Mutacins and their Potential Use in Food Preservation

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ABSTRACT

Mutacins are proteinaceous antibacterial substances produced by Streptococcus mutans, an indigenous bacterial inhabitant of the oral cavity. The metabolism of S. mutans is similar to that of lactic acid bacteria (LAB) used in fermented food. Actually, only a few well-studied mutacins have been described. Mutacins B-Ny266, B-JH1140, I, III, and K8 are linear lantibiotics. Mutacins II and H-29B are globular lantibiotics. Mutacins GS-5/Smb and BHT-Aαβ are dipeptide lantibiotics. Mutacins N and BHT-B are non-lantibiotic peptides while mutacin IV is a non-lantibiotic dipeptide. Some of these mutacins are active against most Gram-positive foodborne pathogens. Nisin is actually the only lantibiotic bacteriocin used as a food additive and pediocin-like bacteriocins are considered to be next in line in case if more antibacterial proteins are to be approved in the future. However, nisin- and pediocin-resistant mutants appear relatively easy while resistant mutants against mutacins B-JH1140 and B-Ny266 could not be obtained. Mutacins thus have potential for controlling foodborne pathogens and spoilage bacteria. New methods for producing and purifying these small peptides will contribute towards developing food grade antimicrobials for use in food products. More research is needed on the applications of bacteriocins in food systems.

Keywords: bacteriocin, biopreservative, biofilm inhibition, food additive, GRAS, hurdle technology, lantibiotic, peptide purification, Streptococcus mutans

INTRODUCTION

The increasing concerns of consumers about the possible adverse health effects from the presence of chemical additives in food associated with the increasing demand for minimally processed food and the need for the industry to preserve the shelf life and safety of food products have stimulated research interest in finding new natural effective food preservatives.

The history of food preservation is associated with the presence of lactic acid bacteria (LAB) in foodstuffs. Today LABs are recognised as producers of a plethora of metabolites involved in preservation properties of a large variety of fermented food products. Among these metabolites are bacteriocins (Chen and Hoover 2003; Gálvez et al. 2007).

Bacteriocins are ribosomally synthesised proteinaceous antibacterial substances produced by bacteria (Jack et al. 1995; Cotter et al. 2005a) with activity directed either against related species (narrow spectrum) or across genera (broad spectrum). They can thus provide a defence mecha-
nism for the producing strain as they kill other competitive bacteria. Discrepancies persist between theories as to whether bacteriocins serve as defence mechanisms or attack weapons (Dykes and Hasting 1997; Riley and Wertz 2002; Gordon et al. 2006). Nevertheless, bacteriocins are able to kill sensitive bacteria. Work on bacteriocins has also been driven by the need to face the fast and continuous increase and widespread bacterial resistance to conventional antibiotics (Bergers et al. 2002; Shamma et al. 2005). This public health problem impels the development and rapid use of new types of antibacterial substances. Bacteriocins and lantibiotics represent candidates with great potential against pathogen infections (Pag and Dahl 2002; Joerger 2003; Chatterjee et al. 2005; Cotter et al. 2005b; Gillor et al. 2005). Similarly in the food industry, the need for more natural food biopreservatives has greatly stimulated research on LAB bacteriocins (Ross et al. 1999; O’Sullivan et al. 2002; Chen and Hoover 2003; Cotter et al. 2005a; Deegan et al. 2006; Gálvez et al. 2007). Particular cautions need to be taken before using new LAB strains in food, and genomics can help by providing new insights into LAB activities and their safety (Konings et al. 2000).

The bacteriocins produced by Streptococcus mutans, an indigenous oral bacterium, were first studied by Kelstrup and Gibbons (1969) and termed mutacins by Hamada and Ooshima (1975a). Although there are many reports showing that S. mutans produces inhibitory substances, only a few such inhibitors have been isolated and characterised as mutacins. The diversity of bacteriocin production by S. mutans has been recently highlighted, as they produce 40% of the listed streptococcal antimiicrobial peptides (Nes et al. 2006). Historically, interest in mutacins was based on their usefulness as possible anti-caries agents and the role they play in the colonization of the oral cavity (Hamada and Ooshima 1975a, 1975b; Weerkamp et al. 1977). They were also used as an epidemiological fingerprinting tool and in determining the distribution of mutacin-producing S. mutans strains (Rogers 1976a; Groonroos et al. 2007).

Despite the fact that S. mutans is physiologically a LAB (Adjic et al. 2002) and is part of the normal human microbiota (Hamada and Slade 1980; Caufield et al. 2007), it is also considered a pathogen (Mitchell 2003; Bannas 2004). In fact, S. mutans should be considered as a pathogen whose virulence is only linked to diet and environment (van Palen-Palmen 1975a, 1975b; Weerkamp et al. 1977). They were also used as an epidemiological fingerprinting tool and in determining the distribution of mutacin-producing S. mutans strains (Rogers 1976a; Groonroos et al. 1998).

