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Review

Antibacterial Peptides "Bacteriocins": An Overview of Their Diverse Characteristics and Applications

MAMI NISHIE[1], JUN-ICHI NAGAO[2], AND KENJI SONOMOTO[1,3]*

[1]*Division of Applied Molecular Microbiology and Biomass Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*
[2]*Section of Infection Biology, Department of Functional Bioscience, Fukuoka Dental College, 2-15-1 Tamura, Sawara-ku, Fukuoka 814-0193, Japan*
[3]*Department of Functional Metabolic Design, Bio-Architecture Center, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*

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Bacteriocins are ribosomally synthesized antibacterial peptides produced by bacteria that inhibit the growth of similar or closely related bacterial strains. A number of bacteriocins from a wide variety of bacteria have been discovered, and their diverse structures have been reported. Growing evidence suggests that bacteriocins have diverse structures, modes of action, mechanisms of biosynthesis and self-immunity, and gene regulation. Bacteriocins are considered as an attractive compound in food and pharmaceutical industries to prevent food spoilage and pathogenic bacterial growth. Furthermore, elucidation of their biosynthesis has led to the use of bacteriocin-controlled gene-expression systems and the biosynthetic enzymes of lantibiotics, a class of bacteriocins, as tools to design novel peptides. In this review, we summarize and discuss currently known information on bacteriocins produced by Gram-positive bacteria and their applications.

Key words : Antibacterial peptide / Peptide biosynthesis / Bacteriocin / Lantibiotic.

Introduction

The discovery of penicillin in 1928 by Alexander Fleming was a revolutionary event in the history of medicine. Penicillin and many other antibiotics discovered in later years have saved countless human lives from infectious diseases previously thought to be incurable. Antibiotics are unarguably one of the major scientific and medical advances of the 20th century. However, the initial widespread and inadequate use of antibiotics has led to the emergence of resistant bacteria, which are presently a public health concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) became a serious problem not only because of their pathogenicity but also because these strains can transfer their antibiotic-resistant genes to other bacteria. There is an urgent need for a new class of antibacterial compounds that are active against the multidrug-resistant strains and would not develop bacterial resistance (Sang and Blecha, 2008; Asaduzzaman and Sonomoto, 2009). Bacteriocins are novel compounds with potential antibacterial applications and are discussed in this review.

Bacteriocins are antibacterial peptides produced by bacteria to compete against bacteria of the same species or other genera (Cotter et al., 2005b). BACTIBASE is an open-access database designed for the characterization of bacteriocins, and the number of entries continues to grow. The database contains 200 bacteriocin sequences (July, 2011), most of which are the products of Gram-positive bacteria, particularly lactic acid bacteria (LAB) (Hammami et al., 2010). LAB are attractive bacteriocin producers

*Corresponding author. Tel/Fax : +81-92-642-3019, E-mail : sonomoto(a)agr.kyushu-u.ac.jp

because of their GRAS (generally recognized as safe) status as designated by the U.S. Food and Drug Administration (FDA), indicating their safe and easy application as food preservatives or pharmaceuticals. Nisin A, a representative and well-studied LAB bacteriocin, was first discovered in 1928 (Rogers and Whittier, 1928) and has been used as a commercial food preservative in more than 50 countries (Cotter et al., 2005b). Non-LAB bacteriocins are also worthy of attention; for instance, some of the bacteriocins produced by the genus *Staphylococcus* were shown to be active against multidrug-resistant *S. aureus* and coagulase-negative staphylococci involved in human infections (Nascimento et al., 2006). Colicins or microcins produced by *Escherichia coli* are able to inhibit the growth of Gram-negative bacteria, while most LAB-derived bacteriocins cannot (Duquesne et al., 2007). However, the term bacteriocin is now used mainly to designate antibacterial peptides produced by Gram-positive bacteria. This review will summarize and discuss bacteriocins produced by Gram-positive bacteria, including their classification, mode of action, biosynthesis mechanism, gene regulation, self-immunity mechanism, and potential for industrial application.

Classification

Although the first identified bacteriocin was colicin produced by *E. coli* in 1925 (Duquesne et al., 2007), LAB bacteriocins have been the most studied thus far. LAB are often found in foods, having unknowingly been utilized in the manufacture of fermented foods such as cheese, yogurt, kimchi, and traditional fermented foods in each various areas of the world. LAB bacteriocins also play an important role in inhibiting the growth of spoilage-causing bacteria.

Cotter et al. (2005b) have classified LAB bacteriocins into 2 distinct categories (Table 1). Class I is comprised of so-called "lantibiotics," which stands for lanthionine-containing antibiotics, and class II includes non-lanthionine-containing bacteriocins. Large, heat-labile proteins, often murein hydrolases, were excluded from previously classified bacteriocins and named bacteriolysins. Here, non-LAB bacteriocins will also be discussed.

Class I bacteriocins (lantibiotics)

Class I bacteriocins, the so-called lantibiotics, are small peptides (19-38 amino acids) that contain unusual amino acids introduced by post-translational enzymatic modifications (Nagao et al., 2006; Field et al., 2010; Lee and Kim, 2011) (Fig. 1). Unusual amino acids such as dehydrated amino acids, lanthionine, and 3-methyllanthionine, which form multiple ring structures, distinguish lantibiotics from other antibacterial peptides and contribute to the lantibiotics' structural stability against heat, a wide range of pH and proteolysis, and to their increased tolerance to oxidation (Sahl et al., 1995; Bierbaum et al., 1996).

