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Application of Bacteriocins in Food Preservation and Safety

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ABSTRACT

Recent developments in the application of bacteriocin-producing bacteria to preserve food and maintain its safety are reviewed. The aim is to create a comprehensive compilation of advances in the applications of bacteriocins or the bacteria that produce them. Bacteria reviewed possess genetic mechanisms to produce, modify and export bacteriocins as well as genetic mechanisms to avoid self destruction by the bacteriocins produced. The actual role of these mechanisms in nature remains unclear. It is hypothesized that there is competitive advantage over bacteria that do not produce bacteriocins, but this has not been convincingly demonstrated. Nevertheless food grade bacteria possessing these mechanisms have been used in food to improve safety and extend shelf life. Driven by increasing consumer demand for more natural yet safer food, research on bacteriocins has yielded several bacteriocins and innovated commercial applications for them. Success in bacteriocin research has initiated commercial, regulatory and consumer acceptance similar or in some cases superior to that of chemical food preservatives currently in use.

Keywords: bacteriocin-producing bacteria, biopreservative, commercial challenges, innovative solutions, lactic acid bacteria

CONTENTS

INTRODUCTION

Bacteria have been used in food processing for a long time. The role of lactic acid bacteria (LAB) in extending shelf life of otherwise perishable foods is especially recognized. Currently, lactic acid bacteria are generally regarded as safe (GRAS status) for use in food processing. Fermentation metabolites of lactic acid bacteria are known to control spoilage bacteria thereby extending the shelf life of the food product. Bioactive metabolites of lactic acid bacteria include hydrogen peroxide, acetoin, organic acids, diacetyl and proteins called bacteriocins.

Despite extensive research on the properties and applications of various bacteriocins, to date, only nisin (produced by *Lactoccocus lactis* subsp. *lactis*) and pediocin (produced by *Pediococcus acidilactici*) are used commerci-

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Invited Review

ally in the food industry (Chikindas and Montville 2002; Drider *et al*. 2006).

Bacteriocins, being protein, are highly attractive to an increasingly chemical-preservative-shy food industry and consumer public. To increase acceptability and applicability of hitherto unexploited bacteriocins, molecular properties and function of the bacteriocins must be fully understood. To this end imaginative applications and commercially viable production schemes continue to emerge. In this review we look at recent advances in food applications and innovative solutions to challenges hindering commercialization of bacteriocins.

DEFINITION AND CLASSIFICATION OF BACTERIOCINS

By definition, bacteriocins are peptides or proteins that are encoded by structural genes (and are therefore ribosomally synthesized, which distinguishes them from enzymatically synthesized peptide antibiotics) and produced by members of the domain *Bacteria* (Dufour *et al*. 2007). It should be noted that bacteriocins from *Escherichia coli* and other Gram-negative bacteria are more specifically referred to as colicins or microcins (Gillor *et al*. 2004). Bacteriocins are produced by many different bacterial species. It has been suggested that 99% of all bacteria produce bacteriocins (Klaenhammer 1988). Unsurprisingly therefore, universal classification of bacteriocins is a highly debated subject. Recently a 'universal' bacteriocin classification scheme that can be applied to most bacteriocins, if not all, regardless of the Gram status of the producer strain' has been proposed (**Table 1**). This scheme includes four classes: I, lantibiotics; II, unmodified peptides; III, large proteins; and IV, cyclic peptides (Heng and Tagg 2006). Class I lantibiotics are small, posttranslationally modified antimicrobial peptides that contain unusual amino acids such as lanthionine. Guder *et al*. (2000) proposed splitting Class I lantibiotics into eight groups (the nisin, epidermin, Pep5, lacticin 481, mersacidin and cinnamycin groups, plus two groups of lantibiotics with incompletely known structures). Class II bacteriocins (unmodified bacteriocins) are small (<10 kDa) heat stable antimicrobial peptides which unlike lantibiotics are not posttranslationally modified. Class II bacteriocins are subdivided into three subclasses, namely, class IIa (pediocin-like bacteriocins), class IIb (two-peptide bacteriocins), and IIc (non-pediocin-like one-peptide bacteriocins) (Drider *et al*. 2006). Class III bacteriocins, the least well characterized so far, are large and heat-labile antimicrobial proteins. Some scientists place the well-characterized colicins produced by *E. coli* in this group (Lazdunski 1995; Nilsen *et al*. 2003). These bacteriocins have a domain-type structure, in which different domains have functions for translocation, receptor binding, and lethal activity (Riley 1993). One major distinction of Class III bacteriocins is that they do not always have specific immunity genes to protect the producer cells from their own bacteriocins, instead producer cells rely on cell wall modifications for protection (Cotter *et al*. 2005). Class IV bacteriocins are cyclic peptides (Heng and Tagg 2006). Their N and C terminal are covalently linked resulting in a cyclic structure. Two bacteriocins belonging to this class, gassericin A and reutericin 6 are the only known non lantibiotic LAB bacteriocins that contain D-amino acids (Kawai *et al*. 2004). It must be mentioned that most of the LAB bacteriocins fall into the first two classes.

MODE OF ACTION OF BACTERIOCINS

The mode of action of bacteriocins produced by gram-positive bacteria, especially those belonging to classes I and IIa, has been explained by cellular and molecular mechanisms (Montville and Chen 1998; Héchard and Sahl 2002). Results from various studies suggest that at cellular level, class IIa bacteriocins induce increases in permeability of the target cell membrane, probably by forming ion-selective pores which cause dissipation of the proton motive force and depletion of intracellular ATP (Bhunia *et al*. 1991; Christensen and Hutkins 1992; Chikindas *et al*. 1993; Drider *et al*. 2006). It is generally accepted that there is need for a docking molecule or target to aid attachment of bacteriocins on the target cell (Cotter *et al*. 2005). At molecular level it has been shown that the presence of certain genes (for example *rpoN* or *mpt* operons) causes sensitivity to bacteriocins while their absence leads to resistance (Robichon *et al*. 1997; Héchard *et al*. 2001). The proteins encoded for by these genes are thought to act as docking molecules. Interruption of these genes, if not detrimental to the target cell, can therefore lead to development of resistant variants (Cotter *et al*. 2005). The docking molecule for nisin (and probably most class I bacteriocins) is thought to be Lipid II. By binding to lipid II, nisin is thought to prevent peptidoglycan synthesis also cause pore formation. Other factors involved in bioactivity of bacteriocins include lipid composition of the membrane, alanine content, and surface charge (Drider *et al*. 2006). Class III bacteriocins, like zoocin A and millericin B, are modular in structure and have an endopeptidase-homologous catalytic domain at the N terminus, and a target recognizing C terminus. They inhibit susceptible bacteria by hydrolysis of specific peptide bonds in the stem and/or inter-peptide bridges in the peptidoglycans (Nilsen *et al*. 2003). Class IV bacteriocins (cyclic antimicrobial peptides) have been reported to act in the cytoplasmic membrane of target cells causing efflux of potassium ions. The cyclic antimicrobial peptides gassericin A and reutericin 6 do not cause efflux of ATP or lysis of susceptible cells (Kawai *et al*. 2004).

