J. Microbiol. Biotechnol. (2011), **21**(3), 229–235
doi: 10.4014/jmb.1010.10017
First published online 15 February 2011

REVIEW

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Lantibiotics, Class I Bacteriocins from the Genus *Bacillus*

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Received: October 11, 2010 / Revised: December 25, 2010 / Accepted: December 27, 2010

Antimicrobial peptides exhibit high levels of antimicrobial activity against a broad range of spoilage and pathogenic microorganisms. Compared with bacteriocins produced by lactic acid bacteria, antimicrobial peptides from the genus *Bacillus* have been relatively less recognized despite their broad antimicrobial spectra. These peptides can be classified into two different groups based on whether they are ribosomally (bacteriocins) or nonribosomally (polymyxins and iturins) synthesized. Because of their broad spectra and high activity, antimicrobial peptides from *Bacillus* spp. may have great potential for applications in the food, agricultural, and pharmaceutical industries to prevent or control spoilage and pathogenic microorganisms. In this review, we introduce ribosomally synthesized antimicrobial peptides, the lantibiotic bacteriocins produced by members of *Bacillus*. In addition, the biosynthesis, genetic organization, mode of action, and regulation of subtilin, a well-investigated lantibiotic from *Bacillus subtilis*, are discussed.

Keywords: Antimicrobial peptides, bacteriocins, lantibiotics, ribosomally synthesized, *Bacillus*

Antimicrobial peptides have been found in most living organisms: prokaryotes, plants, and animals including vertebrates and invertebrates [15, 35, 61]. They have diverse chemical structures and play crucial roles in the innate immunity of early defense systems to protect their hosts from invading pathogens [65]. Among them, a great variety of the peptides are produced by bacteria.. Individual bacteria are subject to competition with either phylogenetically unrelated or closely related microorganisms to survive under limited nutritional conditions. Consequently, antimicrobial peptides, which are indispensible as a component of defensive mechanisms to protect the producers themselves so they may outgrow their competitors, have been extensively investigated [43, 47, 52]. Bacteria produce two different types of antimicrobial peptides that are classified based on biosynthetic mechanisms: ribosomally synthesized peptides, or bacteriocins, that exhibit a relatively narrow range of antimicrobial activity, mainly inhibiting closely related bacteria [20, 43]; and nonribosomally synthesized peptides showing broader spectra of activities, inhibiting bacteria [59] or fungi [36]. The bacteriocins are grouped into four classes (I–IV) based on their biochemical and genetic properties [24]. Both class I and II bacteriocins are small (3–10 kDa), cationic, amphiphilic, membrane-active peptides. Class I bacteriocins, or lantibiotics, contain the unusual amino acids lanthionine and methyllanthionine. In contrast, class II bacteriocins do not contain these modified amino acids. They can be subdivided into three groups: (i) class IIa, *Listeria*-active peptides with the consensus sequence -Y-G-N-G-V-X-C- near the N-terminus; (ii) class IIb, two-peptide bacteriocins in which both components are required for antimicrobial activity; and (iii) class IIc, thiol-activated peptides requiring reduced cysteine residues for activity. Class III bacteriocins are high molecular mass (>30 kDa), heat-labile proteins. Class IV bacteriocins are complex peptides containing lipid or carbohydrate moieties essential for activity.

Fermented foods have been consumed for millennia all over the world. In western countries, dairy products such as cheese, kefir, and yogurt are beneficial microbial reservoirs that contain various groups of lactic acid bacteria (LAB), a number of which produce bacteriocins exhibiting high levels of antibacterial activity [24]. Nisin, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* from dairy products, has been extensively investigated and approved for use as a food preservative for more than 40 years in over 50 countries [11, 49]. In Asia, traditional fermented foods made of vegetables or crops such as *kimchi* or *doenjang* have been served as the main daily meal for several millennia. The genus *Bacillus* has been recognized as a major group of microorganisms that contribute to fermentation of soybean-based fermented foods in East Asia [21–23, 34], producing diverse antimicrobial peptides that show not only antibacterial but antifungal activities

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[55]. Members of this genus produce both types of antimicrobial peptides: ribosomally synthesized bacteriocins such as subtilin [4] and subtilosin A [3]; and nonribosomally synthesized peptides including polymyxins [33, 37] and iturins [31, 39]. Therefore, *Bacillus* spp. can produce more diverse antimicrobial peptide structures than LAB, which produce only ribosomally synthesized bacteriocins. However, antimicrobial peptides from *Bacillus* spp. have received less notice as natural beneficial antimicrobial compounds, which are applicable to the food, agricultural, and pharmaceutical industries.