Lantibiotics were previously classified as either type A or type B according to their structure and function. Elongated amphiphilic peptides such as nisin from *Lactococcus lactis* and Pep5 from *Staphylococcus epidermidis* 5 were type A, whereas globular peptides such as mersacidin from *Bacillus* sp. and actagardine from *Actinoplanes* sp. were type B. Type A lantibiotics were further divided into two subtypes: type A(I), which have an elongated form, such as nisin and subtilin from *Bacillus subtilis* ATCC

TABLE 1. Classification of bacteriocins

Classification	Remarks	Examples
Class I (Lantibiotics)	Lanthionine-containing bacteriocins	
Class I lantibiotics	Unusual amino acids introduced by LanB and LanC	Nisin A, Subtilin, Epidermin, Pep5
Class II lantibiotics	Unusual amino acids introduced by LanM	Lacticin 481, Nukacin ISK-1, Mersacidin, Lacticin 3147 (two-peptide bacteriocin)
Class II	Non-lanthionine-containing bacteriocins	
Class IIa	Pediocin-like bacteriocins Specific against *Listeria monocytogenes*	Pediocin PA-1/AcH, Leucocin A
Class IIb	Two-peptide bacteriocins	Lactococcin G, Lactococcin Q
Class IIc	Cyclic bacteriocins	Enterocin AS-48, Gassericin A, lactocyclicin Q
Class IId	Single-peptide non-pediocin-like linear bacteriocins	Lactococcin A, Lacticin Q

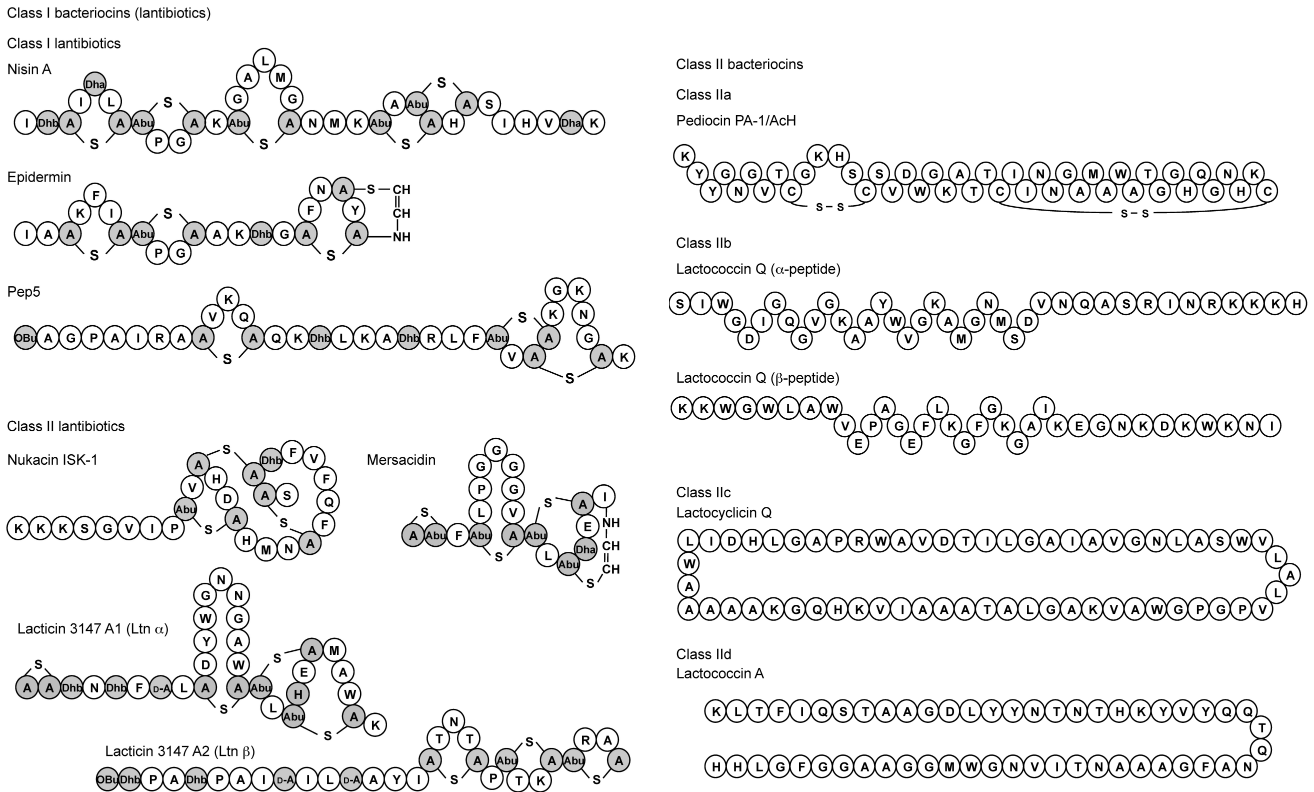

FIG. 1. Structure of bacteriocins. Classification is based on the proposal by Cotter et al. (2005b). Classification of lantibiotics is based on the biosynthesis pathways of peptides proposed by Willey and van der Donk (2007). The shaded residues represent unusual amino acids. Dha, dehydroalanine; Dhb, dehydrobutyrine; A-S-A, lanthionine; Abu-S-A, 3-methyllanthionine; OBu, 2-oxobutyryl; D-A, D-alanine; fM, formylmethionine

6633; type A(II), which have an unbridged N-terminal tail and a globular C-terminal ring, such as lacticin 481 from *L. lactis* and nukacin ISK-1 from *Staphylococcus warneri* ISK-1 (Jung and Sahl, 1991; Dufour et al., 2007). However, as the number of lantibiotics increased and their characteristics were elucidated, this classification became unclear. Recently, on the basis of their structure and mode of action, lantibiotics were divided into 11 subgroups (Nisin, Epidermin, Streptin, Pep5, Lacticin 481, Mersacidin, LtnA, Cytolysin, Lactocin S, Cinnamycin, and Sublancin groups) representing the names of well-known lantibiotics with different features (Cotter et al., 2005a). This classification pointed out that a number of natural variants of lantibiotics exist, and it is likely that there are a large number of other variants that have not yet been isolated. In addition, the existence of natural variants suggests that there is flexibility regarding some of the amino acids at certain positions, and it may thus be possible to engineer peptides without reducing their activity.

Lantibiotics are also classified on the basis of the pathway by which maturation of the peptide occurs (Table 1). This designation mainly incorporates differences in the leader peptide sequence and biosynthetic operon structure (Pag and Sahl, 2002; Willey and van der Donk, 2007). Class I lantibiotics mainly consist of what have been classically called type A(I) lantibiotics, the prepeptides of which are modified by 2 distinct enzymes, LanB and LanC. LanB dehydrates the Ser and Thr residues of prepeptides, and LanC mediates cyclization of dehydrated residues with intramolecular Cys. Class II lantibiotics are classically called type A(II) and type B lantibiotics. Modification enzyme LanM exhibits both dehydration and cyclization activities.

Class II bacteriocins

Class II bacteriocins are non-lanthionine-containing bacteriocins. Unlike lantibiotics, they are not post-translationally modified by dedicated enzymes. The heterogeneous nature of this class of bacteriocins makes further classification difficult, and thus several grouping approaches have been proposed (Nes et al., 1996; van Belkum and Stiles, 2000; Cotter et al., 2005b). Here, we follow the classification by Cotter et

al. (2005b), which subdivides class II bacteriocins into 4 groups (classes IIa, IIb, IIc, and IId) (Table 1).