Table 1 A reflection of recent advances in the classification of bacteriocins.

Group	Properties	Sub grouping	Examples	Classification based on
Class I bacteriocins	Lantibiotics (containing lanthionine and β-lanthionine)	Nisin	Nisin A, Nisin Z, Subtilin	Guder *et al*. 2000
		Epidermin	Epidermin, Gallidermin, MutacinIII	
		Pep5	Pep5, Epilancin K7, Epicidin 280	
		Lacticin 481	Lacticin 481, Salivaricin A Plantaricin C	
		Mersacidin	Mersacidin, Actagardine	
		Cinnamycin	Cinnamycin, Duramycin	
		Structures incomplete	Nukacin ISK-1, Carnocin UI 49	
		Structures incomplete/two component lantibiotics	Lacticin 3147, Cytolysin A1	
Class II bacteriocins	Nonmodified heat-stable bacteriocins containing peptides with molecular masses of <10 kDa	Pediocin-like bacteriocins	Pedioci AcH, Sakacin P	Drider *et al*. 2006
		Two-peptide bacteriocins	Lactococcin, Plantaricin A	
		Non-pediocin-like one-peptide bacteriocins	Carnobacteriocin A, Enterocin B	
Class III bacteriocins	Protein bacteriocins with molecular masses of >30 kDa	Bacteriolytic	Helveticin J, Millericin B	Heng and Tagg 2006
		Non-lytic		
Class IV bacteriocins	Cyclic peptides		Gassericin A, Circularin A	Heng and Tagg 2006

RECENT ADVANCES IN APPLICATION

Application of bacteriocins in food preservation is generally done in three ways (Chen and Hoover 2003). First is direct application of live cells of the bacteriocin-producer to the food product (**Table 2**). The producer must be able to produce the bacteriocin in the food medium for this method to be effective. The second method is application of purified or semi-purified bacteriocins as food preservatives. The third method is application of a product that has been pre-fermented with a bacteriocin-producing strain.

Application of live bacteriocin-producer cells

Meat products

During sausage fermentation, the major microbial hazards to be controlled are *Salmonella*, enterohemorrhagic *E. coli*, *Listeria monocytogenes* and *Staphylococcus aureus* (Hugas *et al*. 2003). Bacteriocin producing strains of *Enterococcus faecium* (RZS C13 and CCM4231) and *Ent. faecalis* AS48 have been shown to inhibit *Listeria* spp. with a decrease of 3 log cycles by the end of ripening in both real or model sausage fermentations (Callewaert *et al*. 2000). Further, slime formation before sell-by date, a common spoilage problem in vacuum-packed cooked meat products, can be controlled with bacteriocinogenic bacteria (Hugas *et al*. 2003). Though no off-odors are detected, slimy meat products are very offensive to consumers. Slime formation is due to the secretion of long-chain, high molecular mass, viscosifying or gelling exocellular polysaccharides into the environment by LAB. *Ent. faecium* CTC492 producing enterocins A and B inhibits *L. monocytogenes* and other pathogenic bacteria (Hugas *et al*. 2003). The application of *Ent. faecium* CTC492 as bioprotective culture in cooked ham after slicing and before packaging inhibits the production of slime due to *L. sakei* CTC746 for up to 7 days of storage (Aymerich *et al*. 2002). Also, Castellano and Vignolo (2006) have studied the potential of lactocin producing *Lactobacillus curvatus* CRL705 for use as a bioprotective culture to control the growth of *L. innocua*, *Brochothrix thermosphacta* and indigenous LAB in fresh meat discs incubated at 2°C. *Lb. curvatus* CRL705 was listeriostatic and also effectively inhibited *B. thermosphacta* in chill stored meat discs. Similarly, Metaxopoulos *et al*. (2002) demonstrated that *Leuc. mesenteroides* L124 and *Lb. curvatus* L442 or their bacteriocins reduced *B. thermosphacta* populations in cooked cured meat products under vacuum or modified atmospheres at 4°C. In another study, the effect of the bacteriocin-producing strain, *Lactobacillus casei* CRL705, in the control of *L. innocua* 7 and *Lb sakei* CRL1424 in meat slurry during chill storage under vacuum conditions was evaluated. *Lb. sakei* CRL 1424 is a predominant indigenous lactic acid bacterium isolated from contaminated vacuum-packaged raw meat. Addition of bacteriocinogenic strain to the meat slurry did not allow the growth of *Lb. sakei* and *L. innocua*, showing a bacteriostatic effect during 21 days of storage at 4°C. Moreover *L. casei* CRL705, as a protective culture, does not significantly change the pH of the meat slurry. The bacteriocinogenic strain *Lb. casei* CRL705 can thus be used to improve microbial stability and safety in commercial meat preservation (Castellano *et al*. 2004). Jacobsen *et al*. (2003) showed that the live cells of *Leuc. carnosum* 4010 were more effective than leucocins alone for the growth inhibition of *L. monocytogenes* in sliced meat products. Bacteriostatic effects were also reported by Katla *et al*. (2002) when comparing the anti-listerial effect of sakacin P and a sakacin P-producing *Lb. sakei* strain on chicken cold cuts. Moreover it is reported that, starter cultures containing lyophilized bacteriocin-producing strains *Lc. lactis* subsp. *lactis* LMG21206 and *Lb. curvatus* LBPE effectively eliminate *L. monocytogenes* in dry-fermented sausages (Benkerrroum *et al*. 2005).

Hugas *et al*. (1998) working on vacuum-packaged fresh meat products, also found that sakacin K producing *Lact. sakei* CTC494 had a bacteriostatic effect on *Listeria* spp.. Similarly, the bacteriocin producer *Lb. plantarum* MCS1 decreased *L. monocytogenes* counts in a naturally contaminated salami sausage (Campanini *et al*. 1993).

Several variables affect growth properties of pathogens and bacteriocin producers. It is important that these are fully understood for the effective utilization of live bacteriocin producing cells for food biopreservation.

Dairy products

Bacteriocin-producing *Streptococcus salivarius* subsp. *thermophilus* B, isolated from bakery yeast, has been successfully used along with *Lb. delbrueckii* subsp. *bulgaricus* CY as yogurt starter cultures to control *L. monocytogenes* ATCC 7644. The combination also resulted in a 5-day shelf life extension in yogurt (Benkerroum *et al*. 2002). Elsewhere bacteriocin-producing *Ent. faecalis* inoculated into Manchego cheese reduced counts of *L. monocytogenes* by 6 logs in 7 days whereas survival of the same pathogen was not affected by commercial starter cultures (Nuñez *et al*. 1997). A recall of 80 tons of *L. monocytogenes*-contaminated soft and semisoft red smear cheeses in Germany in March 2000 prompted renewed concern about the presence of this bacterium in red smear cheese. It has since been demonstrated that pediocin AcH-producing *Lb. plantarum* is a potent measure for combating *Listeria* spp. in a contaminated cheese production line (Loessner *et al*. 2003).

