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RESEARCH REVIEW

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Ribosomally Synthesiszed Antimicrobial Peptides (Bacteriocins) in Lactic Acid Bacteria: A Review

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Abstract Bacteriocins in Gram-positive bacteria have attracted much attention because many have a strong antimicrobial activity also against bacteria outside the genera of the producers. Lantibiotics and the pediocin-like bactericins have attracted most attention since they kill a broad spectrum of Gram-positive bacteria including important pathogens. But many other promising Gram-positive bacteriocins have been thoroughly characterized. Recent studies have shown that bacteriocins may play a role in the intestinal flora to protect us against the food-borne pathogens. Bacterial genome sequencing has demonstrated that there may be an arsenal of such compounds and we are only seeing the top of the iceberg. The present review gives a short outlook of the field of bacteriocins with focus on lactic acid bacteria and includes recent findings.

Keywords: bacteriocin, lactic acid bacteria, classification, modes of action, gene regulation

Introduction

All organisms produce antimicrobial peptides that represent part of the natural and innate immune system that protect them against invading organisms. In the microbial world, the antimicrobial peptides are an important part of the defence system of bacteria and they are referred to as bacteriocins. Traditional antibiotics are also produced by some bacteria but the traditional antibiotics and bacteriocins should be considered as different antimicrobial compounds. An important criterion of being a bacteriocin is that bacteriocins are ribosomaly synthesized, while antibiotics are made by multi-enzyme complexes. Most bacteriocins kill a narrow spectrum of bacteria, as compared to the traditional antibiotics. Moreover, most bacteriocins are more potent against their target bacteria while higher concentrations of traditional antibiotics are needed to kill the target bacteria.

Bacteriocins are produced by both Gram-positive and Gram-negative bacteria and the bacteriocins from Gram-positive bacteria seem to possess a broader range of susceptible organisms. For many bacteriocins from Gram-positive bacteria, the inhibitory activity is not directed against only bacteria within the same species as the producer but also against other species and/or genera different from of the bacteriocin producer. In general, the Gram-positive bacteriocins are not directly active against Gram-negative bacteria, but it has been reported that certain treatments, e.g., with EDTA or lysozyme treatment, can render them susceptible to nisin (1, 2). In addition the circular bacteriocin AS48 has been reported to kill certain isolates of Gram-negative bacteria (3). Furthermore, it has also been shown that Gram-positive bacteria can act synergistically with antimicrobial peptides from eukaryotes to kill Gram-negative bacteria (4).

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As for bacteriocin studies, the colicins found in *Escherichia coli* were the most studied bacteriocins for years (5). During the last 20 years, bacteriocins from Gram-positive bacteria, in particular lactic acid bacteria (LAB) have been thoroughly investigated. Recently, LAB isolates from a variety of the artisan fermented foods have been extensively studied in searching for promising bacteriocin producers in some Asian and those bacteriocins were also examined for their applications (6-8).

Most bacteriocins produced by Gram-negative bacteria are relatively large and consequently heat-labile except for the microcins, while the majority of bacteriocins from Gram-positive bacteria are small consisting of 30-70 amino acids. Present review will focuse on the heat-stable bacteriocins of LAB.

Classification of Bacteriocins

The antimicrobial peptides ribosomally produced by bacteria have been grouped into different classes based on the different criteria such as producer organisms, molecular sizes, physical properties, chemical structures, mode of actions etc., which have sometimes resulted in different names for the same compounds (e.g., thiolbiotics and lantibiotics; microcins and colicins, bacteriocins, just to mention a few) (9). Bacteriocins from Gram-positive bacteria have attracted much interest for many reasons: they are frequently found in many commercially useful LAB (e.g., lactococci, lactobacilli, pediococci) and they are generally regarded as safe (GRAS) for human consumption since they are found or used in food and feed fermented products. Furthermore, they are non-toxic to eukaryotic cells, and they have much broader inhibitory spectra compared to bacteriocins from the Gram-negative bacteria. In addition, some bacteriocins also exert direct activity toward bacterial pathogens including food-borne, and therefore represent a great potential in treatment of certain bacterial infections and food safety. Bacteriocins

from Gram-positive bacteria are presently divided into the four main classes:

i. Lantibiotics
ii. Non-modified heat-stable bacteriocins
iii. Large heat-labile bacteriocins
iv. Cyclic bacteriocins

Recently, this main classification scheme has been disputed and it has been suggested to exclude class III bacteriocins since these include proteins with enzymatic activities that cause cell wall degradation, and this group of antimicrobials should be referred to as bacteriolysins instead (10). Class III bacteriocins will not be included in this review. The cyclic bacteriocins are now included in its own class IV.

Most class I and II bacteriocins are of small and cationic peptides, ranging from 2 to 9 kDa in molecular mass. Some neutral and even anionic peptides have also been identified in the lantibiotic group. Their structures normally contain amino acid stretches with amphiphilic properties and therefore membranes of bacterial cells are believed to be the target of the bacteriocins leading to leakage of cellular solutes and, cell death eventually.

Within class I and II bacteriocins different subgroups have been suggested. New information based on mode of action studies combined with structural features including amino acid sequence homology, transport systems for bacteriocins, has raised some debates about the rationale behind the present bacteriocin subgouping and changes and new subgroups have been suggested. Many bacteriocins lack unifying features to allow them to be included in the present grouping. Since classification of bacteriocions is still controversial, this review will keep to the sub-grouping that is currently most used.

Class I: The Lantibiotics

The current lantibiotic research is presently at an advanced level with great insight in the mechanisms of biosynthesis, regulation, mode of action, structure etc. Numerous excellent reviews have covered this field in great detail and readers are referred to these works for more detailed knowledge (11-20).

A general nomenclature for the genes involved in lantibiotic biosynthesis was suggested: the prefix *lan* should be used to refer to homologous genes of the biosynthesis of different lantibiotics (11, 21, 22).

The lantibiotic class has been subgrouped into type A and type B: the former includes the elongated and positively charged lantibiotics while the latter includes the globular and non-charged lantibiotics (22). Presently, more than 40 lantibiotics have been characterized, and their physicochemical properties have been found to be more diverse than what were originally thought, making it increasingly difficult to unify a common platform for subtyping the various lantibiotics. It has been pointed out that for type A lantibiotics, a further subgrouping (AI and AII) is needed to distinguish those due to their differences in their biosynthetic pathways and modification enzymes (23). Class-AI lantibiotics are modified by two separate enzymes LanB and LanC, and processed by a dedicated serine protease LanP. On the other hand, the class-AII lantibiotics are modified by a single modification enzyme LanM, and simultaneously exported, and activated by an ABC-transporter LanT with an N-terminal associated protease activity. However, not all type A lantibiotics fall within this classification scheme. In an excellent review by Chatterjee *et al.* (13), the type AI lantibiotics were grouped into three subgroups while a more recent report suggested that the present lantibiotics can be divided into 11 subgroups based on the primary pro-peptide sequence (24). While Guder *et al.* (25) suggested to separate the lantibiotics into 8 subtypes based on primary amino acid sequence alignment, Twomey *et al.* (19) divided these peptides into 6 subgroups based on a similar criteria. Finally, the review by Bonelli *et al.* (11) divided all the lantibiotics into 4 groups by taking into account that many new lantibiotics possess intermediate structures being neither A nor B.