Class IIa bacteriocins are pediocin-like peptides that are highly active and specific against the food pathogen *Listeria monocytogenes*. Pediocin PA-1/AcH from *Pediococcus* sp. or *Lactobacillus plantarum* contains the N-terminal consensus sequence YGNGVXC, a characteristic of class IIa bacteriocins (Henderson et al., 1992; Motlagh et al., 1992; Drider et al., 2006).

Class IIb bacteriocins are two-peptide bacteriocins consisting of 2 unmodified peptides, both of which are required for antibacterial activity. When tested individually, these bacteriocins display very low, if any, antibacterial activity. Each peptide pair is encoded by adjacent open reading frames in the same operon, and transcribed and synthesized together (Oppegard et al., 2007). Lactococcin G from *L. lactis* subsp. *lactis* LMG 2081(Nissen-Meyer et al., 1992) was the first to be characterized to belong to this class; other peptides such as ABP-118 (Flynn et al., 2002), plantaricin NC8 (Maldonado et al., 2003), enterocin 1071 (Franz et al., 2002), and lactococcin Q (Zendo et al., 2006) have been identified as class IIb bacteriocins.

Class IIc bacteriocins are cyclic peptides whose N- and C-termini are covalently linked by a peptide bond (van Belkum et al., 2011). Enterocin AS-48 produced by a variety of enterococci was the first discovered class IIc bacteriocin (Martinez-Bueno et al., 1994; Samyn et al., 1994). LAB-derived class IIc bacteriocins include gassericin A from *Lactobacillus gasseri* LA39 (Kawai et al., 1998) and the recently identified uberolysin from *Streptococcus uberis* (Wirawan et al., 2007), and lactocyclicin Q from *Lactococcus* sp. (Sawa et al., 2009). Non-LAB-derived bacteriocins such as butyrivibriocin AR10 from *Butyrivibrio fibrisolvens* AR10 (Kalmokoff et al., 2003) have been recently identified.

The remaining bacteriocins that have no significant sequence similarity with the other class II bacteriocins are categorized as class IId bacteriocins. This group includes single-peptide non-pediocin-like linear bacteriocins (Cotter et al., 2005b; Nissen-Meyer et al., 2009). These bacteriocins are greatly diverse, and there are few proposals to subdivide these peptides according to leader peptide sequences. The leader peptide sequences of divergicin A from *Carnobacterium divergens* (Worobo et al., 1995), lactococcin 972 from *L. lactis* IPLA972 (Martinez et al., 1999), and some other bacteriocins have a cleavage site, which acts as a signal peptide that accesses the general secretory (*sec*-) pathway (Diep and Nes, 2002). On the other hand, some class IId bacteriocins such as enterocin L50 from *Enterococcus faecium* L50 (Cintas et al., 1998), aureocin A70 from *S. aureus* A70 (Netz et al., 2001), and lacticin Q and lacticin Z from *L. lactis* (Fujita et al., 2007; Iwatani et al., 2007) are synthesized without an N-terminal leader sequence, and thus are called "leaderless bacteriocins." However, class IId bacteriocins that do not fit into the *sec*-dependent or leaderless subgroup still remain; however, as long as scientists search for novel bacteriocins, it may be impossible to strictly classify and define them.

Mode of action

Because there is an expectation that bacteriocins will serve as industrial food preservatives or as an alternative to pharmaceutical antibiotics, the risk of developing resistant strains should be avoided by using them intelligently, based on "scientific evidence." Therefore, active research has been dedicated to study the bacteriocin mode of action on the molecular level. The mechanism of bacteriocin activity against target bacteria should provide information on how to prevent the emergence of bacteriocin-resistant bacteria. Bacteriocins superficially resemble each other in that they are often membrane permeabilizing, cationic, and amphiphilic or hydrophobic (Nissen-Meyer et al., 2009). However, research has shown that there are a variety of modes of action; a single bacteriocin possesses more than one mode of action to attack the target bacteria (Wiedemann et al., 2001; Hasper et al., 2006).

The modes of action of bacteriocins are linked to the primary structures of the peptides. It has been reported that several lantibiotics target lipid II, a membrane-bound cell wall precursor, as a docking molecule (Breukink and de Kruijff, 2006). Nisin is a pore-forming lantibiotic that kills the target strain by permeabilizing the plasma membrane, leading to the efflux of intracellular molecules (Fig. 2A). It has been shown that the high activity of nisin in the nanomolar range is the result of a high-affinity binding to lipid II (Breukink et al., 1999). The NMR structure of the nisin-lipid II complex revealed that the N-terminus of nisin binds to the pyrophosphate of lipid II (Hsu et al., 2004). Mutation in the hinge region causes inactivation in pore formation, suggesting the importance of this region in pore formation. This mutant, however, retains antibacterial activity to some extent, showing that nisin can kill bacteria by inhibiting peptidoglycan synthesis through interaction with lipid II (Wiedemann et al., 2001). It was demonstrated that nisin removes lipid II from the cell division site and thus blocks cell wall synthesis (Hasper et al., 2006). Mutacin 1140 is a bactericidal lantibiotic that shares many structural