Interestingly, *Pediococcus* spp. are not ordinarily used as cheese starter cultures and so they are not applied directly to cheese. On the contrary, the plasmid-encoding pediocin has been expressed in bacteria used in dairy fermentation like *Lc. lactis* and *Streptococcus thermophilus* (Buyong *et al*. 1998; Coderre and Somkuti 1999). Cheese made from milk spiked with 10^6 cfu/ml had 10^7 cfu/g after 2 weeks of ripening while that made with *Lc. lactis* strain transformed with the plasmid-encoding pediocin had only 10^2 cfu/g *L. monocytogenes* after 1 week.

Table 2 Examples of food applications of live bacteriocin-producer cells.

Food product	Bioprotective culture	Target	References
Sausage	*Ent. faecium* RZS C13, *Ent. faecium* CCM4231	*Listeria*	Callewaert *et al*. 2000
Cooked ham	*Ent. faecium* CTC492	*Lb. sakei* CTC746	Aymerich *et al*. 2002
Fresh meat	*Lb. curvatus* CRL705	*L. innocua, Brochothrix thermosphacta*	Castellano and Vignolo 2006
Cooked cured meat	*Leuc. mesenteroides* L124, *Lact. curvatus* L442	*B. thermosphacta*	Metaxopoulos *et al*. 2002
Sliced meat products	*Leuc. carnosum* 4010	*L. monocytogenes*	Jacobsen *et al*. 2003
Vacuum-packaged fresh meat products	*Lact. sakei* CTC494	*Listeria* spp.	Hugas *et al*. 1998
Dry fermented sausages	*Staphylococcus xylosus* DD-34	*L. monocytogenes*	Lahti *et al*. 2001
Salami	*Lb. plantarum* MCS1	*L. monocytogenes*	Campanini *et al*. 1993
Meat slurry	*Lb. casei* CRL705	*Lb. sakei, L. innocua*	Castellano *et al*. 2004
Manchego cheese	*Ent. faecalis* INIA4	*L. monocytogenes*	Nunez *et al*. 1997
Red smear cheese	*Lb. plantarum*	*L. monocytogenes* WSLC 1364	Loessner *et al*. 2003
Smoked salmon	*C. divergens* V41, *C. piscicola* V1, *C. piscicola* SF668	*L. monocytogenes*	Brillet *et al*. 2004
	C. piscicola CS526	*L. monocytogenes*	Yamazaki *et al*. 2003

Fish products

Bacteriocin-producing strains have been proved useful in the biopreservation of fishery products (Brillet *et al*. 2004). Brillet *et al*. (2004) screened bacteriocin-producing *Carnobacterium divergens* V41, *C. piscicola* V1 and *C. piscicola* SF668, for their antilisterial activity against a collection of 57 *L. monocytogenes* strains from the French smoked salmon industry. All the *Listeria* strains were inhibited. The antilisterial capacity was then tested in sterile cold smoked salmon (CSS) blocks co-inoculated with *Carnobacterium* spp. and mixtures of *L. monocytogenes* strains. *C. divergens* V41 was the most efficient strain, maintaining the level of *L. monocytogenes* at <50 cfu/g during the 4 weeks of vacuum storage at 4 and 8°C. Brillet *et al*. (2005) further reported no pH acidification was noticed in CSS blocks when inoculated with *C. divergens* V41, *C. piscicola* V1 or SF668, confirming the non-aciduric status of carnobacteria. Total volatile basic nitrogen (TVBN) a major spoilage index for CSS (Leroi *et al*. 2001), was not produced either. Results from organoleptic tests performed on odors by 14 trained panelists showed that none of the three *Carnobacterium* strains had spoiling capacity. Vaz-Velho *et al*. (2005) has further shown that *C. divergens* V41 does not produce off-odors in cold-smoked trout. Concerning safety aspects, none of the three strains produces histamine which is regarded as the main agent for scombroid fish poisoning (Taylor 1986), nor putrescine and cadaverine, often correlated to spoilage (Jorgensen *et al*. 2000a). Another strain *C. piscicola* CS526 isolated from frozen surimi has been shown to inhibit *L. monocytogenes* in cold smoked salmon (Yamazaki *et al*. 2003). It was reported that *C. piscicola* CS526 suppressed the maximum cell number of *L. monocytogenes* by two or three log cycles even at 20°C. For preservation of herring, dipping of fillets into a solution containing 1 x 10^9 cfu/ml of reuterin producing *Lb. reuteri* 16003 and 250 mmol/L glycerol controls Gram negative bacteria and reduces accumulation of TVBN during storage in 100% nitrogen at 2°C (Lindgren and Dobrogosz 1990).

Application of purified or semi-purified bacteriocins

Meat products

The use of purified or semi purified bacteriocins is of special interest in biopreservation of cooked ready to eat meat products. These products have no protection against post-processing contamination as might occur during slicing and packaging (Hugas *et al*. 2003). The pathogenic and spoilage bacteria can therefore grow exponentially even at refrigeration temperatures reaching very high numbers within days due to the absence of competitive flora. In cooked ham, 4800 AU/g of enterocin could limit the growth of *Listeria* spp. by 7.98 log cycles compared to control. A similar growth was observed with pork liver patè; where enterocins completely inhibited *Listeria* spp. outgrowth (<3 MPN/g) (Aymerich *et al*. 2000). Furthermore, enterocins A and B produced by *Ent. faecium* CTC492 could prevent the slime formation in cooked ham for up 21 days of vacuum storage at 8°C (Aymerich *et al*. 2002).

According to Castellano and Vignolo (2006), lactocin 705 produced by *Lb. curvatus* CRL705 effectively inhibits *B. thermosphacta* in chill stored meat discs. It was also noted that part of the naturally growing LAB was suppressed. Lactocin 705 could therefore be an effective way of controlling undesirable organoleptic changes in vacuum-packaged meat. *B. thermosphacta* growth in cooked cured meat products can also be controlled by combinations of bacteriocins produced by *Leuc. mesenteroides* L124 or *Lb. curvatus* L442 and vacuum or modified atmospheres at 4°C (Metaxopoulos *et al*. 2002). Similarly combining nisin spray treatments and refrigerated vacuum packaging inhibits growth of *Listeria* spp and *B. thermosphacta* in fresh beef (Cutter and Siragusa 1996).

Dairy products

Bacteriocins have been directly added to cheese to control *Clostridium* and *Listeria* spp. Nisin inhibits the outgrowth of *Cl. 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). In long-life cottage cheese spiked with 10^4 cfu/g *L. monocytogenes*, the addition of 2000 IU/g nisin 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). On the effect of pediocin PA-1 on the growth of *L. monocytogenes* in cottage cheese, half-and-half cream and cheese sauce systems, it is reported that when 100 AU/ml pediocin was added, counts of the pathogen were 5 logs lower than those in the control (Pucci *et al*. 1988). In another study 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 reported 10-32% loss in nisin activity after 10 weeks (Davies *et al*. 1997). This problem has been overcome by encapsulation of bacteriocins (Benech *et al*. 2002a, 2002b; Were *et al*. 2004).

