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Streptomycetes: Surrogate Hosts for the Genetic Manipulation of Biosynthetic Gene Clusters and Production of Natural Products

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Abstract

Due to the worldwide prevalence of multidrug-resistant pathogens and high incidence of diseases such as cancer, there is an urgent need for the discovery and development of new drugs. Nearly half of the FDA-approved drugs are derived from natural products that are produced by living organisms, mainly bacteria, fungi, and plants. Commercial development is often limited by the low yield of the desired compounds expressed by the native producers. In addition, recent advances in whole genome sequencing and bioinformatics have revealed an abundance of cryptic biosynthetic gene clusters within microbial genomes. Genetic manipulation of clusters in the native host is commonly used to awaken poorly expressed or silent gene clusters, however, the lack of feasible genetic manipulation systems in many strains often hinders our ability to engineer the native producers. The transfer of gene clusters into heterologous hosts for expression of partial or entire biosynthetic pathways is an approach that can be used to overcome this limitation. Heterologous expression also facilitates the chimeric fusion of different biosynthetic pathways, leading to the generation of "unnatural" natural products. The genus *Streptomyces* is especially known to be a prolific source of drugs/antibiotics, its members are often used as heterologous expression hosts. In this review, we summarize recent applications of *Streptomyces* species, *S. coelicolor*, *S. lividans*, *S. albus, S. venezuelae* and *S. avermitilis*, as heterologous expression systems.

Keywords

Streptomyces; Natural Products; Heterologous Expression; Biosynthetic Gene Clusters; Combinatorial Biosynthesis

1. Introduction

Natural products (NPs) have played incredibly important roles in human medicine. Nearly half of all approved therapeutics are derived directly from or can find roots in NPs. Since the discovery of penicillin and streptomycin, drugs derived from NPs have been used for almost all human diseases: infectious, cardiovascular, neurological, oncological, and more recently,

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depressive disorders (Dias et al., 2012; Newman and Cragg, 2016). They are equally important in agriculture, veterinary medicine, and the food industry (Zhou et al., 2008; Bekiesch et al., 2016). NPs are mainly produced by microorganisms and plants. Among the microorganisms, the genus *Streptomyces* is of particular interest as it has been the source of more than half of currently used antibiotics (Watve et al., 2001; Berdy, 2012; Bibb, 2013; Bekiesch et al., 2016). Since the report of the *S. coelicolor* A3(2) genome in 2002 (Bentley et al., 2002), more than 5,200 actinomycete genomes have been registered in the Genome OnLine Database (Reddy et al., 2015). Under current laboratory cultivation conditions, only a few major NPs are usually detected from a microbial source, whereas its genome typically shows the presence of 20~40 biosynthetic gene clusters (BGCs). Over 90% BGCs thus remain unexamined. The uncultivated microorganisms, estimated to represent ~99% of the microbial life on this planet, are an equally important source of NPs. Advances in metagenomics have made it possible to access these vast unexploited regions of "dark matter". New tools and technologies for analyzing and mining genomes/metagenomes are allowing NPs to re-emerge as an attractive resource for drug discovery and are revolutionizing natural product and drug discovery research (Blin et al., 2017a; Douglas et al., 2015; Ziemert et al., 2016).

Heterologous expression plays an indispensable role in the study of NPs and drug discovery (Rappe and Giovannoni, 2003; Craig et al., 2009). Normally, there are several limitations to produce NPs and to study or manipulate their biosynthesis in native strains: (1) low yield; (2) slow-growth; and (3) difficulties in genetic manipulation. In addition, as revealed by recent genomics advances, the majority of BGCs for NPs remain cryptic/unexpressed in native producers. The expression of genes or BGCs in a genetically well-studied, and robustly growing host, i.e., heterologous expression, provides an efficient alternative to overcome these limitations. In addition, heterologous expression has also been widely used to determine the boundaries of a BGC, to create unnatural NPs via combinatorial biosynthesis, and to study the functions of individual genes (Du et al., 2013; Komatsu et al., 2013; Liu et al., 2018; Park et al., 2011a; Waldman et al., 2015). Modifying the architecture, even with a minor structural change, can significantly alter/improve the biological activity of a compound, an important stage in drug discovery (Cummings et al., 2014; O'Connor, 2015). Heterologous expression is an indispensable tool in studying uncultivable microorganisms. It has been estimated that only about 1% of microorganisms can be cultured. With significant advances in next-generation sequencing (NGS), bio informatics tools to assemble and analyze genomes and to predict BGCs, and cloning vectors and techniques for large DNA fragments, it has become possible to access this vast unexplored resource for the discovery of new NPs through heterologous expression (Brady et al., 2009; Zhang et al., 2017a).