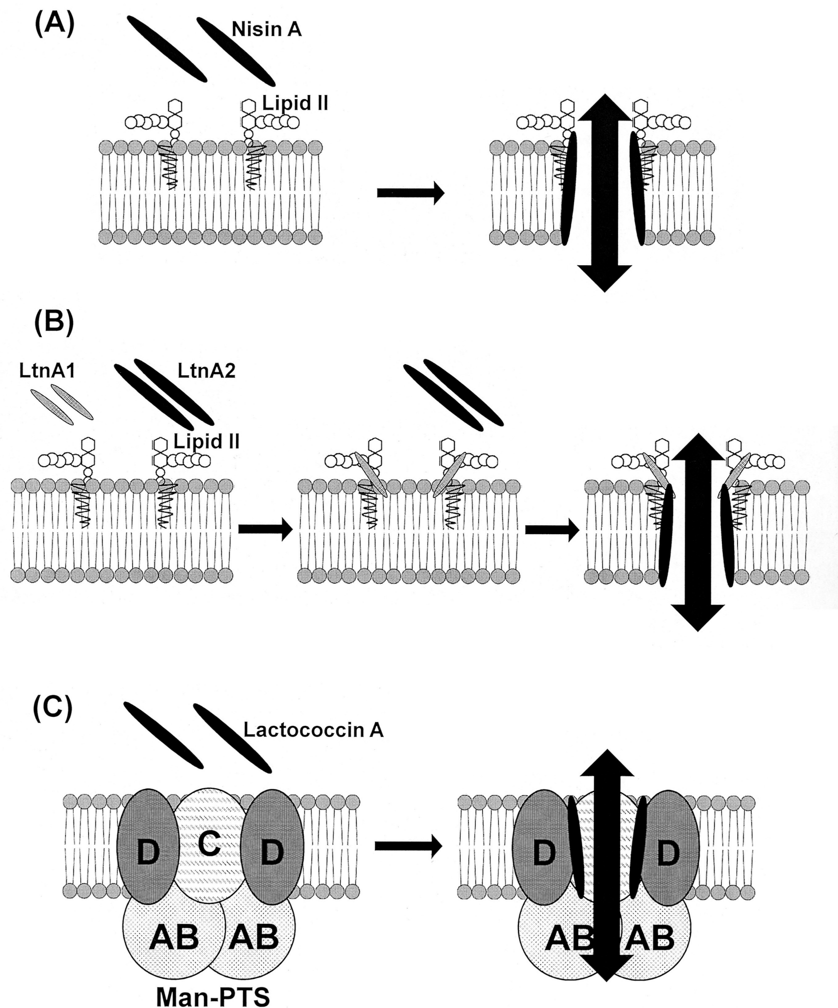

FIG. 2. Model of target-cell killing for nisin A (A), lacticin 3147 (B), and lactococcin A (C). (A) The N-terminus of nisin A binds the pyrophosphate of lipid II and permeabilizes the plasma membrane, resulting in pore formation, which leads to the efflux of intracellular molecules. (B) LtnA1 and LtnA2 interact synergistically to produce antibiotic activity in the two-peptide lantibiotic lacticin 3147. LtnA1 first interacts specifically with lipid II, the lipid II-LtnA1 complex is then able to recruit LtnA2, and finally, a high-affinity 3-component complex forms pores. (C) Lactococcin A employs components IIC and IID of the man-PTS as a receptor, but the cytoplasmic component IIAB is not required for this function. The bound lactococcin A triggers permeabilization of the membrane, causing leakage of intracellular components.

and sequence similarities with the N-terminus of nisin (lipid II-binding domain). Although the mutacin 1140-lipid II complex is as stable as nisin-lipid II, this lantibiotic cannot form pores *in vivo*, thus warranting further study of its mode of action (Smith et al., 2008).

Lacticin 3147 is a two-peptide lantibiotic, with peptides LtnA1 and LtnA2 acting synergistically to produce antibiotic activity. It was proposed that LtnA1 first interacts specifically with lipid II, then the lipid II-LtnA1 complex is able to recruit LtnA2, and finally, a 3-component complex inhibits cell wall biosynthesis and forms pores (Wiedemann et al., 2006) (Fig. 2B). Mersacidin is a lipid II-binding peptide that inhibits cell wall synthesis but does not induce pore formation. The binding site on lipid II was suggested to be the terminal GlcNAc sugar, which is different from nisin (Brötz et al., 1998).

Class IIa bacteriocins such as pediocin PA-1/AcH use components of the mannose phosphotransferase system (man-PTS) of susceptible cells as receptors and exert their activity by dissipation of the proton motive force via membrane pore formation (Ramnath et al., 2004; Diep et al., 2007; Nissen-Meyer et al.,

2009). Man-PTS is the key pathway for mannose and glucose uptake in bacteria. Recently, the extracellular loop of the membrane-located man-PTS was shown to be responsible for specific target recognition by class IIa bacteriocins (Kjos et al., 2010).

Class IIb two-peptide bacteriocins consist of 2 different unmodified peptides, both of which must be present in approximately equal amounts in order to exert antibiotic activity (Cotter et al., 2005b). Lactococcin G (Gα plus Gβ) and lactococcin Q (Qα plus Qβ) are class IIb bacteriocins isolated from *L. lactis*. Peptides α and β show a high level of homology, with only 6 and 3 amino-acid differences in mature peptides, respectively. The hybrid combinations of these peptides such as Gα plus Qβ or Qα plus Gβ showed antibacterial activity, indicating these bacteriocins to be exchangeable (Zendo et al., 2006). Thus far, permeabilization of the target cell membrane is considered as the mode of action of class IIb bacteriocins (Oppegard et al., 2007). They induce the leakage of internal substances such as monovalent cations, phosphate, and ATP (Abee et al., 1994; Moll et al., 1996; Moll et al., 1999; Cuozzo et al., 2003). Secondary structural studies of lactococcin G, plantaricin E/F, and plantaricin J/K have revealed that membrane-like entities induce amphiphilic α-helices of both peptides, and additional structuring was obtained when complementary peptides were exposed simultaneously to the membrane entities (Hauge et al., 1998; Hauge et al., 1999). Although integrated membrane proteins are speculated to be a receptor, recognition targets in the class IIb bacteriocin system have yet to be identified.

Similar to most bacteriocins, class IIc cyclic bacteriocins permeabilize the membrane of susceptible cells, resulting in the leakage of ions, dissipation of the membrane potential, and ultimately cell death (van Belkum et al., 2011). Interestingly, enterocin AS-48, gassericin A, subtilosin A, and carnocyclin A can exert their activity without any receptor (Galvez et al., 1991; Kawai et al., 2004; Thennarasu et al., 2005; Gong et al., 2009). Enterocin AS-48 forms nonselective pores, which cause the leakage of low molecular weight compounds (Galvez et al., 1991). On the other hand, carnocyclin A forms anion-selective ion channels, which result in voltage-dependent pore formation that is abolished upon membrane depolarization (Gong et al., 2009).

