Gram-positive antibiotic biosynthetic clusters: a review

A. Argüelles Arias1,2, M. Craig1 and P. Fickers1

1 Centre d'Ingénierie des Protéines, Université de Liège, Institut de Chimie, Bat B6, B-4000 Liège, Belgium.
2Unité de Biotechnologies et Bioprocédés, Université libre de Bruxelles, Av. F.-D. Roosevelt 50, CP165/61 B-1050 Bruxelles, Belgium.
A. Argüelles Arias and M. Craig have contributed equally to this work.

The worldwide emergence of multidrug-resistant pathogens is a serious medical concern nowadays. The need to discover new bioactive molecules active against these bacteria is crucial and is one of the main fields of research for modern microbiologists. Most natural antibiotics used in medicine are biosynthesised by Gram-positive bacteria. Recent advances in genomics and genome sequencing have shown that the potential of these organisms to produce molecules of pharmacological interest has been greatly underestimated. Full genome sequencing has revealed the three main groups according to their biosynthesis pathways: peptides manufactured by the conventional ribosomal assembly, NRPS metabolites (nonribosomal peptide synthetase) and polyketides (PKS – polyketide synthase). The genera Bacillus, Lactobacillus, Enterococcus, Streptococcus and Lactococcus are responsible for the production of the majority of ribosomal peptides (lantibiotics and bacteriocins) whereas NRPS and PKS metabolites – molecules synthesised by large enzymatic complexes – are mostly produced by bacteria of the Actinomycetales order (the Streptomyces genus produces two-thirds of natural antibiotics, antitumour agents and immunosuppressors used in medicine) and by Bacillus species.

This chapter will survey the three main biosynthesis pathways leading to antibiotic production in Gram-positive bacteria – ribosomal peptide synthetase, NRPS and PKS – and provide insights on recently characterised molecules arising from these antibiotic assembly processes.

Keywords: Gram-positive bacteria, polyketides, nonribosomal peptides, bacteriocins, lantibiotics.

1. Introduction

Following the discovery of the first microbial bioactive metabolites in the 1940s, microbiologists have focused on the isolation, purification and characterisation of molecules of pharmacological interest produced by microorganisms. In 1946, streptomycin, an antibiotic produced by Streptomyces griseus, proved to be the first effective treatment against tuberculosis [1,2]. A large number of bacterial metabolites featuring antibacterial (vancomycin, chloramphenicol, bacitracin) antifungal (natamycin) and antitumour (mitomycin) activities were discovered in the following two decades. By the early 1970s, the rate of discovery of bacterial molecules had decreased sharply and by the 1980s, pharmaceutical companies were of the belief that most of the useful compounds biosynthesised by microorganisms had been discovered (Fig. 1). With the increase in multidrug-resistant pathogens, new antibacterial molecules were required and the focus of antimicrobial research shifted to synthetic chemistry. Although an impressive number of molecules were synthesised, the majority lacked the features essential for interaction with biological targets [3].

At the turn of the millennium, advances in genome sequencing have revealed that Gram-positive bacteria possess an unexpectedly high number of genes potentially implicated in the biosynthesis of antibiotic-like compounds. More than 4% of the Bacillus subtilis genome codes for potential gene clusters of polyketides and bacteriocins [4]. The genome sequences of the model actinomycete strain Streptomyces coelicolor and of the industrial strain Streptomyces avermitilis have disclosed the presence of, respectively, 20 and 30 gene clusters implicated in secondary metabolism, mainly polyketide synthases (PKS) and nonribosomal peptide (NRPS) synthetases [5, 6]. These results were unexpected as only a handful of bioactive compounds had previously been identified for each of these strains. Therefore, many potentially useful compounds have not been identified during routine screening or have not been produced under laboratory conditions. The gene clusters that remain unexpressed have been called silent or “cryptic” and represent a great reserve of novel drugs. These bacteriocins, PKS and NRPS silent clusters have generated a renewed interest in bacterial metabolites as microbiologists aim to awaken these cryptic biosynthetic genes [7, 8].

