Review

Received: 31 May 2013 Revised: 24 June 2013 Accepted article published: 1 July 2013 Published online in Wiley Online Library: 9 August 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6293

Chemical and genetic characterization of bacteriocins: antimicrobial peptides for food safety

Abigail B Snyder and Randy W Worobo*

Abstract

Antimicrobial peptides are produced across all domains of life. Among these diverse compounds, those produced by bacteria have been most successfully applied as agents of biocontrol in food and agriculture. Bacteriocins are ribosomally synthesized, proteinaceous compounds that inhibit the growth of closely related bacteria. Even within the subcategory of bacteriocins, the peptides vary significantly in terms of the gene cluster responsible for expression, and chemical and structural composition. The polycistronic gene cluster generally includes a structural gene and various combinations of immunity, secretion, and regulatory genes and modifying enzymes. Chemical variation can exist in amino acid identity, chain length, secondary and tertiary structural features, as well as specificity of active sites. This diversity posits bacteriocins as potential antimicrobial agents with a range of functions and applications. Those produced by food-grade bacteria and applied in normally occurring concentrations can be used as GRAS-status food additives. However, successful application requires thorough characterization.

Keywords: bacteriocin; antimicrobial peptide; biocontrol

INTRODUCTION

Defining bacteriocins

Numerous antimicrobial peptides (AMPs) have been developed or derivitized into agents useful in agriculture and medicine, making genetic and chemical characterization of these compounds an ongoing endeavor. Perhaps the most relevant AMPs to the food industry are bacteriocins, due in part to the fact that some of the producer microorganisms are already considered food grade by the US Food and Drug Administration (FDA).[1] Bacteriocins are frequently defined as ribosomally synthesized peptides produced by bacteria that inhibit the growth of other closely related bacteria.[1–17] However, exceptions to this general definition certainly exist in terms of inhibitory spectrum and composition. While traditionally defined by antagonism of closely related species, many well-characterized bacteriocins have relatively broad-spectrum inhibitory activity. The basis of this definition may be traced to some of the early work with bacteriocins involving the characterization of colicins, which do have a very narrow inhibitory spectrum.[2] In fact, colicins antagonize only closely related strains within the species *Escherichia coli*.[18] However, nisin, a bacteriocin produced by *Lactococcus lactis* and FDA approved for use within the food industry, is widely active against Gram-positive bacteria. Broadly speaking, the bacteriocins of Gram-negative bacteria have a narrower inhibitory spectrum than their Gram-positive counterparts.[2,4,5,19] Determinants for the spectrum of activity are often based in the mechanism of action for a particular bacteriocin: whether its activity requires binding to a specific cell surface receptor and the relative distribution of that receptor among divergent bacterial species. Colicins require receptor recognition for activity and about half of the peptide's structure is dedicated to this function.[4] Alternatively, nisin binds lipid II, a widely distributed membrane component involved in synthesis of the bacterial cell wall.[20] Other mechanisms, as discussed later, do not require binding to a cell surface receptor at all.[1] Other complications to the conventional bacteriocin definition involve their composition. While all bacteriocins are primarily proteinaceous in nature, some have been identified with carbohydrate and, tentatively, lipid moieties.[21–23] These contested compounds are discussed in more detail under bacteriocin classification strategies. Generally, defining bacteriocins as proteinaceous, however, serves to distinguish them from other small-molecule antibiotics and metabolic byproducts which also inhibit microbial growth.[3] Finally, the general bacteriocin definition describes these compounds as peptides: short chains of amino acid residues. Most known bacteriocins are around 20–60 amino acid residues in length,[1] but there are bacteriocins which are significantly longer and better described as proteins.[24,25]

Despite the exceptions, some of the components of the conventional bacteriocin definition seem to be more universal. Ribosomal synthesis is a trademark distinction between bacteriocins and proteinaceous antibiotics.[1,2,6] Bacteriocins may be modified following translation, but initial synthesis is carried out by the ribosome. Conversely, antibiotics are enzymatically synthesized and have varied structure–function relationships as a result. Antibiotics commonly target cell wall synthesis, degrade nucleic acids, or have other enzymatic functions, while the most common target for bacteriocins is membrane permeabilization,

* *Correspondence to: Randy W Worobo, Department of Food Science, Cornell University, 630 W North St, Geneva, NY 14456, USA E-mail: rww8@cornell.edu*

Department of Food Science, Cornell University, Geneva, NY, 14456, USA

although this is not an absolute. Possibly the most unambiguous component of the bacteriocin definition is the identity of both the producer and target as bacteria. Examples of bacteria producing peptides deleterious to other organisms or other organisms producing peptides which inhibit bacteria can be classified more generally as AMPs, but not as bacteriocins. Well-studied AMPs of other organisms include the defensins, magainins, and melatin, but the cytotoxicity of these compounds makes them less suitable as food preservatives.[19,26]

Bacteriocins are just a small subgroup of an even larger class of compounds – AMPs – which are represented throughout nature. However, many eukaryal AMPs have been shown to be toxic towards mammalian cells as well as deleterious microbes. For this and other reasons, bacteriocins have been the focus of AMP development within the food industry. Bacteriocin research over the past ~50 years has increased our understanding of the diversity of these compounds and opened opportunities for application.

History

Scientists have been aware of the inhibitory activity of certain bacteria against others long before AMPs were officially recognized. Moreover, the effects of bacteriocins were utilized in ancient fermentations long before the identity of even their bacterial producers was known.[2,27] Pasteur is credited with first describing inhibitory activity between bacteria,[2,28] but it was not until the mid 1920s that the antimicrobial activity of secreted peptides was officially recognized. In 1925, Gratia demonstrated the inhibitory activity of what was later determined to be colicin V.[29] Shortly after, in 1928, nisin activity was reported by Rogers.[30] However, the identification of the compound 'nisin' did not occur until 1947 when it was concentrated.[27] The term 'bacteriocin' was instituted in 1953 by Jacob *et al.*[31] and the working definition for these compounds, as described above, arose from these early studies.[2] The same year, nisin was introduced to English markets as a food preservative.[27] Structural elucidation of bacteriocins widely occurred in the 1970s[2] and it was not until 1988 that nisin was approved for use within the US food industry as a GRAS (generally recognized as safe)-status additive.[2,32] The attention bacteriocin research received increased drastically in the 1990s and has continued into recent decades due to a rise in antibiotic resistance and continued interest in developing antibiotics to meet the industry trend towards 'natural' formulations.

Initially, bacteriocins were identified by screening for inhibitory activity using the spot-on-lawn method, further purified, and characterized.[1] Earlier research which attempted to estimate the prevalence of bacteriocin production using these methods gave varied results. In a 1983 paper, Geis *et al.* screened 280 streptococci for bacteriocin production and reported that ~5% showed inhibitory activity due to bacteriocins which were able to be partially purified.[33] A 1998 paper reported that 30% of *E. coli* screened produced some kind of bacteriocin,[13] while another paper screening 52 *Lactobacillus acidophilus* strains found 63% producing bacteriocins.[3] Early screening strategies used to determine what percent of a given set of isolates produced bacteriocins were inherently limited.[18,34] Most notably, to obtain observable activity a sensitive target bacterium is required, but the diversity of inhibitory spectra makes picking a target bacterium challenging without *a priori* knowledge. Klaenhammer in his 1983 paper screening for *L. acidophilus* bacteriocins noted that only four target bacteria were used as screens.[3] This limited range is most problematic for bacteriocins fitting the conventional definition and having a narrow spectrum of activity. The screening method is also limited by regulation of bacteriocin expression. Since activation of the bacteriocin operon is often regulated by particular environmental conditions and pressures, bacteria may not demonstrate inhibitory activity under a given set of laboratory conditions. Colicin production, for example, is part of the SOS regulon and bacteriocin expression is activated by stress.[4] Using genetic screens presents an alternative to methods dependent on expression, but even current genomic methods require *a priori* knowledge about the putative bacteriocin. Early screening methods also required further biochemical characterization to confirm that the inhibitory activity resulting from bacteriocin production. Control of experimental conditions and protease sensitivity are often used to ensure that inhibition of bacterial growth is a result of AMP secretion and not organic acids, small-molecule antibiotics, or ethanol production,[2,3,35] but purification and characterization of the bacteriocin itself, and not the producer or spent growth medium, are ultimately required. Insufficient characterization has resulted in identical bacteriocins being reported and named as novel peptides.[5] Despite limitations, screening for inhibitory activity has its utility, particularly from an application perspective. In fact, screens targeting a particular microbe or fortuitous observations of a previously uncharacterized inhibitory activity have led to the discovery of novel antimicrobials (e.g. penicillin).

Although many bacteriocins are possibly overlooked in the screening process, those which have been identified aid in the iterative process of novel bacteriocin discovery. New bacteriocins have been identified based on well-known structural features in previously characterized bacteriocins. Using biochemical techniques like affinity chromatography and spectrometry,[36–38] unknown bacteriocins can be characterized and extracted from growth media. Furthermore, genetic characterization of bacteriocins has led to genome mining techniques as means for identification.[1,5] This approach is based on sequence homology among bacteriocins and is useful for mapping structural genes similar to known bacteriocins, but unique bacteriocin structural genes which have not been previously characterized would continue to go unrecognized. However, continued investigation of AMPs has led to improved mining strategies. The operon for streptolysin – an AMP but not a bacteriocin – was recently shown to have a great deal of homology with genes associated with the production of known bacteriocins. Using these genes as query sequences in database searches yielded new putative bacteriocin genes, suggesting that the processing machinery for streptolysin may be related to processing enzymes of novel bacteriocins.[39] Nes *et al.* also note that the presence of a bacteriocin structural gene does not definitively indicate that the bacteriocin is actually expressed.[5] The matter is further complicated by the presence of peptide induction factors involved in quorum sensing and regulation of bacteriocin expression. Induction factor sequence is often very similar to the bacteriocin itself and may give a false positive in genome mining exercises.[5,40] New techniques are subject to some of the same limitations plaguing early screening methods, including assumptions about bacteriocin identity and potential for bacteriocins to go unrecognized. But despite these limitations, it has been estimated that most bacteria are bacteriocin producers.[10] It is estimated that up to 99% of bacteria produce at least one bacteriocin.[4] Research in bacteriocin development has continued to evolve and most recently it has been suggested that bacteriocins can be engineered based on known structural features or intended targets, although no preservative derived through this approach is in commercial development as of yet.[8,26,41]

Environmental role

The environmental relevance of bacteriocins is difficult to determine given the great diversity and complexity of these AMPs. While their roles are not well understood, arguments have been made for bacteriocin involvement in cell-to-cell communication, defense against other bacteria, and facilitation of horizontal gene transfer, although none of these are to the absolute exclusion of the others. Surmising their environmental impact is further complicated by the artificial laboratory conditions under which bacteriocins are often studied. By definition, bacteriocins inhibit the growth of other bacteria, which suggests their role in interspecies competition.[6,40,42] Indeed, the narrow spectrum of many bacteriocin activities is given as an example for how bacteriocin production is used to defend an ecological niche.[1,19] However, both the diversity of bacteriocin inhibitory activity and the regulation and production levels of bacteriocin in the environment challenge the notion that most bacteriocins' primary purpose relates to defense. Under idealized laboratory conditions, bacteriocin may be secreted at high levels, providing the inhibitory effect. Under conditions of their native environment, though, expression levels may be too low to have any real antagonistic effect on competitors, or the bacteriocin itself may exhibit only bacteriostatic rather than bacteriocidal activity.[18] In these instances, a generalized role for bacteriocins seems ill fitting. Certain bacteriocins, like colicins, have been shown to provide a competitive advantage in defending an ecological niche, whereas with many other bacteriocins it seems unlikely that defense of the producer bacterium is the primary role.[1,43]

Bacteriocins have also been associated with intercellular communication as a secreted protein which interacts with neighboring cells. The signal can be the bacteriocin itself through low-level constitutive expression, an independently transcribed peptide induction factor which frequently shares structural homology with the bacteriocin but with minimal to no activity, or the transcription of a double leader sequence involved in cell-to-cell communication through quorum sensing as a way of synchronizing production of secondary metabolites.[40] This communication prevents unnecessary energy expenditure in protein synthesis and a shared burden of community protection, suggesting a cooperativity among independent cells analogous to the behavior of cells composing tissue.[40] Finally, the possibility of bacteriocin production facilitating the transfer of DNA via transformation has likewise been supported by the conventional narrow spectrum of antagonistic activity since recombination efficiency decreases with increasing genome diversity. An increase in cellular competence for streptococci and induction of competence-stimulating peptide synthesis has been reported to coincide with bacteriocin synthesis as well.[44,45] As previously stated, the diversity of AMPs which fall into the subcategory of bacteriocins is still so great that determining a defined environmental role is difficult, and it is still probable that the range of bacteriocins which have heretofore been characterized represents only a limited number of bacteriocins that are active within the environment.[7]