Nisin use in cheese manufacture can cause inhibition of cheese starter cultures ultimately affecting the acidification and flavor of the final product. Encapsulation technology has been successfully used for delivery of nisin into cheddar cheese (Benech *et al*. 2002b). Inhibition of *L. innocua* in ripening cheese using liposome-encapsulated nisin Z or nisin Z produced *in-situ* was studied for 6 months. A greater reduction in *L. innocua* was observed in cheese made with liposome-encapsulated nisin Z compared to the cheese made with nisin Z-producing (in-situ) starter. After the six-month ripening phase, cheese made with liposome-encapsulated nisin Z had less than 10 cfu /g of *L. innocua* and nearly 90% of the nisin activity was retained. On the other hand, cheddar cheese made with the nisin-producing starter contained 10^4 cfu/g of *L. innocua* and only 12% of the nisin activity. Use of anti-nisin Z antibodies and Transmission Electron Microscopy to track nisin in the cheddar cheese matrix showed that the liposome immobilized nisin provides an inhibitory effect over a longer period of time due to its slower desorption from the membrane (Benech *et al*. 2002b). The technique of using encapsulation to deliver bacteriocins into food systems is promising. Further research is necessary for optimizing this technology for use in various food products (Gandhi and Chikindas 2007).

Fish products

The shelf life of brined shrimp can be extended using crude bavaricin (cell-free supernatant of *Lb. bavaricus* MI 401) or nisin Z. It is reported that whereas shelf life of shrimp in preservative free controls was 10 days, in the presence of bavaricin and nisin Z it was extend to 16 and 31 days respectively (Einarsson and Lauzon 1995). Combination with benzoate-sorbate solution preserved the brined shrimp for the whole storage period (59 days). In the control, carnocin UI49, and crude bavaricin A treatments, a gram-positive flora dominated towards the end of the storage period while in the nisin Z treatment a gram-negative flora was more pronounced.

Aasen *et al*. (2003) report that growth of *L. monocytogenes* is completely inhibited for at least 3 weeks in cold-smoked salmon by addition of sakacin P (3.5 µg/g). This is despite the proteolytic degradation in the salmon. Zuckermann and Avraham (2002) report that a combination of Nisin and Microgard (natural metabolites produced by fermenting selected food grade cultures on dairy- and/or sugar-based ingredients) reduced the total aerobic bacteria populations of fresh chilled salmon by 2 log (P<0.05) and increased its shelf-life, at 6°C, by 3-4 d, as compared with the control. The bacteriocin combination also reduced the

growth of inoculated *L. monocytogenes* in frozen–thawed salmon and increased its shelf-life from 5 to 10 d at 6°C. The bacteriocin treatment did not affect surface pH values or color of the fish.

Cell adsorbed bacteriocins have been successfully used to control the growth *L. monocytogenes* in cold smoked salmon (Ghalfi *et al*. 2006). In this novel method bacteriocin producer cells (*Lb. curvatus* CWBI-B28) were recovered by centrifugation after overnight fermentation of deMan Rogosa Sharpe (MRS) broth. Heating of the fermentation medium followed by adjustmenting the pH to 6.5 enabled adsorption of the bacteriocin to the producer cells. Cell adsorbed bacteriocin method was reported to be more effective than direct application of the bacteriocin or antimicrobial packaging methods in cold smoked salmon.

In another study, radio frequency heating along with nisin acted synergistically to inactivate *L. innocua* cells and total mesophilic microorganisms in sturgeon caviar or ikura. The products were treated by immersion in 500 IU/ml nisin solution and heat processed (an 8-D process without nisin or a 4-D process with 500 IU/ml nisin) in a newly developed radio frequency (RF; 27 MHz) heating method at 60, 63, and 65°C. In the RF–nisin treatment at 65°C, no surviving *L. innocua* microbes were recovered in sturgeon caviar or ikura. Moreover the visual quality of the caviar products treated by RF with or without nisin was comparable to the untreated control (Al-Holy *et al*. 2004).

Elotmani and Assobhei used the lactoperoxidase (LP) system and nisin to inhibit Gram-negative and Gram-positive bacteria isolated from fresh and ice-stored sardines (*Sardina pilchardus*) (Elotmani and Assobhei 2004). The LP system consists of three primary components: the lactoperoxidase enzyme (LP), thiocyanate (SCN^-) and hydrogen peroxide (H_2O_2) (Wolfson and Sumner 1993; Kussendrager and van Hooijdonk 2000). The combination of nisin (100 IU/ml) and LP system (10 level) was significantly more effective than LP system or nisin alone against all sardine flora, except *Aeromonas salmonicida* subsp. *salmonicida* and *Vibrio alginolyticus*.

Others

The inhibitory effects of nisin on the growth of the thermoacidophilic spoilage bacterium *Alicyclobacillus acidoterrestris* have been investigated for the purpose of preventing flat-sour-type spoilage in acidic drinks (Yamazaki *et al*. 2000). The outgrowth of *A. acidoterrestris* spores was reportedly inhibited by the addition of 25-50 IU/ml nisin in both orange and fruit-mixed drinks, even though inhibitory effect was not observed in clear-apple drinks. Nisin is therefore useful for preventing spoilage caused by *A. acidoterrestris* in orange and fruit-mixed drinks. In another study, enterocin AS-48 inactivation of ropy-forming *Bacillus licheniformis* in two commercial apple ciders was demonstrated (Grande *et al*. 2006).

Recently, Grande *et al*. (2007) reported anti-staphylococcal activity of enterocin AS-48. Enterocin concentrations of 80μg/ml eliminated *Staphylococcus aureus* in vegetable sauces (napoletana, pesto and green sauce for fish). However it is also reported that the effectiveness of enterocin AS-48 against *S. aureus* was reduced in carbonara sauce (less acidic) indicating that efficacy is product dependant. Elsewhere, Jamuna *et al*. (2005) reports that bacteriocins of *Lactobacillus* isolates from vegetable pickles and appam batter inhibit *Staph. aureus* and *L. monocytogenes* in retort processed ready-to-eat vegetable pulav (partially cooked vegetable pulav prepared using rice, vegetable and spices, packed in paper aluminum foil and polypropylene pouches and autoclaved). Combination of the bacteriocins with nisin resulted in maximum inhibition of both pathogens for up to 14 days without bloating of sealed pouches used for food packaging.