Throughout the golden age of bacterial antibiotic discovery, molecules were identified by standard laboratory screening for antimicrobial activity. The molecules responsible for these activities were then purified and characterised in order to assess their suitability as drugs (antibiotic, antifungal, etc). Further research led to the understanding of their biosynthetic gene cluster organisation and regulatory pathways. Currently, available bacterial genomes are screened for bacteriocin, PKS and NRPS gene clusters, as microbiologists and pharmaceutical companies aim to develop strategies (e.g. optimising culture conditions, modulating the expression of secondary metabolite regulators, etc) leading to the production and characterisation of the cryptic metabolites associated with these biosynthetic gene clusters [9].
Fig. 1 Chronology of drug discovery and emergence of resistant strains. The plain curve represents the discovery rate of useful natural secondary metabolites since the 1940s. The dotted curve highlights the increase in pathogen-resistant strains over the same period. Examples of several biologically active compounds have been placed on the graph according to their year of discovery along with the emergence of their natural resistance (underlined) [10, 11].

2. Polyketide synthases and nonribosomal peptide synthetases

Polyketides (PKs) and nonribosomal peptides (NRPs) are biosynthesised from acyl-coenzyme A monomers and amino (or hydroxyl) acid building blocks, respectively, in an assembly line fashion. NRPs are biosynthesised by large multimodular proteins in which each enzymatic module catalyses one step of elongation and modification of the growing polypeptide chain. Bacterial PKSs are subdivided into two groups [12]. Type I PKSs are very similar to NRPSs, consisting as they do of large multifunctional proteins in which each active site is responsible for the catalysis of one cycle of polyketide elongation and modification. Type II PKSs, on the other hand, feature each catalytic site on distinct mono- or bi-functional proteins [13]. The genes coding for these massive enzymatic complexes are clustered in operons that can span over 150 kb. In addition to the biosynthetic genes, the operons often carry genes coding for regulators, PK or NRP export systems (such as ABC transporters), resistance mechanisms for the molecule synthesised, or proteins that will further modify the secondary metabolite.

2.1 Nonribosomal peptide synthetases

The NRPS mechanism was first described in 1971 during research on gramicidin S and tyrocidin biosynthesis [14]. As stated previously, NRPSs are modularly organised with each module responsible for the incorporation of a specific amino acid. The modules consist of at least three core domains catalysing a specific reaction in the incorporation of a monomer. The first, the adenylation (A) domain, selects the cognate amino acid which it activates by transforming it into an amino acyl adenylate. The thiolation or peptidyl carrier protein (PCP) domain covalently binds the activated monomer to the synthetase by a phosphopantetheinyl arm. The condensation (C) domain catalyses the formation of a peptide bond between the amino acids linked onto two adjacent modules. A dedicated loading module, carrying only the A and PCP domains, is the first module of the NRPS, whereas a termination module containing a thioesterase (TE) domain, which releases the peptide from the synthetase, concludes the assembly line (Fig. 2). These synthetase mechanisms can function in two different ways. Linear NRPSs instigate synthesis starting with the loading module, the following modules each adding a specific amino acid, and ending with the cleavage of the peptide by the TE domain (i.e. surfactin). Iterative NRPSs (i.e. gramicidin S) are able to reuse, in succession, one, several or the totality of the modules present on the synthetases [15]. Many NRPSs feature secondary specialised domains within modules that allow residue modifications. Epimerisation (E) domains lead to D-isomer forms of amino acids, methylation (M), oxidation (Ox), reduction (R), formylation (F) and heterocyclisation (Cy) domains enable NRPSs to biosynthesise an impressive number of diversified peptides with broad biological activities that cannot be produced by the classical ribosomal machinery [16].

