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Comparative genome analysis of *Bacillus* spp. and its relationship with bioactive nonribosomal peptide production

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Abstract *Bacillus* genus comprises an important number of species which produce a wide range of secondary metabolites displaying a broad spectrum of activity and great structural diversity. The genome sequences of an important number of species have been published and a large number of orphan genes reported. This review, covering all the literature in this field up to end of 2011, summarizes and compares the genetic potential of these organisms from the point of view of bioactive nonribosomal peptide production and their application as antibiotics, plant pathogen biocontrol, promotion of plant growth, etc. The biological and structural studies of the peptides isolated from *Bacillus* species are revised and some aspects of the biosynthesis of these metabolites and related compounds are discussed.

Keywords *Bacillus* genome · Nonribosomal · Ribosomal · Peptide · Antibiotic · Lipopeptide · Siderophore

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Introduction

Natural products continue to represent an excellent source of lead structures for drug discovery. Traditional methods used to isolate microbial natural products usually involve the collection and cultivation of strains, extraction, bioassay-guided isolation and structure elucidation. Unfortunately, this approach is often frustrating because of a high rediscovery rate. Thus, new concepts are required to increase the probability of discovering new bioactive structures (McAlpine et al. 2005).

However, an ever greater understanding of microbial genomics has provided valuable insights into the principles of natural product biosynthesis offering promising alternatives for the discovery and engineering of new chemical entities. The post-genomic world has shown that the traditional culture-based, bioassay-guided strategies used to discover natural products only provided access to a small fraction of the biosynthetic capacity encoded in genomes. We now know that most biosynthetic pathways are not expressed at all, or just barely, under laboratory conditions, or the products of these pathways have simply been overlooked (Udwary et al. 2007).

Recent whole-genome sequencing programmes have revealed that the biosynthetic potential of microorganisms has been greatly underexplored, relying as it does on traditional approaches. In fact, the number of genes encoding biosynthetic enzymes in various bacteria and fungi clearly outnumbers the

known secondary metabolites of these organisms (Challis 2008).

Bacillus represents just a small fraction of a larger taxonomic consortium of endospore-producing bacteria referred to as *Bacillus* sensu lato. The *Bacillus* genus comprises a very large, diverse set of Gram-positive bacteria that have one common yet distinct feature: the ability to aerobically produce dormant endospores when challenged by unfavorable growth conditions. Thus, Bacilli are endospore-forming bacteria that are characterized by their rod-shaped cell morphology, catalase production and their ubiquitous distribution (Zeigler and Perkins 2009; Logan et al. 2007).

Members of the *Bacillus* genus are bacteria that exploit a wide variety of organic and inorganic substrates as nutrient sources. This ability to survive and grow in such diverse ecosystems is based on the production of robust endospores, their diverse physiological properties, especially the production of antibiotic molecules, and their growth requirements.

Bacilli are often thought of as microbial factories given their production of a wide array of biologically active molecules whose purpose it to suppress competitive bacteria and fungi in plants (Stein 2005), including peptide and lipopeptide antibiotics and bacteriocins. Furthermore, a wide number of complete and draft genome sequences are now available and *Bacillus* may very well be one of the best chemically characterised and most represented genera in the genomic databases.

An overview of *Bacillus* sensu lato genus and its genome sequences

The *Bacillus* sensu lato genus is both taxonomically and metabolically diverse. Their production of antimicrobial substances and sporulation capacity give *Bacillus* strains a dual advantage in terms of their survival in different habitats. They are phenotypically and genotypically heterogenous (Slepecky and Hemphill 2006), and consequently they exhibit quite diverse physiological properties such as the ability to break down many different substrates derived from plant and animal sources, including cellulose, starch, proteins, agar, hydrocarbons and even biofuels (Zeigler and Perkins 2009). Bacilli isolated from a wide range of environments, including plants and soil (Kunst et al. 1997; Priest 1993), hydrothermal vents (Takami et al. 2004), extreme environments (Rey et al. 2004; Takami et al. 2000), sea water (Siefert et al. 2000), and a shallow water column from an oligotrophic environment (Alcaraz et al. 2008) have been reported. The presence of *Bacillus* in these different environments brears witness to the broad metabolic capabilities of this widely distributed genus.

Their primary habitat is the soil and associated plants (*B. amyloliquefaciens*), rivers and estuarine waters (*B. coahuilensis*), and they are well known for their ability to control plant diseases through various mechanisms, including the production of secondary metabolites (Chen et al. 2009a). However, some species are pathogenic to mammals (e.g. *B. anthracis*) and insects (e.g. *B. sphaericus*, *B. thuringiensis*) (Wipat and Harwood 1999).

As *Bacillus* spp. are characteristically omnipresent in soils, exhibit high thermal tolerance and rapid growth in liquid culture, readily produce resistant spores and are considered to be a safe biological agent, their potential as a biocontrol agent is considered to be high (Shoda 2000). Additionally, their uses in insect control, food industry, livestock and environmental applications have been reported (Harwood and Cranenburgh 2008).

The genomic sequences of several *Bacillus* species have been made available over the last several years. Recent research has identified biosynthetic genes that code for a secondary metabolic pathway which are clustered in microbial genomes. This observation has facilitated their study and manipulation. Furthermore, greater insight has been gained into their pathways involving polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) assembly lines which are responsible for the biosynthesis of these two large classes of natural products (Marahiel 1992; Calderone et al. 2006; Marahiel et al. 1993, 1997).

Recently, an interesting study, including a phylogenetic tree (Alcaraz et al. 2010), was carried out using whole genome information to reconstruct their evolutionary history taking advantage of the data set from the complete and draft genomes of twenty *Bacillus* isolated from a wide range of habitats, including a newly sequenced *Bacillus* isolated from an aquatic environment (Alcaraz et al. 2010).

An important number of projects are under way which will contribute to a better understanding of their ecological adaptations so as to be able to correlate

particular sets of genes with their pathogenic potential and the production of bioactive peptides and metabolites in general (Ravel and Fraser 2005).

Bacillus genus: comparative analysis of *Bacillus* genome

The rate at which entire microbial genomes are being sequenced has grown rapidly over the past years and is revolutionising our understanding of microbial molecular biology and genetics.

As already indicated, bacilli can be subgrouped according to their ecophysiology (acidophily, alkaliphily, halophily, psychrophily and thermophily) and in terms of their metabolic diversity, into three major classes: pathogenic, environmental uses and industrial uses (Ravel and Fraser 2005). *B. anthracis*, *B. cereus* and *B. thuringiensis* represent the pathogenic group, while that the environmental bacilli are quite diverse and include *B. subtilis*, *B. amyloliquefaciens*, *B. coahuilensis* and *B. halodurans*. As for the class, the most representative strains with industrial applications are *B. licheniformis* and *B. clausii*. While this classification is useful in characterizing the metabolic diversity of the genus, it provides no guidance for a phylogenetic classification of the *Bacillus* for research purposes (Alcaraz et al. 2010). Thus, four groups of bacilli were defined following a classification system based on orthologous relations among genes: (a) *B. anthracis–cereus–thuringiensis*, (b) *B. subtilis–licheniformis–pumilus*, (c) *B. clausii–halodurans*, and (d) *B. coahuilensis* (Alcaraz et al. 2010).

Comparative analysis of the genome sequence of the related environmental isolates clearly shows that *B. amyloliquefaciens*, *B. subtilis* and *B. licheniformis* are closely related and quite distinct from the *B. cereus* group of organisms (*B. anthracis*, *B. cereus* and *B. thuringiensis*). Despite a high degree of chromosomal similarity among these genomes, significant differences in cell wall and spore coat proteins that contribute to the survival and adaptation in specific hosts were identified (Anderson et al. 2005).

Bacillus clausii–halodurans form the group of alkalophiles and halotolerant strains. Interestingly, this group has a fewer than average number of genes involved in the cell wall/membrane/envelope category. Additionally, there are several known differences in the cell wall composition of *B. clausii–halodurans* compared to *B. subtilis*, such as the presence of the major cell wall component teichurono-peptide (Takami et al. 2000).

Pathogenic *Bacilli* group

Bacillus anthracis, *B. cereus*, and *B. thuringiensis* are genetically very closely related, a fact which has led to a proposal for their consideration as a single species (Radnedge et al. 2003) but no consensus has been reached in this regard. This ambiguous taxonomic state of the *B. cereus* group illustrates the difficulties encountered in species definition within bacterial systematics, especially in the genomic era.

Several interesting reviews and papers have recently been reported on comparative genome analysis of the *B. cereus* group (Rasko et al. 2005; Read et al. 2002, 2003; Ivanova et al. 2003). The analysis of representative members of *Bacillus* genomes has led to the discovery of the shared and singular metabolic and virulence capability of each species. The presence of specific genes with functions related to spore coat, exopolysaccharide biosynthesis and membrane transport has revealed significant differences despite the high level of chromosomal similarity among *B. cereus* group bacteria. In this regard, recent studies have demonstrated differences in gene order, chromosomal rearrangements, nucleotide variations and remnant phages, suggesting that *B. anthracis* diverges from *B. cereus* and *B. thuringiensis* and represents a distinct genetic lineage(Read et al. 2002).

B. cereus

Discussion of the classification of *B. cereus* group strains is not only of academic and taxonomic interest, but is also important from a public health perspective because genes encoding the cytotoxins associated with diarrhoeal disease and other opportunistic *B. cereus* infections are generally chromosomally encoded and are present in all species of the *B. cereus* group, although they are silent in *B. anthracis* (Arnesen et al. 2008). *B. cereus* is frequently isolated as a contaminant of a number of foods and can occasionally be an opportunistic human pathogen (Helgason et al. 2000; Drobniewsk 1993). Thus, *B. cereus* isolates harbouring *B. anthracis* virulence factors have been detected in cases of severe anthrax-like illnesses (Hoffmaster et al. 2004).

B. anthracis

Bacillus anthracis is a potent mammalian pathogen and potential bioterrorist agent which shares so much genetic material with *B. cereus* and *B. thuringiensis* that it is difficult to distinguish it from the other species. All three *Bacillus* species are prevalent in many environments and considerable efforts have been made to define the genetic differences specific to *B. anthracis* in order to design specific DNA-based identification protocols. Thus, a great many comparative analyses of *B. anthracis* to closely related bacteria genome sequences and comparative genome studies of *B. cereus* with *B. subtilis* and *B. anthracis* genomes have been performed by many scientists (Read et al. 2002, 2003; Ivanova et al. 2003; Keim et al. 2009).

A detailed comparative analysis of the genomes of *B. cereus* and *B. anthracis* revealed a small subset of genes unique to either species (Rasko et al. 2005). The majority of these genes are located at the terminus of replication, indicating that genome plasticity mostly occurs in that region. These data suggest a history of insertion and/or deletion in the evolution of the *B. cereus* group. It was observed that, in many cases, genes found at a specific position in one genome were replaced with others at the corresponding loci in another (Rasko et al. 2005).

B. thuringiensis

Bacillus thuringiensis is the most well-known and thoroughly studied example of an entomopathogenic bacterium which produces parasporal protein crystals in association with spore formation (Bode 2009). These protein toxins lyse the insect after ingestion (Zhou et al. 2008). Therefore natural and genetically engineered strains of *B. thuringiensis* have been used commercially to control pests of many representatives of the *Lepidoptera*, *Diptera*, and *Coleoptera* orders. Thus, *B. thuringiensis* has been widely exploited in agriculture as an insecticide, thanks to the presence of plasmid-borne crystal toxin genes (Schnepf et al. 1998).

Virulence against insects is believed to be conferred by multiple factors and the genomic sequence has identified numerous secondary metabolite gene clusters that could also contribute to the insecticidal activity of *B. thuringiensis* (Rasko et al. 2005).

Additionally, the genes encoding the Bt-toxins have been successfully transferred into cotton, corn, soybean, and rice conferring resistance to insect pests thus leading to a significant economic benefit (Zhou et al. 2008). Besides protein toxins, *B. thuringiensis* is also a producer of an acyl homoserine lactone lactonase which can eliminate the pathogenicity of plant pathogenic bacteria, and zwittermicin, a potent antibiotic and antifungal compound (Kevany et al. 2009).

