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REVIEW

Antimicrobial peptides of the genus *Bacillus*: a new era for antibiotics

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Abstract: The rapid onset of resistance reduces the efficacy of most conventional antimicrobial drugs and is a general cause of concern for human well-being. Thus, there is great demand for a continuous supply of novel antibiotics to combat this problem. Bacteria-derived antimicrobial peptides (AMPs) have long been used as food preservatives; moreover, prior to the development of conventional antibiotics, these AMPs served as an efficient source of antibiotics. Recently, peptides produced by members of the genus *Bacillus* were shown to have a broad spectrum of antimicrobial activity against pathogenic microbes. *Bacillus*-derived AMPs can be synthesized both ribosomally and nonribosomally and can be classified according to peptide biosynthesis, structure, and molecular weight. The precise mechanism of action of these AMPs is not yet clear; however, one proposed mechanism is that these AMPs kill bacteria by forming channels in and (or) disrupting the bacterial cell wall. *Bacillus*-derived AMPs have potential in the pharmaceutical industry, as well as the food and agricultural sectors. Here, we focus on *Bacillus*-derived AMPs as a novel alternative approach to antibacterial drug development. We also provide an overview of the biosynthesis, mechanisms of action, applications, and effectiveness of different AMPs produced by members of the *Bacillus* genus, including several recently identified novel AMPs.

Key words: antibiotics, antibiotic resistance, antimicrobial peptides, *Bacillus* species.

Résumé : L'apparition rapide de l'antibiorésistance réduit l'efficacité de la plupart des médicaments antimicrobiens et fait planer une menace sur le bien-être des humains. Pour contrer ce problème, il devient pressant de s'approvisionner en nouveaux antibiotiques. Les peptides antimicrobiens (PAM) dérivés de bactéries sont utilisés depuis longtemps à titre d'agents de conservation. D'ailleurs, avant le développement des antibiotiques conventionnels, ces PAM étaient une source abondante d'antibiotiques. Récemment, on a démontré que des peptides produits par des membres du genre *Bacillus* présentaient une activité antimicrobienne à large spectre contre des microbes pathogènes. Les PAM issus de *Bacillus* peuvent être synthétisés avec ou sans l'intervention du ribosome et peuvent être classés en fonction de leur biosynthèse, leur structure ou leur poids moléculaire. On ne sait pas encore précisément quel est le mécanisme d'action de ces PAM, mais on avance que ces PAM tuent les bactéries par la formation de canaux ou par la perturbation de la paroi cellulaire bactérienne, ou par une combinaison des deux mécanismes. Les PAM issus de *Bacillus* seraient exploitables par l'industrie pharmaceutique de même que par le secteur agroalimentaire. Dans le présent ouvrage, nous abordons le sujet des PAM issus de *Bacillus* sous l'angle d'une approche alternative à l'élaboration de médicaments antibactériens. Nous faisons également un survol de la biosynthèse, des mécanismes d'action, des applications et de l'efficacité de divers PAM produits par des membres du genre *Bacillus*, dont plusieurs PAM nouvellement décrits. [Traduit par la Rédaction]

Mots-clés : antibiotiques, antibiorésistance, peptides antimicrobiens, espèces de *Bacillus*.

Introduction

Infectious diseases are the second most common cause of death worldwide; therefore, it is imperative to discover new drugs to combat microbial pathogens (Lam 2007; Pálffy et al. 2009). The period from the 1940s to the late 1960s is referred to as the "golden era" of antibiotic research because research in this period resulted in the identification of a robust arsenal of effective agents from natural sources to treat microbial infections (Walsh 2003; Brown 2006; Tenover 2006). A recent study of the antibiotics market reported an average annual growth of 4% over the past 5 years, with US$42 billion of antibiotics sold worldwide in 2009 (Hamad 2010). However, many pathogenic bacteria are now resistant to almost all available antimicrobial drugs. The World Health Organization (WHO) has already warned that the world is heading back to the pre-antibiotic era regarding therapy for these multiple antibiotic-resistant pathogens (Parisien et al. 2008). According to the Infectious Disease Society of America, more than 70% of the bacterial pathogens responsible for potentially fatal infections in 2004 were predicted to be resistant to at least one of the antibiotics usually used for the treatment of bacterial infections (Hassan et al. 2012). Additionally, resistant strains rapidly acquire additional resistance to new synthetic derivatives, since these strains are already resistant to the parent agent. The development of novel antibacterial alternatives is the most obvious approach to combat this increase in antimicrobial resistance (Coates et al. 2002; Walsh 2003; Clardy et al. 2006). Among the possibilities, antimicrobial peptides (AMPs), which are ubiquitous gene-encoded natural antibiotics, provide a promising alternative for a new generation of antibiotics (Pálffy et al. 2009; Rotem and Mor 2009; Cotter et al. 2013).

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AMPs have the potential to play a promising role in the fight against the rapid increase in microbial resistance to conventional antibiotics (Rotem and Mor 2009). More than 2000 different AMPs from a multitude of plants, animals, viruses, bacteria, and fungi have been identified to date, and several have entered into clinical trials (Jenssen et al. 2006; Sang and Blecha 2008; Donadio et al. 2010; Brogden and Brogden 2011). Since AMPs are a natural part of the human antimicrobial defense system, the possibility of developing pathogen resistance or unwanted side effects is less likely than with chemical antibiotics. Moreover, compared with conventional antibiotics, which are mostly active against bacteria or fungi, AMPs are considered to be an elementary group of novel antibacterial, antifungal, and antiviral drugs that could be used in the treatment of infectious diseases and parasitic infections and may also be suitable for the treatment of cancer and HIV infection (Jenssen et al. 2006; Pálffy et al. 2009; Rotem and Mor 2009; Brogden and Brogden 2011). In addition, conventional antibiotics generally target metabolic enzymes that may selectively develop resistance, whereas AMPs kill microbes primarily through the generation of membrane pores, thus making it inherently more difficult for the organisms to develop resistance (Sang and Blecha 2008). The genus *Bacillus* is capable of producing a large number of AMPs and is therefore viewed as a promising starting point in the search for new inhibitory substances (Bizani et al. 2005; Xie et al. 2009). Several studies have shown that members of the genus *Bacillus sensulato* produce a wide arsenal of antimicrobial substances, including ribosomally and nonribosomally synthesized lipopeptides, bacteriocins, and other kinds of peptides (Schallmey et al. 2004; Stein 2005; Abriouel et al. 2011). Previous studies have isolated different strains of terrestrial *Bacillus* and identified their inhibitory compounds (Moshafi et al. 2011). In this review, we provide a detailed overview of AMPs that are derived from the *Bacillus* species, with an emphasis on recent findings that highlight their potential as alternatives to conventional antibiotics.

AMPs as an alternative approach for combating antibiotic resistance

The initial enthusiasm over the scientific triumphs against infectious diseases did not last long. The rapid emergence of resistance to current antibiotics has resulted in increased susceptibility to microbial infections and has raised public health concerns worldwide (Overbye and Barrett 2005; Tenover 2006). Many infectious pathogens, especially Gram-negative bacteria, have developed resistance to conventional antibiotics. In rare cases, these pathogens are resistant to all commercially available antibiotics. This resistance phenomenon is not restricted to bacteria and also extends to pathogenic fungi, viruses, and parasites (Alba et al. 2012). The WHO has anticipated that *Mycobacterium tuberculosis* will become more virulent and antibiotic-resistant in the near future and has also estimated that *M. tuberculosis* will infect almost one billion people from 2000 to 2020, leading to approximately 35 million deaths (Hassan et al. 2012). Resistance has developed to every main class of antibiotic, both natural and synthetic. This resistance typically develops over the course of 1 year but can also develop up to more than a decade after the first clinical use (Walsh 2003; Tenover 2006). Initially, the problem of bacterial resistance to antimicrobial drugs was solved by the discovery of new classes of drugs, such as aminoglycosides, macrolides, and glycopeptides, or by the chemical modification of previously existing drugs. Unfortunately, there is no assurance that the development of new antimicrobial drugs will prevent the development of resistant strains of pathogenic bacteria (Gold and Moellering 1996; Levy and Marshall 2004). Moreover, in the last 5 decades, the major pharmaceutical companies have decreased their investment in this important research field (Overbye and Barrett 2005). Only 2 novel classes of antibiotics have been developed over the past 30 years: the oxazolidinone linezolid (Zyvox; Pfizer), which was introduced in 2000, and the cyclic lipopeptide daptomycin (Cubicin; Cubist), which was introduced in 2003 (Hamad 2010; Hassan et al. 2012). Also, hopes were high that resistance to these synthetic antimicrobial agents would be slow to emerge. Unfortunately, however, resistance to synthetic antimicrobial agents has increased quite rapidly (Coates et al. 2002).

Considering the current prevalence of antibiotic-resistant pathogens, there is obviously an unmet need to discover and develop novel classes of potent antibiotics with new inhibitory mechanisms. These novel antibiotics can either replace traditional antibiotics or be used in combinatorial approaches to combat infectious pathogens and their antibiotic-resistant derivatives (Hassan et al. 2012). Thus, bacterially derived AMPs are an option that can no longer be ignored (Cotter et al. 2013). In particular, AMPs isolated from the *Bacillus* species have the potential to be promising candidates for overcoming the shortcomings of current antibiotics (Hassan et al. 2012). The AMPs of various *Bacillus* strains have been extensively characterized, and many of these peptides have been found to be suitable for various applications (Abriouel et al. 2011). The *Bacillus* genus produces a large number of peptide antibiotics with different basic chemical structures (Cladera-Olivera et al. 2004), including bacteriocins, glycopeptides, lipopeptides, and cyclic peptides (Baindara et al. 2013). Moreover, these metabolites also show antagonistic properties and can be used against different pathogens. For example, mersacidin (1.8 kDa), a tetracyclic peptide that belongs to the group of lantibiotics, is produced by *Bacillus* species (Chatterjee et al. 1992; Brötz et al. 1995) and shows bactericidal activity against methicillin-resistant *Staphylococcus aureus* comparable to that of vancomycin or teicoplanin. Importantly, however, mersacidin treatment does not lead to the development of cross resistance (Jenssen et al. 2006). Therefore, AMPs of the genus *Bacillus* may be promising alternatives to conventional antibiotics for the effective treatment of single-drug- and multi-drug-resistant infectious pathogens.

Biosynthesis of *Bacillus* AMPs

Members of the genus *Bacillus* are rod-shaped, endospore-forming, Gram-positive bacteria that are abundant in soil. The *Bacillus* species can produce structurally diverse secondary metabolites, which exhibit a wide spectrum of antibiotic activity (Paik et al. 1997; Motta et al. 2008; Li et al. 2012; Sabaté and Audisio 2013). The peptide antibiotics of the *Bacillus* species can be divided into 2 subgroups based on the synthesis pathway (Nakano and Zuber 1990; Marx et al. 2001). One of these subgroups includes small microbial peptides that are nonribosomally synthesized by large enzymatic complexes, whereas the second subgroup comprises ribosomally synthesized peptides (Marx et al. 2001; Li et al. 2012).