Nisin is so far the most studied bacteriocin and has been permitted in several countries as additive/preservative in certain food commodities. Two nisin variants (nisin A and nisin Z that differ only in one amino acid residue) have been found in *Lactobacillus* (*Lb.*) *lactis*. A new nisin variant, termed nisin U, has been found in *Streptococcus uberis* and nisin U is 78% identical to nisin A (26). In the two fairly new lantibiotic review articles lists of the most common lantibiotics are listed (11, 13)

Genetics and posttranslational modifications of lantibiotics Unlike the structurally more simple class II bacteriocins, the lantibiotics undergo several posttranslational modification steps during their biosynthesis. The genes behind this process are thoroughly characterized not only for the linear type-A but also for type-B globular lantibiotics. The genes responsible for the lantibiotic biosynthesis are most often located on plasmids or other mobile elements, and are organized in operon-like structures. The genetic determinants involved in the biosynthesis of a lantibiotic include genes encoding a precursor of the lantibiotic peptide, a secretion system, modification enzymes, and immunity proteins (27). The biosynthesis of some lantibiotics also includes regulatory genes (28). The antimicrobial activity of a lantibiotic is mostly due to the activity of one peptide. Some lantibiotics require two different peptides to be active, such bacteriocins are referred to as two-peptide lantibiotics. The lantibiotic precursor (the non-modified peptide sequence with an N-terminal leader) is usually encoded by one gene, while two genes are actually involved in the case of the two-peptide lantibiotics. Several two-peptide lantibiotics have been characterized, they include lacticin 3147 (29), cytolysin, plantaricin W (30), bacteriocin Smb (31). The two structural genes of a two-peptide lantibiotic are adjacent to each other in the same operon.

Furthermore, immunity genes are needed to protect the producers against its own bacteriocin. It has been shown that immunity to lantibiotics is encoded by different genes. The immunity factor can be a dedicated protein or an ABC transporter. In some cases including nisin, it has been shown that full immunity is accomplished only in the presence of a dedicated immunity protein and an ABC transporter system (32).

Transport of the lantibiotics is carried out by different types of ABC transporters, depending on the type of N-terminal leaders of the lantibiotic precursors. The transport process is closely associated to the processing of the N-terminal leader and the modifications. Most lantibiotics have a separate ABC transporter and a leader-specific protease. However, a few lantibiotics are encoded with a N-terminal double-glycine leader that varies between 14 and 30 amino residues in length and that possesses a conserved sequence motif (33). In this case the ABC transporter and the protease activity are embedded into one protein (34). The proteolytic domain of the ABC transporter recognizes the N-terminal double-glycine leader that is removed concomitant with the secretion of the mature peptide (34). Among most lantibiotics including nisin the two functions are separated, a specific protease (LanP) removes the N-terminal leader and a separate ABC transporter (LanT) is involved in the externalization.

Modification of a lantibiotic normally starts with the dehydration of selected serines and threonines forming 2,3-dehydroalanines (Dha) and 2,3-dehydrobutyrines (Dhb), respectively. Some of the Dha and Dhb residues will subsequently participate in a covalent S-bridge with cysteines conform lanthionine (Lan) and 3-methyllanthionine (MeLan), respectively. In addition to these four major modifications other posttranslational changes have also been found, such as the unsaturated ring structure (2-aminovinyl-cysteine) in the C-terminus of epidermin, the spontaneous degradation of N-terminal Dha and Dhb by the formation of 2-oxypropionyl and 2-oxo-butyryl, and β-hydroxy aspartate. Dependent on the subtypes of bacteriocins, modification of lantibiotics involves different set of genes: type AI lantibiotics employs the LanB (dehydratase) and LanC (cyclase) proteins in their modifications, while type AII and type B lantibiotics use the alternative and bifunctional enzyme (LanM) processing both dehydratase and cyclase activities. An interesting modified residue found in 2 lantibiotics (lactocin S and lacticin 3147) is D-alanine (35, 36). In the lactocin S prepeptide, it was shown that 3 serines were modified to D-alanine probably through a stereo-specific enzymatic hydrogenation of Dha residues. A recent publication identified an enzyme, LtnJ, responsible for the conversion of Dha to D-alanine in the lacticin 3147 system. Systematically replacing the D-alanine residues by L-alanines in lacticin 3147 confirmed the importance of the chirality of these residues in the antimicrobial activity (37).

Though the enzymatic machinery in the posttranslational maturation process of lantibiotics has been mostly elucidated at the genetic level, the maturation of nisin was recently established on the biochemical level. It was shown that the dehydratase reaction was carried out by NisB (LanB), resulting in Dha and Dhb (38). The stereo-specific coupling of cysteines to the dehydrated amino acid reisdues is performed by NisC (LanC). Li *et al.* (39) demonstrated that NisC is an Zn^{2+}- dependent cyclase which catalyses the covalent binding of the thiol group of cysteine to the α,β-unsaturated bond of Dha or Dhb forming the intracyclic lanthionine and methyllanthionine structures in nisin (39).

The biosynthesis of lactocin S requires the gene *lasM* (*lanM*) that is shown to share some homology with *lanC*. Other lantibiotics, which use a LanC homolog in their maturation process are lacticin 481, cytolysin (CylM), lacticin 481 (LctM), and lacticin 3147 (LtmM9)(11). Functional analysis of LctM (LanM) was performed recently (40). In an *in vitro* reconstitution experiment, it was shown that purified LctM performs a number of posttranslational modification including dehydration of serines and threonines as well as being directly involved in the stereospecific cyclization reaction together with cysteines and in the positioning of the involved residues in the pre-peptide substrate. This was an exciting finding which paves the way for making engineered lantibiotics (40).

