Microbiology (2015), **161**, 683–700 **DOI** 10.1099/mic.0.082289-0

Review

Resistance to bacteriocins produced by Gram-positive bacteria

Maria do Carmo de Freire Bastos,[1] Marcus Lívio Varella Coelho[1,2]† and Olinda Cabral da Silva Santos[1]†

Correspondence
Maria do Carmo de Freire Bastos
mcbastos@micro.ufrj.br *or*
carminhafbastos@gmail.com

[1]Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, CCS, Bloco I, sala I-1-59, Rio de Janeiro

[2]Instituto Nacional da Propriedade Industrial, INPI, Rio de Janeiro, Brazil

Received 23 July 2014
Accepted 13 November 2014

Bacteriocins are prokaryotic proteins or peptides with antimicrobial activity. Most of them exhibit a broad spectrum of activity, inhibiting micro-organisms belonging to different genera and species, including many bacterial pathogens which cause human, animal or plant infections. Therefore, these substances have potential biotechnological applications in either food preservation or prevention and control of bacterial infectious diseases. However, there is concern that continuous exposure of bacteria to bacteriocins may select cells resistant to them, as observed for conventional antimicrobials. Based on the models already investigated, bacteriocin resistance may be either innate or acquired and seems to be a complex phenomenon, arising at different frequencies (generally from 10^{-9} to 10^{-2}) and by different mechanisms, even amongst strains of the same bacterial species. In the present review, we discuss the prevalence, development and molecular mechanisms involved in resistance to bacteriocins produced by Gram-positive bacteria. These mechanisms generally involve changes in the bacterial cell envelope, which result in (i) reduction or loss of bacteriocin binding or insertion, (ii) bacteriocin sequestering, (iii) bacteriocin efflux pumping (export) and (iv) bacteriocin degradation, amongst others. Strategies that can be used to overcome this resistance are also addressed.

Introduction

Bacteriocins are prokaryotic antimicrobial proteins or antimicrobial peptides. As many of these substances reported in the literature have a broad spectrum of action (Heng *et al.*, 2007), exhibiting antagonistic activity against several pathogens, they have potential biotechnological applications (Bastos & Ceotto, 2011; Cotter *et al.*, 2013). Due to the risks derived from the presence of food-borne pathogens or spoilage bacteria in food, and also from the chemical preservatives used in food to control undesirable micro-organisms, the interest in the discovery of new biopreservatives has increased recently (Bastos & Ceotto, 2011). Due to the low availability of new drugs that could be used to control drug-resistant pathogens, bacteriocins have become an important option (Cotter *et al.*, 2013). Therefore, many bacteriocins have been investigated with respect to their potential use in promoting human, plant and animal health, and in enhancing food safety and quality.

Gram-positive bacteria are the major source of bacteriocins examined for biotechnological applications (Bastos & Ceotto, 2011; Cotter *et al.*, 2005, 2013). These substances are ribosomally synthesized, and are generally cationic and hydrophobic. Different schemes of classification have been proposed for bacteriocins produced by these micro-organisms. In this review, we adopt a classification (Table 1) which is an update of the classification proposed by Bastos *et al.* (2009), where bacteriocins are divided into four major classes, combining the propositions of Heng *et al.* (2007), Nes *et al.* (2007), Bierbaum & Sahl (2009), Nissen-Meyer *et al.* (2009) and van Belkum *et al.* (2011), with modifications. Most classes have been subdivided into types or subclasses. Class I bacteriocins, the so-called lantibiotics, are small, heat-stable peptides that contain modified amino acids formed by post-translational modifications (Bierbaum & Sahl, 2009). Class II bacteriocins are small, heat-stable peptides with no modified amino acids (Nissen-Meyer *et al.*, 2009). Heat-labile antimicrobial proteins are included in class III (Heng *et al.*, 2007), whereas class IV is composed of heat-stable cyclic peptides (van Belkum *et al.*, 2011).

†These authors contributed equally to this work.

Abbreviations: 2/3CS, two/three-component signal transduction system; ABC, ATP-binding cassette; AU, arbitrary units; CAMP, cationic antimicrobial peptide; EI, enzyme I; EII, enzyme II; HK, histidine kinase; HPr, histidine phosphocarrier protein; IU, international units; LTA, lipoteichoic acid; Man-PTS, mannose–phosphotransferase system; PBP, penicillin-binding protein; TA, teichoic acid.

Table 1. Classification proposed for bacteriocins produced by Gram-positive bacteria

Classification	Relevant features	Type/subclass
Class I (lantibiotics)	Small, linear peptides (<5 kDa) with modified amino acids	IA (linear) IB (globular) IC (two components) ID (reduced antimicrobial activity)
Class II	Small, linear peptides (<10 kDa) without modified amino acids	IIa (pediocin-like) IIb (two components) IIc* IId (leaderless peptide) IIe (formed by specific degradation of larger proteins) IIf (more than two components)
Class III	Large, heat-labile proteins (>25 kDa)	IIIa (bacteriolysins) IIIb (non-lytic)
Class IV	Small, cyclic peptides (<8 kDa)	–

*This subclass encompasses a miscellaneous collection of bacteriocins with distinct features.

As many bacteriocins exhibit activity at nanomolar concentrations against the target micro-organisms, are bactericidal against drug-resistant bacteria and are non-cytotoxic to eukaryotic cells, biotechnological applications have been proposed for most of them (Bastos *et al.*, 2009, 2010; Bastos & Ceotto, 2011; Cotter *et al.*, 2005, 2013). Examples are given in Table 2.

Mechanisms involved in bacteriocin action

The mechanisms involved in the inhibitory activity of bacteriocins produced by Gram-positive bacteria towards target cells have been shown to be diverse. As most peptide bacteriocins are cationic, interaction between these molecules and anionic components of the bacterial cell surface is considered to play an essential role in the initial stages of bacteriocin activity (Jack *et al.*, 1995). In a general manner, these bacteriocins are presum to cause cell death through membrane permeabilization and pore formation, due to their cationic and amphipathic characteristics. Some bacteriocins are able to impair cell wall formation, thus compromising cell envelope strength (Bastos *et al.*, 2010; Bierbaum & Sahl, 2009).

Class I bacteriocins (lantibiotics) may possess two modes of action, both involving lipid II as a docking molecule. Lipid II is present in the cytoplasmic membrane and plays a central role in building the cell wall through the transport of peptidoglycan monomers from inside out (Breukink *et al.*, 1999). Some type AI lantibiotics, such as nisin, interact with lipid II and then with the membrane, producing a poration complex at this site. Binding of nisin to lipid II also inhibits cell wall synthesis by blocking the lipid II cycle (Breukink *et al.*, 2003; Brötz *et al.*, 1998; van Heusden *et al.*, 2002). With regard to types AII and IB lantibiotics, and to lactococcin 972 (a subclass IIc bacteriocin), their antimicrobial action is due only to the

Table 2. Examples of biotechnological applications of bacteriocins produced by Gram-positive bacteria

Bacteriocin (type/subclass)	Producer micro-organism	Potential application(s)	References
Nisin (IA)	*Lactococcus lactis*	Food biopreservative and bovine mastitis prevention	Cotter *et al.* (2005)
Nukacin 3299 (IA)	*Staphylococcus simulans*	Prevention of streptococcal bovine mastitis	Ceotto *et al.* (2010)
Mutacin 1140 (IA)	*Streptococcus mutans*	Prevention of dental caries	Hillman *et al.* (2007)
Hyicin 3682 (IA)	*Staphylococcus hyicus*	Control of phytopathogens	Fagundes (2014)
Mersacidin (IB)	*Bacillus* spp.	Prevention and control of methicillin-resistant *S. aureus*	Brötz *et al.* (1995)
Lacticin 3147 (IC)	*Lactococcus lactis*	Bovine mastitis prevention	Ryan *et al.* (1999)
Pediocin PA-1 (IIa)	*Pediococcus acidilactici*	Food biopreservative	Cotter *et al.* (2005)
Aureocin A53 (IId)	*Staphylococcus aureus*	Prevention and control of bovine mastitis	Coelho *et al.* (2007)
Lysostaphin (IIIa)	*S. simulans* subsp. *staphylolyticus*	Prevention and control of human and animal infections caused by *S. aureus*	Bastos *et al.* (2010)
Enterocin AS-48 (IV)	*Enterococcus faecalis*	Food biopreservative	Gálvez *et al.* (2007)

inhibition of cell wall synthesis (Bierbaum & Sahl, 2009; Islam *et al.*, 2012; Martínez *et al.*, 2000). Finally, the two-component lantibiotics (type IC) initiate their action with the interaction of peptide A1 (also called α) and the cytoplasmic membrane, resembling the type AI mode of action. Lipid II is then targeted, blocking the transport of the peptidoglycan monomers, whilst the A1 peptide suffers a conformational change, exposing the A2 (also called β) peptide-binding site, thus creating the pore formation complex (Wiedemann *et al.*, 2006).

The best understood mechanism of action of class II bacteriocins is exemplified by subclass IIa – the pediocin-like bacteriocins. These bacteriocins use the mannose–phosphotransferase system (Man-PTS) as a docking molecule through which they exert their activity, leading to permeabilization of the cell membrane, disruption of the proton motive force and depletion of the ATP pool, which, consequently, results in arrest of all cellular biosynthesis (Nissen-Meyer *et al.*, 2009; Ramnath *et al.*, 2000, 2004). Diep *et al.* (2007) also showed that some subclass IIc bacteriocins employ a similar mechanism to kill the target cells. The mode of action of all subclass IIb bacteriocins investigated to date also involves membrane permeabilization to a variety of small molecules (Nissen-Meyer *et al.*, 2009). Aureocin A53 is a subclass IId bacteriocin which rapidly dissipates the membrane potential and simultaneously stops biosynthesis of DNA, polysaccharides and proteins in the target cells. The bacteriolytic activity of aureocin A53 is derived from extensive cell lysis via generalized membrane destruction (Netz *et al.*, 2002).

Lysostaphin is the most studied member of subclass IIIa and its lytic action is due to glycylglycine endopeptidase activity, specifically against the cell wall of some staphylococcal species (Schindler & Schuhardt, 1965). Lysostaphin cleaves the cross-bridges of the peptidoglycan, which are composed of five glycine residues (Browder *et al.*, 1965).

Finally, class IV bacteriocins, similar to most bacteriocins from classes I and II, permeabilize the membrane of the susceptible cells, causing dissipation of the membrane potential, leakage of ions and cell death (van Belkum *et al.*, 2011).

Bacteriocin immunity

Bacteriocin-producer strains are often protected from the activity of their own products by immunity systems, whose encoding genes are generally found in the bacteriocin gene clusters (Bierbaum & Sahl, 2009; Heng *et al.*, 2007; Nissen-Meyer *et al.*, 2009; van Belkum *et al.*, 2011). Consequently, a bacteriocin-producing strain is generally not sensitive to the action of its cognate bacteriocin. Additionally, related bacteriocins may exhibit cross-immunity, as observed for some subclass IIa (Fimland *et al.*, 2002) and subclass IIb (Oppegård *et al.*, 2010) bacteriocins, and for the lantibiotics Pep5 and epicidin 280 (Heidrich *et al.*, 1998), amongst others. However, for at least some subclass IIb bacteriocins, the cross-immunity is target cell dependent, which suggests that its function is dependent on a cellular component, probably the bacteriocin receptor (Oppegård *et al.*, 2010).

The mechanisms involved in immunity are still poorly understood for most bacteriocins. In many systems, bacteriocin producers rely on a single small protein for self-protection. These proteins have been detected either anchored to the membrane surface (Dubois *et al.*, 2009; Heidrich *et al.*, 1998) or embedded in the membrane (Bierbaum & Sahl, 2009; Coelho *et al.*, 2014). Some are largely exported, remaining trapped at the membrane and within the cell wall compartment (Hoffmann *et al.*, 2004). The roles proposed for these proteins include blocking the insertion of the bacteriocin into the membrane or protection of a dedicated target, shielding it from the bacteriocin (Dubois *et al.*, 2009).

For some class II bacteriocins, the immunity protein forms a strong complex with the bacteriocin bound to its receptor, preventing cells from being killed (Diep *et al.*, 2007). In the examples already described, the receptors are components of the Man-PTS, which is generally composed of three proteins: enzyme I (EI), histidine phosphocarrier protein (HPr) and enzyme II (EII). EI and HPr are cytoplasmic proteins involved in the transfer of a phosphate group to EII (Postma *et al.*, 1993). EII consists of four subunits: IIA, IIB, IIC and IID. Subunits IIA and IIB are located in the cytoplasm, whereas subunits IIC and IID form a membrane-bound complex through which the sugar molecules enter the cell. Diep *et al.* (2007) demonstrated that lactococcin A uses the subunits IIC and IID as a receptor on the target cells and that the immunity protein (LciA) binds to the bacteriocin–receptor complex to confer immunity to producer cells. A similar mechanism of immunity has also been shown for subclass IIa bacteriocins (Diep *et al.*, 2007).

For some class II bacteriocins, immunity relies on the activity of putative membrane-bound metalloproteases which belong to the Abi protein family (Kjos *et al.*, 2010a). As these proteins are proteases, Kjos *et al.* (2010a) postulated that Abi-mediated immunity arises by either direct degradation of the bacteriocin or modification of a receptor protein.