Escherichia coli, Pseudomonas putida, Saccharomyces cerevisiae, Streptomyces spp., and *Myxococcus xanthus* are often used as heterologous hosts for the production of exogenous NPs as they are easily cultivable, come with highly developed and easily reachable genetic tools, and are genetically and physiologically well studied. *E. coli* is the most common, fast-growing, and easily manipulated host with comprehensive knowledge of the native metabolic networks; it has been used to produce a large number of metabolites and recombinant proteins (Kim et al., 2015b; Zhang et al., 2016b). The main limitation to using

E. coli as a versatile heterologous host is the lack of specific biosynthetic machineries such as phosphopantetheinyl transferase (PPTase) and precursors such as methylmalonyl-CoA (Kim et al., 2015b), and the need for extensive genetic manipulations for actinomycete derived NPs (Zhang et al., 2016b). *P. putida* is a Gram-negative bacterium characterized by fast growth, well developed genetic tools, xenobiotic tolerance, a high NADPH generation rate and the presence of diverse enzymes; however, it has a low yield for polyketides and non-ribosomal peptides (NRPs) and the limited knowledge of its native metabolic networks largely restrict its applications (Zhang et al., 2016b). *S. cerevisiae* is the best host to produce eukaryotic NPs such as those from fungi and plants. Generally, a host that is phylogenetically close to native producers is advantageous because they may share similar traits including transcriptional and translational machineries, regulatory networks, codon usage, and precursor/substrate availability (Ongley et al., 2013). However, this general assumption has been recently challenged: Moore's group found that the violacein gene cluster from the marine bacterium *Pseudoalteromonas luteoviolacea* 2tal6 was readily expressed, with robust production of violacein in the γ-proteobacterium *P. putida* KT2440 and the α-proteobacterium *Agrobacterium tumefaciens* LBA4404, however, very little was produced in *E. coli* strains (γ-proteobacterium) despite their closer phylogenetic relationship to the native producer (Zhang et al., 2017b).

In addition to host selection, there are many other factors which need to be considered for a successful heterologous production. Unfortunately, the efficacy of heterologous expression of a gene cluster in different hosts varies greatly, and there are no established rules for prediction of the most appropriate system: one can only test various hosts to determine the best one. Representative strategies and factors to be considered for an optimized heterologous production of NPs or unnatural derivatives are summarized in Figure 1.

As the most prolific source of NPs, *Streptomyces* strains have several advantages as heterologous expression hosts: (1) a rich pool of precursors/substrates; (2) available toolkits for genetic manipulation; (3) relatively easy cultivation; and (4) biosafety. With the exception of plant pathogen (Leiminger et al., 2013), streptomycetes are considered to have extremely low health and environmental risks (Shepherd et al., 2010). However, *Streptomyces* do have limitations when compared to *E. coli,* including relatively slow growth rates (usually hours of doubling time), less well-developed genetic engineering tools, and the presence of competing endogenous secondary metabolic pathways (Zhang et al., 2016b). Various *Streptomyces* species including *S. coelicolor*, *S. lividans*, *S. venezuelae, S. avermitilis*, *S. albus*, *S. griseofuscus*, *S. ambofaciens*, *S. fradiae* (tylosin producer), *S. roseosporus,* and *S. toyocaensis* have been used frequently for heterologous expression of foreign BGCs (Baltz, 2010; Thanapipatsiri et al., 2015). They are suitable for the production of NPs derived from both closely related species and distantly related actinomycetes and plants.

In this review, we will focus on recent heterologous expression trials in five strains, *S. coelicolor* A3(2), *S. lividans*, *S. albus*, *S. venezuelae* and *S. avermitilis.* Construction of a 'clean' host, in which endogenous BGCs have been removed, is generally preferred because more precursors, intermediates and energy can be directed towards the production of the heterologous metabolites (Kim et al., 2015b; Komatsu et al., 2010). Recent work on host

engineering is also included. For a better illustration, we have classified trials/examples into different categories based on purposes or outcomes: CA, Cryptic pathway Activation/ awakening; GC, Gene Characterization (individual function); GI, Gene cluster Identification; MG, MetaGenomics; NM, New Metabolite; OP, Over-Production; and P, Plant-derived NPs.

