MicroReview

Bacillus subtilis antibiotics: structures, syntheses and specific functions

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Summary

The endospore-forming rhizobacterium Bacillus subtilis – the model system for Gram-positive organisms, is able to produce more than two dozen antibiotics with an amazing variety of structures. The produced anti-microbial active compounds include predominantly peptides that are either ribosomally synthesized and post-translationally modified (lantibiotics and lantibiotic-like peptides) or non-ribosomally generated, as well as a couple of non-peptidic compounds such as polyketides, an aminosugar, and a phospholipid. Here I summarize the structures of all known B. subtilis antibiotics, their biochemistry and genetic analysis of their biosyntheses. An updated summary of well-studied antibiotic regulation pathways is given. Furthermore, current findings are resumed that show roles for distinct B. subtilis antibiotics beyond the ‘pure’ anti-microbial action: Non-ribosomally produced lipopeptides are involved in biofilm and swarm-ing development, lantibiotics function as pheromones in quorum-sensing, and a ‘killing factor’ effectuates programmed cell death in sister cells. A discussion of how these antibiotics may contribute to the survival of B. subtilis in its natural environment is given.

Introduction

The rhizobacterium Bacillus subtilis (Sonenshein et al. 2001) has been used for genetic and biochemical studies for several decades, and is regarded as paradigm of Gram-positive endospore-forming bacteria (Moszer et al., 2002). Several hundred wild-type B. subtilis strains have been collected, with the potential to produce more than two dozen antibiotics with an amazing variety of structures. All of the genes specifying antibiotic biosyntheses combined amount to 350 kb; however, as no strain possesses them all, an average of about 4–5% of a B. subtilis genome is devoted to antibiotic production. One aim of this review is to give an updated summary of the structures of all B. subtilis antibiotics, the biochemistry and genetic analysis of their biosynthetic pathways, as well as a survey on well-studied regulatory pathways. A further aim is to compile recent findings that demonstrate specific roles for B. subtilis antibiotics beyond the anti-microbial action – distinct antibiotics are involved in the morphology and physiology of B. subtilis and contribute to the survival of this organism in its natural habitat.

The potential of B. subtilis to produce antibiotics has been recognized for 50 years. Peptide antibiotics represent the predominant class. They exhibit highly rigid, hydrophobic and/or cyclic structures with unusual constituents like D-amino acids and are generally resistant to hydrolysis by peptidases and proteases (Katz and Demain, 1977; and references therein). Furthermore, cysteine residues are either oxidized to disulphides and/or are modified to characteristic intramolecular C–S (thioether) linkages, and consequently the peptide antibiotics are insensitive to oxidation. Principally, two different biosynthetic pathways for peptides allow the incorporation of such unusual (non-proteinaceous) constituents: (i) the non-ribosomal synthesis of peptides by large megaenzymes, the non-ribosomal peptide synthetases (NRPSs) and (ii) the ribosomal synthesis of linear precursor peptides that are subjected to post-translational modification and proteolytic processing.

Lantibiotics

Peptide antibiotics with inter-residual thioether bonds as unique feature are outlined as lantibiotics (lanthionine-containing antibiotics) (Schnell et al., 1988). Lanthionine formation occurs through post-translational modification (Fig. 1) of ribosomally synthesized precursor peptides including dehydration of serine and threonine residues, respectively, and subsequent addition of neighbouring cysteine thiol groups (for reviews, see Guder et al., 2000;
is structurally related to the widely utilized biopreservative. The subtilin gene cluster specifies the subtilin prepeptide SpaS, SpaBC for post-translational lanthionine formation, and the translocator SpaT for export of the modified species. The extracellular B. subtilis serine proteases subtilisin (AprE), WpRA and VpR are involved in subtilin processing (Corvey et al., 2003). Subtilin immunity is mediated by the lipoprotein SpaL and the ABC translocator SpaFEG (Klein and Entian, 1994; Stein et al., 2003a). The biosynthesis of subtilin is regulated by a positive feedback mechanism (Stein et al., 2002a; see also a general scheme of B. subtilis regulatory pathways of antibiotic biosynthesis in Fig. 4) in which extracellular subtilin activates the two component regulatory system SpaK (sensor histidine kinase) and SpaR (regulator protein) that binds to a DNA motif (spa-box) promoting the expression of genes for subtilin biosynthesis (spaS and spaBTC) and immunity (spaFEG) (Stein et al., 2003b; Kleerebezem, 2004). SpaRK expression is controlled by the sporulation transcription factor SigH, which itself is repressed during exponential growth by the transition-state regulator AbrB (Fawcett et al., 2000). Thus, subtilin production appears to be dual controlled, to culture density in a quorum-sensing mechanism in which subtilin plays a pheromone-type role and in response to the growth phase (mediated by Abrb/SigH; Stein et al. 2002b).