Activity and mode of action of lantibiotics It was early established that type A lantibiotics permeabilize membranes causing intracellular leakage of low molecular compounds and depleting the membrane potential and causing efflux of low-molecular compounds (41). It was previously shown that nisin could also cause damaging effects on the cell wall of the target bacteria (42). However, a deeper understanding on how the membrane leakages as well as wall damaging effects come upon has for years been a matter of discussion. Today a unifying model based on solid experimental evidence explains how such lantibiotics cause multiple effects. By the initial work of Dr. Sahl's group and others it was shown that nisin actually uses lipid II as a receptor (more frequently referred to as a docking molecule) which was crucial in the initial step of the action of nisin and its similar bacteriocins (43, 44) (Fig. 1). Through the interaction with the membrane-bound cell wall precursor lipid II, nisin inhibits peptidoglycan synthesis and forms highly specific pores that result in the depletion of intracellular components. The combination of two killing mechanisms in one molecule that potentiates the antibiotic activity and results in nanomolar minimum inhibitory concentration (MIC) values, seems to be a promising strategy well worth to be considered for the construction of novel antibiotics (45). Several lantibiotics have shown to target the peptidoglycan precursor lipid II in a similar way as nisin (44). Thus, the positively charged type A lantibiotics (nisin, epidermin, and galidermin) use lipid II as a docking molecule and combine two mechanisms of action; membrane permabilization and inhibition of cell wall

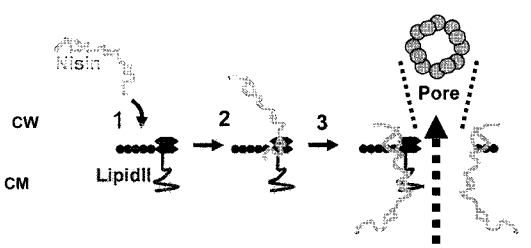

Fig. 1. Model for mechanisms how nisin permeabilize target membranes. Nisin binds to its docking molecule lipid II in the interfase of the cell wall (CW) and cytoplasma membrane (CM) (1 and 2). A stable transmembrane positioning and assembly of such nisin-lipid II complex take place and form membrane pores leading to permeabilization of the cell (43).

biosynthesis.

The mode of action of the globular and noncharged type B lantibiotics mersacidin, has been also studied thoroughly (46, 47). Contrary to nisin, mersacidin does not lead to pore-formation; it inhibits only cell wall biosynthesis. The ability of mersacidin to inhibit synthesis of peptidoglycan is via a strong interaction with lipid II, but not with the tranglycosylation enzyme (48). It should be noted that the mersacidin inhibition of transglycosylation differs from the vancomycin-lipid II complex inhibition of cell wall biosynthesis (48). While vancomycin form complex with the C-terminal moiety of the penta-peptide portion of lipid II, mersacidin binds lipid II via the disaccharide-pyrophosphate moiety (18, 48). Further support to this notion is that binding of mersacidin to cells is not prevented by vancomycin and that mersacidin can kill vancomycin-resistant *Enterococcus faecium* which produces an alternative peptidoglycan precursor.

Plantaricin C, another lantibiotic isolated from *Lb. plantarum* was shown to act on susceptible bacteria through a lipid II-mediated action (49). In contrast to nisin, the lipid II-targeting PlnC could not permeabilize *Leuconostoc* (*Lc.*) *lactis* cells or to form pores in 1,2-dioleoyl-sn-glycero-3-phosphocholine liposomes supplemented with purified lipid II. However, plantaricin C has been shown to be a strong inhibitor of both lipid II synthesis and the FemX reaction, i.e., the addition of the first Gly to the pentapeptide side chain of lipid II (49). This emphasized the diversity in the mechanisms of these lantibiotics for bacterial killing. Pediocin PD-1, produced by *Pediococcus damnosus* shows strong primary sequence homology to plantaricin C and could be another representative for the mode of action observed with plantaricin C (50).

The two-peptide lantibiotics are a growing group of bacteriocins. Presently, 7 two-peptide lantibiotics which are lacticin 3147 (51), staphylococcin C55 (35), plantaricin W (30), cytolysin (52), haloduracin (53), Smb (31) and BHT-A (54) have been identified. It has been pointed out that the ring structures (lanthionines, methyl-lanthionines) among the two-peptide lantibiotics are very much the same between their respective peptides, and in addition high sequence homology is found between some of these bacterioins (30, 55). Recent studies on the mode of activity of the two-peptide lacticin 3147, revealed that a two-step mechanism was involved in the killing action (56, 57). The killing process starts with the binding of A1 peptide of lacticin 3147 to generate a binding site for the A2 peptide which then drives the complex to form pores in the membrane. The pore complex of lactacin 3147 is composed of 4:4:4 molecules of A1, A2, and lipid II, respectively.

Several studies on the mode of action have revealed exciting features of multiple activities of nisin and related lantibiotics making these peptides interesting model systems for the design of new antibiotics (43, 44) A great number of different types of lantibiotics as well as antibiotics interact with lipid II as a docking molecule. Despite of this common theme, the subsequent mechanisms in the mode of killing have evolved differently, which make these peptides attractive to be developed as antibacterial drugs (58).

To summarize, the lantibiotics display a substantial degree of target specificity for particular components of bacterial membrane and cell wall biosynthesis machinery. Mersacidin and actagardin were shown to bind with high affinity to the lipid-coupled peptidoglycan precursor, the so-called lipid II that prevents the polymerization of the cell wall monomers into a functional murein sacculus. The lantibiotics nisin and epidermin also bind tightly to this cell wall precursor; however, for these lantibiotics the binding of lipid II has 2 consequences: (i) like mersacidin, the binding of nisin and epidermin with lipid II inhibits peptidoglycan biosynthesis; and (ii) lipid II is used as a specific docking molecule for the formation of pores. This combination of lethal effects explains the potency of these peptides, which are active in nanomolar concentrations.

The Class II Bacteriocins

Class II bacteriocins constitute a large and diverse group of ribosomally synthesized antimicrobial peptides. Class II bacteriocins should include non-modified heat-stable peptide bacteriocins and have been, like class I lantibiotics, subdivided into different subclasses based on a number of criteria. The class II bacteriocins will in this review be divided into 5 subgroups: class IIa, the pediocin-like and strong antilisterial bacteriocins (e.g., pediocin PA-1); class IIb, the two-peptide bacteriocins (e.g., plantaricin EF); class IIc, the bacteriocins that are not included into any other subclasses (e.g., lactococcin A); class IId, the leaderless bacteriocins (Table 1); class IIe, the peptide bacteriocins that are formed by a specific degradation of larger proteins (Table 2).

The cyclic bacteriocins that have been sugested to comprise a separate class IV (Table 3) and will also be briefly discussed (59).

In general, class II bacteriocins are structurally simpler than lantibiotics because no posttranslational modifications of the peptide chain are taking place, and only genes encoding the bacteriocin, immunity, and transport functions are normally needed. Despite much information is available on different aspects of most class II bacteriocins, the IIe group of bacteriocins is less studied. For instance, how the specific protein degradation is performed and controlled in the formation of a class IIe antimicrobial peptide is yet undiscovered.

Concerning class IV bacteriocins, they differ from the class II bacteriocins in several aspects. The class IV bacteriocins are posttranslationally modified different from the lantibiotics, they are produced by more complex synthesizing machinery and they are closed in a cyclic structure. Such characteristics make it reasonable to place them in a separate class (60).