Nonribosomally synthesized peptide antibiotics

The *Bacillus*-derived peptide antibiotics gramicidin, tyrocidine, bacitracin, surfactin, iturins, and fengycins are synthesized nonribosomally through a multistep mechanism that involves the selection and condensation of amino acid residues by multienzyme thiotemplates. This synthesis mechanism is mediated by nonribosomal peptide synthetases. In this process, large multisubunit enzymes ranging from 100 to more than 1600 kDa play a key role in the synthesis of these peptides (Stachelhaus and Marahiel 1995). The nonribosomally synthesized peptides are assembled from among more than 300 different precursors. These peptides can be linear or cyclic and can also contain cyclic branched structures containing a hydroxyl group, L-amino acids, or D-amino acids. Moreover, these peptides can be further modified by *N*-methylation, acylation, glycosylation, or heterocyclic ring formation (Hancock and Chapple 1999).

Gramicidin S and tyrocidine are small cyclic peptides produced by the *Brevibacillus brevis* (formerly referred to as *Bacillus brevis*)

Table 1. *Bacillus*-species-derived lipopeptides of surfactin, iturin, and fengycin families.

Lipopeptide family and characteristics	AMPs[a]	*Bacillus* species	Reference
Surfactin family			
Ester or peptide bond between a β-OH fatty acid and the C-terminal amino acid carboxyl group; contain 7-amino-acid residues of which the third and sixth are in the D-configuration	Surfactin	*B. subtilis*, *B. polyfermenticus*, *B. megaterium*	Chen et al. 2008; Kim et al. 2009; Goafu et al. 2010; Roongsawang et al. 2010
	Linchenysin	*B. licheniformis*, *B. megaterium*	
	Pumilacidin	*B. pumilus*	
	WH1fungin	*B. amyloliquefaciens*	
Iturin family			
Ester or peptide bond between a β-NH_2 fatty acid and the C-terminal amino acid carboxyl group; contain 7-amino-acid residues of which the second, third, and sixth are always in the D-configuration	Iturin, Bacillomycins, Mycosubtilin, Subtulene (contains a unique Iso C15-long chain β-amino acid)	*B. subtilis*, *B. megaterium*	Chen et al. 2008; Thasana et al. 2010; Roongsawang et al. 2010; Zhang et al. 2013
Fengycin family			
Ester or peptide bond between a β-OH fatty acid and the C-terminal amino acid carboxyl group (lactone linkage: COOH-Ile and OH-Tyr); contain 10-amino-acid residues of which the second, fourth, sixth, and ninth are in the D-configuration	Fengycin, Plipastatin, Agrastatin1	*B. subtilis*, *B. thurigiensis*, *B. circulans*, *B. megaterium*	Chen et al. 2008; Pueyo et al. 2009; Sivapathasekaran et al. 2009; Roongsawang et al. 2010

[a]AMPs, antimicrobial peptides.

strains ATCC 9999 and ATCC 8185, respectively. Synthesis of gramicidin S is catalyzed by the gramicidin S synthetase multifunctional enzyme complex. A multifunctional thiotemplate mechanism is also involved in the biosynthetic pathway of the 12-amino-acid peptide antibiotic bacitracin A, which is produced by certain strains of *Bacillus licheniformis* (Nakano and Zuber 1990). Three multifunctional enzymes, BA1 (335 kDa), BA2 (240 kDa), and BA3 (380 kDa), catalyze the synthesis of bacitracin A (Haddar et al. 2007). However, peptide antibiotic biosynthesis can also occur via alternatives to the thiotemplate mechanism. For example, a 7-amino-acid residue lipopeptide antibiotic that contains surfactin is produced by some *Bacillus subtilis* strains. This antibiotic is synthesized nonribosomally; however, its mechanism of synthesis is apparently distinct from that of the multienzyme thiotemplate (Nakano and Zuber 1990). The regulation of surfactin biosynthesis includes quorum-sensing control mechanisms and the control of fermentation medium components such as glucose and glutamine (Nakano and Zuber 1990; Schallmey et al. 2004).

Antifungal lipopeptides such as iturin, bacillomycin, and fengycin are produced by several strains of *B. subtilis*. These amphipathic lipopeptides have a molecular mass ranging from 1028 to 1084 Da and are synthesized by large multienzyme complexes with modularly arranged catalytic domains (Peypoux et al. 1999; Baindara et al. 2013). Bacilysin, a dipeptide that contains L-alanine, is one of the simplest peptide antibiotics and is produced by the *B. subtilis* Marburg 168 strain. An amino acid ligase (bacilysin synthetase) synthesizes bacilysin from its precursor amino acids in the presence of ATP and Mg^{2+} (Nakano and Zuber 1990). Several *Bacillus*-species-derived lipopeptides in the surfactin, iturin, and fengycin families have been described and are summarized in Table 1.

Ribosomally synthesized peptide antibiotics

Ribosomally synthesized peptide antibiotics are widely distributed in nature and contain between 12 and 50 amino acid residues. They are typically cationic and exhibit great structural diversity (Marx et al. 2001). Bacteriocins are ribosomally synthesized AMPs that are produced by bacteria and are usually active against strains of bacteria that are closely related to the producer strains (Motta et al. 2008; Aunpad and Na-Bangchang 2007). These AMPs comprise a heterogeneous group of amphiphilic and (or) hydrophobic AMPs (Oscáriz et al. 2006). Moreover, bacteriocins produced by *Bacillus* spp. exhibit a broader antimicrobial spectrum than most lactic acid bacteriocins (Wang et al. 2014*a*). These agents include subtilin from *B. subtilis*; coagulin from *Bacillus coagulans*; bacthuricin F4, thuricin 17, entomocin 9, and tochicin from *Bacillus thuringiensis*; cerecin 7 from *Bacillus cereus*; bacillocin 490 from *B. licheniformis*; and other bacteriocins (Aunpad and Na-Bangchang 2007; Cherif et al. 2003; Hammami et al. 2012).

Bacteriocins are classified according to chemical structure, heat stability, molecular mass, enzymatic sensitivity, presence of modified amino acids, and mode of action (Motta et al. 2008). Bacteriocins were first classified by Klaenhammer (1993). He suggested 4 classes for bacteriocins: class I lantibiotics (such as the modified amino acid lanthionine and small peptides <5 kDa), classII cystibiotics (small peptides containing one or more disulfide bonds that are essential for their activity, <10 kDa, heat stable, and membrane-active), class III thiolbiotics (containing an active –SH group, higher molecular mass, <30 kDa, and heat-labile), and class IV (complex proteins containing one or more lipids or carbohydrate moieties) (Klaenhammer 1993; Rea et al. 2011). Abriouel et al. (2011) classified *Bacillus* bacteriocins into 3 categories: class I (the post-translationally modified peptides), class II (the nonmodified peptides), and class III (the large proteins). Furthermore, the class I bacteriocins were divided into 4 subclasses, whereas the class II bacteriocins were divided into 2 subclasses. In 2011, Rea and his co-workers (Rea et al. 2011) also described an updated classification of Gram-positive bacteriocins and bacteriocin-like peptides and proteins: class I, modified peptides containing lantibiotics and lantipeptides, labyrinthopeptins, and sactibiotics; class II, nonmodified peptides containing pediocin-like peptides, 2-peptide bacteriocins, circular bacteriocins, and linear non-pediocin-like 1-peptide bacteriocins; and lastly, bacteriolysins (formerly class III) containing non-bacteriocin lytic peptides. They have also subdivided lantibiotics and pediocin-like peptides into 4 subclasses, and sactibiotics, 2-peptide bacteriocins, and circular bacteriocins into 2 subclasses each. Recently, Cotter and his colleagues (2013) also proposed a classification of bacteriocins based on ribosomally synthesized bacteriocins. In this classification, class I (modified) contain post-translationally modified peptides, including lantibiotics (e.g., mersacidin), sactibiotics (e.g., subtilisin A, thuricin CD), glycocins (e.g., subclancin 168), and so on. On the other hand, class II (unmodified or cyclic) has been divided into more 5 more subclasses.

Table 2. Recently identified antimicrobial peptides (AMPs) produced by different strains of *Bacillus* species.

Bacillus species	Strain	AMPs	Special features	Source	Reference
B. subtilis	JM4	Subpeptin JM4-A and subpeptin JM4-B	Active against a broad spectrum of bacteria, including *Salmonella*, *Bacillus cereus*, *Staphylococcus aureus*	Soil	Wu et al. 2005
	B11	Two AMPs	Effective biocontrol agent against watermelon wilt and rice sheath blight	Rhizosphere of watermelon	Li et al. 2006
		Bacitracin	Neutral, water soluble, and nontoxic antibiotic that is active against Gram-positive bacteria	Soil	Awais et al. 2007
	fmbj	Surfactic and fengycin	Antagonistic to *B. cereus*		Huang et al. 2007
	B-916	Bacisubin	Antifungal protein with ribonuclease and hemagglutinating activities		Liu et al. 2007
	B29	Antifungal protein B29I	Inhibitory activity on mycelial growth in *Fusarium oxysporum*, *Rhizoctonia solani* and other fungi	Soil	Li et al. 2009
	LFB112	Bacteriocin-like substances	Effective against both Gram-positive and Gram-negative bacteria that are involved in domestic animal diseases	Chinese herbs	Xie et al. 2009
	CMB32	Iturin A, fengycin, and surfactin A	Potential to control the anthracnose disease caused by *Colletotrichum gloeosporioides*	Soil	Kim et al. 2010
	EDR4	Antifungal protein E2	High activity against numerous fungal species	Wheat	Liu et al. 2010
	Subsp. *spizizenii* DSM 15029^T	Entianin	Subtilin-like lantibiotic active against Gram-positive pathogens		Fuchs et al. 2011
	SCK-2	AMP IC-1	Antagonistic to *B. cereus*	Kyeopjang	Yeo et al. 2011
	14B	Bac 14B	Useful for seed disinfection	Bitter almond	Hammami et al. 2012
	NT6	AMPNT-6	Active against marine foodborne pathogen *Vibrio parahaemolyticus*, which is commonly found in shrimp	Natto	Xu et al. 2013
B. licheniformis	H1	Bacteriocin-like substance	Antagonistic activity against various species of Gram-positive but not Gram-negative, bacteria	Cow dung	Abdel-Mohsein et al. 2011
B. thuringiensis	Kurstaki BUPM4	Bacthuricin F4	Possesses unique N-terminal sequence, DWTXWSXL	Soil	Kamoun et al. 2005
	SM1	Fengycin	Antifungal activity against *Candida albicans*	Soil	Roy et al. 2013
	Kurstaki Bn-1	Thuricin Bn1	Active against *B. cereus*, *B. weinhenstephenensis*, *Listeria monocytogenes*, and many other *B. thuringiensis* strains	Pest of hazelnut	Ugras et al. 2013
B. amyloliquefaciens	LBM 5006	Iturin and fengycin	Antagonistic against pathogenic bacteria and phytopathogenic fungi, including *Aspergillus* spp.	Soil	Benitez et al. 2010
	GA1	Amylolysin	Antilisterial activity against *L. monocytogenes*	Strawberry	Halimi et al. 2010
	FZB42	Plantazolicin	Thiazole/oxazole modified microcin (TOMM)		Scholz et al. 2011
		Subtilosin	Active against *L. monocytogenes*		van Kuijk et al. 2011
Paenibacillus polymyxa (formerly *B. polymyxa*)	VLB16	Antifungal protein	Inhibits the growth of *Pyricularia grisea* and *Rhizoctonia solani*	Soil	Kavitha et al. 2005
B. pumilus		Bacitracin	Active against *Micrococcus luteus* and *S. aureus*	Soil	Awais et al. 2007
B. vallismortis	ZZ185	Bacillomycin D	Active against phytopathogens	Stems of plant	Zhao et al. 2010
B. halodurans	C-125	Haloduracin	Broad-spectrum 2-peptide lantibiotic	Soil	Lawton et al. 2007
B. mojavensis	A21	Fengycin, surfactin and pumilacidin	Strong activity against *M. luteus* and *S. aureus*	Marine water	Ayed et al. 2014
B. sonorensis	MT39	Sonorensin	Antimicrobial activity against *L. monocytogenes* and *S. aureus*	Marine soil	Chopra et al. 2014
B. coagulans	ATCC 7050	Lactosporin	Strong activity against *M. luteus* and *L. monocytogenes*	Probiotic dietary supplement	Riazi et al. 2009

