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Review

Novel biotechnological applications of bacteriocins: A review

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ABSTRACT

Nowadays, consumers are aware of the health concerns regarding food additives; the health benefits of "natural" and "traditional" foods, processed without any addition of chemical preservatives, are becoming more attractive. One of the alternatives to satisfy this request are bacteriocins, which are antimicrobial peptides produced by a large number of bacteria, including lactic acid bacteria, normally acting against closely related and some spoilage and disease-causing Gram-positive pathogens. For this reason they are used in several applications, among which are biopreservation, shelf-life extension, clinical antimicrobial action and control of fermentation microflora. Toxicological studies showed that nisin intake does not cause any toxic effect to humans having an estimated lethal dose of 6950 mg/kg; thus, it is one of the bacteriocins mostly applied in the food industry as antibotulinic agent in cheese and liquid eggs, sauces and canned foods. It exhibits a wide-spectrum antimicrobial action against *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and other pathogens. Food-grade substrates such as milk or whey can be supplemented with *ex situ* produced bacteriocin preparations obtained by fermentation. Preparations can be added as partially purified or purified concentrates requiring specific approval as preservatives from the legislative viewpoint. Demand for new antibacterial compounds has brought great interest for new technologies able to enhance food microbiological safety. Also the dramatic rise in antibiotic-resistant pathogens has stimulated renewed efforts to identify, develop or redesign antibiotics active against multi-resistant bacteria. Numerous antibacterial agents are now being re-considered for application, among others are bacteriophages, probiotics, antimicrobial peptides and bacteriocins. To optimally exploit their desired activities, chemical or genetic engineering methods are often employed. In this review we focus on recent classification of bacteriocins, their mode of action, biotechnological applications in food and pharmaceutical industries, purification techniques and biosafety, as well as recent attempts to generate custom-designed bacteriocins using genetic engineering techniques.

Contents

1. Introduction 2
2. Classification 3
 2.1. Class I or lantibiotics 3
 2.2. Class II 3
  2.2.1. Subclass IIA 3
  2.2.2. Subclass IIB 3
  2.2.3. Subclass IIC 3
 2.3. Class III 4
3. Mode of action and structure 4
 3.1. Factors affecting bacteriocin efficiency 4

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4. Biotechnological applications 4
4.1. Applications in the food industry 5
4.2. Applications in the pharmaceutical industry 5
5. Differences between bacteriocins and antibiotics 6
6. Resistance to bacteriocins 6
7. Biosafety 7
8. Purification 7
9. Conclusions 7
Acknowledgments 7
References 7

1. Introduction

Lactic acid bacteria (LAB) are a diverse and very useful group of bacteria that, while not adhering to a strict taxonomic group, are gathered on the basis of shared properties (Oguntoyinbo & Narbad, 2012) and have the common trait of producing lactic acid (LA) as a major or sole fermentation product. For these reasons, LAB have historically been associated with the fermentation of foods, and as a result many LAB, like *Lactococcus*, *Oenococcus*, *Lactobacillus, Leuconostoc*, *Pedicoccus* and *Streptococcus* sp., are generally recognized as safe (GRAS) and/or probiotics (Mayo et al., 2010).

The desirable property of a probiotic strain is the ability to produce antimicrobial substances such as bacteriocins that offer the potential to provide an advantage in competition and colonization of the gastrointestinal tract. Bacteriocins are generally defined as peptides produced by bacteria that inhibit or kill other related and unrelated microorganisms. Bacteriocin was firstly identified by Gratia (1925) as an antimicrobial protein produced by *Escherichia coli* and named colicin. The interest in bacteriocins produced by GRAS microorganisms has been leading to considerable interest for nisin, being the first bacteriocin to gain widespread commercial application since 1969. As a result, the field has developed increasingly, resulting in the discovery and detailed characterization of a great number of bacteriocins from LAB in the last few decades (Collins, Cotter, Hill, & Ross, 2010).

Nowadays, consumers are aware of the health concerns regarding food additives; the health benefits of "natural" and "traditional" foods, processed without any addition of chemical preservatives, are becoming more attractive. Thus, because of recent consumer demand for higher quality and natural foods, as well as of strict government requirements to guarantee food safety, food producers have faced conflicting challenges (Franz, Cho, Holzapfel, & Gálvez, 2010). Chemical additives have generally been used to combat specific microorganisms. The application of bacteriocins as biopreservatives for vegetable food matrices started approximately 25 years ago. In these years, a lot of studies have focused on the inhibition of spoilage and/or human pathogens associated with vegetable foods and beverages by bacteriocins, and their application appeared as a good alternative to chemical compounds and antibiotics. When deliberately added or produced *in situ*, bacteriocins have been found to play a fundamental role in the control of pathogenic and undesirable flora, as well as in the establishment of beneficial bacterial populations (Collins et al., 2010).

Traditionally, new bacteriocins have been identified by screening bacterial isolates for antimicrobial activity followed by purification and identification of the bacteriocin and its genetic determinants. Such a strategy is still fundamental for detection and identification of powerful bacteriocins of various subclasses, and recent examples of this include a) a class IIa bacteriocin named avicin A that was identified from *Enterococcus avium* strains isolated from faecal samples of healthy human infants from both Ethiopia and Norway (Birri, Brede, Forberg, Holo, & Nes, 2010), b) a circular bacteriocin named garvicin ML produced by a *Lactococcus garvieae* strain isolated from mallard duck (Borrero et al., 2011), c) a class IIb bacteriocin named enterocin X isolated from an *Enterococcus faecium* strain from sugar apples (Hu, Malaphan, Zendo, Nakayama, & Sonomoto, 2010) and d) a glycosylated bacteriocin (glycocin F) from *Lactobacillus plantarum* isolated from fermented corn (Kelly, Asmundson, & Huang, 1996).

In the next sections, we will present bacteriocin classification, their mode of action and structure, biotechnological applications in food and pharmaceutical industries and problems associated with resistance and purification.

2. Classification

According to Klaenhammer (1993), bacteriocins can be divided into four classes. The class I of lantibiotics, represented by nisin, gathers very low molecular weight (<5 kDa) thermostable peptides characterized by the presence of lanthionine and derivatives. The class II is composed of small thermostable peptides (<10 kDa) divided into three subclasses: IIa (pediocin and enterocin), IIb (lactocin G) and IIc (lactocin B). The class III is represented by high molecular weight (>30 kDa) thermolabile peptides such as the helveticin J, while in the class IV we can find large peptides complexed with carbohydrates or lipids. However, Cleveland, Montville, Nes, and Chikindas (2001) believe that these structures are artifacts of partial purification and not a new class of bacteriocins.

Cotter, Hill, and Ross (2005) suggested a new classification where bacteriocins are divided into two categories: lantibiotics (class I) and not containing lanthionine lantibiotics (class II), while high molecular weight thermolabile peptides, which are formally components of the above class III, would be separately designated as "bacteriolysins". These authors also suggested that the above class IV should be extinguished. Finally, Drider, Fimland, Hechard, Mcmullen, and Prevost (2006) divided bacteriocins into three major classes according to their genetic and biochemical characteristics (Table 1), and we will refer to such a classification in the following.

2.1. Class I or lantibiotics

Lantibiotics are small peptides (19–38 amino acid residues) with rare thermostable amino acids in their composition, which may result from the combination of two alanine linked by a disulfide bond as for lanthionine, or from an amino butyric acid linked to an alanine by a disulfide bond as for β-methyl-lanthionine (Jarvis, Jeffcoat, & Cheeseman, 1968).

The main representative of this class is nisin, which is produced by some strains of *Lactococcus lactis* subsp. *lactis* and is composed of 34 amino acid residues. Two variants of nisin are nisin A and nisin Z, which differ structurally in only one amino acid, but have similar

Table 1
Classification of bacteriocins.