In their diversity, bacteriocins produced by S. mutans present potential for use as food biopreservatives. Even though S. mutans is considered to be a pathogen by the majority of the research community, potentially safe biotechnical application of mutacins may be envisioned with heterologous production by generally recognised as safe hosts or by the addition of cell-free partially purified preparations in foodstuffs.

**Classification of bacteriocins produced by Gram-positive bacteria (adapted from Cotter et al. 2005a, 2006; Heng and Tagg 2006)**

<table>
<thead>
<tr>
<th>Class I-lantibiotics</th>
<th>Class II</th>
<th>Class III</th>
<th>Class IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specific Molecular Weight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 kDa</td>
<td>&lt; 10 kDa</td>
<td>&gt; 10 kDa</td>
<td>No specification</td>
</tr>
<tr>
<td><strong>Subgroups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Type Ia:</strong> Elongated peptides with a net positive charge</td>
<td><strong>Type Ib:</strong> Globular peptides with a net negative charge or no charge</td>
<td><strong>Type Ic:</strong> Multi-component bacteriocins</td>
<td></td>
</tr>
<tr>
<td><strong>Subtype A:</strong> Nisin-like</td>
<td><strong>Subtype SA:</strong> FA-222-like</td>
<td><strong>Examples:</strong> Nisin, lactacin 481, mersacidin, lactacin 3147, mutacins B-Ny266, B-HJ140, H-29B, J-TW (II), I-III, Smhl and BHI-A</td>
<td></td>
</tr>
</tbody>
</table>

**Streptococcus mutans**

S. mutans was first described by J. K. Clarke in 1924 after he isolated it from a carious lesion. Later, serological, protein profiles, cell wall structures and gross DNA composition studies confirmed that there was considerable variation amongst the large number of isolates identified as S. mutans. Based on these studies, S. mutans isolates were sub-divided into a number of distinct species some of which were of animal and some of human origin (Coykendall 1989). Thus the “mutans streptococci” were born and the name S. mutans was retained to describe the more common of the two main human strains, the other being Streptococcus sobrinus. The retention of the name S. mutans has led to some confusion but was necessary to comply with the rules governing scientific nomenclature. S. mutans inhabit the oral cavity of virtually everybody (Hamada and Slade 1980). S. mutans is considered the main microbial aetiological agent of dental caries. However, the high genotypic and phenotypic variation existing within the species influences its cariogenic potential (Balakrishnan et al. 2002; Napimoga et al. 2004; Saxena et al. 2005; Guo et al. 2006; Waterhouse and Russel 2006; Waterhouse et al. 2007). Sucrose is converted by glucosyltransferase enzymes to produce a sticky, extracellular, dextran-based polysaccharide that allows S. mutans to adhere to each other and to the teeth, forming dental plaque. Other primary virulence mechanisms include glucan binding proteins and its combined acidogenicity and acid resistance (Mitchell 2003; Ferretti and McShan 2006).
tibiotic monopeptides (bacteriocin class I type A or type B), lantibiotic dipeptides (bacteriocin class I type C), non-lantibiotic monopeptides (bacteriocin class IIc), and non-lantibiotic dipeptides (bacteriocin class III). The major characteristics of the new mutacins are summarised in Table 2.

**ACTIVITY SPECTRA OF MUTACINS**

Following the characterisation of many mutacins, their biotechnological potential was evaluated by determining their spectrum of activity (Table 2). Mota-Meira et al. (2000) and Morency et al. (2001) have demonstrated that numerous mutacinogenic strains, as for other lantibiotic-producing strains, inhibited the growth of many foodborne pathogenic bacteria such as *Listeria monocytogenes, Bacillus cereus, Clostridium perfringens, Staphylococcus aureus* and *Campylobacter jejuni* and numerous food spoilage bacilli as well as LABs. Mutacins can also inhibit many streptococci and enterococci, including a considerable number of clinical multiresistant strains (Mota-Meira et al. 2000, 2005). The mutacins tested were also active against some Gram-negative pathogens such as *Neisseria gonorrhoeae, Helicobacter pylori* and to a lesser extent against a hyper-permeable *Escherichia coli* strain (Mota-Meira et al. 2000, 2005). Mutacin B-Ny266 is active against many nisin A-resistant strains (*L. monocytogenes, S. aureus, B. subtilis, C. perfringens, C. sporogenes, C. tyrobutyricum*, *S. aureus*, various spoilage LAB)