2. *Streptomyces coelicolor* as a heterologous host

S. coelicolor A3(2) is the most genetically well-studied strain among streptomycetes and is currently one of the best hosts for heterologous production of NPs (Gomez-Escribano and Bibb, 2012; 2014). The major metabolites produced by *S. coelicolor* A3(2) include an aromatic polyketide antibiotic actinorhodin (ACT), the tripyrolle antibiotic undecylprodigiosin (RED), and a non-ribosomal peptide calcium-dependent antibiotic (CDA). In 2002, the 8,667,507 bp genome of *S. coelicolor* A3(2) was reported (see more details in Table 1) (Bentley et al., 2002), the first genome sequence in this group of prolific NP producers. Besides ACT, RED and CDA, many more metabolites were predicted from bioinformatic analysis of potential BGCs, including type I, II and III polyketides, NRPs, terpenes and alkaloids. In addition to the wild-type *S. coelicolor* A3(2), mutants including CH999, M512, M1146, M1152, M1154, and M1317 (as summarized in Table 2) have been generated to better express foreign BGCs. CH999 was engineered to better produce polyketides by diminishing the production of both ACT and RED (McDaniel et al., 1993). For a better production of type III polyketides, a new host M1317 (Thanapipatsiri et al., 2015) was developed by deleting type III polyketide gene clusters (*gcs*, *srsA, rppA*) in the strain M1152 (Gomez-Escribano and Bibb, 2011).

The examples mentioned in this section are summarized in Table 4. *S. coelicolor* A3(2) has proven to be an efficient recipient host for giant clusters. Salinomycin, a polyether antibiotic used to prevent *Coccidioidomycosis* in poultry and to alter gut flora to improve nutrient absorption in ruminants, is generated by an assembly line of nine polyketide synthases (PKSs) (Jiang et al., 2012; Yin et al., 2015). Yin et al. isolated three fragments of the salinomycin gene cluster from *S. albus* DSM 1398, assembled them into a single 106-kb DNA fragment using the Red/ET recombination technique, and successfully expressed the giant cluster in the wild-type *S. coelicolor* A3(2) and restored salinomycin production (Yin et al., 2015). The 141-kb gene cluster of vancoresmycin (Figure 2), a potent antibiotic active against vancomycin-resistant *Enterococcus* spp. (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA), was expressed in *S. coelicolor* M1152 yielding vancoresmycin at 2.2 mg/L (Kepplinger et al., 2018).

Because of the well-understood genetic background and readily available molecular cloning tools, *S. coelicolor* is often used as an alternative host for the expression of genes or gene clusters derived from genetically intractable actinomycetes. Gougerotin (Figure 2), a peptidyl nucleoside antibiotic with antitumor, antiviral, antibacterial, antimycoplasma, anthelmintric and acaricidal activities, was originally isolated from *S. graminearus* (Jiang et al., 2013). The Tan group identified a fosmid D6-4H from a DNA library of *S. graminearus*, which contains a complete gougerotin gene cluster (Du et al., 2013). Expression of D6-4H in *S. coelicolor* M1146 enabled the functional characterization of two genes *gouC* and *gouD,*

as well as the heterologous production of gougerotin (Wei et al., 2016). By replacing native promoters of key structural genes by the *hrdB* promoter, the same group increased the yield of gougerotin in M1146 by 10-fold as compared to its native producer (Du et al., 2013; Niu et al., 2013). Streptothricin (ST), an antibiotic active against both Gram-positive and Gram-negative bacteria and eukaryotes, was isolated from a soil-derived *S.* sp. fdl-xmd (Yu et al., 2018). The verification of ST BGC was accomplished through the expression of the gene cluster in M1146, and the yield was enhanced to 0.5 g/L after an optimization of culture conditions. Though the same level of production of ST is observed in the native producer, production takes 7-12 days, whereas in M1146 it takes only 2 days (Yu et al., 2018). Desotamides A and B, potent antibacterial cyclohexapeptides, were originally isolated from *S. scopuliridis* SCSIO ZJ46 (Song et al., 2014). Expression of the 39-kb *dsa* gene cluster in *S. coelicolor* M1152 afforded desotamides A and B; interestingly, a new structure desotamide G was later identified (Li et al., 2015).

As productivity has often been found to be low in native producers, heterologous expression is frequently used to enhance yield. This approach often involves trials with different hosts and promoter exchange. Two novel aminocoumarins, cacibiocins A and B (Figure 2), were isolated from the very rare actinomycete *Catenulispora acidiphila* DSM 44928. Their production was dramatically increased from 4.9 mg/L in the native strain to 60 mg/L in M1152 (Zettler et al., 2014). A ΦC31-based integration of a newly assembled cosmid containing the 38.6-kb gene cluster of another aminocoumarin antibiotic coumermycin Al into the M512 genome led to a production of coumermycin Al at ~7 mg/L, slightly higher than the native producer *S. rishiriensis* DSM 40489 (5 mg/L) (Wolpert et al., 2008). Production was increased to 52.5 mg/L by simply changing the host to M1146 (Flinspach et al., 2010). In the same set of experiments, production of clorobiocin and its derivatives exceeded 100 mg/L in *S. coelicolor* M512, M1146 and M1154; an optimal production of caprazamycin aglycones reached 152 mg/L in the host M1154 when 0.6 % Q-5247 and 0.2 mg/L $CoCl_2$ were present (Flinspach et al., 2010). Similarly, kocurin, a new thiopeptide antibiotic isolated from *Kocuria rosea* (Schinke et al., 2017), was produced in M1146 by expressing the kocurin BGC (Linares-Otoya et al., 2017).