In recent decades, numerous bioactive molecules assembled by the NRPS pathway have been discovered and their biosynthetic clusters characterised. Recently, a database containing more than a thousand NRPs has been created,
providing users with an interesting computational tool for systematic study of these molecules in numerous microorganism species [17]. The diversity in structure of these compounds is responsible for their wide-ranging activities and different mechanisms of action, and the very diverse biological targets of these molecules has been exploited for pharmaceutical purposes. Daptomycin, for instance, inserts itself into the bacterial cell membrane causing membrane depolarisation and a potassium ion efflux. This is followed by the arrest of DNA, RNA and protein synthesis resulting in bacterial death [18]. Bacitracin disrupts the biosynthesis of the bacterial cell wall by interacting with undecaprenyl pyrophosphate, thereby enabling the bacteria to assemble peptidoglycan [19]. Echinomycin acts as a DNA intercalating agent in both bacteria and eukaryotic cells. It disables DNA replication leading to bacterial death and is also used to prevent DNA replication in tumour cells, thus preventing the propagation of cancers [20]. Table 1 features a few of these compounds, their biological activities and their producing strains.

With the number of bacterial genomes sequenced increasing by the day and with the emergence of bioinformatic tools predicting the structure of NRPs from the arrangements and sequences of the different modules composing the synthetase, identifying new metabolites by genome mining has become a reality [21]. Recently, an NRPS gene cluster was identified in the genome of *Streptomyces coelicolor*. The predicted structure and theoretical biochemical properties of this hypothetical metabolite has led to the identification and characterisation of a novel peptide siderophore, a potent iron chelator named coelichelin [22]. The discovery of new natural products by genome mining is an encouraging sign, suggesting that this methodology could lead to the isolation of novel molecules of pharmacological interest.

![NRPS gene 1](image1.png) ![NRPS gene 2](image2.png)

**Fig. 2** Schematic representation of a hypothetical nonribosomal peptide synthetase composed 4 modules and encoded by two different genes. The hypothetical resulting product is therefore a tetrapeptide. (A, adenylation; PCP, peptidyl carrier protein; C, condensation; TE, thioesterase).

*2.2 Polyketide synthases*

PKSs assemble the core structures, also called aglycons, of polyketides from acyl-coenzyme A monomers in a head-to-tail fashion. Type I PKSs are similar to NRPSs in that the different catalytic domains are found in a single polypeptide and are further subdivided into iterative and modular (linear) synthases. An iterative type I PKS is a monomodular megasynthase in which a single set of catalytic domains is used repeatedly in a highly programmed fashion. Modular PKSs feature several separate modules that do not repeat. Type II PKSs possess a biosynthetic mechanism analogous to iterative type I PKSs but harbour their catalytic domains on mono- or bi-functional proteins. Several bacteria and fungi, but mostly plants, possess type III PKSs. They consist of a single multimodular protein synthesising molecules which, as they do not show exceptional biological activities, will not be the object of further discussion here [23]. Each PKS module consists of at least three core domains: an acyltransferase (AT) domain which selects the appropriate extender unit and transfers it to the acyl carrier protein (ACP) domain, where a thioester bond is formed fixing the growing polyketide to the synthase, and a ketosynthase (KS) domain. The KS domain is responsible for the condensation between the extender unit present on the ACP domain of the same module and the polyketide intermediate bound to the ACP domain of the preceding module. Additional secondary domains such as ketoreductase (KR), oxidation (Ox), dehydratase (DH), methyltransferase (MT), enoylreductase (ER) and methylation (M) domains modify the growing polyketide molecule. Type II PKSs often feature a cyclase (Cy) domain leading to the formation of aromatic structures. The last module possesses a thioesterase (TE) domain catalysing the release of the final product from the enzyme [24] (Fig. 3). Interestingly, the hedamycin gene cluster of *Streptomyces griseoruber* presents an unusual organisation. This antitumour polyketide antibiotic intercalates between DNA oligonucleotides and is assembled by a hybrid type I/type II PKS. The characterisation of the hedamycin gene cluster harbours 6 type II genes and 2 type I genes [25].
Due to their versatile assembly mechanism, polyketides exhibit remarkable diversity both in terms of their structure and their biological activities (Table 1). Erythromycin, for instance, is a macrolide that inhibits protein synthesis by binding to the 50s subunit of the bacterial 70s rRNA complex [26]. Avermectin is an antiparasitic and insecticidal drug that binds selectively to glutamate-gated chloride ion channels in invertebrate muscle and nerve cells, causing an increase in the permeability of the cell membrane to chloride ions and a resulting hyperpolarisation of the cell, leading to paralysis and death of the parasite [27]. Nystatin binds to sterols in fungal cell membrane, resulting in the cell membrane's inability to function as a selective barrier and the subsequent loss of essential cellular constituents [28].

