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REVIEW

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Exploitation of the *Streptomyces coelicolor* A3(2) genome sequence for discovery of new natural products and biosynthetic pathways

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Abstract *Streptomyces*, and related genera of Actinobacteria, are renowned for their ability to produce antibiotics and other bioactive natural products with a wide range of applications in medicine and agriculture. *Streptomyces coelicolor* A3(2) is a model organism that has been used for more than five decades to study the genetic and biochemical basis for the production of bioactive metabolites. In 2002, the complete genome sequence of *S. coelicolor* was published. This greatly accelerated progress in understanding the biosynthesis of metabolites known or suspected to be produced by *S. coelicolor* and revealed that streptomycetes have far greater potential to produce bioactive natural products than suggested by classical bioassay-guided isolation studies. In this article, efforts to exploit the *S. coelicolor* genome sequence for the discovery of novel natural products and biosynthetic pathways are summarized.

Keywords Antibiotic · Siderophore · Polyketide synthase · Nonribosomal peptide synthetase · Terpene synthase

Introduction

Bioactive natural products have many important applications in medicine and agriculture. *Streptomyces* and related genera of filamentous Gram-positive Actinobacteria are a particularly prolific source of such compounds, including antibacterials (e.g., erythromycin A, vancomycin and daptomycin), antifungals (e.g., amphotericin B), immunosuppressants (e.g., FK-506), anticancer agents (e.g., doxorubicin and epoxomicin), anthelmintics (e.g., avermectin B1a), insecticides (e.g., spinosyn A and avermectin B1a) and herbicides (e.g., phosphinothricin) (Fig. 1).

Over the past three decades, numerous research groups across the globe have sought to elucidate the mechanisms responsible for assembly of actinobacterial natural products. A major motivation for such efforts has been the prospect of rational biosynthetic pathway manipulation to generate natural product analogues with novel or superior properties. Understanding the molecular genetics of natural product biosynthesis has been of central importance in this endeavor.

Pioneering research on the molecular genetics underlying the biosynthesis of the actinorhodin complex of pigmented antibiotics in *Streptomyces coelicolor* A3(2) was carried out by Hopwood and coworkers [44, 58]. This culminated in the first example of rational biosynthetic pathway manipulation to produce a novel natural product analogue and stimulated the adoption of *S. coelicolor* as a model organism for studying the biosynthesis of such specialized actinobacterial metabolites [29]. A major advance was the publication in 2002 of the complete genome sequence of *S. coelicolor* M145 [3], a prototrophic derivative of the A3(2) strain that lacks its two plasmids. The complete sequences of SCP1 and SCP2, the giant linear and small circular plasmids, respectively, of *S. coelicolor* soon followed [4, 24].

The *S. coelicolor* genome sequence has provided the complete sequences of the biosynthetic gene clusters for all known specialized metabolites of this model organism. In addition, the genome sequence has been exploited to identify gene clusters encoding cryptic natural product biosynthetic pathways (i.e., those not associated with

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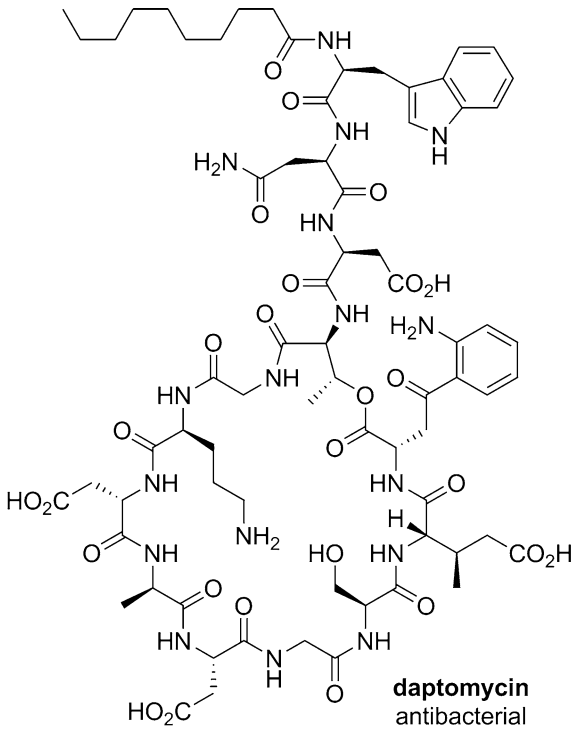
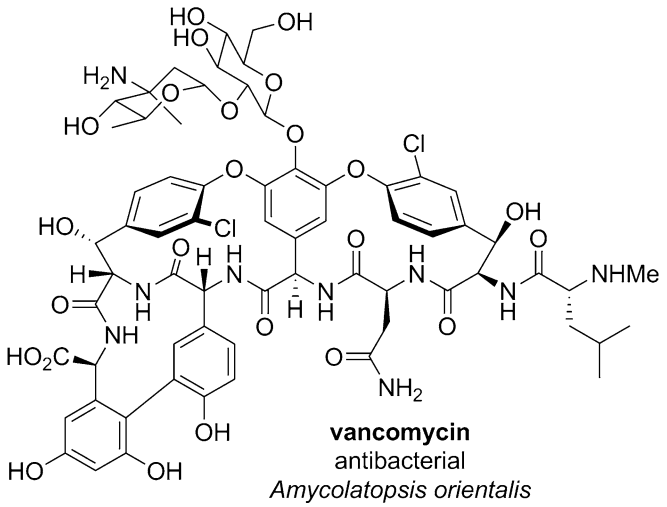
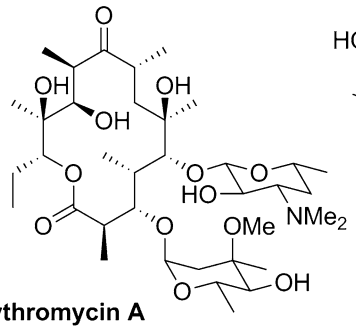


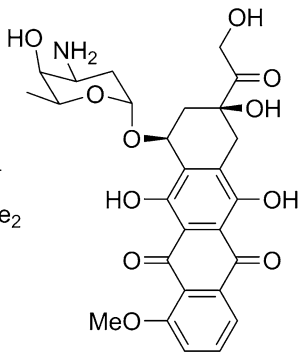
daptomycin
antibacterial
Streptomyces roseosporus



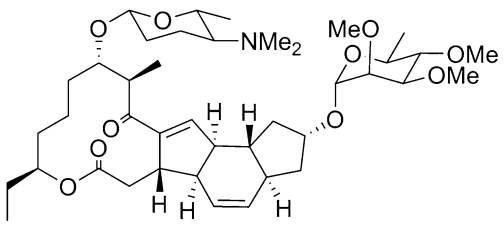
vancomycin
antibacterial
Amycolatopsis orientalis



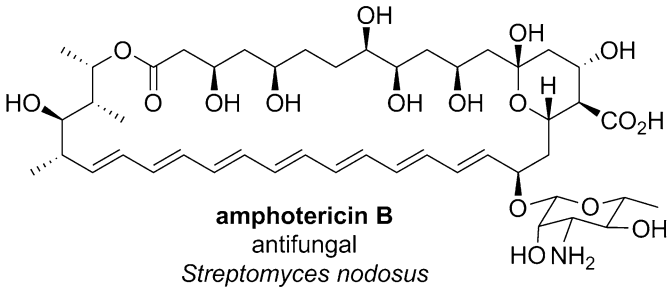
erythromycin A
antibacterial
Saccharopolyspora erythraea