This issue of low yields is particularly profound with marine NPs, which have recently become an attractive source of drug discovery (Gerwick and Moore, 2012). Anthracimycin (Figure 2) is an unusual 14-membered macrolide produced by a trans-acyltransferase (*trans*-AT) PKS system in two marine streptomycetes *S.* sp. CNH365 and *S.* sp. T676 (Jang et al., 2013; Alt and Wilkinson, 2015). It exhibits antibacterial activity (≤0.06 μg/mL) against MRSA and VRE. The heterologous expression of the 53-kb anthracimycin gene cluster in *S. coelicolor* strains M1154, M1152, and M1146 yielded anthracimycin at approximately 9, 10, and 14 μg/mL, respectively (Alt and Wilkinson, 2015). The intact gene cluster of fluostatin, an atypical angucycline isolated from the marine actinomycete *Micromonospora rosaria* SCSIO N160, was heterologously expressed in *S. coelicolor* YF11; when cultured with 3% sea salts, the recombinant *S. coelicolor* strain produced two new fluostatin derivatives, fluo statin L and an unusual heterodimer difluostatin A which exhibit antibacterial activities (Yang et al., 2015).

Tryptophan dimers (TDs) are an important class of NPs with diverse biological activities including antibacterial, antifungal and antiproliferative. The combined use of bio informatics, targeted gene disruption and heterologous expression in *S. coelicolor* YF11 of the *Spm* gene cluster which was isolated from the deep-sea bacterium *S.* sp. SCSIO 03032 confirmed its indispensable role in the biosynthesis of indimicins (IDMs), lynamicins (LNMs) and spiroindimicins (SPMs) (Ma et al., 2017). Although the heterologous expression of the *Spm* gene cluster failed to produce final products, three compounds 6',6"-dichloro-chromopyrrolic acid, demethyl-LNM A and 6',6"-dichloro-bisindolylmaleimide were produced. The latter two compounds, previously known either as a "non-natural" hydrolysis product or a synthetic product, respectively, were produced for the first time in a bacterium (Ma et al., 2017).

S. coelicolor is rich in two-component systems (TCSs), which often play important roles in the physiological differentiation (Bentley et al., 2002). Yepes et al. identified *arbA1/A2* encoding a pleiotropic repressor of antibiotic production in *S. coelicolor* (Yepes et al., 2011). The deletion of *arbA1/A2* in M145 (ΔabrA) not only enhanced the production of endogenous antibiotics such as ACT, RED and CDA, but also foreign NPs; the expression of the entire oviedomycin pathway derived from *S. antibiotics* ATCC 11891 in the ΔabrA mutant duplicated oviedomycin production as compared to the parent host M145 (Rico et al., 2014). Interestingly, expression of a pSET152 construct containing only 12 structural genes controlled by a single *hrdB* promoter in *S. coelicolor* M1146 also produced oviedomycin (Xu et al., 2017b).

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are an emerging group of NPs that are prevalent throughout nature and exhibit potent bioactivities. The elucidation of biosynthetic pathways of RiPPs is challenging as the biosynthesis takes place on a single precursor and intermediates may be rapidly proteolyzed. Bottromycin (BTM) A2 (Figure 2), a potent antibiotic against MRSA and VRE, is a typical RiPP, in which the *N*-terminus of the precursor peptide BtmD undergoes post-translational modifications to decorate a mature BTM scaffold (Crone et al., 2016). The genetic manipulation of the BTM producer *S.* sp. BC16019 has been so far restricted to the single crossover method with very low efficiency, so the BTM gene cluster has been expressed in two different hosts *S. coelicolor* A3(2) and *S. albus* J1074. The native strain produced 100 μg/L of BTM. Heterologous expression of the BTM cluster in *S. coelicolor* and *S. albus* produced very low levels of BTM, 1 and 4 μg/L, respectively, which was increased 20-fold by replacing a ~16-kb DNA fragment of the 5'-end of the BTM gene cluster with a kanamycin resistance gene and by substituting native promoters with the strong *ermE** promoter (Huo et al., 2012). Further engineering of the BTM gene cluster in *S. coelicolor* A3(2) enabled the discovery of 3 novel BTM derivatives B2, D2, and E2 (Figure 2), and functional characterization of an *O*-methyltransferase and three radical SAM-dependent methyltransferases (Huo et al., 2012). Recently, the Truman group identified another BTM gene cluster in *S. scabies* and cloned it into the direct-capture vector pCAP (Yamanaka et al., 2014) to yield pCAPbtm. Expression of pCAPbtm in M1146 produced a very low level of BTMs; further, three constitutive promoters, P_{SEF14}, P_{aac3} and P_{hrdB}, were inserted in front of *btmA, btmB* and *btmC*, respectively, to yield pCAPbtml, expression of which in M1146 increased the productivity 20-fold as compared to pCAPbtm (Eyles et al., 2018). Further expression of pCAPbtm2, in