Furthermore, the complete genome sequence of several strains of *B. thuringiensis*, such as *B. thuringiensis* Al Hakam (Challacombe et al. 2007) and *B. thuringiensis* BMB171 (He et al. 2010), has been reported. A comparative analysis of the genomes of both strains of *B. thuringiensis* has been described (see Table 1).

Environmental *Bacilli* group

The bacterium *B. subtilis* was first described in 1872 by Ferdinand Cohn and is a species that is second only to *Escherichia coli* in terms of the level of detail at which it has been studied (Ravel and Fraser 2005).

The *B. subtilis* group includes the most intensively studied of the bacilli, including species such as *B. subtilis* itself, *B. amyloliquefaciens* and *B. licheniformis* which are of industrial importance (Harwood 1992), and *B. coahuilensis*, one of the most recent *Bacillus* species reported from a marine environment (Cerritos et al. 2008).

B. subtilis

Bacillus subtilis has been a paradigm for the genetics of Gram-positive bacteria for more than 30 years. It

Table 1 Genomic properties of *B. thuringiensis*

Species	Strain	Genome size (Mb)	% GC	No. of protein-coding genes	No. of tRNAs	No. of rRNA operons
B. thuringiensis	Al Hakam	5.31	35	4,969	105	13
	BMB171	5.64	35.3	5,088 plus 276 on plasmids	104	14

has a particularly well-developed natural transformation system that proved invaluable for genetic manipulation and analysis in the pre-recombinant DNA technology era.

The *B. subtilis* genome sequence is the first complete genome of a free-living soil and rhizosphere bacterium which was accomplished by an international consortium of 46 laboratories worldwide (Kunst et al. 1997; Stewart et al. 2009). Its genome, composed of 4,214,810 base pairs comprises 4,100 protein-coding genes. Of these protein-coding genes, 53 % are represented once while a quarter of the genome corresponds to several gene families that have been greatly expanded by gene duplication. The identification of five signal peptidase genes, along with several genes for components of the secretion apparatus (Tjalsma et al. 1997) is important given the capacity of *Bacillus* strains to secrete large amounts of industrially important enzymes. Many of the genes are involved in the synthesis of secondary metabolites, including antibiotics. The genome contains at least ten prophages or remnants of prophages, indicating that bacteriophage infection has played an important evolutionary role in horizontal gene transfer, in particular in the propagation of bacterial pathogenesis (Kunst et al. 1997).

Data derived from the genome sequence (Moszer 1998) and the systematic functional analysis programme (Hunt et al. 2006), together with the wealth of knowledge already available for this organism, has opened the door to new opportunities in studying the behaviour and ecology of this soil and plant growth-promoting rhizobacterium at the molecular level.

B. amyloliquefaciens

Bacteria that are associated with plant roots and exert beneficial effects on plant development are referred to as plant growth-promoting rhizobacteria (Kloepper et al. 1980). They competitively colonize plant roots and can simultaneously act as biofertilizers and as antagonists (biopesticides) of recognized root pathogens including bacteria, fungi and nematodes. There is a large body of literature reporting the potential use of rhizosphere-associated bacteria in stimulating plant growth and biocontrol agents (Argüelles-Arias et al. 2009; Somers et al. 2004; Lugtenberg and Kamilova 2009). Among these, several strains belonging to the *Bacillus* genus, particularly to the *B. subtilis* and *B. amyloliquefaciens* species, were reported as being effective in the biocontrol of multiple plant diseases caused by soilborne (Leclere et al. 2005; Chen et al. 2009b) or post-harvest pathogens (Senghor et al. 2007; Yang et al. 2008; Kotan et al. 2009). Members of the *Bacillus* genus are thus among the beneficial bacteria mostly exploited as microbial biopesticides. *Bacillus*-based products account for approximately half of the commercially available bacterial biocontrol agents (Fravel 2005).

The plant root-colonizing *B. amyloliquefaciens* strain FZB42 is an environmental strain which is distinguished from the domesticated model organism *B. subtilis* 168 by its ability to stimulate plant growth and to suppress plant pathogenic organisms (Idriss et al. 2002).

Bacillus amyloliquefaciens FZB42 genome analysis revealed the presence of numerous gene clusters involved in synthesis of nonribosomally synthesized cyclic lipopeptides (Chen et al. 2009c) and polyketides (Chen et al. 2007; Koumoutsi et al. 2004) with proven antimicrobial action (Koumoutsi et al. 2007).

B. coahuilensis

Bacillus coahuilensis is a sporulating bacterium isolated from the water column of a desiccation lagoon in the Cuatro Ciénegas Basin (CCB) in the central part of the Chihuahan desert (Coahuila, Mexico). A major question yet to be answered is whether bacteria from CCB are ancient marine bacteria that adapted to an oligotrophic system poor in NaCl, rich in sulfates and with extremely low phosphorus levels. The *B. coahuilensis* genome is the smallest genome of a *Bacillus* species (3.35 Megabases) sequenced to date and provides insights into the origin, evolution, and adaptation of *B. coahuilensis* to the CCB environment. The size and complexity of the *B. coahuilensis* genome reflects the adaptation of an ancient marine bacterium to a novel environment, providing support for a "marine isolation origin hypothesis" that is consistent with the geology of CCB. This genomic adaptation includes the acquisition, through horizontal gene transfer, of genes involved in phosphorous utilization efficiency and adaptation to particularly luminous environments (Alcaraz et al. 2008).

Industrial uses *Bacilli* group

B. licheniformis

Bacillus licheniformis is a Gram-positive, spore-forming bacterium widely distributed in the environment as a saprophytic organism. There are numerous commercial and agricultural uses for *B. licheniformis* and its extracellular products (Erickson 1976).

Bacillus subtilis and *B. licheniformis* are closely related species that feature similar saprophytic lifestyles in soils. Both species are known for their ability to secrete numerous proteins into the surrounding medium, some in quite high amounts (Voigt et al. 2006). This enables them to break down a variety of high molecular weight substances found in soils and use the resulting products as nutrients to sustain growth or to ensure their survival under scarce nutrient conditions. In addition, the ability to secrete large amounts of proteins has been exploited by the fermentation industry for some time now, especially for the production of industrial enzymes including several proteases, α-amylase, penicillinase, pentosanase, cycloglucosyltransferase, β-mannanase and several pectinolytic enzymes (Erickson 1976).

A comparative study of the genome sequence of *B. licheniformis* with related environmental isolates clearly shows that *B. licheniformis* is closely related to those of the *B. subtilis* group and is quite distinct from *B. cereus* group organisms (Rey et al. 2004).

Two similar strains of *B. licheniformis* (ATCC 14580 and DSM13) (Rey et al. 2004; Veith et al. 2004) were sequenced by two different groups and published one shortly after the other.

Bacillus subtilis and *B. licheniformis* share approximately 80 % of their proteome. However, there are notable differences in the numbers and locations of prophages, transposable elements and a number of extracellular enzymes and secondary metabolic pathway operons that distinguish these species.

Bacillus licheniformis uses cellulose as it sole source of carbon and energy. Additionally, *B. licheniformis* codes for activities that enable growth on a broad range of polysaccharides, particularly advantageous in the competitive soil environment and also of interest for biotechnological applications. Many new genes of potential interest for biotechnological applications were found in *B. licheniformis*; candidates include proteases, pectate lyases, lipases and various polysaccharide-degrading enzymes (Ravel and Fraser 2005).

B. halodurans

Generally, alkaliphilic *Bacillus* strains cannot grow or grow poorly under neutral pH conditions, but grow well at pH >9.5. The complete genomic sequence of the alkaliphilic bacterium, strain *B. halodurans* C-125, has been determined (Takami et al. 2000) and its genome compared with that of *B. subtilis*.

Alkaliphilic bacteria studies are focused on the enzymology, physiology and molecular genetics of these microorganisms to elucidate their mechanisms of adaptation to alkaline environments. Industrial applications of these microbes have been investigated and some enzymes, such as proteases, amylases, cellulases, and xylanases, have been commercialized (Takami et al. 2000).

B. clausii

Bacillus clausii is a facultative alkalophilic bacillus which is commercially used in the production of alkaline proteases for detergents (Saeki et al. 2007), and as a probiotic (Bozdogan et al. 2003) administered to humans to prevent the gastrointestinal side-effects caused by oral antibiotic therapy. Ingestion of high quantities of spores is thought to restore an intestinal flora following the destruction of normal flora by antibiotics.

Over the last years an important number of papers and reviews on the proteomics of the *Bacillus* genus have appeared providing insights into how proteomics can be used to bring the virtual life of genes to the real life of proteins (Hecker and Voelker 2004).

Peptide metabolites from *Bacillus* spp.

Bacillus species produce a wide array of biologically active molecules including polyketides, lipopeptides, siderophores and peptides. Polyketides (Hamdache et al. 2011) are beyond the scope of this review, which focuses more specifically on nonribosomal bioactive peptides.

Nutritional stress conditions lead to the activation of a number of processes affecting survival rate. Activities induced include the development of genetic

competence, sporulation, and synthesis of degradative enzymes, motility, and antibiotic production. The genes that function in these processes are activated during the transition from exponential to stationary phase and are controlled by mechanisms that operate primarily at the level of transcription initiation (Jedrzejas and Huang 2003).

From the point of view of biosynthesis, *Bacillus* genus species produce two different classes of peptides: ribosomal and nonribosomal peptides, which are biosynthesized by two different biosynthetic pathways allowing the incorporation of such unusual (non-proteinaceous) constituents: (a) the nonribosomal synthesis of peptides by large megaenzymes, nonribosomal peptide synthetases (NRPSs) and (b) the ribosomal synthesis of linear precursor peptides that are subjected to post-translational modification and proteolytic processing.

Ribosomal bioactive peptides

Bacterial antimicrobial peptides produced by ribosomal synthesis are commonly referred to as bacteriocins. This is a heterologous group of proteinaceous antimicrobial substances produced by bacteria from every major lineage. They display a high degree of target specificity against related bacteria although many have a wider spectrum of activity (Abriouel et al. 2011).

Unlike nonribosomal peptides (NRPs), ribosomal peptide (RP) natural products are unable (as far as is known) to explore amino acids beyond the canonical 20 proteinogenic amino acids, limiting their structural diversity to some degree. However, they can be extensively post-translationally modified and such modifications give rise to products with many features resembling those of NRPs. In fact, many of the modifications commonly thought of as "nonribosomal" are also found in ribosomally synthesized peptides. Other RPs has unique modifications that allow them to explore greatly expanded chemical spaces in a manner similar to the NRPs (McIntosh et al. 2009).

A great advantage of the RPs is that their sequences may be modified by simple manipulation of a few codons in contrast to NRPs which require extensive genetic engineering to incorporate changes. Thus, ribosomal natural products have many -but not all- of the structural advantages of the NRPs, and in addition they are easier to modify to produce novel bioactive compounds. Many excellent reviews of individual RP classes have recently come out such as lantibiotics, microcins and others. Especially relevant is the one concerning bacteriocines recently published by A. Gálvez et al. (Abriouel et al. 2011).

Furthermore, the number of known peptide natural products that are synthesized via the ribosomal pathway has grown rapidly and their remarkable structural and functional diversity may lead to novel pharmaceutical applications. The strategies used for the identification of these ribosomally synthesized and posttranslationally modified peptides (RiPPs) and the structures of newly identified compounds have recently been reviewed (Velasquez and van der Donk 2011). The reader is referred to these reviews for a more in-depth look at individual RP classes.

The number of RP families has increased considerably in recent years with the discovery of the biosynthetic origin of cyanobactins, thiopeptides, microviridins and amatoxins. These findings have shown that the ribosomal world is much more diverse than originally thought, and have revealed an enormous structural diversity that is just beginning to be appreciated (McIntosh et al. 2009; Oman and van der Donk 2010).