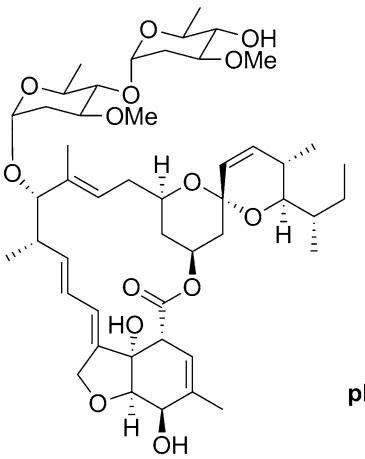
doxorubicin
anticancer
Streptomyces peucetius



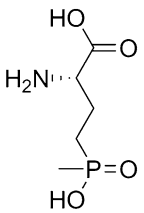
spinosyn A
insecticide
Saccharopolyspora spinosa



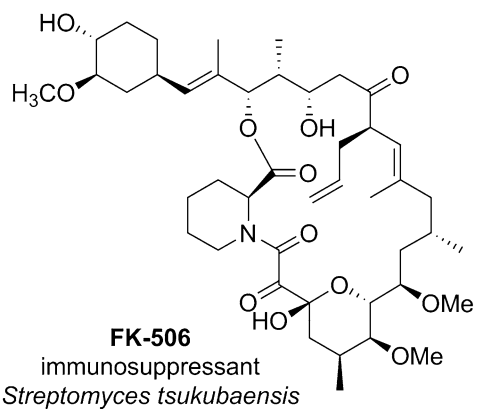
amphotericin B
antifungal
Streptomyces nodosus



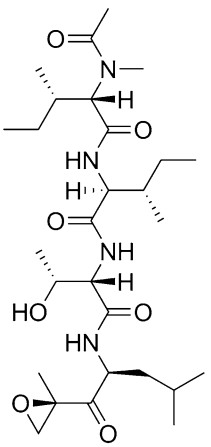
avermectin B1a
anthelmintic/insecticide
Streptomyces avermitilis



phosphinothricin
herbicide
Streptomyces viridochromogenes



FK-506
immunosuppressant
Streptomyces tsukubaensis



epoxomicin
anticancer
actinomycete
strain Q996-17

◀**Fig. 1** Examples of bioactive metabolites used in medicine and agriculture that are produced by Actinobacteria. The application and producing organism for each metabolite is shown below its name. Note that in the case of epoxomicin, it is the synthetic analogue carfilzomib that is used in the clinic

production of known metabolites). This has led to the discovery of several novel metabolic products of *S. coelicolor*. We now know that this famous streptomycete is capable of producing more than 15 distinct families of specialized metabolite (Fig. 2) and complete genome sequences of several other Actinobacteria have shown that, in general, such organisms have the potential to produce a far greater number of bioactive natural products than have thus far been identified by classical bioassay-guided discovery approaches [49].

This article gives an overview of the ways in which the *S. coelicolor* genome sequence has been exploited to enrich our understanding of mechanisms for natural product biosynthesis and to discover novel products of specialized metabolism. The following sections are organized on the basis of the various metabolite families produced by *S. coelicolor*.

Undecylprodigiosin and streptorubin B

The production of a red-pigmented antibiotic by *S. coelicolor* was first discovered during genetic studies of actinorhodin biosynthesis in the 1970s [58]. Subsequently, this red pigment was reported to be a mixture of undecylprodigiosin and its carbocyclic derivative butylcycloheptylprodiginine [68]. More recently, it has been shown that the carbocyclic derivative of undecylprodigiosin produced by *S. coelicolor* is in fact streptorubin B, a regioisomer of butylcycloheptylprodiginine [25, 48] (Fig. 3).

In early genetic studies, strains defective in production of the red pigment were generated by UV mutagenesis and grouped according to their co-synthesis behavior [59]. Mapping of the mutations in these strains to the *S. coelicolor* chromosome indicated that they were clustered. Subsequently, genes for biosynthesis of the red pigment spanning 21 kb of the chromosome were cloned by complementation of these mutants, including a gene encoding an *O*-methyltransferase that was implicated in a late biosynthetic step [15, 16]. Eventually, in the late 1980s the entire biosynthetic gene cluster was cloned and expressed in a heterologous host [45].

The complete sequence of the *red* gene cluster responsible for the biosynthesis of undecylprodigiosin and streptorubin B became available in the late 1990s as a result of the *S. coelicolor* genome sequencing project. This greatly facilitated the application of bioinformatics tools to analysis of the proteins encoded by the cluster [7], as well as a range of genetic and biochemical experiments aimed at elucidating the role played by each enzyme in the biosynthetic pathway [26, 47, 48, 62–64, 67, 71]. In little over a decade, several key intermediates in the assembly of undecylprodigiosin and streptorubin B have been defined and the functions of most of the enzymes encoded by the *red* cluster have been established (Fig. 3).

Methylenomycins and the methylenomycin furans

In 1976, methylenomycin A was identified as an antibiotic determined by SCP1, the 356-kb linear plasmid of *S. coelicolor* [37, 72] (Fig. 4). Subsequently, it was reported that *S. coelicolor* also produces methylenomycin C, a precursor of methylenomycin A [30] (Fig. 4).

Early genetic studies showed that methylenomycin biosynthetic genes are clustered on SCP1 [9]. The complete sequence of the *mmy* cluster of genes responsible for methylenomycin biosynthesis became available through the SCP1 sequencing project [4]. Integration of a cosmid containing the methylenomycin biosynthetic gene cluster into the chromosome of *S. lividans* 1326 resulted in a strain able to produce methylenomycins A and C [12, 23]. This facilitated the incorporation of isotope-labeled precursors into the methylenomycins, revealing that these antibiotics derive from two molecules of acetic acid and a pent(ul)ose [12].

Sequence analysis of the proteins encoded by the *mmy* gene cluster led to the proposal of a pathway for methylenomycin biosynthesis involving MmyC-catalyzed condensation of acetyl-CoA with malonyl-MmyA to afford acetoacetyl-MmyA, which undergoes MmyD-catalyzed condensation with a pentulose to form a butenolide intermediate [12] (Fig. 4). Methylenomycin C is formed from the butenolide intermediate via a series of undefined reactions and is proposed to undergo MmyO-catalyzed epoxidation using molecular oxygen and $FADH_2$, supplied by MmyF, to yield methylenomycin A [12] (Fig. 4).