<table>
<thead>
<tr>
<th>Mutacin</th>
<th>Producing strain</th>
<th>Bacteriocin Class</th>
<th>Similarity with other well described bacteriocins</th>
<th>Activity spectra</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>S. mutans CH43, UA140</td>
<td>Class Ia-lantibiotic</td>
<td>Epidermin</td>
<td>Similar to mutacin B-Ny266</td>
<td>Schnell et al. 1988; Mota-Meira et al. 2000; Qi et al. 2000a, 2000b, 2001</td>
</tr>
<tr>
<td>II or J-T8</td>
<td>S. mutans T8, UA96, 17A2</td>
<td>Class Ia-lantibiotic</td>
<td>Lacticin 481 group (variacin, macedocin among others, see Dufour et al. 2006)</td>
<td>Clostridium spp. (sporogenes), tyrobutyricum, perfringens, Bacillus spp. (subtilis, cereus), Listeria monocytogenes, Staphylococcus aureus, various spoilage LAB</td>
<td>Parrot et al. 1989; Novak et al. 1993; Pridmore et al. 1996; Kroll et al. 2000; Balakrishnan et al. 2002; Dufour et al. 2006; van den Bergh et al. 2006</td>
</tr>
<tr>
<td>III</td>
<td>S. mutans UA787</td>
<td>Class Ia-lantibiotic</td>
<td>Epidermin</td>
<td>Similar to mutacin B-Ny266</td>
<td>Schnell et al. 1988; Qi et al. 1999; Mota-Meira et al. 2000; Qi et al. 2000b</td>
</tr>
<tr>
<td>IVb</td>
<td>NlmA, NlmB</td>
<td>Class Iib or class Iic</td>
<td>Thermophilin 13</td>
<td>Similar to thermophilin 13 (Enteroococcus, Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Listeria, Bacillus spp., and Clostridium spp.)</td>
<td>Marciset et al. 1997; Qi et al. 2001; Hale et al. 2005b; Yonezawa and Kuramitsu 2005</td>
</tr>
<tr>
<td>V</td>
<td>NlmC</td>
<td>Supposed Class Iib</td>
<td>Not known</td>
<td>Non-streptococcal species, Lactococcus lactis</td>
<td>Hale et al. 2005a, 2005b; van der Ploeg 2005</td>
</tr>
<tr>
<td>B-Ny266</td>
<td>S. mutans Ny266</td>
<td>Class Ia-lantibiotic</td>
<td>Epidermin</td>
<td>Similar to mutacin B-Ny266</td>
<td>Schnell et al. 1988; Mota-Meira 1997, 2000</td>
</tr>
<tr>
<td>N</td>
<td>S. mutans N</td>
<td>Class Iib</td>
<td>Partial similarity with mutacin I</td>
<td>Close to that of mutacin I by deferred antagonism tests, Streptococcus pyogenes, oral streptococci</td>
<td>Balakrishnan et al. 2000; Hale et al. 2004</td>
</tr>
<tr>
<td>SmA</td>
<td>S. mutans K34-1, GS-5, S. rattus 67-3, FA1, GF71, IB, LG-1</td>
<td>Class Iib</td>
<td>Aureocin A53</td>
<td>Similar to aureocin A53 (E. faecium, S. aureus)</td>
<td>Netz et al. 2002a, 2002b; Hyink et al. 2005</td>
</tr>
<tr>
<td>H-29B</td>
<td>S. mutans 29B</td>
<td>Class Ia-lantibiotic</td>
<td>Lacticin 481 group</td>
<td>Similar to mutacin II</td>
<td>Dufour et al. 2006; Nicolas et al. 2006</td>
</tr>
<tr>
<td>K6</td>
<td>S. mutans K8</td>
<td>Class Ia-lantibiotic</td>
<td>Lacticin 481 group</td>
<td>Similar to macedocin and variacin (L. monocytogenes, S. aureus, B. cereus, S. subtilis, C. botulinum, C. tyrobutyricum)</td>
<td>Pridmore et al. 1996; Dufour et al. 2006; van den Bergh et al. 2006; Robson et al. 2007</td>
</tr>
</tbody>
</table>

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[a] C. sporogenes belongs to group I (proteolytic) *Clostridium* spp. with the proteolytic strains of the neurotoxin-producing pathogen *Clostridium botulinum*

[b] The first characterisation of mutacin IV included two peptides designated NlmA and NlmB (class Iic bacteriocin) (Qi et al. 2001). However, Hale et al. (2005b) showed that disruption of the nlmB gene has no impact on the activity of mutacin IV, placing mutacin IV in class Iib