Classification	Features	Subcategories	Examples
Class I or lantibiotics	Lantionine or peptides containing β-lantionine	Type A (linear molecules)	Nisin, subtilin, epidermine
		Type B (globular molecule)	Mersacidin
Class II	Heterogeneous class of small thermostable peptides	Subclass IIa (antilisterial-pediocine bacteriocins type)	Pediocin, enterocin, sakacin
		Subclass IIb (composed of two peptides)	Plantaricin, lactacin F
		Subclass IIc (other bacteriocins)	Lactococcin
Class III	Large thermolabile peptides		Helveticin J, millericin B

Source: Adapted from Drider et al. (2006).

activity (Mulders, Boerrigter, Rollema, Siezen, & Vos, 1991). Due to the acidic nature of its molecule, nisin is completely stable in solution at pH 2.0 and can be stored for long time in the temperature range of 2–7 °C, while above pH 7.0 inactivation occurs even at room temperature (Delves-Broughton, 1990).

Toxicological studies showed that nisin intake does not cause any toxic effect to humans with an estimated lethal dose (LD_{50}) as high as 6950 mg/kg (close to that of salt) when administered orally (Jozala, Andrade, Arauz, Pessoa Jr., & Vessoni-Penna, 2007). In general, some authors have ascribed the high LD_{50} values of bacteriocins to digestive enzymes capable of rapidly inactivating trypsin and chymotrypsin produced in the pancreas (Vaucher et al., 2011).

Nisin has been largely using in the food industry as antibotulinic agent in cheese and liquid eggs, sauces and canned foods. It exhibits a wide-spectrum antimicrobial action against *L. monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and other pathogens and LAB species (Rilla, Martinez, & Rodriguez, 2004), which is mediated by a dual action mechanism encompassing interference with cell wall synthesis and promotion of pore formation in cell membrane. The resulting changes in permeability, with outflow of essential compounds (K^+ ion, amino acids and ATP) through the pores, are responsible for cell death (Breukink et al., 1999).

Nisin is the only bacteriocin approved for food applications being considered to be safe by the Food and Agriculture Organization/World Health Organization (FAO/WHO) in 1969. According to Ross, Morgan, and Hill (2002), dairy products can contain nisin as a food additive for processed cheese at concentration up to 12.5 mg/kg pure nisin. In addition, it was also included as biopreservative ingredient in the European food additive list, where it was assigned the number E234.

2.2. Class II

This subclass is composed of small thermostable peptides (<10 kDa) with an amphiphilic helical structure that allows for their insertion in the cytoplasmic membrane of the target cell, thereby promoting membrane depolarization and cell death. Three subdivisions are proposed for this class, according to Drider et al. (2006).

2.2.1. Subclass IIA

The subclass IIa is composed of bacteriocins showing high specificity against *L. monocytogenes*. Its representatives have 37–48 amino acid residues with an N-terminal portion with pleated sheet configuration and a C terminus containing one or two α-helices (Fimland, Johnsen, Dalhus, & Nissen-Meyer, 2005). The bacteriocins of this class fall into the cell membrane of the target microorganism by the C terminus, promoting the formation of pores and consequent dissipation of proton motive force (Kaiser & Montville, 1996). In the attempt to maintain or restore the proton motive force, there is acceleration in the consumption of ATP and consequently cell death.

Pediocin PA-1, which is composed of 44 amino acid residues, is the only bacteriocin belonging to the subclass IIa that is synthesized not only by different species, but also by different genera of LAB. It was initially detected in *Pediococcus acidilactici* (Bhunia, Johnson, & Ray, 1987). Since then, other strains and species of pediococci were described as producers of pediocin (Díez et al., 2012). Ennahar et al. (1996) isolated a strain of *L. plantarum* in Munster cheese able to produce pediocin AcH, a bacteriocin with an antagonistic effect on pathogenic and deteriorating microorganisms, including *L. monocytogenes*, *S. aureus* and *Clostridium perfringens* (Bhunia et al., 1987; Loessner, Guenther, Steffan, & Scherer, 2003).

The first enterocin was identified by Kjems (1955) and subsequently classified as a member of the pediocin family. Since then, several enterocins have been described, that have representatives in more than one class of bacteriocins. Usually they are thermostable (121 °C/15 min) and resistant to lyophilization and storage at −20 °C for long periods. According to Cintas, Casaus, Havarstein, Hernandez, and Nes (1997), these compounds have selective antimicrobial activity, do not show antagonism with *Leuconostoc* and *Lactococcus*, but attack *C. perfringens*, *Clostridium botulinum*, *S. aureus* and especially species of the genus *Listeria*.

2.2.2. Subclass IIB

This subclass includes heterodimeric bacteriocins, i.e. bacteriocins that require the combined activity of two peptides. Normally, genes are located in the same operon and expressed simultaneously, and the two peptides act in combination frequently showing an important synergistic action. Their mechanism of action also involves the dissipation of membrane potential and a decrease in the intracellular ATP concentration. These peptides have very low activity when individually employed (Garneau, Martin, & Vederas, 2002).

2.2.3. Subclass IIC

Bacteriocins belonging to this subclass have a covalent bond between C and N terminals, resulting in a cyclic structure (Kawai et al., 2004). Enterocin AS-48, circularin A and reutericin 6 are representatives of this subclass.

2.3. Class III

This class gathers large thermolabile bacteriocins (>30 kDa) that have complex activity and protein structure. Their action mechanism is different from those of other bacteriocins, in that they promote lysis of the cell wall of the target microorganism. Their N-terminal portion is homologous to an endopeptidase involved in cell wall synthesis, while the C-terminal portion is responsible for recognition of the target cell (Lai, Tran, & Simmonds, 2002).

3. Mode of action and structure

Bacteriocins are usually synthesized as inactive pre-peptides that have an N-terminal sequence guide (Macwana & Muriana, 2012). These precursors are transported to the cell surface during the exponential growth phase and enzymatically converted into their active forms. The carriers contain an N-terminal peptidic portion responsible for the guide peptide cleavage as well as a C-terminal portion responsible for ATP hydrolysis and energy supply

(Aucher, Lacombe, Héquet, Frère, & Berjeaud, 2005). For class II, accessory proteins are used to facilitate the membrane translocation and/or cleave the peptide tab.

The system regulating the production of bacteriocins is composed of three components: an inducing peptide (or pheromone-activating factor), the transmembrane histidine kinase (pheromone receptor) and a response regulator (Nes & Eijsink, 1999). The peptide inducer is synthesized in the ribosome at low levels as a pre-peptide, which is cleaved and secreted in the outer environment by the carrier system. When this compound reaches a threshold concentration, it activates transmembrane histidine kinase, which leads to autophosphorylation of the histidine residue, thus transferring phosphate to a response regulator protein. The phosphorylated regulator activates the transcription of the bacteriocin in addition to the elements that make up the regulatory system, initiating a positive feedback (Nes & Eijsink, 1999). Regulation of the production of lantibiotics such as nisin and subtilin is done by the bacteriocin itself, which acts as a pheromone inducing their production at high levels (Kleerebezem & Quadri, 2001).

The mechanism of immunity of bacteriocin-producing bacteria makes distinction between bacteriocin produced by themselves and by other microorganisms. The protection can be promoted by a specific protein and/or the conveyor system. The mechanism by which they work is similar, by kidnapping the structural protein or by antagonistic competition for receptor of the bacteriocin (Hoffmann, Schneider, Pag, & Sahl, 2004).