which *btmB* gene was deleted, in M1146 produced 60 times more BTMs than M1146::pCAPbtm; expression of pCAPbtm2 in other hosts including *S. laurentii*, *S. lividans* and *S. venezueale* produced BTMs in amounts similar to or slightly higher than in M1146 (Eyles et al., 2018). However, none of the products found so far has shown the *C*-methylation of phenylalanine (Eyles et al., 2018). A new version of pCAPbtml and pCAPbtm2 was constructed by the insertion of theophylline-dependent riboswitches between P_{hrdB}/P_{SEF14} and *btmC* to generate pCAPbtm6 and pCAPbtm7, respectively. While the expression of pCAPbtm6 in M1146 showed almost no production of BTMs, expression of pCAPbtm7 in M1146 increased the production of BTMs 120-fold as compared to the original construct pCAPbtm. Finally, mature BTMs were produced at a similar level as in the native strain and duplicated amounts of total BTMs (Eyles et al., 2018). By studying the heterologous production of another RiPP, Flinspach et al. found that primary metabolic genes can interfere with the expression of foreign DNA. Authors failed to express the GE2270 (*ptb*) gene cluster, which encodes a RiPP thiopeptide antibiotic in *Planobispora rosea* ATCC 53773, in M1146 and it became possible only after the deletion of ribosomal genes flanking the gene cluster. GE2270 production in M1146 was increased 2.5-fold from an initial yield of 0.7 mg/L via co-expression of the tuf^R resistance gene from *P. rosea* under the constitutive *ermE** promoter (Flinspach et al., 2014). Production of albusnodin, a unique RiPP with post-translational acetylation, failed in both the native producer *S. albus* DSM 41398 and the heterologous host *E. coli*; however, expression of the gene cluster under the control of *ermE** promoter in *S. coelicolor* M1146 or *S. lividans* 66 enabled the identification of albusnodin in both cell pellets and culture broth (Zong et al., 2018).

Lantibotics, a group of ribosomally synthesized and post-translationally modified peptides, are produced mainly by Gram-positive bacteria including lactococci, staphylococci and actinomycetes. Due to the lack of sufficient materials for structural characterization of erythreapeptins, a new group of class III lantibiotics, in the natural producer *Saccharopolyspora erythraea* NRRL 2338, Süssmuth and his group achieved the production of erythreapeptins in *S. coelicolor* M1146 and *S. lividans* TK24, which allowed the authors to manipulate the biosynthetic pathway and to elucidate the function of key genes (Völler et al., 2012). Streptocollin, representing the latest member of the venezuelin family lanthipeptides, was identified via an antiSMASH-based genome mining of *S. collinus* Tü 365. However, the native strain produces only trace amounts of streptocollin; by expressing streptocollin biosynthetic genes under the control of a constitutive promoter in *S. collinus* TU365 or heterologously in M1152, preparative amounts of streptocollin were obtained (Iftime et al., 2015). Even though no significant antibacterial or antiviral activity was detected, the compound did show moderate inhibitory activity towards protein tyrosin phosphatase 1B (PTP1B), a negative regulator of the leptin and insulin signaling pathways and its involvement in obesity and diabetes was recently demonstrated (Cho, 2013).

Recent progress in cloning tools and techniques has contributed greatly to natural product research in the post-genome era. Direct capture of BGCs and the use of transformation-associated- recombination (TAR) identified cosmomycin (Figure 2) and its analogues, and ammosamides A-C in the culture of recombinant *S. coelicolor* strains (Jordan and Moore, 2016; Larson et al., 2017). Following the cloning of gene cluster from *S. tsukubaensis* NRRL 18444 using a P1-derived artificial chromosome (PAC) expression vector, Bibb and

his colleagues heterologously expressed the 83.5-kb FK506 gene cluster in a few *S. coelicolor* hosts (Jones et al., 2013); the yield of FK506 was increased from 1.2 mg/L to 5.5 mg/L in M1146 when the LuxR regulatory gene *fkbN* was over-expressed. Using the same PAC vector system, neoabyssomicin A/B and abyssomicin 2/4 were recently identified from a deep-sea isolate *S. koyangensis* SCSIO 5802 via expressing PAC constructs heterologously in M1152 (Tu et al., 2018). Neoabyssomicin A augments human immunodeficiency virus-1 (HIV-1) replication and abyssomicin 2 selectively reactivates latent HIV and is also active against Gram-positive pathogens including MRSA. Co-expression of the gene cluster with *abmH* or *abmI*, two potential pathway-specific regulatory genes, increased the yield of abyssomicin 2 7.6-fold (2.1 g/L) and 3-fold (0.83 g/L), respectively (Tu et al., 2018).