The B. subtilis strain A1/3 produces ericin (Fig. 2; Stein et al., 2002b). Surprisingly, the ericin gene cluster contains two structural genes, eriA and eriS, although the open reading frames (ORFs) are closely related to corresponding genes of the subtilin cluster. Ericin S and subtilin only differ in four amino acid residues, and expectedly the anti-microbial properties of both lantibiotics are comparable. However, ericin A has a different ring organization and 16 amino acid substitutions compared with ericin S. This compound becomes fully matured and is produced in equal quantities as ericin S. The need for only a single synthetase (EriBC) for two different products (ericin A/S) reflects the flexibility of lantibiotic pathways.

The lantibiotic mersacidin (Fig. 2) belongs to the type B lantibiotics which exhibit a more globular structure. It inhibits cell wall biosynthesis by complexing lipid II (Brötz et al., 1997). The mersacidin gene cluster consists of the structural gene mrsA, as well as genes involved in post-translational modification (mrsM and mrsD), transport (mrsT), immunity (mrsFEG) and regulation (mrsR1 mrsR2, mrsK2). Whereas MrsR1 regulates mersacidin biosynthesis, the two-component regulatory system MrsR2/K2 appears to regulate the expression of the mersacidin immunity transporter specifying genes mrsFEG (Guder et al., 2002). Mersacidin production occurs from the beginning of the stationary phase; however, the link between its mersacidin regulatory systems and the cellular regulation network of B. subtilis is yet unknown.

Jack and Jung, 2000; McAuliffe et al., 2001). Based on structural properties two lantibiotic types are distinguishable. Type A lantibiotics (21–38 amino acid residues) exhibit a more linear secondary structure and kill Gram-positive target cells by forming voltage-dependent pores into the cytoplasmic membrane. Remarkably, for the lantibiotic nisin produced by Lactococcus lactis it has been shown that the bactoprenol-bound ultimate peptidoglycan precursor lipid II represents both an important docking/receptor molecule (Breukink et al., 1999) and an intrinsic component of the lethal pore (Hasper et al., 2004). Gram-positive lantibiotic producers exhibit efficient countermeasures to obviate the action of their own products. Self-protection (immunity) against lantibiotics is based on ATP-binding cassette (ABC) transporter homologous proteins (LanFEG) that export the lantibiotic from the cytoplasmic membrane into the extracellular space (Stein et al., 2003a). The biosynthesis of lanthionine is dual controlled, to culture density in a quorum-sensing mechanism in which subtilin plays a pheromone-type role and in response to the growth phase (mediated by Abrb/SigH; Stein et al. 2002b).

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MrsD, a member of the homo-oligomeric flavin-containing cysteine decarboxylases (HFCD) family, catalyses the oxidative decarboxylation of the C-terminal cysteine of the mersacidin prepeptide. The dodecameric MrsD and its closely relative EpiD involved in epidermin biosynthesis of *Streptococcus epidermidis* represent the sole examples of lantibiotic modifying enzymes with known three-dimensional structures (Blaesse et al., 2003).

**Unusual lantibiotics**

Sublancin 168 with a β-methylanthionine bridge and – unusual for lantibiotics, two disulphide bridges (Fig. 2; Paik et al., 1998), acts preferentially against Gram-positive bacteria. Its structural gene *sunA* (formerly *yolG*) belongs to the *B. subtilis* temperate bacteriophage SPβ (Westers et al., 2003) and thus, sublancin and the ‘prophage SPβ-mediated bacteriocin’ (Hemphill et al., 1980) are most probably the same compounds. An ABC transporter (SunT) and two thiol-disulphide oxidoreductases (BdbAB) belong to the sublancin locus (Fig. 2). Only BdbB seems to be dedicated for sublancin production, most probably for the formation of the disulphide bonds (Dorenbos et al., 2002). The BdbB parologue BdbC protein is at least partially able to replace BdbB in sublancin production, but contrariwise BdbB cannot complement the function of BdbC (competence development), showing that these two closely related thiol-disulphide oxidoreductases have different, but partly overlapping substrate specificities (Kunst et al., 1997; Dorenbos et al., 2002). The SPβ locus including the sublancin gene cluster is not essential for *B. subtilis* survival (Westers et al., 2003). However, it contains yet unidentified genes mediating resistance against sublancin action. One attractive hypothesis is that sublancin might contribute to the survival of bacteriophage, e.g. that sublancin kills only non-lysogenized cells and thus, enriching the per cent of a lysogenized *B. subtilis* population.