Lantibiotics are among the best-characterized AMPs. These small microbial peptide antibiotics possess a variety of unusual amino acid residues, genetic determinants, and biosynthesis mechanisms (Paik et al. 1998; McAuliffe et al. 2001; Abriouel et al. 2011). During maturation, the premature peptides undergo post-translational modifications through the introduction of unusual thioether amino acids, such as lanthionine and methyllanthione, together with the proteolytic removal of leader peptides (Lawton et al. 2007; Dischinger et al. 2009). Mature lantibiotics typically contain one or more unusual dehydro residues that do not participate in lanthionine bridges and may thus be useful components in the design of novel biomolecules (Paik et al. 1998). Arias and colleagues (2013) identified amylolysin, a putative lantibiotic that was isolated from the *B. amyloliquefaciens* GA1 strain. A putative lantibiotic gene cluster containing a structural gene (am/A) and genes responsible for modification (am/M), transport (am/T), regulation (am/KR), and immunity (am/FE) has been identified through genome characterization (Arias et al. 2013).

Subtilosin A, which was isolated from the wild-type strain *B. subtilis* 168, is a ribosomally synthesized and post-translationally modified antimicrobial bacteriocin peptide. Its distinctive structure indicates that subtilosin might belong to an exceptional and unique class of bacteriocins (Kawulka et al. 2003; Shelburne et al. 2007; Sutyak et al. 2008). Unlike most membrane-targeting cationic bacteriocins, this unique macrocyclic bacteriocin has an anionic nature (Huang et al. 2009). Subtilosin is a circular molecule of 35 amino acids with a unique post-translational structure that includes 3 sulfur cross-links between its cysteine residues and the α-carbons of 2 phenylalanines and 1 threonine residue (Kawulka et al. 2003; Sutyak et al. 2008).

Interestingly, an exceptionally different class of AMPs from the *Bacillus* species has also been identified. *Brevibacillus brevis* produces a complex mixture of antibiotic peptides, which has been named tyrothricin. In 1944, tyrothricin was first reported to exert antimicrobial activity similar to that of quinine against the chicken pathogen *Plasmodium gallinaceum*. Tyrothricin contains 9 ribosomally produced linear gramicidins (including gramicidin D) and up to 28 different types of nonribosomally produced tyrocidines and tryptocidins (Rautenbach et al. 2007).

Mechanisms of action

Although several theories have been proposed to explain the molecular processes induced by AMPs, it is currently unclear which, if any, of the hypothesized mechanisms is responsible for their biological activity (Pálffy et al. 2009). Multiple models have been proposed, with the exact mechanism probably dependent on the specific peptide, concentration, and bacterium. Bacteria have also been shown to respond to AMPs and even to evolve resistance to their toxic effects (Scott et al. 2008).

Different species of *Bacillus* produce bacteriocins or bacteriocin-like substances with different modes of action. Tochicin (Paik et al. 1997), lichenin (Pattnaik et al. 2001), thuricin 439 (Ahern et al. 2003), and thuricin S (Chehimi et al. 2010) have all been established to exert a bactericidal effect. The antibiotic activity of cerein 8A is most likely due to vesicularization of the protoplasm, pore formation, and complete disintegration of the cells (Bizani et al. 2005). In general, bacteriocins are cationic peptides that display hydrophobic or amphiphilic properties, and in most cases, the bacterial membrane is the target of their activity (Sirtori et al. 2006). Several models have proposed that the mechanism of action of cationic peptides involves the formation of channels through which ions can pass and (or) the disruption of bacterial cytoplasmic membranes (Huang et al. 2009; Pálffy et al. 2009). Killing of bacteria via the formation of pores in the bacterial membrane requires 3 principal steps: binding to the bacterial membrane, aggregation within the membrane, and formation of channels. Channel formation leads to leakage of internal cell contents and, consequently, cell death. In addition, AMPs must cross the negatively charged outer wall of Gram-negative bacteria, which contains lipopolysaccharides (LPS), or the outer cell wall of Gram-positive bacteria, which contains acidic polysaccharides. In many cases, specific metabolic activities of the target microbes provide critical conditions for pore formation (Pálffy et al. 2009). The lantibiotic amylolysin inhibits cell wall biosynthesis by interacting with lipid II, which is the carrier of peptidoglycan monomers across the cytoplasmic membrane (Arias et al. 2013).

Lipopeptides readily bind to the bacterial surface bilayer and alter the local lipid organizational linkages on negatively charged fatty acids, ultimately restructuring the lipid bilayer and thus preventing cellular processes. The fatty acid moiety of lipopeptides also plays an important role in their antimicrobial activity. Members of the iturin family of lipopeptides (e.g., iturin A, bacillomycin D, and bacillomycin F) contain a β-hydroxy fatty acid with a 14-carbon chain and inhibit different species of fungi but have only narrow antibacterial activity (Baindara et al. 2013). Goafu et al. (2010) reported the antifungal mechanism of action of a new member of the surfactin family, named WH1fungin. This AMP is produced by *B. amyloliquefaciens* WH1 and exhibits 2 types of antifungal action: at a high concentration, it forms pores in the cell membrane, and at a low concentration, it induces apoptosis. This antifungal peptide inhibits glucan synthase, resulting in decreased callose synthesis in the fungal cell wall. Moreover, it has been found that this AMP binds to an ATPase on the mitochondrial membrane and decreases its activity in fungal cells (Goafu et al. 2010). Bacillomycin L, which is produced by the *B. amyloliquefaciens* K103 strain, has shown strong antifungal activity against filamentous fungi, including *Rhizoctonia solani* Kühm. This iturinic lipopeptide is believed to act not only through fungal membrane permeabilization, like other iturins, but also by interacting with other intracellular targets such as fungal DNA (Zhang et al. 2013).

Subtilosin A (subtilosin) is one of several AMPs produced by *B. subtilis* (Babasaki et al. 1985). The mechanism by which this anionic peptide kills bacteria is not yet clear. Like the lantibiotic nisin, subtilosin may exert antimicrobial activity by interacting with membrane-associated receptors (Widemann et al. 2001). Moreover, nuclear magnetic resonance and fluorescence experiments on model membranes suggest that this AMP is able to kill bacteria by binding to the outer cell membrane and inducing membrane permeabilization. However, this mechanism is unlike that used by most cationic AMPs, which usually interact directly with the cell membrane and cause membrane disruption, thereby leading to bacterial cell death (Shelburne et al. 2007). The molecular mechanism of action of subtilosin against *Listeria monocytogenes* is yet to be elucidated. However, the mode of action of subtilosin against the vaginal pathogen *Gardnerella vaginalis* has been identified. Noll et al. (2011) reported that subtilosin kills target cells by depleting the transmembrane pH gradient (ΔpH) portion of the proton motive force and causing an efflux of intracellular ATP (Noll et al. 2011; van Kuijk et al. 2011). Thuricin CD, a post-translationally modified AMP isolated from the *B. thuringiensis* DPC 6431 strain, has shown activity against *Clostridium difficile* associated diarrhea in the nanomolar range. Thuricin CD is a 2-component AMP harboring sulfur to α-carbon linkages. These 2 distinct peptides, Trn-α and Trn-β, have been shown to act synergistically to kill clinical isolates of *C. difficile* (Rea et al. 2010).

Resistance to AMPs by Gram-positive and Gram-negative bacteria

In Gram-positive bacteria, with low G+C content, often resistance modules facilitate an antimicrobial resistance mechanism. For instance, the BceRS–BceAB module of *B. subtilis* is responsible for bacitracin and mersacidin resistance mechanism (Kallenberg et al. 2013). *Bacillus subtilis* is also resistant to lantibiotics (e.g., mesacidin and subtilin) by the defensive mechanism of extracyto-

plasmic function σ factors σX, σW, and σM (Kingstone et al. 2013). Moreover, σW provides resistance to AMPs produced by other *Bacillus* spp. Additionally, it has been reported that both σX and σM factors control the BcrC gene, which is responsible for bacitracin resistance (Butcher and Helmann 2006). On the other hand, Gram-negative bacteria can inhibit natural AMPs in several ways (Gruenheid and Moual 2012). For example, firstly, these bacteria can inactivate AMPs by proteolytic degradation. Bacterial proteases secreted or localized at the outer membrane of Gram-negative bacteria can degrade active AMPs into inactive forms. Secondly, resistance to AMPs can be attributable to different types of polysaccharides present in the bacterial cell envelop, such as capsule polysaccharide, biofilm-forming exopolysaccharides, and the O-polysaccharide of LPS. To prevent the AMPs from reaching the bacterial membrane, these polysaccharides bind with the AMPs. Thirdly, LPS play an important role in the resistance to AMPs by modification of outer membrane of Gram-negative bacteria. And finally, resistance is also possible by the pumping out or in of AMPs in the cell through the members of ABC transporters and the resistance nodulation-division efflux pump families.