3.1. Factors affecting bacteriocin efficiency

The activity of bacteriocins produced by different LAB is not uniform and constant and depends on the chemical composition and physical conditions of food; it mainly depends on pH and is reduced by bacteriocin binding to food components, adsorption to cell or protein, activity of proteases and other enzymes (Schillinger, Geisen, & Holzapfel, 1996). A correlation between nisin degradation and extent of proteolysis in pasteurized cream was found by Phillips, Griffths, and Muir (1983). Buyong, Kok, and Luchansky (1998) ascribed the reduction in pediocin activity from 64,000 to 2,000 U/g after six months of maturation of Cheddar cheese to the action of proteases and peptidases. NaCl at certain concentrations can reduce the growth of LAB and consequently the production of bacteriocins, besides protecting the target bacteria such as *L. monocytogenes* from their action (Hugas, Garriga, Pascual, Aymerich, & Monfort, 2002). Sarantinopoulos et al. (2002) observed reductions in bacteriocin activity and *E. faecium* FAIR-E 198 growth rate after addition of 2% NaCl to MRS broth. Nilsen, Nes, and Holo (1998) ascribed this phenomenon to the interference of NaCl in the production factor binding the inductor to the receptor.

Aside from interacting with food components, bacteriocins may be adversely affected by processing and storage conditions such as pH and temperature of the product. According Drosinos, Mataragas, Nasis, Galiotou, and Metaxopoulos (2005), the optimal pH for bacteriocin production (5.5) does not match that for microbial growth (6.5). Because of their maximum stability under acidic conditions, nisin activity is increased when used in acidic foods. Therefore, effective applications of nisin require that the pH of food is less than 7 to ensure satisfactory solubility, stability during processing and storage period (Hernandez et al., 1993). Leroy and De Vuyst (1999) reported that bacteriocin activity decreases with increasing temperature owing to increased activity of proteases.

The inhibitory efficiency of bacteriocins is also related to the level of food contamination by the target organism. If the initial contamination is too high, bacteriocin activity is low and unable to prevent the development of contaminating microorganisms. Rilla et al. (2004) investigated the action of *Lc. lactis* subsp. *lactis* IPLA 729 against *S. aureus* at two different concentrations, specifically 1.8×10^4 and 7.2×10^6 CFU mL^{-1}: after 24 h of incubation, they did not detect *S. aureus* in the more dilute sample, while the other showed a still high count (5.0×10^4 CFU mL^{-1}).

4. Biotechnological applications

There are potentially significant benefits to employing modern cutting-edge bioengineering to progress the traditional peptide discovery, description and production because of the gene-encoded nature of bacteriocins. One of the greatest advantages of bioengineering in the lantibiotic field involves the creation of strains producing larger amounts of lantibiotic peptides (Suda et al., 2010). Another strategy to improve lantibiotic-producing strains is to conjugate multiple large bacteriocin-encoding plasmids into a single strain (Collins et al., 2010), thereby making it able to kill the undesired target more effectively than the wild type (O'Sullivan, Ryan, Ross, & Hill, 2003). It is also possible to achieve this goal through the amplification and cloning of lantibiotic-encoding genes into shuttle vectors and heterologous production in other strains. Such an approach was used to improve the production of lacticin 3147 by an *Enterococcus* host (Ryan, Mcauliffe, Ross, & Hill, 2001).

Bioengineering of existing peptides could also lead to the creation of lantibiotics with improved power and/or suitable for specific applications (Collins et al., 2010). A number of studies allowed for better comprehension of the structure/function relationships of specific lantibiotics and pointed out the significance of nisin and related peptides within the hinge region, whose discrete alterations resulted in mutants with no mutacin II activity (Chen et al., 1998), or improved nisin Z activity, or even enhanced stability at high temperature and/or under neutral or alkaline conditions (Yuan, Zhang, Chen, Yang, & Huan, 2004). In addition, to improve the activity or inhibitory spectrum, peptides were developed with enhanced characteristics. For example, nisin Z studies that solubility and stability were significantly improved by peptide engineering without dramatically reducing specific activity (Rollema, Kuipers, Both, De Vos, & Siezen, 1995).

It is also possible to drastically alter lantibiotic and non-lantibiotic peptides by altering existing or introducing new post-translational modifications through the application of specific enzymes. To provide some examples, the cyclase of nisin (NisC) was utilized to cyclize and protect non-lantibiotic peptides against peptidases and proteases (Rink et al., 2007), a property which is particularly useful from a drug design standpoint, while the dehydratase of nisin (NisB) to introduce dehydro residues making the formation of thioether bridges into various peptides easier (Kluskens et al., 2005).

According to Mills, Stanton, Hill, and Ross (2011), bioengineering of bacteriocins is not limited to lantibiotics. Much effort has been devoted to the subclass IIA of bacteriocins to determine the structure–function relationships. Though variants generated in these types of studies are useful from an academic standpoint, none of them display increased activity against several microorganisms (Kazazic, Nissen-Meyer, & Fimland, 2002).

4.1. Applications in the food industry

Foods products can be supplemented with *ex situ* produced bacteriocin preparations obtained by cultivation of the producer strain in an industrial fermenter followed by adequate recovery. Bacteriocins can be added as partially purified or purified concentrates, which would require specific approval as preservatives from the legislative viewpoint. So far, nisin and pediocin PA-1 are bacteriocins licensed as food preservatives (Simha, Sood, Kumariya, & Garsa, 2012). Many preliminary studies on the activity of bacteriocins *in vitro* or in food

systems are carried out with partially-purified preparations obtained from culture broths, but in the most cases a low concentration of bacteriocin is often recovered (Schillinger et al., 1996; Stiles, 1996), which demonstrates the significance to address many efforts in this direction.

Foods can also be supplemented with bacteriocins *ex situ* produced that can be added in the form of raw concentrates obtained by cultivation of the producer strain in a food-grade substrate (such as milk or whey). The resulting preparations may be regarded as food additives or ingredients from the legal viewpoint, since some of their components may play a recognized function in the food (such as increase in protein content or thickening). They also contain the cell-derived antimicrobial metabolites (such as LA) and bacteriocins, affording an additional bioprotectant function. Other milk-based preparations have been described, in addition to already-marketed concentrates such as ALTA™ 2341 or Microgard™, such as lacticin 3147 (Guinane, Cotter, Hill, & Ross, 2005) and variacin (O'Mahony, Rekhif, Cavadini, & Fitzgerald, 2001). Bacteriocins *ex situ* produced can also be applied in the form of immobilized preparations, in which the partially-purified bacteriocin is bound to a carrier. The carrier acts as a reservoir and diffuser of the concentrated bacteriocin molecules to the food, guaranteeing a gradient-dependent continuous supply of bacteriocin. The carrier may also protect the bacteriocin from inactivation by interaction with food components and enzymatic inactivation. Moreover, the application of bacteriocin molecules on the food surface requires much lower amounts of bacteriocin (compared to application in the whole food volume), decreasing the processing costs. In most cases, immobilized bacteriocin preparations are applied on the surface of the processed food, avoiding post-process contamination and surface proliferation of unwanted bacteria. A recent advance in this field is the use of immobilized bacteriocins in the development of antimicrobial packaging (Ercolini, Storia, Villani, & Mauriello, 2006).

In situ, bacteriocin production offers several advantages compared to *ex situ* production, concerning both legal aspects and costs. Lowering the costs of biopreservation processes may be highly attractive, especially for small economies and developing countries, where food safety may be seriously compromised (Holzapfel, 2002). Several studies demonstrate the effectiveness of these compounds in food biopreservation, as shown in Table 2.

Many studies have also focused on the selection and development of protective bacteriocinogenic cultures for food applications (Leroy, Verluyten, & De Vuyst, 2006; Ross et al., 2002) such as inhibition of spoilage and pathogenic bacteria during the shelf life period of non-fermented foods. A protective culture may grow and produce bacteriocin during refrigerated storage of the food, which must have a neutral impact on its physicochemical and organoleptic properties, and/or during temperature abuse conditions, under which it may even act as the predominant spoiler, ensuring that pathogenic bacteria do not grow and that the spoiled food is not consumed (Holzapfel, Geisen, & Schillinger, 1995).