Class IId (non-categorized) bacteriocins are diverse in their structures as well as modes of action. Lactococcin A and lactococcin B are suggested to utilize the man-PTS as a receptor, permeabilize the cytoplasmic membrane, and cause leakage of solutes across the membrane. Lactococcins A and B do not share sequence similarity to each other or with class IIa bacteriocins. Unlike class IIa bacteriocins, which require a part of the IIC protein of man-PTS, lactococcins A and B require both IIC and IID proteins for man-PTS targeting (Diep et al., 2007) (Fig. 2C). Lacticin Q is a leaderless class IId bacteriocin. Previous studies reported that lacticin Q does not require a receptor for its membrane-permeabilizing activity and acts via a toroidal-pore mechanism (Yoneyama et al., 2009a; Yoneyama et al., 2009b). Lactococcin 972 (Lcn972) was the first identified non-lantibiotic bacteriocin that specifically interacts with lipid II. Lcn972 activity was antagonized by lipid II *in vivo*. It co-precipitates with lipid II micelles and inhibits the activity of 2 enzymes, PBP2 and FemX, which use lipid II as a substrate. In the case of nisin, the N-terminal lanthionine rings are involved in lipid II binding. Lcn972 lacks a lanthionine ring, and hence might display a novel lipid II-binding motif (Martinez et al., 2008).

Resistance to bacteriocins

Nisin A and pediocin PA-1/AcH are thus far the only bacteriocins commercially used as food preservatives. The emergence of bacteriocin-resistant strains should be completely avoided; therefore, understanding the mechanism of bacteriocin resistance is of vital importance.

One important mediator of bacterial stress response is the alternative sigma factor SigB. SigB has been shown to contribute positively to *L. monocytogenes* tolerance of the bacteriocins nisin and lacticin 3147 (Begley et al., 2006). The *L. monocytogenes* two-component signal transduction system, LisRK, plays a significant role in acid, ethanol, and oxidative stress and in murine virulence. It was proposed that LisRK also determines the sensitivity of *L. monocytogenes* to nisin (Cotter et al., 2002). VirRS is a two-component system that regulates the expression of *dltA* and *mprF* in *L. monocytogenes* and influences the bacterium's susceptibility to cationic antibacterial peptides (Mandin et al., 2005). The deletion of *virR* and *mprF* is found to result in 32- and 16-fold reductions in resistance to nisin (Collins et al., 2010a). The penicillin-binding protein gene *pbp* (Gravesen et al., 2001), the tellurite resistance gene *telA* (Collins et al., 2010b), the gene expressing the permease component of an ATP-binding cassette (ABC) transporter, *anrB* (Collins et al., 2010a), and the glutamate decarboxylase gene *gad* (Begley et al., 2010) are also reported to play a role in the innate resistance of *L. monocytogenes* to nisin.

The mechanism of resistance to nisin and class IIa

bacteriocins is related to cell membrane fluidity and cell-surface charge (Vadyvaloo et al., 2002; Vadyvaloo et al., 2004). It was recently demonstrated that there are 2 major resistance mechanisms of man-PTS-targeting lactococcin A in *L. monocytogenes* and *L. lactis* (Kjos et al., 2010). The first involves downregulation of man-PTS gene expression, and the second involves normal expression of the man-PTS system, but the underlying mechanism of resistance for these cells is unknown. However, it has been suggested that cell surface changes that affect the interaction between the bacteriocin and its membrane-located receptor might be involved.

Studies on bacteriocin resistance have focused only on specific bacteriocins such as nisin and some man-PTS-targeting peptides. Extensive use of a variety of bacteriocins would require painstaking research to avoid the possible development of widespread bacteriocin resistance.

Biosynthetic mechanism (biosynthetic enzymes)

Bacteriocins are usually ribosomally synthesized as precursor peptides that consist of an N-terminal leader peptide and a C-terminal pro-region. The leader peptide keeps the bacteriocins inactive and is speculated to protect the producing organisms from being killed by its own bacteriocin. The leader peptide, which is thought to be a scaffold for the biosynthetic enzymes, is cleaved off, and the released pro-region becomes a mature bacteriocin (Cotter et al., 2005b; Dufour et al., 2007; Oppegard et al., 2007; Willey and van der Donk, 2007).

Lantibiotics require post-translational modifications to introduce unusual amino acids into the pro-region. Class I lantibiotics such as nisin, subtilin, and Pep5 are modified by 2 distinct enzymes, LanB and LanC. LanB dehydrates the serine and threonine residues of the pro-region, and LanC cyclizes dehydrated residues with cysteine. LanT is an ABC transporter that transports the modified precursor peptides. Finally, an extracellular serine protease, LanP, cleaves off the N-terminal leader peptide (Fig. 3A). Yeast two-hybrid system and co-immunoprecipitation studies revealed that the modification enzymes (NisB, NisC) and transporter (NisT) of nisin form a membrane-associated multimeric complex (Siegers et al., 1996). Although NisC shows no sequence similarity with any known proteins, a crystal structure analysis of NisC revealed a fold similarity with mammalian farnesyltransferase (Li et al., 2006).

Class II lantibiotics such as lacticin 481 are modified by a single enzyme, LanM (Fig. 3B). LctM, the modification enzyme of lacticin 481, has been proposed to use Mg^{2+} and ATP to phosphorylate the Ser and Thr residues that are targeted for dehydration. Subsequent phosphate elimination then results in net dehydration and the formation of the Dha and Dhb structures. Some of the N-terminal conserved residues of LctM are predicted to be important for phosphorylation and dehydration, whereas the C-terminal zinc ligands are critical for cyclization (Chatterjee et al., 2005; Paul et al., 2007; You and van der Donk, 2007). LanT of class II lantibiotics is an ABC transporter maturation and secretion (AMS) protein that contains a proteolytic enzyme responsible for the cleavage of the leader peptide (Fig. 3B). The leader peptide shares a consensus sequence and a common processing site with 2 conserved glycine residues, called "double-glycine site," at positions -1 and -2 (Håvarstein et al., 1995; Furgerson Ihnken et al., 2008; Nishie et al., 2009). NukT is an ABC transporter of the class II lantibiotic nukacin ISK-1, and it was suggested that the N-terminal peptidase domain and C-terminal ATP-binding domain are both important for the cleavage of the leader peptide (Nishie et al., 2011). Lacticin 3147 is a two-peptide class II lantibiotic. LtnA1 and LtnA2 are modified by the respective modification enzymes LtnM1 and LtnM2. LtnJ catalyzes the conversion of dehydroalanines to D-alanines, and the modified peptides are transported by a single ABC transporter, LtnT (Ryan et al., 1999; McAuliffe et al., 2000; Cotter et al., 2005c).