Genetic features for biosynthesis of most class II bacteriocins are relatively conserved. Genes involved in immunity and in transport are often closely associated with the bacteriocin structural gene(s). For some bacteriocin systems, their biosynthesis is regulated by a 3-component regulatory system consisting of a regulatory peptide-pheromone, a sensor (histidine protein kinase), and a regulator protein (response regulator) (61, 62). For bacterial strains with multiple bacteriocins (e.g., plantaricin EF and plantaricin JK) (63), their genes are often clustered and they share the same regulatory and transport systems but

Table 1. Class IId leaderless bacteriocins

Name	Producer	Number of amino acids residues	Reference
Aureocin A53	*Staph. aureus*	51	(190)
Aureocin A70	*Staph. aureus*	AurA: 31 Aur B: 30 AurC: 31 AurD:31	(191)
Enterocin L50 (Enterocin I)	*E. faecium*	L50A: 43 L50B: 42	(129, 192)
Enterocin Q	*E. faecium*	34	(27)
Lacticin Q	*Lc. lactis*	53	(193)
LsbB	*Lc. lactis*	30	(126)
Enterocin	*E. faecalis*	44	(194)
EJ97 Enterocin RJ-11	*E. faecalis*	44	(195)

Table 2. Class IIe bacteriocins; antimicrobial peptides (AMP) derived from larger proteins

Name	Producer	Protein; precursor of the antimicrobial peptide	Residue position of the formation of AMP (Number of amino acid residues)	Reference
HP	*H. pylori*	Ribosomal protein L1	2-20 (19)	(137)
Propionicin F	*P. freudenrechii*	Orf of 255 aa	102-145 (43)	(138)
Closticin 574	*C. tyrobutyricum*	Orf of 309 aa	228-309 (82)	(158)
PAMP	*P. jensenii*	Orf of 225 aa	162-225 (64)	(196)

Table 3. Class IV cyclic bacteriocins

Name	Producer	Number of amino acid residues	Reference
Gassericin A	*Lb. gasseri*	58	(197)
Reutericin 6	*Lb. reuteri*	58	(157)
Enterocin AS-48 Enterocin 4	*E. faecalis*	70	(146, 149, 197)
Circularin A	*C. beijerinckii*	69	(158, 197)
Butyrivibriocin AR10	*Butyrivibrio fibrisolvens*	51	(198)
Subtilosin	*B. subtilis*	32	(109, 142)
Uberolysin	*S. uberis*	70	(199)

can also be dispersed on different locations (e.g., enterocins P, L50, and Q (64).

Most peptide-bacteriocin genes encode a prebacteriocin with an N-terminal leader which is removed during the exporting process. The most common leader is the so-called double glycine-leader, a peptide sequence varying between 16-30 residues in length embedding some consensus residues including the double-glycine residues at the cleavage site. A few bacteriocins employ a *sec*-like leader and therefore use the more general secretory system (*sec*) for externalization. All the double-glycine leader containing class II bacteriocins are secreted by a dedicated ABC transporter with the support of an accessory protein. The role of the accessory protein in the bacteriocin transport is unknown. It has been shown that the accessory protein (LcnD) of lactococcin A is found together with the transport complex obtained from the membrane fraction, but in addition a truncated cytoplasmically located form of the protein, LcnD is required for the externalization of the bacteriocin (65, 66). The ABC transporter embraces an N-terminal proteolytic domain that specifically removes the double-glycine leader sequence concomitant with the externalization of the bacteriocin as also seen in a few lantibiotics (34). Different from the lantibiotics which involve a dedicated ABC-transporters for export, some classII bacteriocins are secreted by the general *sec* system (67-70).

Like the lantibiotics, the genes for class II bacteriocins are frequently found on plasmids or movable genetic elements, which may explain that similar or identical bacteriocins are found in the different species that includes some pediocin-like bacteriocins such as the two identical bacteriocins curvacin A (71) found in *Lb. curvatus* and sakacin A (72) found in *Lb. sakei.*

In two recent review articles the most common class II bacteriocins are listed (40, 73, 74).

The class IIa: Pediocin-like bacteriocins Class IIa is the largest bacteriocin group and consists of the so-called "pediocin-like" bacteriocins, which have a strong ability to kill listeria (14, 75). They are produced by a variety of lactic acid bacteria and more than 30 different class IIa pediocin-like bacteriocins have been found in different species and genera. The first pediocin-like bacteriocins that were identified and thoroughly characterized were pediocin PA-1 (76-78), enterocin A (79), leucocin A-UAL 187 (80), mesentericin Y105 (81), sakacin P (71), and curvacin A (also called sakacin A) (14). More information of these bacteriocins and others is referred to a recent review (47). The class IIa constitutes a group of bacteriocins that normally share more than 40% amino acid sequence homology, and some conserved motifs that include the sequence (YGNGV) and a cysteine disulfide bridge in their N-terminal half. Apparently they kill the cells by permeabilizing the target membrane (82-85). The necessity of the N-terminal Cys-Cys bridge structure has been experimentally demonstrated and by reducing the disulfide bond or by breaking the same disulfide bond through chemical modification, the antimicrobial activity is abolished almost completely (84, 85). Recently, it was suggested that even the class IIa bacteriocins can be further separated into at least four groups based on structural differences in the C-terminal half of the peptides (86).

Despite the strong amino acid sequence similarities in their N-terminal half of the pediocin-like bacteriocins, they surprisingly differ in their relative activity and target specificity against different organisms (14, 74, 87). The C-terminal half of the molecules is important for the differences in killing ability observed between the different bacteriocins in this group. It has been conclusively shown that the presence of a cystein bridge in the C-terminal half of class IIa bacteriocins stabilize a loop structure that appears to be critical for its activity. Such a forced loop structure in the C-terminal half contributes to enhanced specific activity and heat-stability as well as to broaden the target specificity (88).

The immunity genes of class IIa bacteriocins code for fairly small proteins of about less than 100 and up to approximately 150 amino acid residues. The immunity proteins share less homology than what is observed between their respective bacteriocins. The individual immunity proteins are highy specific for protection against their cognate bacteriocins but a few share homology and display some cross protection for none-cognate class IIa bacteriocins (89). By studying hybrid bacteriocins and their immunity proteins it has been shown that the C-terminal domain of class IIa bacteriocins is involved both in specific recognition of the C-terminal part of cognate immunity proteins and in determining the antimicrobial spectrum (90, 91).

Sequence and structural analysis have concluded that these bacteriocins are composed of a conserved and cationic N-terminal domain and a hydrophobic/amphiphilic and less conserved C-terminal domain which is linked with the N-terminal domain by a flexible hinge. Construction of hybrid bacteriocins concluded that the C-terminal part was involved in the target specificity (88, 90, 92). The 3-dimensional structure of carnobacteriocin B2 (93), leucocin A (94), and 2 variants of sakacin P (95) in membrane mimicking solvent has been analyzed by nuclear magnetic resonance (NMR). It was shown that the conserved N-terminal part was structured in a 3-stranded antiparallel β-sheet-like structure supported by the presence of the conserved N-terminal disulfide bridge. Computer analysis suggested that the β-sheet structure was followed by a central well-conserved amphiphilic α-helix and an extended C-terminal amino acid sequence that folds back onto the helical region. A hinge region exists in the interface between the N-terminal and the central domains. Figure 2 shows a model of the structure and orientation of the class IIa bacteriocins in the membrane-mimicking environment.