Genetic studies implicated the *mmfLHP* operon, which flanks the right extremity of the *mmy* gene cluster, in the biosynthesis of diffusible signaling molecule(s) that induce the production of methylenomycins in *S. coelicolor* [50]. Expression of this operon in *S. coelicolor* M512, which lacks SCP1 and is deficient in the production of actinorhodin and undecylprodigiosin/streptorubin B, led to the discovery of five novel 4-alkyl-2-hydroxymethylfuran-3-carboxylic acids, collectively termed the methylenomycin furans (MMFs) [13] (Fig. 5). The MMFs have been shown to specifically induce the production of methylenomycins in *S. coelicolor* and genetic studies have implicated the putative TetR-like DNA-binding protein encoded by *mmfR* as an MMF-responsive repressor of methylenomycin

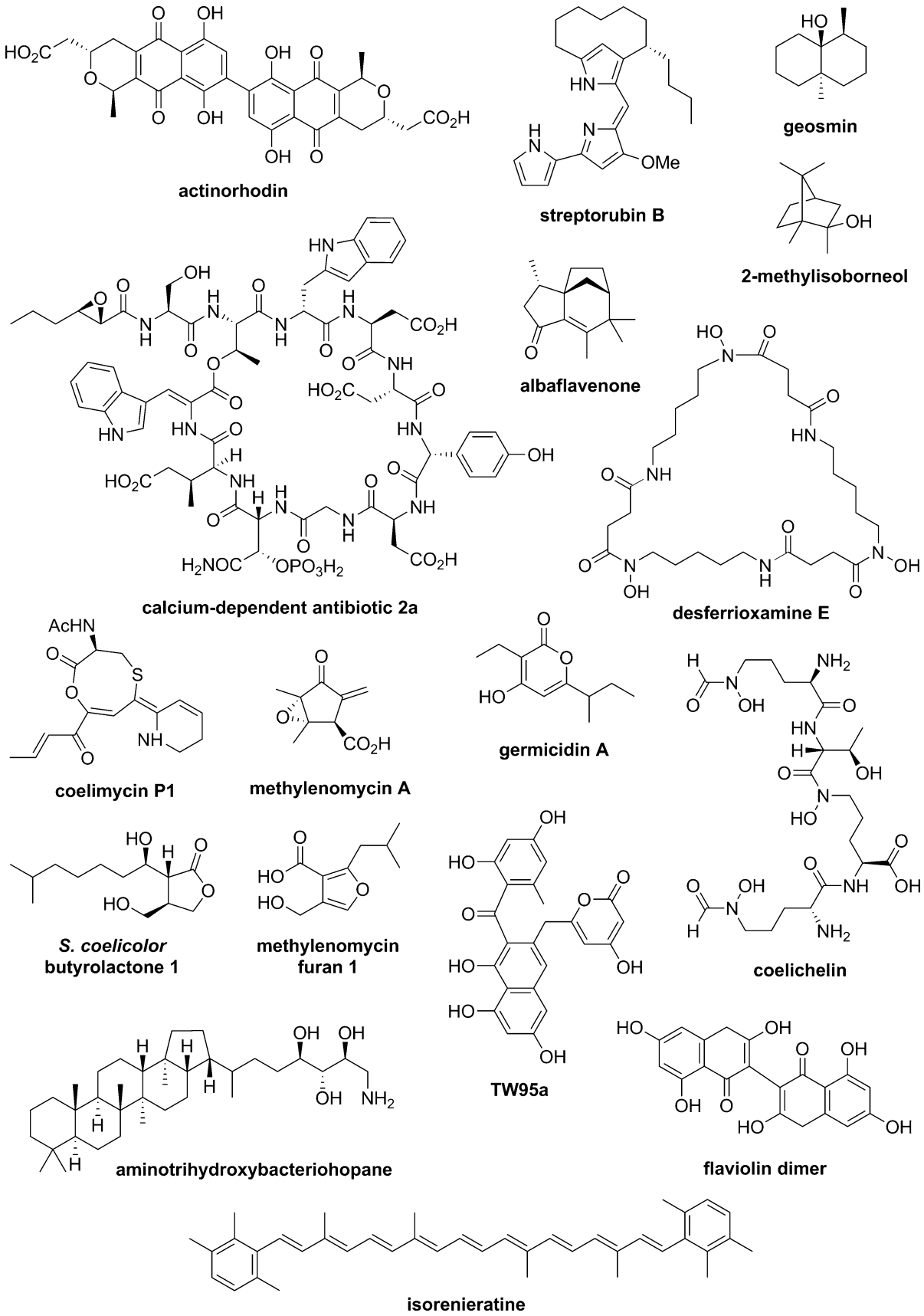


Fig. 2 Representative structures of specialized metabolite families produced by *S. coelicolor*

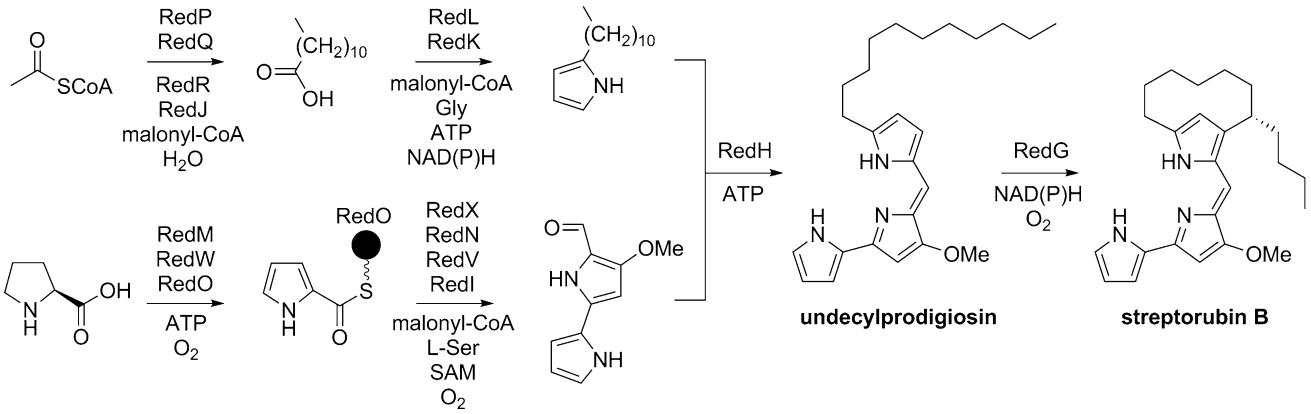


Fig. 3 Biosynthetic pathway for undecylprodigiosin and streptorubin B in *S. coelicolor*. The *black circle* represents an acyl carrier protein

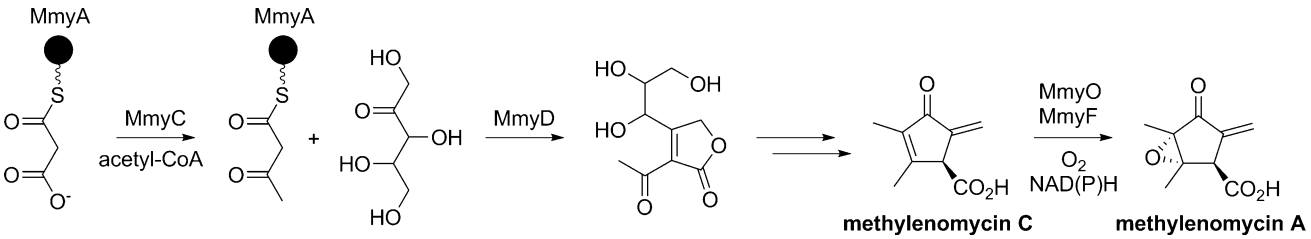


Fig. 4 Proposed pathway for methylenomycin A biosynthesis in *S. coelicolor*. The *black circle* represents and acyl carrier protein

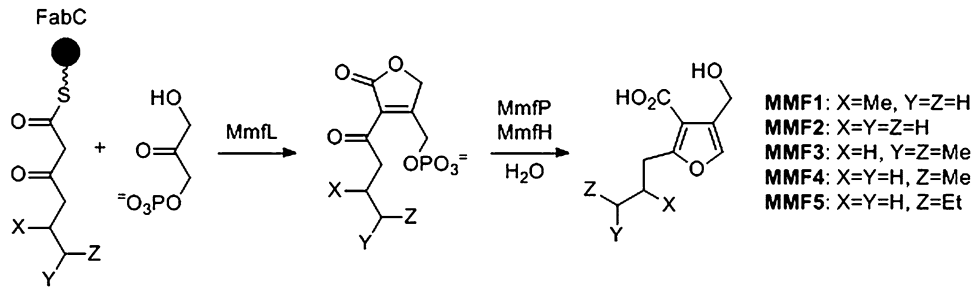


Fig. 5 Proposed pathway for the biosynthesis of the methylenomycin furans (MMFs), signaling molecules that induce production of the methylenomycins in *S. coelicolor*. The *black circle* represents and acyl carrier protein

biosynthesis [13, 50]. Incorporation of stereospecifically ^{13}C-labeled glycerols into the MMFs implies that they are biosynthesized via a pathway involving MmfL-catalyzed condensation of various β-ketoacyl-ACP intermediates in fatty acid biosynthesis with dihydroxyacetone phosphate. The resulting phosphorylated butenolides are proposed to undergo MmfP-catalyzed dephosphorylation, followed by MmfH-catalyzed rearrangement to form the MMFs [14] (Fig. 5).