Another immunity mechanism reported for some class II bacteriocins depends on the activity of multi-drug transporter proteins, which participate in bacteriocin immunity by removing bacteriocin that enters the cytoplasmic membrane from the outside (Gajic *et al.*, 2003).

For some lantibiotics (Bierbaum & Sahl, 2009) and aureocin A53 (Nascimento *et al.*, 2012), immunity results from the combined action of a cognate immunity protein, which binds to bacteriocin molecules on the bacterial cell membrane, and a multi-component ATP-binding cassette (ABC) transporter, which removes the bacteriocin from cells. Proteins involved in immunity to cyclic bacteriocins are probably membrane-associated, but the role of a

cognate immunity protein is also complemented by ABC transporters, required for full immunity to cyclic peptides (van Belkum *et al.*, 2011).

Lysostaphin producers do not have specific immunity genes. However, the peptidoglycan synthesized by the lysostaphin-producer strains is resistant to the hydrolytic activity of the bacteriocin, due to the incorporation of serine residues into the third and fifth positions of the pentapeptide cell wall cross-bridges (Ehlert *et al.*, 2000; Thumm & Götz, 1997). Lysostaphin is unable to hydrolyse glycylserine and serylglycine peptide bonds. The product encoded by the *lif* (lysostaphin immunity factor) gene is required for such incorporation, changing the lysostaphin target in the producer cells (Ehlert *et al.*, 2000; Thumm & Götz, 1997).

Bacteriocin resistance development

Once a new antimicrobial agent is described, and proven to be safe and effective against pathogens, it is crucial to evaluate the potential risks of resistance development upon prolonged exposure to it (Cotter *et al.*, 2013). The frequency at which susceptible organisms can develop resistance to a given bacteriocin is, therefore, a very important issue to consider when bacteriocin-based biocontrol strategies are proposed. Bacteriocins have not been extensively used in the clinical setting (Cotter *et al.*, 2013). Nisin has long been used as a food biopreservative, but nisin resistance has not been reported amongst food-spoilage micro-organisms in the food industry (Blake *et al.*, 2011). Therefore, our understanding of the potential for bacteriocin resistance development has been revealed primarily from experiments performed under laboratory conditions (Cotter *et al.*, 2013).

Bacteriocin resistance is often not defined clearly in the literature (Katla *et al.*, 2003). Enhanced nisin resistance in *Listeria monocytogenes* is generally defined as $<$10-fold increase in MIC (Gravesen *et al.*, 2004). If the mutant is able to grow in the presence of the highest bacteriocin concentration tested, it is generally considered a high-level resistant mutant. In a study published by Blake *et al.* (2011), mutants displaying an eightfold or less decrease in nisin susceptibility were considered to exhibit low resistance, whilst those exhibiting a 32-fold increase in nisin MIC were considered to be highly resistant. However, for subclass IIa bacteriocins, mutants were considered to be highly resistant to a given bacteriocin when (i) they were able to grow on agar plates containing $\geqslant$1600 arbitrary units (AU) bacteriocin ml^{-1} (Rasch & Knøchel, 1998), (ii) the MICs/IC$_{50}$s were 1000 times higher than those for the parental strains (Gravesen *et al.*, 2002b; Katla *et al.*, 2003) or (iii) the IC$_{50}$ was $\geqslant$1 μg ml^{-1} (Tessema *et al.*, 2009). When mutants were inhibited by $<$1600 AU bacteriocin ml^{-1} (Rasch & Knøchel, 1998) or when the IC$_{50}$ was $<$1 μg ml^{-1} (Tessema *et al.*, 2009), they were characterized as having enhanced tolerance or low-level resistance. As can be seen, there is no consensus amongst investigators in relation to what is considered high-, moderate- or low-level resistance to bacteriocins.

One critical factor in susceptibility tests is the bacteriocin solution. In some studies, purified preparations [given in μg or international units (IU)] were used, whereas in others the fermentates (given in AU) of the producer organisms were employed. Therefore, we agree with Katla *et al.* (2003) who pointed out that it is generally difficult to compare studies on susceptibility to bacteriocins due to differences in experimental approaches and terminology.

Several methods have been used to determine differences in bacterial susceptibility to bacteriocins (Katla *et al.*, 2003), including plating of saturated cultures on agar media containing either a single dose or different amounts of the bacteriocins. However, the most sensitive method is the microtitre plate assay, employing bacteriocin solutions of known concentrations (Katla *et al.*, 2003). After incubation at the appropriate conditions, bacterial growth is monitored either visually or by optical denisty.

Bacterial antimicrobial peptides generally act fast, which drastically reduces the possibility of resistance development in their presence in a population containing only susceptible target cells (Cotter *et al.*, 2005). However, mutations resulting in deresistance to bacteriocins may arise in the absence of bacteriocins, being selected in their presence. Bacteriocin resistance may develop on solid media, and in food and animal model systems (Blake *et al.*, 2011; Climo *et al.*, 2001; Gravesen *et al.*, 2002b; Rasch & Knøchel, 1998; Wan *et al.*, 1997). As, to best of our knowledge, there has not been a review published on this subject, we highlight the main findings concerning the resistance to bacteriocins produced by Gram-positive bacteria in the present publication.

According to Collins *et al.* (2012), the mechanisms involved in bacteriocin resistance can be divided into two groups: acquired resistance (that developed by a formerly susceptible strain) and innate resistance (that intrinsically found in particular genera or species). Several genetic loci have been linked with either innate or acquired resistance. Their involvement in bacteriocin resistance has generally been shown by mutations generated by gene knockouts or deletions, complementation or overexpression analysis. A given gene is considered to be involved in innate resistance when mutations in the gene lead to bacteriocin sensitivity, whereas it is considered to be associated with acquired resistance when mutations in the gene lead to bacteriocin resistance. The cell alterations responsible for resistance and resulting from some of these mutations have not yet been elucidated. Nevertheless, descriptions of the genes will be included in the present review as their roles in bacteriocin resistance deserve further investigation and could attract the attention of investigators, as this knowledge is important for optimization of bacteriocin use (Tessema *et al.*, 2011).

The bacteriocins most studied with regard to the development of resistance are nisin, lacticin 3147, pediocin-like bacteriocins and lysostaphin. Both nisin and lacticin 3147 are produced by strains of *Lactococcus lactis*. Nisin A is the most studied lantibiotic, and it has already

found applications as a biopreservative in a broad range of foods worldwide and in the prevention of bovine mastitis (Cotter *et al.*, 2005). Lacticin 3147 is a two-peptide lantibiotic which is bactericidal against many pathogens. Its use as a food preservative has been proposed to control food-spoilage bacteria as well as pathogens (Suda *et al.*, 2012). It has also been used in teat seals for prevention of intramammary infections in dry cows (Suda *et al.*, 2012). Pediocin-like bacteriocins are subclass IIa peptides produced by strains of *Pediococcus* spp. and other lactic acid bacteria, which exhibit a strong anti-listerial activity (Nissen-Meyer *et al.*, 2009). *Listeria monocytogenes* is a food-borne pathogen which causes listeriosis – an infection that can be fatal (Freitag *et al.*, 2009). Pediocin PA-1/AcH is also approved for use in food (Bastos & Ceotto, 2011). Lysostaphin is an enzyme produced by *Staphylococcus simulans* subsp. *staphylolyticus*, which exhibits bacteriolytic activity towards staphylococci, including *Staphylococcus aureus*. It is the most investigated class III bacteriocin in relation to clinical applications (Bastos *et al.*, 2010).

Fig. 1 summarizes some mechanisms involved in resistance to bacteriocins, which will be further explained in the text. However, readers interested in a more detailed description of the experiments performed and the systems investigated in each case are referred to the original publications.

Innate resistance

Innate susceptibility to bacteriocins may vary greatly amongst different bacterial strains (Katla *et al.*, 2003). However, few studies have attempted to compare a large number of strains. In the study performed by Rasch & Knøchel (1998), using 381 strains of *L. monocytogenes*, none of the strains was considered naturally nisin resistant and only two strains (0.52 %) were characterized as exhibiting enhanced nisin tolerance, being able to grow on agar plates containing 500 IU nisin ml^{-1}. However, 20 strains (5.2 %) were considered resistant to pediocin PA-1, growing on 1600 AU ml^{-1}, and 34 strains (8.9 %) were characterized as exhibiting enhanced tolerance to pediocin, growing on 400 AU ml^{-1}. When 200 strains of *L. monocytogenes* were tested by Katla *et al.* (2003) for susceptibility to nisin and three subclass IIa bacteriocins (pediocin PA-1, sakacin P and sakacin A), a 60–360-fold difference in sensitivity was observed. The susceptibility differences measured in the microtiter plates assays were comparable to those observed for the same strains in food model systems (Katla *et al.*, 2002).

Innate bacteriocin resistance may result from different mechanisms. The main mechanisms are summarized in Fig. 2.

Resistance due to immunity mimicry

Immune mimicry is a mechanism which contributes to protection specifically towards bacteriocins. In such cases, non-bacteriocin-producing strains carry genes which encode functional homologues of bacteriocin immunity systems, also designated 'orphan immunity genes'. This phenomenon has been described for lantibiotics (Draper *et al.*, 2009) and class II bacteriocins (Fimland *et al.*, 2002). It has been shown that heterologous expression of these orphan immunity genes confers protection against the cognate bacteriocin (Draper *et al.*, 2012).

Resistance due to bacteriocin degradation

This resistance mechanism also seems to be specific for bacteriocins. Some nisin-resistant strains of *Bacillus* spp. produce an enzyme, nisinase, which degrades nisin (Jarvis, 1967). Nisinase was shown to be produced by strains of *Bacillus cereus* and *Paenibacillus* (formerly *Bacillus*) *polymyxa* during sporulation. Nisinase breaks the C-terminal lanthionine ring (Jarvis & Farr, 1971) and is also active against subtilin, but not against bacitracin, polymyxin and gramicidin (Jarvis, 1967). This absence of cross-resistance towards antibiotics is quite relevant. If bacteriocin-resistant mutants become simultaneously resistant to antibiotics, such findings may create concern with regard to clinical bacteriocin applications.

Nisin degradation has also been observed in some non-nisin-producing *La. lactis* strains. This degradation is catalysed by the nisin resistance protein NSR protease localized on the cell membrane, which removes the nisin C-terminal tail (Sun *et al.*, 2009).

Butcher & Helmann (2006) proposed that the proteolytic activity of YqeZ (a predicted membrane-integrated protease), encoded by the σ^W-regulated *ygeZyqfAB* operon, could provide protection against the lantibiotic sublancin 168 in bacilli.

Resistance associated with growth conditions

Innate bacteriocin resistance seems to depend on various factors. *Listeria monocytogenes* 412 stationary-phase cells exhibited higher resistance to either nisin (300 IU ml^{-1}) or pediocin PA-1 (320 AU ml^{-1}) than exponential-phase cells (Jydegaard *et al.*, 2000). However, no effect of growth phase was observed for a high pediocin concentration (2560 AU ml^{-1}). Tolerance to pediocin PA-1 was also observed in osmotically stressed cultures (6.5 % NaCl) and in cold-stressed cells (5 °C for 60–80 min) (Jydegaard *et al.*, 2000). The former results were probably explained by changes in electrostatic interactions between the bacteriocin and the cell surface in the presence of high ion concentrations. Additionally, cell morphology may change due to increased osmolarity of the growth medium, leading to changes in the cell envelope (Jydegaard *et al.*, 2000).

