computational study of nisin interaction with model membrane. Biochim. Biophys. Acta 1420, 111–120.

Liu, W., 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.

Maisnier-Patin, S., Forni, E., Richard, J., 1996. Purification, partial characterisation and mode of action of enterococcin EFS2, an antilisterial bacteriocin produced by a strain of *Enterococcus faecalis* isolated from a cheese. Int. J. Food Microbiol. 30, 255–270.

Marri, L., Dallai, R., Marchini, D., 1996. The novel antibacterial peptide ceratotoxin A alters permeability of the inner and outer membrane of *Escherichia coli* K-12. Curr. Microbiol. 33, 40–43.

Martinez, B., Rodriguez, A., Suarez, J.E., 2000. Lactococcin 972, a bacteriocin that inhibits septum formation in lactococci. Microbiology 146 (Pt. 4), 949–955.

Marugg, J.D., Gonzalez, C.F., Kunka, B.S., Ledeboer, A.M., Pucci, M.J., Toonen, M.Y., Walker, S.A., Zoetmulder, L.C., Vandenbergh, P.A., 1992. Cloning, expression, and nucleotide sequence of genes involved in production of pediocin PA-1, and bacteriocin from *Pediococcus acidilactici* PAC1.0. Appl. Environ. Microbiol. 58, 2360–2367.

Mazzotta, A.S., Crandall, A.D., Montville, T.J., 1997. Nisin resistance in *Clostridium botulinum* spores and vegetative cells. Appl. Environ. Microbiol. 63, 2654–2659.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.F., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. Emerging Infect. Dis. 5 (5), 607–625.

Ming, X., Daeschel, M., 1993. Nisin resistance of foodborne bacteria and the specific resistance responses of *Listeria monocytogenes* Scott A. J. Food Prot. 56, 944–948.

Miteva, V., Stefanova, T., Budakov, I., Ivanova, I., Mitev, V., Gancheva, A., Ljubenov, M., 1998. Characterization of bacteriocins produced by strains from traditional Bulgarian dairy products. Syst. Appl. Microbiol. 21, 151–161.

Moll, G., Hildeng-Hauge, H., Nissen-Meyer, J., Nes, I.F., Konings, W.N., Driessen, A.J., 1998. Mechanistic properties of the two-component bacteriocin lactococcin G. J. Bacteriol. 180, 96–99.

Moll, G.N., van Den, A.E., Hauge, H.H., Nissen-Meyer, J., Nes, I.F., Konings, W.N., Driessen, A.J., 1999. Complementary and overlapping selectivity of the two-peptide bacteriocins plantaricin EF and JK. J. Bacteriol. 181, 4848–4852.

Moore, A.J., Beazley, W.D., Bibby, M.C., Devine, D.A., 1996. Antimicrobial activity of cecropins. J. Antimicrob. Chemother. 37, 1077–1089.

Muriana, P., 1996. Bacteriocins for control of *Listeria* ssp. in food. J. Food Prot., 54–63.

Nes, I.F., Diep, D.B., Havarstein, L.S., Brurberg, M.B., Eijsink, V., Holo, H., 1996. Biosynthesis of bacteriocins in lactic acid bacteria. Antonie van Leeuwenhoek 70, 113–128.

Nielsen, J.W., Dickson, J.S., 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.

Nilsson, L., Chen, Y., Chikindas, M.L., Huss, H.H., Gram, L., Montville, T.J., 2000. Carbon dioxide and nisin act synergistically on *Listeria monocytogenes*. Appl. Environ. Microbiol. 66, 769–774.

Nissen-Meyer, J., Nes, I.F., 1997. Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. Arch. Microbiol. 167, 67–77.

Nissen-Meyer, J., Havarstein, L.S., Holo, H., Sletten, K., Nes, I.F., 1993. Association of the lactococcin A immunity factor with the cell membrane: purification and characterization of the immunity factor. J. Gen. Microbiol. 139 (Pt. 7), 1503–1509.

Nunez, M., Rodriguez, J.L., Garcia, E., Gaya, P., Medina, M., 1997. Inhibition of *Listeria monocytogenes* by enterocin 4 during the manufacture and ripening of Manchego cheese. J. Appl. Microbiol. 83, 671–677.

Ojcius, D.M., Young, J.D., 1991. Cytolytic pore-forming proteins and peptides: is there a common structural motif? TIBS 16, 225–229.

Okereke, A., Montville, T.J., 1992. Nisin dissipates the proton motive force of the obligate anaerobe *Clostridium sporogenes* PA 3679. Appl. Environ. Microbiol. 58, 2463–2467.

Oren, Z., Shai, Y., 1996. A class of highly potent antibacterial peptides derived from pardaxin, a pore-forming peptide isolated from Moses Sole fish. Eur. J. Biochem. 377, 303–310.