Recently identified AMPs produced by the *Bacillus* species

The soil organism *B. subtilis* is extraordinary among the genus *Bacillus* because it produces so many different potential antibiotics (Stein 2005). Additionally, *B. subtilis* produces a series of peptide antibiotics that include members of both classes: ribosomally synthesized subtilins (e.g., subtilin, ericin A and S, mersacidin, sublancin 168, bacillocin 22, and subtilosin A) (Paik et al. 1998; Zheng and Slavik 1999; Stein 2005; Lawton et al. 2007; Shelburne et al. 2007; Xie et al. 2009) and several kinds of nonribosomally synthesized small antibiotic peptides (<2000 Da) that show antibacterial and antifungal activities (e.g., iturin and lipopeptides such as surfactin, fengycin, mycosubtilin, and mycobacillin) (Peypoux et al. 1999; Marx et al. 2001; Li et al. 2009). We identified an AMP produced by *B. subtilis* SC-8 (BSSC8), which we termed BSAP-254. This AMP was isolated from the traditionally fermented soybean paste Cheonggukjang. Unlike the major surfactin, fengycin, and iturin-like compounds (*m*/*z* 1016 to 1515; 4 to 9 amino acids), this putative lipopeptide-like antagonist is 3.4 kDa in size and contains 14 amino acids (Cys, Asn or Asp, Gln or Glu, Ser, Gly, Arg, Thr, Ala, Pro, Val, Ile, Leu, Trp, and Lys) with likely 36 amino acids and various lipid moieties (Lee et al. 2010, 2011; Yeo et al. 2011). BSAP-254 exhibits narrow-spectrum activity and shows an adequate antagonistic effect against the foodborne pathogen *B. cereus* and other related pathogens, such as *Bacillus anthracis* and *B. thuringiensis* (Yeo et al. 2012). Moreover, we mapped the genome sequence of BSSC8 (http://www.ncbi.nlm.nih.gov/nuccore/AGFW00000000) using the CLC genomics Workbench 4.0 program (CLC Bio, Cambridge, Massachusetts, USA) (Yeo et al. 2012). Another strain, *B. subtilis* subsp. *spizizenii* DSM 15029^T, has been shown to produce a novel unsuccinylated entianin AMP, which differs from subtilin at 3 amino acid positions. This subtilin-like AMP exhibits strong antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and other Gram-positive pathogens (Fuchs et al. 2011). Three surfactin-producing *B. subtilis* strains, C4, M1, and G2III, which were isolated from the honey and intestines of the *Apis mellifera* L. bee, have been shown to exhibit antagonistic activity against 6 different bacteriocin-resistant *L. monocytogenes* strains. Surfactin produced by the *B. subtilis* subsp. *subtilis* C4, G2III, and M1 strains inhibited these pathogens at concentrations of 0.125, 0.25, and 1 mg/mL, respectively (Sabaté and Audisio 2013).

The aerobic Gram-positive spore-forming bacterium *B. cereus* is widely distributed throughout the environment and can be easily isolated from a variety of foods, including dairy products, meats, spices, and cereals. Several AMPs, including cerein 7, cerein 8A, cerein 8B, and cerecidin, are produced by different strains of *B. cereus* (Naclerio et al. 1993; Oscáriz et al. 1999; Abriouel et al. 2011; Wang et al. 2014*a*). Two bacteriocins (cerein 7A and 7B) have been shown to be produced by the *B. cereus* Bc7 strain, which was isolated from a soil sample. Moreover, cerein 7B has been characterized on the molecular level. Mature cerein 7B contains 56 residues (Oscáriz et al. 2006), giving this peptide a molecular mass of 4.8 kDa. Cerein 7B is a small, slightly cationic (net charge of 0.77 at pH 7.0), and very hydrophobic peptide (42.8% apolar residues). No sequence similarity was found between mature cerein 7B and any of the other bacteriocins that are known to be exported via a sec-independent pathway. However, cerein 7B shares some features with enterocin B, carnobacteriocin A, and divergicin A, which belong to the class IId bacteriocins as outlined by Ennahar (Ennahar et al. 2000; Oscáriz et al. 2006). Recently, Wang and colleagues identified the cerecidins, which are a novel class of lantibiotics that are produced by *B. cereus*. These antibiotics possess the *cre* locus, which harbors 7 structural genes and is quite different from those in other class II lantibiotics (Wang et al. 2014*b*).

Bacillus licheniformis is a saprophytic bacterium that is widespread throughout nature. Bacitracin, the first peptide antibiotic derived from cultures of *B. licheniformis*, is widely applied in the medical and veterinary fields and exhibits an excellent safety profile (He et al. 2006). The *B. licheniformis* DSM 13 strain produces an antimicrobial substance that resembles the 2-peptide lantibiotic lichenicidin and also exhibits homology to mersacidin. This AMP shows activity against a wide range of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* strains, but does not cause hemolysis or inhibit the growth of Gram-negative bacteria (Dischinger et al. 2009). Licheniocin 50.2 was isolated from the *B. licheniformis* VPS50.2 strain by Berić and colleagues (2013). This AMP was classified in the bacteriocin subclass II.3 and is effective against *L. monocytogenes*, methicillin-resistant *S. aureus* (MRSA), and β-hemolytic streptococci. Time course analyses of bacterial death have also shown that licheniocin exerts a bactericidal effect against *L. monocytogenes*. The sequence of the N-terminal 15 amino acids of this 3.25 kDa bacteriocin is WEEYNIIXQLGNKGQ (Berić et al. 2013).

Antibiotic, antifungal, and anticancer activities have also been described for bacteriocins produced by *B. thuringiensis* strains (Cherif et al. 2003). A number of *B. thuringiensis* bacteriocins have been partially characterized, such as thuricin, which is produced by *B. thuringiensis* subsp. *thuringiensis* HD2; thuricin B439, which is produced by *B. thuringiensis* B439; chicin, which is produced by *B. thuringiensis* subsp. *tochigiensis* HD868; and bacthuricin F4, which was shown to be produced by a local isolate of *B. thuringiensis* (Paik et al. 1997; Ahern et al. 2003; Kamoun et al. 2005; Cherif et al. 2008). In 2014, Pacheco-Cano and colleagues purified and characterized Tolworthcin 524, which is a bacteriocin synthesized by *B. thuringiensis* subsp. *tolworthi* (Pacheco-Cano et al. 2014). The purified Tolworthcin 524 product included 2 peptides of approximately 9 and 6 kDa. The partial sequences of these peptides (METPVVQPR and DWTCWSCLVCAACS) suggest that they are prebacteriocin and mature Tolworthcin 524, respectively. Sequence analysis revealed that this AMP has high identity with Thuricin H and thuricin 17, thus, it has been classified in subclass II.2 (thuricin-like peptides) of the *Bacillus* bacteriocins.

Many strains of *B. amyloliquefaciens* are known to suppress fungal and bacterial growth in vitro by producing multiple antimicrobial compounds (Caldeira et al. 2008; Sutyak et al. 2008; Benitez et al. 2010). Recently, Torres and colleagues (2013) isolated subtilosin from the *B. amyloliquefaciens* KATMIRA1933 strain and were the first to report the antiviral activity of this peptide. The cell-free supernatants of overnight cultures from *B. amyloliquefaciens* derived from dairy products were active against *L. monocytogenes* (Torres et al. 2013). Moreover, the N3 lipopeptide, which is produced by the *B. amyloliquefaciens* M1 strain, exhibits broad-spectrum antibacterial activity, including activity against multidrug-resistant *Vibrio* species, particularly *Vibrio anguillarum*, and *Shewanella aquimarina*, which was isolated from diseased ma-

rine animals. This lipopeptide contains surfactin isoforms with a common amino acid sequence (GLLVDLL) and hydroxy fatty acids that are 12–15 carbons in length. Treatment with the lipopeptide N3 was also shown to result in damage to the cell membrane of *V. anguillarum* and disruption of the entire bacterial cell (Xu et al. 2014).

Bacillus mojavensis A21, which was isolated from marine water in Tunisia, produces the nonribosomally synthesized lipopeptide biosurfactants surfactin, fengycin, and pumilacidin. The mixture of these lipopeptides has been shown to exhibit significant activity against Gram-positive bacteria, Gram-negative bacteria, and even fungal strains (Ayed et al. 2014). Chopra and colleagues (2014) identified a novel AMP that they termed sonorensin. This AMP is produced by *Bacillus sonorensis* MT93, a new marine soil isolate. Sonorensin belongs to the heterocyclonantracin subfamily of bacteriocins and has a molecular mass of 6.27 kDa. Moreover, this AMP exhibits antimicrobial activity against common foodborne pathogens such as *L. monocytogenes* and *S. aureus*. Furthermore, the ethyl acetate extracts of the cell-free supernatants of 2 *Bacillus* species, SS-12.6 and SS-13.1, have been shown to exhibit strong antifungal and antibacterial activities against phytopathogens in experiments with apple plants. These strains have been proposed to harbor genes for the biosynthesis of the lipopeptides iturin, surfactin, and bacillomycin (Dimkić et al. 2013).

Potential applications and beneficial roles of AMPs of the *Bacillus* species

The *Bacillus* species are industrially important for a variety of reasons, including their excellent safety record; their rapid growth rates, which result in short fermentation cycles; and their high capacity for protein secretion into the extracellular medium (Schallmey et al. 2004; Benitez et al. 2010). *Bacillus*-derived peptides have shown antibacterial, antifungal, antiviral, antitumor, antiamebocytic, and antimycoplasmic activities (Yilmaz et al. 2006; Chen et al. 2008). Several *Bacillus* species, such as *B. subtilis*, *B. clausii*, *B. cereus*, *B. coagulans*, and *B. licheniformis*, have been used as probiotic supplements in both animals and humans (Cutting 2011).

Several peptide antibiotics that are of pharmaceutical importance, such as bacitracin, polymyxin, gramicidin, tyrocidine, subtilin, and bacilysin, are produced by *Bacillus* species (Awais et al. 2007). One of the most important polypeptides is bacitracin, which effectively inhibits the growth of *Streptococcus pyogenes* and *Staphylococcus aureus*. Clinically, bacitracin has been used in combination with other antimicrobial agents (Haddar et al. 2007). In 1980, it was reported that oral administration of 25 000 units of bacitracin 4 times daily for 7–10 days was successful in the treatment of antibiotic-associated colitis and diarrhea caused by *C. difficile* (Chang et al. 1980). Additionally, the cyclic and anionic AMP subtilosin, which is produced by both *B. subtilis* and *B. amyloliquefaciens*, has been demonstrated to exert antimicrobial activity against the bacterial vaginosis-associated pathogens *Gardnerella vaginalis*, *L. monocytogenes*, and other human pathogens (Sutyak et al. 2008; van Kuijk et al. 2011). Furthermore, *B. anthracis* has shown activity against anthrax (Cherif et al. 2003). Polymyxin B and polymyxin E (also known as colistin), isolated from *Bacillus polymyxa*, are a lipopeptide antibiotic. Both of them are rapid bactericidal AMPs with detergent-like mechanism. Polymyxins are not active against Gram-positive bacteria and anaerobes but have strong activity against Gram-negative bacteria including *Enterobacteriaceae* and nonfermentative species (Zavascki et al. 2007). Because of serious side effects, now it is used as an ointment for local surface wounds (Choi et al. 2009).

Bioengineered bacteriocins have been proposed as a promising alternative to existing antibiotics because of their effectiveness and nontoxicity in animals and humans, the availability of both broad-spectrum and narrow-spectrum peptides, and the possibility of in situ production by probiotics (Xie et al. 2009; Cotter et al. 2013). Moreover, bacteriocins are also rapidly digested by proteases in the human digestive tract (Chen and Hoover 2003). The emergence and dissemination of antibiotic-resistant pathogenic bacteria, such as MRSA and vancomycin-resistant *E. coli* (VRE), has become an increasingly serious problem for public health worldwide (Cetinkaya et al. 2000). Thus, new strategies for controlling these bacteria are urgently needed. A search for new bacteriocin-producing bacteria with anti-MRSA activity identified *B. pumilus* WAPB4. This strain produces a novel bacteriocin, termed pumilicin 4, which inhibits several Gram-positive bacteria, including MRSA and VRE (Aunpad and Na-Bangchang 2007; Abriouel et al. 2011). Another emerging pathogen is *L. monocytogenes*, which particularly affects pregnant women, children, elderly people, and patients with AIDS. Fortunately, *Bacillus* species bacteria have been reported to inhibit and control this microorganism (Sabaté and Audisio 2013).