Although class II bacteriocins do not undergo post-translational modification, they are also synthesized as an inactive precursor peptide that contains an N-terminal leader peptide sequence. As in the case of class II lantibiotics, most of class IIa bacteriocins contain a "double-glycine site" at the leader peptide sequence (Håvarstein et al., 1995). Concomitant with the cleavage of the leader peptide, mature bacteriocins are exported across the cytoplasmic membrane by a dedicated AMS protein-type ABC transporter (Fig. 3C). However, some class IIa bacteriocins contain a *sec*-dependent N-terminal leader sequence, such as bacteriocin 31 from *Enterococcus faecalis* 31 (Tomita et al., 1996), enterocin P from *E. faecium* P13 (Cintas et al., 1997), listeriocin 743A from *Listeria innocua* 743 (Kalmokoff et al., 2001), enterocin SE-K4 from *E. faecalis* K-4 (Doi et al., 2002), bacteriocin T8 from *E. faecium* T8 (De Kwaadsteniet et al., 2006), and hiracin JM79 from *Enterococcus hirae* DCH5 (Sanchez et al., 2008).

For all genetically characterized two-peptide class IIb bacteriocins, the 2 structural genes are found next to each other on the same operon, and the 2 peptides

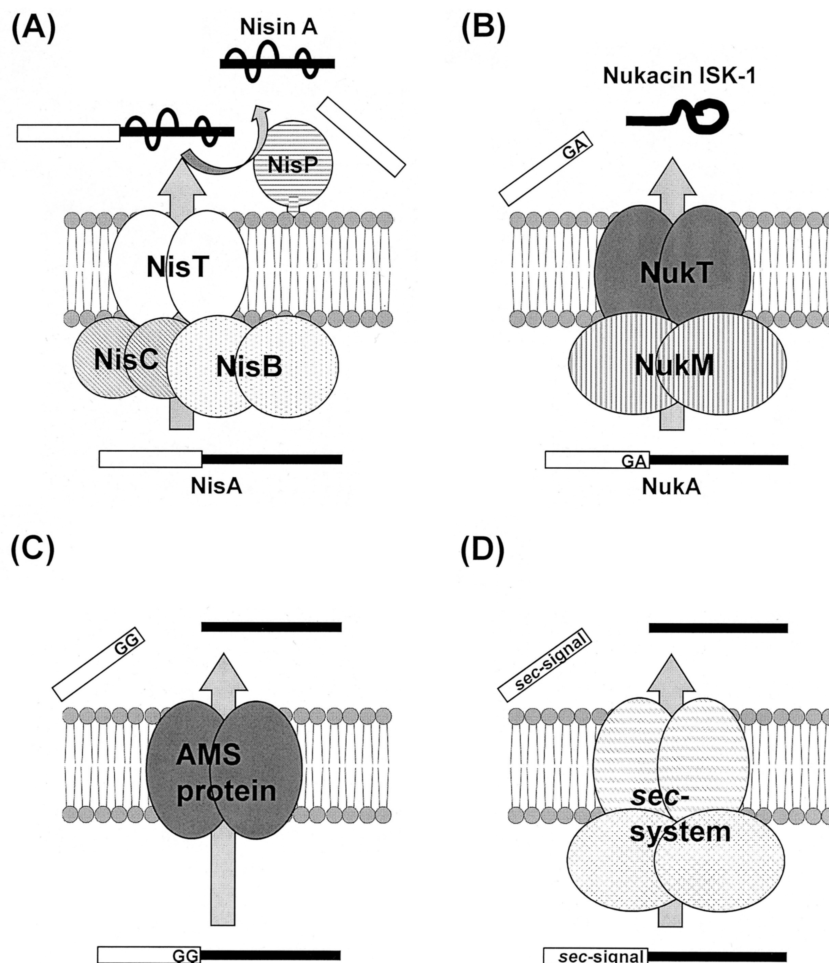

FIG. 3. Biosynthetic enzymes involved in bacteriocin production. Precursor peptides containing an N-terminal leader sequence are ribosomally synthesized, modified, and secreted as mature peptides. (A) Biosynthesis of nisin A (class I lantibiotic). Precursor peptide, NisA, is dehydrated by NisB, cyclized by NisC, and secreted by ABC transporter NisT. The leader peptide is cleaved off by extracellular serine protease NisP. (B) Biosynthesis of nukacin ISK-1 (class II lantibiotic). Precursor peptide NukA is dehydrated and cyclized by NukM, and the leader peptide is cleaved off at a "double-glycine site" concomitantly with transport by NukT. (C) Biosynthesis of AMS protein-dependent class II bacteriocins. The precursor peptide contains a "double-glycine site" at the C-terminus of the leader peptide. The leader peptide is cleaved off by AMS protein concomitantly with transport. (D) Biosynthesis of *sec*-dependent bacteriocins. The precursor peptide is ribosomally synthesized with the N-terminal *sec*-signal peptide. These bacteriocins do not require any dedicated transporter but are secreted by the general *sec*-pathway of the producer cell. The signal peptide is cleaved off during transport.

that constitute the bacteriocin thus appear to be produced in approximately equal amounts. Class IIb bacteriocins also contain a double-glycine site at the leader sequence, and the AMS protein-type ABC transporter is responsible for extracellular transport (Diep et al., 1996; Franz et al., 2002; Zendo et al., 2006; Oppegard et al., 2007).

For class IIc bacteriocins, the mechanism of biosynthesis remains unclear. Although the enzymes responsible for cyclization and leader peptide cleavage are still unknown, the genes that are minimally required for bacteriocin production have been identified for enterocin AS-48, circularin A, gassericin A, and carnocyclin A. They include putative multiple membrane-spanning domains, ATP-binding proteins, and a small hydrophobic peptide that may be involved in immunity (Martinez-Bueno et al., 1998; Kemperman et al., 2003; Kawai et al., 2009; van Belkum et al., 2011).

Divergicin A (Worobo et al., 1995), lactococcin

972 (Martinez et al., 1999), and some other bacteriocins are *sec*-dependent class IId bacteriocins. These bacteriocins have a signal peptide that accesses the general secretory *sec*-pathway and enables translocation across the cytoplasmic membrane without any dedicated transporters (Diep and Nes, 2002) (Fig. 3D). Enterocin L50 (Cintas et al., 1998), aureocin A70 (Netz et al., 2001), lacticin Q (Fujita et al., 2007), and lacticin Z (Iwatani et al., 2007) are leaderless bacteriocins, which are synthesized without an N-terminal leader sequence. Detailed biosynthesis mechanisms of leaderless bacteriocins, such as recognition by transporters, remain unclear. In addition, how the producer strains protect themselves from antibacterial activity without a leader peptide is still an unanswered question.