Calcium-dependent antibiotics (CDAs)

The production of a chromosomally determined CDA by *S. coelicolor* was reported by Hopwood and Wright in the early 1980s [28]. A decade and a half later, CDA was characterized as a complex of structurally related acidic lipopeptides [36]. The following year, the cloning and partial sequencing of an 82-kb region of the *S. coelicolor* chromosome encompassing the CDA biosynthetic gene cluster was reported [11].

The complete sequence of the CDA biosynthetic gene cluster became available through the *S. coelicolor* genome sequencing project, allowing a plausible pathway for CDA biosynthesis to be proposed and mutasynthesis strategies for the production of CDA analogues containing modified arylglycine residues to be developed [27]. Additional CDA congeners containing a C-terminal (*Z*)-2,3-dehydroTrp residue were also identified [27].

The CDA complex is derived from a mixture of proteinogenic and non-proteinogenic amino acids. The latter include L-3-methylglutamate (L-3-MeGlu), L-4-hydroxyphenylglycine (L-4-HPG) and L-3-hydroxyasparagine (3-hAsn). A variety of biochemical and genetic experiments have been employed to elucidate the pathways for the assembly of these amino acids and their incorporation into the CDAs [27, 46] (Fig. 6). Similarly, a mixture of in vivo

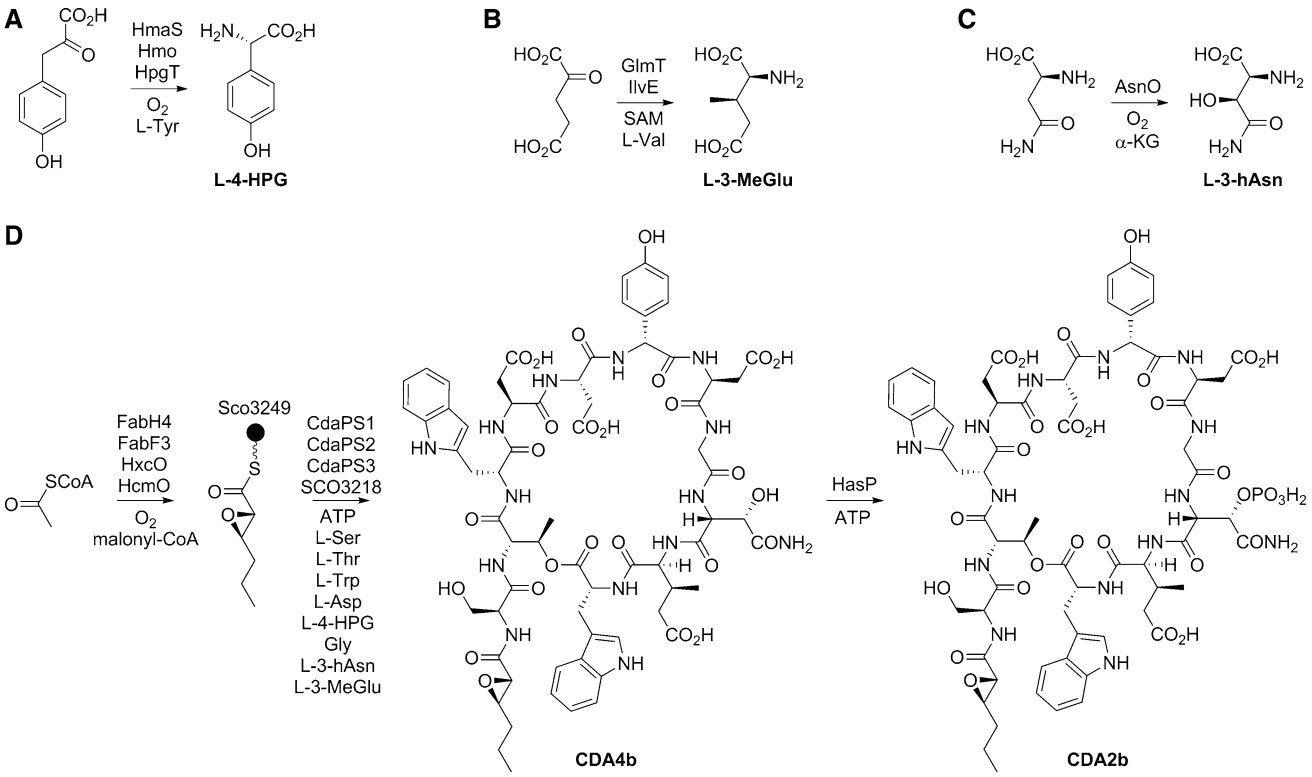


Fig. 6 Pathway for CDA biosynthesis in *S. coelicolor*. **a** Conversion of 4-hydroxyphenylpyruvate to L-4-HPG. **b** β-Methylation and transamination of α-ketoglutarate to produce L-3-MeGlu. **c** Hydroxylation of L-Asn to yield L-3-hAsn. **d** Biosynthesis of the 2,3-epoxyhexanoyl moiety, NRPS-mediated assembly of CDA4b and conversion to CDA2b. The *black circle* represents an acyl carrier protein

and in vitro approaches has been employed to establish the mechanism for assembly of the 2,3-epoxyhexanoyl moiety of the CDAs [46] (Fig. 6). The main engine of CDA biosynthesis is a nonribosomal peptide synthetase (NRPS) consisting of 3 subunits and 11 modules that requires the MbtH-like protein encoded by *sco3218*, or its homolog *cchK*, for catalytic activity [27, 41]. The NRPS successively elongates a 2,3-epoxyhexanoyl thioester with the requisite amino acids to form a peptidyl thioester that undergoes macrolactonization. Phosphorylation of the 3-hAsn residue completes the biosynthesis of CDA2b, the ultimate product of the CDA biosynthetic pathway (Fig. 6). Under certain growth conditions, the C-terminal Trp residue in CDA2b can undergo dehydrogenation by an as yet unidentified enzyme to form CDA2a. The other CDA congeners arise from the utilization of Glu in place of 3-MeGlu by the NRPS and/or omission of the 3-hAsn phosphorylation step and/or Trp dehydrogenation.

Geosmin, albaflavenone, and 2-methylisoborneol

Analysis of the *S. coelicolor* genome sequence identified two genes (*sco6073* and *sco5222*) encoding putative sesquiterpene synthases, neither of which was associated with the production of known metabolites [3]. Deletion of *sco6073* abolished the production of geosmin, a sesquiterpene derivative with a characteristic earthy odor that is known to be produced by many Actinobacteria [22]. The protein encoded by *sco6073* contains two sesquiterpene synthase-like domains. The N-terminal domain of the protein was shown to catalyze the Mg^{2+}-dependent conversion of farnesyl diphosphate to germacradienol and germacrene D [6]. In contrast, the C-terminal domain lacked activity towards farnesyl diphosphate, but catalyzed the Mg^{2+}-independent conversion of germacradienol to geosmin [34] (Fig. 7).