The increasing trend of limiting the use of chemical food preservatives has generated considerable interest in the use of natural alternatives. Recently, antimicrobial lipopeptide microcapsules prepared from the spray drying of the *B. amyloliquefaciens* ES-2 strain were tested as food additives (Wang et al. 2014*c*). Subtilosin is also an attractive food preservative alternative to nisin because of its efficacy against *L. monocytogenes* and other foodborne pathogens (Jung et al. 2008; Sutyak et al. 2008). Amylolysin, a novel bacteriocin produced by the *B. amyloliquefaciens* GA1 strain, has been reported to exhibit activity against *L. monocytogenes* strains, which are responsible for food-related listeriosis. At concentrations of 5–10 μg/g, this bacteriocin has been shown to inhibit the proliferation of different *L. monocytogenes* isolates in poultry meat. Thus, this bacteriocin can be used as a food preservative in poultry meat (Halimi et al. 2010).

Lipopeptides such as fengycin and iturins have antifungal activity; moreover, surfactin has exceptional surfactant activity and emulsification properties, indicating that these AMPs have potential applications in bioremediation. The surfactin lipopeptide has also demonstrated potential as an antitumor, antiviral, antibacterial, and hypocholesterolemic agent (Guo et al. 2014; Schallmey et al. 2004). Several *Bacillus*-species-derived AMPs can be used in the agricultural sector to inhibit plant pathogens and preserve grain. Moreover, the *B. subtilis* species is widely used in the biocontrol of plant diseases. Recently, Guo and colleagues (2014) discovered that the *B. subtilis* NCD-2 strain secretes fengycin-type lipopeptides that exhibited antifungal activity against *Rhizoctonia solani*, which is the causative agent of cotton damping-off disease.

Current and future perspectives

In the effort to overcome the increasing threat of infectious bacteria to humans, natural products from microbial sources appear to be the most favorable alternative to current antibiotics. In this respect, AMPs from the *Bacillus* species are ideal therapeutic tools because of their broad specificity and specific and rapid killing activity against various pathogens. However, to conclusively establish the safe and effective use of *Bacillus*-derived AMPs such as bacteriocins, lantibiotics, lipopeptides, and biosurfactants in medical settings, further studies on their modes of action, toxicities, and immunogenicities need to be carried out in humans.

During the last 20 years, bacteriocins of lactic acid bacteria (LAB) have attracted more attention than *Bacillus*-derived AMPs. This may be because the LAB AMPs appear to be nontoxic in a variety of hosts, and some of these AMPs have exhibited strong therapeutic activity against pathogenic organisms. Furthermore, these AMPs have traditionally been developed as intravenously administered antibiotics. However, studies on *Bacillus*-derived bacteriocins and bacteriocin-like substances have already yielded promising results in the field of human health. Researchers are currently aiming to identify the mechanisms of antimicrobial ac-

tivity of these AMPs in model membrane systems and to evaluate the cytotoxicity of these agents against eukaryotic cells and erythrocytes.

Drug discovery from natural products presents a major challenge. Thus, it will be difficult to accomplish this goal without extensive efforts from pharmaceutical and biotechnological industries. Inappropriate funding, strict laws and regulations for promoting new drugs, and societal reservations regarding the use of novel antimicrobial agents are the most common reasons for the pharmaceutical industry to be reluctant to become involved. As a result, naturally occurring AMPs are generally assumed to be unsuitable for pharmaceutical development. Few detailed investigations have been performed of *Bacillus*-derived AMPs; moreover, most AMPs tested did not succeed in clinical trials, and no AMP has yet received FDA approval for clinical use.

Despite these challenges to the development of *Bacillus*-derived AMPs as alternatives for conventional antibiotics, the emergence of drug-resistant bacteria has necessitated a constant search for new AMPs. More studies focusing on AMPs are urgently required to bring these potential antibiotics into practical use and to fully realize their commercial and biotechnological applications. We are optimistic that the coming years will see comprehensive research efforts into the chemical properties, biological mechanisms of action, and biosynthesis of these *Bacillus*-derived AMPs. These studies will ultimately lead to preclinical studies and clinical trials of their safety and efficacy in humans, in addition to patent applications for novel AMPs. Microbial peptides have the potential to combat the escalating problem of single-drug- and multi-drug-resistant infectious pathogens in the foreseeable future and may constitute a new generation of antibiotics.

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References

Abdel-Mohsein, H., Sasaki, T., Tada, C., and Nakai, Y. 2011. Characterization and partial purification of a bacteriocin-like substance produced by thermophilic *Bacillus licheniformis* H1 isolated from cow manure compost. Anim. Sci. J. **82**(2): 340–351. doi:10.1111/j.1740-0929.2010.00835.x. PMID:21729216.

Abriouel, H., Franz, C.M., Omar, N.B., and Gálvez, A. 2011. Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiol. Rev. **35**(1): 201–232. doi:10.1111/j.1574-6976.2010.00244.x. PMID:20695901.

Ahern, M., Verschueren, S., and van Sinderen, D. 2003. Isolation and characterisation of a novel bacteriocin produced by *Bacillus thuringiensis* strain B439. FEMS Microbiol. Lett. **220**(1): 127–131. doi:10.1016/S0378-1097(03)00086-7. PMID:12644238.

Alba, A., López-Abarrategui, C., and Otero-González, A.J. 2012. Host defense peptides: an alternatives as antiinfective and immunomodulatory therapeutics. Pept. Sci. **98**(4): 251–267. doi:10.1002/bip.22076. PMID:23193590.

Arias, A.A., Ongena, M., Devreese, B., Terrak, M., Joris, B., and Fickers, P. 2013. Characterization of amylolysin, a novel lantibiotic from *Bacillus amyloliquefaciens* GA1. PLoS One, **8**: e83037. doi:10.1371/journal.pone.0083037. PMID:24349428.

Aunpad, R., and Na-Bangchang, K. 2007. Pumilicin 4, a novel bacteriocin with anti-MRSA and anti-VRE activity produced by newly isolated bacteria *Bacillus pumilus* strain WAPB4. Curr. Microbiol. **55**(4): 308–313. doi:10.1007/s00284-006-0632-2. PMID:17700984.

Awais, M., Shah, A.A., Hameed, A., and Hasan, F. 2007. Isolation, identification and optimization of bacitracin produced by *Bacillus* sp. Pak. J. Bot. **39**(4): 1303–1312. Available from http://www.pakbs.org/pjbot/PDFs/39(4)/PJB39(4)1303.pdf.

Ayed, H.B., Hmidet, N., Béchet, M., Chollet, M., Chataigné, G., Leclére, V., et al. 2014. Identification and biochemical characteristics of lipopeptides from *Bacillus mojavensis* A21. Process Biochem. **49**(10): 1699–1707. doi:10.1016/j.procbio.2014.07.001.

Babasaki, K., Takao, T., Shimonishi, Y., and Kurahashi, K. 1985. Subtilosin A, a new antibiotic peptide produced by *Bacillus subtilis* 168: isolation, structural analysis, and biogenesis. J. Biochem. (Tokyo), **98**(3): 585–603. PMID:3936839.

Baindara, P., Mandal, S.M., Chawla, N., Singh, P.K., Pinnaka, A.K., and Korpole, S. 2013. Characterization of two antimicrobial peptides produced by a halotolerant *Bacillus subtilis* strain SK.DU.4 isolated from a rhizosphere soil sample. AMB Express, **3**(1): 2. doi:10.1186/2191-0855-3-2. PMID:23289832.

Benitez, L.B., Velho, R.V., Lisboa, M.P., Medina, L.F., and Brandelli, A. 2010. Isolation and characterization of antifungal peptides produced by *Bacillus amyloliquefaciens* LBM5006. J. Microbiol. **48**(6): 791–797. doi:10.1007/s12275-010-0164-0. PMID:21221936.

Berić, T., Stanković, S., Draganić, M., Kojić, M., Lozo, J., and Fira, D. 2013. Novel antilisterial bacteriocin licheniocin 50.2 from *Bacillus licheniformis* VPS50.2 isolated from soil sample. J. Appl. Microbiol. **116**(3): 502–510. doi:10.1111/jam.12393. PMID:24238327.

Bizani, D., Motta, A.S., Morrissy, J.A., Terra, R.M., Souto, A.A., and Brandeii, A. 2005. Antibacterial activity of cerein 8A, a bacteriocin-like peptide produced by *Bacillus cereus*. Int. Microbiol. **8**(2): 125–131. PMID:16052461.

Brogden, N.K., and Brogden, K.A. 2011. Will new generations of modified antimicrobial peptides improve their potential as pharmaceuticals? Int. J. Antimicrob. Agents, **38**(3): 217–225. doi:10.1016/j.ijantimicag.2011.05.004. PMID:21733662.

Brötz, H., Bierbaum, G., Markus, A., Molitor, E., and Sahl, H.G. 1995. Mode of action of the lantibiotic mersacidin: Inhibition of peptidoglycan biosynthesis via a novel mechanism? Antimicrob. Agents Chemother. **39**(3): 714–719. doi:10.1128/AAC.39.3.714. PMID:7793878.

Brown, E.D. 2006. Antibiotic stops 'ping-pong' match. Nature, **441**(7091): 293–294. doi:10.1038/441293a. PMID:16710404.

Butcher, B.G., and Helmann, J.D. 2006. Identification of *Bacillus subtilis* σ^{W}-dependent genes that provide intrinsic resistance to antimicrobial compounds produced by *Bacilli*. Mol. Microbiol. **60**(3): 765–782. doi:10.1111/j.1365-2958.2006.05131.x. PMID:16629676.

Caldeira, A.T., Feio, S.S., Arteiro, J.M., Coelho, A.V., and Roseiro, J.C. 2008. Environmental dynamics of *Bacillus amyloliquefaciens* CCMI 1051 antifungal activity under different nitrogen patterns. J. Appl. Microbiol. **104**(3): 808–816. doi:10.1111/j.1365-2672.2007.03601.x. PMID:17953685.

Cetinkaya, Y., Falk, P., and Mayhall, C.G. 2000. Vancomycin-resistant enterococci. Clin. Microbiol. Rev. **13**(4): 686–707. doi:10.1128/CMR.13.4.686-707.2000. PMID:11023964.

Chang, T.W., Gorbach, S.L., Bartlett, J.G., and Saginur, R. 1980. Bacitracin treatment of antibiotic-associated colitis and diarrhea caused by *Clostridium difficile* toxin. Gastroenterology, **78**(6): 1584–1586. PMID:7372074.

Chatterjee, S., Chatterjee, S., Lad, S.J., Phansalkar, M.S., Rupp, R.H., Ganguli, B.N., et al. 1992. Mersacidin, a new antibiotic from *Bacillus*. Fermentation, isolation, purification and chemical characterization. J. Antibiot. (Tokyo), **45**(6): 832–838. doi:10.7164/antibiotics.45.832. PMID:1500347.

Chehimi, S., Pons, A.M., Sablé, S., Hajlaoui, M.R., and Limam, F. 2010. Mode of action of thuricin S, a new class IId bacteriocin from *Bacillus thuringiensis*. Can. J. Microbiol. **56**(2): 162–167. doi:10.1139/W09-125. PMID:20237578.