4.2. Applications in the pharmaceutical industry

With the availability of a powerful and effective arsenal of drugs, most pharmaceutical companies abandoned their antimicrobial drug development programs, as there seemed to be little need for new drug compounds (Knowles, 1997). Bacterial resistance to antimicrobials was observed right after their initial wide-scale use (Levin et al., 1998). Since then, the levels of resistance have continued to rise dramatically. It has reached the point that by 2000 the World Health Organization cautioned that infectious diseases might become untreatable as a result of high levels of multiply resistant pathogens. At first, antibiotic resistance was thought to be confined to hospital settings, where the use of antibiotics was most intensive; approximately one third of all hospitalized patients receive antibiotics with at least half of those prescriptions being unnecessary, poorly chosen or incorrectly administered (Van Houten, Luinge, Laseur, & Kimpen, 1998).

Compounding the problem further, an almost exclusive reliance on broad-spectrum antibiotic agents has contributed to a rapid emergence of multiresistant pathogens (Wester et al., 2002). The increasing threat of antibiotic resistance is also the result of antibiotic use in agricultural and food production settings. In the agricultural industry, the use of antibiotics for disease control, prophylactic agents and growth promotion, has contributed significantly to the emergence of resistant bacteria pathogenic to animals (Barton & Hart, 2001) and plants (Mcmanus, Stockwell, Sundin, & Jones, 2002). Additionally, bacteria isolated from animals in environments unrelated to clinical or agricultural management settings have been shown to naturally acquire high levels of antibiotic resistance (Sherley, Gordon, & Collignon, 2000). Ironically, it is likely that the extensive benefits of antibiotic use has contributed to the limited array of effective drugs available today for treating multi-resistant bacteria.

Only recently the alarming nature of this problem has motivated research efforts to find alternatives to our increasingly limited antibiotic resources. Numerous antibacterial agents are now being considered such as bacteriophages (Alisky, Iczkowski, Rapoport, & Troitsky, 1998), probiotic bacteria (Macfarlane & Cummings, 2002), antimicrobial peptides (Joerger, 2003), and bacteriocins (Twomey, Ross, Ryan, Meaney, & Hill, 2002). In order to optimally exploit the desired activities of these varied antimicrobial leads, researchers often employ chemical or genetic engineering methods (Lien & Lowman, 2003). Examples of some bacteriocins and their pharmaceutical applications are shown in Table 3.

The use of microcins is a possible alternative to control Gram-negative bacteria (Duquesne, Destoumieux-Garzón, Peduzzi, & Rebuffat, 2007). Similarly to pediocin-like bacteriocins, microcins belonging to class IIa such as microcin V are linear polypeptides, and the removal of the leader peptide is the unique post-translational modification that they undergo before being secreted by the cells (Duquesne et al., 2007; Pons, Lanneluc, Cottenceau, & Sable, 2002). Three different proteins may serve as

Table 2
Application of bacteriocins in food biopreservation.

Bacteriocin	Culture producer	Target microorganism	Food	Reduction (log CFU g^{-1})	References
Nisin	*Lactococcus lactis*	*Brochothrix thermosphacta*	Pork	3.5	Nattress, Yost, & Baker, 2001
Nisin	*L. lactis*	*Listeria monocytogenes*	Fermented milk	6.0	Benkerroum et al. (2002)
AcH Pediocin	*Lactobacillus plantarum*	*L. monocytogenes*	Cheese	1.0–2.0	Loessner et al., 2003
Enterocin	*Enterococcus faecium*	*L. monocytogenes*	Milk	2.0	Elotmani, Revol-Junelles, Assobhei, & Milliére, 2002
Enterocin	*Enterococcus faecalis*	*Staphylococcus aureus*	Sausage	5.3	Ananou, Maqueda, Martínéz-Bueno, Gálvéz, & Valdivia, 2005
Nisin Z	*Lactococcus lactis lactis*	*S. aureus*	*Afuega'l pitu* cheese	2.0	Rilla et al. (2004)

Source: Adapted from Nascimento, Moreno, and Kuaye (2008).

Table 3
Examples of some bacteriocins and their pharmaceutical applications.

Group of bacteriocins	Pharmaceutical applications
Lantibiotics	Blood pressure treatment Inflammations and allergies treatment Skin infections treatment Mastitis infections treatment Herpes treatment Dental carries treatment Peptic ulcer treatment
Colicins	Urinogenital infection Hemorrhagic colitis treatment Hemolytic uremic syndrome treatment
Microcins	Antibacterial agent Salmonellosis treatment

Source: Adapted from Gillor, Nigro, and Riley (2005).

a specific receptor for linear microcins, namely the membrane component F_0 of the ATP synthase, SdaC, and the mannose permease, required by MccH47, MccV, and MccE492, respectively (Biéler, Silva, & Belin, 2010; Gérard, Pradel, & Wu, 2005). Because of the Gram-negative envelope structure, an additional step is required by class IIa microcins, i.e. an OM transporter system is used for these peptides to reach the plasma membrane receptor. The enterocin CRL35, a pediocin-like bacteriocin isolated from Argentinean regional cheese, has a potent antilisterial activity but is inactive against Gram-negative bacteria (Farías, Farías, de Ruiz Holgado, & Sesma, 1996). On the other hand, microcin V, previously known as colicin V, is specifically active against Gram-negative bacteria (Gratia, 1925). In order to obtain a peptide with a broader antimicrobial spectrum, Acuña, Picariello, Sesma, Morero, and Bellomio (2012) fused by asymmetrical PCR the required portions of genes encoding enterocin CRL35 and microcin V, namely *munA* and *cvaC*. The hybrid bacteriocin purified from *E. coli* extracts, named Ent35–MccV, showed inhibitory activity against enterohemorrhagic *E. coli*, *L. monocytogenes*, and other pathogenic Gram-positive and Gram-negative bacteria (Acuña et al., 2012).

5. Differences between bacteriocins and antibiotics

In contrast to the currently used antibiotics, bacteriocins are often considered more natural because they are thought to have been present in many of the foods eaten since ancient times. Bacteriocins are inactivated by enzymes, such as trypsin and pepsin, found in the gastrointestinal tract and therefore, they do not alter the microbiota of the digestive tract (Cleveland et al., 2001). If bacteriocins are considered antibiotics, they may not be used in human food, since the use of antibiotics in food is illegal (Collins et al., 2010). Nisin is the only bacteriocin considered by the Codex Alimentarius committee FAO (Food and Agriculture Organization) as GRAS (Generally Regarded As Safe) and can be used as a food additive in the inhibition of post-germination spores and toxin formation by *C. botulinum* in pasteurized processed cheese. Antibiotics for use in animal feed have been first approved in 1951 by the U.S. Food and Drug Administration that now maintains a list of currently approved products. Over the years and especially more recently, a number of strategies for improvements in animal health, productivity, and microbial food safety that did not involve antibiotics have been explored, like probiotics and bacteriocins (Joerger, 2003).

Antibiotics and bacteriocins have different mechanisms of action. When nisin is combined with some antibiotics, antimicrobial synergy may occur. The mechanisms of resistance to nisin and antibiotics are different. Antibiotic-resistant cells are sensitive to nisin and nisin-resistant cells are sensitive to antibiotics (Cleveland et al., 2001; Fernández, Delgado, Herrero, Maldonado, & Rodríguez, 2008). More recently, microencapsulated nisin in nanovesicles prepared from partially purified soy lecithin was shown to be as effective as free nisin to inhibit *L. monocytogenes* growth in whole and skim milk at low temperatures over 14 days (da Silva-Malheiros, Daroit, da Silveira, & Brandelli, 2010). Naghmouchi, Le Lay, Baah, and Drider (2012) determined the synergistic effect of bacteriocins and antibiotics on sensitive and resistant variants of strains. In particular, a synergistic effect against *Pseudomonas fluorescens* was observed with 90% of the combinations of the class I or subclass IIa bacteriocins with antibiotics and 60% of the combinations of colistin with antibiotics. So, in the future, combination of antibiotics with antimicrobial peptides could allow for reduced use of antibiotics in medical applications and could help to prevent the emergence of bacteria resistant to antibiotics.