The purified recombinant protein encoded by *sco5222* was shown to catalyze the Mg^{2+}-dependent conversion of farnesyl diphosphate to *epi*-isozizaene [43] (Fig. 7). The *sco5223* gene encodes a cyctochrome P450 that catalyzes the oxidation of *epi*-isozizaene to albaflavenone, a known metabolite of *S. albidoflavus* [74] (Fig. 7). Stereoisomeric albaflavenols are intermediates in the oxidation process. Albaflavenone and the albaflavenols were identified as metabolites of *S. coelicolor* and deletion of *sco5223* was shown to abolish their production, resulting in the accumulation of *epi*-isozizaene [74].

Cane and coworkers identified 2-methylisoborneol, another terpenoid with an earthy odor, as the product of

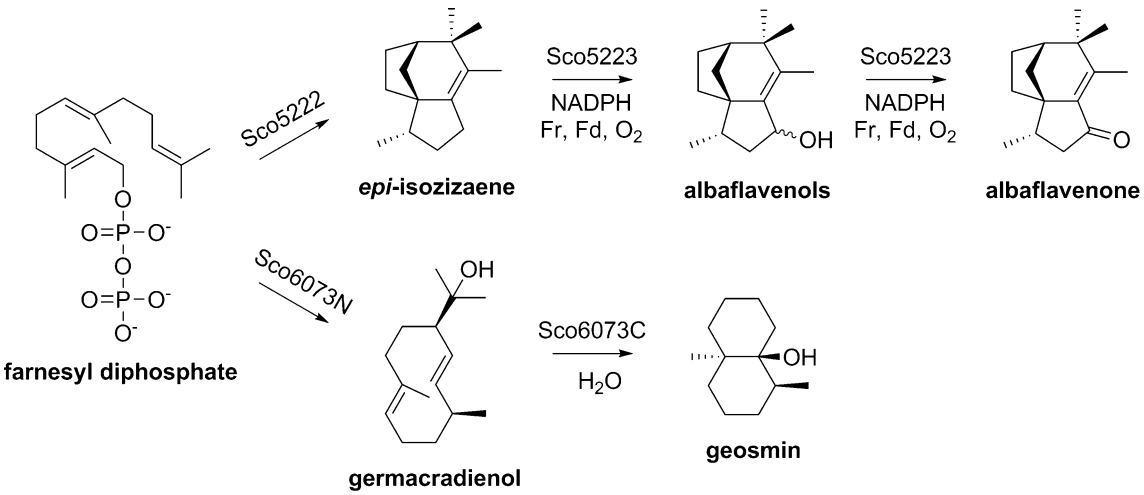


Fig. 7 Biosynthetic pathways for geosmin and albaflavenone in *S. coelicolor*, involving sesquiterpene synthases encoded by the *sco6073* and *sco5222* genes. Fd and Fr are ferredoxin and ferredoxin reductase, respectively. Sco6073N and Sco6073C refer to the N- and C-terminal domains of Sco6073, respectively

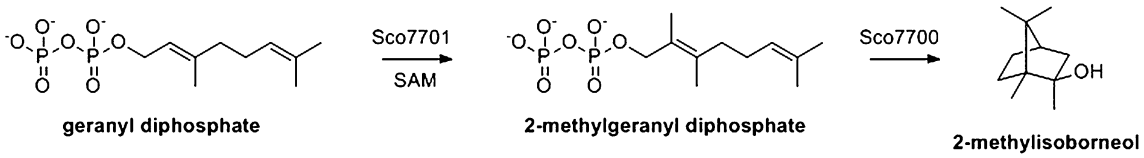


Fig. 8 The *S. coelicolor* pathway for the biosynthesis of 2-methylisoborneol

sco7700 and *sco7701* [70]. The *sco7701* gene was shown to encode an *S*-adenosylmethionine (SAM)-dependent methyltransferase that catalyzes the C-2 methylation of geranyl diphosphate (Fig. 8). The resulting 2-methylgeranyl diphosphate is converted to 2-methylisoborneol by an Mg^{2+}-dependent reaction catalyzed by Sco7700 (Fig. 8).

Carotenoids and hopanoids

Analysis of the *S. coelicolor* complete genome sequence identified a cluster of putative carotenoid biosynthetic genes (*sco0185-sco0191*) [3]. Subsequently, *S. coelicolor* was shown, upon illumination, to produce five distinct yellow carotenoid pigments, two of which were identified as β-carotene and isorenieratene on the basis of chromatographic comparisons with authentic standards (Fig. 9) [66]. Deletion of the *crtEIBV* (*sco0185–0188*) operon abolished the production of carotenoids, consistent with the proposal that the enzymes encoded by these genes are involved in carotenoid biosynthesis [66]. Genetic analysis of the roles played by genes within an essentially identical carotenoid biosynthetic gene cluster in *S. griseus* has led to a proposed pathway for isorenieratene biosynthesis (Fig. 9) [38]. No carotenoids are produced when *S. coelicolor* is grown in the dark and the *sco0192–0194* genes have been implicated in the regulation of illumination-dependent expression of the carotenoid biosynthetic gene cluster [66].

The *sco6759–sco6771* cluster of genes has been predicted to direct the biosynthesis of hopanoids and *S. coelicolor* has been reported to produce a mixture of hopene and aminotrihydroxybacteriohopane (ATBH) (Fig. 10) [56]. However, definitive evidence to support the hypothesis that hopene and ATBH are the metabolic products of the *sco6759–sco6771* gene cluster is currently lacking. Nevertheless, it is tempting to speculate that the aminotrihydroxy moiety of ATBH originates from the addition of an adenosyl radical to the C=C of hopene catalyzed by the putative radical SAM enzyme encoded by *sco6766*. Subsequent phosphorolysis of the resulting adenine derivative catalyzed by the putative nucleotide phosphorylase encoded by *sco6765*, followed by dephosphorylation, would yield the cyclic hemiacetal form of a hopanoid containing a trihydroxyaldehyde moiety. Ring opening of the hemiacetal followed by transamination catalyzed by Sco6769, a putative pyridoxal-dependent aminotransferase, would yield ATBH (Fig. 10).