Nonribosomal bioactive peptides

Nonribosomal peptides are a structurally diverse class of peptides with various functions, such as cytostatic, immunosuppressive, antibacterial, or antitumor properties. These secondary metabolites differ from peptides of ribosomal origin in several ways. Their length is limited to a mere 20 building blocks, roughly, and mostly a circular or branched cyclic connectivity is found. Furthermore, aside from the proteinogenic amino acids, a larger variety of chemical groups is found in these bioactive compounds: D-configurated amino acids, fatty acids, methylated, oxidized, halogenated, and glycosylated building blocks. These functional and structural features are known to be important for bioactivity, and often natural defense mechanisms are thus evaded (Schoenafinger and Marahiel 2009).

The genes encoding the multifunctional enzymes that catalyse nonribosomal synthesis of the peptide antibiotics are organized in operons that are transcriptionally induced in response to nutritional stress. The regulation of antibiotic biosynthesis operons is accomplished through the interaction of diverse factors

which also function in controlling other stationary-phase-induced processes in *Bacillus*. Peptide antibiotic biosynthesis genes are regulated by factors as diverse as the early sporulation gene product SpoOA, the transition-state regulator AbrB, and gene products (ComA, ComP, and ComQ) required for the initiation of the competence developmental pathway (Marahiel et al. 1993).

In spite of their structural heterogeneity, the nonribosomal peptide antibiotics are biosynthesized by the iterative coupling of amino acids and share a common mode of synthesis, the multicarrier thiotemplate mechanism which is carried out by the large multidomain enzymatic machineries, the so-called nonribosomal peptide synthetases (NRPSs). NRPS are megaenzymes organized in iterative functional units called modules that catalyze the different reactions leading to polyketide or peptide transformation. The first crystal structure of an entire module of domains from an NRPS system has recently been reported (Weissman and Mueller 2008). These megasynthetases build the growing chains as a series of elongating acyl-*S*-proteins, covalently tied to the terminal thiol of phosphopantetheinyl arms, with themselves hitched to side chains of serine residues of carrier protein domains in the assembly line (Fischbach and Walsh 2006; Walsh 2008).

Each module is subdivided into several catalytic domains responsible for each biochemical reaction. A typical NRPS module usually comprises 1,000 amino acid residues approximately and is responsible for one reaction cycle. Each elongation cycle in nonribosomal peptide biosynthesis needs the cooperation of three basic domains:

(a) adenylation domain, which generates an enzymatically stabilized aminoacyl adenylation.
(b) peptidyl carrier domain, which transfer and bound the adenylated amino acid substrate by a thioester bond.
(c) condensation domain catalyzes formation of a new peptide bond. The linear organization of such core units (a–c) ensures the coordinated elongation of the peptide product. The basic set of domains within a module can be extended by substrate modifying domains, including domains for substrate epimerization, hydroxylation, methylation and heterocyclic ring formation, which act as independent catalytic units (Finking and Marahiel 2004).

Three major chemical routes for disconnection of the covalent thioester linkages of NRPS acyl chains when they reach the most downstream carrier protein domains on assembly lines have been observed. A thioesterase domain, which in some cases is responsible for the cyclization of the peptide, is usually present in the last module to ensure the cleavage of the thioester bond between the emerging peptide and the last PCP domain (Walsh 2008).

The logic of NRPS assembly lines is modular (Marahiel et al. 1997), and the encoding genes in microbial genomes are almost always clustered. The molecular organization of these peptides synthetases and the regulation of the corresponding biosynthesis genes have been analyzed and reported (Marahiel et al. 1993). This basic mechanism can result in great chemical variety of peptide products containing hydroxy-, L-, D-, or unusual amino acids, which can be further modified by N methylation, acylation, glycosylation or heterocyclic ring formation. More than 300 different residues are known to be incorporated into these peptide secondary structures (Hancock and Chapple 1999).

The therapeutic potential of peptides of nonribosomal origin is well recognized. In addition, recent years have seen the discovery of a growing number and wide diversity of peptides that are biosynthesized by a nonribosomal pathway (Frueh et al. 2008).

However, in spite of the high number, wide structural diversity and growing number of nonribosomal peptides, to our knowledge no classification scheme has been devised for this class of *Bacillus* peptides. This is probably a result of the confusing information about structures of some reported peptides, lack of information on many of these peptides amino acid sequences or the vast diversity of nonribosomal peptides produced by bacilli. This review has been organized on the base of the peptides/amino acid sequences molecular structure point of view. Two major groups can be differenced, cyclic and linear peptides, and both groups have been arranged on the base of the structure-peptides number.

Cyclic peptides

Tridecapeptides

The antifungal peptide antibiotic, mycobacillin (**1**) (Fig. 1), was isolated from the B_3 strain of *B. subtilis*. The study of the physicochemical properties of

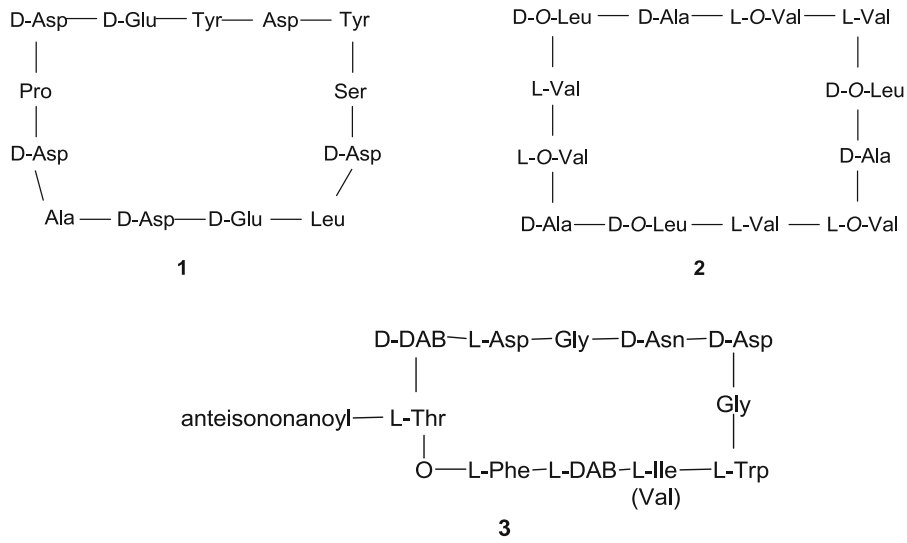


Fig. 1 Trideca-, dodeca- and undecapeptides reported from Bacilli

mycobacillin (**1**) shows that it is a polypeptide consisting of five aspartic acid residues, two glutamic acid and two tyrosine residues and one residue each of serine, alanine, leucine and proline (Majumdar and Bose 1960).

The biosynthesis of the cyclic mycobacillin (**1**) is an alternative to the thiotemplate mechanism of peptide antibiotic biosynthesis. Instead of two-step activation of amino acids, a single-step mechanism with formation of aminoacylphosphate was proposed. The enzyme complex that catalyzes the synthesis of mycobacillin (**1**) is separated into three fractions: (A) which operates during synthesis of the first pentapeptide; (B) which catalyzes the synthesis of the nonapeptide; and (C) which catalyzes synthesis of the final product (Mannanov and Sattarova 2001).

Dodecapeptides

Cereulide (**2**) (Fig. 1), is a cyclic dodecadepsipeptide produced by *B. cereus* which acts as a potassium ionophore. Cereulide (**2**), an emetic toxin, is heat, acid and alkaline stable as well as resistant to proteolysis by trypsin and pepsin, making it of particular concern to the food industry, as existing emetic toxin is not inactivated by processing methods (Ehling-Schulz et al. 2004). The emetic syndrome is usually characterized by nausea and vomiting, however, more severe cases have also been reported, leading for instance to acute liver failure (Posfay-Barbe et al. 2008).

In light of the increasing number of serious food borne outbreaks caused by emetic *B. cereus*, transcriptional kinetic analyses of cereulide synthetase genes with respect to growth, sporulation and emetic toxin production have been reported (Dommel et al. 2011).

Cereulide (**2**) is closely related to the antibiotic valinomycin (Agata et al. 1994). Both compounds are known as K^+-ionselective ionophores and cause a potassium-dependent drop in the transmembrane potential of mitochondria arising from the uptake of a K^+-ioncharged ionophore complex (Makarasen et al. 2009). The cereulide peptide synthetase of the emetic *B. cereus* reference strain F4810/72 was found to be located on a pXO1-like plasmid, designated pBCE, and was identified as a 24 kb ces gene cluster comprising seven codingDNA sequences(Ehling-Schulz et al. 2006).

Homocereulide, isolated from the marine bacterium *B. cereus* SCRC, is very similar to cereulide (**2**). It differs only in the presence of a methyl group. Thus, the stereochemistry of homocereulide was deduced to be cyclo[-(D-Hic-D-Ala-L-Hiv-L-Val-)$_2$-D-Hic-D-Ala-L-*allo*-Hmv-D-Val-]. Both homocereulide and cereulide (**2**), exhibited extremely potent cytotoxicity and activity against the murine leukemia cell line P388 and the colon 26 tumor cell line (Sheng et al. 1995).

Undecapeptides

In 1976, Shoji et al. (1976a) reported the isolation of an amphoteric acylpeptide antibiotic named brevistin (**3**) (Fig. 1) from the culture broth of *B. brevis* 342-14 which is active against Gram-positive bacteria in vitro and in vivo. Brevistin (**3**) is thought to be a complex of undecapeptides, consisting of a major peptide (80 %)

containing isoleucine and a minor (20 %) containing valine: isoleucine or valine is present in the same position of the sequence. The constituent fatty acid was elucidated to be anteisononanoic acid by gas chromatography-mass spectrometry (Shoji and Kato 1976a).

Esein and bresein are two cyclopeptides produced by *B. brevis* (Zharikova et al. 1975). Esein is amphoteric and has one free NH_2 group and 11 amino acids (Radzhapov et al. 1968). Bresein is basic and it has two NH_2 groups. The biosynthesis of these antibiotics was studied in different media (Kherat et al. 1974).

Decapeptides

Lipodecapeptides

Fengycin and related compounds

Bacillus strains produce a broad spectrum of bioactive nonribosomal lipopeptides with great potential for biotechnological and biocontrol applications and include lipopeptides of the iturin, surfactin and fengycin families. All these lipopeptides feature a lipophilic fatty acid chain and a hydrophilic peptide ring and exhibit powerful biocontrol activities (Touré et al. 2004). They are amphiphilic cyclic peptides composed of 7 α-amino acids (surfactins and iturins) or 10 α-amino acids (fengycins) linked to one unique β-amino fatty acid (iturins) or β-hydroxy fatty acid (surfactins and fengycins). The length of the fatty acid chain varies from C_{13} to C_{16} for surfactins and from C_{14} to C_{17} for iturins. Lipopeptides have low toxicity, high biodegradability and are environmentally friendly compared with chemical pesticides. Furthermore, some lipopeptides show great promise as antitumour, antiviral and antimycoplasma agents (Yang et al. 2006).

Fengycin (**4**) and the closely related plipastatin (Fig. 2) are cyclic lipodecapeptides containing a β-hydroxy fatty acid with a side chain length of 16–19 carbon atoms. Four D-amino acids and ornithine (a non-proteinogenic residue) have been identified in the peptide portion of fengycin (**4**). It is specifically active against filamentous fungi and inhibits phospholipase A_2 (Nishikiori et al. 1986a).