Application of products pre-fermented with a bacteriocin-producer

Meat products

Freeze-dried whey powder, fermented with bacteriocin producing *Carnobacterium piscicola* CS526, is reported to be effective for the inhibition of *L. monocytogenes* in ground meat (Azuma *et al*. 2007). In the presence of 10% fermented whey powder *L. monocytogenes* in ground meat rapidly decreased from 10^5 cfu/g to less than the detection limit ($3{\cdot}0 \times 10^3$ cfu/g) within 5 and 1 days at 4 and 12°C, and was bacteriostatically inhibited for 25 and 4 days at 4 and 12°C respectively. It is also reported that in real sausage fermentations spiked with *Listeria* spp. addition of enterocins significantly diminished *Listeria* spp. counts compared with the control batch from the third day of fermentation until the end of the drying period (Parente and Ricciardi 1994).

CURRENT CHALLENGES

Narrow activity spectra and bacteriocin resistance

Narrow activity spectra and moderate antibacterial effects are some of the major limitations for application of bacteriocins in food (Chen and Hoover 2003). Most bacteriocins of LAB are effective only against closely related species and ineffective against Gram negative bacteria. Bacteriocin resistance is another limitation for application of bacteriocins. Similar to the use of antibiotics, the concern with the use of bacteriocins is the development of resistance in foodborne pathogens. Hayashi (2007) reports that piscicocin resistance levels in *L. monocytogenes* and *Ent. hirae* are 10^{-5} and 10^{-2} respectively. Martínez *et al*. (2005) also reports the survival and multiplication of nisin-resistant variants in artisanal cheeses fermented by a nisin-producing *Lactococcus* strain. Variants showed different phenotypes including cross-resistance to lysozyme. These studies and others highlight the unpredictability of the consequences of the development of bacteriocin resistance in food borne pathogens and spoilage organisms.

Gravesen *et al*. (2002) investigated the frequency of resistance development in *L. monocytogenes* to two bacteriocins, pediocin PA-1 and nisin A, along with the effects of strain differences and environmental conditions. The resistance frequencies for pediocin investigated in about 20 strains were approximately 10^{-6}, irrespective of the environmental conditions, while the frequency of resistance to nisin was strain-specific and varied with environmental conditions from 10^{-7} to 10^{-2}. High-level resistance to class IIa bacteriocins (at least a 10^3-fold increase in the MIC) in *L. monocytogenes* and some other Gram-positive bacteria involves eradication of the docking molecule (Gravesen *et al*. 2004) a mannose-specific phosphoenolpyruvate-dependent phosphotransferase system (PTS) encoded by *mptACD*. Only intermediately resistant nisin mutants occur because elimination of the docking molecule for nisin (a peptidoglycan precursor called lipid II) is not possible. The pathogen can only modify the cell envelope charge or density to reduce the accessibility of the docking molecule. This results in intermediate level resistance to nisin at best (Gravesen *et al*. 2004). Furthermore Vadyvaloo *et al*. (2004) deduces that the class IIa bacteriocin resistant strains have: (1) a more positively charged cell wall, (2) possibly a more neutral cell membrane, (3) a more fluid cell membrane, and (4) a highly decreased *mptA* expression. Moreover Schaik *et al*. reports that stress mechanisms induced in *L. monocytogenes* exposed to mildly acidic conditions, increase tolerance of the pathogen to bacteriocins. However, tolerance to nisin is more pronounced than tolerance to lacticin 3147 (Schaik *et al*. 1999). It is therefore thought that, the combination structurally varied bacteriocins as part of a hurdle concept may constitute a good approach to avoid the outgrowth of resistant cells (Loessner *et al*. 2003). The fore-

going results help us understand the ways in which food pathogens evade bacteriocins in food substances. Effective bacteriocin based food biopreservation hurdle technology systems need to be based on a careful understanding of the food properties and the evasion potential of the pathogens to be controlled.

Inactivation of bacteriocins in food

Inactivation of bacteriocins by food components is another challenge to be considered in biopreservation applications. Bacteriocins are susceptible to adsorption by food macromolecules and proteolytic degradation. These properties may limit their use as preservation agents (Jung *et al*. 1992; Blom *et al*. 1997; Benech *et al*. 2002b; Aasen *et al*. 2003). A study on the use of cell adsorbed bacteriocins to inhibit growth of *L. monocytogenes* in pork, it was reported that bacteriocin concentration dropped by more than 70% with in the first week of incubation at 4°C (Ghalfi *et al*. 2007). Similar results were reported in a study on the growth of *L. monocytogenes* in ricotta cheese. In this study a 10-32% loss in nisin activity occurred after 10 weeks (Davies *et al*. 1997). This loss was slower than the loss of 50% lacticin activity that has been reported in 10 day old smear-ripened cheese (O'Sullivan *et al*. 2006). Aasen *et al*. (2003) also reports that in homogenates of cold-smoked salmon, chicken cold cuts and raw chicken more than 80% of added sakacin P and nisin are quickly adsorbed to proteins in the food matrix. In foods that had not been heat-treated, proteolytic activity caused a rapid degradation of the bacteriocins, with less than 1% of the total activity left after 1 week in cold-smoked salmon, and even less in raw chicken. It is worth noting that despite the proteolytic degradation in salmon, growth of *L. monocytogenes* was still completely inhibited for at least 3 weeks in both chicken cold cuts and cold-smoked salmon by addition of sakacin P (3.5 μg/g) (Aasen *et al*. 2003). Ghalfi *et al*. also successfully extended the effectiveness of a cell adsorbed bacteriocin from 4 weeks to 6 weeks by addition of oregano essential oil (Ghalfi *et al*. 2007).

In sausages, growth of bacteriocinogenic *Ent. faecium* CTC492 (enterocin A and B producing) is not affected by the concentration of sodium chloride, sodium nitrite or pepper. Even then these ingredients reduce bacteriocin production by 50%. These ingredients, though important for the salt-spice taste of sausages, may lower bacteriocin production 16-fold if combined. The sodium ions of the salt and the manganese content of pepper apparently compete with enterocin F, the induction factor, for the binding sites of the sensor protein, blocking the production of enterocins A and B (Nilsen *et al*. 1998; Aymerich *et al*. 2000; Hugas *et al*. 2003). Moreover enterocin production *in vitro* by *Ent. faecium* CTC492 is reported to be inhibited when pH falls below 5.5 (Parente and Ricciardi 1994). It is therefore important to optimize the in vitro bacteriocin production with other metabolites or antimicrobial agents for effectiveness to be achieved.

Food poisoning

Food poisoning as a result of inoculation of food with bacteriocin producers is a minimal risk. Tyramine production by *Carnobacterium* spp. has been reported by Edwards *et al*. (1987), Leisner *et al*. (1994) and Jorgensen *et al*. (2000b). Tyramine may cause migraine headaches and hypertensive effects, and in some cases can act as a potentiator of histamine effects (Ten Brink *et al*. 1990). Three bacteriocin producing carnobacteria *Carnobacterium divergens* V41, *C. piscicola* V1 and *C. piscicola* SF668 are reported to produce small amounts of tyramine (Brillet *et al*. 2005). Tyramine concentrations were (122 μg/g for *C. divergens* V41, 63 μg/g for *C. piscicola* SF668and 37 μg/g for *C. piscicola* V1). However, tyramine concentration found in CSS blocks inoculated with *C. divergens* V41 is lower than natural tyramine concentrations currently reported in commercial CSS (Jorgensen *et al*. 2000b; Connil *et al*. 2002). Moreover, no legal upper limit exists for tyramine in fish products in the European legislation and unlike histamine, tyramine has never been associated with fish poisoning. Thus though present, the risk is minimal in comparison to the efficacy in controlling *L. monocytogenes*.