2.3 Hybrid polyketide synthase and nonribosomal peptide synthetase

Several compounds isolated from bacteria are biosynthesised by NRPS - PKS hybrid synthetases. These metabolites are composed of a polyketide backbone featuring incorporated amino acids in the case of a PKS/NRPS hybrid or a peptidyl chain harbouring ketone groups characteristic of an NRPS-PKS hybrid. The characterisation of several of these secondary metabolite clusters has led the scientific community to assume that these hybrid systems are relatively frequent. The gene cluster coding for the anticancer drug bleomycin is considered the model for hybrid peptide-polyketide synthetases. Its megasynthase consists of 9 NRPS modules and a single PKS module (Fig. 4). The same catalytic sites appear to be conserved in the hybrid PKS-NRPS, with the exception of the KS domain that remains unique [29]. Interestingly, the N-terminal module of the bleomycin biosynthetic cluster features an acyl-CoA ligase (AL) domain instead of the classic adenylation domain. These hybrid metabolite biosynthesis mechanisms greatly expand the structural diversity and functions of natural products (Table 1). Bleomycin is an antibiotic possessing antitumour properties that forms an intermediate iron complex reacting with oxygen to produce superoxide free radicals that cleave DNA [30].
organism specific regulatory genes has led to the production and characterisation of two novel PKs. The deletion, in the model precursors, which could be supplemented to the media leading to the production of the natural compound [40]. Metabolites [7]. Predicting the hypothetical structure of the molecule makes it possible to determine its potential carbon and nitrogen sources. The appropriate medium or a precise nutrient could trigger the production of cryptic morphological development, etc [44, 45].

Molecules could play extremely important roles in cell-cell signalling, transcriptional and metabolism regulation, antimicrobial and antifungal properties) legitimising the important energetic cost required for their synthesis. These growth of bacteria and fungi), PK and NRP metabolites evince a plethora of functions (other than laboratory-induced activity [42]. This approach appears to be a powerful strategy to awaken cryptic secondary metabolite gene clusters that could lead to the discovery of numerous potentially bioactive compounds.

A growing understanding of PKS and NRPS mechanisms has led to the development of a technique yielding "unnatural" natural compounds by genetic engineering. This method consists of adding, subtracting or permuting modules or catalytic domains of a known natural synthase to obtain NRPs and PKs presenting modified structures and "unnatural" natural compounds by genetic engineering. This method consists of adding, subtracting or permuting modules or catalytic domains of a known natural synthase to obtain NRPs and PKs presenting modified structures and functions. Some of these modified metabolites exhibit more potent biological activities than the natural compound [43].

Finally, there is an emerging consensus that, in an environmental concentration range (usually too low to inhibit growth of bacteria and fungi), PK and NRP metabolites evince a plethora of functions (other than laboratory-induced antimicrobial and antifungal properties) legitimising the important energetic cost required for their synthesis. These molecules could play extremely important roles in cell-cell signalling, transcriptional and metabolism regulation, morphological development, etc [44, 45].

### Table 1 Examples of metabolites produced by Gram-positive bacteria used in human medicine along with their biosynthetic mechanisms, therapeutical uses and producing strains.