Pol, I.E., Mastwijk, H.C., Bartels, P.V., Smid, E.J., 2000. Pulsed-electric field treatment enhances the bactericidal action of nisin against *Bacillus cereus*. Appl. Environ. Microbiol. 66, 428–430.

Post, R., 1996. Regulatory perspective of the USDA on the use of antimicrobials and inhibitors in foods. J. Food Prot. 78–81.

Pucci, M.J., Vedamuthu, E.R., Kunka, B.S., Vandenbergh, P.A., 1988. Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. Appl. Environ. Microbiol. 54, 2349–2353.

Qiao, M., Ye, S., Koponen, O., Ra, R., Usabiaga, M., Immonen, T., Saris, P.E., 1996. Regulation of the nisin operons in *Lactococcus lactis* N8. J. Appl. Bacteriol. 80, 626–634.

Rasch, M., Knochel, S., 1998. Variations in tolerance of *Listeria monocytogenes* to nisin, pediocin PA-1 and bavaricin A. Lett. Appl. Microbiol. 27, 275–278.

Rauch, P.J., de Vos, W.M., 1992. Characterization of the novel nisin-sucrose conjugative transposon Tn5276 and its insertion in *Lactococcus lactis*. J. Bacteriol. 174, 1280–1287.

Rayman, M.K., Aris, B., Hurst, A., 1981. Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. Appl. Environ. Microbiol. 41, 375–380.

Rayman, K., Malik, N., Hurst, A., 1983. Failure of nisin to inhibit outgrowth of *Clostridium botulinum* in a model cured meat system. Appl. Environ. Microbiol. 46, 1450–1452.

Rekhif, N., Atrih, A., Lefebvre, G., 1995. Activity of plantaricin SA6, a bacteriocin produced by *Lactobacillus plantarum* SA6 isolated from fermented sausage. J. Appl. Bacteriol. 78, 349–358.

Robichon, D., Gouin, E., Debarbouille, M., Cossart, P., Cenatiempo, Y., Hechard, Y., 1997. The rpoN (sigma54) gene from *Listeria monocytogenes* is involved in resistance to mesentericin Y105, an antibacterial peptide from *Leuconostoc mesenteroides*. J. Bacteriol. 179, 7591–7594.

Rodriguez, J.M., Cintas, L.M., Casaus, P., Horn, N., Dodd, H.M., Hernandez, P.E., Gasson, M.J., 1995. Isolation of nisin-producing *Lactococcus lactis* strains from dry fermented sausages. J. Appl. Bacteriol. 78, 109–115.

Rodriguez, E., Tomillo, J., Nunez, M., Medina, M., 1997. Combined effect of bacteriocin-producing lactic acid bacteria and lactoperoxidase system activation on *Listeria monocytogenes* in refrigerated raw milk. J. Appl. Microbiol. 83, 389–395.

Ryan, M.P., Rea, M.C., Hill, C., Ross, R.P., 1996. An application in cheddar cheese manufacture for a strain of *Lactococcus lactis* producing a novel broad-spectrum bacteriocin, lacticin 3147. Appl. Environ. Microbiol. 62, 612–619.

Sahl, H.G., Bierbaum, G., 1998. Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria. Annu. Rev. Microbiol. 52, 41–79.

Saris, P.E., Immonen, T., Reis, M., Sahl, H.G., 1996. Immunity to lantibiotics. Antonie van Leeuwenhoek 69, 151–159.

Schlyter, J.H., Glass, K.A., Loeffelholz, J., Degnan, A.J., Luchansky, J.B., 1993. The effects of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. Int. J. Food Microbiol. 19, 271–281.

Schoeman, H., Vivier, M.A., Du, T.M., Dicks, L.M., Pretorius, I.S., 1999. The development of bactericidal yeast strains by expressing the *Pediococcus acidilactici* pediocin gene (*ped*A) in *Saccharomyces cerevisiae*. Yeast 15, 647–656.

Severina, E., Severin, A., Tomasz, A., 1998. Antibacterial efficacy of nisin against multidrug-resistant Gram-positive pathogens. J. Antimicrob. Chemother. 41, 341–347.

Shtenberg, A.I., 1973. Toxicity of nisin. Food Cosmet. Toxicol. 11, 352.

Siegers, K., Entian, K.D., 1995. Genes involved in immunity to the lantibiotic nisin produced by *Lactococcus lactis* 6F3. Appl. Environ. Microbiol. 61, 1082–1089.

Siezen, R.J., Kuipers, O.P., de Vos, W.M., 1996. Comparison of lantibiotic gene clusters and encoded proteins. Antonie van Leeuwenhoek 69, 171–184.