Inhibition of bacteriocins by other bacteria

Inhibition of bacteriocins by metabolites of other bacteria may disrupt effectiveness of bacteriocins in mixed fermentations. For example, supernatant fluids from the yeast *Pichia pastoris* were reported to inhibit pediocin PA-1 (Beaulieu *et al*. 2005). Inhibition was thought to be due to "collagen-like" material which accumulated during growth and became tightly associated, most probably via covalent binding, with pediocin PA-1. The binding was most probably covalent in nature since SDS–PAGE was unable to resolve the mixture. The formation of the covalent bond was probably favored by the oxidative conditions found in aerated *P. pastoris* cultures. Though this phenomenon has not been fully elucidated, it raises questions about the effectiveness of pediocins in foods containing collagen.

Organoleptic concerns

Bacteriocins may affect organoleptic properties of food products (Drider *et al*. 2007). Samples of vacuum packed cooked meat products treated with enterocins developed tart and floral odors that caused panelists to give a low score to the samples (Aymerich *et al*. 2002). Hugas *et al*. (2003) suggests that the odors observed could be avoided by improving the purification methods of the bacteriocins applied.

Many of the challenges facing application of bacteriocins in food substances can be overcome or at least minimized by synergistic approaches. For example, Yamazaki *et al*. (2004) reports that nisin and diglycerol monolaurate can be used to enhance the antilisterial activity of essential oils, allowing for a reduction in the dosage used in food preservation and thereby resulting in the reduction of undesirable flavors.

Regulatory concerns

Regulatory concerns still remain a hindrance to the application of bacteriocins (Lauková *et al*. 1999). Although LAB are GRAS (generally regarded as safe) for the production of fermented foods, their bacteriocins are not automatically GRAS. The GRAS status granted by the U.S. Food and Drug Administration (FDA) allows for the use of a compound without need for additional approval. LAB are not GRAS for non-fermented food applications. But a food fermented by bacteriocin producing starters can be used as an ingredient in a second food product. But if this ingredient was added for the purpose of extending shelf life then it is considered to be an additive requiring pre-market clearance and label declaration (Montville and Matthews 2005). Although in 1969, the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives gave international approval for the use of nisin, the bacteriocin was only affirmed as GRAS for use in pasteurized processed cheese in the U.S. in 1988. This affirmation was based on published and unpublished data regarding its safety, not on history of common use. Acute, sub-chronic, 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 (WHO 1969; U.S. Food and Drug Administration 1988). In Australia, France and Great Britain use of nisin in processed cheeses is permitted without limit (Chikindas and Montville 2002). According to WHO the maximum daily intake of pure nisin for a 70 kg person is 60 mg (33000 units) (WHO 1969).

The 1988 FDA approval for the use of nisin in cheese

products established the legal precedent for application of bacteriocins as food additives in the United States. In the European Union, authorized food additives have to fulfill the criteria laid down in Directive 89/107 EC. Before accepting an additive, the Commission would have to verify that these criteria are fulfilled by consulting the Scientific Panel on food additives, flavorings, processing aids and materials in contact with food of the European Food Safety Authority to ensure the substance is safe for human health. Safety information should be provided according to specific guidelines (Hugas *et al*. 2003). Nisin (E 234) is listed as an accepted food additive in the European Union according to Commission directive 96/77/EC. Furthermore, in Australia, France and Great Britain the use of nisin in processed cheeses is permitted without limit (Chikindas and Montville 2002). In Europe, pediocin is reportedly available from Quest International as ALTA 2341 (Drider *et al*. 2006). The use of bacteriocins including nisin in Japan has not yet been permitted.

Consumer acceptance challenges

Consumer acceptance of bacteriocin-preserved foods is another challenge that is yet to be sufficiently handled. 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). This information includes studies by Frazer *et al*. (1962), Hara *et al*. (1962), Claypool *et al*. (1966), Fowler (1973), and Shtenberg (1973). It is likely that more information regarding nisin safety exists, but is not available to the public. When patents for new bacteriocins are submitted, often full toxicological data is not complete (Vedamuthu *et al*. 1992). Recently however, Naidu *et al*. (1999) reported that probiotic LAB were well tolerated and proven safe in 143 human clinical trials involving 7,526 human subjects. These trails were performed between 1961 and 1998. Markedly, half of the number of trails increased sharply in the last 8 years indicating that clincians, basic scientists and corporate R&D personnel developed a high degree of confidence in the safety of administering of LAB to humans (Naidu and Clemens 2000). In fact probiotic LAB are now widely used in commercially available dairy products in the U.S., Europe and Japan.

OVERCOMING THE CHALLENGES

Hurdle technology

Narrow activity spectra and moderate antibacterial effects are some of the major limitations for application of bacteriocins in food (Chen and Hoover 2003). This challenge can be overcome by hurdle technology. Hurdle technology refers to the intelligent combination of hurdles to secure safety, stability, sensory, nutritive and economic aspects of a food product (Leistner 1999). It is a fact that even ordinary food ingredients can enhance activity of bacteriocins, broadening their activity spectra (**Table 3**). For example, the presence of salt increases the activity of some bacteriocins like pediocins and sakacins (Gänzle *et al*. 1999; Bagenda 2006). Sashihara *et al*. (2001) reported that osmotic stress (1.4M NaCl) stimulated nukacin production by activating the transcription of the nukacin ISK-1 structural gene (*nukA*) in bacteriocinogenic *S. warneri* ISK-1.

Hurdles in a food system may be composed of factors like high temperature during processing, low temperature during storage, water activity, acidity, redox potential of the product as well as preservatives. Combinations of these hurdles in food are difficult for pathogens to overcome thus the food is microbiologically safe (Leistner 1999). Depending on the risk and the type of pathogen, the intensity of the various hurdles may be adjusted to suit consumer tastes and economic regimes without sacrificing safety aspects of the product. Multiple hurdle technology targets the bacterial cell in different ways resulting in better control of the pathogen.

Nisin is very useful as a part of hurdle technology (Cleveland *et al*. 2001; Ghandi and Chikindas 2007). Calderon-Miranda *et al*. (1999a, 1999b) investigated the use of pulsed electric fields (PEF) and nisin in combination to inactivate *L. innocua* in liquid whole egg and skim milk. The results showed an additive effect on the inactivation of *L. innocua* in both foods when the pathogen was exposed to PEF and the sensitized cells treated with nisin. The combined action of nisin and carbon dioxide on *L. monocytogenes* cells grown at 4°C has been investigated (Nilsson *et al*. 2000). Nisin brought about a two-log reduction in wild type *L. monocytogenes* cells and acted synergistically with carbon dioxide to give a four-log reduction in cell count. Nisin had no effect on nisin-resistant cells grown in the presence of air or carbon dioxide. Carbon dioxide increased the lag phase of *L. monocytogenes* by six days and was more effective against nisin-resistant cells compared to the wild type strain. The presence of carbon dioxide increases the

Table 3 Examples of food applications of bacteriocins in hurdle technology systems.