6. Resistance to bacteriocins

The resistance of spontaneous mutants to bacteriocins may be related to changes in membrane and cell wall, such as alterations in the electrical potential, fluidity, membrane lipid composition and load or cell wall thickness (Mantovani & Russel, 2001), or even a combination of all factors. According to Van Schaik, Gahan, and Hill (1999), these changes may occur following cell exposure to low concentrations of bacteriocins or as part of an adaptive response to some other stress. The mechanism of resistance of cells to nisin is not yet well understood. According to Abee (1995), the resistance of *L. monocytogenes* to nisin is related to variation in fatty acid composition of cell membranes, reducing the concentration of phospholipids, hindering the formation of pores.

Gravesen, Axelsen, Silva, Hansen, and Knochel (2002) reported that the frequency of resistance may vary between 10^{-2} and 10^{-7}, depending on the strain of *L. monocytogenes*. The mechanism of resistance to subclass IIa bacteriocins appears to be linked to reduced expression of mannose permease of the phosphotransferase system (Vadyvaloo, Hastings, Van Der Merwe, & Rautenbach, 2002).

7. Biosafety

Microorganisms like *Lactobacillus* spp., *Lactococcus* spp. and *Streptococcus thermophilus* have been used in food processing, and consumption of foods containing them or their metabolites has taken place for a long time (Ishibashi & Yamazaki, 2001). The safety of these microorganisms has not been questioned and reports of harmful effects of these bacteria have been very rare. Some LAB have proven to be associated with human infections, like endocarditis by *Lactobacillus fermentum* isolated in the mitral valve (Gallemore, Mohon, & Ferguson, 1995), pancreatitis by *Lactobacillus rhamnosus* isolated in the intra-abdomen and blood (Brahimi, Mathern, Fascia, Afchain, & Lucht, 2008), urinary tract infection by *P. acidilactici*, *Lactobacillus gasseri* and *Leuconostoc mesenteroides* (Taneja et al., 2005), and several other diseases. In addition, some LAB has been associated with resistance to antibiotics, but according to Songisepp et al. (2012), *L. plantarum* Tensia is not resistant to tetracycline.

However, various clinical studies have been conducted to assess the safety of probiotics in small groups of specific HIV infected patients, and the findings of these studies support the safety of probiotics consumed by such groups (Cunningham-Rundles et al., 2000).

8. Purification

Bacteriocin-producers are LAB that need complex nutritional exigencies to grow, and this not only increases the production cost, but also makes the purification of bacteriocins more difficult (Li, Bai, Cai, & Ouyang, 2002). Since bacteriocins form an extremely heterogeneous group of substances, specific purification protocols generally need to be designed for each of them, which may explain why only few bacteriocins have been purified to homogeneity like nisin. Three major methods for the purification of LAB bacteriocins can be distinguished according to their biochemical structure. First, purification can be done by a conventional method that is based on a rather laborious series of subsequent steps of ammonium sulfate precipitation, ion exchange, hydrophobic interaction, gel filtration, and reversed-phase high-pressure liquid chromatography (Parente & Ricciardi, 1999). Second, a simple three-step protocol has been developed, including (1) ammonium sulfate precipitation, (2) chloroform/methanol extraction/precipitation, and (3) reversed-phase high-pressure liquid chromatography, as the sole chromatographic step involved (Callewaert et al., 1999). Third, bacteriocins can be isolated through a unique unit operation, i.e. expanded bed adsorption, using a hydrophobic interaction gel, after maximizing the bioavailable bacteriocin titer through pH adjustment of the crude fermentation medium (Foulquié-Moreno, Callewaert, & De Vuyst, 2001).

Following the last two methods, which are more rapid and successful than the first conventional one, several bacteriocins with interesting industrial potential have been purified such as amylovorin L (produced by *Lactobacillus amylovorus* DCE 471 and belonging to the class II), several enterocins (produced by the *E. faecium* RZS C5, RZS C13 and FAIR-E 406 strains) and the lantibiotic macedocin (produced by *Streptococcus macedonicus* ACA-DC 198) (Callewaert et al., 1999; Georgalaki et al., 2002). Nisin, for example, has been purified using immunoaffinity chromatography (Prioult, Turcotte, Labarre, Lacroix, & Fliss, 2000), expanded bed ion exchange (Cheigh, Kook, Kim, Hong, & Pyun, 2004) and reversed-phase high-performance liquid chromatography (López et al., 2007). However, these methodologies greatly increase the cost of nisin, which is the most consumed bacteriocin in the world.

9. Conclusions

Bacteriocins have the potential to cover a very broad field of application, including both the food industry and the medical sector. They are a diverse group of antimicrobial proteins/peptides; therefore, they are expected to behave differently on different target bacteria and under different environmental conditions. Since the efficacy of bacteriocins is dictated by environmental factors, there is a need to determine more precisely the most effective conditions for application of each particular bacteriocin. For uses involving purified bacteriocins, cost of the compounds can become a significant barrier. Production of all but the smallest bacteriocins is currently only imaginable by culture of natural or genetically engineered producer organisms. Investments in research and development can be expected to be high, and the size of the market is difficult to predict, but the fact that nisin has found commercial uses indicates that economic aspects are not insurmountable barriers to bacteriocin applications.

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References

Abee, T. (1995). Pore-forming bacteriocins of Gram-positive bacteria and self-protection mechanisms of producer organism. *FEMS Microbiology Letters, 129*, 1–9.

Acuña, L., Picariello, G., Sesma, F., Morero, R. D., & Bellomio, A. (2012). A new hybrid bacteriocin, Ent35–MccV, displays antimicrobial activity against pathogenic Gram-positive and Gram-negative bacteria. *Federation of European Biochemical Societies Open BIO*, 12–19.

Alisky, J., Iczkowski, K., Rapoport, A., & Troitsky, N. (1998). Bacteriophages show promise as antimicrobial agents. *Journal of Infection, 36*, 5–15.

Ananou, S., Maqueda, M., Martínéz-Bueno, M., Gálvéz, A., & Valdivia, E. (2005). Control of *Staphylococcus aureus* in sausages by enterocin AS-48. *Meat Science, 71*, 549–556.

Aucher, W., Lacombe, C., Héquet, A., Frère, J., & Berjeaud, J. M. (2005). Influence of amino acid substitutions in the leader peptide on maturation and secretion of mesentericin Y105 by Leuconostoc mesenteroides. *Journal of Bacteriology, 187*, 2218–2223.

Barton, M. D., & Hart, W. S. (2001). Public health risks: antibiotic resistance – Review. *Asian-Australasian Journal of Animal Sciences, 14*, 414–422.

Benkerroum, N., Ghouati, Y., Ghalfi, H., Elmejdoub, T., Roblain, D., Jacques, P., et al. (2002). Biocontrol of *Listeria monocytogenes* in a model cultured milk (Iben) by *in situ* bacteriocin production from *Lactococcus lactis* ssp. *lactis*. *International Journal of Dairy Technology, 55*, 145–151.

Bhunia, A. K., Johnson, M. C., & Ray, B. (1987). Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Journal of Industrial Microbiology & Biotechnology, 2*, 319–322.

Biéler, S., Silva, F., & Belin, D. (2010). The polypeptide core of microcin E492 stably associates with the mannose permease and interferes with mannose metabolism. *Research in Microbiology, 161*, 706–710.