<table>
<thead>
<tr>
<th>Name</th>
<th>Cluster</th>
<th>Clinical use</th>
<th>Producing strain(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactin</td>
<td>NRPS</td>
<td>Antibiotic/Antiviral</td>
<td><em>Bacillus subtilis</em></td>
<td>[31]</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>NRPS</td>
<td>Antibiotic</td>
<td><em>B. subtilis / B. licheniformis</em></td>
<td>[31]</td>
</tr>
<tr>
<td>Mycosubtilin</td>
<td>NRPS</td>
<td>Antifungal</td>
<td><em>B. subtilis</em></td>
<td>[31]</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>NRPS</td>
<td>Antibiotic</td>
<td><em>Nocardia orientalis</em></td>
<td>[32]</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>NRPS</td>
<td>Antibiotic</td>
<td><em>Streptomyces roseosporus</em></td>
<td>[18]</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>NRPS</td>
<td>Antibiotic</td>
<td><em>S. pristinaespiralis</em></td>
<td>[33]</td>
</tr>
<tr>
<td>Actinomycin</td>
<td>NRPS</td>
<td>Antibiotic/Antitumour</td>
<td><em>S. parvulus</em></td>
<td>[34]</td>
</tr>
<tr>
<td>Echinomycin</td>
<td>NRPS</td>
<td>Antibiotic/Antitumour</td>
<td><em>S. echinatus</em></td>
<td>[20]</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Type I PKS</td>
<td>Antibiotic</td>
<td><em>Saccharopolyspora erythraea</em></td>
<td>[35]</td>
</tr>
<tr>
<td>Nystatin</td>
<td>Type I PKS</td>
<td>Antifungal</td>
<td><em>Streptomyces noursei</em></td>
<td>[24]</td>
</tr>
<tr>
<td>Avermectin</td>
<td>Type I PKS</td>
<td>Antiparasitic</td>
<td><em>S. avermitilis</em></td>
<td>[23]</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Type II PKS</td>
<td>Antibiotic</td>
<td><em>S. aureofaciens</em></td>
<td>[36]</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>Type II PKS</td>
<td>Antitumour</td>
<td><em>S. peucetius</em></td>
<td>[37]</td>
</tr>
<tr>
<td>FK-506</td>
<td>PKS/NRPS</td>
<td>Immunosuppressor</td>
<td><em>S. tsukubaensis</em></td>
<td>[38]</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>NRPS/PKS</td>
<td>Antibiotic/Antitumor</td>
<td><em>S. verticillus</em></td>
<td>[30]</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>PKS/NRPS</td>
<td>Immunosuppressor/Antifungal</td>
<td><em>S. hygrosopicus</em></td>
<td>[39]</td>
</tr>
</tbody>
</table>

### 2.4 Awakening cryptic secondary metabolites and genetic engineering

Table 1 is not an exhaustive list, as many more natural compounds produced by Gram-positive bacteria play an important role in human and veterinary medicine. This highlights the pharmacological importance of these molecules. With the development of resistance mechanisms to these molecules by human and/or animal pathogens and the concomitant decline in the discovery of natural metabolites (exhibited in Fig. 1), new methodologies have been developed to take advantage of the ability of microorganisms to produce these useful bioactive compounds. Genome mining was discussed above in relation to the discovery of the new catecholate siderophore coelichelin. A strategy in vogue nowadays is to try to awaken silent PKS and NRPS clusters. Several approaches have been proposed to express these silent genes and identify their related biosynthetic products. Considering that the biosynthesis of most secondary metabolites is medium-dependent, an easy approach is to test numerous producing media supplemented by different carbon and nitrogen sources. The appropriate medium or a precise nutrient could trigger the production of cryptic metabolites [7]. Predicting the hypothetical structure of the molecule makes it possible to determine its potential precursors, which could be supplemented to the media leading to the production of the natural compound [40].

A novel approach to activating secondary metabolite clusters has proven to be successful. Modulating antibiotic-specific regulatory genes has led to the production and characterisation of two novel PKs. The deletion, in the model organism *S. coelicolor*, of the *schR2* gene encoding a gamma-butyrolactone receptor induced the expression of the orphan *cpk* biosynthetic pathway. The novel polyketide associated with the type I PKS exhibits antibacterial activity [8, 41]. The partial genome sequence of *Streptomyces ambofaciens* reveals the presence of a type I PKS gene cluster spanning 150 kb. These genes are not expressed under laboratory conditions and their metabolomic product has yet to be identified. The constitutive overexpression of a putative LAL (Large ATP binding of the LuxR family) regulator triggered the expression of the gene cluster leading to the production of a giant macrolide with promising antitumour activity [42]. This approach appears to be a powerful strategy to awaken cryptic secondary metabolite gene clusters which could lead to the discovery of numerous potentially bioactive compounds.