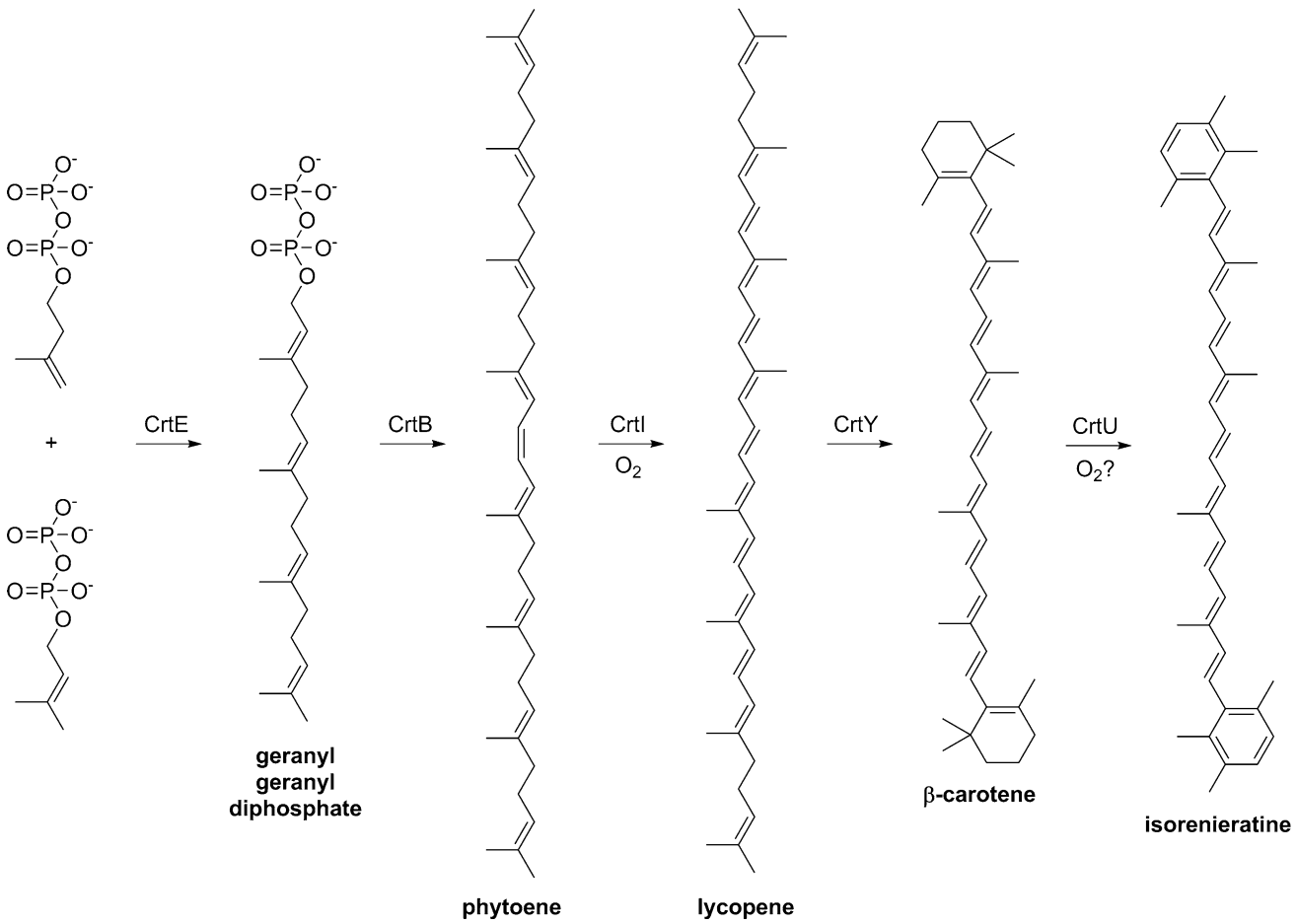


Fig. 9 Proposed pathway for isorenieratene biosynthesis in *S. coelicolor*

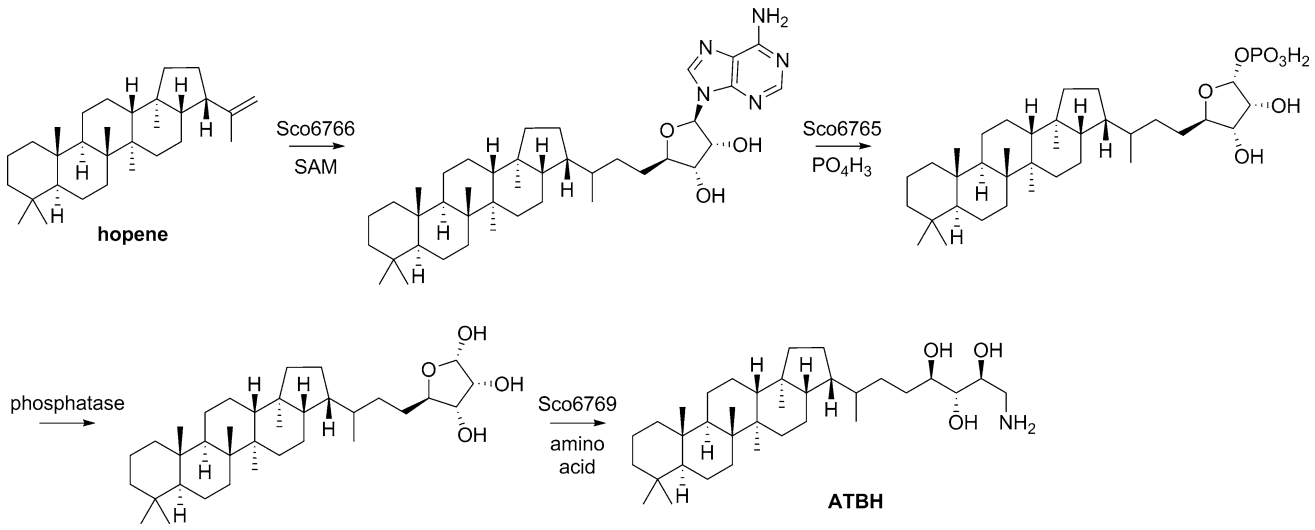


Fig. 10 Proposed pathway for ATBH biosynthesis from hopene in *S. coelicolor*

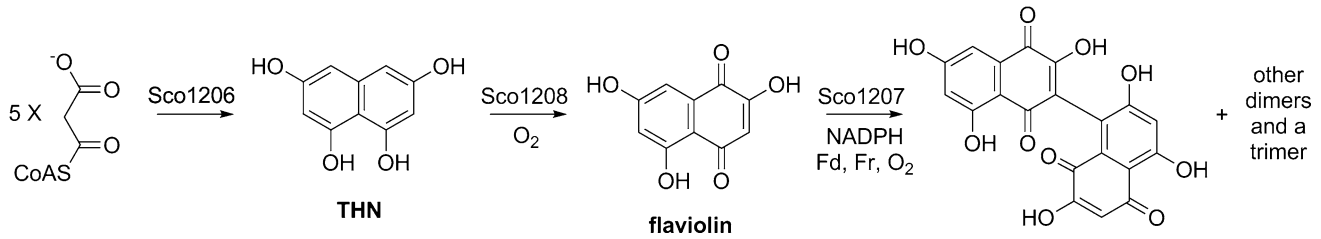


Fig. 11 Biosynthesis of flaviolin dimers/trimer in *S. coelicolor* catalyzed by a type III PKS (Sco1206), a cupin-like monooxygenase (Sco1208) and a cytochrome P450 (Sco1207). Fd and Fr are flavodoxin and flavodoxin reductase, respectively

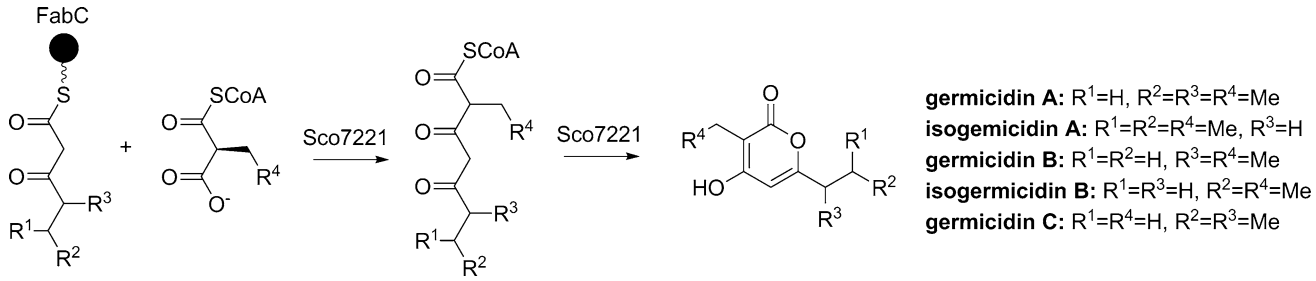


Fig. 12 Reactions catalyzed by the type III PKS Sco7221 in *S. coelicolor* germicidin biosynthesis. The *black circle* represents the fatty acid synthase ACP FabC

Germicidins and flaviolin oligomers

Three genes (*sco1206*, *sco7221*, and *sco7671*) encoding type III polyketide synthases (PKSs) have been identified within the *S. coelicolor* genome. In contrast to most other varieties of PKS, type III PKSs typically utilize malonyl-CoA directly as a starter/extender unit, rather than first loading it (or a derivative—as is often the case for type I modular PKSs) onto an acyl carrier protein (ACP).