[c] Multi-component mutacin

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Table 2: Activity of known mutacins against spoilage and foodborne pathogenic bacteria.
determined for some mutacins. Meta-Meira et al. (2000) compared MICs of mutacin B-Ny266 to the lantibiotic nisin A, and the antibiotics vancomycin and oxacillin. The lantibiotics were found to be as active as these antibiotics against most of the strains tested (actinobacilli, bacilli, clostridia, corynebacteria, enterococci, listeria, mycobacteria, neisseria, staphylococci, streptococci, H. pylori, and C. jejuni). Mutacin B-Ny266 was even active against strains resistant to nisin A, oxacillin, and vancomycin. Its wide spectrum of activity in the nanomolar range makes mutacin B-Ny266 an excellent candidate for antibiotic therapy or to be used as biopreservative in the food industry. Mutacins I and III were shown to be more efficient than nisin against methicillin-resistant S. aureus (MRSA), vancomycin-resistant Enterococcus faecium (VRE) and E. faecalis, showing MICs lower than 1 μg/mL (Qi et al. 1999, 2000b). Mutacins I, II, III and IV are very active against group A streptococci and penicillin-resistant Streptococcus pneumoniae with MICs lower than 1 μg/mL (Qi et al. 2000b).

MODE OF ACTION OF MUTACINS

Although four types of mutacin have been described (monopeptide lantibiotic, dipeptide lantibiotic, monopeptide non-lantibiotic, and dipeptide non-lantibiotic), the mode of action of the monopeptide lantibiotic mutacin II has been experimentally studied. Mutacin II was grouped into the type AII lantibiotics by biochemical tests (Krull et al. 2000). Generally, lantibiotics of type AII (Jung 1991; Pag and Sahl 2002) were found to kill sensitive cells by forming pores that perturb the cellular membrane. Mutacin II was shown rather to inhibit energy metabolism of sensitive cells by depolymerizing, in a transient manner, the electrical transmembrane potential, the transmembrane pH gradient and by partially inhibiting amino acid transport (Chikindas et al. 1995). This observed mode of action of mutacin II is related to that of the type B lantibiotics. However, mutacin II shares partial sequence similarities with lantibiotics of related to that of the type B lantibiotics. However, mutacins from group B share with mutacin II a Glu-Cys motif which is proposed to be responsible for the inhibition of murein biosynthesis (Zimmermann and Jung 1997; Sahl and Bierbaum 2000). Generally, lantibiotics of type AII (Jung 1991; pag and Sahl 2002) were found to kill sensitive cells by forming pores that perturb the cellular membrane. Mutacin II was shown that the loss of a mannose permease component in the bridging of lactacin 481 (van den Hooven et al. 1996) and mutacin II (Krull et al. 2000). Actagardin and mersacidin also share with mutacin II a Glu-Cys motif which is proposed to be responsible for the inhibition of murein biosynthesis (Zimmermann and Jung 1997; Sahl and Bierbaum 1998). Lantibiotics are known to possess a dual activity against Gram-positive and Gram-negative bacteria. First they block peptidoglycan synthesis by forming pores leading to dissipation of membrane potential and efflux of small metabolites. Second, they block peptidoglycan synthesis (Bauer and Dicks 2005). These two functions are ensured by their link to the peptidoglycan precursor lipid II serving as docking or target molecule (Brötz et al. 1998; Breukink et al. 1999; Wiedemann et al. 2001). For mutacin B-Ny266, binding to membrane via lipid II is required as its mode of action. A membrane-spanning domain was found in the N-terminal region of the peptide sequence of mutacin N by comparison to the transmembrane domain of the E. coli PTS enzyme IIC and also observed in the C-terminus of the peptide (Balakrishnan et al. 2000). A membrane disruptive mechanism similar to that of aureocin A53 was predicted for mutacin BHFT-B, which has similarities with this tryptophan-rich and amphipathic bacteriocin (Netz et al. 2002a; Hyink et al. 2005). Similarly, the mode of action of SmbAB and BHT-Aβ peptides were predicted based on sequence comparison with the two-component lactacin 3147β. The proposed mechanism is that the α peptide binds to the docking molecule lipid II and then recruits the β peptide, which forms pores in the membrane of the target cell (Hyink et al. 2005; Morgan et al. 2005; Yonezawa and Kuramitsu 2005; Wiedemann et al. 2006).

MUTACIN RESISTANCE

Many bacteriocin resistance mechanisms have been reported among usually sensitive bacteria, associated with a different phenotypic and genetic traits (Ennahar et al. 2000; Rammath et al. 2000; Dalet et al. 2001; Héchard et al. 2001; Gravesen et al. 2002; Vadyvaloo et al. 2002; Rammath et al. 2004; Vadyvaloo et al. 2004a, 2004b; Xue et al. 2005; Kramer et al. 2006). While resistant mutant strains were easily obtained for nisin (Mazzotta et al. 1997; Crandall and Montville 1998), no mutacin-resistant mutants have been reported in the literature (Dagry 1996; Smith et al. 2003).