The major difference in the primary structure is found in their C-terminal part; this difference causes great variations in their antimicrobial efficiency as well as inhibitory spectra. As mentioned already, the strongest antibacterial bacteriocins have an additional C-terminal disulfide bridge. The absence of the C-terminal disulfide bridge makes the antimicrobial activity more temperature-dependent, such bacteriocins are consequently compromised at the elevated temperatures (above 35°C). The importance for a strong antimicrobial activity by the presence of the C-terminal cysteine bridging was conclusively demonstrated by removal or introduction of such a bridge in pediocin PA-1 and sakacin P, respectively (88). It was noted that the introduction of the C-terminal bridge into sakacin P (which lacks the bridge) not only broadened the target cell specificity but also made it 10 to 20 times more potent than the wild-type and the antimicrobial activity was less influenced by temperature variation (same potency at 20 and 37°C), Reciprocal effects on target cell specificity and the temperature-dependent potency were observed by removing the C-terminal disulfide bridge from pediocin PA-1 (which naturally contains the bridge) (88). Since most class IIa bacteriocins do not have this C-terminal bridge, many of them instead have replaced it with a tryptophan residue located near the C-terminal end that seems also to stabilize the proposed C-terminal fold-back structure in a manner similar to the disulphide bridge, suggesting that the tryptophan residue can position itself in the membrane water interface and thereby enforce the important C-terminal hairpin structure (96).

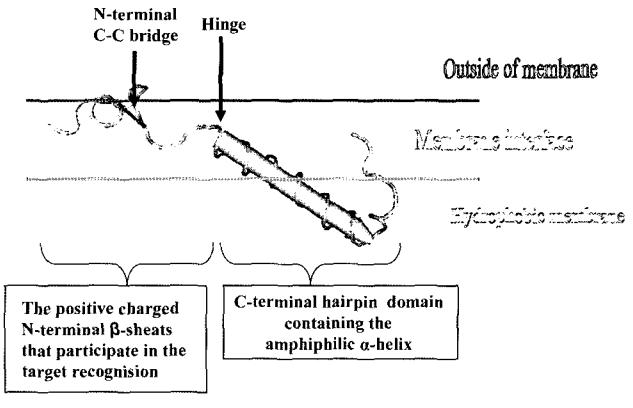

Fig. 2. Model of the insertion of pediocin-like bacteriocins (class IIa) into target membranes.

Random mutagenesis has been performed on mesentericin Y105. Ten single-mutants randomly derived covering over the entire length of the molecule were obtained and all showed reduced activity compared to the wild-type molecule, implying that most part of mesentericin Y105 is important for the antimicrobial activity (97). The use of various 15-mer fragments of pediocin PA-1, identified a specific fragment from the hinge region (residue 20) to the C-terminal end (residue 34) which strongly inhibited the antimicrobial activity of pediocin PA-1, suggesting that this fragment somehow specifically prevented the full-length bacteriocin to interact with the target bacterium. We could envisage that this inhibition could be due to competition for receptor site as seen with some of the lantibiotics. The existence of a specific receptor on target cells for one-peptide bacteriocins that include the class IIa bacteriocins has now been demonstrated and will be discussed in another section of this review (98).

The class IIb: Two-peptide bacteriocins As mentioned already, several two-peptide bacteriocins have been identified for both lantibiotics (class I) and non-lantibiotics (class II) (99). In this section, only two-peptide class II bacteriocins are discussed.

In order to identify a two-peptide bacteriocin, three criteria should be met: (i) both peptides are required to obtain full activity and the individual peptides display little or no antimicrobial activity, ii) one immunity protein is sufficient to confer immunity, and iii) the genetic organization of a two-peptide bacteriocin system includes two consecutive bacteriocin structural genes encoding the individual peptides, followed by a single immunity gene.

Although the presence of both peptides is importance for full activity for two-peptide bacteriocins (100-112) the individual peptides can, in some cases, exert modest residual antimicrobial activity. Purification of the individual peptides of a class IIb bacteriocin is frequently troublesome as they often share several physicochemical properties, e.g., being small, cationic, and hydrophobic/amphiphilic, causing contamination of both peptides in the individual purified peptide fractions. In order to confirm residual activity in one of the peptides such experiments should be performed with individually cloned entities or chemically synthesized ones before testing.

Lactococcin G was the first bacteriocin that was identified and proven to be a two-peptide bacteriocin (113). Its activity depends on the complementary action of two peptides, termed α- and β-peptides. The α- and β-peptides can bind independently and strongly to the target cell surface but the antimicrobial activity requires the complementary action of both peptides in a stoichiometric molar ratio of 1:1 in order to exert maximum antimicrobial activity. The individual peptides of lactococcin G do not exert any measurable antimicrobial activity (113, 114). Biologically active and synthetic lactococcin G was used to study the mode of action on sensitive *Lc. lactis* cells. Once bound to a bacterial cell surface, the peptides cannot be displaced from the surface of one cell to another cell (114).

Displaying the peptide sequences on an Edmundson α-helical wheel analysis, suggests that the individual peptides contain an amphiphilic stretch in their N-terminal and mid-regions as observed for other classs II bacteriocins (113). Circular dichroism (CD) studies of these peptides strongly suggest that they are unstructured in water but are converted to α-helical structures in membrane-mimicking solvents (115). Other two-peptide bacteriocins such as plantaricin EF and plantaricin JK show very much the same physicochemical and activity properties as seen with plantaricin G peptides although none of the peptides share any significant sequence homology (101, 111, 116)

It has been shown that the lactococcin G peptides show significant sequence homology with the 2 two-peptide enterocin 1071 from *E. faecalis* and lactococcin Q from *Lc. lactis* (106, 117). However, it was surprising to observe that these two-peptide bacteriocins varied so much in target specificity. An analysis of enterocin 1071 and lactococcin G by site-directed mutagenesis concluded that both the α- and β-peptides influenced the target specificity but the N-terminal amino acid residues in β-peptides were especially important (118).

The individual peptides of class IIb bacteriocins share many physicochemical properties of the one-peptide bacteriocins by being amphiphilic/hydrophobic and cationic and also possess a similar mechanism of bacterial killing, i.e., through permeabilization of the target membrane. The complementary activity of both peptides of some class IIb bacteriocins is quite potent and can kill selected target bacteria at pico- to nano-molar concentrations, while the individual peptides are not active at all or only display some activity at micromolar concentrations. It is tempting to suggest that the two-peptide bacteriocins do act specifically on a target site in a manner as seen with lantibiotics and the one-peptide class II bacteriocins (see below). A complex of α- and β-peptides probably forms a transmembrane pore that conducts monovalent cations including potassium ions but not protons, and eventually leads to cell death of target bacteria (114, 119). Today, we do not fully understand the mode of action of the two-peptide bacteriocins but hopefully more information will be available in the near future.