Song, H.J., Richard, J., 1997. Antilisterial activity of three bacteriocins used at sub minimal inhibitory concentrations and cross-resistance of the survivors. Int. J. Food Microbiol. 36, 155–161.

Stevens, K.A., Sheldon, B.W., Klapes, N.A., Klaenhammer, T.R., 1991. Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. Appl. Environ. Microbiol. 57, 3613–3615.

Szabo, E.A., Cahill, M.E., 1998. The combined affects of modi-

fied atmosphere, temperature, nisin and ALTA 2341 on the growth of *Listeria monocytogenes*. Int. J. Food Microbiol. 43, 21–31.

Szybalski, W., 1953. Genetic studies on microbial cross resistance to toxic agents. Antibiot. Chemother. 3 (11), 1095–1103.

Tchikindas, M., Cleveland, J., Li, J., Montville, T., 2000. Unrelatedness of nisin resistance and antibiotic resistance in *Listeria monocytogenes*. Program and Abstract Book. IAFP, p. 55.

Terebiznik, M.R., Jagus, R.J., Cerrutti, P., de Huergo, M.S., Pilosof, A.M., 2000. Combined effect of nisin and pulsed electric fields on the inactivation of *Escherichia coli*. J. Food Prot. 63, 741–746.

Thomas, L.V., Davies, E.A., Delves-Broughton, J., Wimpenny, J.W., 1998. Synergist effect of sucrose fatty acid esters on nisin inhibition of gram-positive bacteria. J. Appl. Microbiol. 85, 1013–1022.

U.S. Food and Drug Administration, 1988. Nisin Preparation: Affirmation of GRAS status as direct human food ingredient. Federal Register. 53, April 6.

U.S. Food and Drug Administration, 1993. Toxilogical principles for the safety assessment of direct food additives and color additives used in food. (Washington, D.C.: U.S. FDA).

U.S. Government Printing Office, 1990. Food Additives. pp. 5–23, Washington, DC.

Valdes-Stauber, N., Scherer, S., 1994. Isolation and characterization of Linocin M18, a bacteriocin produced by *Brevibacterium linens*. Appl. Environ. Microbiol. 60, 3809–3814.

van Belkum, M.J., Kok, J., Venema, G., Holo, H., Nes, I.F., Konings, W.N., Abee, T., 1991. The bacteriocin lactococcin A specifically increases permeability of lactococcal cytoplasmic membranes in a voltage-independent, protein-mediated manner. J. Bacteriol. 173, 7934–7941.

Vedamuthu, E.R., Henderson, J., Marugg, J., VanWassenar, P., 1992. Bacteriocin from *Lactococcus lactis* subspecies lactis. Quest International Flavor and Food Ingredient Company, Bridgewater, NJ, USA, 721774 5,173,297.

Venema, K., Haverkort, R.E., Abee, T., Haandrikman, A.J., Leenhouts, K.J., de Leij, L., Venema, G., Kok, J., 1994. Mode of action of LciA, the lactococcin A immunity protein. Mol. Microbiol. 14, 521–532.

Venema, K., Venema, G., Kok, J., 1995. Lactococcal bacteriocins: mode of action and immunity. Trends Microbiol. 3, 299–304.

Vignolo, G., Fadda, S., de Kairuz, M.N., Ruiz Holgado, A.A., Oliver, G., 1996. Control of *Listeria monocytogenes* in ground beef by 'Lactocin 705', a bacteriocin produced by *Lactobacillus casei* CRL 705). Int. J. Food Microbiol. 29, 397–402.

Villani, F., Salzano, G., Sorrentino, E., Pepe, O., Marino, P., Coppola, S., 1993. Enterocin 226NWC, a bacteriocin produced by *Enterococcus faecalis* 226, active against *Listeria monocytogenes*. J. Appl. Bacteriol. 74, 380–387.

Wandling, L.R., Sheldon, B.W., Foegeding, P.M., 1999. Nisin in milk sensitizes *Bacillus* spores to heat and prevents recovery of survivors. J. Food Prot. 62, 492–498.

Wessels, S., Jelle, B., Nes, I., 1998. Bacteriocins of lactic acid bacteria. Report of the Danish Toxicology Centre, Denmark.

Yildirim, Z., Johnson, M.G., 1998. Detection and characterization of a bacteriocin produced by *Lactococcus lactis* subsp. cremoris R isolated from radish. Lett. Appl. Microbiol. 26, 297–304.

Zhang, S., Mustapha, A., 1999. Reduction of *Listeria monocytogenes* and *Escherichia coli* O157:H7 numbers on vacuum-packaged fresh beef treated with nisin or nisin combined with EDTA. J. Food Prot. 62, 1123–1127.