Nisin resistance of *L. monocytogenes* has also been associated with acid stress response (Begley *et al.*, 2010; van Schaik *et al.*, 1999). The glutamate decarboxylase system is considered the most important system employed by this species to withstand low pH stress (Begley *et al.*, 2010). In this system, glutamate undergoes decarboxylation to

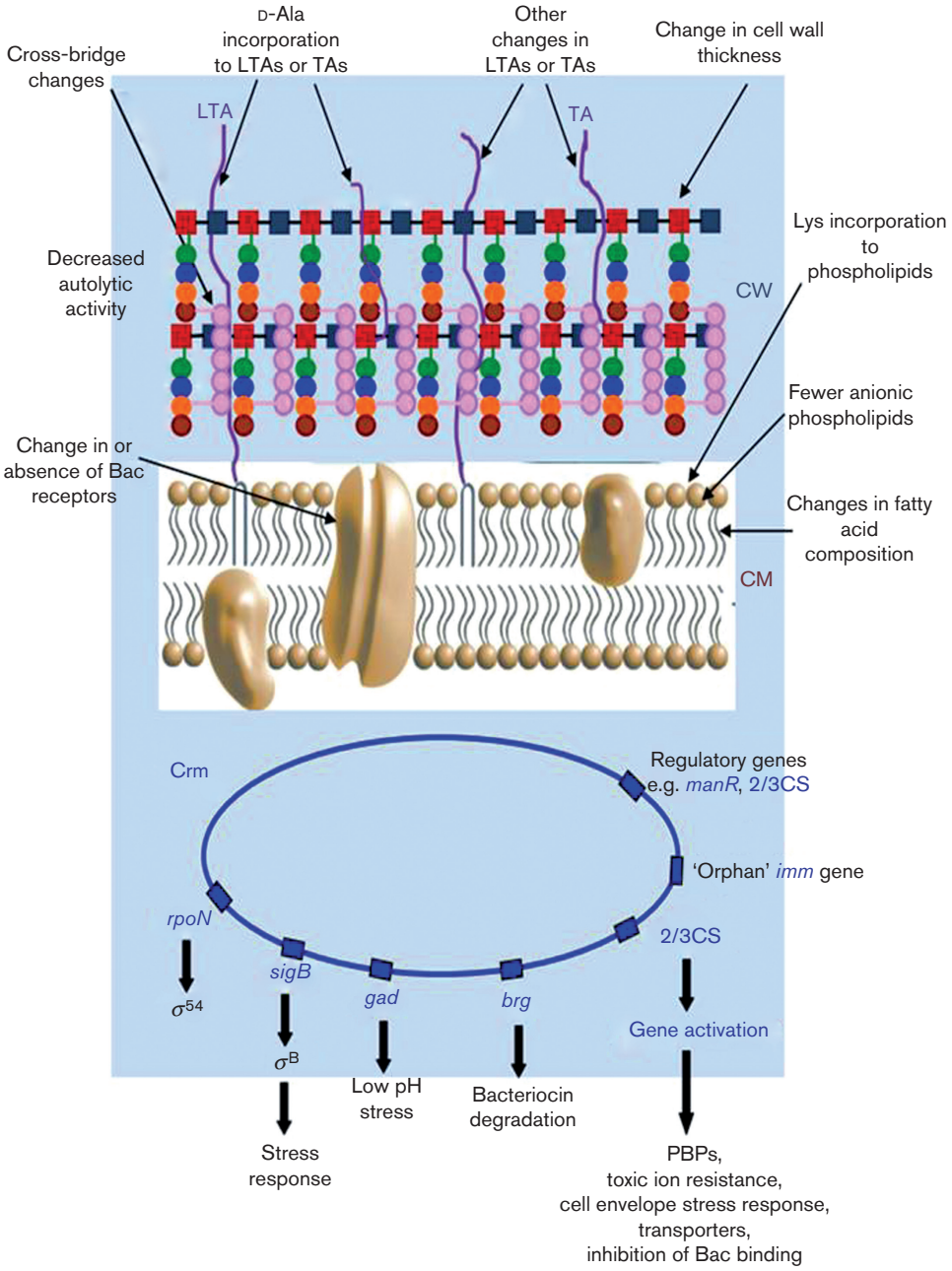

Fig. 1. Some mechanisms and genes involved in bacterial resistance to bacteriocins produced by Gram-positive bacteria. Bac, bacteriocin; *brg*, bacteriocin resistance gene; CM, cytoplasmic membrane; Crm, chromosome; CW, cell wall; gad, glutamate decarboxylase; *imm*, immunity; LTA, lipoteichoic acid; PBP, penicillin-binding protein; *sig*, sigma factor; TA, teichoic acid; 2/3CS, two/three-component signal transduction system.

γ-aminobutyrate and carbon dioxide. In this reaction, catalysed by GadD1, GadD2 and GadD3, ATP is formed, contributing to ATP pools within the cell. γ-Aminobutyrate is then transported by the antiporters GadT1 and GadT2 (Begley *et al.*, 2010). Begley *et al.* (2010) investigated a complete set of *gad* deletion mutants of *L. monocytogenes* LO28 and found that only the Δ*gadD1* mutant was susceptible to nisin, exhibiting a 10^2-fold reduction survival in the presence of 300 μg nisin ml^{-1}. They also observed a 40 % reduction in the intracellular ATP levels found in the Δ*gadD1* mutant. Based on these data, Begley *et al.* (2010) proposed that, as nisin activity ultimately leads to release of ATP and cell death, GadD1 may restore the intracellular ATP pools, leading to nisin resistance. Similar results were observed for *gadB* mutants of *La. lactis* (Begley *et al.*, 2010).

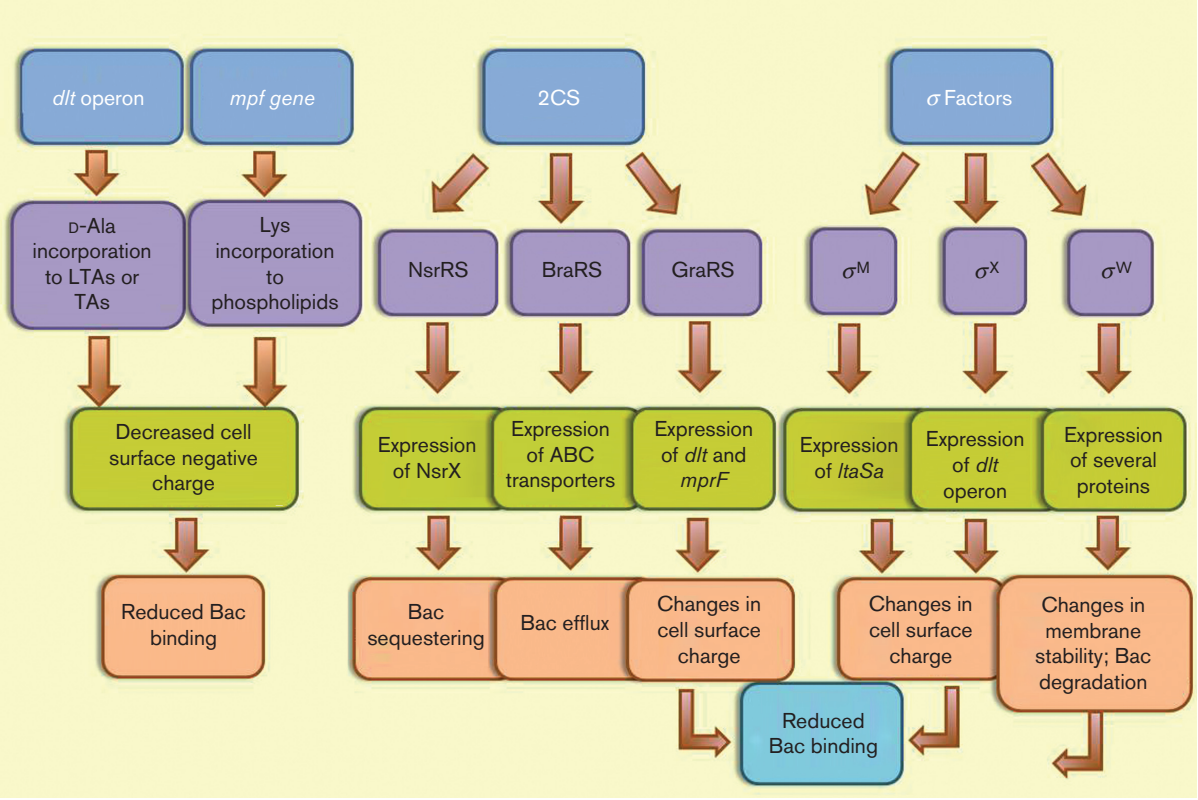

Fig. 2. Examples of mechanisms involved in innate bacteriocin resistance. ltaSa, stress-activated LTA synthase.

Resistance due to changes in the bacterial cell envelope

Many Gram-positive bacteria have teichoic acids (TAs) or lipoteichoic acids (LTAs) as major constituents of the cell wall (Fischer, 1988). These molecules extend through the peptidoglycan to the outer cell surface and their backbone is highly charged by deprotonized phosphate groups. However, backbone esterification with D-alanine reduces the cell wall net negative charge by the introduction of basic amino groups (Fischer, 1988). An example of a system contributing to innate bacteriocin resistance in Gram-positive bacteria is the *dlt* operon, which codes for proteins required for the incorporation of D-alanine to TAs and LTAs (Peschel *et al.*, 1999). Mutations in the *dltA* gene result in increased sensitivity to bacteriocins due to a defective D-alanine incorporation to TAs or LTAs. Consequently, the mutant cells carry an increased negative surface charge, resulting in increased interaction of bacteriocins with the bacterial surface and, therefore, in cell inhibition (Peschel *et al.*, 1999).

The involvement of the *dlt* operon in innate resistance to bacteriocins and other cationic antimicrobial peptides (CAMPs) is probably a common occurrence amongst Gram-positive bacteria, and it has been experimentally proven for *S. aureus* (Peschel *et al.*, 1999), *Clostridium difficile* (McBride & Sonenshein, 2011a) and *Bacillus cereus* (Abi Khattar *et al.*, 2009), amongst other species. Moreover, the expression of the *dltDABC* genes was shown to increase when *Clostridium difficile* WT cells were grown in the presence of bacteriocins (McBride & Sonenshein, 2011a).

A functional MprF protein is required for the biosynthesis of lysylphosphatidylglycerols (Peschel *et al.*, 2001), whose presence in the bacterial cytoplasmic membrane also reduces the net negative charge of the cell envelope, contributing to bacteriocin resistance. An alteration in the cell envelope charge due to defective incorporation of lysine to membrane phospholipids is also responsible for the increased sensitivity to bacteriocins and other CAMPs exhibited by *mprF* gene mutants (Peschel *et al.*, 2001; Samant *et al.*, 2009; Thedieck *et al.*, 2006).

The response regulator VirR, which is part of the two-component signal transduction system (2CS) VirRS, positively controls the expression of both *dltA* and *mprF* in *L. monocytogenes* (Mandin *et al.*, 2005). In *S. aureus*, the role of VirRS in the regulation of the *dlt* operon and the *mprF* gene is played by the 2CS GraRS (Falord *et al.*, 2011).Therefore, as expected, inactivation of VirR or GraR results in enhanced bacterial susceptibility to bacteriocins and other CAMPs (Falord *et al.*, 2011; Kawada-Matsuo *et al.*, 2013a; Thedieck *et al.*, 2006).

Alternative sigma factors, which control cell envelope structure and charge, may also play a role in innate

resistance, as reported by Kingston *et al.* (2013) for *Bacillus subtilis*. In this micro-organism, the sigma factors σ^M and σ^X contribute to resistance against lantibiotics, as mutants lacking σ^M and σ^X exhibit an increased susceptibility to nisin, mersacidin, gallidermin and subtilin. σ^M contributes to resistance through expression of *ltaSa*, which encodes a stress-activated LTA synthase, whereas σ^X activates the synthesis of phosphatidylethanolamine, which decreases the net negative charge of the cell envelope (Cao & Helmann, 2004) and the expression of the *dlt* operon (Cao & Helmann, 2004; Kingston *et al.*, 2013).

The *sigB* gene is an important mediator of the bacterial stress response and is also involved in the resistance of *L. monocytogenes* to bacteriocins. In the studies performed by Begley *et al.* (2006), a mutation of *sigB* of *L. monocytogenes* resulted in a reduced tolerance to nisin (300 μg ml^{-1}) and lacticin 3147 (100 AU ml^{-1}) in broth survival experiments, suggesting the role of SigB in innate resistance. A total of 55 genes, including those encoding putative efflux pumps, penicillin-binding proteins (PBPs), autolysins and proteins involved in the modification of the bacterial cell envelope, were shown to be positively regulated by SigB using microarray analysis. However, the exact role of SigB in *L. monocytogenes* bacteriocin resistance has not yet been determined, although Begley *et al.* (2006) postulated that SigB may control membrane charge or lipid composition, as alteration of these characteristics affects bacteriocin binding or insertion. SigB may also regulate transporters involved in bacteriocin efflux. However, contradictory results were reported by Palmer *et al.* (2009), who showed that a Δ*sigB* mutant of *L. monocytogenes* was more resistant to nisin in broth survival assays, suggesting a role for SigB in acquired resistance. Such discordance may, however, be explained by differences in the experiments performed, such as assay procedures, bacteriocin concentrations and growth phase of *L. monocytogenes* (Laursen *et al.*, 2014).

Kawada-Matsuo *et al.* (2013b), studying the 2CS NsrRS found in *Streptococcus mutans*, observed that mutants affected in the *nsrRS* genes exhibited an increased susceptibility to nisin. NsrRS regulates the expression of NsrX, which is predicted to be a membrane-bound protein. The results of a nisin-binding assay showed that a Δ*nsrX* mutant exhibiting increased sensitivity to nisin could only bind significantly to nisin when complemented with a functional *nsrX* gene. Based on these results, the authors proposed that NsrX, or an unidentified factor modified through NsrX, binds to nisin and inhibits its binding to lipid II, preventing nisin activity.

Several other 2CSs have been implicated in innate bacteriocin resistance. These are encoded by an operon which codes for a response regulator and a histidine kinase (HK). This operon lies upstream of a second operon which encodes a putative ABC transporter, generally composed of two separate domains: an ATP-binding domain and a permease domain (Blake *et al.*, 2011). The ABC transporter is regulated by the 2CS and is believed to participate in bacteriocin resistance by mediating its export (Blake *et al.*, 2011). Examples of 2CSs with such a role in innate resistance include *graRS*/*vraFG* (Kawada-Matsuo *et al.*, 2013a) and *braRS* (also called *nsaRS* or *bceRS*)/*vraDE* (Hiron *et al.*, 2011; Kawada-Matsuo *et al.*, 2013a), both found in *S. aureus*, and *lcrRS*/*lctFEG* (in this case, a three-component ABC transporter), in *Streptococcus mutans* (Kawada-Matsuo *et al.*, 2013b). The BraRS system was shown to be specific for nisin and nukacin ISK-1. Conversely, GraRS confers broad-spectrum resistance against CAMPs (Kawada-Matsuo *et al.*, 2013b).