The growing understanding of PKS and NRPS mechanisms has led to the development of a technique yielding "unnatural" natural compounds by genetic engineering. This method consists of adding, subtracting or permuting modules or catalytic domains of a known natural synthase to obtain NRPs and PKs presenting modified structures and functions. Some of these modified metabolites exhibit more potent biological activities than the natural compound [43].

Finally, there is an emerging consensus that, in an environmental concentration range (usually too low to inhibit growth of bacteria and fungi), PK and NRP metabolites evince a plethora of functions (other than laboratory-induced antimicrobial and antifungal properties) legitimising the important energetic cost required for their synthesis. These molecules could play extremely important roles in cell-cell signalling, transcriptional and metabolism regulation, morphological development, etc [44, 45].

©FORMATEX 2011
3. Ribosomally synthesised peptides

Beside NRP and PK molecules, bacteria from the *Bacillus* and *Streptomyces* genus also produce ribosomally synthesised peptides with biological activity, the so-called bacteriocin. Based on their structure and biological activities, bacteriocins are divided into three main groups.

Class I bacteriocins, also known as lantibiotics, are characterised by their unusual amino acids such as lanthionine, methyllanthionine and dehydrated residues. Lantionines and methyllanthionines residues are enzymatically synthesised by the cyclisation of a free cysteine and a dehydrated residue, namely dehydroalanine (Dha) and didehydrobutyryne (Dhb), respectively. Dha and Dhb are obtained beforehand by the dehydration of a serine or a threonine [46]. Lantibiotics can be further subdivided into two types based on their structure and mode of posttranslational modifications (see below) (Table 1). Type A lantibiotics exhibit linear secondary structures and are positively charged (Fig. 5A). Upon synthesis, they are modified by two distinct LanB and LanC enzymes and processed by the LanP protease. Type B lantibiotics, conversely, exhibit a globular structure and are non-charged (Fig. 5B). They are modified by a single modification LanM enzyme and processed by a LanT ABC transporter that has N-terminal-associated protease activity [47]. Two-component lantibiotics consisting of two post-translationally modified peptides that act synergistically could be classified into a third group but instead are classified as type B lantibiotics because each subunit is processed by a single modifying LanM-type enzyme [48]. Lantibiotics are mostly produced by *Bacillus* sp.

Class II bacteriocins include small ribosomally synthesised peptides (> 5kDa) that do not display any modification in their amino acids except for, in some cases, the presence of disulfide bridges. Class II bacteriocins can be further subdivided into three subclasses (IIa, IIb, IIc) based on structural properties, activity and mode of action [49, 50]. Class IIa bacteriocins are characterized by an hydrophobic N-termini containing the YGNGV consensus sequence and a disulfide bridge. Class IIb feature two-component non-modified bacteriocin whereas class IIc regroups all the other molecules that do not correspond to class IIa and IIb.

Class III bacteriocins are heat sensitive molecules with a molecular weight superior to 30 kDa. Its only representative among the *Bacillus* genera is the megacin A-216 produced by *B. megaterium*. This 293 amino acids peptide only exhibits a phospholipase A2 activity [51].