Genetic organization and gene regulation

The genetic determinants of bacteriocins are located either on the chromosome or on plasmids, and often associated with transferable elements. The genes related to bacteriocin production, including structural sequence, regulatory, modification, transporter, and immunity genes, are generally found in clusters. Lantibiotic gene clusters are composed of the structure gene (*lanA*), modification enzymes (*lanB*, *lanC*, or *lanM*), ABC transporter (*lanT*), proteolytic enzyme (*lanP*), self-protection protein (*lanFEG*, *lanI*, or *lanH*), and transcriptional regulators (*lanR*, *lanK*, *lanQ*, or *lanX*) (Willey and van der Donk, 2007). Nisin production is encoded by a gene cluster, *nisABTCIPRKFEG*. Nisin acts as an extracellular peptide pheromone signal involved in the regulation of its own biosynthesis. The response regulator NisR and the histidine kinase NisK are also involved in the regulation of nisin biosynthesis (Buchman et al., 1988; Kuipers et al., 1995a; Kleerebezem and Quadri, 2001). Subtilin, a lantibiotic structurally related to nisin, follows the nisin model, which is subject to strict autoregulation (Kleerebezem, 2004; Kleerebezem et al., 2004). Mersacidin is a globular shaped class II lantibiotic and is also regulated by an auto-induction mechanism (Schmitz et al., 2006). The regulatory characteristics of nisin biosynthesis were exploited to develop a *ni*sin *c*ontrolled *e*xpression (NICE) system for *L. lactis*. Several *nis*-promoter vectors were constructed that appeared suitable for tightly controlled expression of both homologous and heterologous genes in *L. lactis* (Kuipers et al., 1995b).

Class IIa bacteriocins require at least 4 genes including structural, immunity, ABC transporter, and accessory protein genes (Ennahar et al., 2000; Fimland et al., 2005). Some members of class IIa bacteriocins, such as curvacin A, sakacin P, carnobacteriocin B2, and enterocin A, are transcriptionally regulated through a three-component signal transduction system. Peptide-pheromone induction factor interacts with a membrane-associated histidine kinase, thereby triggering the kinase to phosphorylate the intracellular response regulators to activate the genes needed for bacteriocin production (Kleerebezem and Quadri, 2001). Some class IIb bacteriocins, such as ABP-118, plantaricin E/F, and plantaricin J/K, are also known to be transcriptionally regulated through a three-component regulatory system. Plantaricin A is a peptide pheromone that induces the production of plantaricin E/F and plantaricin J/K (Anderssen et al., 1998; Flynn et al., 2002).

Self-immunity mechanism

Bacteriocin producers usually produce self-immunity proteins that protect the bacteria from being killed by their own bacteriocins. In the case of lantibiotics, the ABC transporter-type protein LanFEG and specific immunity protein LanI or LanH are responsible for self-immunity (Willey and van der Donk, 2007; Okuda and Sonomoto, 2011) (Fig. 4A). LanFEG proteins scavenge membrane-bound lantibiotics from the membrane to protect cells from attack by self-produced lantibiotics. SpaFEG of the subtilin producer (Klein and Entian, 1994) and NisFEG of the nisin producer (Siegers and Entian, 1995) were identified as immunity proteins at the early stage of lantibiotic immunity study. LanF is a nucleotide-binding domain (NBD) of the ABC transporter, and multiple sequence alignment analysis of LanF proteins and NBDs of other ABC transporters revealed that LanF proteins have the E-loop as a variant of the Q-loop conserved in general ABC transporters, and E-loop was suggested to have an important role in the function of LanFEG (Okuda et al., 2010). LanI proteins are divided into 3 groups, including lipoproteins (NisI and SpaI), immunity peptides existing on the membrane and extracellular space (PepI and EciI), and transmembrane proteins (LtnI, CylI, and SunI) (Engelke et al., 1994). NukH is a transmembrane protein found to be involved in self-immunity through its binding activity against nukacin ISK-1 (Aso et al., 2005). LanI or LanH alone cannot confer full immunity, and functions cooperatively with LanFEG (Okuda et al., 2008).

The self-immunity proteins of class IIa bacteriocins are well structured α-helical proteins that consist of between 88 and 115 amino acid residues and display 5% to 85% sequence similarities (Dalhus et al., 2003; Sprules et al., 2004; Fimland et al., 2005; Johnsen et al., 2005). Lactococcin A is a bacteriocin that targets

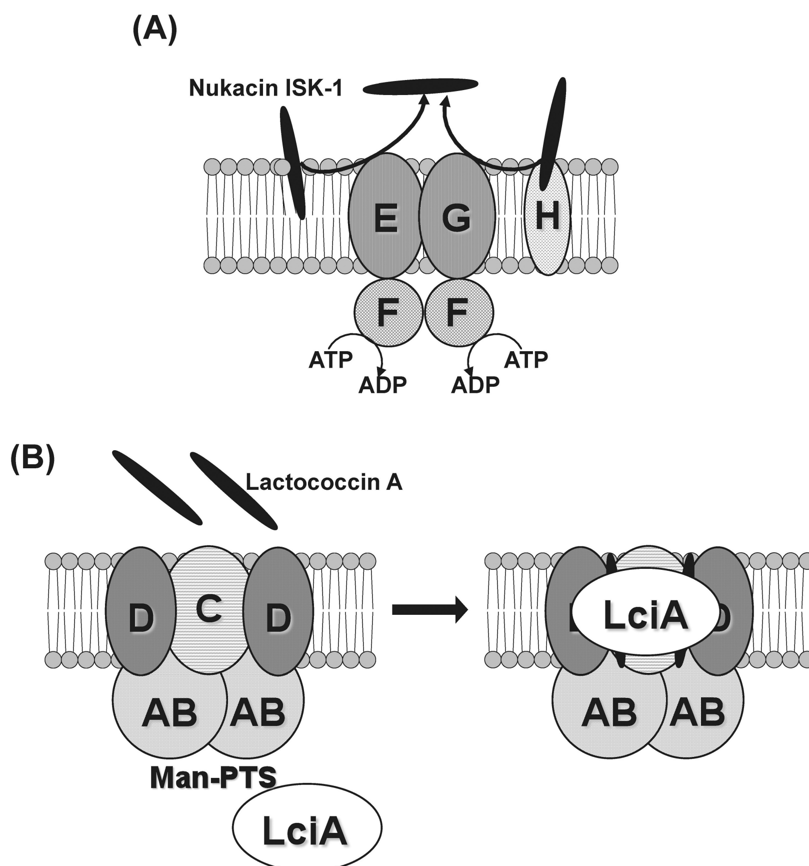

FIG. 4. Self-immunity mechanism of nukacin ISK-1 (A) and lactococcin A (B). (A) NukFEG is an ABC transporter that transports cell-associated nukacin ISK-1, and NukH exhibits binding activity against nukacin ISK-1. Nukacin ISK-1, captured by NukH, is transported to the extracellular space by NukFEG in an energy-dependent manner. (B) LciA is a self-immunity protein for lactococcin A. LciA forms a strong complex with lactococcin A and its receptor proteins (man-PTS), and thereby the cells are protected through the blocking of the bound lactococcin A from advancing to the subsequent steps that lead to cell death.