Classification strategies

Similar to the challenges facing determination of environmental relevance, development of a classification strategy generalized to all bacteriocins is difficult given their widespread diversity. No such overarching classification has been applied, although modified versions of the classification system for the bacteriocins of lactic acid bacteria have been proposed.[5,25,46] The LAB system was proposed by Klaenhammer in 1993[47] and more recently revisions and residual debate over the definition and inclusion of certain divisions has arisen. The original system split LAB bacteriocins into four divisions based on structure. Class I bacteriocins are the lantibiotics, a name which comes from 'lanthionine-containing antibiotics', of which nisin is the most widely recognized. Characteristic post-translational modifications of lantibiotics include lanthionine and methyllanthionine bridges, 2,3-dehydroalanine (Dha) and 2,3-dehydrobutyrine (Dhb), which result from condensation of serine or threonine residues.[47] Class I was broken into two subclasses: A type, which are flexible, linear peptides with a net cationic charge; and B type, which are rigid, globular, and are more typically anionic or contain no net charge.[6,48,49] Later, discovery of lantibiotics containing domains typical of both subgroups prompted creation of a third subtype, C, which contains multicomponent lantibiotics.[5,46,50] Class II LAB bacteriocins have been defined as non-lantibiotic or unmodified peptides, of which pediocins are the most recognizable example. While the class has long been recognized, modifications of what is included in the subgroups within Class II have occurred over time, establishing it as a kind of catch-all for miscellaneous bacteriocins.[5] Klaenhammer's original subclasses included: IIa, defined as pediocin-like, antilisterial bacteriocins; IIb, two-component bacteriocins comprised of separate peptide domains; and IIc, thiol-containing bacteriocins lacking a leader sequence and characteristically secreted through a general *sec*-pathway.[5,47] Later discovery of subtype IIa bacteriocins secreted via the *sec*-pathway challenged the definition of the IIc subtype and the thiol group was determined not to be required for activity.[5,7,51] Cotter *et al.* later proposed that subtype IIc should be reserved for cyclic bacteriocins,[52] but most recently it has been proposed that cyclic bacteriocins merit their own class, as proposed by Heng and Tagg with Class IV.[53] Cotter's revision also proposed the addition of a IId subtype for non-pediocin, miscellaneous peptides including lactoccin A.[7,52] Major revisions suggested by Cotter in 2005 left only the first two original classes, but Klaenhammer's system had two additional divisions. Class III was defined by large, heat-labile bacteriocins and was later subdivided into two subgroups, for bacteriolytic (IIIa) and non-bacteriolytic (IIIb) activities.[53] Finally, the original system included Class IV – complex bacteriocins containing carbohydrate or lipid moieties[47] – which was eliminated totally from classifications due to lack of substantiation that such bacteriocins existed. Such compounds had been predicted but were not successfully purified. Additionally, some of the original complex bacteriocins had been isolated without their carbohydrate or lipid moieties but retained their inhibitory activity, suggesting these components were not necessary for activity. It was suggested that the net charge of the bacteriocin had led to a complex with the hetero-group, which was not covalently linked.[5,7,52] Recent studies, however, have confirmed the existence of glycocins – glycosylated bacteriocins – and identified the enzyme responsible for the covalently linked carbohydrate moiety.[21,22,54] Many lipopeptides are still nebulously categorized as bacteriocin-like substances without purification for confirmation.[23] While recent classifications have maintained the exclusion of complex bacteriocins, the confirmation of glycocin F perhaps indicates that they should be re-established in the accepted classification strategy. Now, Class IV bacteriocins are described as the aforementioned cyclic peptide group. A summary of a current classification system generalized to all bacteriocins is summarized in Table 1. Classification strategies

Table 1. Bacteriocin classification scheme, originally proposed by Klaenhammer[47] and recently revised by Heng and Tagg[53]

Class I		Lantibiotics
IA	Linear, rigid	
IB	Globular, flexible	
IC	Multi-component	
Class II		Non-lantibiotics
IIa	Pediocin-like	
IIb	Miscellaneous	
IIc	Multi-component	
Class III		Large, heat stable
IIIa	Bacteriolytic	
IIIb	Non-bacteriolytic	
Class IV		Cyclic

offer appealing and concise groupings for bacteriocin research, but the diversity of bacteriocins and lack of sufficient structural information for many bacteriocins render these systems imperfect.

GENETIC ORGANIZATION

Genes associated with bacteriocin expression are often described as being arranged in operons variously found in different elements of the bacterial genome.[12,55–58] Frequently, though, bacteriocin-associated genes are described as being arranged in gene clusters. Since many bacteriocin genes are under the regulation of multiple promoters, their structure does not technically match the description of an operon, although the term is often used. Nisin-associated genes, for instance, are regulated by three distinct promoters, all auto-regulated by extracellular nisin,[59] making nisin expression more accurately described as under transcriptional regulation of a regulon. Gene clusters are often complex, with divergently transcribed genes encoded on complimentary strands of DNA[60] and ancillary genes encoded in different loci from the structural gene.[12] And, as was noted in the discussion on genome mining for identification of bacteriocin expression, the presence of bacteriocin operon components does not necessarily indicate that functional expression, and partial or inactive operons have been identified.[11] Functional operons have been identified on the chromosome, as in the case of sakacin[11] and mersacidin;[49] on plasmids, both conjugative and mobilizable, as is the case of many colicins[10] and pediocin PA1;[61] and transposon encoded, for which nisin A is a prime example,[2] as is lacticin A.[1] Bacteriocin gene clusters are commonly associated with transferable elements like plasmids (or conjugative transposon in the case of nisin A) because of their wide distribution in nature, non-essential function, and conferrable advantage.[10,14] However, many examples of chromosomally encoded bacteriocins exist. Some of these potentially resulted from the introduction of transferrable elements into the chromosome, including the transposon containing the nisin A structural gene, which is found in the chromosome of *Lactococcus lactis*.[16,62,63] In all cases, the bacteriocin operon contains a structural gene, encoding either the bacteriocin itself or a pre-peptide subject to further modification. Additional ancillary genes may include immunity peptides, modifying enzymes, secretion machinery, and an inducing factor – a bacteriocin-like substance. The exact makeup of the operon is dependent on the structure, regulation, and expression of the bacteriocin, but commonalities have been observed among distinct groups of bacteriocin producers. There are exceptions to these general schemes in terms of both regulation and genetic organization, for which carnobacteriocin B2 serves as an example for further reading.[64,65]

Regulation

Placing the bacteriocin structural and modification genes under the control of a single promoter, or in a regulon, allows for induction of expression only under conditions when bacteriocin secretion is advantageous to the cell. As described in the section on environmental relevance, bacteriocin expression in native environments generally occurs at a low level and, under all proposed reasons for expression, is involved in interactions with other bacteria in close proximity. Therefore, expression is only useful when a certain density, or quorum, of neighboring cells has been achieved and is observed during late lag growth phase.[66] Quorum sensing is the primary mechanism for regulation of bacteriocin expression in most Gram-positive bacteria, and either the bacteriocin itself or some closely related peptide analog serves to induce expression via a signal transduction pathway. Two regulation systems represent generalized pathways for transcriptional control of bacteriocins in Gram-positive and Gram-negative bacteria.

Although Gram-negative bacteria make use of quorum sensing, which is not regulated by peptides as is the case for Gram-positive bacteria, the regulatory system for bacteriocin expression in Gram-negative bacteria discussed here is stress induced.[45] Many Gram-negative bacteriocins like the nuclease active colicins, produced by some *E. coli* and pyocins, produced by pseudomonads, are under the regulation of an SOS promoter which is induced by DNA damage and derepression of the SOS response. Cellular recognition of single-stranded DNA leads to disruption of LexA binding and expression of genes involved in DNA repair and recombination. The same promoter governs expression of many Gram-negative bacteriocin operons and is recognized by the $\sigma 70$ subunit of RNA polymerase. An SOS box of approximately 16 nucleotides is located around the promoter region and is the binding site for LexA, the binding of which prevents the association of RNA polymerase and transcription.[67] When the SOS response is induced as a result of DNA damage initially affecting only a fraction of the producer cells, the bacteriocin is expressed in those injured cells. Autolysis of the producer cells results in bacteriocin release, which promotes antagonism of neighboring, sensitive cells.[10,68] This system contrasts significantly with bacteriocin regulation in Gram-positive bacteria like lactic acid bacteria, which are well studied and are often taken to represent the whole of bacteriocin research. The differences in regulation between nuclease colicins and those like them and bacteriocins of lactic acid bacteria can be taken as an indication of the potential differences in their environmental role, as previously suggested.

The regulation of Gram-positive bacteria follows different pathways, and those utilized by Class I and II bacteriocins have been well characterized. Both rely on signal transduction systems and are mostly differentiated based on their inducer peptide. This difference distinguishes them as either two- or three-component regulatory systems. Class I bacteriocins are under transcriptional regulation of a two-component regulatory system, indicating that they are auto-regulated and the bacteriocin itself activates expression.[1,10,65] The bacteriocin interacts with the first component, a membrane-bound histidine protein kinase. ATP is hydrolyzed and the internal portion of the kinase transfers a phosphoryl group to the second component, a response regulator located in the cellular cytoplasm. This causes a change in the

structure of the response regulator which activates transcription.[65] However, the complete set of genes associated with secretion of lantibiotics is often transcribed in multiple (3–4) units and are separated by several promoters which are activated by the same signal response.[12,69] Activation of these various promoters often occurs at different concentrations of extracellular bacteriocin, which is to say that the promoters vary in their sensitivity towards activation. Moreover, the region downstream of the structural gene in the nisin operon contains a leaky terminator site, so that many more transcripts for the bacteriocin are generated per complete transcript containing the structural gene as well as modification and immunity genes.[70] This additional level of organization with multiple promoters of different sensitivities and variability in transcription rates improves expression efficiency and decreases unnecessary energy costs. Class II regulation is almost identical to Class I regulation pathways except that it is generally associated with peptide pheromone induction as opposed to autoregulation.[12,15,40] A gene unique from the structural gene encodes a bacteriocin-like substance which is the signal recognized by the extracellular domain of the histidine protein kinase. The induction factor is often structurally similar to the bacteriocin, is synthesized as a pre-peptide and undergoes similar proteolytic processing, and is secreted through the same dedicated transporter.[16] Both Class I and Class II bacteriocins are therefore subject to positive feedback once expression is initiated. Both classes utilize Rho-independent termination[14–16] and are frequently flanked by regulatory sequences like inverted repeats and palindromes, which serve not only a regulatory function but can often indicate a recombination or transposition event.[12,69] Characterization of bacteriocin regulation systems has led to their development and exploitation in commercial settings. As an example, the nisin-controlled expression system (NICE) contains modified regulatory elements which allow for well-controlled and optimized expression of the peptide.[70]

The Gram-negative gene cluster and its function

Despite the diversity among bacteriocin structure and sequence, the operon structure and sequence may show homology among distant bacterial species.[39] Lagos *et al.* determined that the dedicated secretion machinery utilized by microcins shared a high degree of similarity with pore-forming colicins despite being unrelated.[71] Recent bioinformatic work has even suggested that Gram-negative AMPs which utilize Class II-like expression systems may exist.[45] The microcin-processing machinery also shows functional similarity to the AMP streptolysin enzymes, as discussed in the Introduction.[39] In nuclease-active colicin operons regulated by the SOS promoter, there are typically fewer genes present, as many of the functions of regulatory machinery or proteolytic processing are not required by these systems. Although this varies depending on the specific bacteriocin, this review will discuss the well-studied nuclease colicin operons as a representative of Gram-negative bacteriocins, which contrast significantly with bacteriocins of lactic acid bacteria. Colicins themselves are a somewhat heterologous group in terms of target receptor specificities and mechanisms of action, although the major difference among operonic structures may simply result in the directionality of the immunity gene.[72] Colicins that act by permeabilizing the membrane of the target cell have an immunity gene (*cxi*) encoded on the DNA strand complementary to the colicin structural gene. This is in contrast to colicins with enzymatic activities wherein the immunity gene is co-transcribed with the structural gene.[73] The immunity gene is immediately downstream of the structural gene as is the organizational arrangement in Class II bacteriocins produced by Gram-positive bacteria. The structural gene is the first cistron following the promoter and is termed *cxa*, where the '*x*' stands for the respective letter code for a given colicin (e.g. colicin V, colicin E1) based on its cell surface receptor. Some operons may contain an additional processing gene(s), but the final gene within the colicin operon encodes the lysin, *cxl*, which initiates the release of the bacteriocin from the producer cell.[10] Figure 1 is a schematic of the colicin operon.

The Gram-positive gene cluster and its function

For bacteriocins produced by Gram-positive bacteria, nisin A and pediocin PA1 will be discussed as examples of Class I and Class II operonic structures. The nisin A gene cluster is analogous to many other well-characterized lantibiotics and contains 11 open reading frames. The first is the structural gene for the bacteriocin, *nisA*, followed by a series of ancillary functions regulated by the same promoter. These include genes for modification enzymes (*nisB*, *nisC*), a translocating protein (*nisT*), the immunity protein (*nisI*), and a peptidase (*nisP*), while the additional genes are placed under the control of one of two other promoters. The modification enzymes NisB and NisC are involved in post-translational modification. NisB facilitates dehydration reactions in the generation of Dha and Dhb while NisC is involved in formation of lanthionine bridges.[74] NisT transports the fully modified peptide across the cell membrane,[65] while NisP cleaves the leader sequence from the pre-peptide.[75] The immunity protein NisI is a lipopeptide and is associated with producer immunity for intracellular nisin (nisin 'interception') whereas the three immunity genes *nisFEG* regulated by the most downstream promoter are affiliated with disruption of nisin interaction with the producer cell membrane.[76] The genes regulated by the middle promoter encode the regulatory machinery NisR and NisK, the response regulator and histidine protein kinase necessary for signal transduction.[65] A point of variability among lantibiotic gene clusters concerns the modification enzymes. In some bacteriocins, including lacticin A, the modification is carried out by a single enzyme (generalized as LacM) as opposed to proteins analogous to NisB and NisC – although, in the lacticin operon, there happens to be a duplication of the *lanM* gene, but this is not a general rule.[14]

The pediocin operon is exemplary of those for Class II bacteriocins, is expressed in multiple species of *Pediococcus* and even across genera in the case of *Lactobacillus plantarum*,[77] and has demonstrated homology with coagulin (producer: *Bacillus coagulans*).[78] Like the colicin operon, the operons of Class II bacteriocins can be somewhat streamlined compared to those of the lantibiotics since some enzymes involved in post-translational modifications are unnecessary. However, many of the remaining genes are homologous to their lantibiotic counterparts, including genes involved in signal transduction.[79] The structural gene *papA* is the first to follow the promoter and the gene encoding the immunity protein *papB* typically immediately follows. The peptidase PapC cleaves the leader sequence and PapD is the system's dedicated transporter.[12,80,81] The diversity of pediocin-producing bacteria is an indication of horizontal transfer of these plasmid-encoded genes. In the *L. plantarum* gene cluster, the pediocin operon is homologous to that found in various *Pediococcus* species, but after a distance of only a few hundred base pairs on either side of the operon the sequence diverges significantly. The pediocin operon is generally defined