Fengycin (**4**) is a lipopeptide biosurfactant (Vater et al. 2002) produced by several *Bacillus* species. This

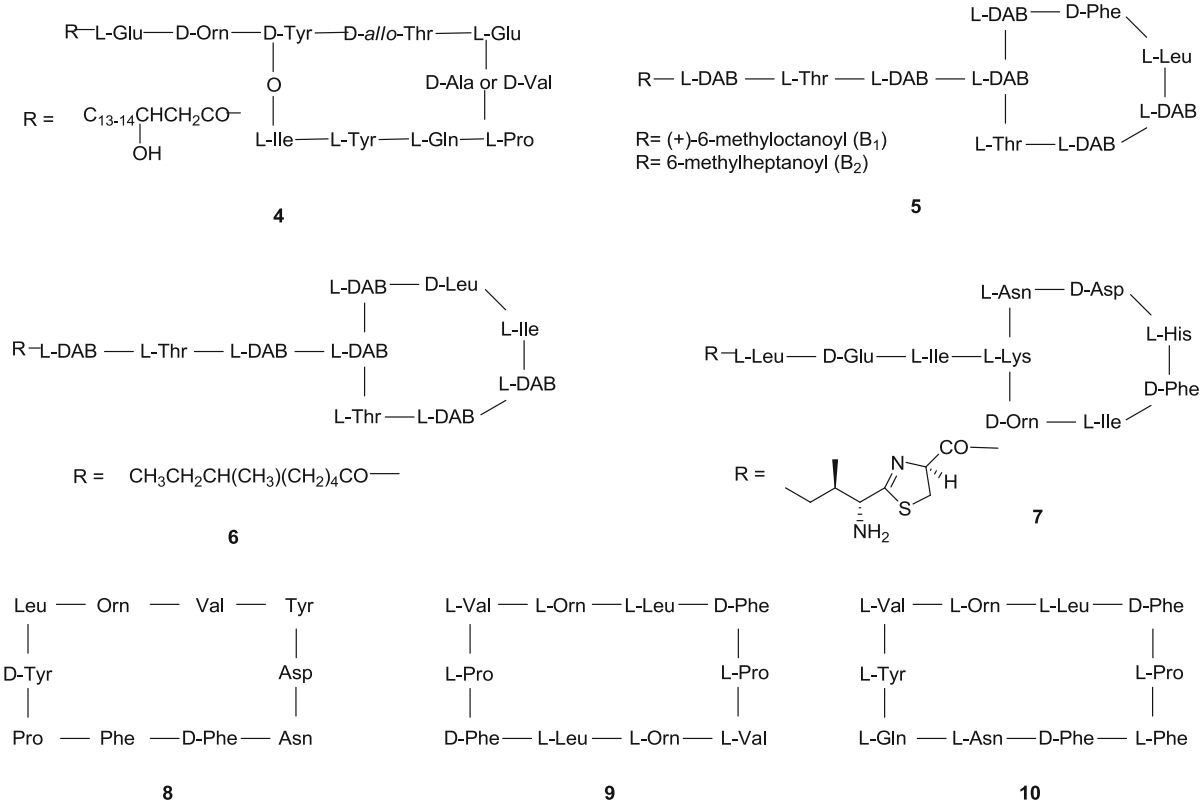


Fig. 2 Decapeptides

lipopeptide can function as a biocontrol agent, and exhibits fungicidal, bactericidal, and insecticidal activity (Vanittanakom et al. 1986). It is known to develop antifungal activity against phytopathogen fungi and to exhibit hemolytic activity. Like most of the natural antimicrobial peptides, fengycin (**4**) likely acts by increasing the permeability of the target cell's plasma membrane (Deleu et al. 2008). The inhibition is antagonized by sterols, phospholipids and oleic acid, whereas two other unsaturated fatty acids increase the antifungal effect (Vater et al. 2002).

The plipastatins, isolated from *B. subtilis* (Tsuge et al. 1996, 1999) and *B. cereus* (Umezawa et al. 1986), are very similar to fengycins. They differ only in the stereochemistry of the Tyr residues (L to D diastereoisomers) (Volpon et al. 2000). The antimicrobial spectrum of plipastatins is still mostly unknown but was shown to inhibit the phospholipase A_2 (Umezawa et al. 1986), an enzyme involved in a number of physiologically important cellular processes such as inflammation, acute hypersensitivity and blood platelet aggregation (Hirata and Axelrod 1980). As for the other lipopeptides produced by *B. subtilis*, plipastatins are biosynthesised as a mixture of isoforms characterised by variations in both the nature of the hydrophobic tail and the amino acid composition (Nishikiori et al. 1986b). The hydrophobic tail is a 3(*R*)-hydroxy hexadecanoic acid (plipastatins A_1 and B_1) or a 14(*S*)-methyl-3(*R*)-hydroxy hexadecanoic acid (plipastatins A_2 and B_2) while the amino acid sequence differ in position 6 with a D-Ala (plipastatin A_1 and A_2) substituted by a D-Val (plipastatin B_1 and B_2) (Volpon et al. 2000).

Maltacines comprise a family of peptide lactone antibiotics related to fengycin (**4**), produced by a strain of *B. subtilis*, of which 13 of the molecules have been studied, B1a, B1b, B2a, B2b (Hagelin 2005a, 527–538), C1a, C1b, C2a, C2b (Hagelin 2005b, 1276–1286), D1a, D1b, D1c (Hagelin 2005c, 1287–1299), E1a and E1b (Hagelin 2005d). These are suggested to be cyclo-4,12 peptide lactones whose members have major parts of their primary sequences in common. Position 4 in all members was thought to be occupied by a hydroxyamino-acid that is part of the lactone ring (Hagelin et al. 2007). Maltacines present activity against Gram-positive bacteria such as *Staphylococcus aureus*, some Gram-negative bacteria and the opportunistic fungi *Candida albicans*, *Tricophyton mentagrophytes* and *Aspergillus fumigatus* (Hagelin, 2005d).

Polymyxin family and related compounds

Antimicrobial polycationic peptides have been isolated from a wide variety of species and include the microbial polymyxins (Vogler and Studer 1966). Polymyxins, including polymyxins A (Wilkinson and Lowe 1966), B (**5**), C (Charbonneau et al. 1998), D (Brownlee et al. 1949), E, F, M, T_1, S_1 and P, are a wide family of closely related peptide antibiotics containing a cycloheptapeptide ring with a C-8 or C-9 fatty acid attached through an amide bond (Fig. 2) (Storm et al. 1977). These antibiotics are active against many Gram-negative and a few Gram-positive microorganisms (Newton 1956), but their mode of action is still not precisely known.

The cationic surfactant polymyxin B (**5**) (a mixture of polymyxins B_1 and B_2) is an α,γ-diaminobutyric acid (DAB)-rich decapeptide from *Paenibacillus polymyxa* exhibiting antimicrobial and lipopolysaccharide (LPS)-antagonistic activities. It is effective against Gram-negative bacteria (Rosenberg and Ron 1999). Polymyxin B nonapeptide is a derivative of polymyxin B (**5**) (Bhattacharjya et al. 1997).

Polymyxin E, a mixture of polymyxins E_1 and E_2 isolated from *P. polymyxa*, designates a multicomponent branch of the polymyxin family that is commonly known as colistin. Polymyxin E_1, the major constituent, is considered to be the active pharmaceutical ingredient for therapeutic indications. It contains one D-leucine, one L-leucine, six L-α,γ-diaminobutyric acid, and two L-threonine residues. The cyclic structures of the polymyxins E_1 and theirs analogs were selective for Gram-negative bacteria and showed potent inhibitory activity against *E. coli* and against *Pseudomonas aeruginosa*. In contrast, the acyclic analogs of these peptides showed no antimicrobial activity (Kline et al. 2001).

Polymyxin F is an antibiotic produced by *B. circulans* ATCC No 31228, which is comprised of 2,4-diaminobutyric acid (DAB), Thr, Ser, Ile and Leu at a ratio of 5:1:1:1:2. The hydrolysate also contains three extractable fatty acids identified as 6-methyloctanoic acid, isooctanoic acid and octanoic acid, with relative abundances of 78:19:3, respectively. Polymyxin F possesses a pattern of antimicrobial activity that is similar to that of polymyxin B (**5**) (Parker et al. 1977).

Mattacin (polymyxin M) possesses an amide linkage between the C-terminal threonine and the side chain amino group of the diaminobutyric acid residue

at position 4. It contains an (*S*)-6-methyloctanoic acid moiety attached as an amide at the N-terminal amino group, one D-leucine, six L-α,γ-diaminobutyric acid and three L-threonine residues. Mattacin was capable of inhibiting the growth of a wide variety of Gram-positive and Gram-negative bacteria, including several human and plant pathogens with activity comparable with purified polymyxin B (**5**), a commercial antibiotic (Martin et al. 2003).

Two members of the polymyxin group of antibiotics, named polymyxins S_1 and T_1, have been isolated from *B. polymyxa* Rs-6 and *B. polymyxa* E-12, respectively. These antibiotics are strong basic substances soluble in water, and are primarily active against Gram-negative bacteria in vitro and in vivo, though polymyxin T_1 exhibits somewhat higher activities against Gram-positive bacteria than other polymyxin group antibiotics do (Shoji et al. 1977a). In both polymyxins, S_1 (Shoji et al. 1977b) and T_1, the constituent fatty acid proved to be anteisononanoic acid. Another antibiotic complex, polymyxin P, was also isolated from *B. polymyxa* T-39 (Kimura et al. 1969).

Circulins are a group of basic polypeptides related to polymyxins which are produced by *B. circulans* ATCC 14040 (Fig. 2) (Fujikawa et al. 1965). The isooctanoyl group is the terminal group of the side-chain. Circulin A (**6**) (Vaara and Vaara 2009) and circulin B are similar to polymyxin insofar as their ability to inhibit the growth of Gram-negative more strongly than Gram-positive bacteria, but have a somewhat different bacterial spectrum. They are inactivated by crude trypsin while polymyxin is not and are more toxic than polymyxin (Murray et al. 1949).

A strain of *B. polymyxa* (BP1), isolated from cauliflower seeds, inhibited the growth of microbial phytopathogens. Two antibacterial substances were isolated and purified from culture broth. The first compound was named gavaserin because it contained glutamic acid, alanine, valine, serine and 2,4-diaminobutyric acid, and octanoic acid. No fatty acid was detected in the second compound, which was named saltavalin because it contained serine, alanine, leucine, threonine, valine, and 2,4-diaminobutyric acid (Pichard et al. 1995).

Bacitracin family

Bacitracins are synthesized by certain strains of *B. licheniformis* or *B. subtilis* (Pfaender et al. 1973; Ming and Epperson 2002). Although several bacitracins have been described in the literature, the most thoroughly researched is bacitracin A (**7**), a cyclic decapeptide with four of the aminoacids (glutamic, ornithine, phenylalanine, and asparagine) in the D-configuration. The molecule contains a thiazoline ring structure and exhibits potent antibiotic activity against Gram-positive bacteria (Pavli and Kmetec 2006).

Other decapeptides

Microorganisms isolated from marine habitats have emerged as a promising source of new bioactive metabolites with potential for development into drugs for treating human diseases (Davidson 1995). Loloatins A (**8**) to D (Fig. 2) is a family of cationic cyclic decapeptide antibiotics isolated from a tropical marine *B. laterosporus* collected in Papua New Guinea. Loloatins exhibit in vitro antimicrobial activity against methicillin-resistant *S. aureus*, vancomycin-resistant *enterococci*, and drug-resistant *Streptococcus pneumoniae* (Gerard et al. 1999). In addition to being the most potent of the four family members, loloatin C is also the only one active against the Gram-negative strain *E. coli*, making it an interesting lead compound for further development towards broad spectrum antibiotics (Tuin et al. 2009).

The soil bacterium *Aneurinibacillus migulanus* (formerly known as *B. brevis*) secretes the antibiotic tyrothricin, a mixture of linear pentadecapeptides (gramicidins A, B, and C) and backbone-cyclized cationic decapeptides [gramicidin S (GS) (**9**) and tyrocidins] (Mogi and Kita 2009).

GS (**9**) is an extremely powerful antibiotic drug against a broad spectrum of both Gram-negative and Gram-positive bacteria (Kondejewski et al. 1996). Regrettably, **9** is very hemolytic which presently restricts its use to topical applications (Xu et al. 1995). GS (**9**) is also effective against several pathogenic fungi, and Otoguro et al. (1988) reported nematocidal activities of GS (**9**) and polymyxins against the pine wood nematode *Bursaphelenchus lignicolus*. The gramicidin S (**9**) was also studied for its efficiency in inhibiting insect larvae (Nickerson and Schnell 1983).

Gramicidin S (**9**) and tyrocidines produced by different strains of *B. brevis* share two identical sequences of five different amino acids. Studies in various laboratories have led to suggestions of a possible regulatory function of tyrocidines in the induction of sporulation through a selective inhibition of RNA synthesis during cell differentiation. Despite

its structural similarity to tyrocidine, gramicidin S (**9**) does not inhibit RNA synthesis at the transcription level but does inhibit the uptake of RNA precursors during germination and, as a consequence, it may delay spore outgrowth (Danders et al. 1982).