3. *Streptomyces lividans* as a heterologous host

As a close relative of *S. coelicolor* A3(2), *S. lividans* is another genetically well-studied *Streptomyces,* and has been used extensively as a host for heterologous expression of foreign genes or gene clusters (Ruckert et al., 2015). Notably, *S. lividans* accepts methylated foreign DNA, and shows low protease activity (Baltz, 2010). The genome sequence of *S. lividans* TK24 has been determined, indicating a size of approximately 8.3-Mb, slightly smaller than that of *S. coelicolor* A3(2) (details listed in Table 1) (Jayapal et al., 2007; Ruckert et al., 2015). *S. lividans* has gene clusters encoding for the synthesis of ACT, RED and CDA but their production is very rare and depends on the specific conditions (Kim et al., 2001; Meschke et al., 2012).

The examples discussed in this section are summarized in Table 4. Paromamine and 2-deoxystreptamine (2-DOS), key intermediates in kanamycin biosynthesis, were produced by expressing two constructs pSK-2 and pSK-7 in *S. lividans* TK24, thus confirming the involvement of genes in kanamycin production (Nepal et al., 2009). Ribostamycin (Figure 3) was produced in *S. lividans* TK24 through the expression of a cosmid pRBM4 which harbors a 31.8-kb DNA fragment isolated from the ribostamycin producer *S. ribosidificus* ATCC 21294 (Subba et al., 2007).

Using a similar strategy with *S. lividans* TK24, a DNA locus of 33.3-kb cloned in a cosmid cosRav32 is proven sufficient to produce a antitumor angucycline ravidomycin (Kharel et al., 2010). Likewise, a recombinant *S. lividans* strain carrying 25 biosynthetic genes of hatomarubigins, *hrbR1-hrbX* and *hrbY,* was able to produce all known hatomarubigins including the dimer hatomarubigin D (Izawa et al., 2014). A new compound, 5-hydroxyhatomarubigin E, was detected when the *hrbF* gene was removed from the expressing cassette; the *hrbF* gene has no homology to any known angucycline biosynthetic genes (Izawa et al., 2014). Asukamycin (Figure 3), a potent antimicrobial and antitumor polyketide, was produced in *S. lividans* through heterologously expressing the entire gene cluster isolated from *S. nodosus* subsp. *asukaensis* (Rui et al., 2010). Leptomycin, isolated from *S.* sp. ATCC 39366, exerts its antifungal and antitumor activity via the inhibition of nucleo-cytoplasmic translocation in eukaryotic cells. The completeness of the 90-kb leptomycin gene cluster was confirmed by a successful production of leptomycins A and B in *S. lividans* (Hu et al., 2005). The BGC of mithramycin A, a potent antitumor polyketide from *S. argillaceus,* was heterologously expressed in *S. lividans* TK24 using the TAR

cloning. Mithramycin A was produced at 0.86 g/L in a standard fermentation system; but after a successive engineering of *S. lividans* TK24 and an optimization of fermentation conditions, the yield was elevated to 3 g/L (Novakova et al., 2018). Clethramycin and mediomycin A, two linear polyene polyketide (LPP) family antibiotics with potent antifungal activity, were reported from *S. mediocidicus* ATCC 23936. The later contains an amino moiety substituted for the guanidino moiety. The draft genome sequence and bioinformatic analysis revealed only one gene cluster (*cle*) for clethramycin. Further, genome mining analysis found a remotely located amidinohydrolase MedX. The co-expression of the *cle* gene cluster and *medX* in *S. lividans* indeed restored the production of mediomycin (Sun et al., 2018).

Deng et al. recently constructed a BAC library of *S. avermitilis* ATCC 31267 with an average insert size of 100~130-kb. Heterologous expression of five clones in *S. lividans* produced three novel avermectin analogs A2a, B1a and A1a (Deng et al., 2017). Using the similar BAC vector, streptothricin, borrelidin, and two novel linear lipopeptides 8D1-1 and 8D1-2 were produced in *S. lividans* by expressing cryptic gene clusters which were isolated from *S. rochei* (Xu et al., 2016).