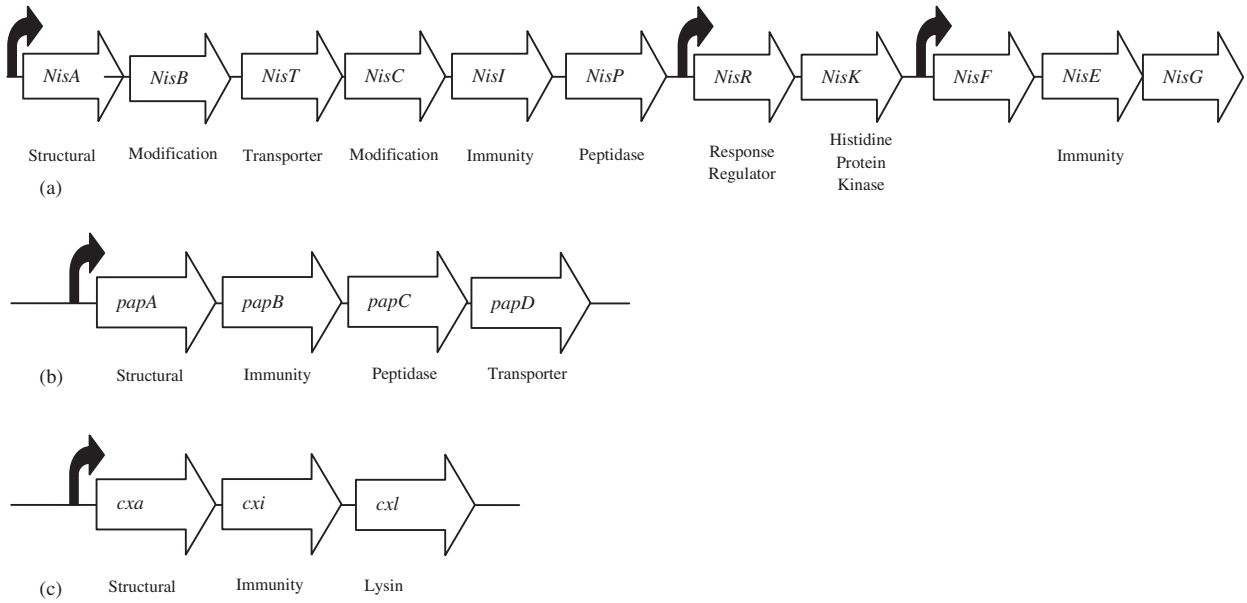

Figure 1. Variability in gene clusters for (a) nisin, (b) pediocin, and (c) colicin.

as containing the four genes *papABCD* previously described, but additional ancillary genes may be located elsewhere in the genome as a result of genetic exchange.[12] For further reading on the operon structure for Gram-positive bacteriocins, see the review by Chen and Hoover[1] and for Gram-negative bacteriocins see the review by Riley.[10]

PEPTIDE STRUCTURE ANALYSIS

The structural gene for all bacteriocins encodes the primary amino acid sequence and is the single greatest determinant of subsequent activity. The original sequence may be enzymatically modified, proteolytically processed during secretion, and folded into secondary and tertiary structures which collectively contribute to important structure–function relationships relevant to a given bacteriocin's mechanism of action. Structural analysis of the bacteriocin macromolecule yields valuable insight into the target and specificity for antimicrobial activity, potential mammalian toxicity, and requirements for generating variants or engineering new antimicrobials. Structural analysis is often dependent on a combination of biochemical assays, genetic experiments for both point mutations and peptide fusions/deletions, in addition to nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography, and circular dichroism (CD). Structural analysis is often difficult and dependent on the ease with which a bacteriocin can be purified or crystallized, and data, once obtained, may be subject to interpretation and inference in the absence of direct evidence for structure–function relationships. The results of such analysis, though, provide information regarding the role of specific peptide domains and determination of active sites within the bacteriocin.

Post-translational modifications

In the case of lantibiotics, primary sequence structure is altered by post-translational modification prior to secretion and is necessary in the generation of a functional peptide. Modification reactions for nisin are mediated by the enzyme NisB and involve dehydration of specific serine or threonine residues to form Dha or Dhb – amino acid derivatives containing an alkene in resonance with the carbonyl.[5] The specificity of the modification enzymes for their peptide substrate and generation of products with the correct stereochemistry is an important component of functional lantibiotic biosynthesis, and is one of the challenges of synthetic engineering.[82,83] The second modification enzyme, NisC, is involved in the formation of cyclic structures from the dehydration products of the NisB reaction.[74] The Dha or Dhb residue along with a neighboring cystine undergoes a Michael addition to form a lanthionine or β-methyllanthionine bridge – a covalent modification which plays a significant role in structure determination for lantibiotics. The thiol group involved in bridging could be contributed from cystines throughout the primary structure since a 'neighboring' thiol group is defined only as one for which torsion angles and steric hindrance are conducive for bridging, but enzyme specificity exactly determines these modifications.[83,84] Moreover, nisin contains unbridged Dha and Dhb, indicating that the enzyme does not act on all Dha/Dhb residues as substrates. In fact, the unbridged Dha located at the 5-residue position in nisin is essential for its interaction with the target cell membrane and its antibacterial effects.[82] The specificity of modification enzymes (LanB, LanC, or LanM) varies, so that a recombinant enzyme may function in a different bacteriocin expression vector. However, enzyme activity retains enough specificity to selectively dehydrate only certain cystine/threonine residues and form bridges between exact Dha/Dhb and cystine pairs.[85] For structural determination of novel lantibiotics, lanthione bridges must be determined using spectroscopic methods (3D NMR, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) and sequence analysis since oxidation of thioether bridges results in two alanine residues with no indication of involvement in lanthionine/β-methyllanthionine bridges.[86,87] Post-translational modifications are involved in stabilizing the molecule, an important feature for consideration in application, and contribute to structural elements necessary for antimicrobial activity. Figure 2(a) depicts the post-translationally modified nisin peptide interacting with a lipid membrane.

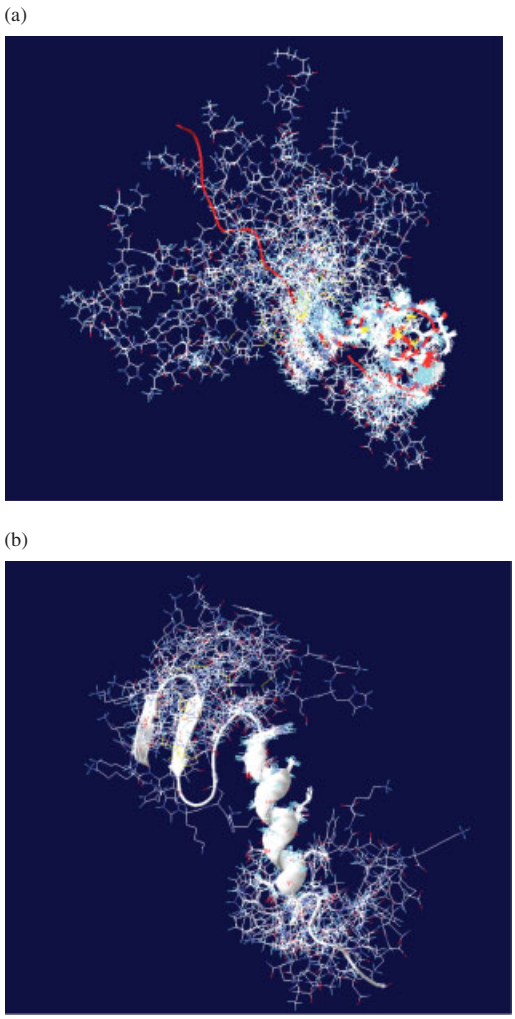

Figure 2. Structural analysis of Class I nisin and Class II sakacin P. (a) Nisin structure (red) in complex with the lipid II receptor molecule elucidated by Hsu *et al*.[118] Image generated using Swiss-PDB viewer 4.0.4. (b) Sakacin P structure in micelles as elucidated by Uteng *et al*.[92] Image generated using Swiss-PDB viewer 4.0.4.

Leader sequences

Many characterized bacteriocins are first synthesized with a pre-peptide leader sequence attached to the N-terminus of the bacteriocin.[88] Whether the structural gene encodes for a leader sequence is in keeping with the respective bacteriocin's secretion system.[89] Unlike colicin V, which utilizes a secretion system, other colicins with nuclease activity are not transcribed as a pre-peptide and do not require subsequent proteolytic processing since they do not utilize a dedicated transport system.[10,72,90] Similarly, Class II bacteriocins which utilize the general *sec*- pathway have leader sequences which differ from those that utilize ABC transporters. The structural gene encodes for the pro-peptide in Class I and II bacteriocins. It includes an additional sequence extension which encodes for 18–28 amino acids not present in the functional bacteriocin expressed in the culture supernatant.[2] The protease encoded in Class I and Class II bacteriocin gene clusters is responsible for cleavage of the pro-peptide, which occurs in tandem with secretion. The antimicrobial activity of the bacteriocin is greatly attenuated by the leader sequence, which has led to speculation that the leader peptide protects the producer bacterium prior to secretion.[1,88] Additionally, the leader sequence is recognized by the domain of the secretion machinery located in the cytosol of the cell by its characteristic hydrophobic charge, which aids in export from the cell. The pre-peptide sequence is conserved among related bacteriocins with a double glycine motif characteristic of Class IIa bacteriocins serving as the recognition site for proteolytic cleavage.[89]

Primary sequence

Amino acid sequence dictates the formation of various 3D structural elements that are determinants for bacteriocin activity. In addition to the overall structure and charge of various peptide domains, individual amino acids are involved in specific binding as active sites, so that substitution of particular residues with even residues of similar size and charge will result in a decrease in function.[88,91–94] Ascribing function to particular amino acids or particular structural elements can be tenuous as the two are closely tied; however, defining activity determinants is necessary for the generation of derivatized or engineered bacteriocins. Sequence identity contributes general characteristics such as net surface charge, which for many bacteriocins is cationic and contributes to cell wall affinity.[95] Class IIa bacteriocins have been well characterized structurally and analysis of the sequence of the N-terminal domain reveals the highly conserved sequence: YGNGV.[96,97] The N-terminal of pediocin, and bacteriocins like it, is associated with binding the target cell receptor, but it is predicted that this interaction is facilitated by the general hydrophobicity of the residues as opposed to the specificity of the YVGNGV (referred to as the 'pediocin box') sequence.[88,97] See Fig. 2(b) for a depiction of the pediocin-like bacteriocin sakacin P in lipid micelles. Three amino acid residues are of particular significance as determined by analysis of mutant IIa bacteriocins altered in only a single amino acid residue, and they are cystine, tryptophan, and proline. Proline is rendered significant mostly by its absence in much of the primary sequence. A survey of the primary sequences available for a diverse set of bacteriocins (+100) indicates that the relative abundance of proline in these compounds is proportionally very low. Proline's unique structure, wherein the amino group is part of a heterocyclic R-group, explains this phenomenon. Replacing a native amino acid with proline consistently resulted in a decrease in activity, since proline restricts torsion angles and hydrogen bonding potential in peptides. In contrast, substitution of a pre-existing proline with another amino acid also resulted in a decrease in activity that was not associated with structural changes based on structural analysis and location of the residue near the terminus of the peptide. Taken generally, these data suggest that, while proline may be found in low abundance in bacteriocins, the presence of proline when it does occur serves a specific purpose in bacteriocin functionality.[91,98] Among Class IIa bacteriocins, specific tryptophan residues have been found to be highly conserved and necessary for activity. Modification of any of the tryptophan residues has been found to be deleterious to activity, as was the modification of many other of the aromatic compounds. In particular, this class of bacteriocins has a conserved tryptophan residue located near position 18/19 which serves as a junction between the aqueous-facing N-terminal domain and the membrane-embedded C-terminal domain. This tryptophan is essential and does not tolerate substitutions – even substitution with another aromatic residue.[91,97] Rihikova *et al.* noted that several residues following this tryptophan are also essential, prompting a description of this region as a kind of active site required to maintain function of the C-terminal domain.[91] Substitution of amino acid residues at

positions 19, 21, or 23, even if the substitution was similar in size and charge, decreased the inhibitory activity. Furthermore, none of the substitutions greatly impact the higher orders of peptide structure. Tryptophan's characteristic hydrophobic and hydrophilic sides have been associated with transitional positions at membrane interfaces and are predicted to serve a crucial function in domain orientation which cannot be achieved by other aromatic compounds.[88]

Finally, the role of cystine has been shown to be significant in the generation of disulfide bridges for this same class of bacteriocins. Disulfide bridges contribute to the stability and target cell recognition of pediocin-like AMPs. In the N-terminal domain, a single disulfide bridge is generally found and serves a stabilizing function, while the presence of a cystine residue and subsequent disulfide bridge C-terminal domain is more variable. However, elimination of either of the cystine residues generally proves deleterious, with the activity of pediocin itself being one of the most sensitive to the loss.[99] It is the formation of the disulfide bridge form cystine and its stabilizing effect on the larger peptide domains which causes has such a deleterious effect on bacteriocin activity and specificity.[88,91,97] However, this effect has been shown to be a result of structure as opposed to direct interaction of the cystine residue with any particular receptor.[100]

Many of the sequence features which play a large role in defining activity for lantibiotics are discussed in the above section on post-translational modifications. Lanthionine bridges, as an example, maintain a characteristic 5-ring structure, which is necessary for nisin activity. Nisin has two naturally occurring variants, nisin A and nisin Z, which are distinguished by a single amino acid difference (A27H). While this change does not have an impact on activity, the nisin Z variant has been reportedly more soluble at neutral pH, which is a desirable characteristic for applications in food.[93]