Another example of a transporter involved in innate bacteriocin resistance is AnrAB, described in *L. monocytogenes* (Collins *et al.*, 2010a). This transporter was also shown to provide protection against bacitracin and β-lactam antibiotics (Collins *et al.*, 2010a).

Other genetic loci involved in innate resistance

Collins *et al.* (2010b) described a locus, *lmo1967*, required for innate nisin resistance in *L. monocytogenes*. This locus is a homologue of the tellurite resistance gene *telA*, and its role in innate nisin resistance was shown by analysing different types of mutant. All mutants became fourfold more susceptible to the lantibiotics nisin and gallidermin, and also to cefuroxime, cefotaxime and bacitracin. This was the first report associating a *telA* gene with innate bacteriocin resistance, although no mechanism has yet been proposed to explain the resistance phenotype.

Acquired resistance

There is substantial variation in the frequency of spontaneous mutations resulting in bacteriocin resistance, depending on the micro-organism, the bacteriocin and the strain tested, and the assay employed, including the bacteria/bacteriocin ratio and environmental conditions (Blake *et al.*, 2011; Gravesen *et al.*, 2002a; Mazzotta *et al.*, 1997; Ming & Daeschel, 1993). The mechanisms involved in acquired resistance are also quite diverse (Fig. 3).

Resistance to lantibiotics

The frequency of spontaneous mutations resulting in nisin resistance varies from $<10^{-9}$ to 10^{-2} in *L. monocytogenes* (de Martinis *et al.*, 1997; Gravesen *et al.*, 2002a; Ming & Daeschel, 1993) and from 10^{-8} to 10^{-2} in other Gram-positive organisms (Mazzotta *et al.*, 1997). Strains with moderate resistance (an eightfold increase in MIC) are isolated more frequently (Blake *et al.*, 2011; Gravesen *et al.*, 2002a). However, mutants exhibiting a higher-level resistance (a 32-fold increase in MIC) could be selected by plating mutants with moderate resistance onto media containing higher nisin concentrations (Blake *et al.*, 2011). The development of nisin resistance could be reduced by increasing the nisin concentration (Gravesen *et al.*, 2002a).

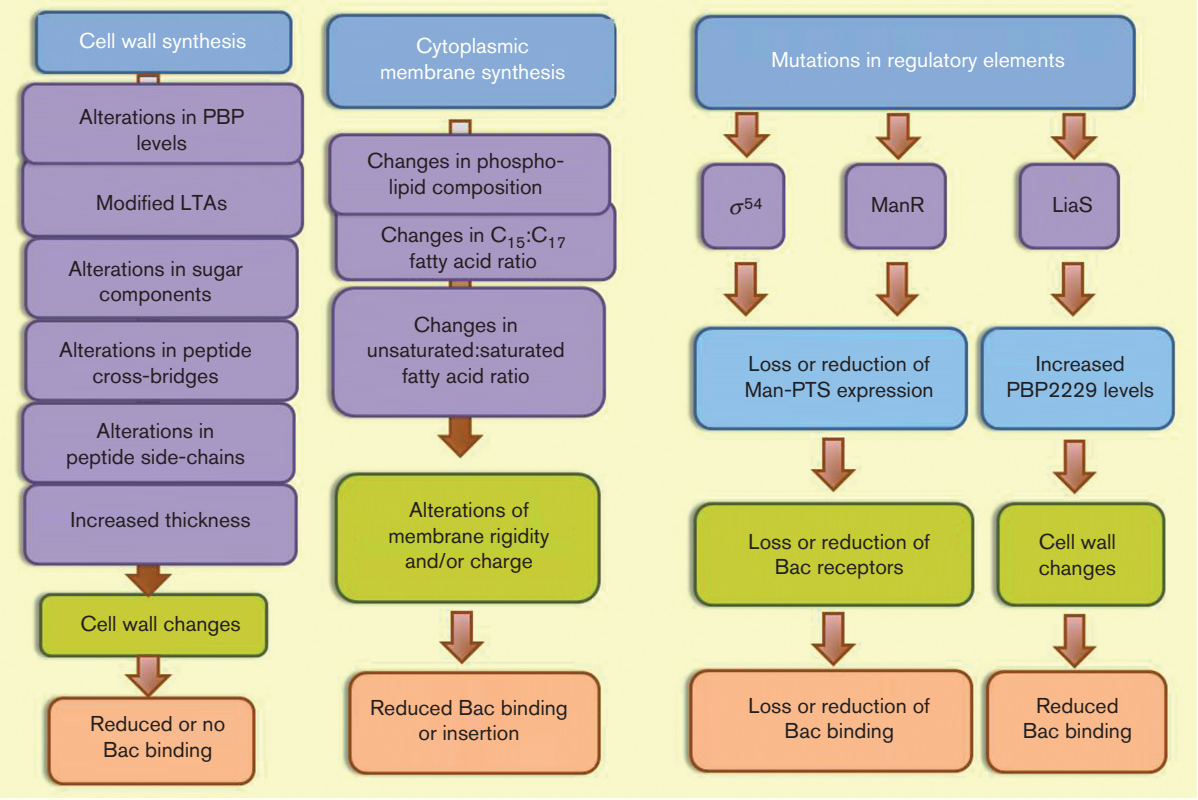

Fig. 3. Examples of mechanisms involved in acquired bacteriocin resistance.

The frequency of nisin (100 IU ml^{-1}) resistance development in *L. monocytogenes* strain ScottA was found to drop (at least 100-fold) when the strain was grown at 10 °C and in the presence of decreasing pH (pH 5.5 and 6.0) and decreasing NaCl concentration (2.0 and 0.5 %) (de Martinis *et al.*, 1997). Growth on NaCl (6.5 %) at 30 °C also reduced the frequency of nisin (500 IU ml^{-1}) resistance development ($<5\times10^{-8}$) (Gravesen *et al.*, 2002a). Nisin resistance seems to have little impact on bacterial fitness, and resistance seems to be stable in most mutants in the absence of selection (Blake *et al.*, 2011; Gravesen *et al.*, 2002a; Mantovani & Russell, 2001).

An interesting phenomenon described by Blake *et al.* (2011) was the isolation of a small percentage (5 %) of nisin-resistant mutants of *S. aureus* (with a fourfold increase in MIC) which formed small-colony variants on solid medium. These mutants displayed a 32-fold increase in gentamicin susceptibility and were menadione auxotrophs.

Mutants spontaneously resistant to lacticin 3147 do not arise at high levels of bacteriocin or at high frequencies (generally $<10^{-7}$) (Coakley *et al.*, 1997; Guinane *et al.*, 2006; Ryan *et al.*, 1998), and the few mutants isolated were only able to withstand low levels of bacteriocin (100 AU ml^{-1}) (Guinane *et al.*, 2006). However, mutants resistant to moderate levels of lacticin 3147 (up to 600 AU ml^{-1}) could be selected by repeated exposure to bacteriocin (Guinane *et al.*, 2006). The resistance phenotype was stable.

Guinane *et al.* (2006) also investigated the antibiotic sensitivity of lacticin 3147 spontaneously resistant mutants and reported that no change in susceptibility was observed for the majority of antibiotics tested. The only exceptions were enhanced neomycin resistance and increased cephalosporin sensitivity exhibited by some mutants. Based on these results, it can be concluded that lacticin 3147 resistance is not expected to be of concern if this bacteriocin is used commercially. Moreover, if resistance develops, conventional antimicrobials could be employed in the clinical setting for bacterial control (Guinane *et al.*, 2006).

The mechanisms responsible for acquired resistance to lantibiotics vary amongst strains and species. In many micro-organisms investigated, the resistance phenotype was associated with cell wall alterations, such as abnormal cell wall synthesis and autolysin inhibition (Maisnier-Patin & Richard, 1996), the presence of higher amounts of modified LTAs (Kramer *et al.*, 2008; Mantovani & Russell, 2001) and increased thickness at the septum (Kramer *et al.*, 2008) or content of rhamnose (Xuanyuan *et al.*, 2010).

Changes in cytoplasmic membrane composition may also render a bacterium resistant to lantibiotics. A nisin-resistant

variant of *L. monocytogenes* Scott A, isolated by exposure to increasing concentrations of nisin, was shown to produce less diphosphatidylglycerol and more phosphatidylglycerol than the parental strain (Verheul *et al.*, 1997). As nisin penetrates more deeply into lipid monolayers of diphosphatidylglycerol than those of other lipids, including phosphatidylglycerol, the resistance exhibited by the mutant was attributed to a reduction in the diphosphatidylglycerol content of the cytoplasmic membrane (Verheul *et al.*, 1997). Crandall & Montville (1998) also reported that nisin resistance in *L. monocytogenes* ATCC 700302 involved alterations in cellular membrane composition, such as a lower ratio of $C_{15}:C_{17}$ fatty acids and the presence of more phosphatidylethanolamine and less phosphatidylglycerol and cardiolipin. All these changes may result in a less fluid cytoplasmic membrane and increased rigidity, which may prevent nisin from inserting into its target. Moreover, the decrease in phosphatidylglycerol content and, therefore, in the net negative charge of the lipid bilayer may also hamper nisin's ability to bind to and interact with the membrane (Crandall & Montville, 1998).

Regulators have also been shown to contribute to acquired resistance. Cotter *et al.* (2002) reported that the LisRK 2CS is involved in listerial susceptibility to nisin. A Δ*lisK* mutant (lacking the HK sensor component) of strain LO28 displayed enhanced resistance to nisin, manifested by a significantly shorter lag phase in the presence of 300 μg nisin ml^{-1}. The expression of some genes, including *lmo1021* which encodes another HK (subsequently named LiaS), was significantly reduced in the mutant. The LisRK 2CS regulates the $LiaFSR_{Lm}$ 3CS, which in turn regulates the listerial response to environmental stresses (Fritsch *et al.*, 2011). The $LiaFSR_{Lm}$ 3CS is induced in the presence of antibiotics and CAMPs which target the cell wall or the membrane (Fritsch *et al.*, 2011; Mascher *et al.*, 2004). Expression of a functional $LiaFSR_{Lm}$ seems to result in extensive remodelling of the protein composition of the cytoplasmic membrane (Fritsch *et al.*, 2011) and in a decreased transcription of *lmo2229*/*pbp2229*, which codes for a protein similar to PBPs (named PBP2229) (Collins *et al.*, 2012), rendering the cell more susceptible to nisin.

A Δ*liaS* mutant exhibited an increased tolerance to nisin (a twofold increase in MIC), but an increased susceptibility to cephalosporins and disinfectants (Collins *et al.*, 2012). This link between nisin resistance and sensitivity to antimicrobials acting on the cell wall had been observed previously by others (Crandall & Montville, 1998; Gravesen *et al.*, 2001). The nisin resistance of the double-mutant Δ*liaS* Δ*lisK* was only marginally greater than that of the Δ*liaS* mutant. However, *lmo2229*/*pbp2229* transcription increased in the Δ*liaS* mutant and, even more intensely, in the Δ*liaS* Δ*lisK* double mutant. Mutation of *lmo2229* in the Δ*liaS* Δ*lisK* mutant resulted in a large increase in nisin susceptibility (a 32-fold reduction in MIC) (Collins *et al.*, 2012), suggesting that PBP2229 plays an important role in the nisin-resistant phenotype of this mutant. Collins *et al.* (2012) then proposed that, as PBPs catalyze the incorporation of the disaccharide–pentapeptide moiety of lipid II by the growing peptidoglycan chain (Gravesen *et al.*, 2004), PBP2229 may contribute strongly to nisin resistance by shielding lipid II.

In a nisin-resistant mutant of *Clostridium difficile*, exhibiting a fourfold increase in MIC, the resistance phenotype was attributed to an increased expression of a three-component ABC transporter encoded by the operon *cprABC* and probably involved in peptide export (McBride & Sonenshein, 2011b). This operon is regulated by a putative orphan HK (*cprK*). This mutant was also resistant to gallidermin (McBride & Sonenshein, 2011b).

Resistance to class II bacteriocins

To date, only cells resistant to subclass IIa bacteriocins, lactococcin A and lactococcin 972 (both belonging to subclass IIc) have been investigated. For class II bacteriocins, resistance has been found to be either spontaneous (Katla *et al.*, 2003; Rasch & Knøchel, 1998) or induced by exposure. Resistance frequencies from 10^{-9} to 10^{-3} have been reported for subclass IIa bacteriocins (Dykes & Hastings, 1998; Gravesen *et al.*, 2002a; Rekhif *et al.*, 1994; Tessema *et al.*, 2009; Vadyvaloo *et al.*, 2002) and from 10^{-7} to 10^{-4} for lactococcin A (Kjos *et al.*, 2011). Environmental stress in the form of low temperature (10 °C), reduced pH (5.5) or the presence of NaCl (6.5 %) did not influence the frequency of pediocin PA-1 resistance (Gravesen *et al.*, 2002a). The resistance developed proved to be stable in some mutants (Gravesen *et al.*, 2002a; Kjos *et al.*, 2011; Rekhif *et al.*, 1994; Tessema *et al.*, 2011) but unstable in others (Dykes & Hastings, 1998; Tessema *et al.*, 2011).

Different mechanisms are associated with resistance development to class II bacteriocins and the level of resistance depends on the mechanism involved.