Chen, H., and Hoover, D.G. 2003. Bacteriocins and their food application. Compr. Rev. Food Sci. Food Safe. **2**(3): 82–100. doi:10.1111/j.1541-4337.2003.tb00016.x.

Chen, H., Wang, L., Su, C.X., Gong, G.H., Wang, P., and Yu, Z.L. 2008. Isolation and characterization of lipopeptide antibiotics produced by *Bacillus subtilis*. Lett. Appl. Microbiol. **47**(3): 180–186. doi:10.1111/j.1472-765X.2008.02412.x. PMID:19552782.

Cherif, A., Chehimi, S., Limem, F., Hansen, B.M., Hendriksen, N.B., Daffonchio, D., and Boudabous, A. 2003. Detection and characterization of the novel bacteriocin entomocin 9, and safety evaluation of its producer, *Bacillus thuringiensis* ssp. *entomocidus* HD9. J. Appl. Microbiol. **95**(5): 990–1000. doi:10.1046/j.1365-2672.2003.02089.x. PMID:14633027.

Cherif, A., Rezgui, W., Raddadi, N., Daffonchio, D., and Boudabous, A. 2008. Characterization and partial purification of entomocin110, a newly identified bacteriocin from *Bacillus thuringiensis* subsp. *entomocidus* HD110. Microbiol. Res. **163**(6): 684–692. doi:10.1016/j.micres.2006.10.005. PMID:19216106.

Choi, S.K., Park, S.Y., Kim, R., Kim, S.B., Lee, C.H., Kim, J.F., and Park, S.H. 2009. Identification of a polymyxin sythetase gene cluster of *Paenibacillus polymyxa* and heterologous expression of the gene in *Bacillus subtilis*. J. Bacteriol. **191**(10): 3350–3358. doi:10.1128/JB.01728-08. PMID:19304848.

Chopra, L., Singh, G., Choudhury, V., and Shaoo, D.K. 2014. Sonorensin: an antimicrobial peptide, belonging to the heterocycloanthracin subfamily of bacteriocins, from a new marine isolate, *Bacillus sonorensis* MT93. Appl. Environ. Microbiol. **80**(10): 2981–2990. doi:10.1128/AEM.04259-13. PMID:24610839.

Cladera-Olivera, F., Caron, G.R., and Brandelli, A. 2004. Bacteriocin-like substance production by *Bacillus licheniformis* strain P40. Lett. Appl. Microbiol. **38**(4): 251–256. doi:10.1111/j.1472-765X.2004.01478.x. PMID:15214721.

Clardy, J., Fischbach, M.A., and Walsh, C.T. 2006. New antibiotics from bacterial natural products. Nat. Biotechnol. **24**(12): 1541–1550. doi:10.1038/nbt1266. PMID:17160060.

Coates, A., Hu, Y., Bax, R., and Page, C. 2002. The future challenges facing the development of new antimicrobial drugs. Nat. Drug Discov. Rev. **1**(11): 895–910. doi:10.1038/nrd940. PMID:12415249.

Cotter, P.D., Ross, R.P., and Hill, C. 2013. Bacteriocins — A viable alternative to antibiotics? Nat. Rev. Microbiol. **11**(2): 95–105. doi:10.1038/nrmicro2937. PMID:23268227.

Cutting, S.M. 2011. *Bacillus* probiotics. Food Microbiol. **28**(2): 214–220. doi:10.1016/j.fm.2010.03.007. PMID:21315976.

Dimkić, I., Žicković, S., Berić, T., Ivanović, Ž., Gavrilović, V., Stanković, S., and

Fira, D. 2013. Characterization and evaluation of two *Bacillus* strains, SS-12.6 and SS-13.1, as potential agents for the control of phytopathogenic bacteria and fungi. Biol. Control, **65**(3): 312–331. doi:10.1016/j.biocontrol.2013.03.012.

Dischinger, J., Josten, M., Szekat, C., Sahl, H.G., and Bierbaum, G. 2009. Production of the novel two-peptide lantibiotic lichenicidin by *Bacillus licheniformis* DSM 13. PLoS One, **4**(8): e6788. doi:10.1371/journal.pone.0006788. PMID:19707558.

Donadio, S., Maffioli, S., Monciardini, P., Sosio, M., and Jabes, D. 2010. Antibiotic discovery in the twenty-first century: current trends and future perspectives. J. Antibiot. (Tokyo), **63**(8): 423–430. doi:10.1038/ja.2010.62. PMID:20551985.

Ennahar, S., Sashihara, T., Sonomoto, K., and Ishizaki, A. 2000. Class IIa bacteriocins: biosynthesis, structure and activity. FEMS Microbiol. Rev. **24**(1): 85–106. doi:10.1111/j.1574-6976.2000.tb00534.x.

Fuchs, S.W., Jaskolla, T.W., Bochmann, S., Kötter, P., Wichelhaus, T., Karas, M., et al. 2011. Entianin, a novel subtilin-like lantibiotic from *Bacillus subtilis* subsp. *spizizenii* DSM 15029^{T} with high antimicrobial activity. Appl. Environ. Microbiol. **77**(5): 1698–1707. doi:10.1128/AEM.01962-10. PMID:21239550.

Goafu, Q., Fayin, Z., Peng, D., Xiufen, Y., Dewen, Q., Ziniu, Y., et al. 2010. Lipopeptide induces apoptosis in fungal cells by a mitochondria-dependent pathway. Peptides, **31**(11): 1978–1986. doi:10.1016/j.peptides.2010.08.003. PMID:20713103.

Gold, H.S., and Moellering, R.C. 1996. Antimicrobial-drug resistance. N. Engl. J. Med. **335**: 1445–1453. doi:10.1056/NEJM199611073351907. PMID:8875923.

Gruenheid, S., and Moual, H.L. 2012. Resistance to antimicrobial peptide in Gram-negative bacteria. FEMS Microbiol Lett. **330**: 81–89. doi:10.1111/j.1574-6968.2012.02528.x. PMID:22339775.

Guo, Q., Dong, W., Li, S., Lu, X., Wang, P., Zhang, X., et al. 2014. Fengycin produced by *Bacillus subtilis* NCD-2 plays a major role in biocontrol of cotton seedling damping-off disease. Microbiol. Res. **169**(7–8): 533–540. doi:10.1016/j.micres.2013.12.001. PMID:24380713.

Haddar, H.O., Aziz, G.M., and Al-Gelawi, M.H. 2007. Optimization of bacitracin production by *Bacillus licheniformis* B5. Pak. J. Biol. Sci. **10**(6): 972–976. doi:10.3923/pjbs.2007.972.976. PMID:19069901.

Halimi, B., Dortu, C., Arguelles-Arias, A., Thonart, P., Joris, B., and Fickers, P. 2010. Antilisterial activity on poultry meat of amylolysin, a bacteriocin from *Bacillus amyloliquefaciens* GA1. Probiotics Antimicrob. Proteins, **2**(2): 120–125. doi:10.1007/s12602-010-9040-9.

Hamad, B. 2010. The antibiotics market. Nat. Drug Discov. Rev. **9**(9): 675–676. doi:10.1038/nrd3267. PMID:20811374.

Hammami, I., Jaouadi, B., Bacha, A.B., Rebai, A., Bejar, S., Nesme, X., and Rhouma, A. 2012. *Bacillus subtilis* bacteriocin Bac 14B with a broad inhibitory spectrum: purification, amino acid sequence analysis, and physicochemical characterization. Biotechnol. Bioprocess Eng. **17**(1): 41–49. doi:10.1007/s12257-010-0401-8.

Hancock, R.E.W., and Chapple, D.S. 1999. Peptide antibiotics. Antimicrob. Agents Chemother. **43**(6): 1317–1323. Available from http://aac.asm.org/content/43/6/1317.short. PMID:10348745.

Hassan, M., Kjos, M., Nes, I.F., Diep, D.B., and Lotfipour, F. 2012. Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. J. Appl. Microbiol. **113**(4): 723–736. doi:10.1111/j.1365-2672.2012.05338.x. PMID:22583565.

He, L., Chen, W., and Liu, Y. 2006. Production and partial characterization of bacteriocin-like peptides by *Bacillus licheniformis* ZJU12. Microbiol. Res. **161**(4): 321–326. doi:10.1016/j.micres.2005.12.002. PMID:16427261.

Huang, T., Geng, H., Miyyapuram, V.R., Sit, C.S., Vederas, J.C., and Nakano, M.M. 2009. Isolation of a variant of subtilosin A with hemolytic activity. J. Bacteriol. **191**(18): 5690–5696. doi:10.1128/JB.00541-09. PMID:19633086.

Huang, X., Lu, Z., Bie, X., Lü, F., Zhao, H., and Yang, S. 2007. Optimization of inactivation of endospores of *Bacillus cereus* by antimicrobial lipopeptides from *Bacillus subtilis* fmbj strains using a response surface method. Appl. Microbiol. Biotechnol. **74**(2): 454–461. doi:10.1007/s00253-006-0674-1. PMID:17043814.

Jenssen, H., Hamill, P., and Hancock, R.E. 2006. Peptide antimicrobial agents. Clin. Microbiol. Rev. **19**(3): 491–511. doi:10.1128/CMR.00056-05. PMID:16847082.

Jung, W.J., Mabood, F., Souleimanov, A., Zhou, X., Jaoua, S., Kamoun, F., and Smith, D.L. 2008. Stability and antibacterial activity of bacteriocins produced by *Bacillus thuringiensis* and *Bacillus thuringiensis* ssp. *kurstaki*. J. Microbiol. Biotechnol. **18**(11): 1836–1840. doi:10.4014/jmb.0800.120. PMID:19047829.

Kallenberg, F., Dintner, S., Schmitz, R., and Gebhard, S. 2013. Identification of regions important for resistance and signaling within the antimicrobial peptide transporter BceAB of *Bacillus subtilis*. J. Bacteriol. **195**(14): 3287–3297. doi:10.1128/JB.00419-13. PMID:23687272.

Kamoun, F., Mejdoub, H., Aouissaoui, H., Reinbolt, J., Hammami, A., and Jaoua, S. 2005. Purification, amino acid sequence and characterization of Bacthuricin F4, a new bacteriocin produced by *Bacillus thuringiensis*. J. Appl. Microbiol. **98**(4): 881–888. doi:10.1111/j.1365-2672.2004.02513.x. PMID:15752334.

Kavitha, S., Senthilkumar, S., Gnanamanickam, S., Inayathullah, M., and Jayakumar, R. 2005. Isolation and partial characterization of antifungal protein from *Bacillus polymyxa* strain VLB16. Process Biochem. **40**(10): 3236–3243. doi:10.1016/j.procbio.2005.03.060.

Kawulka, K., Sprules, T., McKay, R.T., Mercier, P., Diaper, C.M., Zuber, P., and Vederas, J.C. 2003. Structure of Subtilosin A, an antimicrobial peptide from *Bacillus subtilis* with unusual posttranslational modifications linking cysteine sulfurs to α-carbons of phenylalanine and threonine. J. Am. Chem. Soc. **125**(16): 4726–4727. doi:10.1021/ja029654t. PMID:12696888.