For lantibiotic resistance, only nisin resistance has been investigated in detail. Nisin-resistant variants of many organisms have been isolated after exposure to the lantibiotic (Mazzotta and Montville 1997; Gravesen et al. 2002 for L. monocytogenes; Mazzotta et al. 1997 for Clostridium botulinum; Garde et al. 2004 for Streptococcus thermophilus; Mantovani and Russell 2001 for Streptococcus bovis; Peschel et al. 1999 for S. aureus). Nisin resistance has been shown to be related to alterations in the cell wall (Garde et al. 2004), membrane fatty acid and/or phospholipid composition (Mazzotta and Montville 1997), and the amount of lipoteichoic acids in the membrane composition (Peschel et al. 1999; Mantovani and Russell 2001). A nisin resistant profile is acquired through alterations in the expression of genes that are involved in cell wall and cytoplasmic membrane biosynthesis (Kramer et al. 2006). According to Kramer et al. (2006) four major mechanisms of acquired resistance have been defined (i) preventing nisin from reaching the cytoplasmic membrane, (ii) reducing the acidity of the extracellular medium, thereby stimulating the binding of nisin to the cell wall, (iii) preventing the insertion of nisin into the membrane, and (iv) possibly transporting nisin across the membrane or extruding nisin out of the membrane.

Class Ia bacteriocin resistance has been reported for normally sensitive bacteria (Ennahar et al. 2000). In Listeria species, low resistance levels (two- to four-fold) to class Ia bacteriocins are caused by alterations in the membrane lipid composition (Crandal and Montville 1998; Vadyvaloo et al. 2002, 2004a). An increase in positive charges in the membrane decreases the sensitivity to the class Ila bacteriocins (Vadyvaloo et al. 2004b). High resistance levels (1000-fold) in L. monocytogenes and E. faecalis result primarily from the loss of a mannos permease component (Rammath et al. 2000; Dalet et al. 2001; Héchard et al. 2001; Gravesen et al. 2002). High resistance levels in L. monocytogenes are also observed in the mannos transporter complex (Crandal and Montville 1998; Vadyvaloo et al. 2002, 2004a). Similarly, the mode of action of SmbAB and BHT-Aβ peptides predicted based on sequence comparison with the two-component lactacin 3147β. The proposed mechanism is that the α peptide binds to the docking molecule lipid II and then recruits the β peptide, which forms pores in the membrane of the target cell (Hyink et al. 2005; Morgan et al. 2005; Yonezawa and Kuramitsu 2005; Wiedemann et al. 2006).
ARE MUTACINS SUITABLE FOR USE IN THE FOOD INDUSTRY?

Limitations to produce and use mutacin in food systems

Recently, Gálvez and co-workers (2007) reviewed the most important factors influencing the efficacy of bacteriocins in food systems. In this chapter, virtual pathogenicity of S. mutans, interactions of bacteriocins with food matrices and factors related to the obtaining of pure mutacins will be discussed. Also, conceivable solutions based on intrinsic properties and test procedures concerning bacteriocin applications in food are proposed for mutacins.

Pathogenicity

The major objection to the use of S. mutans and/or its products in the food industry is that S. mutans is recognised as a “pathogen”. Nobody wants to put S. mutans in food to increase risk of dental caries, do they? However, is S. mutans really a “pathogen”? S. mutans is considered as the major cause of dental caries, but what is S. mutans really guilty of?

S. mutans produces acid from sugars, but so do all the LAB used in milk fermentation processes. It produces water-soluble exopolysaccharides, which is often a desired trait in the food industry (Tettelin 2004) but also water-insoluble glucans, which promote adherence to the cells and are referred to as unwanted exopolysaccharides in food (de Vuyst and Degeest 1999). S. mutans is considered a “pathogen” because production of soluble and insoluble glucan by extracellular and cell-associated glycosyltransferase promotes accumulation of S. mutans cells on the surface of the teeth while carbohydrate metabolism leads to production of lactic acid destroying the tooth enamel (Mitchell 2003). However, who is the real culprit? Is it S. mutans, or the refined sugar, particularly sucrose, in the diet? In fact, S. mutans should not be considered as a pathogen to be afraid of, but as one whose only light virulence is linked to the diet (Rolla 1989; van Palenstein Helderman et al. 1998; Tettelin 2004). This appears only after oral exposure when given to mice (Reese et al. 2007; Uteng et al. 1998). Methanol could also be replaced by ethanol in the process of bacteriocin purification often involves a straight-forward four-step process (Berjeaud and Cenatiempo 2004). The methods most frequently used for isolation, concentration and purification include salt precipitation of bacteriocin from culture supernatant, cationic exchange chromatography and reverse-phase high performance liquid chromatography (Sauvedra et al. 2004). Large scale food grade purification of bacteriocin still requires improvement (Coventry et al. 1996). However, the use of toxic organic solvents. Use of food grade mutacins could also be enabled by applying food grade purification procedures developed by Coventry et al. (1996) and Wan et al. (1996), which use diatomite calcium silicate or other ingestible silica compounds to purify different bacteriocins to homogeneity. This purification process involved both electrostatic and hydrophobic interactions of bacteriocins with food grade adsorbing resins. The use of food grade emulsifier under limited concentrations for human consumption to desorb bacteriocins is also possible (Daeschel 1993; Coventry et al. 1996; Janes et al. 1998). We previously described a technique of purification using hydrochloric acid instead of trifluorooric acid (Gaussier et al. 2002). Methanol could also be replaced by ethanol in the elution process. Another possible avenue to explore would be to use shrimp exoskeleton (mainly made of chitin) as a solid phase in the chromatography process (Casal et al. 2006). However, although chitin is closely related structurally to chitosan (poly-D-glucosamine), which shows no toxicity to mammals, and is approved by FDA as a food additive, it was recently shown to induce the accumulation in tissue of IL-4 expressing innate immune cells, including eosinophils and basophils responsible for allergic reactions, when given to mice (Reese et al. 2007). Potential food grade alternatives to these processes also include immuno-
affinity chromatography (Suarez et al. 1997).