Subtilosin A produced by several *B. subtilis* strains (Zheng et al., 1999; Stein et al., 2004) has a macrocyclic
structure (Fig. 2) with three inter-residuak linkages (Marx et al., 2001) that have been elucidated as thioether bonds between cysteine sulphurs and amino acid alpha-carbons (Kawulka et al., 2004). It acts against a variety of Gram-positive bacteria, including Listeria (Zheng et al., 1999). The sbo-alb (anti-lijisterial bacteriocin) cluster encodes proteins AlbA (YwiA) most probably involved in post-translational modification of presubtilisin, AlbF (YwhH) probably acting in subtilisin processing and the subtilisin immunity proteins AlbB-D (YwhOPO) (Zheng et al., 2000). Expression of the sbo-alb genes occurs under stress conditions (Nakano et al., 2000) under AbrB control (Zheng et al., 1999; see also Fig. 4).

Non-ribosomal biosynthesized peptides

The non-ribosomal synthesis of peptide antibiotics is widespread among bacteria and fungi (for recent reviews, see Sieber and Marahiel, 2003; Finking and Marahiel, 2004; Walsh, 2004; and references therein). Large multi-enzymes, the NRPSs, that are composed of modularly arranged catalytic domains (Fig. 3A), catalyse all necessary steps in peptide biosynthesis including the selection and ordered condensation of amino acid residues. Each elongation cycle in non-ribosomal peptide biosynthesis needs the cooperation of three core domains. (i) The adenylation domain (550 amino acid residues) selects its cognate amino acid and generates an enzymatically stabilized aminoacyl adenylate. This mechanism resembles the amino-acylation of tRNA synthetases during ribosomal peptide biosynthesis. (ii) The thiolation or peptidyl carrier domain (80 aa) is equipped with a 4'-phosphopantetheine (PPan) prosthetic group to which the adenylated amino acid substrate is transferred and thiosterified under release of AMP. Thus, the PPan cofactor acts as thio template and as a swinging arm to transport intermediates between the various catalytic centres. The peptidyl carrier proteins are post-translationally converted from inactive apoforms to their active holoforms by dedicated PPan transferases (Lambalot et al., 1996). (iii) The formation of a new peptide bond is catalysed by condensation domains (450 aa) located between each pair of adenylation and peptidyl carrier domains. The linear assembly line-like arrangement of multiple of such core units (i–iii) ensure the co-ordinated elongation of the peptide product. In most of the cases the non-ribosomal peptide biosynthesis is terminated by macrocyclization of the peptide product, whereby parts of the molecule distant in the constructed linear peptide chain are covalently linked to one another (Kohli and Walsh 2003). Typically, such reactions are catalysed by thioesterases domains at the C-terminal end of the NRPS assembly line. The depicted mechanism of peptide biosynthesis has been outlined in the concept of the ‘Multiple Carrier Model of Nonribosomal Peptide Biosynthesis at Modular Multienzymatic Templates’ (Stein et al., 1996). Mechanistically, NRPSs are closely related to polyketide synthetases (PKSs), as both modular systems utilize multiple Ppan carriers for covalent binding of monomers and growing chains. Both systems are highly flexible in which naturally rearrangements can be easily achieved within a relatively short period, permitting the random evolution of compounds that provide selective advantages. Striking examples for such flexibility are the systems specifying the biosynthesis of the closely

Fig. 3. Summary of B. subtilis antibiotics.
A. Non-ribosomally synthesized peptide antibiotics. In each line the producing B. subtilis strains, the genetic organization of the NRPSs (boxed), and schematic representations of produced peptide antibiotics and their possible isoforms are given. Amino acid residues, usually in L-configuration, are shown in the single-letter code, and residues in D-configuration are underlined; the fatty acid moieties are hatched and the positions of their carbon atoms are indexed (C). For mycosubtilin synthetase the denotation of the NRPSs symbols is explicitly shown: mycA codes for an NRPS (449 kDa) encompassing domains for an acyl- ligase (AL), a ketosynthase (KS) and an acylmethyltransferase (AMT) followed by an elongation unit for asparagine (N). Each modularly arranged elongation unit contains a domain for adenylation of the amino acid substrate, a peptidyl carrier protein (PCP) and a condensation domain where the formation of a new peptide bond occurs. In the case of amino acids in D-configuration, the NRPSs contain an additional epimerase domain. Numbers correspond either to the size of the gene clusters (in kb) or to the derived molecular mass of the NRPSs (in kDa).
1Surfactin consists of a heptapeptide moiety bonded to the carboxyl and hydroxyl groups of a β-hydroxy fatty acid. Its production is widely distributed among B. subtilis, pumilus, licheniformis and amyloidefaciens strains and thus, a disconcerting variety of surfactin isoforms have been described under different synonyms such as bacirince, halo- and isohalobactin, lichenysin A/G, daitocin and pumilacidin (summarized in Peypoux et al., 1999; Kalinovskaya et al., 2002).
2The iturine lipopeptide family share a β-amino fatty acid as integral constituent, positions 1–3 of the peptide moiety (L-Asx-D-Tyr-D-Asx) and an additional Ω-amino acid at position 6.
3Fengycin (plipastatin) consists of a β-hydroxy fatty acid connected to the N-terminus of a decapeptide including four D-amino acid residues and the rare amino acid L-ornithine. The C-terminal residue of the peptide moiety is linked to the tyrosine residue at position 3, forming the branching point of the acylpeptide and the eight-membered cyclic lactone.
4NRPSs can be involved in producing compounds other than antibiotics: Corynebactin (DHB-Gly-Thr), produced by Corynebacterium glutamicum (Budzikiewicz et al., 1997) is a 12-membered trilactone macrocyclic ring composed of three threonine residues, each connected to dihydroxybutyrate (DHB) via glycin spacers; the B. subtilis product has been renamed to bacillibactin (May et al., 2001). Corynebactin/bacillibactin acts as a siderophore; complexing of ferric iron occurs by the six hydroxyl groups of the DHB moieties.
B. Structure representations of further non-ribosomally synthesized B. subtilis peptide antibiotics and miscellaneous antibiotics (Wilson et al., 1987; Hilton et al., 1988; Kitajima et al., 1990; Majumder et al., 1988; Pinchuk et al., 2002; Tamehiro et al., 2002; Inaoka et al., 2004).