Polycyclic tetramate macrolactams (PTMs) are hybrid PKS/NRPS compounds. The PTM gene clusters are conserved and widely distributed among bacterial genomes but normally remain silent. The activation of six new PTM congeners, pactamides A-F (Figure 3), was achieved through the expression of PTM cluster isolated from a deep-sea *S. pactum* strain SCSIO 02999 (Saha et al., 2017). A hybrid PKS/NRPS gene cluster of approximately 90-kb was captured in a single pSBAC clone through a straightforward restriction enzyme digestion and cloning approach; expression of the construct in *S. lividans* produced meridamycin (Figure 3); its productivity was enhanced by feeding precursors and using the strong constitutive *ermE** promoter (Liu et al., 2009). Heterologous expression of the pacidamycin BGC in *S. lividans* TK24 significantly increased the production of pacidamycin D (Figure 3) than in wild-type strain *S. coeruleorubidus*; a new compound pacidamycin S which differs from D by substituting a C-terminal tryptophan with phenylalanine was also identified (Rackham et al., 2010).

A diazo group has been found in a wide range of NPs and is extremely important due to its unique capability to facilitate 1,3-dipolar cyclo additions, carbine insertions, and alkylations. The heterologous production of cremeomycin (Figure 3) in *S. lividans* TK64 was the first report of heterologous production of a diazo-containing NP (Waldman et al., 2015). This heterologous expression not only enabled mechanistic studies of genes *creABDAM* in the diazo formation, but also identified a diazo metabolite, 2-hydroxy-4-methoxybenzoic acid (2,4-HMBA) (Waldman et al., 2015). A unique and unusual phosphonotripeptide antibacterial compound, dehyrophos, was identified in *S. lividans* 66 through the expression of fosmid clones isolated from the wild-type producer *S. luridus* NRRL 15101 (Circello et al., 2010). The heterologous expression system further determined minimal genes required for dehydrophos production. A *d*-cycloserine (DCS) non-producing *S. lividans* 66 could produce DCS through the expression of a 21-kb DNA fragment encoding 10 ORFs, DcsA-DcsJ (Kumagai et al., 2010).

Thioviridamide (Figure 3) contains five thioamide bonds and was originally isolated from *S. olivoviridis* NA 05001 (Hayakawa et al., 2006). Introduction of a 14.5-kb *Eco*RI fragment containing genes *tvaB-tvaO* together with an additional gene *tvaA* into *S. lividans* TK23 produced thioviridamide; further, the authors also confirmed the compound as a RiPP (Izawa et al., 2013). The antitumor agent YM-216391 is a new cyclic peptide containing a polyoxazole-thiazole moiety, originally isolated from *S. nobilis* (Sohda et al., 2005). Gene cluster analysis suggested it is a RiPP involving multistep posttranslational modifications. Since the strain is hard to manipulate, the entire gene cluster was expressed in *S. lividans*, producing ~0.2 mg/L of YM-216391, and if *ymR3* gene encoding a pathway-specific repressor was deleted, the yield increased to 3.84 mg/L (Jian et al., 2012).

Low yield, particularly in the less-studied actinomycetes, is a major bottleneck to access the biosynthesis of new NPs in their native producers. In addition, these strains are often not amenable for genetic manipulation. A desert isolate *Actinomadura namibiensis* produces type III lantibiotics labyrinthopeptin A1 and A2. In addition to its unique structure, A2 possesses remarkable antiviral and antiallodynic activities. Due to the genetic intractability of *A. namibiensis*, the BGC was heterologously expressed in *S. lividans* enabling the identification of novel labyrinthopeptins and harnessing the flexibility of the biosynthetic machinery (Krawczyk et al., 2013). The anti-tuberculosis antibiotic capreomycin was produced at 50 mg/L when simply expressing the gene cluster in *S. lividans* 1326 without any modification, in comparison to a trace level production in the native strain *Saccharothrix mntabilis* (Fenlnagle et al., 2017). Platensimycin and platensin (Figure 3) exhibit antibacterial activity against Gram-positive bacteria by selectively inhibiting cellular lipid biosynthesis through the selective targeting of β-keto-acyl (ACP) synthase I/II (FabF/FabB) in the fatty acid pathway (Wang et al., 2006; Wang et al., 2007). PtmRl and PtnRl have been identified as pathway-specific repressors of platensimycin and platensin production in *S. platensis* MA7327 and *S. platensis* MA7339, respectively (Smanski et al., 2009). Since the strain is difficult to manipulate genetically, heterologous expression of the platensin gene cluster without the *ptn*R1 gene in *S. lividans* K4-114 enabled the production of platensin with 6 congeners, which were not detected in the native producer (Smanski et al., 2012). Similarly, due to the lack of a genetic manipulation system in *S. antibioticus*, heterologous production of 8,8a-dihydroxy-6-deoxyerythronolide B in *S. lividans* provided a useful platform to study and engineer the biosynthesis of oleandomycin, a group of important macrolide antibiotics (Shah et al., 2000).