**Heterologous production of mutacins in GRAS bacteria**

Heterologous expression systems for bacteriocins production may offer advantages over the original producer, such as allowing control of bacteriocin gene expression and achieving higher production levels for selected food grade LAB. Heterologous expression may also contribute to the production of hybrid bacteriocins with improved properties and effectiveness in food systems (Rodriguez et al. 2002; Ingham and Moore 2007). Construction of multibacteriocinogenic strains is also possible in order to contravene bacteriocin resistance development. With the advances in knowledge acquired on the genetics of mutacin production, we can envision some cocktails of mutacins targeting a broader spectrum of pathogens could be produced by one food grade LAB strain. Research efforts are still necessary to improve the production levels of heterologous expression of bacteriocin-lantibiotic in genetically modified GRAS (Generally Recognised As Safe) organisms.

**Stability**

The biochemical properties of lantibiotics make them resistant to a wide range of temperatures (up to 100°C for 15-30 min and some up to 121°C for 15 min) and pH (2 to 12) (Delves-Broughton et al. 1996; Lawton et al. 2007). The high stability of lantibiotics is explained by their thioether bridges which lock the molecule into the biologically active conformation as well as the presence of D-amino acids that protect them against the activity of certain proteases (Ryan et al. 1999). However, from our mutacin producing strains, all mutacins were sensitive to pronase E, proving their digestibility, and a few demonstrated reduced activity after heat treatment (80°C, 1 h) suggesting their potential use in pasteurized food products (Parrot et al. 1990; Morency et al. 1995; Nicolas et al. 2006). Mutacins H-29B and B-Ny266 also showed resistance to autoclaving (121°C, 15 min) (Nicolas et al. 2006).

**APPLICATIONS**

**Biopreservatives**

Can pure mutacin be used as a food biopreservative? Bacteriocins produced by *S. mutans* have interesting properties suitable to use as food biopreservative. Similar to bacteriocins produced by LAB, mutacins are inactivated by enzymes from the gastrointestinal tract, they are heat and pH-tolerant, they have a bactericidal effect against many foodborne pathogenic and spoilage bacteria. However, unlike LAB bacteriocins, no mutacin-resistant mutants have been reported in the literature. Other bacteriocins produced by GRAS streptococcal species are currently intentionally or unintentionally used either in the food and feed industries or in various probiotic treatments and are seriously considered for a number of other applications (Kirkup 2006; Dief-Gonzalez 2007). By their homology with well-characterized bacteriocins, some mutacins could find application in food products.

Mutacin II (H-29B) and mutacin K8 are related to the well-described lacticin 481 and to other lantibiotics of the lactacin 481 group (Dufour et al. 2006). Many studies have reported the effectiveness of this type of lantibiotic in food products (O’Sullivan et al. 2003; Avila et al. 2006; Dufour et al. 2006). For example, proposed applications are varia-
cin powder preparation to inhibit *B. cereus* growth in chilled dairy products (O’Mahony et al. 2001), warnericin RB4 against spoilage bacteria in acidic fruit juice-based drinks (Minamikawa et al. 2005), macedocin-producing strains as bioprotective cultures in cheese making (van den Berghe et al. 2006). Mutacins Smb and BHT-A are related to the well-studied lacticin 3147, which has demonstrated its potential as a food preservative by inhibiting many pathogens and spoilage bacteria such as *L. monocytogenes*, *B. cereus* and *S. aureus* (Morgan et al. 1999, 2000; Scannell et al. 2000; Morgan et al. 2001; Cotter et al. 2005a).