In many cases, resistance to subclass IIa bacteriocins and lactococcin A involves their receptors (encoded by the *mpt*/*ptn* operon) and downregulation of their expression (Gravesen *et al.*, 2002b; Kjos *et al.*, 2011; Opsata *et al.*, 2010; Ramnath *et al.*, 2000, 2004). High-level resistance of Gram-positive bacteria to subclass IIa bacteriocins has been reported to result from either loss or reduction of expression of Man-PTS, either in naturally resistant isolates or in induced resistant mutants (Gravesen *et al.*, 2002b; Kjos *et al.*, 2011; Opsata *et al.*, 2010; Tessema *et al.*, 2011). In many instances, the mutants no longer synthesized the MptA subunit from Man-PTS (Gravesen *et al.*, 2002b) or its synthesis was found to be reduced (Tessema *et al.*, 2009). Reduced transcription of the *mptC* or *mptD* gene was observed in mutants of *Enterococcus faecalis* (Opsata *et al.*, 2010) or *L. monocytogenes* (Kjos *et al.*, 2011). Site-directed mutagenesis in the extracellular loop of MptC, the main determinant for specific targeting by subclass IIa bacteriocins (Kjos *et al.*, 2010b), was performed and two mutations caused a significant reduction in receptor activity for eight subclass IIa bacteriocins, resulting in

bacteriocin resistance. The point mutation Q88F caused MIC values to increase six- to 500-fold, depending on the bacteriocin tested and the G89H mutation totally disrupted the receptor function for all tested bacteriocins (Kjos *et al.*, 2010b). In-frame deletions in the *mptD* gene also led to resistance of *L. monocytogenes* to mesentericin Y105 (Dalet *et al.*, 2001). Moreover, the introduction of the *L. monocytogenes mptC* gene or the *La. lactis ptnABCD* rendered a resistant strain of *La. lactis* sensitive to subclass IIa bacteriocins (Ramnath *et al.*, 2004) or to lactococcin A (Kjos *et al.*, 2011), respectively.

In other studies performed with *L. monocytogenes* (Robichon *et al.*, 1997) and *Enterococcus faecalis* (Dalet *et al.*, 2000), resistance to subclass IIa bacteriocins was linked to the regulatory gene *rpoN*. *rpoN* encodes the alternative sigma factor σ^{54} (SigL). SigL is involved in activation of the *mptA/BCD* operon, which codes for EIIAB, EIIC and EIID in *L. monocytogenes* and *Enterococcus faecalis* (Héchard *et al.*, 2001), in conjunction with ManR (the transcriptional activator for σ^{54}) (Dalet *et al.*, 2001). Mutations in *rpoN* resulted in loss of *mpt* expression, which in turn led to mesentericin Y105 resistance in both micro-organisms (Dalet *et al.*, 2000; Héchard *et al.*, 2001). Mutations in ManR of *L. monocytogenes* (Dalet *et al.*, 2001; Gravesen *et al.*, 2002b) or in its homologue of *Enterococcus faecalis* (Opsata *et al.*, 2010) also rendered the cells resistant to subclass IIa bacteriocins. As both regulatory proteins RpoN and ManR are required for active transcription of the genes encoding the subclass IIa bacteriocin receptor, the bacteria become resistant if the receptor is not synthesized (Diep *et al.*, 2007). The molecular switch that turns off or downregulates Man-PTS expression in resistant cells is reportedly a stable phenomenon (Gravesen *et al.*, 2002b; Kjos *et al.*, 2011; Rekhif *et al.*, 1994).

Opsata *et al.* (2010) performed transcriptome analyses in order to compare *Enterococcus faecalis* cells sensitive and resistant (with mutation in the *mpt* operon) to pediocin PA-1. About 200 genes exhibited a significantly altered transcription in the mutant cells. Most of these genes encoded functions involved in energy metabolism and transport. Some genes were downregulated (e.g. genes involved in glycolysis), whereas others were upregulated (e.g. genes involved in transportation and degradation of secondary sugar sources). The metabolism was shifted from lactic acid fermentation to mixed fermentation in the mutant. Similar findings have also been reported in resistant mutants of *L. monocytogenes* (Naghmouchi *et al.*, 2007; Vadyvaloo *et al.*, 2004). As discussed by Vadyvaloo *et al.* (2004), the shift in metabolism and the subsequent change in the end products will profoundly influence both the organoleptic properties and spoilage potential of the food product in which pediocin is used as a biopreservative. Moreover, these changes generally exhibit fitness costs, such as reduced growth rate in broth (Gravesen *et al.*, 2002a; Vadyvaloo *et al.*, 2004), but this fitness cost was not observed when the pediocin PA-1-resistant mutants were grown in a meat model at 5 °C (Gravesen *et al.*, 2002a).

A strain with a high resistance to subclass IIa bacteriocins also displayed a reduced ability to grow on 0.4 % glucose (Man-PTS dependent), but grew normally on cellobiose (Man-PTS independent) relative to the growth of parental cells (Kjos *et al.*, 2011). Similar results were reported for lactococcin A-resistant mutants, although in this case the alternative sugar used was galactose (Man-PTS independent) instead of cellobiose (Kjos *et al.*, 2011).

Although the downregulation of Man-PTS expression is the main resistance mechanism associated with high-level resistance to various class II bacteriocins, there are other mechanisms which normally occur in mutants with low resistance. In the latter, normal or high Man-PTS gene expression is generally observed (Kjos *et al.*, 2011). Although the exact nature of these mechanisms is still unknown, there is evidence that suggests the involvement of changes in the cell envelope, such as: (i) phospholipids with increased ratios of unsaturated to saturated fatty acids and short- to long-acyl chain species (Vadyvaloo *et al.*, 2002), which result in an increased membrane fluidity; (ii) increased ratios of saturated fatty acids, leading to a decrease in membrane fluidity (Limonet *et al.*, 2002); (iii) a decrease in the cell wall negative charge by an increase in D-alanine content in TAs (Vadyvaloo *et al.*, 2004); (iv) a higher content of peptidoglycan with tripeptide side-chains and a reduced content of pentapeptide side-chains, leading to a profound change in the peptidoglycan structure (Roces *et al.*, 2012a); and (v) efflux of the bacteriocin by the twin-arginine translocase system (Tessema *et al.*, 2011).

Recently, Laursen *et al.* (2014) reported that *L. monocytogenes* stress caused by exposure to a single sublethal pediocin concentration resulted in increased expression of LisRK, and SigB- and SigL-dependent genes, implicating these regulators in acquired subclass IIa bacteriocin resistance. These authors proposed that, in the presence of the bacteriocin: (i) the LisRK system senses the cell envelope damage caused by pediocin and initiates a response which results in cell envelope alterations in an attempt to maintain its integrity; (ii) SigB-dependent genes participate in a more general defence mechanism, whose role in bacteriocin sensitivity has not yet been elucidated, and (iii) SigL-dependent genes (involved in protein synthesis, nutrient transport and motility) participate in a phase of cell reconstruction. Consequently, decreased bacteriocin susceptibility is observed.

High induction of *llmg2447* was observed in a lactococcin 972-resistant mutant of *La. lactis*. *llmg2447* is predicted to encode a putative anti-sigma factor (Roces *et al.*, 2012b). An elevated expression of this gene led to high lactococcin 972 resistance (a 400-fold decrease in susceptibility) in *La. lactis*, with no cross-resistance to nisin, bacitracin, penicillin G and vancomycin (Roces *et al.*, 2012b). However, the way in which Llmg2447 protects *La. lactis* from lactococcin 972 remains to be elucidated (Roces *et al.*, 2012b).

Resistance to class III bacteriocins

Lysostaphin is the only class III bacteriocin investigated in relation to resistance development. Resistance to lysostaphin (a five- to 15-fold increase in MIC) occurred both *in vitro* (at frequencies of $<10^{-9}$ to 10^{-1}) and in a rabbit model of experimental endocarditis (at a frequency of 10^{-6}) (Climo *et al.*, 2001; Kusuma & Kokai-Kun, 2005) following exposure to low doses of lysostaphin (0.25–0.50 MIC). This resistance has generally been associated with mutations in *femAB* and results in an alteration of the peptidoglycan cross-bridges, in which one or more serine residues replace glycine (Gargis *et al.*, 2010; Strandén *et al.*, 1997). Moreover, this resistance mechanism is generally accompanied by increased β-lactam susceptibility (Climo *et al.*, 2001; Guignard *et al.*, 2005; Kiri *et al.*, 2002; Rohrer & Berger-Bächi, 2003) and by loss of fitness in the mutants (Kusuma *et al.*, 2007). Therefore, Kusuma *et al.* (2007) recommended the inclusion of β-lactam antibiotics in all therapeutic trials with lysostaphin to both suppress resistance and promote synergy.

Resistance to cyclic bacteriocins

To date, at least 12 cyclic bacteriocins have been described (Potter *et al.*, 2014; van Belkum *et al.*, 2011). Enterocin AS-48, a bacteriocin produced by *Enterococcus faecalis* and the first reported to be cyclic, is the prototype of this group and is the best characterized class IV bacteriocin (van Belkum *et al.*, 2011).

With regard to resistance mechanisms against cyclic bacteriocins, it is not surprising that not many studies have been undertaken, and that the only models investigated are enterocin AS-48, which has potential application as a food preservative (Gálvez *et al.*, 2007), and garvicin ML.

A mutant of *L. monocytogenes* with higher tolerance to enterocin AS-48 (MIC 0.5 μg ml^{-1}) was studied by Mendoza *et al.* (1999). The resistance phenotype was, however, unstable as it was lost after 11 steps of subcultivation of the mutant without enterocin AS-48. Cell envelope changes were implicated in the resistance, such as a higher proportion of branched fatty acids as well as a higher C_{15}:C_{17} ratio. The cell wall thickness was also increased, although it seemed to be less dense.

Later, Grande Burgos *et al.* (2009) investigated the effect of enterocin AS-48 against vegetative cells of *Bacillus cereus* by transcriptome analysis. Expression of 24 genes changed significantly after exposure to a subinhibitory bacteriocin concentration (0.5 μg ml^{-1}). Most genes (n=20) were upregulated and encoded membrane-associated or secreted proteins. The genes which are part of an operon that encodes a PadR-type transcriptional regulator and a hypothetical membrane protein were found to be the most upregulated. The upregulation was only observed in the presence of enterocin AS-48, but not of nisin. *Bacillus subtilis* 168 lacks that operon, and is sensitive to enterocin AS-48 and nisin. However, when the gene encoding the membrane protein was introduced and expressed in this strain, the transformant exhibited increased resistance to enterocin AS-48, but not to nisin (Grande Burgos *et al.*, 2009). Therefore, this membrane protein is involved in the resistance mechanism of *Bacillus cereus* cells against enterocin AS-48 by a hitherto unknown mechanism.

In relation to garvicin ML, a bacteriocin produced by *Lactococcus garvieae* DCC43, Gabrielsen *et al.* (2012) characterized various spontaneous mutants of *La. lactis* IL1403 with lower susceptibility to garvicin ML (with a six- to 11-fold decreased sensitivity). These mutants lost the ability to metabolize starch and maltose, and carried a 13.5 kb chromosomal deletion. The *malEFG* operon, which encodes a maltose ABC transporter, was included in the deleted region. When the mutants were complemented with these three genes, they recovered the susceptibility to garvicin ML. Higher expression levels of the operon resulted in higher bacteriocin sensitivity, suggesting an important role of the maltose ABC transporter in the antagonistic activity of garvicin ML, potentially as a target receptor for the bacteriocin (Gabrielsen *et al.*, 2012).

Cross-resistance amongst bacteriocins

As the combined use of bacteriocins, especially of those with different mechanisms of action, may reduce (Gravesen *et al.*, 2002a) or prevent (Coelho *et al.*, 2007; Vignolo *et al.*, 2000) the development of bacteriocin resistance, it is critical to determine whether cross-resistance will occur following the spontaneous development of resistance to one of the two types of bacteriocins (Gravesen *et al.*, 2004). In this respect, contradictory reports are found in the literature. No cross-resistance between nisin and pediocin PA-1 was found by Rasch & Knøchel (1998) in *L. monocytogenes* strains which were shown to exhibit naturally enhanced tolerance to either nisin or pediocin PA-1. Similar results have been reported by others. Rekhif *et al.* (1994) reported that mutants of *L. monocytogenes* resistant to one of three class II bacteriocins tested [mesenterocin 52B (IIc), curvaticin 13 (IIa) and plantaricin C19 (IIa)] exhibited some degree of cross-resistance to the other two, but not to nisin. Cross-resistance between lacticin 3147, nisin and lacticin 481(all lantibiotics) was reported by van Schaik *et al.* (1999) and by Guinane *et al.* (2006), but cross-resistance to the class II bacteriocin lactococcin ABM was not observed (Guinane *et al.*, 2006). In one case, resistance to a subclass IIa bacteriocin was reported to confer increased susceptibility to nisin (Bouttefroy & Millière, 2000). Conversely, cross-resistance between bacteriocins belonging to different classes has also been observed. Crandall & Montville (1998) reported that nisin resistance in *L. monocytogenes* conferred cross-resistance to pediocin PA-1. In another study, a strain of *L. monocytogenes* displayed resistance to nisin, pediocin PA-1 and leuconocin S – bacteriocins belonging to at least two distinct classes (Bruno & Montville, 1993). Gravesen *et al.*

(2004) found a degree of cross-resistance between nisin and subclass IIa bacteriocins (pediocin PA-1 and leucocin A). They also observed complete cross-resistance amongst subclass IIa bacteriocins (pediocin PA-1, leucocin A and carnobacteriocin B2). Indeed, cross-resistance between different subclass IIa bacteriocins has been reported frequently (Dykes & Hastings, 1998; Gravesen *et al.*, 2002a, b; Ramnath *et al.*, 2004; Rasch & Knøchel, 1998).