Sco1206 has 83 % sequence similarity to RppA of *S. griseus*, the first reported example of a bacterial type III PKS, which assembles 1,3,6,8-tetrahydroxynaphthalene (THN) from 5 units of malonyl-CoA [17]. It has also been shown to assemble THN, which undergoes spontaneous or Sco1208-catalyzed oxidation to flaviolin [18, 33] (Fig. 11). The cytochrome P450 encoded by *sco1207* catalyzes oxidative coupling of flaviolin molecules to produce a mixture of dimers and a trimer [73] (Fig. 11).

A gene knockout/comparative metabolite profiling approach implicated *sco7221* in the production of a mixture of germicidins [61], some of which had previously been reported as metabolites of *S. viridochromogenes* NRRL B-1551 [54] (Fig. 12). Heterologous expression of *sco7221* in *S. venezuelae* ISP5230 demonstrated that it is the only gene required for germicidin biosynthesis [61]. Incorporation of labeled precursors into the germicidins, coupled with alterations to the profile of germicidins produced by a strain of *S. coelicolor* in which the FabH fatty acid biosynthetic enzyme is replaced by its *Escherichia coli* homolog, suggested a pathway for germicidin biosynthesis involving elongation of β-ketoacyl-ACP intermediates in fatty acid biosynthesis with ethyl or methylmalonyl-CoA, followed by cyclization to form the pyrone [61] (Fig. 12). Studies with purified recombinant enzymes have subsequently confirmed this proposed biosynthetic pathway [10].

Based on studies of an ortholog from *S. griseus*, the type III PKS encoded by *sco7671* appears to be involved in the biosynthesis of phenolic lipids [19].

Desferrioxamines and coelichelin

Streptomyces coelicolor was reported to produce a mixture of desferrioxamines E and G1 in the mid-1990s [32]. Prior to this, studies of desferrioxamine B biosynthesis in *S. pilosus* had shown that the first step is decarboxylation of L-lysine [60]. Analysis of the *S. coelicolor* genome sequence identified a cluster of four genes (*sco2782–sco2785*) that were proposed to direct desferrioxamine biosynthesis [3]. The *desA* (*sco2782*) gene encodes a putative pyridoxal phosphate-dependent decarboxylase and *desB* (*sco2783*) encodes a putative flavin-dependent monooxygenase [1]. This is consistent with the involvement of these genes in the conversion of L-lysine to *N*-hydroxycadaverine. Deletion of *desA* abolishes the production of desferrioxamines in *S. coelicolor* [69].

DesC (encoded by *sco2784*) was hypothesized to catalyze the acylation of *N*-hydroxycadaverine with succinyl-CoA and DesD (encoded by *sco2785*) was proposed to

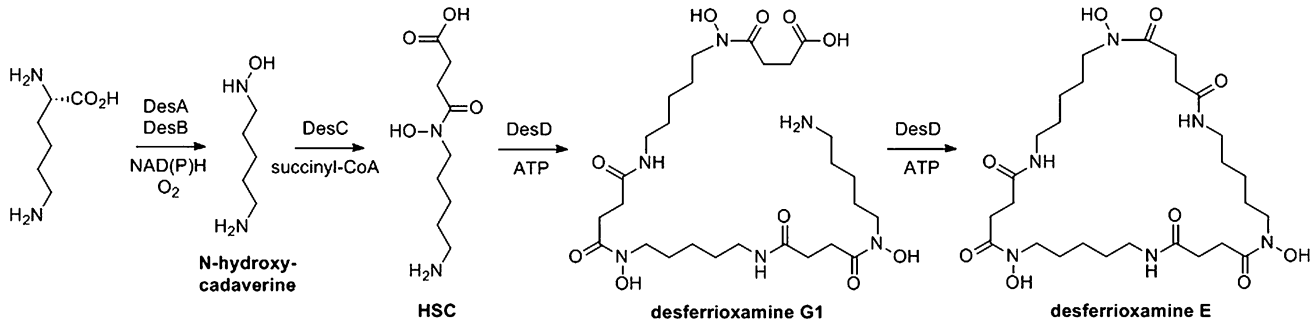


Fig. 13 Biosynthetic pathway for desferrioxamines G1 and E in *S. coelicolor*. Desferrioxamine B derives from DesD-catalyzed condensation of two molecules of HSC with a molecule of *N*-hydroxy-*N*-acetyl-cadaverine

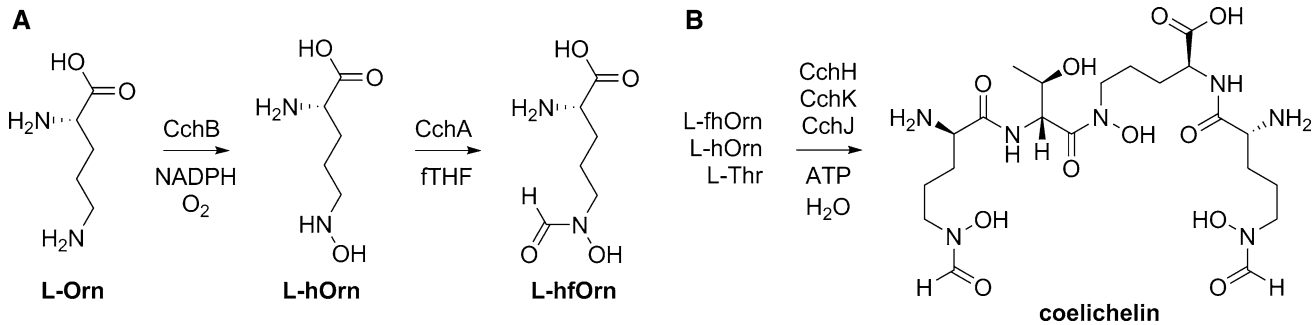


Fig. 14 Proposed pathway for coelichelin biosynthesis in *S. coelicolor*. **a** Biosynthesis of the non-proteinogenic amino acids L-hOrn and L-hfOrn from L-Orn (fTHF = 10-formyltetrahydrofolate). **b** NRPS-mediated assembly of coelichelin from amino acid precursors

catalyze nucleotide triphosphate-dependent trimerization of *N*-hydroxy-*N*-succinyl-cadaverine (HSC) (Fig. 13) [1]. Genetic and biochemical experiments confirmed the proposed role of DesD in desferrioxamine biosynthesis, identifying it as the founding member of a new class of ATP-dependent oligomerization-macrocyclization biocatalysts [35]. *S. coelicolor* was also shown to produce desferrioxamine B [2], which is assembled by DesD from two molecules of HSC and a molecule of *N*-hydroxy-*N*-acetylcadaverine [35]. Desferrioxamines were shown to function as siderophores in *S. coelicolor* and the *desE* gene (*sco2780*) upstream of the *desABCD* operon was found to encode a lipoprotein receptor that selectively binds ferrioxamines and initiates their transport into the cell [1, 51]