Peptide A from mutacin IV shows homology with peptide A (ThmA) of thermophilin 13, a bacteriocin that is naturally found in yoghurt. Furthermore, the *S. mutans* genome appears to code for additional ThmA-like peptides with GenBank accession numbers AE141322 and AE141316 in the *S. mutans* genome (Neergaard et al. 2005). Thermophilin 13 is a dipeptide non-lantibiotic bacteriocin able to inhibit growth of different species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Listeria*, *Bacillus* spp. and *Clostridium* spp. It even prevents outgrowth of spores of *B. cereus* and *Clostridium botulinum* (Marciset et al. 1997). Thermophilin 13 was shown to act by dissipating the membrane potential and the pH gradient in liposomes by forming pores and no component from the membrane sensitive strains (e.g. lipid or proteinaceous receptor) seems to be required for its activity (Marciset et al. 1997).

Mutacin B-Ny266 is basically related to the lantibiotic nisin, which is the only purified bacteriocin approved for use as a food additive. It has been used for more than thirty years against foodborne and spoilage bacteria in several food systems such as low-acid canned foods and dairy products (Delves-Broughton et al. 1996; Chen and Hoover 2003). Toxicology of pure nisin has been tested and found harmless for human consumption (Delves-Broughton et al. 1996). Mutacin B-Ny266 shows no haemolytic activity against blood erythrocytes and no apparent deleterious effect in a mouse model (Mota-Meira et al. 1997, 2005). As for nisin, mutacin B-Ny266 has a broad activity spectrum against important foodborne and food spoilage Gram-positive bacteria and its activity is enhanced at acidic pH (Mota-meira et al. 2000; Nicolas et al. 2004). Furthermore, muta-
cin B-Ny266 remained active against bacterial strains that are resistant to nisin A (Mota-Meira et al. 2000), placing this mutacin as a good candidate to be used when nisin re-
sistant mutants are present in a food system.

As certain food spoilage and foodborne pathogen strains can be naturally resistant or develop resistance to one particular bacteriocin (Ennahar et al. 2000), combinations of bacteriocins represent valuable strategies to eliminate the propagation of resistant bacterial contaminants in food pro-
ducts (Hanlin et al. 1993; Bouttefroy and Milliere 2000; Vignolo et al. 2000).

**Cheese ripening**

Enzymatic coagulation, draining, and ripening ensure trans-
formation of milk into cheese. Ripening represents a critical step in the maturation of cheese and development of cheese flavour and texture. Many complex biochemical reactions of proteolysis, lipolysis and glycolysis take place during the maturation period. These reactions are generally performed by starter cultures and their enzymes. To reduce the cost of cheese production, different methods have been developed to accelerate the cheese ripening step, such as the use of re-
combinant enzymes or microencapsulated enzymes (Azarnia et al. 2006). Mutacins could be used to increase the rate of starter culture lysis and enhance the release of intracel-
ular enzymes such as aminopeptidase during cheese ripening, as has been proposed for other bacteriocins (Ryan et al. 1996; Morgan et al. 1997; Ross et al. 1999; O’Sullivan et al. 2003; Avila et al. 2006). The oral cavity naturally contains bacteriocin-producing strains that show broad spectra inhib-
itory activity not only against pathogenic and food spoilage microorganisms, but also against starter and non-starter LAB. Under the proper conditions, pure mutacins could be used to accelerate cell lysis during cheese ripening (Table 3).

**Biofilm inhibition**

Biofilm formation is an emerging problem in sanitation and
Table 3 Summary of proposed applications for some mutacins.

<table>
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<tr>
<th>APPLICATIONS</th>
<th>Mutacin as food preservatives. Nicolas et al.</th>
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| In combination with other bacteriocins to eliminate the propagation of resistant bacterial contaminants in food products | - Inactivation of pathogenic bacteria in cheese (L. monocytogenes, S. aureus) - Inactivation of C. tyrobutyricum (late-blowing defect in cheese) - Inactivation of L. monocytogenes in ready-to-eat foods (e.g. hot dog sausage) - Inhibition of spoilage bacteria in processed foods (e.g. Bacillus spp.) - Inhibition of spoilage bacteria in acidic fruit-juice-based drinks - Inhibition of spoilage bacteria in beer fermentation processes Immobilized mutacins in food contact surfaces - Equipment and surfaces in the food plant to avoid bacterial biofilm formation - Food packaging, e.g. in meat packaging for inhibition of pathogenic (L. monocytogenes) and food spoilage bacteria Adjunct in raw milk products Acceleration of cheese ripening
| Safety of the food environment (reviewed by Kumar and Anand 1998; Lyndsay and von Holy 2006). Biofilms are complex communities of microorganisms irreversibly attached to a substratum, interface, or to each other, producing an extracellular polymeric matrix (Costerton 1995; Kumar and Anand 1998; O’Toole and Ghanoum 2004). Cells in biofilms are often more resistant to treatment with antimicrobial compounds, such as disinfectants, than planktonic cells (Costerton et al. 1995). Cells of pathogenic or spoilage microorganisms may survive cleaning and disinfection, detach and contaminate the food products resulting in post-processing contamination (Kumar and Anand 1998). Undesirable biofilms and their decomposition products on surfaces may lead to reduced heat transfer, increased resistance to flow, and corrosion of food processing equipment (Kumar and Anand 1998). Biofilms in the food industry can be eliminated by physical and chemical methods. In addition, the use of antimicrobial compounds such as bacteriocins adsorbed onto surfaces has been proposed to inhibit biofilm formation (Kumar and Anand 1998). Daeschel et al. (1992) and Bower et al. (1995) showed that nisin adsorbed to food contact surfaces lowered the incidence of surface contamination by L. monocytogenes. Ming et al. (1997) proposed the use of bacteriocins on food packaging materials for the biocontrol of L. monocytogenes on meats. Ercolini et al. (2006) used polythene films coated with a solution of bacteriocin 32Y from Lactobacillus curvatus to package frankfurters artificially contaminated with L. monocytogenes. The antimicrobial package was effective in inhibiting the growth and survival of the pathogen on the surface of the food product during storage. Joerger (2007) reviewed the effectiveness of films to which antimicrobials, such as nisin, food-grade acids and salts, chitosan, plant extracts, and enzymes such as lysozyme and lactoperoxidase, were incorporated for food applications. He pointed out that antimicrobial films still face limitations and suggested that they be used as part of a hurdle strategy to provide safe foods. Mutacins such as mutacin B-Ny266 having specific or broad activity spectra against several foodborne bacteria could find similar applications (Table 2 and Table 3).