Consecutive single amino acid substitutions of tyrocidin result in the A (**10**), B and C analogues (King and Craig 1955). Tyrocidin is active against Gram-negative bacteria and is used in a wide variety of pharmaceutical compositions such as dental care products, mouth wash, cough medicines and eye-drops.

Gramicidin S (**9**), tyrocidine A (**10**) and gratisin (GR) [cyclo(-Val^1-Orn^2-Leu^3-D-Phe^4-Pro^5-D-Tyr^6-)$_2$] (Fig. 2) are potent cyclopeptide antibiotics with the amphiphilic β-sheet conformation. In view of widespread resistance to antibiotics that has become a serious threat to public health, amphiphilic antibiotics are attractive targets for drug discovery. It has been proposed that the principal modes of antibiotic action result from an interaction of these antibiotics with the cell membrane of the target microorganisms. Moreover, so far no resistance to the antibiotics has been detected because it requires significant alteration of the lipid composition of the cell membrane. Gramicidin S (**9**) and tyrocidine A (**10**) have very high hemolytic activity, preventing their direct use in combating microbial resistance. However, gratisin showed significantly reduced toxicity against human blood cells and high antimicrobial activity thus proving to be a good drug candidate (Tamaki et al. 2009).

Nonapeptides

Polypeptin-permetin family and related compounds

The cyclic depsipeptide permetin A (**11**) is one member of the polypeptin-permetin family of antibiotics produced by *B. circulans*. The members of this family, polypeptin A, permetin A (**11**) and BMY-28160 have closely related structure, they have the same β-hydroxyl fatty acid constituent and differ from one other only by one or two amino acids (Fig. 3) (Murai et al. 1985). Permetin A (**11**) was isolated from *B. circulans* AJ 3902 and showed activity in vitro against Gram-negative, Gram-positive and some anaerobic bacteria (Yoko et al. 1979).

In the course of screening for new antibiotics with broad antibacterial spectrum, *B. circulans* strain H913-B4 was found to produce a novel antibiotic coded as BMY-28160. Antibiotic BMY-28160 is active against Gram-positive and Gram-negative bacteria, anaerobes and fungi. BMY-28160 is closely related to permetin A (**11**), the difference being the L-valine content in place of L-isoleucine in permetin A (**11**) (Sugawara et al. 1984).

Polypeptin, a basic peptide antibiotic isolated from *B. circulans*, contained 2 components, polypeptin A and polypeptin B, which have identical amino acid components but varied in the structure of the hydroxy acid constituent attached to the α-amino group of the peptide chain. Polypeptin A contained 3-hydroxy-4-methylhexanoic acid and polypeptin B contained 3-hydroxy-5-methylhexanoic acid (Sogn 1976).

Octapeptides

Lipooctapeptides: octapeptin and related compounds

Octapeptins are antibiotics, all octapeptides acylated with a fatty acid residue, which differ with respect to the ratio and the chirality of the constituent amino acids and also in the nature of the fatty acid substituent. Most, if not all, are broad-spectrum antibiotics exhibiting good activity against *P. aeruginosa* (Meyers et al. 1976).

Octapeptins A and B, previously named EM49, have two different peptides: one comprised of five 2,4-diaminobutyric acid (DAB), one Phe, and two Leu residues and the other comprised of five DAB and three Leu residues. Each of these variants is acylated

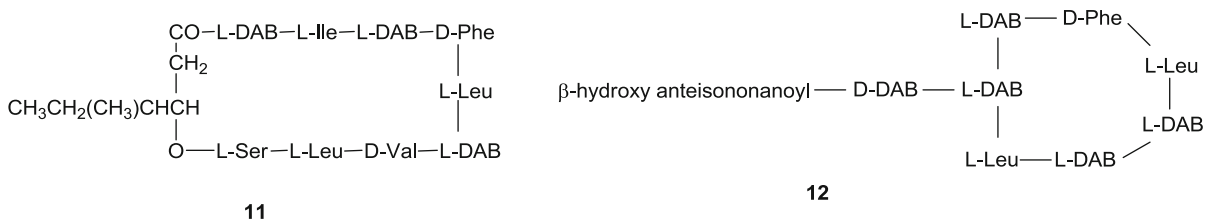


Fig. 3 Nona- and Octapeptides

with a C-10 or a C-11 β-hydroxy fatty acid. Moreover, the EM49 antibiotics have been reported to have moderate activity against yeasts and filamentous fungi (Parker and Rathnum 1975). The antibiotic octapeptin C_1 (**12**), previously named 333-25 (Shoji et al. 1976b), is an acylpeptide produced by a strain of *B. circulans* (Fig. 3). It is active against Gram-positive and Gram-negative bacteria (Kato and Shoji 1976).

Octapeptin D is another member of the octapeptin group of antibiotics, produced by *Bacillus* strain JP-301 (Shoji 1978). Octapeptin D is assumed to be a complex of components which differ at the fatty acyl residue. It appears to be an octapeptide in which the γ-amino groups of the three DAB residues were uncovered and the N-terminal amino groups were masked. Furthermore, a ring structure with a branched chain was also suggested in which one DAB residue was present at the branching point (Kato and Shoji 1980).

Heptapeptides

Lipoheptapeptides: iturins and surfactins families

Iturin family

The iturin family is of great biotechnological and pharmaceutical interest. These are polar cyclic heptapeptides extracted from various strains of *Bacillus* spp. These compounds contain one β-amino fatty acid and seven α-amino-acids with D-tyrosin as the second amino acid and two additional D-amino acids at positions 3 and 6 (Fig. 4) (Chen et al. 2009c). Numerous pharmacological properties have been reported for various iturins, including potent antifungal, antibiotic and antitumor activities (Trischman et al. 1994). They exhibit strong antifungal activities against a wide variety of pathogenic yeasts and fungi but their antibacterial activities are restricted to some bacteria such as *Micrococcus luteus* (Maget-Dana and Peypoux 1994). The iturin group contains iturins A, C, D and E, bacillomycin D, F and L, bacillopeptin and mycosubtilin (Moyne et al. 2004).

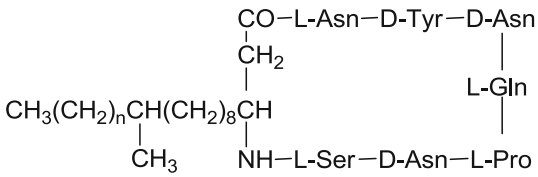
Iturin A (**13**) is a cyclic lipopeptide produced nonribosomaly by several strains of *Bacillus* spp. with an aliphatic chain of the β-amino acid with 14 or 15 carbon atoms (Fig. 4) (Peypoux et al. 1978a). Compound **13** exhibits antibacterial activity on *M. luteus* (Maget-Dana and Peypoux 1994) but is more effective against fungi and yeasts, passing through the cell wall and disrupting the plasma membrane with the formation of small vesicles and aggregation of intramembranous particles (Thimon et al. 1995). The antibiotic strength of iturin A (**13**) is related to change in the permeability of the membrane cells which leads to a leakage of K^+ from the intracellular medium (Harnois et al. 1989). It has recently been reported that iturin A (**13**) is the principal inhibitor in the biocontrol activity of *B. amyloliquefaciens* PPCB004 against postharvest fungal pathogens (Arrebola et al. 2010). In addition, the lipopeptide iturin A (**13**) is able to cause dose-dependent hemolysis in human erythrocytes (Aranda et al. 2005).

Iturin C, normally found together with iturin A (**13**), differs from it by the presence of an L-aspartyl residue linked to the carboxyl group of the β-aminoacid in spite of the L-asparaginyl residue in iturin A (**13**). In contrast to iturin A (**13**), iturin C exhibits no antibiotic activity (Peypoux et al. 1978b). Two novel metabolites with long chain β-amino acid moieties of different length were isolated from a strain of *Bacillus* sp. as inhibitors of oxidized low density lipoprotein (oxidized LDL) binding. These metabolites were shown to be related to the cyclic lipopeptide iturin C (Park et al. 1995).

Iturin D and iturin E, isolated from a strain of *B. subtilis* producing iturin A (**13**), differ from iturin A (**13**) by the presence of a free carboxyl group in iturin D and a carboxymethyl group in iturin E. Iturin D and iturin E exhibited strong antifungal activity against yeasts and fungi but failed to exhibit any antibacterial activity (Besson and Michel 1987).

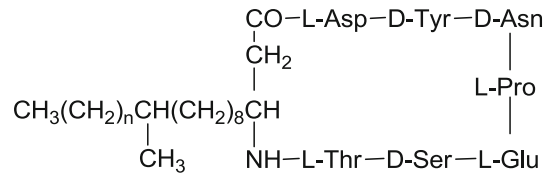
Bacillomycin D (**14**), previously reported by Raubitschek and Dostrovsky (1950) as an antifungal crude preparation isolated from a strain of *B. subtilis*, was later classified in the iturin group (Besson et al. 1976). Bacillomycin D (**14**) is a mixture of two homologous lipopeptides: the lipid moiety consists of 3-amino-12-methyltridecanoic or 3-amino-12-methyltetradecanoic acid; the peptide moiety contains one residue of each of the following seven amino acids: D-asparagine, L-aspartate, L-glutamate, L-proline, D-serine, L-threonine and D-tyrosine (Peypoux et al. 1981). Bacillomycin D (**14**) has also been isolated from an endophytic *B. vallismortis* ZZ185 which exhibited strong inhibition of the growth of several phytopathogenic fungi, including *Fusarium graminearum*, *Alternaria alternata*, *Phytophthora capsici* and *Rhizoctonia solani* (Zhenzhen et al. 2010).

Bacillomycin F is a cyclic peptidolipid isolated from *B. subtilis*. Preliminary studies have shown that bacillomycin F belongs to the iturin group



n=0 or 1

13



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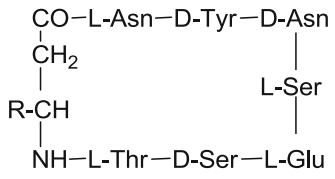

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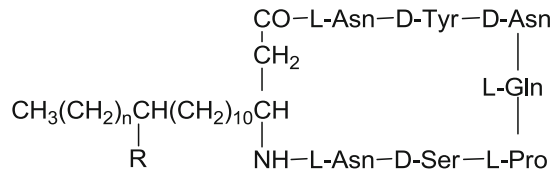
16 R=$(CH_2)_9CH(CH_3)CH_3$
17 R=$(CH_2)_{10}CH(CH_3)CH_3$



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R=H or CH_3

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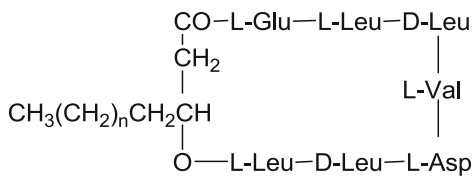
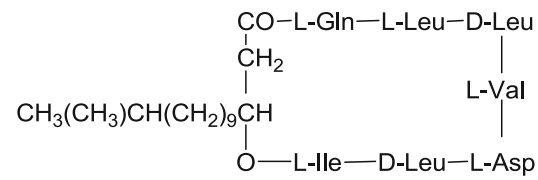


n= 8, 9 or 10

19



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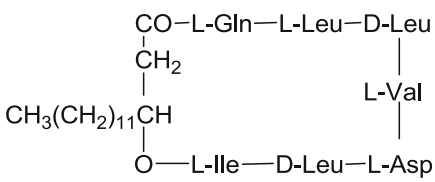

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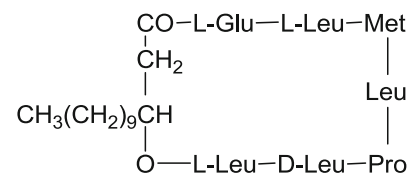

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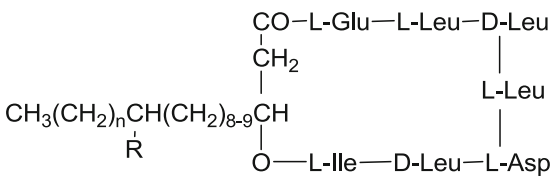
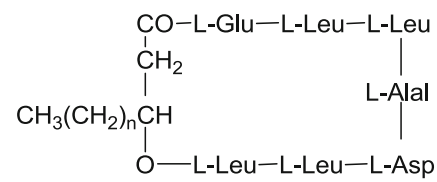


n= 0 or 1
R=H or CH_3

23



24 n= 12
25 n= 13
26 n= 14

Fig. 4 Heptapeptides

(Mhammedi et al. 1982), containing a peptide moiety with L and D α-amino acids and a lipid moiety principally consisting of a mixture of C_{16} and C_{17} β-amino acids. This antibiotic is related to iturin A (**13**) and possesses the same sequence except for the presence of an L-threonine instead of an L-serine residue which is linked to the β-amino acid (Peypoux et al. 1985).