Kim, K.M., Lee, J.Y., Kim, C.K., and Kang, J.S. 2009. Isolation and characterization of surfactin produced by *Bacillus polyfermenticus* KJS-2. Arch. Pharm. Res. **32**(5): 711–715. doi:10.1007/s12272-009-1509-2. PMID:19471885.

Kim, P.I., Ryu, J., Kim, Y.H., and Chi, Y.T. 2010. Production of biosurfactant lipopeptides iturin A, fengycin, and surfactin A from *Bacillus subtilis* CMB32 for control of *Colletotrichum gloeosporioides*. J. Microbiol. Biotechnol. **20**(1): 138–145. doi:10.4014/jmb.0905.05007. PMID:20134245.

Kingstone, A.W., Liao, X., and Helmann, J.D. 2013. Contributions of the σ^{W}, σ^{M} and σ^{X} regulons to the lantibiotic resistome of *Bacillus subtilis*. Mol. Microbiol. **90**(3): 502–518. doi:10.1111/mmi.12380. PMID:23980836.

Klaenhammer, T.R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev. **12**(1–3): 39–86. doi:10.1016/0168-6445(93)90057-G. PMID:8398217.

Lam, K.S. 2007. New aspects of natural products in drug discovery. Trends Microbiol. **15**(6): 279–289. doi:10.1016/j.tim.2007.04.001. PMID:17433686.

Lawton, E.M., Cotter, P.D., Hill, C., and Ross, R.P. 2007. Identification of a novel two-peptide lantibiotic, Haloduracin, produced by the alkaliphile *Bacillus halodurans* C-125. FEMS Microbiol. Lett. **267**(1): 64–71. doi:10.1111/j.1574-6968.2006.00539.x. PMID:17233677.

Lee, N.K., Yeo, I.C., Park, J.W., Kang, B.S., and Hahm, Y.T. 2010. Isolation and characterization of a novel analyte from *Bacillus subtilis* SC-8 antagonistic to *Bacillus cereus*. J. Biosci. Bioeng. **110**(3): 298–303. doi:10.1016/j.jbiosc.2010.03.002. PMID:20547349.

Lee, N.K., Yeo, I.C., Park, J.W., and Hahm, Y.T. 2011. Growth inhibition and induction of stress protein, GroEL, of *Bacillus cereus* exposed to antibacterial peptide isolated from *Bacillus subtilis* SC-8. Appl. Biochem. Biotechnol. **165**(1): 235–242. doi:10.1007/s12010-011-9246-7. PMID:21544555.

Levy, S.B., and Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. **10**(Suppl. 12): S122–S129. doi:10.1038/nm1145. PMID:15577930.

Li, G., Liu, B., Shang, Y., Yu, Z., and Zhang, R. 2012. Novel activity evaluation and subsequent partial purification of antimicrobial peptides produced by *Bacillus subtilis* LFB112. Ann. Microbiol. **62**(2): 667–674. doi:10.1007/s13213-011-0303-9.

Li, J., Yang, Q., Zhao, L.H., Zhang, S.M., Wang, Y.X., and Zhao, X.Y. 2009. Purification and characterization of a novel antifungal protein from *Bacillus subtilis* strain B29. J. Zhejiang Univ. Sci. B, **10**(4): 264–272. doi:10.1631/jzus.B0820341. PMID:19353744.

Li, Q.Q., Meng, X.Y., Wu, X., Lin, W., Duan, C.J., Feng, J.X., and Tang, J.L. 2006. Purification of two antimicrobial substances produced by *Bacillus subtilis* strain B11 and their properties. Agr. Sci. China, **5**(5): 363–369. doi:10.1016/S1671-2927(06)60062-X.

Liu, B., Huang, L., Buchenauer, H., and Kang, Z. 2010. Isolation and partial characterization of an antifungal protein from the endophytic *Bacillus subtilis* strain EDR4. Pestic. Biochem. Physiol. **98**(2): 305–311. doi:10.1016/j.pestbp.2010.07.001.

Liu, Y., Chen, Z., Hg, T.B., Zhang, J., Zhou, M., Song, F., et al. 2007. Bacisubin, an antifungal protein with ribonuclease and hemagglutinating activities from *Bacillus subtilis* strain B-916. Peptides, **28**(3): 553–559. doi:10.1016/j.peptides.2006.10.009. PMID:17129637.

Marx, R., Stein, T., Entian, K.D., and Glaser, S.J. 2001. Structure of the *Bacillus subtilis* peptide antibiotic subtilosin A determined by 1H-NMR and matrix assisted laser desorption/ionization time-of-flight mass spectrometry. J. Protein Chem. **20**(6): 501–506. doi:10.1023/A:1012562631268. PMID:11760125.

McAuliffe, O., Ross, R.P., and Hill, C. 2001. Lantibiotics: structure, biosynthesis and mode of action. FEMS Microbiol. Rev. **25**(3): 285–308. doi:10.1111/j.1574-6976.2001.tb00579.x. PMID:11348686.

Moshafi, M.S., Forootanfar., H., Ameri, A., Shakibaie, M, Dehghan-Naudeh, G., and Razavi, M. 2011. Antimicrobial activity of *Bacillus* sp. strain FAS1 isolated from soil. Pak. J. Pharm. Sci. **24**(3): 269–275. PMID:21715259.

Motta, A.S., Flores, F.S., Souto, A.A., and Brandelli, A. 2008. Antibacterial activity of a bacteriocin-like substance produced by *Bacillus* sp. P34 that targets the bacterial cell envelope. Antonie Van Leeuwenhoek, **93**(3): 275–284. doi:10.1007/s10482-007-9202-2. PMID:17906937.

Naclerio, G., Ricca, E., Sacco, M., and De Felice, M. 1993. Antimicrobial activity of a newly identified bacteriocin of *Bacillus cereus*. Appl. Environ. Microbiol. **59**(12): 4313–4316. PMID:8285719.

Nakano, M.M., and Zuber, P. 1990. Molecular biology of antibiotic production in *Bacillus*. Crit. Rev. Biotechnol. **10**(3): 223–240. doi:10.3109/07388559009038209. PMID:1702690.

Noll, K.S., Sinko, P.J., and Chikindas, M.L. 2011. Elucidation of the molecular mechanisms of action of the natural antimicrobial peptide subtilosin against the bacterial vaginosis-associated pathogen *Gardnerella vaginalis*. Probiotics Antimicrob. Proteins, **3**(1): 41–47. doi:10.1007/s12602-010-9061-4. PMID:21949544.

Oscáriz, J.C., Lasa, N., and Pisabarro, A.G. 1999. Detection and characterization of cerein 7, a new bacteriocin produced by *Bacillus cereus* with a broad spectrum of activity. FEMS Microbiol. Lett. **178**(2): 337–341. doi:10.1016/S0378-1097(99)00370-5. PMID:10499284.

Oscáriz, J.C., Cintas, L., Holo, H., Lasa, I., Nes, I.F., and Pisabarro, A.G. 2006. Purification and sequencing of cerein 7B, a novel bacteriocin produced by

Bacillus cereus Bc7. FEMS Microbiol. Lett. **254**(1): 108–115. doi:10.1111/j.1574-6968.2005.00009.x. PMID:16451187.

Overbye, K.M., and Barrett, J.F. 2005. Antibiotics: Where did we go wrong? Drug Discov. Today, **10**(1): 45–52. doi:10.1016/S1359-6446(04)03285-4. PMID:15676298.

Pacheco-Cano, R., de la Fuente-Salcido, N.M., Salcedo-Hernández, R., León-Galván, M.F., Bideshi, D.K., Hernández-Guzmán, G., and Barboza-Corona, J.E. 2014. Characterization, N-terminal sequencing and classification of Tolworthcin 524: a bacteriocin produced by *Bacillus thuringiensis* subsp. *tolworthi*. Microbiol. Res. **169**(12): 948–953. doi:10.1016/j.micres.2014.04.005. PMID:24880804.

Paik, H.D., Bae, S.S., Park, S.H., and Pan, J.G. 1997. Identification and partial characterization of tochicin, a bacteriocin produced by *Bacillus thuringiensis* subsp. *tochigiensis*. J. Ind. Microbiol. Biotechnol. **19**(4): 294–298. doi:10.1038/sj.jim.2900462. PMID:9439004.

Paik, S.H., Chakicherla, A., and Hansan, J.H. 1998. Identification and characterization of the structural and transporter genes for, and the chemical and biological properties of, subclancin 168, a novel lantibiotic produced by *Bacillus subtilis* 168. J. Biol. Chem. **273**(36): 23134–23142. doi:10.1074/jbc.273.36.23134. PMID:9722542.

Pálffy, R., Gardlík, R., Behuliak, M., Kadasi, L., Turna, J., and Celec, P. 2009. On the physiology and pathophysiology of antimicrobial peptides. Mol. Med. **15**(1–2): 51–59. doi:10.2119/molmed.2008.00087. PMID:19015736.

Parisien, A., Allain, B., Zhang, J., Mandeville, R., and Lan, C.Q. 2007. Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. J. Appl. Microbiol. **104**(1): 1–13. doi:10.1111/j.1365-2672.2007.03498.x. PMID:18171378.

Pattnaik, P., Kaushik, J.K., Grover, S., and Batish, V.K. 2001. Purification and characterization of a bacteriocin-like compound (Lichenin) produced anaerobically by *Bacillus licheniformis* isolated from water buffalo. J. Appl. Microbiol. **91**(4): 636–645. doi:10.1046/j.1365-2672.2001.01429.x. PMID:11576300.

Peypoux, F., Bonmatin, J.M., and Wallach, J. 1999. Recent trends in the biochemistry of surfactin. Appl. Microbiol. Biotechnol. **51**(5): 553–563. doi:10.1007/s002530051432. PMID:10390813.

Pueyo, M.T., Bolch, C., Jr., Carmona-Ribeiro, A.M., and di Mascio, P. 2009. Lipopeptides produced by a soil *Bacillus megaterium* strain. Microb. Ecol. **57**(2): 367–378. doi:10.1007/s00248-008-9464-x. PMID:18958512.

Rautenbach, M., Vlok, N.M., Stander, M., and Hoppe, H.C. 2007. Inhibition of malaria parasite blood stages by tyrocidines, membrane-active cyclic peptide antibiotics from *Bacillus brevis*. Biochim. Biophy. Acta, **1768**(6): 1488–1497. doi:10.1016/j.bbamem.2007.01.015. PMID:17462586.

Rea, M.C., Sit, C.S., Clayton, E., O'Connor, P.M., Whittal, R.M., Zheng, J., et al. 2010. Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. Proc. Natl. Acad. Sci. U.S.A. **107**(20): 9352–9357. doi:10.1073/pnas.0913554107. PMID:20435915.

Rea, M.C., Ross, R.P., Cotter, P.D., and Hill, C. 2011. Classification of bacteriocins from Gram-positive bacteria. *In* Prokaryotic antimicrobial peptides: from genes to application. *Edited by* D. Drider and S. Rebuffat. Springer. pp. 29–47. doi:10.1007/978-1-4419-7692-5.

Riazi, S., Wirawan, R.E., Badmaev, V., and Chikindas, M.L. 2009. Characterization of lactosporin, a novel antimicrobial protein produced by *Bacillus coagulans* ATCC 7050. J. Appl. Microbiol. **106**(4): 1370–1377. doi:10.1111/j.1365-2672.2008.04105.x. PMID:19191946.