Strategies to overcome bacteriocin resistance

Resistance to a given bacteriocin arises by different mechanisms, even amongst strains of the same bacterial species. Moreover, similar resistance mechanisms are shared by bacteriocins belonging to different classes. From the examples described above, it can be concluded that the bacterial cell envelope plays an important role in both innate and acquired resistance. However, the development of bacteriocin resistance does not hamper the intended use of a given bacteriocin as there are strategies that can be used to prevent or overcome this resistance.

In food technology, the risk of developing bacteriocin resistance may be reduced by the judicious use of a multiple-hurdle preservation strategy, combining several methods to preserve food (Bastos & Ceotto, 2011; Cotter *et al.*, 2005). Kaur *et al.* (2013) showed that *L. monocytogenes* mutants resistant to nisin, pediocin 34 and enterocin FH99 did not become resistant to low pH, sodium chloride, potassium sorbate or sodium nitrite. Resistant variants were equally or more sensitive than the WT strain. Therefore, according to these authors, bacteriocin resistance should not affect the use of hurdle preservation technologies employing bacteriocins to improve food safety. However, other studies have shown that salt stress at low temperature could provide cross-protection to *L. monocytogenes* against nisin (Bergholz *et al.*, 2013). Thus, it is advisable to take into consideration the potential for cross-protective effects between different hurdle technologies used for food preservation.

Another approach to avoid the emergence of bacteriocin-resistant bacteria may be the combined use of bacteriocins with different mechanisms of action, which may additionally allow the use of lower bacteriocin doses (Bastos & Ceotto, 2011). Such an approach can be illustrated by the combination of nisin and curvaticin 13 (subclass IIa) to control *L. monocytogenes* (Bouttefroy & Millière, 2000). The bactericidal activity against this pathogen resulting from this combination was higher than that of either bacteriocin alone. Similar results were observed by Kaur *et al.* (2013), who combined nisin, pediocin 34 and enterocin FH99 (both suclass IIa bacteriocins) to control *L. monocytogenes*. Moreover, the combination of nisin (500 IU ml^{-1}) and pediocin PA-1 (1720 AU ml^{-1}) reduced the frequency of resistance development (a 10^2–10^4 reduction, depending on the strain tested) in *L. monocytogenes* (Gravesen *et al.*, 2002a).

As resistance to a given bacteriocin may extend to other bacteriocins, especially of the same subclass, it is advisable that combinations should be tested with bacteriocins belonging to different classes or, at least, subclasses. Even so, preliminary tests aiming to investigate the development of cross-resistance should be performed before combining distinct bacteriocins, so that the emergence of resistance can be minimized. Macwana & Muriana (2012) published a study in which they proposed a method, based on mode of action, to classify bacteriocins into functional groups that could be used for identifying the most adequate combination of bacteriocins for use in biocontrol.

Complementary use of conventional drugs and bacteriocins may be another potential approach to prevent the emergence of resistant micro-organisms (Coelho *et al.*, 2007; Cotter *et al.*, 2013). Most traditional antimicrobials generally act as enzyme inhibitors. Most bacteriocins, however, target the membranes of sensitive cells leading to cell death. Therefore, development of cross-resistance to antimicrobials with different modes of action is expected to occur less frequently (Coelho *et al.*, 2007).

Conclusions

The studies reported in the literature suggest that bacteriocin resistance may develop during bacteriocin exploitation for biotechnological applications. Most bacteriocin resistance does not result in great fitness costs. If resistance makes the cells sick, the bacteriocin-sensitive cells are expected to exhibit a higher growth rate and predominate in the culture or in the foods, and the mutants will be outnumbered and presumably eliminated. Therefore, such sick mutants would not have any impact on microbial control by bacteriocins. As pointed out by Gravesen *et al.* (2002a), the frequency of development of resistance to bacteriocins cannot be predicted, as it will depend on the specific strain and growth conditions. The main proposed application for most bacteriocins is food preservation. Although experiments performed *in vitro* are relevant to measure important parameters, such as an assessment of the ease with which resistance arises, the impact that resistance exerts upon bacterial fitness and the stability of the resistance in the absence of selection (Blake *et al.*, 2011), more studies on bacteriocin resistance development in food model systems are required in order to measure its frequency and assess its impact on microbial control. Food systems are heterogeneous in composition, and their intrinsic factors may affect bacteriocin activity and the development of bacteriocin resistance (Bastos & Ceotto, 2011). According to Gravesen *et al.* (2002a), the presence of gradients of food components may create local areas which may favour the development of, presumably, low-level bacteriocin resistance. As in many cases these resistant mutants can be stable, it is conceivable that the occurrence of further mutations in these mutants may result in the emergence of high-level bacteriocin resistance (Gravesen *et al.*, 2002a). However, such assumptions deserve investigation.

Finally, considering the importance of bacteriocins in preventing and controlling bacterial infections, investigation on the functions involved in bacteriocin resistance should also continue, as this knowledge could facilitate the development of strategies to increase bacterial susceptibility to bacteriocins (Begley *et al.*, 2006; Collins *et al.*, 2012). However, we would like to point out, as already proposed by Katla *et al.* (2003), the urgent need for guidelines for the classification of different micro-organisms according to their susceptibilities to various bacteriocins, which would not only facilitate the comparison of different studies, but also help to identify new lines of investigation on this topic.

Acknowledgements

O. C. S. S. is recipient of a post-doctoral fellowship from CAPES/FAPERJ. The research developed by the group is supported by grants from CNPq, FAPERJ and CAPES to M. C. F. B.

References

Abi Khattar, Z., Rejasse, A., Destoumieux-Garzón, D., Escoubas, J. M., Sanchis, V., Lereclus, D., Givaudan, A., Kallassy, M., Nielsen-Leroux, C. & Gaudriault, S. (2009). The *dlt* operon of *Bacillus cereus* is required for resistance to cationic antimicrobial peptides and for virulence in insects. *J Bacteriol* **191**, 7063–7073.

Bastos, M. C. F. & Ceotto, H. (2011). Bacterial antimicrobial peptides and food preservation. In *Natural Antimicrobials in Food Safety and Quality*, pp. 62–76. Edited by M. Ray & M. Chikindas. Wallingford: CAB International.

Bastos, M. C. F., Ceotto, H., Coelho, M. L. V. & Nascimento, J. S. (2009). Staphylococcal antimicrobial peptides: relevant properties and potential biotechnological applications. *Curr Pharm Biotechnol* **10**, 38–61.

Bastos, M. C. F., Coutinho, B. G. & Coelho, M. L. V. (2010). Lysostaphin: a staphylococcal bacteriolysin with potential clinical applications. *Pharmaceuticals (Basel)* **3**, 1139–1161.

Begley, M., Hill, C. & Ross, R. P. (2006). Tolerance of *Listeria monocytogenes* to cell envelope-acting antimicrobial agents is dependent on SigB. *Appl Environ Microbiol* **72**, 2231–2234.

Begley, M., Cotter, P. D., Hill, C. & Ross, R. P. (2010). Glutamate decarboxylase-mediated nisin resistance in *Listeria monocytogenes*. *Appl Environ Microbiol* **76**, 6541–6546.

Bergholz, T. M., Tang, S., Wiedmann, M. & Boor, K. J. (2013). Nisin resistance of *Listeria monocytogenes* is increased by exposure to salt stress and is mediated via LiaR. *Appl Environ Microbiol* **79**, 5682–5688.

Bierbaum, G. & Sahl, H.-G. (2009). Lantibiotics: mode of action, biosynthesis and bioengineering. *Curr Pharm Biotechnol* **10**, 2–18.

Blake, K. L., Randall, C. P. & O'Neill, A. J. (2011). *In vitro* studies indicate a high resistance potential for the lantibiotic nisin in *Staphylococcus aureus* and define a genetic basis for nisin resistance. *Antimicrob Agents Chemother* **55**, 2362–2368.

Bouttefroy, A. & Millière, J.-B. (2000). Nisin-curvaticin 13 combinations for avoiding the regrowth of bacteriocin resistant cells of *Listeria monocytogenes* ATCC 15313. *Int J Food Microbiol* **62**, 65–75.

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

Breukink, E., van Heusden, H. E., Vollmerhaus, P. J., Swiezewska, E., Brunner, L., Walker, S., Heck, A. J. & de Kruijff, B. (2003). Lipid II is an intrinsic component of the pore induced by nisin in bacterial membranes. *J Biol Chem* **278**, 19898–19903.

Brötz, H., Bierbaum, G., Markus, A., Molitor, E. & Sahl, H.-G. (1995). Mode of action of the lantibiotic mersacidin: inhibition of peptidoglycan biosynthesis via a novel mechanism? *Antimicrob Agents Chemother* **39**, 714–719.

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

Browder, H. P., Zygmunt, W. A., Young, J. R. & Tavormina, P. A. (1965). Lysostaphin: enzymatic mode of action. *Biochem Biophys Res Commun* **19**, 383–389.

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

Butcher, B. G. & Helmann, J. D. (2006). Identification of *Bacillus subtilis* σ^{W}-dependent genes that provide intrinsic resistance to antimicrobial compounds produced by *Bacilli*. *Mol Microbiol* **60**, 765–782.

Cao, M. & Helmann, J. D. (2004). The *Bacillus subtilis* extracytoplasmic-function σ^{X} factor regulates modification of the cell envelope and resistance to cationic antimicrobial peptides. *J Bacteriol* **186**, 1136–1146.

Ceotto, H., Holo, H., da Costa, K. F., Nascimento, J. S., Salehian, Z., Nes, I. F. & Bastos, M. C. F. (2010). Nukacin 3299, a lantibiotic produced by *Staphylococcus simulans* 3299 identical to nukacin ISK-1. *Vet Microbiol* **146**, 124–131.

Climo, M. W., Ehlert, K. & Archer, G. L. (2001). Mechanism and suppression of lysostaphin resistance in oxacillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **45**, 1431–1437.

Coakley, M., Fitzgerald, G. F. & Ros, R. P. (1997). Application and evaluation of the phage resistance- and bacteriocin-encoding plasmid pMRC01 for the improvement of dairy starter cultures. *Appl Environ Microbiol* **63**, 1434–1440.

Coelho, M. L. V., Nascimento, J. S., Fagundes, P. C., Madureira, D. J., Oliveira, S. S., Brito, M. A. V. P. & Bastos, M. C. F. (2007). Activity of staphylococcal bacteriocins against *Staphylococcus aureus* and *Streptococcus agalactiae* involved in bovine mastitis. *Res Microbiol* **158**, 625–630.

Coelho, M. L. V., Coutinho, B. G., Santos, O. C. S., Nes, I. F. & Bastos, M. C. F. (2014). Immunity to the *Staphylococcus aureus* leaderless four-peptide bacteriocin aureocin A70 is conferred by AurI, an integral membrane protein. *Res Microbiol* **165**, 50–59.

Collins, B., Curtis, N., Cotter, P. D., Hill, C. & Ross, R. P. (2010a). The ABC transporter AnrAB contributes to the innate resistance of *Listeria monocytogenes* to nisin, bacitracin, and various beta-lactam antibiotics. *Antimicrob Agents Chemother* **54**, 4416–4423.

Collins, B., Joyce, S., Hill, C., Cotter, P. D. & Ross, R. P. (2010b). TelA contributes to the innate resistance of *Listeria monocytogenes* to nisin and other cell wall-acting antibiotics. *Antimicrob Agents Chemother* **54**, 4658–4663.

Collins, B., Guinane, C. M., Cotter, P. D., Hill, C. & Ross, R. P. (2012). Assessing the contributions of the LiaS histidine kinase to the innate resistance of *Listeria monocytogenes* to nisin, cephalosporins, and disinfectants. *Appl Environ Microbiol* **78**, 2923–2929.

Cotter, P. D., Guinane, C. M. & Hill, C. (2002). The LisRK signal transduction system determines the sensitivity of *Listeria monocytogenes* to nisin and cephalosporins. *Antimicrob Agents Chemother* **46**, 2784–2790.

Cotter, P. D., Hill, C. & Ross, R. P. (2005). Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* **3**, 777–788.

Cotter, P. D., Ross, R. P. & Hill, C. (2013). Bacteriocins – a viable alternative to antibiotics? *Nat Rev Microbiol* **11**, 95–105.

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

Dalet, K., Briand, C., Cenatiempo, Y. & Héchard, Y. (2000). The *rpoN* gene of *Enterococcus faecalis* directs sensitivity to subclass IIa bacteriocins. *Curr Microbiol* **41**, 441–443.

Dalet, K., Cenatiempo, Y., Cossart, P., Héchard, Y. & European Listeria Genome Consortium (2001). A σ^{54}-dependent PTS permease of the mannose family is responsible for sensitivity of *Listeria monocytogenes* to mesentericin Y105. *Microbiology* **147**, 3263–3269.

de Martinis, E. C. P., Crandall, A. D., Mazzotta, A. S. & Montville, T. J. (1997). Influence of pH, salt, and temperature on nisin resistance in *Listeria monocytogenes*. *J Food Prot* **60**, 420–423.