Bacillomycin L is an antifungal agent isolated from a strain of *B. subtilis*. The peptide moiety contains D-aspartic acid, L-aspartic acid, L-glutamine, L-serine, D-serine, L-threonine, and D-tyrosine. The lipid moiety is a mixture of 3-amino-12-methyltridecanoic acid (46 %), 3-amino-12-methyltetradecanoic acid (38 %), 3-amino-14-methylpentadecanoic acid (11 %) and 2 minor homologs (Besson et al. 1977). This antibiotic lipopeptide belongs to the iturinic family of antifungal agents and acts with a strict sterol-phospholipid dependence on biomembranes (Volpon et al. 1999).

Bacillomycin Lc, a new antifungal antibiotic of the iturin class, was isolated from a strain of *B. subtilis* as a set of five congeners. The structure has been shown to differ from that of bacillomycin L by sequence changes from aspartate-1 to asparagine-1 and from glutamine-5 to glutamate-5. The five congeners differ from one other only in the structure of the aliphatic side chain of the constituent β-amino acid (Eshita et al. 1995).

Bacillopeptins A (**15**), B (**16**), and C (**17**) (Fig. 4) were identified as iturin-group antifungal antibiotics, isolated from *B. subtilis* FR-2 (Kajimura et al. 1995). Their structures were elucidated as cyclic lipopeptides analogous to bacillomycin L, containing long-chain β-amino acids and L-Asn and L-Glu instead of the L-Asp and L-Gln residues found in bacillomycin L. Bacillopeptin C (**17**) proved active against yeasts and fungi, whereas bacillopeptins A (**15**) and B (**16**) did not exhibit any activity at 100 μg/mL.

Mycosubtilin (**18**), isolated from *B. subtilis*, is a cyclopeptide consisting of seven α-amino acids closed by a β-amino acid linkage similar to that found in other antibiotics of the iturin group (Peypoux et al. 1986). This compound has a strong lytic action on erythrocytes (Besson et al. 1989). Mycosubtilins B and C were isolated from *B. subtilis* and differ by the presence of a carboxyl group and carboxymethyl group respectively instead of a carboxamide group in previously described mycosubtilin (**18**) (Besson and Michel 1990).

Surfactin family

Surfactins, powerful amphiphilic membrane-active biosurfactant lipoheptapeptides with a structure similar to that of iturins, are not fungitoxic by themselves but do have some synergistic effects on the antifungal activity of iturin A (**13**) (Maget-Dana et al. 1992). Surfactin (**19**), produced by *Bacillus* species like *B. subtilis* (Rahman et al. 2006), *B. amyloliquefaciens* (Sun et al. 2006), *B. licheniformis* (Tendulkar et al. 2007), and *B. natto* (Kameda et al. 1974), is a heptapeptide with an LLDLLDL chiral sequence linked by a β-hydroxy fatty acid consisting of 13–15 carbon atoms to form a cyclic lactone structure (Fig. 4) (Chen et al. 2009c).

The antagonistic lipopeptide surfactin (**19**), secreted by *B. licheniformis* BC98, had significant activity against different phytopathogenic fungi including *Magnoporthe grisea* (Tendulkar et al. 2007).

Surfactin (**19**) possessed antimicrobial (Vater 1986), antiviral (Kracht et al. 1999; Vollenbroich et al. 1997a), antitumor (Kameda et al. 1974), hemolytic (Kikuchi and Hasumi 2002), blood anticoagulant and fibrinolytic (Kim et al. 2006) activities. As one of the strongest biosurfactants (Nicolas 2003), surfactin (**19**) has numerous environmental and biotechnological applications (Solaiman 2005) and has proven to be particularly useful in oil recovery (Youssef et al. 2007), remediation of soil contaminated by heavy metals (Mulligan 2005) and biocontrol against phytopathogens (Bais et al. 2004) and insects (Assie et al. 2002). Diverse new properties have been identified including emulsification, foaming (Razafindralambo et al. 1998) inhibition of star-fish oocyte maturation (Toraya et al. 1995) and antimycoplasmic activities (Vollenbroich et al. 1997b).

The biological role of surfactin (**19**) is believed to support the colonization of surfaces and acquisition of nutrients through their surface-wetting and detergent properties. Surfactin (**19**) synthesized by *B. amyloliquefaciens* FZB42 protects it against bacteria and enables it to form biofilms thus equipping bacterium with powerful antagonistic advantages during surface colonization (Chen et al. 2009a).

The production of the lipopeptide biosurfactants lichenysins by various strains of *B. licheniformis* has been reported during the past few years (Jenny et al. 1993). Interest in such compounds has increased because of their successful applications for in situ microbial oil recovery and dispersion of oil spills

(Clark et al. 1981). Elucidation of their structures has shown that the basal structure is similar to that of surfactin (**19**), the basic difference between surfactin (**19**) and lichenysin turning out to be an amide residue at position 1 (Gln) or 5 (Asn) in lichenysin instead of an acidic one in the case of surfactin (Grangemard et al. 1999).

Eight types of lichenysin commonly produced by *B. licheniformis* are lichenysin A (**20**), lichenysin B, lichenysin C, lichenysin D, lichenysin G, [Val7] lichenysin G, [Ile4] lichenysin G and [Ile2,4] lichenysin G (Yakimov et al. 1995; Konz et al. 1999; Danders et al. 1982). Differences in lichenysin types are due to the kinds and sequences of amino acids in the lactone ring (Li et al. 2008).

Lichenysin A (**20**) is a cyclic halotolerant lipoheptapeptide (Yakimov et al. 1997) produced by several *B. licheniformis* strains (Yakimov et al. 1995; Grangemard et al. 2001; Yakimov et al. 1999). The antibiotic activity of lichenysins was shown to be lower than that of surfactin (**19**) (Yakimov et al. 1995) but they are more potent surfactants than surfactins, thus emphasizing the predominant role of the carboxyl group in the aggregation process (Grangemard et al. 1999). Lichenysins are also produced by other *Bacillus* species such as a soil microorganism identified as *B. megaterium* (Pueyo et al. 2009).

Halobacillin (**21**) is an acylpeptide similar to surfactin (**19**) and is produced by a *Bacillus* species; culture CND-914, isolated from a deep-sea sediment core. Halobacillin (**21**) exhibited moderate human cancer cell cytotoxicity but, in contrast to the iturins, no antifungal or antibiotic activity (Trischman et al. 1994).

A complex of metabolites consisting of two isomeric cyclic acylpeptides identified as isomers of halobacillin (**21**) was isolated from a culture of *Bacillus* sp. A1238. The complex was designated isohalobacillin. Each molecule of isohalobacillin subcomponents contains either a 3-hydroxy-1-oxo-13-methyltetradecyl or a 3-hydroxy-1-oxo-12-methyltetradecyl moiety instead of the 3-hydroxy-1-oxopentadecyl moiety found in the halobacillin (**21**). It inhibited Acyl-CoA: cholesterol acyltransferase activity: cholesterol acyltransferase, a microsomal enzyme that catalyzes the synthesis of cholesteryl esters from acyl-CoA and cholesterol, plays key roles in both intestinal absorption of cholesterol and cholesteryl ester accumulation in macrophages (Hasumi et al. 1995).

Bamylocin A (**22**), a novel lipopeptide related to surfactin (**19**) from *B. amyloliquefaciens* LP03 having antagonistic and crude oil-emulsifying activity, was isolated recently. As constituents of the peptide and lipophilic part, seven amino acids and β-hydroxy-C13 fatty acid were determined (Lee et al. 2007).

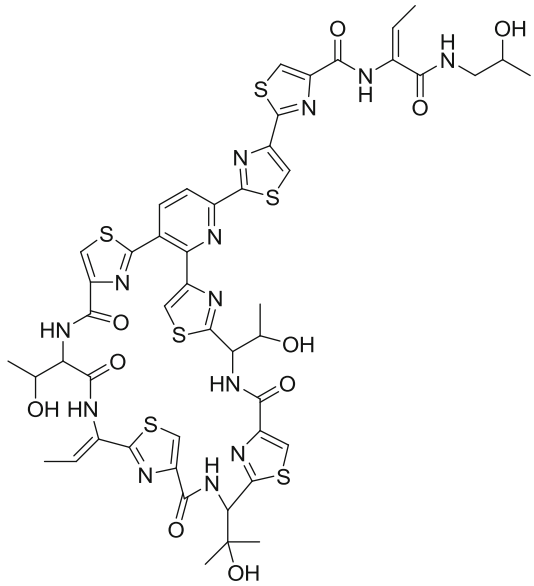
Other cyclic lipopeptides related to surfactin (**19**) are pumilacidins A (**23**), B, C, D, E, F and G, which were isolated from the culture broth of a strain of *B. pumilus*. They are cyclic acylheptapeptides composed of a β-hydroxy fatty acid, two L-leucine, two D-leucine, L-glutamic acid, L-aspartic acid and L-isoleucine (or L-valine). Pumilacidins A (**23**), C and E contain L-isoleucine at the C-terminal, while pumilacidins B, D, F and G have L-valine at this position. Pumilacidin screened as an antiviral antibiotic active against herpes simplex virus was found to have inhibitory activity against gastric H^+, K^+-ATPase and to be protective against gastric ulcers in vivo (Naruse et al. 1990). A strong inhibitory activity against the fungi *R. solani*, *Pythium aphanidermatum* and *Sclerotium rolfsii* was exhibited by pumilacidin isolated from the cassava endophyte *B. pumilus* MAIIIM4a in Brazil (Pereira de Melo et al. 2009).

The implication of pumilacidin was also reported in food poisoning from a *B. pumilus* strain. The ability of pumilacidin to inactivate oxidative phosphorylation in mitochondria due to destruction of the cell membrane was suggested to explain some of the symptoms (From et al. 2007).

Three new lipopeptides (**24**–**26**), members of the surfactins family, were recently isolated from *B. amyloliquefaciens* BO7 (Fig. 4). These newly characterized lipopeptides exhibited a strong inhibitory activity against *Fusarium oxysporum*, an economically important fungal pathogen affecting a large number of different crop plants, highlighting their potential role in biocontrol activity of *B. amyloliquefaciens* (Romano et al. 2011).

Hexapeptides

Fusaricidins A (**27**), B, C and D, antifungal antibiotics more potent than bacillopeptins, were found to be produced by *B. polymyxa* KT-8 (Kajimura and Kaneda 1997). They are cyclic hexadepsipeptides all containing 15-guanidino-3-hydroxypentadecanoic acid as a side chain (Fig. 5). Fusaricidins strongly inhibit the growth of various kinds of fungi and show strong inhibitory activity against Gram-positive bacteria (Kaneda and Kajimura 2002).



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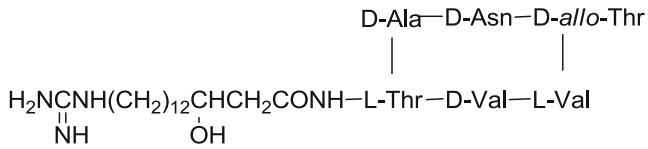

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Fig. 5 Hexapeptides

A group of antibiotics characterized by high sulfur content were found to be produced by a variety of *Bacillus* species. Thiocillins I (**28**) and II were isolated from *B. cereus* G-15 and thiocillins II and III from *B. badius* AR-91. Also, the former two were probably produced by *B. megatherium* I-13. Thiocillins I (**28**), II and III show antimicrobial activity against Gram-positive bacteria in vitro but not against Gram-negative bacteria (Shoji et al. 1976c). The differences between these antibiotics were clarified in their structural units (Shoji et al. 1981).