Structural domains

Folding of the primary sequence yields secondary structures (α-helices, β-sheets, turns) and tertiary structures largely defined as orientation of secondary structures in particular domains. Quaternary structure is sometimes relevant for bacteriocins since peptides may oligomerize to form subunits of an active inhibitory complex. Bacteriocin AS-48, as an example, was characterized as a helical bundle that dimerizes in membrane-mimicking conditions to form a torridal pore complex that causes membrane permeabilization.[94] Structural analysis has depended on circular dichroism analysis for prediction of secondary structural elements when purified bacteriocin is in an aqueous solvent, hydrophobic solvent, or in the presence of micelles.[92] For pediocin-like bacteriocins, an undefined structure has been observed in aqueous solutions, while more defined α-helix and β-sheet structures are formed in a hydrophobic environment or when added to micelles.[88,94,101,102] Characterization of over 25 Class IIa bacteriocins has indicated that these compounds share similar sequence identity, similar secondary structural elements, but different global domain orientation. These data have indicated that the mechanism and dedicated purpose for the structural features is common across this group, but that differences in domain structure have resulted in the range of target specificities.[103] While this is true for the Class IIa bacteriocins that have been characterized, other bacteriocins have more specific binding requirements based on recognition by an active site.[10,72,80,88,91] Class IIa bacteriocins are composed of two distinct domains. The N-terminal domain is highly conserved and composed of three antiparallel β-sheets, which are stabilized by a disulfide bond as previously described.[104] This domain's hydrophobic regions extend outside the cell membrane and are associated with receptor recognition. Since this region is so highly conserved, the diversity of target spectra for different Class IIa bacteriocins is explained by variations in the orientation of the β-sheets imposed by the exchange of only a few different amino acid residues. The cystine involved in bridging in this region, as previously described, is necessary for activity and may orient the N-terminal domain for receptor site recognition.[8] The more hydrophobic C-terminal domain is known for having slightly more sequence divergence. Consistently, though, this domain forms one or more membrane-embedded α-helices which may or may not be stabilized by an additional cystine residue, depending on the particular bacteriocin. The remainder of the C-terminal domain extension folds back in a hairpin turn in the hydrophobic membrane bilayer and remains antiparallel to the α-helix.[105] The number and length of α-helices and length of the hairpin turn account for much of the variability observed within this domain.[88,91] This C-terminal domain is associated with spatial orientation of the bacteriocin within the cell membrane and membrane permeabilization of the target, and the active site discussed in the previous section, including amino acid residues 19–23, are within this central α-helical domain. It has been proposed that pediocin-like bacteriocins act as pore formers and the hydrophobic C-terminal domain interacts with the cell membrane, while the N-terminal domain eventually forms an interior portion of the pore.[8] The two domains are separated by a flexible hinge, inclusive of a conserved tryptophan residue.[80] The hinge allows movement of the two domains relative to one another, facilitating the mechanism of action.[80] Among several lantibiotics, similar features crucial for activity have been identified and are comparable to the flexible hinge and conserved, hydrophobic helix identified in Class IIa bacteriocins.[106] Structural differences between subtype A and B lantibiotics have also been associated with differences in activity. Class IA bacteriocins are viewed as rigid, cationic peptides which often bind a target receptor and induce membrane permeabilization, whereas type B are globular, neutral anionic peptides which bind a target receptor and prevent cell wall synthesis.[93]

Functional analysis

Determination of the structural features that contribute to antimicrobial activity is an integral part of bacteriocin characterization. Functionality that stems primarily from structural domains can often tolerate amino acid substitution given that the altered residue has similar properties. Nisin variants have been successfully generated by selective mutations using similarly charged amino acids that maintain the cationic charge of the peptide. The significance to the net charge of the molecule was further illustrated by showing a decrease in antimicrobial activity when the net negative charge on the micelle surface was reduced.[107] Interestingly, modification of surface charge is one of the methods of target cell resistance when sensitive cells are exposed to cationic AMPs.[108] Modifications to particular amino acid residues can be used to modify characteristics like solubility, pI, and target specificity. Recent studies have shown that even required amino acid residues like cystine can be modified if the modification retains the native structural features of the disulfide bridge. Multiple examples of alkene groups substituted for cystines in Class IIa bacteriocins have successfully maintained the antilisterial characteristics of the bacteriocin, since the olefin groups retain the native structure without

introducing cyclization.[109,110] Much of this research benefits from comparison with known structures and homology modeling, making continued contributions to protein databases of significant value.[111] Structural analysis is often coupled with determination of the mechanism of action. Indeed, concomitant experiments involving mutagenesis, structural analysis, and biochemical assays are almost required for prediction of either.[105]

MECHANISM OF ACTION

Bacteriocins antagonize sensitive cells through different and distinctive mechanisms. Although structure–function relationships have only been determined for particular bacteriocins and to varying degrees, examples of bacteriocins targeting the cell wall, cell membrane, nucleic acids, or enzymes have been established. Some colicins, as discussed above, have enzymatic activity and target nucleic acids.[10,112] Examples include colicin E2 and colicin DF13, which have ribonuclease activity, and colicin E2, which has endonuclease activity, requiring DNA as a substrate.[17] Alternatively, many bacteriocins produced by lactic acid bacteria act by pore formation or inhibition of cell wall biosynthesis, and have been known to bind specifically to lipid II moieties on the cell surface. Mersacidin and other lantibiotics belonging to Class IB bind lipid II and inhibit cell wall biosynthesis by preventing transglycosylation.[113] Similarly, the glycopeptide antibiotic vancomycin binds lipid II and blocks the enzymatic function required for cell wall synthesis.[114] Nisin also binds lipid II and at sufficiently high concentrations has been shown to significantly impede cell wall synthesis.[115] However, the primary killing mechanism resulting from nisin treatment is cell membrane permeabilization. The pore formation steps involved in permeabilization of the cytoplasmic membrane by nisin have been well studied. The initial interaction between AMP and the cell is mediated by charge attractions between the cationic peptide and the negatively charged cell envelope, and decreasing the net charge in either of these entities results in decreased activity.[114,116] This particularly concerns the positively charged amino acids near the C-terminus of nisin, which are attracted to the negatively charged phospholipid membrane during initial interaction.[117] Specific binding subsequently occurs between the N-terminus of nisin and the cell surface receptor, lipid II. Multiple rings generated by the formation of lanthionine bridges established a corkscrew-like structure in the N-terminus that binds the disaccharide–pyrophosphate of the peptidoglycan precursor lipid II.[118] While several bacteriocins have been found to utilize lipid II as a docking molecule in their mechanisms of action, as mentioned above, distinctive differences have been observed in the region of lipid II targeted by the various peptides. For instance, mersacidin, actagardine, and the AMP vancomycin have different lipid II binding sites compared to nisin, so that a target cell which has acquired resistance to one of these AMPs may still be sensitive to the inhibitory effects of the others.[119] For nisin and many other bacteriocins of lactic acid bacteria, binding to the docking molecule is followed by the generation of membrane pores via one of several models, described below.

However, it should be noted that binding to a specific target receptor is not a universal step in bacteriocin mechanisms of action. Some bacteriocins only require electrostatic interaction between the AMP and the host cell surface to induce activity.[120] Many eukaryotic AMPs do not require such binding, while it is more common for bacteriocins to utilize a target receptor – a step that is associated with their high degree of target specificity. A higher MIC is an indication of a bacteriocin that does not require receptor binding for activity. This can be confirmed experimentally by enantiomeric analysis. If activity is observed in both enantiomeric forms of the peptide, then receptor binding may not be needed.[121] The attraction of cationic peptides for anionic cell surfaces has also explained the lack of bacteriocin cytoxicity towards mammalian cells. Bacterial cell envelopes, either Gram negative or Gram positive, have a net negative charge from either lipopolysaccharide (LPS) or teichoic acid and phospholipids, respectively. This is in contrast to the cell surface of a mammalian cell, which is composed of phosphatydilcholine and sphingomyelin phospholipids that establish a net positive charge.[122] Although these surface charges are the basis for non-receptor-mediated interaction, it has been observed that the density of LPS content in the target cell membrane is not directly correlated with activity to the same degree that activity has been correlated to the density of receptor content in target cell membranes for receptor-mediated bacteriocins.[123]

Challenges with determining mechanism of action

Pore formation is generally regarded as the primary killing mechanism for nisin, but a secondary mechanism of action has been observed at high concentrations and over longer periods of incubation.[124] Since nisin binds the peptidoglycan precursor undecaprenylpyrophosphoryl-MurNAc(pentapeptide)-GlcNAc, cell wall synthesis can be inhibited by the presence of high levels of nisin.[119] During cell division, nisin treatments impede cell wall synthesis at the septum and resulting daughter cells show abhorrent cell morphologies.[14] These results led to reports that nisin antagonized cells by inhibition of cell wall synthesis,[125,126] while alternative studies that focused on the leakage of cellular contents suggested pore formation as a primary mechanism.[14,119,127,128] However, pore formation is induced at a lower peptide concentration than is required for inhibition of cell wall synthesis and occurs more rapidly, which implicates it as the primary killing mechanism.[14] Indeed, the role of peptide concentration in studying the mechanism of action can prove significant. Harvard *et al.* noted that all AMPs would probably permeabilize the cell membrane at a high enough concentration because at least a part of the peptide would likely be involved with membrane interactions to facilitate translocation into the cell for subsequent interaction with the intended target, be it enzymes, nucleic acids, etc.[129] In fact, high concentrations of nisin have even been known to eliminate the dependence of inhibitory activity on the density of docking molecule within the membrane.[124] These secondary mechanisms and alternative effects that emerge at elevated peptide concentrations, which are often used experimentally, underscore the challenge of accurately determining the primary mechanism of action for a given bacteriocin.

Cellular targets and mechanism of action are frequently determined *in vitro* by assessing biochemical changes resulting from the treatment of sensitive cells with bacteriocins. Often indirect assessments, these studies have led to the development of several different mechanistic models for pore formation and limited the general acceptance of any individual model.[14] Liposomes, or other synthetic membranes, may be alternatively used to assess the effect of different membrane components on permeabilization efficiency and provide a simplified model wherein a specific membrane-contained compound can be assayed in the liposome suspension.[95,120] Leakage of the target compound suggests membrane damage, and liposomes

containing compounds of different molecular sizes may be assayed as a way of estimating pore size. These data suggest whether cell death is a result of either proton motive force (PMF) depletion, as is the case for relatively small pores, or immediate loss of metabolites, suggested by the formation of larger pores.[14] It has been reported that nisin and other Class IIA bacteriocins antagonize cells by depleting the PMF, while pediocin-like bacteriocins cause the efflux of larger molecules. Moreover, some bacteriocin-induced pores may cause leakage of only specific molecules or those with a specific charge.[95] Other characteristics defined by *in vitro* analysis of pore-forming bacteriocins include pore lifetime and membrane conductivity.[38] General analysis of membrane permeability has often been assessed with several of the experimental methods mentioned in the previous section on peptide structure and function. CD experiments have indicated conformational shifts and membrane insertion, while site-directed mutagenesis and spectroscopy have been used to determine the peptide domain which spans the membrane in pore formation.[14,123,125] Collectively, these features are used in predicting the pore-forming mechanism, whether multiple copies of the bacteriocin are required for formation of a single pore, and to further characterize the events leading to cell death.

However, as previously stated, these conclusions are limited by their dependence on experimental conditions since conflicting results may be obtained from different assays, membrane systems, or with the use of different bacteriocin concentrations. It has even been suggested that these conflicting results may indicate different biological activity under different environmental conditions, while other ambiguities have been retrospectively taken as experimental artifact.[129] The secondary effects of nisin treatment, as an example, represent the importance of peptide concentration in assessing mechanism of action. The exclusive use of only artificially high peptide concentration as definitive in pore formation studies should be proscribed. These experiments may be inadvertently designed to only recognize pore formation as the mechanism of action as a consequence of high levels of amphipathic bacteriocin, regardless of any subsequent activity.[129] A tell-tale indication for secondary effects is if the putative activity is only observed at concentrations above the MIC. Different detection methods have also contributed to the contrasting conclusions concerning the nisin mechanism of action. Mutagensis with fluorescently labeled tryptophan residues and spectroscopy suggested cell wall synthesis was the primary mechanism, while studies with liposomes indicated membrane permeabilization was the primary cause.[14,125] In an analogous example, mechanistic details concerning the pore formation mechanism for magainin were originally debated due to conflicting results concerning peptide orientation during pore formation at various peptide concentration levels. Both results were eventually explained by a universal model in which peptide orientation is concentration dependent.[123] Shai *et al.* divided experimental methods into three categories based on their limitations.[123] The first category is comprised of methods which are highly sensitive and report on initial steps in the mechanism of action, prior to obvious damage to the cell or vesicle, and includes highly sensitive fluorescent-labeled or radiolabeled probes. The second group is substantially less sensitive and unable to effectively monitor initial binding reactions; however, the majority of cellular structures maintain their integrity. This group includes the majority of mutagenesis and spectroscopic analysis. The final category includes the least sensitive assays – those which quantify the effects of cellular damage such as leakage of intracellular contents from liposomes. The results from these three types of studies may be contradictory and a lack of clarity regarding bacteriocin mechanisms remains. However, current research suggests that pore formation and membrane permeabilization is the most common mechanism among known bacteriocins, and it is certainly the most studied.[124,125]

Pore formation

Many AMPs act by inducing pore formation. Some bacteriocins associated with pore formation include nisin A, pediocin PA1, subtilin, epidermin, and streptococcin A-M57.[14] Other two-component lantibiotics are also known pore formers: lactococcin Q and lactacin A are membrane active. For these bacteriocins, one of the peptides binds a cell surface receptor and the other peptide interacts with the membrane. Consequently, optimum activity requires approximately equal quantities of the peptide components and activity may be attenuated by the exclusion of either.[95] A peptide domain with an α-helical secondary structure is often associated with transmembrane localization, whether or not that domain is a peptide acting in a two-component system or encompassed in a single, fully active peptide.[121] This same α-helical confirmation is not associated with other mechanisms of action such as inhibition of DNA or cell wall synthesis.[112]

Pore formation can lead to cell death as a consequence of PMF depletion through dissipation of the pH gradient or membrane potential. Alternatively, cell death can result from efflux of phosphate, amino acids, or other metabolites.[113] Liposome experiments to determine pore size and assays quantifying membrane potential, as mentioned above, are used to resolve the different causes of cell death resulting from pore formation. The two-component bacteriocin lactococcin dissipates membrane potential, which depletes the PMF. However, the pH gradient component of the PMF remains intact as lactococcin-generated pores do not allow for the translocation of protons. Alternatively, potassium ions are rapidly effluxed by sensitive cells treated with lactoccin and cellular ATP levels are significantly reduced, indicating that potassium-selective pores result in the depletion of the PMF, which diminishes the cell's ATP levels and prohibits ATP-dependent reactions.[95] Lactacin also induces potassium ion efflux as well as phosphate efflux, but the activity is not dependent on depletion of the PMF. The ATP hydrolysis equilibrium is shifted due to the loss of cellular phosphate, which results in a decrease of available ATP.[116] Conversely, nisin- and pediocin-induced pores are nonspecific and result in efflux of protons and other charged molecules.[124,130–133] While pore specificity may vary among bacteriocins that act by pore formation, collapse of PMF generally results from efflux of cellular contents, which destroys either the pH gradient or membrane potential or both and that loss, in turn, reduces ATP levels within the cell and restricts energy-requiring biosynthetic processes.[14] The process by which peptides assemble and form pores is another source of variability among bacteriocin mechanisms of action.