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related compounds of the iturin family (see Fig. 3A). Thus, NRPSs and PKSs are per se extremely amenable to genetic manipulations, providing powerful tools for future development and production of novel peptides, polyketides and hybrid compounds with new properties. The huge potential of NRPSs and PKSs in the generation of novel drugs has been excellently reviewed elsewhere (Sieber and Marahiel, 2003; Finking and Marahiel, 2004; Walsh, 2004).

Non-ribosomally generated amphipathic lipopeptide antibiotics with condensed β-hydroxyl or β-amino fatty acids are widespread in B. subtilis. Variations in length and branching of the fatty acid chains and amino acid substitutions lead to remarkable product microheterogeneity (Kowall et al., 1998). The lipopeptidetripeptide surfactin (Fig. 3A) is the most powerful biosurfactant known – a 20 μM solution lowers the surface tension of water from 72 to 27 mN m⁻¹; it exerts a detergent-like action on biological membranes (Carrillo et al., 2003), and is distinguished by its exceptional emulsifying, foaming, anti-viral and anti-myco-plasmas activities (reviewed by Peypoux et al., 1999). Surfactin is biosynthesized by the three NRPSs SrfA–C (Peypoux et al., 1999); the thioesterase/cytochrome enzyme SrfD stimulates the initiation of this process (Steller et al., 2004). The mechanism of surfactin excretion is fully unknown, as an active transporter has not been found, implying passive diffusion across the cytoplasmic membrane. Surfactin resistance is provided by YerP, the first example of a RND (resistance, nodulation and cell division) family multidrug efflux pump in Gram-positive bacteria (Tsuge et al., 2001a). The regulation of surfactin biosynthesis is closely connected to the competence development pathway (Marahiel et al., 1993; reviewed in Hamoen et al., 2003; see also Fig. 4). Natural competence defines the ability for exogenous DNA uptake. Remarkably, the comS gene involved in B. subtilis competence development is located within and out of frame of the srfA gene that specifies surfactin synthetase (Fig. 3A). The expression of both srfA and comS is regulated via a complex network that governs cellular differentiation, including quorum sensing via extracellular ComX and the two-component regulatory system ComPA (reviewed in Hamoen et al., 2003). Thus, B. subtilis elegantly uses a single quorum-sensing pathway for the DNA-uptake system and surfactin production. It is conceivable that competence development in order to assimilate external DNA is a microbial attempt to ensure the maintenance of genetic information beyond the individual cell. Additionally, uptake of external DNA can be used to increase the genetic diversity of the bacterial population.

The iturin family encompasses the closely related cyclic lipopeptidetripeptides mycosubtilin, the iturines and the bacillomycins (Fig. 3A) with strong anti-fungal and haemolytic but only limited anti-bacterial activities (Thimon et al., 1995). They are synthesized by the closely related NRPSs mycosubtilin (Duitman et al., 1999), iturin (Tsuge et al., 2001b) and bacillomycin (Mooyne et al., 2004) synthetase.

Fengycin (synonymous to plipastatin) combines several exceptional structural properties: cyclization, branching and unusual constituents (Fig. 3A). Fengycin specifically acting against filamentous fungi (Vanittanakom et al., 1986) is biosynthesized by fengycin synthetase encompassing the five NRPSs Fen1–Fen5 encoded by ppsA–E (Steller et al., 1999).