Class IIc: The unsorted bacteriocins As mentioned earlier a great variety of bacteriocins exist within class II bacteriocins and it has therefore not been possible to find unifying criteria for subgrouping all bacteriocins. Consequently, those class II bacteriocins that do not fall within any of the other 4 class II subgroups, are grouped together in class IIc (120). Lactococcin A was one the very first bacteriocins in *Lc. lactis* that was biochemically characterized. Lactococcin A does not share any significant sequence homology with other bacteriocins (121). Lactococcin A is abundant among lactococci and has very narrow target specificity, it kills only other lactococci but the lethal potency can vary at least 100-fold between different target lactococci. This makes it even more surprising that the target (receptor) for the activity of lactococcin A is the same as for the class IIa bacteriocins, namely the mannose PTS. It has been conclusively shown that lactococcin A does kill exclusively other lactococci, while pediocin-like bacteriocins do not kill lactococci at all (98). A number of class IIc bacteriocins are listed in a recent review (74).

Class IId: Leaderless bacteriocins Leaderless bacteriocins have been found among several Gram-positive bacteria. Such peptides were first identified in *Streptococcus* and *Staphylococcus* (122, 123) as hemolytic peptides but they are apparently also bactericidal as seen with the lantibiotic cytolysin found in *Enterococcus faecalis* (124). Since then other leaderless bacteriocins have been identified in *S. aureus*, *E. faecium*, and *Lc. lactis* (125-127) (see Table 1). Sometimes homologous leaderless bacteriocins are found in multiple but not identical copies (2-4 homologous peptides) encoded by consecutive genes located in an operon structure. The leaderless peptides are somehow secreted by ABC transporters and the producers are protected from their leaderless bacteriocins by a dedicated immunity protein or by the ABC transporter that is also involved in the bacteriocin secretion (126, 128, 129).

It is interesting to note that the individual peptides exert some antimicrobial activity but by adding the complementary peptides the antimicrobial activity increases in a synergistic way not just additional (64, 127). This is also the case for aureocin 70 constituting four complementary peptides. The antimicrobial activity of synthesized enterocin L50 (wrongly named as pediocin L50 in the first publication (125)) was tested against four susceptible bacteria from four different genera. Enterocin L50 encompasses 2 peptides L50A and L505 that share 72% sequence identity. Both peptides showed low but significant antibacterial activity that varied considerably between the four tested target strains, but L50A was consistently the most active one. When the 2 peptides were combined in equal molar concentration, the antimicrobial activity increased 5- to 80-fold, compared with the activity of the L50A peptide alone (128, 129).

The leaderless bacteriocins are found in different genera among the Gram-positive bacteria, they have a fairly broad antimicrobial activity and some may also have hemolytic activity. An interesting feature is that some of these peptides are found in multiple but not identical copies encoded by consecutive *orfs*. This observation strongly suggests that gene duplication has taken place, and the minor sequence alterations evolved have made the bacterium capable to produce stronger and more efficient antimicrobial active and synergistically acting peptides.

Class IIe: Larger protein-derived bacteriocins Antimicrobial peptides produced by a specific degradation of larger proteins have been known for years. In eukaryotes, it has been known that lactoferrin (130), and different histones (131-136) are sources of antimicrobial peptides.

In recent years it has also been shown that certain bacterial proteins can be processed or degraded proteins to defined antimicrobial peptides. In *Helicobacter pylori* a ribosomal protein is processed to antimicrobial peptides (137). Also, it has been shown in two cases that degradation of proteins produces antimicrobial peptides in propionic acid bacteria (138, 139).

One of the best studied bacteriocins derived from large proteins is propionicin F that is produced by *Propionibacterium freudenreichii* (140). The maturation of propionicin F involves both N- and C-terminal processing of a 255-residue large protein (PcfA) of which the amino acids 102-145 (43 residues) represent the mature bacteriocin. Mature propionicin F is hydrophobic with a net negative charge which is unusual for bacteriocins. It is possible that the hydrophobic nature of propionicin F enables binding to the cell envelope, but it is tempting to speculate that the bacteriocin may attack *P. freudenreichii* cells by binding to a specific receptor molecule. In the downstream region of *pcfA*, genes encoding a radical S-adenosyl-methionine transferase (*pcfB*), a proline peptidase (*pcfC*), and a bacteriocin-type ABC-transporter (*pcfD*) were identified (140). Transcriptional analysis shows that the *pcfABCD* genes constitute an operon. The presence of an S-adenosyl-methionine transferase gene (*pcfB*) in the propionicin F locus is of special interest, since the involvement of such an enzyme has only been reported for one other bacteriocin system, subtilosin A, where the AlbE protein is suggested to be responsible for thioether-bridge formation (141, 142) However, we have no evidence that propionicin F contains any ring structure. Recently we have shown that a small membrane protein encoding *orf* (termed *pcfI*) in this operon accounts for propionicin F immunity (submitted for publication).

Currently, little information is available about the biological significance of these antimicrobial peptides however it is plausible that they somehow serve a role in the ecology of their producers. It might also be beneficial to an organism if the degradation product of a protein could provide a second and new biological function of the producer as seen for eukaryotic histones (4, 133, 143, 144).

Class IV: Circular Bacteriocins

Due to the unique property as being circularized (head to tail), these bacteriocins have been proposed to be encompassed in a new class of bacteriocins, class IV (145) (Table 3). The circular antimicrobial bacteriocins should not be confused with the non-ribosomally synthesized antibiotics such as gramicidin S.

These circular peptide-bacteriocins are covalently linked head to tail and bacteriocin AS-48 from *E. faecalis* is so far the most thoroughly studied one (61). Bacteriocins identical to AS-48 have been reported, these include enterocin 4 (146) and bacteriocin 21 (147). The genetic information for AS-48 bacteriocin is located in 7.8 kb region on a 68 kb pheromone-responsive conjugative plasmid. The 10 genes involved are organized in two operon structures (148, 149): a four-gene operon (*as-48 EFGH*) encodes a new multi-component ABC transporter that is involved in bacteriocin export and also in producer self-protection (immunity), and a six-gene operon (*as-48-A,B,C,C1,D,D1*) encodes the biosynthesis apparatus for bacteriocin AS-48, including part of the self protecting system. Genetic search by PCR indicates that genes encoding AS-48-like bacteriocins are abundant among enterococci (150). Most interestingly, AS-48 is known to be one of the very few bacteriocins from Gram-positive bacteria that act bactericidal not only on a great variety of Gram-positive bacteria but also on some Gram-negative bacteria (3).

AS-48 permeabilizes the cytoplasmic membrane of sensitive cells, leading to dissipation of the proton motive force and eventually cell death, through a mechanism similar to that proposed for many other bacteriocins from

Gram-positive bacteria and other cationic antibacterial peptides. Details of its mechanism are presently not known.

AS-48 is one of the very few bacteriocins of which the 3-dimensional structure has been resolved. It was shown that AS-48 in organic solvents consists of a globular arrangement of 5 α-helices enclosing a compact hydrophobic core (151). The compact structure may explain why the molecule is so inert to physical stress (152-154).