Bacteriocin	Combination factors	Application against	References
Nisin	Pulsed electric fields	*L. innocua* in liquid whole egg and skim milk	Calderon-Miranda *et al*. 1999a, 1999b
	Lactoperoxidase (LP) system	Bacteria from fresh and ice-stored sardines	Elotmani and Assobhei 2004
	Bacteriocins of *Lactobacillus* isolates from vegetable pickles	*S. aureus* and *L. monocytogenes*	Jamuna *et al*. 2005
	Carbon dioxide	*L. monocytogenes*	Nilsson *et al*. 2000
	Sub-lethal heat treatment	*L. monocytogenes*	Modi *et al*. 2000
	Phytic acid	*L. monocytogenes*	Bari *et al*. 2005
	Reuterin	Gram-negative organisms in milk	Arques *et al*. 2004
	Lactoferrin	*E. coli* O157:H7	Murdock *et al*. 2007
	Lactoferrin	*L. monocytogenes*	Branen and Davidson 2004
	Smoking and salting (dolphin fish)	*L. monocytogenes*	Montero *et al*. 2007
	Sodium diacetate and sodium lactate	*L. monocytogenes*	Lungu and Johnson 2005
	Lauric acid	*L. monocytogenes*	Dawson *et al*. 2002
	Microgard fermentate	*L. monocytogenes*	Zuckermann and Avraham 2002
	Thymol	*L. monocytogenes*, *B. subtilis*	Ettayebi *et al*. 2000
Pediocin	Freezing	*E. coli*, *Ps. Fluorescens*, *Y. enterocolitica*	Osmanagaoglu 2005
	Sublethal heat, EDTA	*Salmonella* spp.	
	Acetic acid	*L. monocytogenes* in cheese	Davies *et al*. 1997
Sakacin	Fish smoking	*L. monocytogenes*	Aasen *et al*. 2003
Enterocin	Carvacrol	*S. aureus*	Grande *et al*. 2007
	Heat 95°C	*Bacillus licheniformis* spores	Grande *et al*. 2006

membrane permeability and the proportion of short-chain fatty acids in the cell membrane, which helps in the pore formation by nisin (Nilsson *et al*. 2000). Modi *et al*. (2000) studied the combined effect of heat and nisin on wild type and nisin-resistant *L. monocytogenes* cells. The heat sensitivity of wild type and nisin-resistant strains was the same in the absence of nisin. The synergistic effect of heat and nisin on nisin-resistant cells caused a 3.7 log reduction in the first 7 minutes of treatment. The sub-lethal heat treatment alters the membrane permeability along with nisin that causes poration of the cell membrane. Similarly, the effectiveness of reuterin, an antimicrobial compound produced by *Lb. reuteri*, along with nisin against Gram-positive and Gram-negative organisms in milk has been studied. At the concentrations tested, reuterin exhibited bacteriostatic activity against *L. monocytogenes*. When reuterin was used in combination with nisin, it acted synergistically to inhibit *L. monocytogenes* (Arques *et al*. 2004). The co-expression of bacteriocins from different classes in a single bacterial cell therefore has bright prospects in the food industry. For example pediocin PA-1 and nisin, bacteriocins of different classes that have both been shown to be safe and effective, were co-expressed in *Lc. lactis* (Horn *et al*. 1999). Though the transformed cells produced only 11.8% total pediocin in comparison to the wild type pediocin producer, the co-production of bacteriocins may have major applications in improving food safety and minimizing the likelihood of resistant organisms.

Bacteriocins such as nisin do not have a significant inhibitory effect on Gram-negative organisms; however, the use of nisin with other agents could probably serve as a method to enhance its effectiveness against a wider range of organisms. Recently a combination of 500 µg/ml of lactoferrin and 250 IU/ml of nisin effectively inhibited the growth of *E. coli* O157:H7, as compared to, 250 µg /ml of lactoferrin and 10 IU/ml of nisin required to inhibit to *L. monocytogenes*. The result provides an alternative way to apply nisin against Gram negative bacteria (Murdock *et al*. 2007). Branen and Davidson (2004) investigated the effect of ethylenediaminetetraacetic acid (EDTA) and lactoferrin on the antimicrobial activity of nisin. Low levels of EDTA synergistically enhanced the activity of nisin against *L. monocytogenes*. EDTA also increased the effectiveness of nisin against *E. coli*, a Gram-negative organism. Furthermore, lactoferrin alone did not show any bacteriostatic effect against *L. monocytogenes*, but in a combination with low doses of nisin, lactoferrin totally inhibited *L. monocytogenes*. This effectiveness of bacteriocins at low concentrations to achieve food preservation helps to prevent the risk of development of bacteriocin-resistant populations of cells (Branen and Davidson 2004). Nisin Z and thymol, when used alone only partially inhibit the pathogens *L. monocytogenes* and *B. subtilis*. But the two agents act synergistically at sub-inhibitory concentrations of both nisin Z and thymol to reduce the growth of both pathogens. Thymol alters the bacterial membrane structure resulting in greater permeability for nisin. This results in a higher concentration of nisin within the bacterial cells, thus permitting the use of lower nisin concentrations to obtain the same level of antibacterial activity (Ettayebi *et al*. 2000). Further, Osmanagaoglu (2005) reports that when subjected to sublethal stress (exposure to physical and chemical stresses such as freezing, heating and acid treatment), Gram-negative bacterial cells that were normally resistant to pediocin P became sensitive and pediocin P reduced the viability of bacterial cells surviving sub-lethal stress. The extent of reduction varied with strain and type of treatment. When the combined effect of ethylenediaminetetraacetic acid (EDTA), sub-lethal heat and pediocin P was studied by adding EDTA and pediocin P to cell suspensions before heating them, there was a considerable reduction in the number of viable cells, even at the lowest concentration of pediocin P tested. Elsewhere Grande *et al*. (2007) reports that the inhibitory effect of enterocin AS-48 against *S. aureus* in vegetable sauces is significantly potentiated by phenolic compounds like carvacrol and geraniol. Phenolic compounds are highly hydrophobic at low pH values, enabling them to dissolve into and probably interfere with the lipids of the cell membrane, increasing sensitivity of target bacteria to bacteriocins.

The importance of careful analysis of the functioning of each individual hurdle and compatibility of different hurdles cannot be overemphasized. 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). Nisin is actually 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. Although the remaining, active nisin increased the lethality of PEF against *E. coli*, the effect was additive, not synergistic. Also, it has been demonstrated that though bacteriocinogenic *Lb. sakei* starter cultures reduce *Listeria* populations in sausages, when combined with water activity or pH reduction, the efficacy of either hurdle depends on the fermentation temperature. At fermentation temperatures of 15^{o}C water activity decrease and *Lb. sakei* combination is more effective while at 25°C, pH decrease combines better with *Lb. sakei* to reduce *L. monocytogenes* (Drosinos *et al*. 2006).