Linear peptides

Gramicidins A, B, and C are linear pentadecapeptides isolated from the soil bacterium *A. migulanus* (formerly known as *B. brevis*) together with the backbone-cyclized cationic decapeptides gramicidin S (**9**) and tyrocidins (Mogi and Kita 2009).

In contrast to gramicidin S (**9**) (Fig. 2), the structure of linear gramicidins like gramicidin A is an unconventional β-helix (6.3 amino acid residues per turn) with the alternating L- and D-amino acid composition except for position 2 (Gly) (Wallace 1986). All side chains point outward and linear gramicidins form N-to-N termini dimeric ion channels (Wallace 1998) which selectively transport alkaline metal cations and protons across the lipid bilayer (Kelkar and Chattopadhyay 2007).

Five analogues of gramicidin A are also available from the bacterial extracts. Whereas gramicidin B differs from gramicidin A by a single Trp11 → Phe substitution, gramicidin C exhibits a Trp11 → Tyr replacement. In addition, a Val1 → Ile modification exists for each of these analogues (Vogt et al. 2003). Another analogue, gramicidin K, contains formyl and ethanolamine blocking groups and can be resolved into two components, one of which contains tyrosine. In lipid bilayer membranes, both components form channels of considerably longer lifetime and somewhat lower conductance than gramicidin A. Gramicidin K appears to be a lipopeptide that consists of a fatty acyl chain attached to the ethanolamine of gramicidin A (Koeppe et al. 1985).

Four compounds belonging to the class of lower mass lipopeptides were isolated from *B. thuringiensis kurstaki* HD-1. The four kurstakins were found to have the same amino acid sequence, Thr-Gly-Ala-Ser-His-Gln-Gln, but different fatty acids. Each lipopeptide has a lactone linkage between the carboxyl terminal amino acid and the hydroxyl group in the side chain of the serine residue. Their antifungal activity was demonstrated against *Stachybotrys charatum* (Hathout et al. 2000).

Cerexins is a family of antibiotic peptides produced by *B. cereus*. Cerexins A (**29**) (Fig. 6) and C have been reported to be single entities whereas cerexins B and D are complexes of four acylpeptides whose only difference is a fatty acyl residue (Shoji et al. 1979).

The constituent fatty acid of cerexin A (**29**), an antibiotic active against Gram-positive bacteria, was elucidated to be β-hydroxy isoundecanoic acid. The asparaginyl asparagine linkage in the amino acid sequence of the antibiotic proved to be a normal α-carboxyl peptide bond (Shoji et al. 1976d).

Cerexin B is another amphoteric antibiotic produced by *B. cereus*, closely related to cerexin A (**29**). The essential difference between them was shown to be the replacement of serine and one valine residue in cerexin A (**29**) by glycine and phenylalanine in cerexin B (Shoji and Hinoo 1975). The constituent fatty acids for cerexin B were elucidated to be β-hydroxy isodecanoic acid, β-hydroxy decanoic acid, β-hydroxy isoundecanoic acid and β-hydroxy anteisoundecanoic acid (Shoji and Kato 1976b).

Cerexins C and D are closely related to cerexins A (**29**) and B in their physical–chemical and antimicrobial properties. While active against Gram-positive bacteria, they are somewhat less active than cerexin A (**29**). The fatty acid constituents of cerexins A (**29**) and C and cerexins B and D are essentially the same. It was concluded that cerexins C and D are the acylpeptides analogous to cerexins A (**29**) and B, respectively, in which the γ-hydroxylysine residue is replaced by a lysine residue (Shoji et al. 1976e).

The tridecaptin group of antibiotics produced by strains of *B. polymyxa* contains tridecaptins A (**30**), B and C differing from one other in their fatty acid components and amino acid residues (Fig. 6) (Kato et al. 1978). Tridecaptins are assumed to be linear acyl tridecapeptides active against Gram-negative and Gram-positive bacteria in vitro and in vivo (Kato et al. 1979). Each one of the three tridecaptins has been reported to be a complex (Shoji et al. 1979).

Edeines is a mixture of closely related basic peptide antibiotics produced by the *B. brevis* Vm4 strain. These compounds are linear pentapeptide amides with

Fig. 6 Linear peptides isolated from *Bacillus* genus

β-hydroxy isoundecanoyl—D-Asn—D-Val—D-Val—L-Asn—D-Asn—L-γ-Hyl—D-α-Thr—L-Ser—D-Trp—D-α-Ile

L-γ-Hyl = L-*threo*-γ-hydroxylysine

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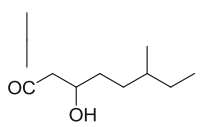
D-Val—D-DAB—Gly—D-Ser—D-Trp—L-Ser—L-DAB—D-DAB—L-Phe—L-Glu—L-Val—D-α-Ile—L-Ala



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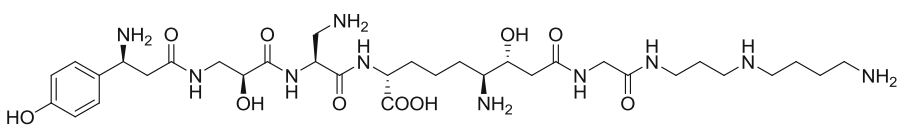

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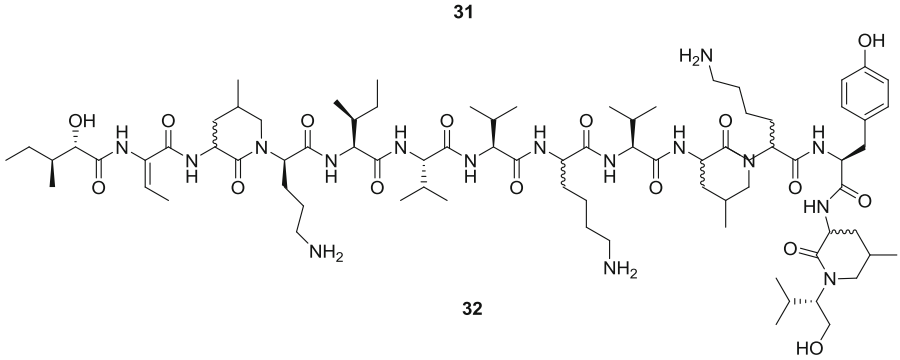

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an unusual amino acid composition; in addition to glycine, the molecule consists of isoserine, β-tyrosine, α,β-diaminopropionic acid, and 2,6-diamino-7-hydroxyazaleic acid. The glycine residue in edeine A (**31**) is attached to the basic constituent, spermidine. In the case of edeine B, the base is guanylspermidine (*N*-guanyl-*N*′-(3-aminopropyl)-1,4-diaminobutane). The structure of edeine D is similar to edeine A (**31**) with β-phenyl-β-alanine substituting for β-tyrosine. Edeine F is composed of amino acids: (*S*)-β-phenyl-β-alanine, (*S*)-isoserine, (*S*)-2,3-diaminopropionic acid, (2*R*,6*S*)-diamino-(7*R*)-hydroxyazaleic acid, glycine and a polyamine guanidylspermidine (Fig. 6) (Wojciechowska et al. 1983). It was shown that the free ionizable carboxy group in the (2*R*,6*S*,7*R*)-2,6-diamino-7-hydroxyazaleic acid moiety is not essential for biological activity in these compounds (Czajgucki et al. 2006).

Edeines exhibit a broad spectrum of antimicrobial activity including Gram-positive and Gram-negative bacteria, yeasts, mould (Chmara and Borowski 1968), and mycoplasmas (Borysiewicz 1966), but their high animal toxicity does not permit their application in chemotherapy as antimicrobial agents. These peptides have the ability to eliminate plasmids determining antibiotic resistance from bacterial cells (Borowski et al. 1977a) and they demonstrate considerable immunosuppressive effects in mice (Borowski et al. 1977b). Edeine antibiotics specifically and reversibly inhibit the biosynthesis of DNA (Kuryło-Borowska and Szer 1972) and have the ability to differentiate prokaryotic and eukaryotic microorganisms based on differences in functioning and structure of the DNA replicating systems (Woynarowska et al. 1979). Edeines are also universal inhibitors of protein synthesis (Obrig et al. 1971).

The strain *B. licheniformis* M-4 produces a hydrophilic peptide with antifungal activity named fungicin M4. It contains the amino acids Glu (8), Arg (5), Pro (4), Tyr (8), Val (3), Met (2) and Orn (4). Its inhibitory spectrum is limited to *Microsporum canis* CECT 2797, *Mucor mucedo* CECT 2653, *Mucor plumbeus* CCM F 443, *Sporothrix schenckii* CECT 2799, *B. megaterium* and *Corynebacterium glutamicum* CECT 78 (Lebbadi et al. 1994).

Bogorol cationic peptide antibiotics contain a number of unusual structural features which include the reduction of the C-terminal residue to valinol, an N-terminal residue of 2-hydroxy-3-methylpentanoic acid, the incorporation of four D-amino acids and the presence of a dehydroamino acid. Bogorols show selective and relatively potent activity against methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus* spp., as well as moderate activity against *E. coli* (Barsby et al. 2006). The most representative compound of this family, bogorol A (**32**), isolated from a strain of *B. laterosporus* obtained from a marine habitat, was shown to have antimicrobial activity against several human pathogens including *S. aureus, E. coli, Enterococcus faecalis*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* and *C. albicans* (Andersen et al. 2002; Barsby et al. 2001).

Esperin is an antibiotic isolated from a strain of *B. mesentericus* (Ogawa and Ito 1951). Esperin contains the sequence R-CH(OH)CH_2CO-Glu-Leu-Leu-Val-Asp-Leu-Leu(Val) where R is a mixture of $C_{10}H_{21}$, $C_{11}H_{23}$, and $C_{12}H_{25}$, and the C-terminal residue contains a 30 % replacement by valine, with a lactone formed by the OH group of the fatty acid constituent and the carboxylic acid function of the aspartic acid residue (Thomas and Ito 1969).

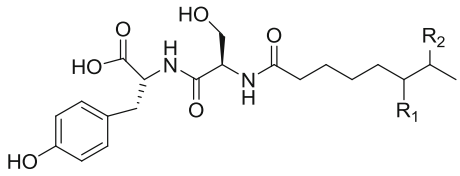
Laterosporin A and laterosporin B are two very similar antibiotics produced by *B. laterosporus* which were active against *Corynebacterium xerosis*, *S. aureus* and *Mycobacterium phlei* (Barnes 1949).

Di- and tripeptides

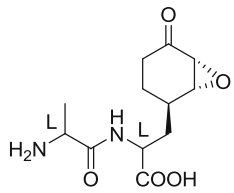
Two acyldipeptides, tupuseleiamides A (**33**) and B (**34**) (Fig. 7), in which the amino acids both have the nonprotein D configuration, are produced by a *B. laterosporus* isolate obtained from a tropical marine habitat. Studies on tupuseleiamide A (**33**) revealed the presence of D-tyrosine and D-serine with a 7-methyloctanoyl fragment which accounted for the remaining site of unsaturation. Analysis for tupuseleiamide B (**34**) showed that it differed from tupuseleiamide A (**33**) only in the methyloctanoyl fragment (Barsby et al. 2002).

Bacilysin (**35**), also named tetaine (Kaminski and Sokolowska 1973), is a dipeptide antibiotic containing an epoxyaminoacid moiety, and L-alanine residue at the N-terminus and a non-proteinogenic amino acid, L-anticapsin, at the C-terminus (Rogers et al. 1965). Bacilysin (**35**), produced by *B. subtilis* (Loeffler et al. 1986; Abraham 1965; Newton 1949), *B. pumilus* (Chmara and Borowski, 1973; Steinborn et al. 2005)

Fig. 7 Di- and Tripeptides



33 R_1=H; R_2=CH_3
34 R_1=CH_3; R_2=H



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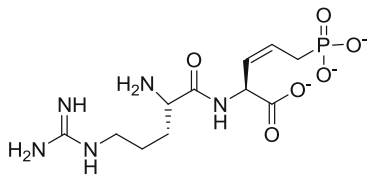

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and *B. amyloliquefaciens* (Chen et al. 2009b), is active against a wide range of bacteria like *S. aureus*, *Corynebacterium xerose* and *E. coli* and against yeast *C. albicans* due to the anticapsin moiety which is released after uptake into susceptible cells (Chmara et al. 1980).