In two different *Lactobacillus* species, the two almost identical cyclic bacteriocins are found; they have been termed gassericin A and reutericin 6. They are produced by *Lb. gasseri* and *Lb. reutrii* respectively (155, 156). They were found to be identical with regard to molecular weights and primary sequences but differ from each in the number of D-ala residues present in their structures. Two D-ala residues are found in gassericin A but only one in reutericin 6 (157). Probably, the D-ala content plays a direct role in the bacteriocin activity as these two bacteriocins differ from each other in the mode of action. Also, *Clostridium* species produce circular bacteriocins and these bacteriocins are termed circularin A and closticin 574 (157, 158).

Target/Receptor of Class II One-peptide Bacteriocins

The non-modified heat stable class II bacteriocins constitute a large and diverse group of antimicrobial peptides (AMP). Among this group it is certainly the pediocin-like (strong antilisterial) bacteriocins that are most studied. Numerous studies have described the mode of action of the pediocin-like bacteriocins and it boils down to that most class II bacteriocins permeabilize the membrane of target organisms and consequently lead to an efflux of low molecular compounds from the cytoplasm which eventually kills the cell. A common denominator of many such mechanistical studies is the potassium ion efflux.

One of the key questions during decades of bacteriocin research is: do the bacteriocins need a receptor/target for their activity? This is the crucial question that needs to be answered in order to understand the mechanism of action, resistance development as well as to rationally develop such compounds into efficient antimicrobial agents for medical or other pathogen-combating applications. During the last few years various genetic studies have shown that bacteria susceptible to pediocin-like bacteriocins need the presence a mannose phosphotransferase system (PTS) on the target bacterium to be active. Initially it was shown that isolation of a mesentericin Y105 resistant mutant actually had an inactivated *rpoN* gene (encoding sigma 54) which is required for the expression of the mannose-PTS operon (159, 160). It was shown that the absence of enzyme IIAB component in a putative mannose-specific PTS in *Listeria monocytogenes* made it resistant to leucocin A (161). Later it was experimentally demonstrated that inactivation of the man-PTS system of a susceptible strain turned it insensitive to mesentericinY105 and leucocin A. Cloning of the *man-PTS* genes from a pediocin-like sensitive bacterium into an insensitive *Lb. lactis* strain rendered the bacterium sensitive to leucocin A, pediocin PA-1, and enterocin A (162).

The presence of glucose or mannose in the growth medium of *L. monocytogenes* simultaneously induces its expression of EII_t^{Man} (a sigma 54-dependent PTS permease) and sensitivity to mesentericin Y105, suggesting that the expression level of EII_t^{Man} affects the sensitivity (38). The correlation of resistance to mesentericin Y105 or leucocin A and efficiency in expression of a mannose-PTS subunit EII_t^{Man} was first described in a leucocin-resistant *L. monocytogenes* by Ramnath *et al.* (161). Another study strongly points out that leucocin A actually requires an interaction with a chiral receptor at the surface of the target cell to be active (162). Indeed, the D-enantiomer of leucocin A is not active against ten different strains, whereas all the strains were sensitive to the L-form of leucocin A. Taken together, these results strongly indicate that EII_t^{Man} could be the chiral receptor needed for bacteriocin interaction at the surface of target cells. If true, the membrane-associated IIC and/or IID subunits would interact directly with the bacteriocins. The IID subunit was finally proposed to be the putative target molecule.

In a recent study, it was presented biochemical evidence for a specific interaction between pediocin-like bacteriocins (class IIa) and the mannose-PTS system (98). Furthermore, it was shown that the same interaction is taking place between the class IIc lactococcin A and the dedicated mannose PTS-system. This study implies strongly that there exists a common mechanism which involves a specific interaction between the mannose PTS and these one-peptide bacteriocins. This interaction seems to be quite species-specific since the lactococcinA-targeted mannose-PTS from lactococci was not targeted by pediocin-like bacteriocins, and similarly the pediocin-like bacteriocin-targeted manose-PTS (e.g., from *Lb. sakei*) was not recognized as a receptor for lactococcin A. The interaction complex between the bacteriocins and the PTS has not been isolated and is probably weak. However, when a bacteriocin-susceptible bacterium has been made immune by cloning of an immunity gene; the bacteriocin, the cognate immunity protein and manose-PTS system (components IIIAB, IIC, and IID) form a strong complex that can be isolated. Probably, this strong complex prevents the bacteriocin to proceed further to form lethal pores in the membrane of the immune bacteria, thus no permeablization and no bacteriocin-caused cell death are observed. It should be emphasized that the formation of this complex has a negative effect on the bacterial growth since it apparently compromises transport of mannose and glucose thereby causing a reduced cell growth on these sugars as sole carbohydrates, a process normally referred to as fitness-cost (98). Our suggested model on how such one-peptide bacteriocins act on their target (man-PTS) is presented in Fig. 3.

Regulation of Class I and II Bacteriocin Production

It has been known for some years that production of many class I and II bacteriocins is controlled at the transcriptional level by a quorum sensing-based 3-component regulatory system. It was first documented at the molecular level with nisin synthesis in *Lc. lactis* (118) and with the synthesis of plantaricins in *Lb. plantarum* C11 (61, 63). The core mechanism of a quorum sensing-based

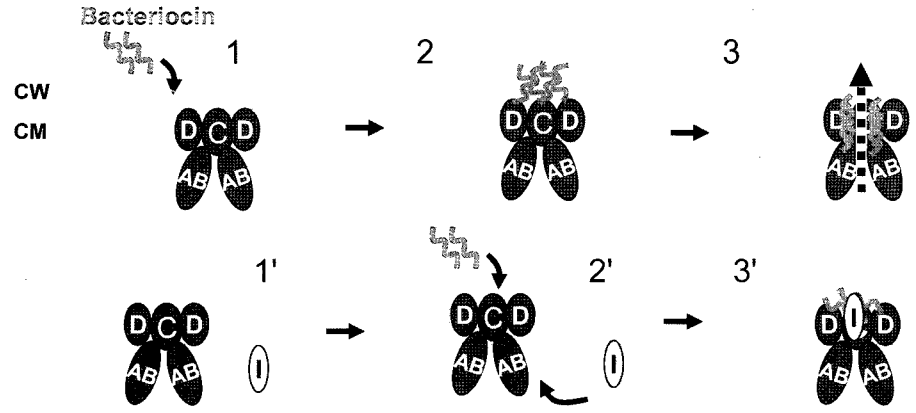

Fig. 3. Model for the mechanism on how one-peptide class II bacteriocins interact with their receptor and permeabilize bacterial membranes (1-3), and on how the bacteriocin immunity interacts with the receptor to block the permeabilizing effect of the bacteriocin (1′-3′). The receptor mannose-PTS complex comprises the proteins AB, C, and D. The immunity protein is shown as I in the figure.

regulation is dependent on a signal (in this case a peptide pheromone) in the environment. Prior to gene activation, the pheromone is produced constitutively at a low level, and as the cell number increases the pheromone will accumulate extracellularly. Eventually, the pheromone reaches a certain critical threshold concentration, monitored by a membrane-located sensor called histidine protein kinase (HPK). Activation of HPK triggers a series of phosphorylation reactions ending up with an intracellularly phosphorylated response regulator (RR) which subsequently binds to the regulated promoters in order to activate a defined set of genes (62, 163). In most bacteriocin regulated systems, the pheromone is usually a bacteriocin-like peptide (smaller than an average bacteriocin molecule with no or little antimicrobial activity) or the bacteriocin itself. Both the nisin and the plantaricin regulatory systems have been scrutinized in detail (164, 165). The most striking difference between the two systems is that while the plantaricin A system employs a dedicated peptide pheromone (plantaricin A) to regulate gene expression of at least two different bacteriocins, the nisin molecule serves a dual function being both an induction peptide (peptide pheromone) as well as being the bacteriocin.