Pore formation models

Several models of the mechanisms of action for pore-forming compounds have been proposed. The models are not specific to bacteriocins and many were originally developed to explain the activity of other AMPs. In these different models, the peptide enters the membrane by one of two general principles. Bacteriocins are often described as containing two domains: one responsible for binding the target receptor and a second for pore formation.

Pore formation requires conformation changes in the peptide as it moves from a hydrophilic environment to the hydrophobic environment of the membrane. This is accomplished by either a structural shift in the individual peptide to adopt a more hydrophobic surface once in contact with the membrane or by oligomerization of peptides such that hydrophobic amino acids are on the exterior of the channel in solution, but an inversion of the arrangement once in contact with the membrane so that hydrophobic regions are then on the inside of the channel.[122] An additional factor which diversifies the pore formation models even further is the permeabilization event itself. The AMP may form temporary pores or channels of varying lifetimes; the AMP may form micelles around membrane fractions; or the AMP may cause dissolution of the membrane.[129] The step-wise arrangement, orientation, and permeabilization mechanisms of AMPs have been described in five models: barrel-stave, wedge, toroidal pore, carpet, and aggregate. It is not clearly understood whether or not one of these models predominantly represents pore formation, or whether certain models hold for particular bacteriocins or under certain environmental conditions. Moreover, these models are based on the activity of α-helical AMPs with little research indicating which model describes the activity of β-sheet forming AMPs.[129]

The barrel-stave model

The earliest of the models proposed for pore formation is the barrel-stave mechanism. It was originally used to describe the activity of the bacteriocin nisin and the AMP melittin, but both have been more recently characterized by the wedge and toroidal pore models, respectively.[132,134] The barrel-stave model was originally proposed by Sahl *et al.* and was generally recognized as the mechanism by which pore formation occurred,[135,136] but has lost favor in recent years.[134] The wedge model, described below, has been reported as a modified version of barrel-stave and is instead the predominant model currently used for lantibiotics.[124] Nonetheless, the barrel-stave is still descriptive of the activity for the AMPs gramicidin, alamethicin, and other cytolytic toxins.[38,124,137] The barrel-stave mechanism is unique compared to other models in that the peptides themselves enter into the hydrophobic core of the membrane to form the pore instead of 'tempting' a change in lipid orientation.[121] In this way, the peptide serves as a stave supporting the barrel-shaped cluster of the pore, an activity from which the model's name is derived. This model is specific for peptides which are amphipathic α-helices such that hydrophobic regions of the secondary structure will orient themselves towards the hydrophobic lipid membrane, while hydrophilic regions will line the lumen of the water-filled channel formed by the barrel of α-helical bundles.[123] Peptides in this model are originally attracted to cell membrane based on electrostatic interactions, and parallel aggregation of peptides on the membrane surface causes thinning of the phospholipids in a density-dependent manner. Once a threshold level is reached, cooperative binding is initiated between at least two of the peptides, which can then integrate into the hydrophobic lipid membrane.[137] This small peptide oligomer is required prior to insertion into the membrane in order to satisfy hydrophobic interactions with the membrane by an amphipathic peptide.[122] Once the initial helical bundle has permeated the lipid bilayer, additional peptides are recruited to form the channel. Based on this mechanism, pores formed by the barrel-stave model should be a consistent size.[129] An unknown number of peptides serve as staves for the fully formed pore. However, predictions can be made about how many peptides are required, and the potential of a peptide to utilize the barrel-stave mechanism based on an AMPs size and length.[14] That is, a minimum length of 22 amino acids has been proposed for an α-helix capable of spanning the length of the cell membrane. Subsequently, AMPs smaller than 22 amino acids may utilize a different mechanism for pore formation.[123] The pore formed through the barrel-stave mechanism is illustrated in Fig. 3(A).

The wedge model

A variation of barrel-stave pore formation is the wedge model, which differs primarily in its lack of peptide exposure to the hydrophobic core of the membrane. Although nisin A was originally described using the barrel-stave model,[125] this has been replaced by the wedge model, which is currently favored to describe pore-forming activity by lantibiotics. An additional distinction between wedge and barrel-stave is the role of PMF. PMF potential increases the killing activity of peptides in the barrel-stave model, while PMF decreases the ability of the peptides to insert into the membrane in the wedge model.[125] The wedge model was originally proposed by Moll *et al.*[125] and is the current mechanism favored for nisin activity. This model was predicted based on NMR findings indicating that nisin remained parallel to the lipid bilayer, which is to say that the peptide never penetrated the hydrophobic core of the membrane and instead the phospholipids themselves reorient in response to nisin binding.[38] Hydrophilic residues are, as in the barrel-stave model, predicted to line the internal channel while hydrophobic side chains are predicted to be shallowly embedded in the outer leaflet of the membrane.[125] The initial binding of nisin to the membrane creates strain, which tempts the phospholipids into reorientation.[114] Several nisin molecules are involved in formation of a single pore, transiently formed and unstable due to the hydrophobic forces driving the restoration of the native bilayer arrangement.[95] This is in keeping with the short lifetime observed for nisin-mediated pores which have been measured to transiently exist for only a number of milliseconds.[117] The size of the pore has been estimated to have a diameter of about 1 nm, which supports the model's proposed involvement of multiple nisin molecules.[38] The pore formed through the barrel-stave mechanism is illustrated in Fig. 3(B).

The toroidal pore model

The toroidal pore model, also known as the wormhole model, can be thought of as a hybrid between barrel-stave and wedge mechanisms. Like the barrel-stave, peptides orient themselves perpendicularly in respect to the membrane and are directed towards the lipid core. Like the wedge model, the orientation of the phospholipids in the membrane is corrupted by interactions with the AMP, introducing curvature into the bilayer, and the resulting strain aids in pore formation.[129] The shape the membrane takes as it folds back on itself is said to resemble a torus, from which this model's name is derived.[121] Several AMPs are predicted to use this mechanism, including melittin, protegrins, and magainin.[124,134] Although less common, some bacteriocins have also been predicted to follow the toroidal pore model. Lacticin Q is reportedly the only known bacteriocin from a Gram-positive bacterium to follow this mechanism, and colicin E1 is the only reported bacteriocin from a Gram-negative bacterium.[120] In this model, the AMP aggregates on the membrane surface and begins to tempt the membrane into adopting a positive curvature.[112] This induces transbilayer movement known as lipid flip-flop, which results in a channel formed by both the perpendicularly oriented peptides as well as the head groups of the phospholipids.[138] This pore structure

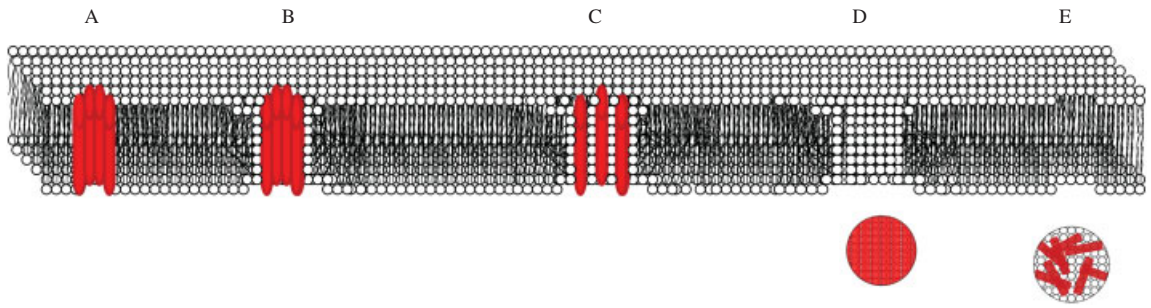

Figure 3. Models for pore formation by AMPs: (A) barrel-stave; (B) wedge; (C) toroidal pore; (D) carpet; (E) aggregate channel.

is unique to the toroidal pore model where the lumen of the pore is lined with both peptide and lipid membrane. This model is also associated with the formation of larger pores, those which have been shown to cause leakage of dextran, and even small peptides from model membrane systems.[120] The pore formed through the barrel-stave mechanism is illustrated in Fig. 3(C).

The carpet model

Two models have been developed which predict AMP activity by micellization of the lipid membrane. The first of these is the carpet model, in which peptides solubilize the membrane and lead to dissolution of the lipid bilayer. In contrast to the aggregate channel model, described below, the carpet model involves total peptide coverage, as with a carpet, of regions of the membrane. Peptides align parallel to the membrane based on electrostatic interactions.[129] Once a threshold concentration has been achieved, the detergent-like activity breaks up regions of the membrane and leads to micelle formation. This requires the rotation of phospholipids.[121] This model has been proposed as the primary mechanism by which AMPs act against Gram-negative bacteria. AMPs which have been predicted to utilize the carpet mechanism include cathelicidin and melittin.[112] In the initial steps of the model, or at low AMP concentration, membrane channels like those formed in a toroidal pore mechanism may be formed.[121,122] Oren *et al.* suggest that a carpet-like mechanism better represents the activity of most α-helical AMPs which do not principally self-aggregate prior to insertion into the membrane, a requirement of other models. Moreover, electron microscopy has indicated that many α-helical AMPs cause drastic permeabilization, beyond what is anticipated by simple pore formation, at high concentrations.[121] The pore formed through the barrel-stave mechanism is illustrated in Fig. 3(D).

The aggregate channel model

The proposed aggregate channel model also results in micelle formation. Membrane perturbation through this mechanism is a function not only of membrane–lipid interaction, but also from displacement of divalent cations which compete with the AMP for interaction with the membrane. This additional factor is proposed to increase the level of lytic activity observed with many bacteriocidal AMPs.[121] This model proposes a less ordered peptide orientation and activity. Peptides aggregate randomly on the membrane surface, regardless of orientation, and form channels in the membrane of variable sizes by generating micelles. Penetration into the hydrophobic core of the membrane is not required. Peptide aggregates induce negative strain in the phospholipids directly adjacent and micelle-like complexes containing a mixture of peptide and phospholipid head groups on the surface are formed.[120] The AMP maculatin is reported to use this mechanism.[112]

Shai *et al.* proposed that the fundamental differences among the described pore-forming mechanisms of action are based on a few fundamental principles. The first relates to the binding interactions of the peptide, whether or not the peptide uses a docking molecule or if activity is guided by electrostatic interactions alone. The second difference concerns association among individual peptides. Some models predict that peptides independently interact with the membrane, while others propose oligomerization and cooperative binding. A related factor is whether or not the mechanism is concentration dependent. The authors also propose the insertion of the peptide into the hydrophobic core of the membrane versus limited interactions with the phospholipid head groups on the surface of the membrane is a definitive difference. Finally, the structure of the peptide in aqueous solution compared to its structure in the membrane-bound state also differentiates mechanisms for different AMPs.[123] These features collectively describe the five models for pore-forming AMPS discussed above, and a representative pore formed as described in each of these models is illustrated in Fig. 3.

APPLICATIONS AND CONCLUSIONS

Within the food industry, bacteriocins or other bacterially synthesized peptides have found the most widespread application among AMPs. Bacteriocins can be integrated into a food product in three different ways: addition of the purified compound as a food additive; addition of a bacterial fermentate as a GRAS substance; or addition of the producer bacterium to the product or as part of the starter culture. Each approach has specific advantages and limitations and may be more or less appropriate in particular foods.

Protein purification is an expensive process, and complete purification of the bacteriocin from the crude fermentate is often unnecessary if the producer is considered a food-grade bacterium.[139] Consequently, purified bacteriocins have not been utilized in commercial applications to the extent other routes of bacteriocin integration have been.[140] Purified bacteriocins are also subject to different regulatory processes, as discussed below, which disincentivize the use of purified bacteriocins in food as well. It should be noted that, while purification is prohibitively costly in the food industry, AMPs that have application as pharmaceuticals are commonly purified and the ease with which an AMP can be purified from its fermentation medium may be a desirable attribute in drug development applications.[141] Purification is accomplished through a series of partial purification steps which include salt precipitations, filtrations, and various forms of chromatography. The isoelectric point of the bacteriocin and conformational

changes that result from adjustment of the solvent pH can be manipulated to increase separation efficiency. Depending on the intended use, bacteriocin concentration can be increased several fold to near total purity.[142,143]

Alternatively, bacteriocins can be added as lyophilized end products of a bacterial fermentation. The producer bacterium is grown in a milk-based, or similar but non-allergenic, medium. The end product contains many of the secondary metabolites produced by the bacteria that have known antimicrobial functions, including bacteriocins and organic acids.[144] Powdered preparations of the fermentate can also serve other functions within processed foods such as flavor and textural enhancements due to the high protein content.[145] Integration techniques in which the bacteriocin is produced *ex situ* can be better controlled and production optimized compared with the *in situ* production resulting from integration of the producer bacteria. Expression of the bacteriocin structural gene may be repressed during growth in a food system and expression levels have shown more variability in systems where the bacteriocin is produced *in situ*.[146] Homogeneous distribution may also be better achieved by adding the bacteriocin as an ingredient during processing. While the same limitations for the peptide itself within a given food system are valid regardless of the integration method, challenges with the producer microorganism are avoided by adding the bacteriocin directly.