Birri, D. J., Brede, D. A., Forberg, T., Holo, H., & Nes, I. F. (2010). Molecular and genetic characterization of a novel bacteriocin locus in *Enterococcus avium* isolates from infants. *Applied and Environmental Microbiology, 76*, 483–492.

Borrero, J., Brede, D. A., Skaugen, M., Diep, D. B., Herranz, C., Nes, I. F., et al. (2011). Characterization of garvicin ML, a novel circular bacteriocin produced by *Lactococcus garvieae* DCC43, isolated from mallard ducks (*Anas platyrhynchos*). *Applied and Environmental Microbiology, 77*, 369–373.

Brahimi, M., Mathern, P., Fascia, P., Afchain, J. M., & Lucht, F. (2008). Two cases of *Lactobacillus rhamnosus* infection and panctreatitis. *Médecine et Maladies Infectieuses, 38*, 29–31.

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

Buyong, N., Kok, J., & Luchansky, J. B. (1998). Use of a genetically enhanced, pediocin-producing starter culture, *Lactococcus lactis* subsp. *lactis* MM 217 to control *Listeria monocytogenes* in cheddar cheese. *Applied and Environmental Microbiology, 64*, 4842–4845.

Callewaert, R., Holo, H., Devreese, B., Van Beeumen, J., Nes, I., & De Vuyst, L. (1999). Characterization and production of amylovorin L471, a bacteriocin purified from *Lactobacillus amylovorus* DCE 471 by a novel three-step method. *Microbiology, 145*, 2559–2568.

Cheigh, C. I., Kook, M. C., Kim, S. B., Hong, Y. H., & Pyun, Y. R. (2004). Simple one-step purification of nisin Z from unclarified culture broth of *Lactococcus lactis* subsp. *lactis* A164 using expanded bed ion exchange chromatography. *Biotechnology Letters, 26*, 1341–1345.

Chen, P., Novak, J., Kirk, M., Barnes, S., Qi, F., & Caufield, P. W. (1998). Structure-activity study of the lantibiotic mutacin II from *Streptococcus mutans* T8 by a gene replacement strategy. *Applied and Environmental Microbiology, 64*, 2335–2340.

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

Cleveland, J., Montville, T. J., Nes, I. F., & Chikindas, M. L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology, 71*, 1–20.

Collins, B., Cotter, P. D., Hill, C., & Ross, R. P. (2010). Applications of lactic acid bacteria-produced bacteriocins. In F. Mozzi, R. R. Raya, & G. M. Vignolo (Eds.), *Biotechnology of lactic acid bacteria: Novel applications* (pp. 89–109).

Cotter, P. D., Hill, C., & Ross, P. (2005). Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology, 3*, 777–788.

Cunningham-Rundles, S., Ahrne, S., Bengmark, S., Johann-Liang, R., Marshall, F., Metakis, L., et al. (2000). Probiotics and immune response. *American Journal of Gastroenterology, 95*, 22–25.

Delves-Broughton, J. (1990). Nisin and its application as a food preservative. *Journal of the Society of Dairy Technology, 43*, 73–76.

Díez, L., Rojo-Bezares, B., Zarazaga, M., Rodríguez, J. M., Torres, C., & Ruiz-Larrea, F. (2012). Antimicrobial activity of pediocin PA-1 against *Oenococcus oeni* and other wine bacteria. *Food Microbiology, 31*, 167–172.

Drider, D., Fimland, G., Hechard, Y., Mcmullen, L. M., & Prevost, H. (2006). The continuing story of class IIa bacteriocins. *Microbiology and Molecular Biology Reviews, 70*, 564–582.

Drosinos, E. H., Mataragas, M., Nasis, P., Galiotou, M., & Metaxopoulos, J. (2005). Growth and bacteriocin production kinetics of *Leuconostoc mesenteroides* E131. *Journal of Applied Microbiology, 99*, 1314–1323.

Duquesne, S., Destoumieux-Garzón, D., Peduzzi, J., & Rebuffat, S. (2007). Microcins, gene-encoded antibacterial peptides from enterobacteria. *Natural Product Reports, 24*, 708–734.

Elotmani, F., Revol-Junelles, A. M., Assobhei, O., & Milliére, J. (2002). Characterization of anti-*Listeria monocytogenes* bacteriocins from *Enterococcus faecalis, Enterococcus faecium* and *Lactococcus lactis* strains isolated from Raib, a Moroccan traditional fermented milk. *Current Microbiology, 44*, 10–17.

Ennahar, S., Aoude-Werner, D., Sorokine, O., Dorsselaer, A. V., Bringel, F., Hubert, J. C., et al. (1996). Production of pediocin AcH by *Lactobacillus plantarum* WHE92 isolated from cheese. *Applied and Environmental Microbiology, 62*, 4381–4387.

Ercolini, D., Storia, A., Villani, F., & Mauriello, G. (2006). Effect of a bacteriocin-activated polythene film on *Listeria monocytogenes* as evaluated by viable staining and epifluorescence microscopy. *Journal of Applied Microbiology, 100*, 765–772.

Farías, M. E., Farías, R. N., de Ruiz Holgado, A. P., & Sesma, F. (1996). Purification and N-terminal amino acid sequence of enterocin CRL 35, a "pediocin-like" bacteriocin produced by *Enterococcus faecium* CRL 35. *Letters in Applied Microbiology, 22*, 417–419.

Fernández, L., Delgado, S., Herrero, H., Maldonado, A., & Rodríguez, J. M. (2008). The bacteriocin nisin, as effective agent for the treatment of Staphylococcal mastitis during lactation. *Journal of Human Lactation, 24*, 311–316.

Fimland, G., Johnsen, L., Dalhus, B., & Nissen-Meyer, J. (2005). Pediocin-like antimicrobial peptides (class IIa bacteriocins) and their immunity proteins: biosynthesis, structure and mode of action. *Journal of Peptide Science, 11*, 688–696.

Foulquié-Moreno, M. R., Callewaert, R., & De Vuyst, L. (2001). Isolation of bacteriocins through expanded bed adsorption using a hydrophobic interaction medium. *Bioseparation, 10*, 45–50.

Franz, C. M. A. P., Cho, G. S., Holzapfel, W. H., & Gálvez, A. (2010). Safety of lactic acid bacteria. In F. Mozzi, R. R. Raya, & G. M. Vignolo (Eds.), *Biotechnology of lactic acid bacteria: Novel applications* (pp. 341–359).

Gallemore, G. H., Mohon, R. T., & Ferguson, D. A. (1995). *Lactobacillus fermentum* endocarditis involving a native mitral valve. *Journal of the Tennessee Medical Association, 88*, 306–308.

Garneau, S., Martin, N. I., & Vederas, J. C. (2002). Two-peptide bacteriocins produced by lactic acid bacteria. *Biochimie, 84*, 577–592.

Georgalaki, M. D., Van Den Berghe, E., Kritikos, D., Devreese, B., Van Beeumen, J., Kalantzopoulos, G., et al. (2002). Macedocin, a food-grade lantibiotic produced by *Streptococcus macedonicus* ACA-DC 198. *Applied and Environmental Microbiology, 68*, 5891–5903.

Gérard, F., Pradel, N., & Wu, L. F. (2005). Bactericidal activity of colicin V is mediated by an inner membrane protein, SdaC, of *Escherichia coli*. *Journal of Bacteriology, 187*, 1945–1950.

Gillor, O., Nigro, L. M., & Riley, M. A. (2005). Genetically engineered bacteriocins and their potential as the next generation of antimicrobials. *Current pharmaceutical design, 11*, 1067–1075.

Gratia, A. (1925). Sur un remarquable exemple d'antagonisme entre deux souches de coilbacille. *Comptes Rendus des Séances et Mémoires de la Société de Biologie, 93*, 1040–1041.

Gravesen, A., Axelsen, A. M. J., Silva, J. M., Hansen, T. B., & Knochel, S. (2002). Frequency of bacteriocin resistance development and associated fitness costs in *Listeria monocytogenes*. *Applied and Environmental Microbiology, 68*, 756–764.