<table>
<thead>
<tr>
<th>Class or Subclass</th>
<th>Name</th>
<th>Producing strain</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I Subclass IA</td>
<td>Subtilin</td>
<td><em>B. subtilis</em> ATCC6633</td>
<td>[52]</td>
</tr>
<tr>
<td>Ericin S</td>
<td><em>B. subtilis</em> A1/3</td>
<td>[53]</td>
<td></td>
</tr>
<tr>
<td>Ericin A</td>
<td><em>B. subtilis</em> A1/3</td>
<td>[53]</td>
<td></td>
</tr>
<tr>
<td>Subclass IB</td>
<td>Sublancin 168</td>
<td><em>B. subtilis</em> 168</td>
<td>[54]</td>
</tr>
<tr>
<td>Mersacidin</td>
<td><em>B. subtilis</em> HILY-85</td>
<td>[55]</td>
<td></td>
</tr>
<tr>
<td>Haloduracin</td>
<td><em>B. halodurans</em> C-125</td>
<td>[56]</td>
<td></td>
</tr>
<tr>
<td>Lichenicidin</td>
<td><em>B. licheniformis</em> DSM13</td>
<td>[57]</td>
<td></td>
</tr>
<tr>
<td>Class II Subclass IIA (Pediocin-like)</td>
<td>Coagulin</td>
<td><em>B. coagulans</em> I4</td>
<td>[58]</td>
</tr>
<tr>
<td>SRCAM1580</td>
<td><em>B. citrulans</em></td>
<td>[59]</td>
<td></td>
</tr>
<tr>
<td>Subclass IIB (Thuricin-like)</td>
<td>Thuricin HD2</td>
<td><em>B. thuringiensis</em></td>
<td>[60]</td>
</tr>
<tr>
<td>Cerein MRX1</td>
<td><em>B. cereus</em></td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td>Class III</td>
<td>Magacin A-216</td>
<td><em>B. megaterium</em></td>
<td>[51, 62]</td>
</tr>
</tbody>
</table>

3.1 Genetic loci for lantibiotic production, regulation of production and mode of action

Subtilin, produced by *B. subtilis* ATCC6633, is the most studied class of bacteriocin produced by members of the *Bacillus* genus. Active subtilin is composed of 32 amino acids, eight of which are post-transcriptionally modified: four methyllanthionines (Abu-S-Ala), one didehydrobutyryine, one dehydroalanine and one lanthionine (Ala-S-Ala) and [52]. Despite the relative phylogenetic distance between the producer strains, the structure, biosynthetic pathway, regulation and mode of action of all lantibiotics are highly conserved. Subtilin is a cationic pentacyclic lantibiotic belonging to the
class of type IA lantibiotics. The gene cluster, which is approximately 12 kb for class I bacteriocin synthesis, is composed of a structural gene as well as other genes necessary for the modification, transport, regulation and immunity for self-protection of the producer strain. The subtilin gene cluster is composed of 10 ORFs, *spaBTCSIFEGRK* whose expressions are governed by four distinct promoters (Fig. 5C). Subtilin is synthesised as a 56-residue inactive peptide encoded by structure gene *spaS*, which contains a signal sequence of 24 residues. SpaB and SpaC are involved in the dehydration of serine and threonine, which are required for the formation of Dha and Dhb, respectively, as well as that of lanthionine, which is the result of the cyclisation of Dha with thiol group of a free cysteine. Presubtilin is exported by the ABC transporter protein SpaT and is then processed by the serine protease subtilisin to form a bioactive subtilin. Self-protection of the producer strain from the synthesised lantibiotics is ensured by different immunity mechanisms. The lipoprotein SpaI, a membrane-bound protein, interacts specifically with subtilin causing its inactivation. Another immunity complex formed by SpaF, SpaE and SpaG acts like an ABC transporter which pumps subtilin out of the producer strain.

The production of lantibiotics appears to be regulated at the transcriptional level in a cell-density-dependent manner (i.e. by quorum sensing) and was found to act as an auto-inducing agent [63]. Two proteins, spaR and spaK, corresponding to a response regulator protein and a sensor kinase, respectively, form the regulatory system. After subtilin reaches a specific threshold, it activates the membrane-bound SpaK protein which then autophosphorylates. This leads to the phosphorylation of SpaR, which in phosphorylated form can recognize the binding domain on three promoters upstream of *spaS, spaBTC* and *spaIFEG*, resulting in subtilin production. (Fig. 5C). In addition, *spaRK* expression is controlled by the sporulation transcription factor Sigma H [64]. Therefore, subtilin production is closely related to both to cell density and to sporulation mechanism. Lantibiotics are exclusively active against Gram-positive bacteria by forming pores in target cell membranes. This pore formation could be mediated through an interaction with the so-called lipid II [undecaprenylpyrophosphoryl-MurNAc-(pentapeptide)-GlcNAc] or bactoprenyl pyrophosphate inhibiting, thus, the peptidoglycan synthesis [65].