man-PTS of the susceptible cells. The immunity protein LciA has been demonstrated to form a strong complex with the receptor protein and the bacteriocin, thereby preventing cells from being killed. The complex is formed only in the presence of bacteriocin, and the membrane components IIC and IID of man-PTS are sufficient for sensitivity to lactococcin A as well as complex formation with LciA (Diep et al., 2007) (Fig. 4B). For class IIb bacteriocin, it is hypothesized that the lactococcin G immunity protein also interacts with the lactococcin G receptor (Nissen-Meyer et al., 2010). The gene clusters of class IIc and IId bacteriocins contain ABC transporter-like or small hydrophobic peptide genes that may be involved in immunity, but further studies are required to understand their self-protection mechanism in detail (Nissen-Meyer et al., 2009; van Belkum et al., 2011).

Applications of bacteriocins

LAB-derived bacteriocins are promising as food preservatives given their characteristics of having no taste, no odor, high stability, and specific activity against foodborne pathogenic and spoilage-causing bacteria. LAB bacteriocins are considered as safe compounds for humans because of their nontoxicity and the GRAS status of the producer, which is a food-grade organism. Nisin and pediocin PA-1 can be purchased for use as food additives as "Nisaplin" and "ALTA 2341," respectively (Cotter et al., 2005b). The combination of bacteriocins and other treatments, such as pulsed electric fields, high hydrostatic pressure, and organic acids, enhances the potency of bacteriocins and prevents the growth of Gram-negative bacteria such as *Escherichia* and *Pseudomonas*, which cannot be inhibited by simple treatment with bacteriocins (Ananou et al., 2004; Grande et al., 2007; Martinez Viedma et al., 2008).

Some lantibiotics such as nisin, mersacidin, mutacin 1140, and lacticin 3147 are reported to be active against drug-resistant pathogens such as MRSA and VRE, which makes them an attractive option as possible therapeutic agents. Nisin F was injected into *S. aureus*-infected mice and was able to control the growth of *S. aureus* for at least 15 min *in vivo* (Brand et al., 2010). Previous studies evaluated the activity of nisin in the treatment of bovine mastitis. Because treatment of subclinical mastitis is not suggested for lactating cows to avoid the risk of milk contamination, nisin might be a potentially safe compound to treat this disease (Cao et al., 2007; Wu et al., 2007).

In addition to the use of bacteriocins as novel antibacterial compounds in food or pharmaceutical products, the application of bacteriocins, in particular lantibiotic biosynthetic enzymes, in peptide engineering has recently attracted attention (Cotter et al., 2005a; Nagao et al., 2006; Rink et al., 2010; Nagao et al., 2011). Lantibiotics are gene-encoded and, therefore, can be readily manipulated by genetic engineering. Several *in vivo* and *in vitro* approaches have been explored to engineer novel lantibiotics. A number of different systems have been used to mutate specific amino acids in lantibiotics, and some of the produced mutants displayed improved activity (Cotter et al., 2006; Field et al., 2007; Lubelski et al., 2008; Islam et al., 2009). The use of lantibiotic modification enzymes to introduce unusual amino acids in *E. coli* has many advantages, such as easy genetic manipulation and rapid and instant use (Nagao et al., 2005; Caetano et al., 2011). Lantibiotic biosynthetic enzymes could introduce unusual amino acids into not only the variants of parental peptides but also nonrelated peptides fused to the leader peptide (Kluskens et al., 2005; Chatterjee et al., 2006; Rink et al., 2007). Introducing unusual amino acids into non-lantibiotic peptides would be a powerful tool to improve the stability of peptides of interest.

Concluding remarks

We have reviewed recent progress in the characterization of bacteriocins produced by Gram-positive bacteria with regard to their diversity in structure, modes of action, biosynthetic mechanism, gene regulation, self-immunity mechanism, and applications. The number of newly discovered bacteriocins continues to grow, as well as the expectation for their potential use as alternative antibiotics. In parallel with screening novel bacteriocins, there is a pressing need to understand their mode of action and self-immunity systems, which should relate to the mode of action of bacteriocins, to meet the future challenges of developing antibiotics that can combat emerging drug-resistant pathogens. The features of bacteriocins that it is ribosomally synthesized, enable their manipulation through genetic engineering. Engineered bacteriocins may acquire a higher antibacterial activity, work over a wider spectrum, develop improved structural stability, or acquire other unexpected characteristics. The diversity of bacteriocins makes it difficult for scientists to fully understand their mechanism. However, the use of bacteriocins in combination can have wider applications compared with the use of single bacteriocins. Additionally, knowledge of the diversity of bacteriocins can pave the way for designing highly effective bacteriocins.

Recent applications of lantibiotics include the use of the regulatory system of nisin biosynthesis to control gene expressions, such as the NICE system in *L. lactis*. Lantibiotic engineering is a novel peptide-designing tool that takes advantage of the biosynthetic machinery of lantibiotics. Some of the lantibiotic enzymes, such as NisB (Kluskens et al., 2005) and LctM (Chatterjee et al., 2006), are applied to introduce unusual amino acids into unrelated peptides fused to their cognate leader peptide. Introduction of unusual amino acids into potent therapeutic peptides may increase their bioactivity or tolerance against protease.

Bacteriocins are promising compounds with potential applications in the food and pharmaceutical industries. Moreover, with increasing knowledge of the biosynthetic mechanisms of bacteriocins, new application tools can be developed for purposes of controlled gene regulation and peptide engineering. It is our hope that further studies of bacteriocins will lead to wider applications of these compounds in many aspects of everyday life.

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