Combination of mutacins with other hurdle technologies

The concept of hurdle technology implies the activity of various antimicrobial factors to which foodborne microorganisms are exposed to reduce or control their population in food products. Many hurdles have been developed to enhance the control of foodborne and spoilage microorganisms found in food products (Leistner 2000). Recently, bacteriocins have gained great interest as part of one hurdle since they can be easily combined with other selected preservation technologies without losing their antibacterial activity. Combinations of bacteriocins with chemical substances, heat treatment, modified atmosphere packaging, pulsed electric field, high hydrostatic pressure, irradiation and pulsed magnetic fields have often been studied, showing a synergistic effect in inhibitory activity against target microorganisms (reviewed by Gálvez et al. 2007). Indeed, by their particular physical and chemical properties, bacteriocins can be applied to food and can resist treatments such as temperature, addition of chemicals, high pressure processing or pulsed electric fields. The combination of different kinds of hurdle technologies avoids development of resistant bacteria. It also widens the inhibitory activity spectra of the antimicrobial factors targeting Gram-positive and Gram-negative spoilage bacteria thus improving the preservation of foodstuff (Chen and Hoover 2003; Gálvez et al. 2007). The counterpart of this kind of process is often the reduction of the organoleptic properties of food products. Bacteriocin efficiency in food matrices depends also on several food-related factors to which one particular bacteriocin molecule could be less susceptible. Impacts of such food-related factors on mutacin activity are yet unknown and remain to be studied.

CONCLUDING REMARKS

Over the last five years, advances in bacteriocin research have been spectacular, contributing to the better understanding of the molecular mechanism of action and structure-function relationships. Some crucial studies have clarified the enzymatic activity responsible for the modification reactions in the biosynthesis of lantibiotics given the possibility of synthesizing dehydrated and unsaturated residues in vitro (Xie et al. 2004; Li et al. 2006). A more thorough understanding of the mechanisms involved in bacteriocin immunity is emerging ( Cotter et al. 2005a). However, effective application of bacteriocins in food will be restricted until comprehensive studies of their activity, solubility, stability in food products and more importantly, their toxicity are carried out. While a plethora of bacteriocins have gained interest in food applications, to date only nisin is legally approved for use in the food industry (Delves-Broughton et al. 1996; Chen and Hoover 2003). Lacticin 3147, class Ia bacteriocin and lacticin 3147 (class Ic) exhibiting broad activity spectra, are prototypes of new potentially applicable bacteriocins with properties more specific than nisin for application in particular food products. Mutacins, in their diversity, possess the general properties of these bacteriocins. They are active against various Gram-positive and Gram-negative bacteriocin-resistant foodborne pathogens and

MAP: modified atmosphere packing
VP: vacuum packing
spoilage bacteria (Mota-Meira et al. 2000, 2005). Due to the cautions over the use of enterococci and streptococci in the food industry, heterologous production of mutacins constitutes an attractive mean of exploiting their broad antibacterial activity. Finally, application of bacteriocins in food should not be seen as the cure to food spoilage and contamination, but rather as a valuable tool contributing to the hurdle concept for food preservation and food safety.

ACKNOWLEDGEMENTS
Guillaume Nicolas is supported by a University-Industry Ph.D. Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) and Microbio LCA Inc.

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Food 1(2), 161-171 ©2007 Global Science Books


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