Despite some challenges, addition of the producer microorganism has been proposed for particular use in fermented foods. In fermented foods, the producer can be included as part of the starter culture.[147] The addition of the producer bacteria directly to the food product offers advantages in cost and regulatory requirements, but the producer used in this approach must meet certain parameters.[142] Besides being a food-grade bacterium that is compatible with the starter culture bacteria, the producer must express bacteriocin under the storage conditions relevant to the product.[145] The efficient production of bacteriocin *in situ* is also dependent on the relative sensitivity of the producer to phage infection and the frequency at which spontaneous loss of bacteriocin expression occurs.[148] Any bacteriocin integration method, *in situ* or *ex situ*, is subject to limiting factors based on the peptide in the food system. The bacteriocin may be inactivated due to adsorption to food components, proteases, processing conditions, or instability in the native pH of the food matrix.[149,150] The sensory changes that occur as a result of bacteriocin addition may also limit application, as do selective toxicity levels of the bacteriocin. A desirable bacteriocin would exhibit inhibitory activity at low concentrations among spoilage or pathogenic bacteria, but with limited toxic effects to mammalian cells.[151] The normal microbiota of the food matrix also bears on the relative efficacy of the bacteriocin. Bacteriocins have often shown stronger activity against cells that are actively replicating as opposed to stationary or stressed cells, making the physiological state of the microbiota a relevant consideration. The relative frequency at which resistant mutants occur and the spectrum of activity of a bacteriocin against different strains of the target species can impact the value of the bacteriocin to the food industry. Additionally, the diversity and density of the microbiota in foods and whether they are protected in biofilms or by slime layers impact the inhibitory activity of the bacteriocin.[144] Downstream consequences of these factors may also impact the safety of the product. For instance, if many bacteria present in a given food are sensitive to the bacteriocin, the peptide may be adsorbed to commensalistic bacteria, reducing the relative quantity of peptide available for inhibition of the target pathogen. Moreover, elimination of competitor species may result in an unintentional advantage to the target pathogen.

Potential solutions have been proposed to the above limitations. However, some of the solutions would affect the regulation of these compounds within foods, detracting from the advantages of bacteriocins as natural, GRAS status, and potentially low cost to produce. Suggested improvements to the efficacy of bacteriocins as biopreservatives include using them as part of a hurdle system and increasing the concentration of peptide in fermentate preparations by modification of the expression system. Hurdle technologies imply the use of multiple controls, each with a low level of antagonism that in combination reduce the population of the target bacterium by a sufficient level.[152] Many bacteriocins applied in food systems reduce *Listeria* by only 1 or 2 logs.[153] Subsequently, additional controls in the form of mild processing treatments or other additives may be necessary to achieve a sufficient kill.[154–156] Hurdle technologies must be carefully reviewed prior to application for the possibility of adaptation of the target pathogen since any single control is assumed to kill only a portion of the target population.[144,152] Increasing the concentration of bacteriocin within the fermentate through genetic engineering has also been proposed.[144,155] Heterologous expression or inducible expression vectors could increase the content of bacteriocin within the fermentate. However, genetic modification of the producer changes the regulatory status of the fermentate in a similar fashion to concentration and purification of the compound. These changes necessitate pre-market approval of the compound as a novel food additive.[157]

Regulation of commercial applications

Currently, nisin is approved for use in ~50 countries.[157] In the USA, nisin has been approved for use as a food additive in select foods at levels of 2.9 mg per person per day according to the FDA.[32] Toxicological assessment of AMPs is based on exposure levels via the intended administration route, and many bacteriocins are assumed to be largely inactivated in the digestive process, which rapidly decreases the exposure levels experienced by consumers.[157,158] The primary legislation regulating bacteriocins comes from the Food Additives Amendment of 1958. The amendment demanded pre-market approval for food additives and created a practical need for exemption from pre-market approval for foodstuffs that were recognized as safe. Within Section 201(s) is a provision for foods generally recognized as safe (GRAS). Compounds are granted GRAS status based on empirical evidence or precedence of safe consumption and include food products such as sugar or salt, as well as ingredients of biological origin (e.g. processed starches or enzyme preparations).[159] When bacteriocins produced by food-grade bacteria are normally expressed, lyophilized, and added to food products in concentrations occurring in fermentations, the bacteriocin can be considered GRAS. If the producer is not food grade, has been genetically modified, or if the bacteriocin is added in elevated concentrations, then it is not considered GRAS and is treated as a 'food additive' subject to pre-market approval. GRAS status is true only for an FDA-approved use for a given compound, which includes specifications on concentration limits and particular food applications.[157]

Regulatory authority over the use of bacteriocins in foods is dependent on which foods it will be used in and may fall to the Center for Food Safety and Applied Nutrition or Center for Veterinary Medicine in the FDA, the Food Safety Inspection

Service in the USDA, or to the EPA under regulations for pesticides covered in the Federal Insecticide, Fungicide, and Rodenticide Act in the case of a bacteriocin to be used on whole fruits or vegetables.[159] For AMPs used in meat and poultry products, an additional level of approval beyond regulation by the FDA is obtained from the FSIS of USDA. Initial approval of the bacteriocin by the FDA is mandatory; afterwards, a review by FSIS will lead to either acceptance or rejection of the formal request for use of a particular bacteriocin as a food additive.[160]

Application in foods

Bacteriocins as biopreservatives have been explored in fermented foods,[127,161] in particular for application in cheese.[162,163] Raw and cured meat products are also potential applications for bacteriocins, and their utility in the anaerobic environments of sealed meat casings or integrated within the packaging itself has also been investigated.[148,164] Although commercial application has not been fully realized, the growing interest in ensuring the safety of fresh produce has raised interest in using bacteriocins in fresh or minimally processed fruits or vegetables.[154] Even more unusual applications for AMPs include the introduction of the producer to animals for the prevention of zoonosis[165] or the use of bacteriocin-producing probiotics.[142] In commercial applications, 'natural antimicrobial blends' have been widely marketed by DuPont through the Danisco brand acquired by the company in 2011. Although their exact formulation is proprietary, the NovaGard® solutions are reportedly blends of microbial fermentates designed to target a broader range of spoilage microorganisms or pathogens. Other commercial products include AvGard®, MicroGard®, Natamax®, and Nisaplin®.[166] Nisaplin® and Natamax® are the commercial form of nisin and natamycin, respectively, the latter being a bacterially synthesized AMP which inhibits mold and yeast and is commercially applied in shredded cheese.[163] The product AvGard® is inhibitory to Gram-negative pathogens for application on meat and poultry. Different formulations of each of these products are marketed for use in particular food items including cheese, breads, beverages, soups and sauces, meat and seafood.[166] In many applications within the USA, these products offer a clean-label alternative to other antimicrobial agents.

CONCLUSIONS

AMPs, particularly those produced by food-grade bacteria, are intriguing sources of novel antimicrobial agents for use within the food industry. Many have already been successfully applied but represent only a small fraction of the diversity of AMPs. Continued research geared toward the discovery of novel AMPs and the rapid research and development required to characterize these peptides will lead to the commercialization of additional AMPs for use in food and agriculture. Advances in basic science have been applied to the field of antimicrobial development for genetic and chemical characterization of AMPs. Continued use of these tools will lead to more diverse and robust antimicrobials.

REFERENCES

1 Chen H and Hoover DG, Bacteriocins and their food applications. *Compr Rev Food Sci Food Saf* **2**:82–100 (2006).
2 Jack RW, Tagg JR and Ray B, Bacteriocins of Gram-positive bacteria. *Microbiol Mol Biol Rev* **59**:171–200 (1995).
3 Brefoot SF and Klaenhammer TR, Detection and activity of lacticin B, a bacteriocin produced by *Lactobacillus acidophilus*. *Appl Environ Microbiol* **45**:1808–1815 (1983).
4 Riley MA and Wertz JE, Bacteriocin diversity: ecological and evolutionary perspectives. *Biochimie* **84**:357–364 (2002).
5 Nes IF, Diep DB and Holo H, Bacteriocin diversity in *Streptococcus* and *Enterococcus*. *J Bacteriol* **189**:1189–1198 (2007).
6 Cleveland J, Montville TJ, Nes IF and Chikindas ML, Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol* **1**:1–20 (2001).
7 Masuda Y, Ono H, Kitagawa H, Ito H, Mu F, Sawa N *et al.*, Identification and characterization of leucocyclin Q, a novel cyclic bacteriocin produced by *Leuconostoc mesenteroides* TK41401. *Appl Environ Microbiol* **77**:8164–8170.
8 Sit CS and Vederas JC, Approaches to the discovery of new antibacterial agents based on bacteriocins. *Biochem Cell Biol* **86**:116–123 (2008).
9 Muriana PM and Klaenhammer TR, Purification and partial characterization of lacticin F, a bacteriocin produced by *Lactococcus acidophilus* 11088. *Appl Environ Microbiol* **57**:114–121 (1991).
10 Riley MA, Bacteriocin biology, ecology, and evolution. *Encyclopedia Microbiol* 32–44 (2009).
11 Huhne K, Axelsson L, Holck A and Krockel L, Analysis of the sakacin P gene cluster from *Lactobacillus sake* Lb674 and its expression in sakacin-negative *Lb. sake* strains. *Microbiology* **142**:1437–1448 (1996).
12 Kotelnikova EA and Gelfand MS, Bacteriocin production by Gram positive bacteria and the mechanism of transcriptional regulation. *Russian J Genet* **38**:628–641 (2002).
13 Riley MA, Molecular mechanisms of bacteriocin evolution. *Annu Rev Genet* **32**:255–278 (1998).
14 McAilluffe O, Ross RP and Hill C, Lantibiotics: structure, biosynthesis, and mode of action. *FEMS* **25**:285–308 (2000).
15 Feng G, Guron GKP, Churey JJ and Worobo RW, Characterization of mundticin L, a Class IIa anti-*Listeria* bacteriocin from *Enterococcus mundtii* CUGF08. *Appl Environ Microbiol* **75**:5708–5713 (2009).
16 Belguesmia Y, Naghmouchi K, Chihib NE and Drider D, Class IIa bacteriocins: current knowledge and perspectives, in *Prokaryotic Antimicrobial Peptides: From Genes to Applications*, ed. by Drider D and Rebuffat S. Springer, Berlin, pp. 171–194 (2011).
17 Konisky J, Colicins and other bacteriocins with established modes of action. *Annu Rev Microbiol* **36**:125–144 (1982).
18 Kolter R and Moreno F, Genetics of ribosomally synthesized antimicrobial peptides. *Annu Rev Microbiol* **46**:141–163 (1992),
19 Nissen-Meyer J and Nes IF, Ribosomally synthesized antimicrobial peptides: their structure, function, biogenesis, and mechanism of action. *Arch Microbiol* **167**:67–77 (1997).
20 Breukink E and Kruijff B, Lipid II as a target for antibiotics. *Nat Rev Drug Discov* **10**:1–12 (2006).
21 Stepper J, Shastri S, Loo TS, Preston JC, Novak P, Man P *et al.*, Cysteine *S*-glycosylation, a new post translational modification found in glycopeptides bacteriocins. *FEBS Lett* **585**:645–650 (2011).
22 Wang H, Fewer DP and Sivonen K, Genome mining demonstrates the widespread occurrence of gene clusters encoding bacteriocins in cyanobacteria. *PLoS ONE* **6**: e22384.
23 Guo Y, Yu Z, Xie J and Zhang R, Identification of a new *Bacillus licheniformis* strain producing a bacteriocin-like substance. *J Microbiol* **50**:452–458 (2011).
24 Heng NCK, Ragland NL, Swe PM, Baird HJ, Inglis MA, Tagg JR *et al.*, Dysgalacticin: a novel, plasmid-encoded antimicrobial protein (bacteriocin) produced by *Streptococcus dysgalactiae* subsp. *equisimilis*. *Microbiology* **152**:1991–2001 (2006).
25 Abriouel H, Franze CMAP, Omar NB and Galvez A, Diversity and applications of *Bacillus* bacteriocins. *FEMS Microbiol Rev* **35**:201–232 (2011).
26 Hancock REW and Chapple DS, Peptide antibiotics. *Antimicrob Agents Chemother* **43**:1317–1323 (1999).
27 Ross RP, Morgan S and Hill C, Preservation and fermentation: past, present, and future. *Int J Food Microbiol* **79**:3–16 (2002).
28 Rauch PJG, Beerthuyzen MM and de Vos WM, Nucleotide sequence of IS*904* from *Lactococcus lactis* subsp. *lactis* strain Nizo R5. *Nucleic Acids Res* **18**:4253–4254.
29 Gratia A, A remarkable example of antagonism between species. *French Soc Biol Fil* **93**:1040–1041 (1925).
30 Rogers LA, The inhibiting effect of *Streptococcus lactis* on *Lactobacillus bulgaricus* . *J Bacteriol* **16**:321–325 (1928).
31 Jacob FA, Lwoff A. Siminovitch and Wollman E, Définition de quelques termes relatifs à la lysogénie. *Ann Inst Pasteur (Paris)* **84**:222–224 (1953).