Diep, D. B., Skaugen, M., Salehian, Z., Holo, H. & Nes, I. F. (2007). Common mechanisms of target cell recognition and immunity for class II bacteriocins. *Proc Natl Acad Sci U S A* **104**, 2384–2389.

Draper, L. A., Grainger, K., Deegan, L. H., Cotter, P. D., Hill, C. & Ross, R. P. (2009). Cross-immunity and immune mimicry as mechanisms of resistance to the lantibiotic lacticin 3147. *Mol Microbiol* **71**, 1043–1054.

Draper, L. A., Tagg, J. R., Hill, C., Cotter, P. D. & Ross, R. P. (2012). The *spiFEG* locus in *Streptococcus infantarius* subsp. *infantarius* BAA-102 confers protection against nisin U. *Antimicrob Agents Chemother* **56**, 573–578.

Dubois, J.-Y. F., Kouwen, T. R. H. M., Schurich, A. K., Reis, C. R., Ensing, H. T., Trip, E. N., Zweers, J. C. & van Dijl, J. M. (2009). Immunity to the bacteriocin sublancin 168 is determined by the SunI (YolF) protein of *Bacillus subtilis*. *Antimicrob Agents Chemother* **53**, 651–661.

Dykes, G. A. & Hastings, J. W. (1998). Fitness costs associated with class IIa bacteriocin resistance in *Listeria monocytogenes* B73. *Lett Appl Microbiol* **26**, 5–8.

Ehlert, K., Tschierske, M., Mori, C., Schröder, W. & Berger-Bächi, B. (2000). Site-specific serine incorporation by Lif and Epr into positions 3 and 5 of the Staphylococcal peptidoglycan interpeptide bridge. *J Bacteriol* **182**, 2635–2638.

Fagundes, P. C. (2014). *Characterization of hyicin 3682 and its potential application in the control of a phytopathogen*. PhD thesis, Federal University of Rio de Janeiro, Brazil.

Falord, M., Mäder, U., Hiron, A., Débarbouillé, M. & Msadek, T. (2011). Investigation of the *Staphylococcus aureus* GraSR regulon reveals novel links to virulence, stress response and cell wall signal transduction pathways. *PLoS ONE* **6**, e21323.

Fimland, G., Eijsink, V. G. & Nissen-Meyer, J. (2002). Comparative studies of immunity proteins of pediocin-like bacteriocins. *Microbiology* **148**, 3661–3670.

Fischer, W. (1988). Physiology of lipoteichoic acids in bacteria. *Adv Microb Physiol* **29**, 233–302.

Freitag, N. E., Port, G. C. & Miner, M. D. (2009). *Listeria monocytogenes* – from saprophyte to intracellular pathogen. *Nat Rev Microbiol* **7**, 623–628.

Fritsch, F., Mauder, N., Williams, T., Weiser, J., Oberle, M. & Beier, D. (2011). The cell envelope stress response mediated by the LiaFSR$_{Lm}$ three-component system of *Listeria monocytogenes* is controlled via the phosphatase activity of the bifunctional histidine kinase LiaS$_{Lm}$. *Microbiology* **157**, 373–386.

Gabrielsen, C., Brede, D. A., Hernández, P. E., Nes, I. F. & Diep, D. B. (2012). The maltose ABC transporter in *Lactococcus lactis* facilitates high-level sensitivity to the circular bacteriocin garvicin ML. *Antimicrob Agents Chemother* **56**, 2908–2915.

Gajic, O., Buist, G., Kojic, M., Topisirovic, L., Kuipers, O. P. & Kok, J. (2003). Novel mechanism of bacteriocin secretion and immunity carried out by lactococcal multidrug resistance proteins. *J Biol Chem* **278**, 34291–34298.

Gálvez, A., Abriouel, H., López, R. L. & Ben Omar, N. (2007). Bacteriocin-based strategies for food biopreservation. *Int J Food Microbiol* **120**, 51–70.

Gargis, S. R., Heath, H. E., LeBlanc, P. A., Dekker, L., Simmonds, R. S. & Sloan, G. L. (2010). Inhibition of the activity of both domains of lysostaphin through peptidoglycan modification by the lysostaphin immunity protein. *Appl Environ Microbiol* **76**, 6944–6946.

Grande Burgos, M. J., Kovács, A. T., Mirończuk, A. M., Abriouel, H., Gálvez, A. & Kuipers, O. P. (2009). Response of *Bacillus cereus* ATCC 14579 to challenges with sublethal concentrations of enterocin AS-48. *BMC Microbiol* **9**, 227–234.

Gravesen, A., Sørensen, K., Aarestrup, F. M. & Knøchel, S. (2001). Spontaneous nisin-resistant *Listeria monocytogenes* mutants with increased expression of a putative penicillin-binding protein and their sensitivity to various antibiotics. *Microb Drug Resist* **7**, 127–135.

Gravesen, A., Jydegaard Axelsen, A. M., Mendes da Silva, J., Hansen, T. B. & Knøchel, S. (2002a). Frequency of bacteriocin resistance development and associated fitness costs in *Listeria monocytogenes*. *Appl Environ Microbiol* **68**, 756–764.

Gravesen, A., Ramnath, M., Rechinger, K. B., Andersen, N., Jänsch, L., Héchard, Y., Hastings, J. W. & Knøchel, S. (2002b). High-level resistance to class IIa bacteriocins is associated with one general mechanism in *Listeria monocytogenes*. *Microbiology* **148**, 2361–2369.

Gravesen, A., Kallipolitis, B., Holmstrøm, K., Høiby, P. E., Ramnath, M. & Knøchel, S. (2004). *pbp2229*-mediated nisin resistance mechanism in *Listeria monocytogenes* confers cross-protection to class IIa bacteriocins and affects virulence gene expression. *Appl Environ Microbiol* **70**, 1669–1679.

Guignard, B., Entenza, J. M. & Moreillon, P. (2005). Beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Curr Opin Pharmacol* **5**, 479–489.

Guinane, C. M., Cotter, P. D., Hill, C. & Ross, R. P. (2006). Spontaneous resistance in *Lactococcus lactis* IL1403 to the lantibiotic lacticin 3147. *FEMS Microbiol Lett* **260**, 77–83.

Héchard, Y., Pelletier, C., Cenatiempo, Y. & Frère, J. (2001). Analysis of σ^{54}-dependent genes in *Enterococcus faecalis*: a mannose PTS permease (EII^{Man}) is involved in sensitivity to a bacteriocin, mesentericin Y105. *Microbiology* **147**, 1575–1580.

Heidrich, C., Pag, U., Josten, M., Metzger, J., Jack, R. W., Bierbaum, G., Jung, G. & Sahl, H.-G. (1998). Isolation, characterization, and heterologous expression of the novel lantibiotic epicidin 280 and analysis of its biosynthetic gene cluster. *Appl Environ Microbiol* **64**, 3140–3146.

Heng, N. C. K., Wescombe, P. A., Burton, J. P., Jack, R. W. & Tagg, J. R. (2007). The diversity of bacteriocins in Gram positive bacteria. In *Bacteriocins: Ecology and Evolution*, pp. 45–92. Edited by M. A. Riley & M. A. Chavan. New York: Springer.

Hillman, J. D., Mo, J., McDonell, E., Cvitkovitch, D. & Hillman, C. H. (2007). Modification of an effector strain for replacement therapy of dental caries to enable clinical safety trials. *J Appl Microbiol* **102**, 1209–1219.

Hiron, A., Falord, M., Valle, J., Débarbouillé, M. & Msadek, T. (2011). Bacitracin and nisin resistance in *Staphylococcus aureus*: a novel pathway involving the BraS/BraR two-component system (SA2417/

SA2418) and both the BraD/BraE and VraD/VraE ABC transporters. *Mol Microbiol* **81**, 602–622.

Hoffmann, A., Schneider, T., Pag, U. & Sahl, H.-G. (2004). Localization and functional analysis of PepI, the immunity peptide of Pep5-producing *Staphylococcus epidermidis* strain 5. *Appl Environ Microbiol* **70**, 3263–3271.

Islam, M. R., Nishie, M., Nagao, J., Zendo, T., Keller, S., Nakayama, J., Kohda, D., Sahl, H.-G. & Sonomoto, K. (2012). Ring A of nukacin ISK-1: a lipid II-binding motif for type-A(II) lantibiotic. *J Am Chem Soc* **134**, 3687–3690.

Jack, R. W., Tagg, J. R. & Ray, B. (1995). Bacteriocins of gram-positive bacteria. *Microbiol Rev* **59**, 171–200.

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

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

Jydegaard, A. M., Gravesen, A. & Knøchel, S. (2000). Growth condition-related response of *Listeria monocytogenes* 412 to bacteriocin inactivation. *Lett Appl Microbiol* **31**, 68–72.

Katla, T., Møretrø, T., Sveen, I., Aasen, I. M., Axelsson, L., Rørvik, L. M. & Naterstad, K. (2002). Inhibition of *Listeria monocytogenes* in chicken cold cuts by addition of sakacin P and sakacin P-producing *Lactobacillus sakei*. *J Appl Microbiol* **93**, 191–196.

Katla, T., Naterstad, K., Vancanneyt, M., Swings, J. & Axelsson, L. (2003). Differences in susceptibility of *Listeria monocytogenes* strains to sakacin P, sakacin A, pediocin PA-1, and nisin. *Appl Environ Microbiol* **69**, 4431–4437.

Kaur, G., Singh, T. P. & Malik, R. K. (2013). Antibacterial efficacy of Nisin, Pediocin 34 and Enterocin FH99 against *Listeria monocytogenes* and cross resistance of its bacteriocin resistant variants to common food preservatives. *Braz J Microbiol* **44**, 63–71.

Kawada-Matsuo, M., Yoshida, Y., Zendo, T., Nagao, J., Oogai, Y., Nakamura, Y., Sonomoto, K., Nakamura, N. & Komatsuzawa, H. (2013a). Three distinct two-component systems are involved in resistance to the class I bacteriocins, nukacin ISK-1 and nisin A, in *Staphylococcus aureus*. *PLoS ONE* **8**, e69455.

Kawada-Matsuo, M., Oogai, Y., Zendo, T., Nagao, J., Shibata, Y., Yamashita, Y., Ogura, Y., Hayashi, T., Sonomoto, K. & Komatsuzawa, H. (2013b). Involvement of the novel two-component NsrRS and LcrRS systems in distinct resistance pathways against nisin A and nukacin ISK-1 in *Streptococcus mutans*. *Appl Environ Microbiol* **79**, 4751–4755.

Kingston, A. W., Liao, X. & Helmann, J. D. (2013). Contributions of the σ^{W}, σ^{M} and σ^{X} regulons to the lantibiotic resistome of *Bacillus subtilis*. *Mol Microbiol* **90**, 502–518.

Kiri, N., Archer, G. & Climo, M. W. (2002). Combinations of lysostaphin with β-lactams are synergistic against oxacillin-resistant *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* **46**, 2017–2020.

Kjos, M., Snipen, L., Salehian, Z., Nes, I. F. & Diep, D. B. (2010a). The Abi proteins and their involvement in bacteriocin self-immunity. *J Bacteriol* **192**, 2068–2076.

Kjos, M., Salehian, Z., Nes, I. F. & Diep, D. B. (2010b). An extracellular loop of the mannose phosphotransferase system component IIC is responsible for specific targeting by class IIa bacteriocins. *J Bacteriol* **192**, 5906–5913.

Kjos, M., Nes, I. F. & Diep, D. B. (2011). Mechanisms of resistance to bacteriocins targeting the mannose phosphotransferase system. *Appl Environ Microbiol* **77**, 3335–3342.

Kramer, N. E., Hasper, H. E., van den Bogaard, P. T. C., Morath, S., de Kruijff, B., Hartung, T., Smid, E. J., Breukink, E., Kok, J. & Kuipers, O. P. (2008). Increased D-alanylation of lipoteichoic acid and a thickened septum are main determinants in the nisin resistance mechanism of *Lactococcus lactis*. *Microbiology* **154**, 1755–1762.

Kusuma, C. M. & Kokai-Kun, J. F. (2005). Comparison of four methods for determining lysostaphin susceptibility of various strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother* **49**, 3256–3263.

Kusuma, C. M., Jadanova, A., Chanturiya, T. & Kokai-Kun, J. F. (2007). Lysostaphin-resistant variants of *Staphylococcus aureus* demonstrate reduced fitness *in vitro* and *in vivo*. *Antimicrob Agents Chemother* **51**, 475–482.

Laursen, M. F., Bahl, M. I., Licht, T. R., Gram, L. & Knudsen, G. M. (2014). A single exposure to a sublethal pediocin concentration initiates a resistance-associated temporal cell envelope and general stress response in *Listeria monocytogenes*. *Environ Microbiol* n/a.

Limonet, M., Revol-Junelles, A. M. & Millière, J. B. (2002). Variations in the membrane fatty acid composition of resistant or susceptible *Leuconostoc* or *Weissella* strains in the presence or absence of Mesenterocin 52A and Mesenterocin 52B produced by *Leuconostoc mesenteroides* subsp. *mesenteroides* FR52. *Appl Environ Microbiol* **68**, 2910–2916.