Bacilipins A and B, unsaturated aliphatic N-containing acids related to bacilysin (**35**), produced by *B. subtilis* A14, are active against *M. phlei*. The action of these antibiotics was enhanced when used as mixture (Newton 1949).

Chlorotetaine is a natural product isolated from the *B. subtilis* strain BGSC1E2 (Rapp et al. 1988a) and *B. amyloliquefaciens* (Argüelles-Arias et al. 2009). It is related to the longer known dipeptide bacilysin (**35**) containing the new amino acid (*R*)-3′-chloro-4′-oxo-2′-hexenylalanine (Fig. 7). Chlorotetaine inhibited various fungi and, at higher concentrations, Gram-positive and Gram-negative bacteria. It is the first Cl-containing dipeptide identified as a metabolite of *B. subtilis* (Rapp et al. 1988a).

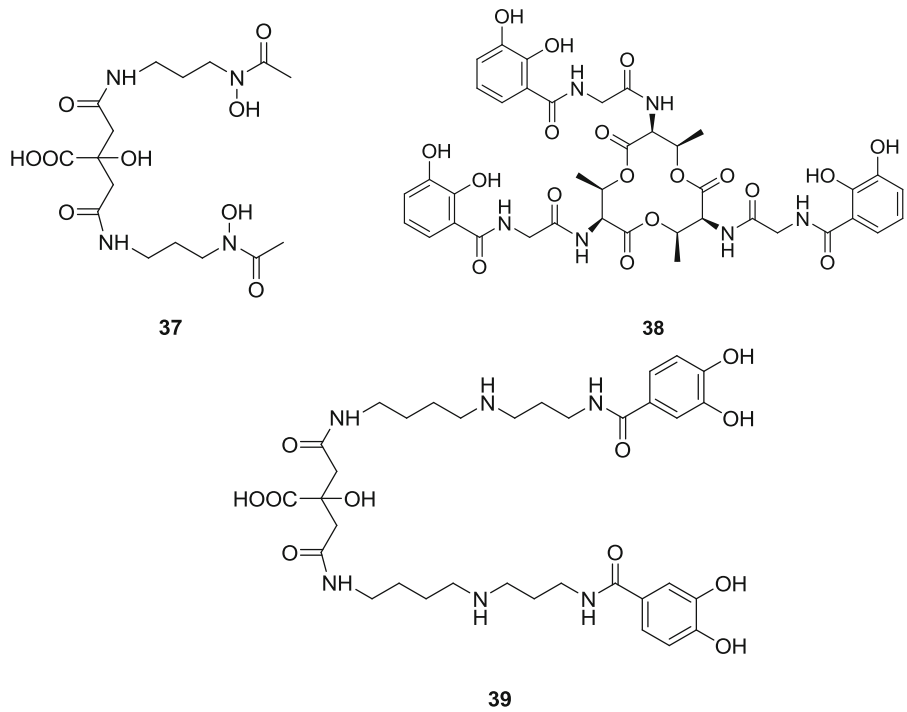
Rhizocticins are phosphonate-containing oligopeptide antibiotics produced by the Gram-positive bacterium *B. subtilis* ATCC6633 (Michener and Snell 1949; Rapp et al. 1988b). They are di- and tripeptide antibiotics consisting of a variable amino acid at the N terminus followed by arginine and the nonproteinogenic amino acid (*Z*)-L-2-amino-5-phosphono-3-pentenoic acid (APPA) (Borisova et al. 2010). Rhizocticins consist of four antifungal hydrophilic peptide antibiotics: L-arginyl-L-2-amino-5-phosphono-3-*cis*-pentenoic acid (rhizocticin A (**36**)), L-valyl-L-arginyl-L-2-amino-5-phosphono-3-*cis*-pentenoic acid (rhizocticin B); and rhizocticin C and D which are the same as rhizocticin but contain L-Ile and L-Leu, respectively, in place of L-Val (Fig. 7) (Kino et al. 2009). Filamentous fungi as well as the cultivated nematode *Caenorhabditis elegans* were found to be sensitive to rhizocticin A (**36**), while bacteria and the protozoan *Paramecium caudatum* were not (Kugler et al. 1990).

Siderophores

Many organisms produce highly specific chelators called siderophores in response to iron limitation. These siderophores chelate and transport iron via specific transport systems to promote cell growth and primarily utilize either hydroxamic acids or catechols for the chelating ligands. Most of the hydroxamate-containing siderophores, including ferrichrome, aerobactin, and mycobactins, contain ω-*N*-hydroxy L-amino acids. However, others such as schizokinen (**37**) (Fig. 8) and ferrioxamine B, utilize 1-amino-ω-(hydroxyamino) alkane residues to bind ferric ion (Lee and Miller 1983).

A specific siderophore is often synthesized by more than one species of microbes. *B. subtilis* utilized 3 types of hydroxamate siderophores, ferrichromes (Takimura et al. 2004), ferrioxamines (Ollinger et al. 2006) and shizokinen (**37**), each of which is taken up by different transport systems (Schneider and Hantke 1993).

Fig. 8 Siderophores isolated from *Bacillus* genus



Bacillus megaterium ATCC 19213 produces the dihydroxamate siderophore schizokinen (**37**). This compound was originally isolated as a factor active in the initiation of cell division (Lankford et al. 1966; Byers et al. 1967) and later shown to be important in iron uptake (Mullis et al. 1971). Schizokinen (**37**) consists of two residues of L-amino-3-(*N*-acetylhydroxyamino) propane linked to the two terminal carboxyl groups of a citric acid residue by amide bonds (Fig. 8). The two hydroxamate groups and the hydroxyl and carboxyl groups on the citrate backbone form the six coordination sites for ferric iron (Plowman et al. 1984).

The monohydroxamate siderophore *N*-deoxyschizokinen was isolated from *B. megaterium* ATCC 19213 and identified as 4-[(3-(acetylhydroxyamino) propyl) amino]-2-[2-[(3-(acetylamino) propyl) amino]-2-oxoethyl]-2-hydroxy-4-oxo-butanoic acid. It has the same carbon skeleton as schizokinen (**37**) but the hydroxyl group on one hydroxamate is replaced by hydrogen (Hu and Boyer 1995).

In addition to their high affinity for ferric ions, these siderophores, schizokinen (**37**) and N-deoxyschizokinen, chelate aluminum. Aluminum was absorbed by *B. megaterium* ATCC 19213 through the siderophore transport receptor providing an extra pathway for aluminum accumulation into iron-deficient aluminum accumulation (Hu and Boyer 1996).

The classical catecholate siderophores contain 2,3-dihydroxybenzoic acid (2,3-DHB) at the iron binding center of the molecule and siderophores based on this moiety have been found in many bacterial species. These microorganisms use orthologs of a common four-gene operon for synthesis of 2,3-DHB from the bacterial aromatic biosynthetic pathway. Thus, the siderophore of *B. licheniformis* K11 (siderophore K11) was determined to be a catechol type siderophore which is produced generally by *Bacillus* spp. (Woo et al. 2007).

The first example of a bacterial catecholate siderophore to be structurally characterized was itoic acid (2,3-dihydroxybenzoylglycine) isolated from low iron fermentation cultures of *B. subtilis* (Ito 1993).

Bacillibactin (**38**), a catecholic trilactone (2,3-dihydroxybenzoate-glycinethreonine) (Fig. 8) (Miethke et al. 2006), is a hexadentate siderophore produced by *B. subtilis* (Raza et al. 2008), *B. anthracis*, *B. cereus*, *B. thuringiensis* (Wilson et al. 2006), *B. amyloliquefaciens* (Chen et al. 2009b) and *B. licheniformis* (Temirov et al. 2003). Bacillibactin (**38**) and many of the siderophores are synthesized nonribosomally by large, multidomain enzymes termed as

nonribosomal peptide synthetases (NRPS) that can assemble peptides of wide structural diversity and broad biological activity (Konz and Marahiel 1999). Bacillibactin (**38**) binds iron through three 2,3-catecholamide moieties linked to a trithreonine scaffold via glycine spacers (Abergel et al. 2009).

The majority of all catecholate siderophores which have been structurally characterized are 2,3-dihydroxybenzoate-containing species. In fact, only petrobactin (**39**) and a related siderophore, petrobactin sulfate, have been reported to contain the 3,4-dihydroxyl substitution pattern (Garner et al. 2004).

The hexadentate 3,4-catecholate/citrate siderophore petrobactin (**39**) is produced by the mammalian pathogens *B. anthracis* (Koppisch et al. 2005) and *B. cereus* (Wilson et al. 2006), apparently to evade the immune protein siderocalin and mediate their iron acquisition during infection; it is essential for the pathogenicity of these organisms.

Bacillus anthracis, the causative agent of anthrax, is a Gram-positive, spore-forming bacterium that readily infects a variety of mammals. Targeted inhibition of the biosynthesis of the essential siderophore petrobactin (**39**), may hold promise as a potential therapeutic treatment against anthrax. Several studies have focused on characterizing the enzymes responsible for petrobactin (**39**) biosynthesis and designing inhibitors for key biosynthetic steps (Pfleger et al. 2009).

Concluding remarks

Bacilli have been known for their biosynthetic potential in the production of ribosomal and nonribosomal bioactive natural products. In recent years, great efforts have been made to elucidate the molecular structures of an important number of bioactive nonribosomal peptides isolated from *Bacillus* species in order to discover the enzymatic mechanisms involved in biosynthesis and to decipher the role played by the genes involved and their regulation. While knowledge is already available on the production and characterization of a vast number of nonribosomal peptides by bacilli, as indicated in this review an important number of peptides structures remain uncharacterized and a great deal of confusing data about peptides abound in the literature.

Genomics has transformed the way in which we think about these microorganisms and has greatly influenced the manner in which we probe their chemistry and biology. A large number of complete and draft genome sequences are now available and *Bacillus* is probably one of the best characterised chemically and one of the most frequently represented genera in the genomic databases bearing witness to the importance of this genus in environmental processes, industrial applications and medicine.

Hopefully, this information will contribute to a better understanding of the features that divide this family into pathogens and environmental and industrial isolates. Furthermore, the genomic data will contribute to a deeper understanding of ecological adaptations when correlating genes with pathogenic potential and with the production of bioactive peptides and metabolites in general, and will help to characterize the vast number of bioactive nonribosomal peptides whose structures remain unresolved.

Although a vast number of bioactive peptides from *Bacillus* genus have been reported, we now know that, in general, conventional natural product isolation programs vastly underestimated their biosynthetic ability, in many cases by upwards of 80–90 % (Nett et al. 2009). As a consequence, orphan secondary metabolic gene clusters deduced from genome sequencing efforts represent an unexplored resource of new chemical entities that promise to provide a novel source of bioactive peptides. Hence, one of the greatest future challenges in this field will be to develop general methods to trigger these silent gene clusters and characterize the chemical biology of their pathway products (Williams et al. 2008).

Further investigation of new antibiotic peptides and their complex regulation of biosynthesis genes will continue to be a fruitful approach to developing a more complete understanding of the biology and metabolite production by these bacteria (Harwood and Cranenburgh 2008). Future sequencing efforts will stimulate the broader scientific community to search for new peptides and lead structures for industrial applications: biosurfactancts (Raaijmakers et al. 2010), plant bioprotectants (Romano et al. 2011), pharmaceutical and agrochemical development (Seydlova and Svobodova 2008), etc., and to exploit commercial strains optimized for the biocontrol of pathogens (in plants and animals) (Harwood and Cranenburgh 2008; Jourdan et al. 2009), in order to prevent the post-harvest loss of fruits and vegetables, such as probiotics (Hong et al. 2005) and for the production of other molecules of

industrial interest such as enzymes (amylases or proteases) or native and heterologous proteins (Ongena and Jacques 2008).

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