32 Federal Register, Nisin preparation: affirmation of GRAS status as a direct human food ingredient. *Fed Regist* **54**:11247–11251 (1988).
33 Geis A, Singh J, and Teuber M, Potential of lactic streptococci to produce bacteriocin. *Appl Environ Microbiol* **45**:205–211 (1983).
34 Bernhard K, Schrempf H and Goebel W, Bacteriocin and antibiotic resistance plasmids in *Bacillus cereus* and *Bacillus subtilis*. *J Bacteriol* **133**:897–903 (1978).
35 Liao CC, Yousef E, Chism GW and Richter EW, Inhibition of *Staphylococcus aureus* in buffer, culture media, and foods by lacidin A, a bacteriocin produced by *Lactobacillus acidophilus* OSU133. *J Food Prot* **14**:87–101(1994).
36 Clintas LM, Rodriguez JM, Fernandez MF, Sletten K, Nes IF, Hernandez PE *et al.*, Isolation and Characterization of pediocin L50, a new bacteriocin from *Pediococcus acidilactici* with a broad inhibitory spectrum. *Appl Environ Microbiol* **61**:2643–2648 (1995).
37 Rouse S, Sun F, Vaughan A and Sinderen D, High-throughput isolation of bacteriocin-producing lactic acid bacteria, with potential application in brewing industry. *J Inst Brew* **113**:256–262 (2007).
38 Hechard Y, Derijard B, Letellier F and Cenatiempo Y, Characterization and purification of mesentericin Y105, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*. *J Gen Microbiol* **138**:2725–2731 (1992).
39 Lee SW, Mitchell DA, Markley AL, Hensler ME, Gonzalez D, Wohlrab A *et al.*, Discovery of widely distributed toxin biosynthetic gene cluster. *Proc Natl Acad Sci USA* **15**:5879–5884 (2008).
40 Eijsink VGH, Axelsson L, Diep DB, Havarstein LS, Holo H and Nes IF, Production of class II bacteriocins by lactic acid bacteria: an example of biological warfare and communication. *Antonie van Leeuwenhoek* **81**:639–654 (2002).
41 Gillor O, Nigro ML and Riley MA, Genetically engineered bacteriocins and their potential as the next generation of antimicrobials. *Curr Pharm Design* **11**:1–9 (2005).
42 Dykes GA, Bacteriocins: ecological and evolutionary significance. *Trends Ecol Evol* **10**:186–189 (1995).
43 Majeed H, Gillor O, Kerr B and Riley MA, Competitive interaction in *Escherichia coli* populations: the role of bacteriocins. *ISME J* **5**:71–81 (2011).
44 Schlegel R and Slade HD, Bacteriocin production by transformable group H streptococci. *J Bacteriol* **112**:824–829 (1972).
45 Dirix G, Monsieurs P, Dombrecht B, Daniels R, Marchal K, Vanderleyden J *et al.*, Peptide signal molecules and bacteriocins in Gram-negative bacteria: a genome-wide in silico screening for peptides containing a double-glycine leader sequence and their cognate transporters. *Peptides* **25**:1425–1440 (2004).
46 Brogden KA, Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* **3**:239–250.
47 Klaenhammer TR, Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev* **12**:39–85 (1993).
48 Sahl HG, Jack RW and Bierbaum G, Biosynthesis and biological activities of lantibiotics with unique post-translational modifications. *Eur J Biochem* **230**:827–853 (1995),
49 Altena K, Guder A, Cramer C and Bierbaum G, Biosynthesis of the lantibiotic mersacidin: organization of a Type B lantibiotic gene cluster. *Appl Environ Microbiol* **66**:2565–2571 (2000).
50 Turner DL, Brennan L, Meyer HE, Lohaus C, Siethoff C, Costa HS *et al.*, Solution structure of plantaricin C, a novel lantibiotic. *Eur J Biochem* **264**:833–839 (1999).
51 Enhaner S, Sashihara T, Sonomoto K and Ishizaki A, Class IIa bacteriocins: biosynthesis, structure and activity. *FEMS Microbiol Rev* **24**:83–100 (2006).
52 Cotter PD, Hill C and Ross RP, Food microbiology: bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* **3**:777–788 (2005).
53 Heng NCK and Tagg JR, What's in a name? Class distinction for bacteriocins. *Nat Rev Microbiol* **4**: doi:10.1038/nrmicro1273-c1 (2006).
54 Venugopal H, Edwards PJB, Schwalbe M, Claridge JK, Libich DS, Stepper J *et al.*, Structural, dynamic, and chemical characterization of a novel *S*-glycosylated bacteriocin. *Biochemistry* **50**:2748–2755 (2011).
55 Stoddard GW, Petzel JP, Belkum MJ, Kok J and McKay LL, Molecular analyses of the lactococcin A gene cluster from *Lactococcus lactis* subsp. *lactis* biovar diacetylactis WM4. *Appl Environ Microbiol* **58**:1952–1961 (1992).
56 Engelke G, Gutowski-Eckel Z, Hammelmann M and Entian KD, Biosynthesis of the lantibiotic nisin: genomic organization and membrane localization of the NisB protein. *Appl Environ Microbiol* **58**:3730–3743 (1992).
57 Klein C, Kaletta C, Schnell N and Entian KD, Analysis of genes involved in biosynthesis of the lantibiotic subtilin. *Suppl Environ Microbiol* **58**:132–142 (1992).
58 Sahl HG and Bierbaum G, Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from Gram positive bacteria. *Annu Rev Microbiol* **52**:41–79 (1998).
59 Cheigh CI and Pyun YR, Nisin biosynthesis and its properties. *Biotech Lett* **27**:1641–1648 (2005).
60 Diep DB, Havarstein LS and Nes IF, Characterization of the locus responsible for the bacteriocin production in *Lactobacillus plantarum* C11. *J Bacteriol* **178**:4472–4483 (1996).
61 Bukhtiyarova M, Yang R and Ray B, Analysis of the pediocin AcH gene cluster from plasmid pSMB74 and its expression in a pediocin-negative *Pediococcus acidilactici* strain. *Appl Environ Microbiol* **60**:3405–3408 (1994).
62 Muriana PM and Klaenhammer TR, Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. *Appl Environ Microbiol* **53**:553–560 (1987).
63 Entian KD and Vos WM, Genetics of subtilin and nisin biosyntheses. *Antonie van Leeuwenhoek* **69**:109–117 (1996).
64 Quadri LEN, Kleerebezem M, Kuipers OP, Vos WM, Roy KL, Vederas JC *et al.*, Characterization of locus from *Carnobacterium piscicola* LV1B Involved in bacteriocin production and immunity: evidence for global inducer-mediated transcriptional regulation. *J Bacteriol* **179**:6163–6171 (1997).
65 Kuipers OP, Ruyter PGGA, Kleerebezem M and Vos WM, Quorum sensing-controlled gene expression in lactic acid bacteria. *J Biotech* **64**:15–21 (1998).
66 Jack RW and Sahl HG, Unique peptide modifications involved in the biosynthesis of lantibiotics. *TIBTECH* **13**:269–278 (1995).
67 Davies EO, Dullaghan EM and Rand L, Definition of the mycobacterial SOS box and use to identify LexA-regulated genes in *Mycobacterium tuberculosis*. *J Bacteriol* **184**:328–3295 (2002).
68 Gillor O, Kirkup BC and Riley MA, Colicins and microcins: the next generation of antimicrobials. *Adv Appl Microbiol* **54**:129–146 (2004).
69 Skaugen M, Abildgaard CIM and Nes IF, Organization and expression of a gene cluster involved in the biosynthesis of the lantibiotic lactocin S. *Mol Gen Genet* **253**:674–686 (1997).
70 Kleerebeezem M, Quorum sensing control of lantibiotic production: nisin and subtilin autoregulate their own biosynthesis. *Peptides* **25**:1405–1414 (2004).
71 Lagos R, Baeza M, Corsini G, Hetz C, Sstrahsburger E, Castillo JA *et al.*, Structure, organization, and characterization of the gene cluster involved in the production of microcin E492, a channel-forming bacteriocin. *Mol Microbiol* **42**:229–243 (2001).
72 Cascales E, Buchanan SK, Duche D, Kleanthous C, Lloubes R, Postle K *et al.*, Colicin biology. *Microbiol Mol Biol Rev* **71**:158–229 (2007).
73 Riley MA and Chavan MA, *Bacteriocins Ecology and Evolution*. Springer, Berlin (2007).
74 Koponen O, Tolonen M, Qiao M, Wahlstrom G, Helin J and Saris PEJ, NisB is required for the dehydration and NisC for the lanthionine formation in the post-translational modification of nisin. *Microbiology* **148**:3561–3568 (2002).
75 Seizen R, Rollema HS, Kuipers OP and Vos WM, Homology modeling of the *Lactococcus lactis* leader peptides NisP and its interaction with the precursor of the lantibiotic nisin. *Prot Eng* **8**:117–125 (1995).
76 Stein T, Heinzmann S, Solovieva I and Entian KD, Function of *Lactococcus lactis* nisin immunity genes *nisI* and *nisFEG* coordinated expression in the surrogate host *Bacillus subtilis*. *J Biol Chem* **278**:89–94 (2003).
77 Ennahar S, Aoude-Werner D, Sorokine O, Dorsselaer AV, Bringel F, Hubert JC *et al.*, Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. *Appl Environ Microbiol* **62**:4381–4387 (1996).
78 Miller KW, Ray P, Steinmetz T, Hanekamp T and Ray B, Gene organization and sequences of pediocin AcH/PA-1 production operons in *Pediococcus* and *Lactobacillus* plasmids. *Lett Appl Microbiol* **40**:56–62 (2005).
79 Giacomini A, Squartini A and Nuti MP, Nucleotide sequence and analysis of plasmid pMD136 from *Pediococcus pentosaceus* FBB61 (ATCC43200) involved in pediocin A production. *Plasmid* **43**:111–122 (2000).