Remarkably, although genes specifying surfactin and fengycin synthetase are conserved within the B. subtilis 168 genome (Kunst et al., 1997), the corresponding antibiotics are not produced. Surfactin production depends on the P Pan transferase Sfp (Nakano et al., 1992) which converts the inactive apoforms of surfactin and fengycin synthetase to their active holoforms (Lambalot et al., 1996). However, the sfp allele of the 168 strain specifies an inactive protein due to a frameshift mutation (Mootz et al., 2001). Accordingly, the introduction of a native sfp allele into B. subtilis 168 provoked surfactin (Nakano et al., 1992) and fengycin (plipastatin) (Tsuge et al., 1999) production.

The biosynthesis of the dipeptide bacilysin (Fig. 3B; L-alanine-[2,3-epoxy cyclohexano-4]-L-alanine) depends on the ywIBCDEFGH cluster (Inaoka et al., 2003). The unusual epoxy-modified amino acid anti-capisin is probably generated through the action of a prephenate dehydratase and an amindo transferase encoded by ywBG, respectively, as a branching off from prephenate of the aromatic amino acid pathway (Hilton et al., 1988). Genes

Fig. 4. Regulatory pathways of antibiotic biosynthesis in B. subtilis. Survey of the regulatory pathways for the biosynthesis of the B. subtilis antibiotics subtilin, subtilosin, bacilysin, surfactin, the killing factor Skf and the spore-associated anti-microbial polypeptide TasA. The scheme is simplified in terms of the regulation of competence development, which has been elaborately summarized by Hamoen et al. (2003); for details, see the corresponding text. A B. subtilis cell is symbolized by a lipid bilayer; compounds acting as pheromones are boxed; membrane-localized sensor histidine kinases are symbolized as circles. Positive and negative regulation of gene expression is indicated by arrows and T-bars respectively. For clarity, the repression of AbrB on sbo-alb and tasA was omitted.
bicDE (ywfEF) have been shown to encode the functions of amino acid ligation and bacilysin immunity respectively (Steinborn et al., 2004). Bacilysin production is regulated on different levels (see also Fig. 4), negatively by GTP via the transcriptional regulator CodY (Inaoka et al., 2003) and AbrB (Yazgan et al., 2003). Positive regulation occurs by guanosine 5’-diphosphate 3’-diphosphate (ppGpp) (Inaoka et al., 2003) and a quorum-sensing mechanism through the peptide pheromone PhrC (Yazgan et al., 2003).

Miscellaneous antibiotic compounds

The genome of B. subtilis 168 contains the pksA–S locus with a remarkable size of 76 kb, that specifies a PKS–homologous system (Kunst et al., 1997). Speculative products might be the polyketides difficidin (Fig. 3B; Wilson et al., 1987) or bacillaene (empirical formula C_{30}H_{48}O_{7}; Patel et al., 1995). However, B. subtilis 168 does not produce polyketides, presumably due to the mutated sfp gene (see above). It has been very recently shown that the biosynthesis of difficidin and bacillaene in B. subtilis A1/3 is dependent on a Sfp-homologous PPan transferase (Hofemeister et al., 2004). Thus, Sfp in B. subtilis 168 might also be involved in the phosphopantetheinylation of polyketide synthase acyl carrier domains.

A series of new antibiotics have been recently isolated from well-known B. subtilis strains. These include bacilysocin (Fig. 3B), an anti-microbial phospholipid, that can be isolated from B. subtilis 168 cells by extraction with butanol (Tamehiro et al., 2002). Most probably bacilysocin is derived from the major B. subtilis phospholipid phosphatidylglycerol through YtpA-catalysed acyl ester hydrolysis (Tamehiro et al., 2002). Amicoumacins (Fig. 3B) are produced by several B. subtilis strains excluding the 168 strain (Pinchuk et al., 2002). Their anti-bacterial and anti-inflammatory activities, as well as their action on Helicobacter pylori make the amicoumacins attractive for the treatment of chronic gastritis and peptic ulcer in humans (Pinchuk et al., 2001). Very recently, Inaoka et al. (2004) showed the production of the aminosugar antibiotic 3,3’-neotrehalosadiamine (NTD), dormant in the wild-type strain, that can be induced by a rifampicin-resistant phenotype of the RNA polymerase. The operon specifying NTD biosynthesis encompasses the genes ntdABC (yjhLKn). NTD acts as an autoinducer for its own biosynthesis genes via the regulator protein NtdR encoded by ntdR (yjhM) (Inaoka et al., 2004). The transition-phase, spore-associated 31 kDa TasA protein exhibits a broad spectrum of anti-microbial activity. TasA together with yqxM and sipW constitutes a transition-phase operon (under positive control of Spo0A/SigH, and under repression of AbrB; see Fig. 4) that could play a protective role during B. subtilis sporulation (Stover and Driks, 1999). Further B. subtilis antibiotics are summarized in Fig. 3B.