Macwana, S. & Muriana, P. M. (2012). Spontaneous bacteriocin resistance in *Listeria monocytogenes* as a susceptibility screen for identifying different mechanisms of resistance and modes of action by bacteriocins of lactic acid bacteria. *J Microbiol Methods* **88**, 7–13.

Maisnier-Patin, S. & Richard, J. (1996). Cell wall changes in nisin-resistant variants of *Listeria innocua* grown in the presence of high nisin concentrations. *FEMS Microbiol Lett* **140**, 29–35.

Mandin, P., Fsihi, H., Dussurget, O., Vergassola, M., Milohanic, E., Toledo-Arana, A., Lasa, I., Johansson, J. & Cossart, P. (2005). VirR, a response regulator critical for *Listeria monocytogenes* virulence. *Mol Microbiol* **57**, 1367–1380.

Mantovani, H. C. & Russell, J. B. (2001). Nisin resistance of *Streptococcus bovis*. *Appl Environ Microbiol* **67**, 808–813.

Martínez, B., Rodríguez, A. & Suárez, J. E. (2000). Lactococcin 972, a bacteriocin that inhibits septum formation in lactococci. *Microbiology* **146**, 949–955.

Mascher, T., Zimmer, S. L., Smith, T.-A. & Helmann, J. D. (2004). Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*. *Antimicrob Agents Chemother* **48**, 2888–2896.

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

McBride, S. M. & Sonenshein, A. L. (2011a). The *dlt* operon confers resistance to cationic antimicrobial peptides in *Clostridium difficile*. *Microbiology* **157**, 1457–1465.

McBride, S. M. & Sonenshein, A. L. (2011b). Identification of a genetic locus responsible for antimicrobial peptide resistance in *Clostridium difficile*. *Infect Immun* **79**, 167–176.

Mendoza, F., Maqueda, M., Gálvez, A., Martínez-Bueno, M. & Valdivia, E. (1999). Antilisterial activity of peptide AS-48 and study of changes induced in the cell envelope properties of an AS-48-adapted strain of *Listeria monocytogenes*. *Appl Environ Microbiol* **65**, 618–625.

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

Naghmouchi, K., Kheadr, E., Lacroix, C. & Fliss, I. (2007). Class I/Class IIa bacteriocin cross-resistance phenomenon in *Listeria monocytogenes*. *Food Microbiol* **24**, 718–727.

Nascimento, J. S., Coelho, M. L. V., Ceotto, H., Potter, A., Fleming, L. R., Salehian, Z., Nes, I. F. & Bastos, M. C. F. (2012). Genes involved in immunity to and secretion of aureocin A53, an atypical class II bacteriocin produced by *Staphylococcus aureus* A53. *J Bacteriol* **194**, 875–883.

Nes, I. F., Yoon, S.-S. & Diep, D. B. (2007). Ribosomally synthesized antimicrobial peptides (bacteriocins) in lactic acid bacteria: a review. *Food Sci Biotechnol* **16**, 675–690.

Netz, D. J. A., Bastos, M. C. F. & Sahl, H.-G. (2002). Mode of action of the antimicrobial peptide aureocin A53 from *Staphylococcus aureus*. *Appl Environ Microbiol* **68**, 5274–5280.

Nissen-Meyer, J., Rogne, P., Oppegård, C., Haugen, H. S. & Kristiansen, P. E. (2009). Structure-function relationships of the non-lanthionine-containing peptide (class II) bacteriocins produced by gram-positive bacteria. *Curr Pharm Biotechnol* **10**, 19–37.

Oppegård, C., Emanuelsen, L., Thorbek, L., Fimland, G. & Nissen-Meyer, J. (2010). The lactococcin G immunity protein recognizes specific regions in both peptides constituting the two-peptide bacteriocin lactococcin G. *Appl Environ Microbiol* **76**, 1267–1273.

Opsata, M., Nes, I. F. & Holo, H. (2010). Class IIa bacteriocin resistance in *Enterococcus faecalis* V583: the mannose PTS operon mediates global transcriptional responses. *BMC Microbiol* **10**, 224.

Palmer, M. E., Wiedmann, M. & Boor, K. J. (2009). σ^B and σ^L contribute to *Listeria monocytogenes* 10403S response to the antimicrobial peptides SdpC and nisin. *Foodborne Pathog Dis* **6**, 1057–1065.

Peschel, A., Otto, M., Jack, R. W., Kalbacher, H., Jung, G. & Götz, F. (1999). Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. *J Biol Chem* **274**, 8405–8410.

Peschel, A., Jack, R. W., Otto, M., Collins, L. V., Staubitz, P., Nicholson, G., Kalbacher, H., Nieuwenhuizen, W. F., Jung, G. & other authors (2001). *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine. *J Exp Med* **193**, 1067–1076.

Postma, P. W., Lengeler, J. W. & Jacobson, G. R. (1993). Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol Rev* **57**, 543–594.

Potter, A., Ceotto, H., Coelho, M. L. V., Guimarães, A. J. & Bastos, M. C. F. (2014). The gene cluster of aureocyclicin 4185: the first cyclic bacteriocin of *Staphylococcus aureus*. *Microbiology* **160**, 917–928.

Ramnath, M., Beukes, M., Tamura, K. & Hastings, J. W. (2000). Absence of a putative mannose-specific phosphotransferase system enzyme IIAB component in a leucocin A-resistant strain of *Listeria monocytogenes*, as shown by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Appl Environ Microbiol* **66**, 3098–3101.

Ramnath, M., Arous, S., Gravesen, A., Hastings, J. W. & Héchard, Y. (2004). Expression of *mptC* of *Listeria monocytogenes* induces sensitivity to class IIa bacteriocins in *Lactococcus lactis*. *Microbiology* **150**, 2663–2668.

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

Rekhif, N., Atrih, A. & Lefebvre, G. (1994). Selection and properties of spontaneous mutants of *Listeria monocytogenes* ATCC 15313 resistant to different bacteriocins produced by lactic acid bacteria strains. *Curr Microbiol* **28**, 237–241.

Robichon, D., Gouin, E., Débarbouillé, M., Cossart, P., Cenatiempo, Y. & Héchard, Y. (1997). The *rpoN* (σ^{54}) gene from *Listeria monocytogenes* is involved in resistance to mesentericin Y105, an antibacterial peptide from *Leuconostoc mesenteroides*. *J Bacteriol* **179**, 7591–7594.

Roces, C., Courtin, P., Kulakauskas, S., Rodríguez, A., Chapot-Chartier, M. P. & Martínez, B. (2012a). Isolation of *Lactococcus lactis* mutants simultaneously resistant to the cell wall-active bacteriocin Lcn972, lysozyme, nisin, and bacteriophage c2. *Appl Environ Microbiol* **78**, 4157–4163.

Roces, C., Pérez, V., Campelo, A. B., Blanco, D., Kok, J., Kuipers, O. P., Rodríguez, A. & Martínez, B. (2012b). The putative lactococcal extracytoplasmic function anti-sigma factor Llmg2447 determines resistance to the cell wall-active bacteriocin Lcn972. *Antimicrob Agents Chemother* **56**, 5520–5527.

Rohrer, S. & Berger-Bächi, B. (2003). FemABX peptidyl transferases: a link between branched-chain cell wall peptide formation and β-lactam resistance in gram-positive cocci. *Antimicrob Agents Chemother* **47**, 837–846.

Ryan, M. P., Meaney, W. J., Ross, R. P. & Hill, C. (1998). Evaluation of lacticin 3147 and a teat seal containing this bacteriocin for inhibition of mastitis pathogens. *Appl Environ Microbiol* **64**, 2287–2290.

Ryan, M. P., Flynn, J., Hill, C., Ross, R. P. & Meaney, W. J. (1999). The natural food grade inhibitor, lacticin 3147, reduced the incidence of mastitis after experimental challenge with *Streptococcus dysgalactiae* in nonlactating dairy cows. *J Dairy Sci* **82**, 2108–2114.

Samant, S., Hsu, F. F., Neyfakh, A. A. & Lee, H. (2009). The *Bacillus anthracis* protein MprF is required for synthesis of lysylphosphatidylglycerols and for resistance to cationic antimicrobial peptides. *J Bacteriol* **191**, 1311–1319.

Schindler, C. A. & Schuhardt, V. T. (1965). Purification and properties of lysostaphin – a lytic agent for *Staphylococcus aureus*. *Biochim Biophys Acta* **97**, 242–250.

Strandén, A. M., Ehlert, K., Labischinski, H. & Berger-Bächi, B. (1997). Cell wall monoglycine cross-bridges and methicillin hypersusceptibility in a *femAB* null mutant of methicillin-resistant *Staphylococcus aureus*. *J Bacteriol* **179**, 9–16.

Suda, S., Cotter, P. D., Hill, C. & Ross, R. P. (2012). Lacticin 3147 – biosynthesis, molecular analysis, immunity, bioengineering and applications. *Curr Protein Pept Sci* **13**, 193–204.

Sun, Z., Zhong, J., Liang, X., Liu, J., Chen, X. & Huan, L. (2009). Novel mechanism for nisin resistance via proteolytic degradation of nisin by the nisin resistance protein NSR. *Antimicrob Agents Chemother* **53**, 1964–1973.

Tessema, G. T., Møretrø, T., Kohler, A., Axelsson, L. & Naterstad, K. (2009). Complex phenotypic and genotypic responses of *Listeria monocytogenes* strains exposed to the class IIa bacteriocin sakacin P. *Appl Environ Microbiol* **75**, 6973–6980.

Tessema, G. T., Møretrø, T., Snipen, L., Axelsson, L. & Naterstad, K. (2011). Global transcriptional analysis of spontaneous sakacin P-resistant mutant strains of *Listeria monocytogenes* during growth on different sugars. *PLoS ONE* **6**, e16192.

Thedieck, K., Hain, T., Mohamed, W., Tindall, B. J., Nimtz, M., Chakraborty, T., Wehland, J. & Jänsch, L. (2006). The MprF protein is required for lysinylation of phospholipids in listerial membranes and confers resistance to cationic antimicrobial peptides (CAMPs) on *Listeria monocytogenes*. *Mol Microbiol* **62**, 1325–1339.

Thumm, G. & Götz, F. (1997). Studies on prolysostaphin processing and characterization of the lysostaphin immunity factor (Lif) of *Staphylococcus simulans* biovar *staphylolyticus*. *Mol Microbiol* **23**, 1251–1265.

Vadyvaloo, V., Hastings, J. W., van der Merwe, M. J. & Rautenbach, M. (2002). Membranes of class IIa bacteriocin-resistant *Listeria monocytogenes* cells contain increased levels of desaturated and

short-acyl-chain phosphatidylglycerols. *Appl Environ Microbiol* **68**, 5223–5230.

Vadyvaloo, V., Arous, S., Gravesen, A., Héchard, Y., Chauhan-Haubrock, R., Hastings, J. W. & Rautenbach, M. (2004). Cell-surface alterations in class IIa bacteriocin-resistant *Listeria monocytogenes* strains. *Microbiology* **150**, 3025–3033.

van Belkum, M. J., Martin-Visscher, L. A. & Vederas, J. C. (2011). Structure and genetics of circular bacteriocins. *Trends Microbiol* **19**, 411–418.

van Heusden, H. E., de Kruijff, B. & Breukink, E. (2002). Lipid II induces a transmembrane orientation of the pore-forming peptide lantibiotic nisin. *Biochemistry* **41**, 12171–12178.

van Schaik, W., Gahan, C. G. & Hill, C. (1999). Acid-adapted *Listeria monocytogenes* displays enhanced tolerance against the lantibiotics nisin and lacticin 3147. *J Food Prot* **62**, 536–539.

Verheul, A., Russell, N. J., Van'T Hof, R., Rombouts, F. M. & Abee, T. (1997). Modifications of membrane phospholipid composition in nisin-resistant *Listeria monocytogenes* Scott A. *Appl Environ Microbiol* **63**, 3451–3457.

Vignolo, G., Palacios, J., Farías, M. E., Sesma, F., Schillinger, U., Holzapfel, W. & Oliver, G. (2000). Combined effect of bacteriocins on the survival of various *Listeria* species in broth and meat system. *Curr Microbiol* **41**, 410–416.

Wan, J., Harmark, K., Davidson, B. E., Hillier, A. J., Gordon, J. B., Wilcock, A., Hickey, M. W. & Coventry, M. J. (1997). Inhibition of *Listeria monocytogenes* by piscicolin 126 in milk and Camembert cheese manufactured with a thermophilic starter. *J Appl Microbiol* **82**, 273–280.

Wiedemann, I., Böttiger, T., Bonelli, R. R., Wiese, A., Hagge, S. O., Gutsmann, T., Seydel, U., Deegan, L., Hill, C. & other authors (2006). The mode of action of the lantibiotic lacticin 3147 – a complex mechanism involving specific interaction of two peptides and the cell wall precursor lipid II. *Mol Microbiol* **61**, 285–296.

Xuanyuan, Z., Wu, Z., Li, R., Jiang, D., Su, J., Xu, H., Bai, Y., Zhang, X., Saris, P. E. J. & Qiao, M. (2010). Loss of IrpT function in *Lactococcus lactis* subsp. *lactis* N8 results in increased nisin resistance. *Curr Microbiol* **61**, 329–334.

Edited by: S. Spiro