80 Fimland F, Johnsen L, Dalhus B and Nissen-Meyer J, Pediocin-like antimicrobial peptides (class IIa bacteriocins) and their immunity proteins: biosynthesis, structure, and mode of action. *J Peptide Sci* **11**:688–696 (2005).
81 Gebard S, ABC transporters of antimicrobial peptides in Firmicutes bacteria: phylogeny, function and regulation. *Mol Microbiol* **86**:1295–1317 (2012).
82 Kluskens LD, Kuipers A, Rink R, Boef E, Fekken S, Driessen AJM *et al.*, Post-translational modification of therapeutic peptides by nis B, the dehydratase of the lantibiotic nisin. *Biochemistry* **44**:12827–12834 (2005).
83 Levengood MR, Kerwood CC, Chatterjee C and van der Donk WA, Investigation of the substrate specificity of lacticin 481 synthetase using nonproteinogenic amino acids. *Chembiochem* **10**:911–919 (2009).
84 Ihnken LAF, Chatterjee C and van der Donk WA, *In vitro* reconstitution and substrate specificity of a lantibiotic protease. *Biochemie* **47**:7352–7363 (2008).
85 Cheeterje C, Patton GC, Cooper L, Paul M and van der Donk WA, Engineering dehydro amino acids and thioethers into peptides using lacticin 481 synthetase. *Chem Biol* **13**:1109–1117 (2006).
86 Wilson-Stanford S, Kalli A, Hakansson K, Kastrantas J, Oruguntry RS and Smith L, Oxidation of lanthionines renders the lantibiotic nisin inactive, *Appl Environ Microbiol* **75**: 1381–1387 (2009).
87 Martin NI, Sprules T, Carpenter MR, Cotter PD, Hill C, Ross RP *et al.*, Structural characterization of lacticin 3147, a two-peptide lantibiotic with synergistic activity. *Biochemistry* **43**:3049–3056 (2004).
88 Drider D, Fimland G, Hechard Y, McMullen LM and Prevost H, The continuing story of Class IIa bacteriocins. *Microbiol Mol Biol Rev* **70**:564–582 (2006).
89 van Belkum MJ, Worobo RW and Stiles ME, Double-glycine-type leader peptides direct secretion of bacteriocins by ABC transporters: colicin V secretion in *Lactococcus lactis*. *Mol Microbiol* **23**:1293–1301 (1997).
90 McCormick JK, Klaenhammer TR and Stiles ME, Colicin V can be produced by lactic acid bacteria. *Lett Appl Microbiol* **29**:3–41 (1999).
91 Rihikova J, Petit VW, Demnerova K, Prevost H, Rebuffat S and Drider D, Insights into structure–activity relationships in the C-terminal region of divercin V41, a Class IIa bacteriocin with high-level antilisterial activity. *Appl Environ Microbiol* **75**:1811–1819 (2009).
92 Uteng M, Hauge HH, Markwick PRL, Fimland G, Mantzilas D, Nissen-Meyer J *et al.*, Three-dimensional structure in lipid micelles of the pediocin-like antimicrobial peptide sakacin P and a sakacin P variant that is structurally stabilized by an inserted C-terminal disulfide bridge. *Biochemistry* **42**:1141–11426 (2003).
93 Twomey D, Ross RP, Ryan M, Meaney B and Hill C, Lantibiotics produced by lactic acid bacteria: structure, function and applications. *Antonie van Leeuwenhoak* **82**:165–185 (2002).
94 Sanchez-Barrenna MJ, Martinez-Ripoll M, Galvez A, Valdivia E, Maqueda M, Cruz V *et al.*, Structure of bacteriocin AS-48: from soluble state to membrane bound state. *J Mol Biol* **334**:541–549 (1998).
95 Moll GN, Konings WN and Driessen AJM, Bacteriocins: mechanism of membrane insertion and pore formation. *Antonie van Leeuwenhoek* **76**:185–198 (1999).
96 Heng NCK, Burtenshaw GA, Jack RW and Tagg JR, Ubercin A, a Class IIa bacteriocin produced by *Streptococcus uberis*. *Appl Environ Microbiol* **73**:7763–7766 (2007).
97 Fimland G, Pirneskoski J, Kaewsrichan J, Arimatti J, Kristiansen PE, Kinnunen PKJ *et al.*, Mutational analysis and membrane-interactions of the β-sheet-like N-terminal domain of the pediocin-like antimicrobial peptide sakacin P. *Biochim Biophys Acta* **1762**:1132–1140 (2006).
98 Johnson VG, Nicholls PJ, Habig WH and Youle RJ, The role of proline 345 in diphtheria toxin translocation. *J Biol Chem* **268**:3514–3519 (1993).
99 Eijsink VGH, Skeie M, Middelhoven PH, Brurberg MB and Nes IF, Comparative studies of Class IIa bacteriocins of lactic acid bacteria. *Appl Environ Microbiol* **64**:3275–3281 (1998).
100 Derksen DJ, Stymiest JL and Vederas JC, Antimicrobial leucocin analogues with disulfide bridge replaced by a carbocycle or by noncovalent interactions of allyl glycine residues. *J Agric Chem Comm* **128**:14252–14253 (2006).
101 Fluery Y, Dayem MA, Montagnes JJ, Chaboisseau E, Caier JPL, Nicolas P *et al.*, Covalent structure, synthesis, and structure–function studies of mesentericin Y 105, a defensive peptide from Gram-positive bacteria *Leuconostoc mesenteroides*. *J Biol Chem* **271**:14421–14429 (1996).
102 Gallagher NLF, Sailer M, Niemczura WP, Nakashima TT, Stiles ME and Vederas JC, Three-dimensional structure of leucocin A in trifluoroethanol and dodecylphosphocholine micelles: spatial location of residues critical for biological activity in Type IIa bacteriocins from lactic acid bacteria. *Biochemistry* **36**:15062–15072 (1997).
103 Johnsen L, Fimland G and Nissen-Meyer J, The C-terminal domain of pediocin-like antimicrobial peptides (Class IIs bacteriocins) is involved in specific recognition of the C-terminal part of cognate immunity proteins and in determining the antimicronial spectrum. *J Biol Chem* **280**:9243–9250 (2005).
104 Bhugaloo-Vail P, Dousset X, Metivier A, Sorokine O, Anglade P, Boyaval R *et al.*, Purification and amino acid sequence of piscicocins V1a and V1b, two class IIa bacteriocins secreted by *Carnobacterium piscicola* V1 that display significantly different levels of specific inhibitory activity. *Appl Environ Microbiol* **62**:4410–4416 (1996).
105 Haugen HS, Kristiansen PE, Fimland G and Nissen-Meyer J, Mutational analysis of the Class LLa bacteriocin curvacin A and its orientation in target cell membranes. *Appl Environ Microbiol* **74**:6766–6773 (2008).
106 Kellner R, Jung G and Sahl HG, Structure elucidation of the tricyclic lantibiotic Pep5 containing eight positively charged amino acids, in *Nisin and Novel Lantibiotics*, ed. by Jung G, Sahl H-G. Escom, Leiden, pp. 141–158l (1991).
107 Giffard CJ, Dodd HM, Horn N, Ladha S, Mackie AR, Parr A *et al.*, Structure–function relations of variants and fragment nisins studied with model membrane systems. *Biochemistry* **36**:3802–3810 (1997).
108 Kristian SA, Datta V, Weidenmaier C, Kansal R, Fedtke I, Peschel A *et al.*, D-Alanylation of teichoic acids promotes group A *Streptococcus* antimicrobial peptide resistance, neutrophil survival, and epithelial cell invasion. *J Bacteriol* **187**:6719–6725 (2005).
109 Stymiest JL, Mitchell BF, Wong S and Vederas JC, Synthesis of oxytocin analogues with replacement of sulfur by carbon gives potent antagonists with increased stability. *J Org Chem* **70**:7799–7809 (2005).
110 Sprules T, Kawulka KA and Vederas JC, NMR solution structure of ImB2, a protein conferring immunity to antimicrobial activity of the Type IIa bacteriocin, carnobacteriocin B2. *Biochemistry* **43**:11740–11749 (2005).
111 Kauer K, Andrew LC, Wishart DS and Vederas JC, Dynamic relationships among Type IIa bacteriocins: temperature effects on antimicrobial activity and on structure of the C-terminal amphipathic α helix a receptor-binding region. *Biochemistry* **43**:9009–9020 (2004).
112 Li Y, Xiang Q, Zhang Q, Huang Y and Su Z, Overview on the recent study of antimicrobial peptides: origins, functions, relative mechanisms and application. *Peptides* **37**:207–215 (2012).
113 Brotz H, Beirbaum G, Markus A, Moliter E and Sahl HG, Mode of action of the lantibiotic mersacidin: inhibition of peptidoglycan biosynthesis via a novel mechanism? *Antimicrob Agents Chemother* **39**:714–719 (1995).
114 Asaduzzaman SM and Sonomoto K, Lantibiotics: diverse activities and unique modes of action. *J Biosci Bioeng* **107**:475–487 (2009).
115 Brotz K, Josten M, Wiedemann I, Schneider U, Gotz F, Bierbaum G *et al.*, Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. *Mol Microbiol* **30**:317–327 (1998).
116 Abee T, Krockel L and Hill C, Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *Int J Food Microbiol* **28**:169–185 (1995).
117 Breukink E and Kruijff B, The lantibiotic nisin, a special case or not? *Biochim Biophys Acta* **1462**:223–234 (1999).
118 Hsu STD, Breukink E, Tischenko E, Lutters MAG, Kriujff B, Kaptein R *et al.*, The nisin–lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. *Nat Struct Mol Biol* **11**:963–967 (2004).
119 Hecahrd Y and Sahl HG, Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. *Biochemie* **84**:545–55 (2002).
120 Yoneyama F, Imura Y, Ohno K, Zendo T, Nakayama J, Matsuzaki K *et al.*, Peptide–lipid huge toroidal pore, a new antimicrobial mechanism mediated by a lactococcal bacteriocin, lacticin Q. *Antimicrob Agents Chemother* **53**:3211–3217 (2009).

121 Oren Z and Shai Y, Mode of action of linear amphipathic α-helical antimicrobial peptides. *Peptide Sci* **47**:451–463 (1999).
122 Shai Y, Mode of action of membrane active antimicrobial peptides. *Peptide Sci* **66**:236–248 (2002).
123 Shai Y, Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochim Biophys Acta* **1462**:55–70 (1999).
124 Bauer R and Dicks LMT, Mode of action of lipid II-targeting lantibiotics. *Int J Food Microbiol* **101**:201–216 (2005).
125 Moll GN, Konings WN and Driessen AJM, Bacteriocins: mechanism of membrane insertion and pore formation. *Antonie van Leeuwenhoek* **76**:185–198 (1996).
126 Linnett PE and Strominger JL, Additional antibiotic inhibitors of peptidoglycan synthesis. *Antimicrob Agents Chemother* **4**:231–236 (1973).
127 Hasper HE, Kramer NE, Smith JL, Killman JD, Zachariah C, Kuipers OP *et al.*, An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. *Science* **313**:1636–1637 (2006).
128 Gao FH, Abee T and Konings WN, Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome C oxidase-containing proteoliposomes. *Appl Environ Microbiol* **57**:2164–2170 (1991).
129 Havard J, Hamill P and Hancock EW, Peptide antimicrobial agents. *Clin Microbiol Rev* **19**:491–511 (2006).
130 Montville TJ, Emilia M and Bruno MEC, Evidence that dissipation of proton motive force is a common mechanism of action for bacteriocins and other antimicrobial proteins. *Int J Food Microbiol* **24**:53–74 (1994).
131 Abee T, Klaenhammer TR and Letellier L, Kinetic studies of the action of lactacin F, a bacteriocin produced by *Lactobacillus johnsonii* that forms poration complexes in the cytoplasmic membrane. *Appl Environ Microbiol* **60**:1006–1013 (1994).
132 Montville TJ and Chen Y, Mechanistic action of pediocin and nisin: recent progress and unresolved questions. *Appl Microbiol Biotechnol* **50**:511–519 (1998).
133 Bruno MEC and Montville TJ, Common mechanistic action of bacteriocins from lactic acid bacteria. *Appl Environ Microbiol* **59**:3003–3010 (1993).
134 Yang L, Harroun TA, Weiss TM, Ding L and Juang HW, Barrel-stave model or toroidal model? *A case study on melittin pores. Biophys J* **81**:1475–1485 (2001).
135 Freund S, Jung G, Gibbons WA and Sahl HG, NMR and circular dichroism studies on Pep5, in *Nisin and Novel Lantibiotics: ESCOM Science*, ed. by Jung G and Sahl HG, pp. 103–113 (1991).
136 Tahara T, Oshimura M, Umezawa C and Kanatani K, Isolation, partial characterization, and mode of action of acidocin J1132, a two-component bacteriocin produced by *Lactobacillus acidophilus* JCM 1132. *Appl Environ Microbiol* **62**:892–897 (1996).
137 Tossi A, Sandri L and Giangaspero A, Amphipathic, α-helical antimicrobial peptides. *Peptide Sci* **55**:4–30 (2000).
138 Sobko AA, Kotova EA, Antonenko YN, Zakharov SD and Cramer WA, Lipid dependence of the channel properties of a colicin E1–lipid toroidal pore. *J Biol Chem* **281**:14408–14416 (2005).
139 Saitoh H, Kiba A, Nishihara M, Yamamura S, Suzuki K and Terauchi R, Production of antimicrobial defensin in *Nicotiana benthamiana* with a potato virus X vector. *Mol Plant–Microbe Interact* **14**:111–115 (2001).
140 Deegan LH, Cotter PD, Hill C and Ross P, Bacteriocins: biological tools for bio-preservation and shelf-life extension. *Int Dairy J* **16**:1058–1071 (2006).
141 Lehrer Ri and Ganz T, Cathelicidins: a family of endogenous antimicrobial peptides. *Curr Opin Hematol* **9**:18–22 (2002).
142 De Vuyst L and Leroy F, Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J Mol Microbiol Biotechnol* **13**:194–199 (2007).
143 Joshi VK, Sharma S and Rana NS, Production, purification, stability and efficacy of bacteriocin from isolates of natural lactic acid fermentation of vegetables. *Food Technol Biotechnol* **44**:435–439 (2006).
144 Galvez A, Abriouel H, Lopez RL and Omar NB, Bacteriocin-based strategies for food biopreservation. *Int J Food Microbiol* **120**:51–70 (2007).
145 O'Sullivan L, Ross RP and Hill C, Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochimie* **84**:593–604 (2002).
146 McMullen LM and Stiles ME, Potential of use of bacteriocin-producing lactic acid bacteria in the preservation of meats. *J Food Prot* (Suppl.):64–71 (1996).
147 Callewaert R, Hugas M and De Vuyst L, Competitiveness and bacteriocin production of Enterococci in the production of Spanish-style dry fermented sausage. *Int J Food Microbiol* **57**:33–42 (2000).
148 Schilinger U, Geisen R and Holzapfel WH, Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends Food Sci Technol* **7**:58–64 (1996).
149 Stiles ME, Potential for biological control of agents of foodborne disease. *Food Res Int* **27**:245–250 (1994).
150 Hugas M, Pages F, Garriga M and Monfort JM, Application of the bacteriocinogenic *Lactobacillus saki* CTC494 to prevent growth of *Listeria* in fresh and cooked meat products packed with different atmospheres. *Food Microbiol* **15**:639–650 (1998).
151 Schobitz R, Zaror T, Leon O and Costa M, A bacteriocin from *Carnobacterium piscicola* for the control of *Listeria monocytogenes* in vacuum-packaged meat. *Food Microbiol* **16**:249–255 (1999).
152 Lou Y and Ahmed AE, Adaptation to sublethal environmental stresses protects *Listeria monocytogenes* against lethal preservation factors. *Appl Environ Microbiol* **63**:1252–1255 (1997).
153 Eppert I, Valdes-Stauber N, Gotz H, Busse M and Scherer S, Growth reduction of *Listeria* spp. caused by undefined industrial red smear cheese cultures and bacteriocin-producing *Brevibacterium* lines as evaluated *in situ* on soft cheese. *Appl Environ Microbiol* **63**:4812–4817 (1997).
154 Leverntz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M *et al.*, Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and bacteriocin. *Appl Environ Microbiol* **69**:4519-4526 (2003).
155 Kreth J, Merritt J, Shi W and Qi F, Coordinated bacteriocin production and competence development: a possible mechanism for taking up DNA from neighbouring species. *Mol Microbiol* **57**:392–404 (2005).
156 Muriana PM, Bacteriocins for control of *Listeria* spp. in food. *J Food Prot* (Suppl.):54–63 (1996).
157 Gillor P, Etzion A and Riley MA, The dual role of bacteriocins as anti- and probiotics. *Appl Microbiol Biotechnol* **81**:591–606 (2008).
158 Kroes R, Kleiner J and Renwick A, The threshold of toxicological concern concept in risk assessment. *Toxicol Sci* **86**:226–230 (2005).
159 Fields FO, Use of bacteriocins in food: regulatory considerations. *J Food Prot* (Suppl.):72–77 (1996).
160 Post RC, Regulatory perspective of the USDA on the use of antimicrobials and inhibitors in foods. *J Food Prot* (Suppl.): 78–81 (1996).
161 Sharma N, Kapoor R, Gautam N, and Kumari R, Purification and characterization of bacteriocin produced by *Bacillus subtilis* R75 isolated from fermented chunks of mung bean. *Food Technol Biotechnol* **49**:169–176 (2011).
162 Samelis J, Bleicher A, Delbes-Paus C, Kakouri A, Neuhaus K and Montel MC, FTIR-based polyphasic identification of lactic acid bacteria isolated from traditional Greek Graviera cheese. *Food Microbiol* **28**:76–83 (2010).
163 Daview EA, Bevis HE and Delves-Broughton J, The use of the bacteriocin, nisin, as a preservative in ricotta-type cheeses to control the food-borne pathogen *Listeria monocytogenes*. *Lett Appl Microbiol* **24**:343–346 (1997).
164 Siragusa GR, Cutter CN and Willett JL, Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. *Food Microbiol* **16**:229–235 (1999).
165 Stern NJ, Svetoch EA, Eruslanov BV, Kovalev YN, Volodina LI, Perelygin VV *et al.*, *Paenibacillus polymyxa* purified bacteriocin to control *Campylobacter jejuni* in chickens. *J Food Prot* **68**:1450–1453 (2005).
166 Danisco website, Antimicrobials. [Online]. Available: http://www.danisco.com/product-range/antimicrobials/ [May 2013].