Specific biological functions of distinct B. subtilis antibiotics

Microbes produce an amazing variety of antibiotics and, moreover, possess multidrug-type resistance genes, both suggesting dynamic ‘intermicrobial warfares’. Consequently, the classification of anti-microbials as competitive weapons against other microorganisms has influenced our view for several decades. However, antibiotics are often produced by specific strains and, thus, are not obligatory for the general survival of the genera per se. Two important questions that arise are: (i) why antibiotics are biosynthesized and (ii) are there any biological roles for antibiotics beyond the ‘pure’ anti-microbial action? The efforts for antibiotic production are enormous, in particular if one reminds that most of antibiotic biosyntheses are regulated by mechanisms shared with other starvation-induced activities (see also Fig. 4) such as sporulation, genetic competence development and production of extracellular degradative enzymes (Katze and Demain, 1977; Losick et al., 1986; Marahiel et al., 1993). Therefore, it is inconceivable that the intricate reaction sequences of antibiotic biosyntheses would have been retained in nature without benefit to the organism.

Rhizobacteria are present in the soil in an average of about 10^8 cells per gram, and from the soil, they are transferred to various associated environments including plants, foods, animals, marine and freshwater habitats (Priet, 1993). One of the main representative, the ‘hay-bacterium’ B. subtilis produces more than two dozen antibiotics. If all pathways are considered, their production requires more than 350 kb (NRPSs, 200 kb; PKSs, 76 kb; lantibiotics, 50 kb; others >20 kb), corresponding to a remarkable 10% of the annotated ORFs. It should be emphasized that all investigated B. subtilis strains produce individual antibiotic cocktails encompassing only a portion of the compounds depicted above; the average of a B. subtilis genome that is devoted to antibiotic production is about 4–5%. The potential of a given B. subtilis strain for antibiotic syntheses is comparable with Bacillus amyloliquefaciens (six operons of 306 kb, 7.5% of the genome; Koumoutsi et al., 2004) but stays behind the potential of Streptomyces such as Streptomyces avermitilis (25 operons of 560 kb corresponding to 6.4% of the genome; Omura et al., 2001). The marked differences of B. subtilis strains with regards to their produced antibiotic spectra suggest that the antibiotic specifying loci must have been recent acquisitions. Horizontal exchange of genetic material enabled via uptake of phage, plasmid or naked DNA by genetically competent cells is a feasible possibility for this divergence. Presumably, accommoda-
tion of genes specifying antibiotic biosyntheses and/or resistance determinants would be beneficial for the *B. subtilis* cells and thus, enriching the fraction of a population that is comprised of antibiotic producing and/or tolerant cells. One example of *B. subtilis* for the acquisition of phage DNA is the subblancin specifying gene cluster within the prophage SPβ locus (Dorenbos et al., 2002; Westers et al., 2003). Remarkably, the closely related gene cluster for subtilin and ericin biosynthesis inhabit identical gene loci in *B. subtilis* strains ATCC 6633 and A1/3 (Fig. 2), suggesting that they have evolved from a common ancestor (Stein et al., 2002b) and/or that they might be interchangeable genetic elements. Presumably also NRPSs specifying genes might be interchangeable among different *B. subtilis* strains, as for example mycosubtilin and fengycin synthetase genes in *B. subtilis* ATCC 6633 (Duitman et al., 1999) and A1/3 (Hofemeister et al., 2004) have been found in identical loci respectively. Furthermore, the *srf* loci of *B. subtilis* 168 (Kunst et al., 1997) and *B. amyloliquefaciens* (Koumoutsi et al., 2004) are identical, supporting the idea that NRPSs are also interchangeable among different *Bacilli*.

A couple of antibiotics have been found to be produced by a great variety of *B. subtilis* strains (subtilosin, surfactin, bacilysin); others are produced strain-specifically (lantibiotics subtilin, ericin and mersacidin). However, systematic studies that survey the complete spectrum of antibiotic activities by different *B. subtilis* strains (e.g. in the A1/3 strain; Hofemeister et al., 2004) are rare. Pinchuk et al. (2002) investigated 51 *Bacillus* strains isolated from different habitats, from which 47 have been identified as *B. subtilis*, among them 11 amicoumacin producer. Surfactin production is widely spread among *B. subtilis* (Leenders et al., 1999; Peypoux et al., 1999; Vater et al., 2002; Hofemeister et al., 2004), a property that is shared with closely related *Bacilli* such as *amyloliquefaciens* (Koumoutsi et al., 2004), *circulans* (Hsieh et al., 2004) and *pumilus* (Kalinochkina et al., 2002) strains.