Guinane, C. M., Cotter, P. D., Hill, C., & Ross, R. P. (2005). Microbial solutions to microbial problems; lactococcal bacteriocins for the control of undesirable biota in food. *Journal of Applied Microbiology, 98*, 1316–1325.

Hernandez, P. E., Rodriguez, J. M., Cintas, L. M., Moreira, W. L., Sobrino, O. J., Fernandez, M. F., et al. (1993). Utilización de bactérias lácticas en el control de microorganismos patógenos de los alimentos. *Microbiologia SEM, 9*, 37–48.

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

Holzapfel, W. H. (2002). Appropriate starter culture technologies for small-scale fermentation in developing countries. *International Journal of Food Microbiology, 75*, 197–212.

Holzapfel, W. H., Geisen, R., & Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology, 24*, 343–362.

Hu, C. B., Malaphan, W., Zendo, T., Nakayama, J., & Sonomoto, K. (2010). Enterocin X, a novel twopeptide bacteriocin from *Enterococcus faecium* KU-B5, has an antibacterial spectrum entirely different from those of its component peptides. *Applied and Environmental Microbiology, 76*, 4542–4545.

Hugas, M., Garriga, M., Pascual, M., Aymerich, M. T., & Monfort, J. M. (2002). Enhancement of sakacin K activity against *Listeria monocytogenes* in fermented sausages with pepper or manganese as ingredients. *Food Microbiology, 19*, 519–528.

Ishibashi, N., & Yamazaki, S. (2001). Probiotics and safety. *American Journal of Clinical Nutrition, 73*, 465–470.

Jarvis, B., Jeffcoat, J., & Cheeseman, G. C. (1968). Molecular weight distribution of nisin. *Biochemical Biophysiologie Acta, 168*, 153–155.

Joerger, R. D. (2003). Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. *Poultry Science, 82*, 640–647.

Jozala, A. F., Andrade, M. S., Arauz, L. J., Pessoa, A., Jr., & Vessoni-Penna, T. C. (2007). Nisin production utilizing skimmed milk aiming to reduce process cost. *Applied Biochemical Biotechnology, 136*, 515–528.

Kaiser, A. L., & Montville, T. J. (1996). Purification of the bacteriocin bavaricin MN and characterization of its mode of action against *Listeria monocytogenes* Scott A cells and lipid vesicles. *Applied and Environmental Microbiology, 62*, 4529–4535.

Kawai, Y., Ishii, Y., Arakawa, K., Uemura, K., Saitoh, B., Nishimura, J., et al. (2004). Structural and functional differences in two cyclic bacteriocins with the same sequences produced by lactobacilli. *Applied and Environmental Microbiology, 70*, 2906–2911.

Kazazic, M., Nissen-Meyer, J., & Fimland, G. (2002). Mutational analysis of the role of changed residues in target-cell binding, potency and specificity of the pediocin-like bacteriocin sakacin P. *Microbiology, 148*, 2019–2027.

Kelly, W. J., Asmundson, R. V., & Huang, C. M. (1996). Characterization of plantaricin KW30, a bacteriocin produced by *Lactobacillus plantarum*. *Journal of Applied Bacteriology, 81*, 657–662.

Kjems, E. (1955). Studies on streptococcal bacteriophages: I. Techniques for isolating phage producing strains. *Pathology and Microbiology Scandinavia, 36*, 433–440.

Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiological Review, 12*, 39–85.

Kleerebezem, M., & Quadri, L. E. (2001). Peptide pheromone-dependent regulation of antimicrobial peptide production in Gram-positive bacteria: a case of multicellular behavior. *Peptides, 22*, 1579–1596.

Kluskens, L. D., Kuipers, A., Rink, R., De Boef, E., Fekken, S., Driessen, A. J., et al. (2005). Posttranslational modification of therapeutic peptides by NisB, the dehydratase of the lantibiotic nisin. *Biochemistry, 44*, 12827–12834.

Knowles, D. J. C. (1997). New strategies for antibacterial drug design. *Trends in Microbiology, 5*, 379–383.

Lai, A. C., Tran, S., & Simmonds, R. S. (2002). Functional characterization of domains found within a lytic enzyme produced by *Streptococcus equi* subsp. zooepidemicus. *FEMS Microbiology Letters, 215*, 133–138.

Leroy, F., & De Vuyst, L. (1999). The presence of salt and a curing agent reduces bacteriocin production by *Lactobacillus sakei* CTC 494, a potential starter culture for sausage fermentation. *Applied and Environmental Microbiology, 65*, 5350–5356.

Leroy, F., Verluyten, J., & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. *International Journal of Food Microbiology, 106*, 270–285.

Levin, B. R., Antia, R., Berliner, E., Bloland, P., Bonhoeffer, S., & Cohen, M. (1998). Resistance to antimicrobial chemotherapy: a prescription for research and action. *American Journal of the Medical Sciences, 315*, 87–94.

Li, C., Bai, J., Cai, Z., & Ouyang, F. (2002). Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. *Journal of Biotechnology, 93*, 27–34.

Lien, S., & Lowman, H. B. (2003). Therapeutic peptides. *Trends in Biotechnology, 21*, 556–562.

Loessner, M., Guenther, S., Steffan, S., & Scherer, S. (2003). A pediocin-producing *Lactobacillus plantarum* strain inhibits *Listeria monocytogenes* in a multispecies cheese surface microbial ripening consortium. *Applied and Environmental Microbiology, 69*, 1854–1857.

López, R. L., García, M. T., Abriouel, H., Omar, N. B., Grande, M. J., Martínez-Cañamero, M., et al. (2007). Semi-preparative scale purification of enterococcal bacteriocin enterocin EJ97, and evaluation of substrates for its production. *Journal of Industrial Microbiology & Biotechnology, 34*, 779–785.

Macfarlane, G. T., & Cummings, J. H. (2002). Probiotics, infection and immunity. *Current Opinion in Infectious Diseases, 15*, 501–506.

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. *Journal of Microbiological Methods, 88*, 7–13.

Mantovani, H. C., & Russel, J. B. (2001). Nisin resistance of *Sreptococcus bovis*. *Applied and Environmental Microbiology, 67*, 808–813.

Mayo, B., Aleksandrzak-Piekarczyk, T., Fernández, M., Kowalczyk, M., Álvarez-Martín, P., & Bardowski, J. (2010). Updates in the metabolism of lactic acid bacteria. In F. Mozzi, R. R. Raya, & G. M. Vignolo (Eds.), *Biotechnology of lactic acid bacteria: Novel applications* (pp. 3–33). Iowa, USA: Wiley-Blackwell.

Mcmanus, P. S., Stockwell, V. O., Sundin, G. W., & Jones, A. L. (2002). Antibiotic use in plant agriculture. *Annual Review of Phytopathology, 40*, 443–465.

Mills, S., Stanton, C., Hill, C., & Ross, R. P. (2011). New developments and applications of bacteriocins and peptides in foods. *Annual Review of Food Science and Technology, 2*, 299–329.

Mulders, J. W., Boerrigter, I. J., Rollema, H. S., Siezen, R. J., & Vos, W. M. (1991). Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. *European Journal of Biochemistry, 201*, 581–584.

Naghmouchi, K., Le Lay, C., Baah, J., & Drider, D. (2012). Antibiotic and antimicrobial peptide combinations: synergistic inhibition of *Pseudomonas fluorescens* and antibiotic-resistant variants. *Research in Microbiology*. http://dx.doi.org/10.1016/j.resmic.2011.11.002.

Nascimento, M. S., Moreno, I., & Kuaye, A. Y. (2008). Bacteriocinas em alimentos: uma revisão. *Brazilian Journal of Food Technology, 11*, 120–127.