In this review, we summarize the current knowledge regarding lantibiotics, class I bacteriocins produced by members of the genus *Bacillus* exhibiting antimicrobial activity against foodborne pathogens. Moreover, the biosynthetic mechanism, mode of action, genetic organization, and regulation of the class I bacteriocin subtilin are described, and the potential application of antimicrobial peptides in the food, agricultural, and pharmaceutical industries is discussed.

Bacteriocins: Ribosomally Synthesized Antimicrobial Peptides

Bacteriocins, ribosomally synthesized antimicrobial peptides from bacteria, have been extensively investigated [19]. Bacteriocins typically exhibit their antimicrobial activity against closely related bacteria. Gram-negative bacteria are known to produce colicins and microcins [12, 13, 51]. In Gram-positive bacteria, nisin and subtilin have been the most thoroughly studied [14]. Bacteriocins have been widely studied because they are generally recognized as safe. Bacteriocins are small (3–10 kDa) and are inactivated by conventional proteolytic enzymes. Despite having different spectra of antibacterial activity, most bacteriocins are cationic and amphiphilic, implying that a common mode of action is shared among different types. Bacteriocins from the genus *Bacillus* that have been mainly investigated are subtilin [14, 48] and subtilosin A [41, 66]. In addition, *Bacillus subtilis*, *B. cereus*, *B. thuringiensis*, and other *Bacillus* spp. have been reported to produce a variety of bacteriocins showing a broad range of antibacterial activity against food spoilage and pathogenic microorganisms [2, 6–8, 32, 40, 44–46, 50, 55]. Thus far, all of the bacteriocins from *Bacillus* spp. whose structures have been elucidated are members of class I, with the exception of a class II pediocin-like bacteriocin, coagulin [30]. Here, we focus on subtilin to introduce the genetic locus, mode of action, and regulatory system of class I bacteriocins produced by *Bacillus* spp.

Lantibiotics. Class I bacteriocins, called lantibiotics, contain the unusual amino acids including lanthionine and β-methyllanthionine as well as dehydrated residues formed by enzymatic reaction. Lantibiotics are subjected to posttranslational modification during maturation, resulting in the presence of the modified amino acids in the mature peptide [17]. Class I bacteriocins exhibit antibacterial activity against closely related Gram-positive bacteria but typically do not inhibit Gram-negative bacteria.

Subtilin, the most extensively studied class I bacteriocin produced by members of the genus *Bacillus* [38], is composed of 32 amino acids, eight of which are modified (Fig. 1A). Subtilin is produced by *B. subtilis* ATCC 6633, an endospore-forming bacterium inhibiting a broad range of Gram-positive bacteria including other *Bacillus* and *Listeria* spp. The structure of subtilin is very similar to that of nisin, which has been applied as a food preservative in dairy products including cheese.

Genetic loci for bacteriocin production. The gene cluster for bacteriocin synthesis is composed of structural gene(s) and accessory genes necessary for bacteriocin transport, immunity, regulation, and processing to produce the mature form; bacteriocin producers require additional proteins involved in immunity for self-protection, enzymatic processing, transport, and regulatory systems composed of induction factors, sensor kinases, and response regulators [42, 62]. Bacteriocins are initially synthesized as premature peptides with a leader or signal sequence at the N-terminus. Immunity proteins are required to protect the producers themselves from the activity of their own bacteriocins. Class I bacteriocins are subject to further posttranslational modification to produce the mature peptide form, resulting in the formation of unusual amino acids such as lanthionines and intramolecular disulfide linkage by enzymatic reaction. Dedicated ATP-binding cassette (ABC) transporters, containing hydrophobic membrane spanning motifs, are required for either dual functioning that includes proteolytic activity on the N-terminus and translocation of the peptides (nisin) or bacteriocin transport only (subtilin). After being modified and transported out of producer cells, bacteriocins are enzymatically cleaved to remove the leader sequence; this step yields mature, active subtilin. Transcription of accessory proteins is co-regulated with the production of bacteriocins by a signal transduction system in a cell density-dependent manner, as discussed in a later section.

The subtilin gene cluster is composed of 10 genes, *spaBTCSIFEGRK*, with a total length of approximately 12 kb [54], and four promoters are involved in its transcription (Fig. 1B). The structural gene encoding presubtilin, composed of 56 amino acids, is *spaS*. Presubtilin requires processing, which yields mature, active subtilin containing 32 amino acids. The mature subtilin structure contains one dehydrobutyrine (Dhb) and two dehydroalanine (Dha) residues as well as one *meso*-lanthionine (Ala-S-Ala) and four 3-methyllanthionine (Abu-S-Ala) ring structures. SpaB and SpaC were predicted to be involved in posttranslational modification on the cytosolic side of the membrane [18, 29]. It has been suggested that SpaT is an ABC transporter protein localized on the membrane for export of presubtilin [29], forming a complex with SpaB