Roongsawang, N., Washio, K., and Morikawa, M. 2010. Diversity of nonribosomal peptide synthetases involved in the biosynthesis of lipopeptide biosurfactants. Int. J. Mol. Sci. **12**(1): 141–172. doi:10.3390/ijms12010141. PMID:21339982.

Rotem, S., and Mor, A. 2009. Antimicrobial peptide mimics for improved therapeutic properties. Biochim. Biophys. Acta, **1788**(8): 1582–1592. doi:10.1016/j.bbamem.2008.10.020. PMID:19028449.

Roy, A., Mahata, D., Paul, D., Korpole, S., Franco, O.L., and Mandal, S.M. 2013. Purification, biochemical characterization and self-assembled structure of a fengycin-like antifungal peptide from *Bacillus thuringiensis* strain SM1. Front Microbiol. **4**: 1–6. doi:10.3389/fmicb.2013.00332. PMID:23346082.

Sabaté, D.C., and Audisio, M.C. 2013. Inhibitory activity of surfactin, produced by different *Bacillus subtilis* subsp. subtilis strains, against *Listeria monocytogenes* sensitive and bacteriocin-resistant strains. Microbiol. Res. **168**(3): 125–129. doi:10.1016/j.micres.2012.11.004. PMID:23265790.

Sang, Y., and Blecha, F. 2008. Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. Anim. Health Res. Rev. **9**(2): 227–235. doi:10.1017/S1466252308001497. PMID:18983725.

Schallmey, M., Singh, A., and Ward, O.P. 2004. Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol. **50**(1): 1–17. doi:10.1139/w03-076. PMID:15052317.

Scholz, R., Molohon, K.J., Nachtigall, J., Vacter, J., Markley, A.L., Süssuth, R.D., et al. 2011. Plantazolicin, a novel microcin B17/Streptolysin S-like natural product from *Bacillus amyloliquefaciens* FZB42. J. Bacteriol. **193**(1): 215–224. doi:10.1128/JB.00784-10. PMID:20971906.

Scott, R.W., DeGrado, W.F., and Tew, G.N. 2008. De novo designed synthetic mimics of antimicrobial peptides. Curr. Opin. Biotechnol. **19**(6): 620–627. doi:10.1016/j.copbio.2008.10.013. PMID:18996193.

Shelburne, C.E., An, F.Y., Dholpe, V., Ramamoorthy, A., Lopatin, D.E., and Lantz, M.S. 2007. The spectrum of antimicrobial activity of the bacteriocin subtilosin A. J. Antimicrob. Chemother. **59**(2): 297–300. doi:10.1093/jac/dkl495. PMID:17213266.

Sirtori, L.R., Cladera-Olivera, F., Lorenzini, D.M., Tsai, S.M., and Brandelli, A. 2006. Purification and partial characterization of an antimicrobial peptide produced by *Bacillus* sp. strain P45, a bacterium from the Amazon basin fish *Piaractus mesopotamicus*. J. Gen. Appl. Microbiol. **52**(6): 357–363. doi:10.2323/jgam.52.357. PMID:17325449.

Sivapathasekaran, C., Mukherjee, S., Samanta, R., and Sen, R. 2009. High-performance liquid chromatography purification of biosurfactant isoforms produced by a marine bacterium. Anal. Bioanal. Chem. **395**(3): 845–854. doi:10.1007/s00216-009-3023-2. PMID:19688340.

Stachelhaus, T., and Marahiel, M.A. 1995. Modular structure of genes encoding multifunctional peptide synthetases required for non-ribosomal peptide synthesis. FEMS Microbiol. Lett. **125**(1): 3–14. doi:10.1111/j.1574-6968.1995.tb07328.x. PMID:7867917.

Stein, T. 2005. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. Mol. Microbiol. **56**(4): 845–857. doi:10.1111/j.1365-2958.2005.04587.x. PMID:15853875.

Sutyak, K.E., Wirawan, R.E., Aroutcheva, A.A., and Chikindas, M.L. 2008. Isolation of the *Bacillus subtilis* antimicrobial peptide subtilosin from the dairy product-derived *Bacillus amyloliquefaciens*. J. Appl. Microbiol. **104**(4): 1067–1074. doi:10.1111/j.1365-2672.2007.03626.x. PMID:17976171.

Tenover, F.C. 2006. Mechanisms of antimicrobial resistance in bacteria. Am. J. Med. **119**(6 Suppl. 1): S3–S10. doi:10.1016/j.amjmed.2006.03.011. PMID:16813980.

Thasana, N., Prapagdee, B., Rangkadilok, N., Sallabhan, R., Aye, S.L., Ruchirawat, S., Loprasert . 2010. *Bacillus subtilis* SSE4 produces subtulene A, a new lipopeptide antibiotic possessing an unusual C15 unsaturated β-amino acid. FEBS Lett. **584**(14): 3209–3214. doi:10.1016/j.febslet.2010.06.005. PMID:20541548.

Torres, N.I., Noll, K.S., Xu, S., Li, J., Huang, Q., Sinko., P.J., et al. 2013. Safety, formulation and in vitro antiviral activity of the antimicrobial peptide subtilosin against herpes simplex virus type 1. Probiotics Antimicrob. Proteins, **5**(1): 26–35. doi:10.1007/s12602-012-9123-x. PMID:23637711.

Ugras, S., Sezen, K., Kati, H., and Demirbag, Z. 2013. Purification and characterization of the bacteriocin thuricin Bn1 produced by *Bacillus thuringiensis* subsp. kurstaki Bn1 isolated from a hazelnut pest. J. Microbiol. Biotechnol. **23**(2): 167–176. doi:10.4014/jmb.1209.09056. PMID:23412058.

van Kuijk, S., Noll, K.S., and Chikindas, M.L. 2011. The species-specific mode of action of the antimicrobial peptide subtilosin against *Listeria monocytogenes* Scott A. Lett. Appl. Microbiol. **54**(1): 52–58. doi:10.1111/j.1472-765X.2011.03170.x. PMID:22040458.

Walsh, C. 2003. Where will new antibiotics come from? Nat. Rev. Microbiol. **1**(1): 65–70. doi:10.1038/nrmicro727. PMID:15040181.

Wang, G., Manns, D.C., Churey, J.J., and Worobo, R.W. 2014*a*. Development of a homologous expression system development and the systematic site-directed mutagenesis analysis of thurincin H, a bacteriocin produced by *Bacillus thuringiensis* SF361. Appl. Environ. Microbiol. **80**(12): 3576–3584. doi:10.1128/AEM.00433-14. PMID:24682301.

Wang, J., Zhang, L., Teng, K., Sun, S., Sun, Z., and Zhong, J. 2014*b*. Cerecedins, novel lantibiotics from *Bacillus cereus* with potent antimicrobial activity. Appl. Environ. Microbiol. **80**(8): 2633–2643. doi:10.1128/AEM.03751-13. PMID:24532070.

Wang, Y., Zhu, X., Bie, X., Lu, F., Zhang, C., Yao, S., and Lu, Z. 2014*c*. Preparation of microcapsules containing antimicrobial lipopeptide from *Bacillus amyloliquefaciens* ES-2 by spray drying. Food Sci. Technol. **56**(2): 502–507. doi:10.1016/j.lwt.2013.11.041.

Widemann, I., Breukink, E., van Kraaji, C., Kuipers, O.P., Bierbaum, G., de Kruijff, B., and Sahl, H.G. 2001. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J. Biol. Chem. **276**(3): 1172–1179. doi:10.1074/jbc.M006770200. PMID:11038353.

Wu, S., Jia, S., Sun, D., Chen, M., Chen, X., Zhong, J., and Huan, L. 2005. Purification and characterization of two novel antimicrobial peptides Subpeptin JM4-A and Subpeptin JM4-B produced by *Bacillus subtilis* JM4. Curr. Microbiol. **51**(2): 292–296. doi:10.1007/s00284-005-0004-3. PMID:16211432.

Xie, J., Zhang, R., Shang, C., and Guo, Y. 2009. Isolation and characterization of a bacteriocin produced by an isolated *Bacillus subtilis* LFB112 that exhibits antimicrobial activity against domestic animal pathogens. Afr. J. Biotechnol. **8**(20): 5611–5619. Available from http://www.academicjournals.org/AJB.

Xu, D., Wang, Y., Sun, L., Liu, H., and Li, J. 2013. Inhibitory activity of a novel antibacterial peptide AMPNT-6 from *Bacillus subtilis* against *Vibrio parahaemolyticus* in shrimp. Food Control, **30**(1): 58–61. doi:10.1016/j.foodcont.2012.07.025.

Xu, M.H., Rong, Y.J., Zhao, M.X., Song, B., and Chi, Z.M. 2014. Antibacterial activity of the lipopetides produced by *Bacillus amyloliquefaciens* M1 against multidrug-resistant *Vibrio* spp. isolated from diseased marine animals. Appl. Microbiol. Biotechnol. **98**(1): 127–136. doi:10.1007/s00253-013-5291-1. PMID:24132666.

Yeo, I.C., Lee, N.K., Cha, C.J., and Hahm, Y.T. 2011. Narrow antagonistic activity of antimicrobial peptide from *Bacillus subtilis* SCK-2 against *Bacillus cereus*. J. Biosci. Bioeng. **112**(4): 338–344. doi:10.1016/j.jbiosc.2011.06.011. PMID:21783410.

Yeo, I.C., Lee, N.K., and Hahm, Y.T. 2012. Genome sequencing of *Bacillus subtilis* SC-8, antagonistic to the *Bacillus cereus* group, isolated from traditional Korean fermented-soyabean food. J. Bacteriol. **194**(2): 536–537. doi:10.1128/JB.06442-11. PMID:22207744.

Yilmaz, M., Soran, H., and Beyatli, Y. 2006. Antimicrobial activities of some

Bacillus spp. strains isolated from the soil. Microbiol. Res. **161**(2): 127–131. doi:10.1016/j.micres.2005.07.001. PMID:16427515.

Zavascki, A.P., Goldani, L.Z., Li, J., and Nation, R.L. 2007. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. J. Antimicrob. Chemother. **60**: 1206–1215. doi:10.1093/jac/dkm357. PMID:17878146.

Zhang, B., Dong, C., Shang, Q., Han, Y., and Li, P. 2013. New insights into membrane-active action in plasma membrane of fungal hyphae by the lipopeptide antibiotic bacillomycin L. Biochim. Biophys. Acta, **1828**(9): 2230–2237. doi:10.1016/j.bbamem.2013.05.033. PMID:23756779.

Zhao, Z., Wang, Q., Wang, K., Brian, K., Liu, C., and Gu, Y. 2010. Study of the antifungal activity of *Bacillus vallismortis* ZZ185 in vitro and identification of its antifungal components. Bioresour. Technol. **101**(1): 292–297. doi:10.1016/j.biortech.2009.07.071. PMID:19717300.

Zheng, G., and Slavik, M.F. 1999. Isolation, partial purification and characterization of a bacteriocin produced by a newly isolated *Bacillus subtilis* strain. Lett. Appl. Microbiol. **28**(5): 363–367. doi:10.1046/j.1365-2672.1999.00545.x. PMID:10347890.