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Editors

Prokaryotic Antimicrobial Peptides

From Genes to Applications

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Part I
Introduction: History, Current Knowledge, and Future Research on Antimicrobial Peptides

Chapter 1
History, Current Knowledge, and Future Directions on Bacteriocin Research in Lactic Acid Bacteria

Ingolf F. Nes

All organisms, both eukaryotic organisms and bacteria, are able to produce ribosomally antimicrobial peptides. In bacteria, such compounds are referred to as bacteriocins. The history of bacteriocins goes back to the early 1920s. One has experienced many disappointments in the efforts how to put these compounds into practical use despite being one of the most promising groups of antimicrobial agents to fight bacterial pathogens. However, today, we see new possibilities how to take advantage of such peptides for the benefit of man and animals. Bacteriocin production has become an important property of probiotic bacteria, and targeted use of bacteriocins to fight certain pathogens may have a future.

We should separate bacteriocins from our traditional peptide antibiotics. First, the peptide antibiotics differ from ribosomally synthesized peptides because peptide antibiotics are synthesized by enzymes. Second, bacteriocins are targeted at a narrow spectrum of bacteria often within the species of the producer or closely related ones, while the classical antibiotics are active against broad spectra of bacteria. Another feature that separates bacteriocins from antibiotics is their potency against susceptible bacteria; bacteriocins are unique because they can kill bacteria at nanomolar concentrations, while antibiotics are needed in much higher concentrations.

For many reasons, it is meaningful to separate the bacteriocins of gram-positive and gram-negative bacteria, and this short overview focuses on bacteriocins from gram-positive bacteria. It is most fruitful to divide G+ bacteriocins into two major groups: the heat-stable lantibiotics (Class I) and the nonmodified (some minor modifications may exist) and heat-stable bacteriocins (Class II). These two major classes are further divided into subclasses (Chatterjee et al. 2005; Cotter et al. 2005; Nes et al. 2006). Numerous excellent reviews on bacteriocins have been published

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in recent years (Breukink and de Kruijff 2006; Chatterjee et al. 2005; Cotter et al. 2005; Diep et al. 2009; Drider et al. 2006; Nes et al. 2007; Nissen-Meyer et al. 2009; Oppegard et al. 2007; Willey and van der Donk 2007).

The focus of bacteriocins in gram-positive bacteria has for the most part been on lactic acid bacteria (LAB) due to their apparent importance in food and feed fermentation, and also by being considered as GRAS organisms by FDA, and not least because of good funding in the 1990s and into the twenty-first century by the European Union. An important reason for research on bacteriocin has been and still is their extreme potency as antimicrobials as observed with some bacteriocins that are active at nanomolar concentrations against a number of bacteria including pathogens such as *Listeria monocytogenes*. Some bacteriocins exhibit activity against multidrug-resistant nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE). Thus, it is believed that they could have a potential in medical and veterinary applications.

Fermented food and feed and plant material have been a source for isolation of bacteriocin-producing LAB, but intestinal and fecal sampling of LAB from animals and humans has become an increasingly important source for such bacteria due to an increased awareness of their importance as probiotic bacteria.

Modification and Structure of Bacteriocins

Lantibiotics are gene-encoded peptides that contain intramolecular ring structures by the formation the thioether bridges between dehydrated serine or threonine and cysteines that confer lanthionine and methyllanthionine residues, respectively. Additional but less frequent modifications have been identified in some lantibiotics. Such modifications include lysinoalanine, 2-oxybutyrate, 2-oxopropionate, 2-hydroxypropionate, β-hydroxy-aspartate, S-aminovinyl-D-cysteine, S-aminovinyl-D-methylcysteine, and D-alanine.

After the discovery of lantibiotic bacteriocins that goes back to the early 1920s, it took more than 50 years for the structure of nisin, the first identified lantibiotic, to be determined, i.e., in 1971 (de Klerk and Smit 1967; Gross and Morell 1971). In the 1960s, it was reported that lactobacilli produced antimicrobial substances different from the organic acids (de Klerk and Smit 1967; Sabine 1963; Tramer 1966). It was a slow start, and the area of lantibiotic research did not take off before the 1970s. Since then, numerous lantibiotics have been identified and characterized with respect to structure, mode of action, genetics, regulation, synthesis, and modification. Many excellent and comprehensive review articles have been published in recent years on these topics of lantibiotics (Bonelli et al. 2006; Chatterjee et al. 2005; Dufour et al. 2007; Guder et al. 2000; Pag and Sahl 2002; Twomey et al. 2002; Willey and van der Donk 2007; Xie and van der Donk 2004).

There is presently focus on development of bioengineered lantibiotics, to reveal the location of essential and variable domains therein and to create derivatives with

broader specificity, increased stability, and even higher activities against specific target organisms for both in vivo and in vitro use.

In vitro modification systems have successfully been used to introduce thioether rings into other biologically active peptides. These enzymes have been the focus of recent bioengineering studies.

Of particular significance with respect to posttranlational modification is the new bacteriocin thuricin CD, a strong anticlostridial bacteriocin that is particularly effective against *Clostridium difficile* (Rea et al. 2010), produced by *Bacillus thuringiensis*. This two-peptide bacteriocin is quite unique not just because of its anticlostridial activity but also because both peptides feature three posttranslationally modified sulfur to alpha carbon in thioether linkages. It should also be added that such modification has previously been identified in the circular bacteriocin.

Also, class II bacteriocins can be structured by inducing certain posttranslational changes, and the most common modification is the conserved N-terminal cysteine-bridge formation that has shown to be of crucial importance for the antimicrobial activity of class IIa bacteriocin (Eijsink et al. 1998).

Also, circular bacteriocins are posttranslationally modified through a head-to-tail backbone covalent linkage (Maqueda et al. 2008).

Structures of many bacteriocins have been resolved by NMR analysis. Such studies include both classes of lantibiotics, Class II bacteriocins (Kristiansen et al. 2005; Oppegard et al. 2007; Rogne et al. 2009; Sprules et al. 2004) and cyclic bacteriocins (Gonzalez et al. 2000; Martin-Visscher et al. 2009). Combined with functional analysis, important structural features important for the antimicrobial activity have been determined. In addition, immunity proteins have also been structurally determined (Johnsen et al. 2005; Martin-Visscher et al. 2008). Hopefully, these structural studies combined with functional studies will bring together how the bacteriocins work and how the immunity interacts with its bacteriocin to prevent self-destruction of the host.

Genetics

The genes required for biosynthetic machinery of lantibiotics are complex and are often organized in operons. Together with the structural gene(s) (*lanA*), genes encoding modification enzymes, externalization system of the bacteriocins as well as immunity genes to protect the producer for self-destruction are needed. In addition, it has been shown that the production of some lantibiotics is also regulated by a two-component regulatory system (Kleerebezem et al. 2001), although alternative regulatory systems are identified in a few lantibiotics as seen for lactocin S (Rawlinson et al. 2005).

The modifications are introduced either by one biosynthetic enzyme (LanM) or by a dehydratase (LanB) in combination with a cyclase (LanC). The structure of NisC has been resolved; the reaction mechanism of LctM has been studied, and the amino-acid residues in the active site were identified by mutagenesis studies