Antimicrobial packaging film

To further extend the bioactive life of bacteriocins in the food matrix, alternative methods of introducing bacteriocins into food have been suggested. For example the gradual release of bacteriocins from an antimicrobial packaging film onto the food surface (Chen and Hoover 2003). Antimicrobial packaging is a form of active packaging. Materials used for active packaging interact with the product or the headspace between the package and the food system, to obtain a desired outcome (Labuza and Breene 1989; Rooney 1995; Brody *et al*. 2001). The aim of antimicrobial packaging is to reduce, inhibit or retard the growth of microorganisms in the packed food or packaging material itself.

Bacteriocins are amphiphilic and so can be adsorbed onto surfaces. Based on this property, bacteriocins have successfully been coated onto the packaging material after it has been formed or merely added to cast films. Such antimicrobial packaging films prevent microbial growth on the food surface by direct contact (Appendini and Hotchkiss 2002). Examples include nisin/methylcellulose coatings for polyethylene films (Cooksey 2000); pediocin-containing milk-based powders adsorbed onto cellulose casings and barrier bags (Ming *et al*. 1997); nisin/EDTA/citric solutions coated onto PVC, nylon and LLDPE films (Natrajan and Sheldon 2000), corn zein films containing nisin (Hoffman *et al*. 2001).

Recently scientists have also investigated the use of a variety of antimicrobial edible films and coatings for meat products. Successful examples include, Nisaplin (Aplin & Barrett)-containing cellophane-based coating for controlling total aerobic bacteria in chopped meat (Guerra *et al*. 2005); nisin and lauric acid impregnated soy-based films for inhibiting *L. monocytogenes* on turkey bologna (Dawson *et al*. 2002); inhibition of *L. monocytogenes* on turkey frankfurters coated with zein films containing nisin, sodium diacetate and sodium lactate (Lungu and Johnson 2005). In another study a polyethylene film activated using a bacteriocin produced by *Lactobacillus curvatus* 32Y was used to reduce *L. monocytogenes* in pork steak and ground beef. The antimicrobial packaging was developed by the coating method. Results showed about a one log reduction in cell numbers, with the highest antimicrobial activity after 24 h at 4°C (Mauriello *et al*. 2004).

However processes used in the making of the film may affect the activity of the bacteriocin. It has been reported that antimicrobial activity of some bacteriocins is higher when heat is not used in the making of the film. In nisin-containing films made of methylcellulose, hydroxypropylmethylcellulose, carrageenan and chitosan, the activity of

cast films was thrice that of heat-pressed films (Cha *et al*. 2001).

Moreover surface characteristics and diffusion kinetics become crucial when using this mode of application. Studies on the diffusion of antimicrobials from packaging have been reviewed by Han (2000). It is understood that antimicrobial substance release from the polymer has to be maintained at a minimum rate so that the surface concentration is above a critical inhibitory concentration. To achieve appropriate controlled release to the food surface, the use of multilayer films (control layer/matrix layer/barrier layer) has been proposed (Floros *et al*. 2000). The inner layer controls the rate of diffusion of the active substance while the matrix layer contains the active substance and the barrier layer prevents migration of the agent towards the outside of a package. Buonocore *et al*. (2003) successfully developed a mathematical model to describe the release kinetics of antimicrobial agents (lysozyme, nisin and sodium benzoate) from cross-linked polyvinyl alcohol into water. They determined the diffusion of water molecules into the polymeric matrix and the reverse diffusion of the antimicrobial agent from the film into the water to develop the model.

Cayré *et al*. (2005) studied the relationship between oxygen permeability of packaging films and the ability of LAB to inhibit *B. thermosphacta* in vacuum-packaged cooked meat emulsions. This study showed that *B. thermosphacta* was better inhibited when highly oxygen permeable films were used. Furthermore, biodegradable antimicrobial packaging has been created using nisin and stearic acid, which serve as antimicrobial and moisture barrier agents, respectively. The pH of the hydroxyl propyl methyl cellulose (HPMC) film was adjusted to 3 to prevent the nisin and stearic acid from interacting. The packaging showed high inhibitory activity against *L. monocytogenes* and *S. aureus* (Sebti *et al*. 2002).

For bacteriocins to be successfully applied in antimicrobial packaging, the target microorganism(s) and the food composition must be considered. Bacteriocins to be used must be selected based on their spectrum of activity, mode of action, chemical composition, and the rate of growth and physiological state of the targeted microorganisms (Appendini and Hotchkiss 2002). The activity of antimicrobials that diffuse from packaging to the food will be determined at least in part by diffusion kinetics (Han 2000). It must be ensured that the bacteriocin does not alter physical and mechanical properties of the packaging material like heat sealing strength, adhesion and printing properties (Appendini and Hotchkiss 2002).

Microencapsulation

Several recent studies have investigated the use of encapsulation as another method to deliver antimicrobial agents such as nisin into food systems (Gibbs *et al*. 1999; Benech *et al*. 2002a, 2002b; Were *et al*. 2004). Encapsulated materials are protected from adverse effects of heat, moisture, pH changes and their activity is maintained for prolonged periods of time (Gibbs *et al*. 1999). Substances such as fats, starches, proteins and lipids are commonly used to encapsulate materials using techniques such as spray drying, extrusion coating and entrapment in liposomes. The release of encapsulated materials can be initiated by various conditions such as temperature, pH or moisture (Gibbs *et al*. 1999).

Benech *et al*. (2002b) reported that nearly 90% of the nisin activity was retained in cheese made with encapsulated nisin Z-liposomes after 6 months compared to 12% of the nisin activity in cheese made with the nisin-producing starter. Moreover in heat-treated foods, the bacteriocin activity of nisin and Sakacin P is stable for more than 4 weeks as compared to less than 1% of the total activity left after 1 week in raw cold-smoked salmon, and chicken (Aasen *et al*. 2003). It can be deduced that heat treatment of food products, where applicable, inactivates proteolytic enzymes and extends the life of the bacteriocin.

CONCLUDING REMARKS

Although currently underexploited, bacteriocins are set to become more prominent in the biopreservation of food. The major driving force for this "waiting-to-happen-revolution" will be increasing demands for "more natural" and "less chemical" food products by modern and better informed 21st century markets. Recent advances in understanding the molecular aspects, modes of action, potential and limitations of bacteriocins have set the stage for increased customer acceptance and lifting of regulatory limitations against use of bacteriocins. Moreover, unlike most chemical preservatives, man, by using bacteria, may have used bacteriocins in food preservation for many centuries. A food industry challenged with increasing consumer health awareness as well as interest in probiotic, nutraceutics and cosmeceutics will certainly find the current arsenal of bacteriocin related information valuable.

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