Altogether, it seems to be that *B. subtilis* is outstanding in the genus *Bacillus* with regards to its potential to produce so many different antibiotics. However, *B. subtilis* is by far the most commonly investigated *Bacillus* genus, and the large number of known *B. subtilis* antibiotics might reflect the numerosness of natural isolates and studies. Also other *Bacilli* such as *Bacillus brevis* (brevistin, edeines, gramicidines, tyrocidin) or *B. amyloliquefaciens* (Koumoutsi et al., 2004) produce a couple of antibiotics, although their number seems to minor as compared with *B. subtilis*. Otherwise, it is tempting to speculate that the frequent occurrence of *B. subtilis* among other *Bacillus* strains in natural isolates might be also a consequence of the benefits of the produced compounds. Unfortunately, the originally *B. subtilis* 168 Marburg strain systematically investigated and used as a model system for Gram-positive organisms has been cultivated in the laboratory for several decades, and more alarmingly, was exposed to X-rays in the mid-1940s (Burkholder and Giles, 1947). This strain does not produce lipopeptides or polyketides, and consequently, important contributions of these compounds to the morphology of *B. subtilis* might have been overlooked or underestimated in previous studies.

Lipopeptide antibiotics are among the most frequently produced *B. subtilis* antibiotics. They as well as other amphiphilic compounds such as the phospholipid bacilysin are low-molecular-mass surfactants that are able to alter the physical and/or chemical properties at interfaces. Three possible roles for such bioemulsifiers have been proposed: (i) an increase of the surface area of hydrophobic water-insoluble growth substrates, (ii) an increase in the bioavailability of hydrophobic substrates by increasing their apparent solubility and (iii) an influence on the attachment and detachment of microorganisms to and from surfaces (Rosenberg and Ron, 1999). It is easy to imagine that these roles would have strong influence on the survival of *B. subtilis* in its natural habitat, the soil and the rhizosphere. In this respect, the non-ribosomally generated anionic lipopeptidapeptide surfactin is by far the most prominent and best-investigated representative.

Many bacteria exhibit two distinct lifestyles, a free-floating planktonic mode for rapid proliferation and spread into new territories and a sessile biofilm mode. Biofilms are highly structured microbial communities that adhere to surfaces and constitute the majority of bacteria in most natural and pathogenic ecosystems (for recent reviews, see Harshey, 2003; Hall-Stoodley et al., 2004; Stanley and Lazazzera, 2004). Cell motility in colonies, swarming, involves differentiation of vegetative cells into hyperflagellated ‘swarmer cells’ that undergo rapid and co-ordinated population migration across solid surfaces (Shapiro, 1998; Fraser and Hughes, 1999). The swarming motility of *B. subtilis* is strictly dependent on the production of surfactin (Kinsinger et al., 2003), an observation made with undomesticated strains (Kearns and Losick, 2003; Kearns et al., 2004). However, surfactin production is necessary but not sufficient for swarming, in which at least the factors swrAB, swrC (synonymous to the surfactin resistance gene yerP) and efp are additionally involved (Kearns et al., 2004). *B. subtilis* biofilm formation (Branda et al., 2004) is dependent on the transcription factors SpoOA (Hamon and Lazazzera, 2001), sigma-H and AbrB (Hamon et al., 2004). As these transcription factors are also involved in the regulation of several antibiotic biosyntheses (Fig. 4), antibiotic production in a biofilm is conceivable. It has been recently documented that the colonization of plant roots by *B. subtilis* is associated with surfactin production and biofilm formation, and strikingly, surfactin protected the plant against the infection by the pathogen *Pseudomonas syringae* (Bais et al., 2004).
Results from a very recent study imply that the surfactins, and not other lipopeptides like the bacillomycins, enable the natural isolate B. subtilis A1/3 to form biofilms (Hofmeister et al., 2004). A close correlation between antibiotic production and biofilm formation in other bacilli (Yan et al., 2003) or the observation that surface-active rhamnolipid surfactants affect the architecture of biofilms in Pseudomonas aeruginosa (Davey et al., 2003) suggests that biofilm-associated antibiotic/surfactant production is more widely distributed than previously thought. Interestingly, surfactin is also able to inhibit biofilm formation of other bacteria (Bais et al., 2004), and even the human pathogen Salmonella enterica (Mireles et al., 2001). The anti-microbial and fungicidal action of lipopeptides in addition to surfactin (fengycin, iturin, bacillomycin) might be advantageous for B. subtilis cells to eliminate competitors in the same habitat. It seems to be that the production of these lipopeptides (e.g. bacillomycin in B. subtilis A1/3; Hofemeister et al., 2004) is articulately delayed (late stationary phase) as compared with surfactin (transition between exponential and stationary growth). Altogether, it is worth to further consider the use of B. subtilis, an ubiquitously occurring ‘safe’ microorganism, in agriculture as natural fungicide and plant growth-promoting microorganism (reviewed in Nicholson, 2002) and/or decontamination of solid surfaces (Rosenberg and Ron, 1999).