3.2 Unusual antibiotic peptides.

Members of the *Streptomyces* genus are known to produce unusual lantibiotics which do not exhibit antibiotic activity, one of which is the morphogenetic surface active peptide SapB. This lantibiotic is required for aerial mycelium formation by *Streptomyces coelicolor* [66]. The *ram* gene cluster is made up of five genes: *ramCSABR*. The structure gene *ramS* encodes a non-modified peptide. All modifications required to produce mature peptides are specified by
RamC. SapB is exported from the cell by the ABC transporter encoded by ramAB. These four genes are under the control of the regulatory gene ramR.

Rhizocticins are phosphonate oligopeptide antibiotics containing the C-terminal nonproteinogenic amino acid (Z)-l-2-amino-5-phosphono-3-pentenoic acid (APPA). The molecules are synthesised by the so-called L-amino acid ligase. L-Amino acid ligase is a microbial enzyme catalysing formation of an alpha-peptide bond from unprotected L-amino acids in an ATP-dependent manner. The YwIIE protein from Bacillus subtilis 168, the first reported L-amino acid ligase, synthesises various dipeptides. However, these L-amino acid ligases synthesise only dipeptide and no longer peptide. A novel L-amino acid ligase capable of catalysing oligopeptide synthesis is required to enhance the variety of peptides [67]. The most abundant component, Rhizocticin A, is known to inhibit the growth of yeast and filamentous fungi but is not active against bacteria. The rhizocticin constituent APPA is also a part of the tripeptide antibiotics plumbecmycin A and B produced by Streptomyces plumbeus [68]. Whereas rhizocticins are antifungals, plumbeymycins exhibit antibacterial activity. These molecules enter the target cell via the oligopeptide transport system and must be cleaved by a peptidase in order to release the active APPA [69].

Some strains of B. subtilis produce the dipeptide bacilysin composed of L-alanine and the unusual amino acid L-anticapain [70]. Anticapain inhibits glucosamine synthetase. This inhibition of glucosamine formation causes a failure in the bacterial wall synthesis and is responsible for the ability of bacilysin to inhibit the growth of bacteria [71].

### 4. Industrial Applications

In addition to their obvious antitumour, antibiotic or antifungal activities, natural compounds produced by bacteria feature properties which have been exploited in industry. For example, the lantibiotic nisin, produced by Lactococcus lactis, is used in the alimentary industry as a preservative (E234) in dairy products, including cheese [72]. Furthermore, the very potent antimicrobial activities of Bacillus lantibiotics against pathogenic microorganisms such as B. cereus, Listeria monocytogenes and Staphylococcus spp. make them good candidates for application in the food industry. For example, the antisternal effect on poultry meat of amylolysin, a bacteriocin produced by B. amyloliquefaciens GA1, demonstrates its potent use as a biopreservative [73].

Streptomyces viridochromogenes possesses an NRPS cluster responsible for the biosynthesis of a tripeptide called bialaphos. This molecule is used as a potent herbicide [74]. S. neyagawaensis produces a compound with an antialgal activity which may have, after further characterisation, potential application in controlling cyanobacterial blooms in freshwaters [75]. Natamycin has been used in the food industry for decades to prevent fungal contamination of dairy products and meat [76]. These are only a few examples of industrial applications of PKs, NRPs and bacteriocins. The pharmacological and industrial applications of these bacterial compounds are numerous and diversified. As just a small fraction of the molecules potentially produced by Gram-positive bacteria has been identified, research to discover these compounds is sure to be ongoing for many more years.

### 5. References.