Detailed information of the various steps in the regulatory pathway has been elucidated in the plantaricin system (61, 63, 166-168). In addition to the pheromone (plantaricin A), the HPK PlnB, this sytem involves to highly homologous RRs, PlnC, and PlnD, for gene regulation. Both regulators bind as dimers to the regulated promoters but while PlnC activates gene expression the other regulator PlnD represses expression of these genes (168-170). In the plantaricin regulatory system as well as in similar systems of class II bacteriocins, each promoter contains a pair of 9-base direct repeats that serves as binding sites for gene regulators (RR) (62). While the regulation of class II bacteriocins is well understood, little is known on how regulators of lanthibiotic systems bind their target promoters to activate gene expression.

More recently, another down-regulation mechanism has also been established for the class IIb plantaricin system (171). The gene regulator *plnC* encodes both the full-length protein and truncated species with alternative start codons within the gene. While the full-length PlnC serves as a gene activator, its truncated species which have a depleted receiver domain and an intact DNA-binding domain, somehow repress genes involved in bacteriocin biosynthesis. Such a down-regulation mechansism involving truncated species of a gene regulator was also established in the class IIa sakacin P system and in the class IIb plantaricin system of *Lb. plantarum* NC8.

Among the two-peptide lantibiotics it has also been shown that cytolysin from *E. faecalis* responds to a quorum–sensing autoinduction (172). In this system, products of the two genes *cylR1* and *cylR2* work together in order to repress the expression of cytolysin genes. Repression occurs when one of the cytolysin peptides reaches a critical theshold level in the extracellular environment. The cytolysin system does not involve an HPK or an RR in its quorum sensing-based regulation as normally found in other regulated systems.

The gene cluster needed for production of the two-peptide lantibiotic lacticin 3147 consists of two divergently transcribed operons $ltnA_1A_2M_1TM_2D$ and *ltnRIFE* encoding the bacteriocin biosynthesis machinery and its dedicated immunity system, respectively. The immunity operon of lacticin 3147 is autoregulated at the transcriptional level by the repressor LtnR which establishes a balance between the level of immunity and bacteriocin production in the cell. The bacteriocin producing operon is constitutively transcribed but a stem-loop structure seems to act as an attenuator to regulate production of the active bacteriocin (173).

The Rgg family of transcription regulators are widely but exclusively distributed among Gram-positive bacteria. Rgg was originally discovered in *Streptococcus gordonii*, where it was demonstrated to regulate the expression of the glucosyltransferase gene *gtfG* (174, 175). Since then, a number of Rgg-like regulators have been described: the *Streptococcus oralis* Rgg regulates the expression of the glucosyltransferase gene (gtfR) (176) and, RopB (also known as Rgg) regulates the expression of the virulence gene *speB* in *S. pyogenes* (177, 178). It has conclusively been shown that the Rgg-like regulator LasX regulated the lantibiotic lactocin S biosynthesis in *Lb. sakei* (162, 163)

and the Rgg-like MutR seems also to be involved in the regulation of the lantibiotic mutacin II biosynthesis in *S. mutans* (179).

Lactocin S is a 37-residue lantibiotic produced by *Lb. sakei* L45 (180, 181). The *las* gene cluster is organized into two divergently oriented operons, *lasAMNTUVPJW* (*lasA–W*) and *lasXY*; the former contains the biosynthetic, immunity, and transport genes (181). Two directly repeated heptanucleotide motifs (TTATCCC) located in the vicinity and common DNA region of the two divergent promoters could play a regulatory role for these two operons. It has been established that *lasA–W* and *lasXY* are regulated by LasX (182). Using reporter-gene fusions, it was demonstrated that LasX, supplied in *trans*, affected the transcription from both promoters P_{lasA-W} and P_{lasXY}. LasX was shown to activate P_{lasA-W}, while it repressed its own promoter P_{lasXY} (182).

Also alternative sigma factors can be involved in the regulation of bacteriocin expression in Gram-positive bacteria. It has been shown that a UV-inducible *Clostridium perfringens* bacteriocin is activated by a novel sigma factor (183, 184), however this bacteriocins belong to class III (large heat labile bacteriocins) and such regulation has not been found in class I and II bacteriocins so far.

Perspectives

Ribosomally synthesized antimicrobial peptides (bacteriocins) comprise an interesting group of antimicrobial compounds and may in certain medical treatments replace for or complement with traditional antibiotics. Even more interesting is the role bacteriocins may play in probiotic bacteria. A recent and exciting study demonstrated that a probiotic strain *Lb. salivarius* that produces the bacteriocin Abp118 protected mice against infection with invasive *L. monocytogenes*. The protection was primarily mediated by the bacteriocin because when the listerial strain expressed the cognate *abp118* immunity gene the bacteriocin Abp118 did not give any protection against the listeria infection (185). This finding should trigger a broader survey of bacteriocin producing probiotic LAB as a protection agent against pathogenic bacteria.

Identification of targets for many lantibiotics and many one-peptide class II bacteriocins is also another important research theme as it is necessary for further development of peptide-bacteriocins in medical applications. When the molecular mechanism behind the target recognition is understood, a more rational approach to design of novel or more efficient antimicrobials would be possible. Use of DNA shuffling has been proven possible to develope new class IIa bacteriocins (181). By shuffling the gene sequences of 10 pediocin-like bacteriocins, the resulting bacteriocin mutants were screened for bacteriocin activity and the active mutants showed increased activity against target strains of various species.The most interesting observation was that some of the new mutants showed activity against *Lc. lactis*, a bacterium to which most of the original bacteriocins did not affect. Sequence analysis disclosed that the change in target specificity was found, not unexpected, in the N-terminal domain (186).

The advent of bacterial genome sequences (more than 500 bacterial genomes have been finished) represents a source of discovering new peptide-bacteriocins (187, 188). In order to make an efficient search for such peptides efficient search tools have to be developed and a few are currently available (189).

One of the main obstacles for more efficient use of bacteriocins in food or medicine-related treatments is the emergence of bacteriocin resistance. Bacteriocin resistance can arise at varying frequencies depending on the bacteriocins while some bacteriocins creat low level of resistance, other bacteriocins such as the pediocin-like ones can cause bacteria to appear resistant at a frequency of as high as 0.1-0.01% when sensitive bacteria are exposed. The mechanism behind such a resistance development is one of the most important challanges needed to be adressed.

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