Nutrient-limited B. subtilis cells are able to sporulate, an elaborate process that results in the release of an endospore from the terminally differentiated, apoptotic mother cell (Errington, 2003). Strikingly, Branda et al. (2001) documented that sporulation is tightly intertwined with the development of highly ordered and surface-associated cell clots, ‘fruiting-bodies’, that are characterized by spore-specific gene expression. The formation of similar aerial hyphae in multicellular organism like fungi need the generation of surface-active molecules (Wösten et al., 1999; Kodani et al., 2004). Three genes are involved in B. subtilis ‘fruiting body’ formation (Branda et al., 2001): yveQ and yveF specify exopolysaccharide biosynthetic enzymes, and sfp specifies a PPane transferase. As Sfp can modify the surfactin and fengycin NRPSs and the PKS synthase (see above), its influence on fruiting-body formation is most probably exerted by one or more surface-active products of these NRPS and/or PKS systems. Importantly, fruiting bodies are only formed by undomesticated, natural B. subtilis isolates, which again emphasizes the importance of carrying out investigations with other than laboratory or laboratory-acclimatized strains (general aspects are reviewed in Palkova, 2004).

Bacillus subtilis sporulation is governed by the regulatory protein Spo0A. Gonzalez-Pastor et al. (2003) discovered that Spo0A is also involved in the regulation of two highly interesting operons, namely skf (sporulation killing factor) and sdp (sporulation delay protein) (Fawcett et al., 2000; Molle et al., 2003). Early sporing B. subtilis cells (Spo0A-active) produce and export the antibiotic-like killing factor Skf, to which they are immune, and that causes lysis of non-sporulating (Spo0A-inactive) sister cells – a mechanism designated as ‘cannibalism of siblings’ (Gonzalez-Pastor et al., 2003). Remarkably, Skf (YbcO) exhibits also anti-microbial activity, in particular against the rice pathogen Xanthomonads (Lin et al., 2001). The sporulation delay protein Sdp acts cooperatively with Skf and effectuates programmed cell death in Spo0A-inactive cells, and furthermore, Sdp holds up sporulation within Spo0A producer cells (Gonzalez-Pastor et al., 2003). The nutrient scavange of lysed sister cells is beneficial for Spo0A-active Skf/Sdp-producing cells, a mechanism that allows them to keep growing rather than to complete the energy-consuming last resort sporulation pathway.

We become increasingly aware that single-cell microorganisms display sophisticated social behaviours: prokaryotic B. subtilis cells live in complex communities where they co-ordinate gene expression and group behaviour through different quorum-sensing pathways (Shapiro, 1998). The collective cell death of a subpopulation can be seen as ‘altruistic suicide’, as a consequence of developmental processes which would ensure the survival of the remaining unharmed and/or better-adapted cells. Such a mechanism might be one of the clues to understand the classical question: why are antibiotic production and sporulation so often related to one another (Katz and Demain, 1977; Marahiel et al., 1993). Although antibiotics are not obligatory for sporulation, the biosyntheses of a couple of them are regulated by factors shared with the sporulation process (Fig. 4). It is conceivable that AbrB-regulated antibiotics that are consequently induced in Spo0A-active cells (e.g. subtilin, subtilosin, bacilysin, surfactin) are also involved in the action against non-sporulating (Spo0A-inactive) sister cells. However, the direct regulation of the skf cluster by Spo0A (Fawcett et al., 2000; Gonzalez-Pastor et al., 2003) clearly distinguishes Skf from other B. subtilis antibiotics. It is remarkable that the B. subtilis lantibiotics subtilin (Stein et al., 2002a) and ercin (J. Hofemeister, pers. comm.), both autoregulated via two-component regulatory systems, function as pheromones for quorum sensing (Stein et al., 2002a; Kleerebezem, 2004). It has to be elucidated whether quorum sensing via lantibiotics is restricted to only a handful B. subtilis strains or whether it is wider distributed than actually known. Notably, we have begun to understand that distinct B. subtilis antibiotics and antibiotic-like compounds play crucial roles in communal development and contribute to the survival of B. subtilis in its natural habitat. It is to be expected that future studies will give us a detailed and more integrated understanding of the challenging biological functions of anti-microbial compounds of Bacillus and other organisms.
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