Nattress, F. M., Yost, C. K., & Baker, L. P. (2001). Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. *International Journal of Food Microbiology, 70*, 111–119.

Nes, I. F., & Eijsink, V. G. H. (1999). Regulation of group II peptide bacteriocin synthesis by quorum-sensing mechanisms. In G. M. Dunny, & S. C. Winans (Eds.), *Cell-cell signalling in bacteria* (pp. 175–192). American Society for Microbiology.

Nilsen, T., Nes, I. F., & Holo, H. (1998). Na exported inducer peptide regulates bacteriocin production in *Enterococcus faecium* CTC 492. *Journal of Bacteriology, 180*, 1848–1854.

Oguntoyinbo, F. A., & Narbad, A. (2012). Molecular characterization of lactic acid bacteria and in situ amylase expression during traditional fermentation of cereal foods. *Food Microbiology, 31*, 254–262.

O'Mahony, T., Rekhif, N., Cavadini, C., & Fitzgerald, G. F. (2001). The application of a fermented food ingredient containing 'variacin', a novel antimicrobial produced by *Kocuria varians*, to control the growth of *Bacillus cereus* in chilled dairy products. *Journal of Applied Microbiology, 90*, 106–114.

O'Sullivan, L., Ryan, M. P., Ross, R. P., & Hill, C. (2003). Generation of food-grade lactoccal starters which produce the lantibiotics lacticin 3147 and lacticin 481. *Journal of Applied and Environmental Microbiology, 69*, 3681–3685.

Parente, E., & Ricciardi, A. (1999). Production, recovery and purification of bacteriocins from lactic acid bacteria. *Journal of Applied Microbiology and Biotechnology, 52*, 628–638.

Phillips, J. D., Griffths, M. W., & Muir, D. D. (1983). Effect of nisin on the shelf-life of pasteurized double cream. *The Journal of the Society of the Dairy Technology, 36*, 17–21.

Pons, A. M., Lanneluc, I., Cottenceau, G., & Sable, S. (2002). New developments in non-post translationally modified microcins. *Biochimie, 84*, 531–537.

Prioult, G., Turcotte, C., Labarre, L., Lacroix, C., & Fliss, I. (2000). Rapid purification of nisin Z using specific monoclonal antibody-coated magnetic beads. *International Dairy Journal, 10*, 627–633.

Rilla, N., Martinez, B., & Rodriguez, A. (2004). Inhibition of a methicillin-resistant *Staphylococcus aureus* strain in Afuega'l Pitu cheese by the nisin Z producing strain *Lactococcus lactis lactis* IPLA 729. *Journal of Food Protection, 67*, 928–933.

Rink, R., Kluskens, L. D., Kuipers, A., Driessen, A. J., Kuipers, O. P., & Moll, G. N. (2007). NisC, the cyclase of the lantibiotic nisin, can catalyze cyclization of designed nonlantibiotic peptides. *Biochemistry, 46*, 13179–13189.

Rollema, H. S., Kuipers, O. P., Both, P., De Vos, W. M., & Siezen, R. J. (1995). Improvement of solubility and stability of the antimicrobial peptide nisin by protein engineering. *Journal of Applied and Environmental Microbiology, 61*, 2873–2878.

Ross, R. P., Morgan, S., & Hill, C. (2002). Preservation and fermentation: past, present and future. *International Journal of Food Microbiology, 79*, 3–16.

Ryan, M. P., Mcauliffe, O., Ross, R. P., & Hill, C. (2001). Heterologous expression of lacticin 3147 in *Enterococcus faecalis*: comparison of biological activity with cytolysin. *Letters in Applied Microbiology, 32*, 71–77.

Sarantinopoulos, P., Leroy, F., Leontopoulou, E., Georgalaki, M. D., Kalantzopoulos, G., Tsakalidou, E., et al. (2002). Bacteriocin production by *Enterococcus faecium* FAIR-E 198 in view of its application as adjunct starter in Greek Feta cheese making. *International Journal of Food Microbiology, 72*, 125–136.

Schillinger, U., Geisen, R., & Holzapfel, W. H. (1996). Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends Food Science and Technology, 7*(5), 158–164.

Sherley, M., Gordon, D. M., & Collignon, P. J. (2000). Variations in antibiotic resistance profile in Enterobacteriaceae isolated from wild Australian mammals. *Environmental Microbiology, 2*, 620–631.

da Silva-Malheiros, P., Daroit, D. J., da Silveira, N. P., & Brandelli, A. (2010). Effect of nanovesicle-encapsulated nisin on growth of *Listeria monocytogenes* in milk. *Food Microbiology, 27*, 175–178.

Simha, B. V., Sood, S. K., Kumariya, R., & Garsa, A. K. (2012). Simple and rapid purification of pediocin PA-1 from *Pediococcus pentosaceous* NCDC 273 suitable for industrial application. *Microbiological Research*, . http://dx.doi.org/10.1016/j.micres.2012.01.001.

Songisepp, E., Hütt, P., Rätsep, M., Shkut, E., Kõljalg, S., Truusalu, K., et al. (2012). Safety of a probiotic cheese containing *Lactobacillus plantarum* Tensia according to a variety of health indices in different age groups. *Journal of Dairy Science, 95*, 5495–5509.

Stiles, M. E. (1996). Biopreservation by lactic acid bacteria. *Antonie Van Leeuwenhoek, 70*, 331–345.

Suda, S., Westerbeek, A., O'Connor, P. M., Ross, R. P., Hill, C., & Cotter, P. D. (2010). Effect of bioengineering lacticin 3147 lanthionine bridges on specific activity and resistance to heat and proteases. *Chemistry & Biology, 17*, 1151–1160.

Taneja, N., Rani, P., Emmanuel, R., Khudaier, B. Y., Sharma, S. K., Tewari, R., et al. (2005). Nosocomial urinary tract infection due to *Leuconostoc mesenteroides* at a tertiary care centre in north india. *Indian Journal of Medical Research, 122*, 178–179.

Twomey, D., Ross, R. P., Ryan, M., Meaney, B., & Hill, C. (2002). Lantibiotics produced by lactic acid bacteria: structure, function and applications. *Antonie Van Leeuwenhoek, 82*, 165–185.

Vadyvaloo, V., Hastings, J. W., Van Der Merwe, M. J., & Rautenbach, M. (2002). Membranes of class IIa bacteriocin-resistant *L. monocytogenes* cells contain increased levels of desaturated and snort-acyl-chain phosphatidylglycerols. *Applied and Environmental Microbiology, 68*, 5223–5230.

Van Houten, M. A., Luinge, K., Laseur, M., & Kimpen, J. L. (1998). Antibiotic utilisation for hospitalised paediatric patients. *International Journal of Antimicrobial Agents, 10*, 161–164.

Van Schaik, W., Gahan, C. G., & Hill, C. (1999). Acid-adapted *Listeria monocytogenes* displays enhanced tolerance against the lantiobiotics nisin and lacticin 3147. *Journal of Food Protection, 62*, 536–540.

Vaucher, R. A., Gewehr, C. C. V., Correa, A. P. F., Sant'Anna, V., Ferreira, J., & Brandelli, A. (2011). Evaluation of the immunogenicity and in vivo toxicity of the antimicrobial peptide P34. *International Journal of Pharmaceutics, 421*, 94–98.

Wester, C. W., Durairaj, L., Evans, A. T., Schwartz, D. N., Husain, S., & Martinez, E. (2002). Antibiotic resistance – a survey of physician perceptions. *Archives of Internal Medicine, 162*, 2210–2216.

Yuan, J., Zhang, Z. Z., Chen, X. Z., Yang, W., & Huan, L. D. (2004). Site-directed mutagenesis of the hinge region of nisin Z and properties of nisin Z mutants. *Applied Microbiology and Biotechnology, 64*, 806–815.