The *cchH* (*sco0496*) gene uncovered by the genome sequencing project encodes a trimodular NRPS that was not associated with the production of a known *S. coelicolor* metabolite [3]. Predictive sequence analysis provided considerable insight into the structural features of the peptide assembled by this multienzyme, suggesting that it may function as a siderophore [8]. Comparative metabolite profiling of supernatants from iron-deficient cultures of wild-type *S. coelicolor* and a mutant in which the *cchH* gene had been disrupted led to identification of the tetrapeptide coelichelin as the metabolic product of CchH (Fig. 14) [41]. Further genetic experiments showed that *cchJ* (*sco0498*) is also required for coelichelin biosynthesis [41]. Expression of a cosmid clone containing the *cchA–cchK* (*sco0489–sco0499*) gene cluster in *S. fungicidicus* resulted in coelichelin production [41]. Taken together, the above results suggested an unusual pathway for coelichelin biosynthesis involving iterative module use, module skipping and a standalone thioesterase (CchJ) in the assembly of a tetrapeptide by the trimodular NRPS CchH (Fig. 14).

CchA and CchB were proposed, and subsequently shown, to catalyze hydroxylation and formylation, respectively, of L-ornithine to provide the non-proteinogenic amino acids L-N5-hydroxyornithine (hOrn) and L-N5-hydroxy-N5-formylornithine (hfOrn) incorporated by CchH into coelichelin (Fig. 14) [5, 8, 55]. The MbtH-like protein encoded by *cchK*, or its homolog in the CDA biosynthetic gene cluster, was also shown to be required for coelichelin biosynthesis [42].

Coelimycin P1

In the mid-1990s, Kuczek and coworkers reported the identification of *S. coelicolor* genomic DNA fragments encoding type I modular PKS acyltransferase (AT) and ketosynthase (KS) domains that were not associated with production of any known *S. coelicolor* specialized

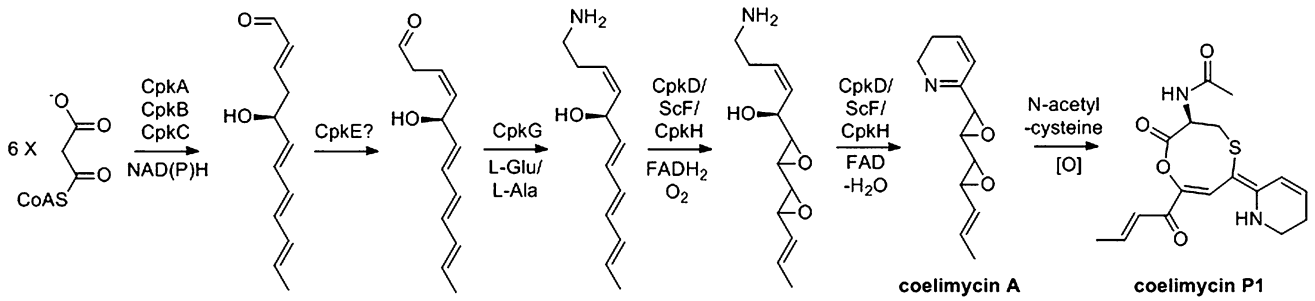


Fig. 15 Proposed pathway for assembly of coelimycin P1 via non-enzymatic trapping of coelimycin A, a putative antibacterial metabolic product of the *S. coelicolor cpk* gene cluster, with *N*-acetylcysteine and subsequent oxidation

metabolites [39, 40]. Subsequent analysis of the *S. coelicolor* genome sequence showed that these DNA fragments are located within the *cpk* gene cluster, which encodes a hexamodular PKS that is predicted to assemble a 4-hydroxy-2,6,8,10-dodecatetraenoyl thioester [52]. Interestingly, *S. coelicolor* γ-butanolide (SCB) signaling molecules have been shown to directly control the expression of the *cpk* gene cluster [65]. The *scbA* gene, which has been implicated in SCB biosynthesis [31], lies at the left extremity of the *cpk* cluster.

Two groups independently reported in 2010 that a diffusible yellow pigment is a metabolic product of *cpk* cluster [21, 53]. In one case, antibiotic activity was found to be associated with production of the pigment [21]. However, neither report provided any structural insight into these metabolites. The production of a diffusible yellow-orange pigment and associated antibiotic activity by *S. coelicolor* was first observed by Rudd in the late 1970s during genetic studies of antibiotic biosynthesis [57]. Mapping of the locus responsible for production of this pigment by Rudd corresponds precisely to the location of the *cpk* gene cluster on the *S. coelicolor* chromosome.

In 2012, the discovery of coelimycin P1 was reported [20] (Fig. 15). This novel yellow alkaloid was identified as a metabolic product of the *cpk* cluster utilizing a genetic engineering strategy aimed at maximizing flux through the biosynthetic pathway. In line with previous reports, antibacterial activity was observed in cultures of *S. coelicolor* producing coelimycin P1. However, purified coelimycin P1 was found to possess no antibacterial activity and was hypothesized to derive from the putative antibacterial *bis*-epoxide coelimycin A via reaction with *N*-acetyl-cysteine in the culture medium (Fig. 15). The incorporation patterns for labeled *N*-acetyl-cysteine and molecular oxygen into coelimycin P1 are consistent with this hypothesis [20]. A plausible pathway for the biosynthesis of coelimycin A involving reductive release of the 4-hydroxy-2,6,8,10-dodecatetraenoyl thioester assembled by the modular PKS to afford the corresponding aldehyde, followed by double-bond isomerization (*E*-Δ2 to *Z*-Δ3), transamination, bis-epoxidation, oxidation of the C-4 hydroxyl group and dehydration, has been proposed (Fig. 15) [20].

Conclusions

Clearly, the availability of a complete genome sequence has greatly facilitated the discovery and characterization of pathways for specialized metabolite biosynthesis in *S. coelicolor*. We now know that this model actinomycete is able to produce at least 17 chemically distinct classes of specialized metabolite and in many cases the pathways for assembly of these are now reasonably well understood. Many novel enzymes have been discovered in the course of elucidating the biosynthetic pathways for such metabolites. This has contributed significantly to our growing appreciation of the remarkably diverse enzymology involved in bioactive natural product biosynthesis and has provided novel "parts" for synthetic biology approaches to the production of fine, commodity, and specialty chemicals, as well as novel biocatalysts with potential applications in industrial biotechnology.

Despite these dramatic advances, there is still much to be learned about *S. coelicolor* specialized metabolism. The metabolic products of several putative specialized metabolic pathways remain unidentified and numerous exciting questions regarding the mechanisms for specialized metabolite assembly are still unresolved. Moreover, the biological functions of the majority of *S. coelicolor* specialized metabolites are far from clear. Thus, *S. coelicolor* specialized metabolism is likely to remain an important topic of research for the foreseeable future. As the cost of generating near-complete bacterial genome sequences continues to drop, the methodology developed to mine the *S. coelicolor* genome for new natural products and biosynthetic pathways is increasingly being applied to other Actinobacteria. Ultimately, such approaches will uncover the full metabolic potential of this remarkable phylum.

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