Expression of BGCs in heterologous hosts sometimes modifies the final products due to an unexpected involvement of host catalysts. When the gene cluster of blasticidin S, a strong fungicidal agent isolated from *S. griseochromogenes,* was engrafted into the chromosome of *S. lividans* 66, an inactive deaminohydroxylblasticidin S (Figure 3) was isolated instead of blasticidin S, likely due to the action of a host deaminase in *S. lividans* 66 (Li et al., 2013b). Another intriguing example is the heterologous production of a cyanotoxin lyngbyatoxin A (Figure 4). Genes *ltxABC* were identified to direct the biosynthesis of lyngbyatoxin A in cyanobacterium *Moorea producens* (Edwards and Gerwick, 2004); a similar gene cluster *tleABC* has been recently found in *S. blastmyceticus* NBRC 12747 (Awakawa et al., 2014). Expression of *tleABC* in three different hosts *S. albus* G153, *S. lividans* TK21 and *S. avermitilis* SUKA22 revealed unexpected host-pathway interactions. Although two

intermediates valindolmycin and indolactam V and the final product lyngbyatoxin A were detected in all hosts, new analogues that are absent in both native producer and hosts were produced (Zhang et al., 2016a). Interestingly, these analogues were produced in a host-specific manner; different host preferably recognizes different compounds in the lyngbyatoxin pathway. For example, *S. lividans* used indolactam V to generate analogues; while *S. albus* and *S. avermitilis* interacted with the early intermediate valindolmycin and the final product lyngbyatoxin A, respectively. The host specific generation of lyngbyatoxin A analogues was summarized in Figure 4 (Zhang et al., 2016a).

It has been widely recognized that secondary metabolism in *Streptomyces* is controlled by a complex regulatory network, which includes pathway-specific regulators (activators or repressors), global regulators (controlling multiple pathways), and small signaling molecules (van Wezel and McDowall, 2011). Overexpression of activators and knock-out of repressors are effective strategies to achieve a better production of NPs. The *ppk* and *pstS* genes encode a polyphosphate kinase that down-regulates antibiotic production and a high-affinity phosphate binding protein in *S. lividans,* respectively. The *ppk/pstS* double knock-out mutant of *S. lividans* showed a higher level of ACT production (Chouayekh and Virolle, 2002; Ramos et al., 2008). The expression of the cosmid cos16F4 in the mutant enhanced the production of 8-methyl-tetracenomycin C by 10-fold (Diaz et al., 2013).

The aromatic compound cinnamic acid (Figure 3) is a precursor of various phenylpropanoids including lignins, flavonoids, and coumarins, and is of industrial importance in diverse areas such as perfume production, the food industry, and pharmaceutical production. The precursor can be produced either through chemical synthesis or through microbial fermentation using *E. coli* or *P. putida,* however, the former is energy intensive and the latter usually ends with a low-yield (Nijkamp et al., 2005; Vannelli et al., 2007). Alternatively, the ubiquitous enzyme phenylalamine ammonia lyse (PAL) catalyzes the conversion of L-phenylalanine to ammonia and cinnamic acid (Cui et al., 2014). A PAL was recently identified in *S. maritimus* (PAL_{Sm}), which is much more robust and flexible with substrates than those found in plant such as *Petroselinum crispum* or yeast such as *Rhodosporidium toruloides.* The overexpression of PALsm in *S. coelicolor* led to the production of cinnamic acid at 0.2~0.5 mg per 100 mL; notably, expressing the same PAL in *S. lividans* enhanced the production to 210 mg per L with glucose as the carbon source and to 450 mg/L, if glucose was replaced by glycerol (Xiang and Moore, 2002). A similar yield of cinnamic acid was obtained when cheap carbon sources such as raw starch or xylose were used, indicating that *S. lividans* represents an economically friendly alternative to supply this industrially important precursor.

It has been estimated that only about 1% of all microbes have been cultured for conventional microbial studies (Torsvik et al., 1990). The vast majority of uncultivable microorganisms are unexploited. Efficient harnessing of biosynthetic potential and discovery of new NPs have only become possible recently due to the advances in genomics and metagenomics together with heterologous expression. Five novel compounds, terragines A (Figure 3), B, C, D and E, were identified through expressing soil DNA in the surrogate host *S. lividans* (Wang et al., 2000). The assembly and functional analysis of individual genomes in an environmental sample is an alternative method for accessing the metabolic potential of the

majority of uncultured organisms (Handelsman et al., 1998; Gillespie et al., 2002; Daniel, 2004). Most functional metagenomic studies have been limited by the poor expression of genes derived from metagenomic DNA in *E. coli*. To overcome this limitation, a new tool has been developed for the construction and functional screening of metagenomics libraries in *S. lividans.* These studies demonstrated that functionally screening metagenomic libraries in *Streptomyces* hosts provides access to metabolic potential different from those expressed in *E. coli* alone (McMahon et al., 2012).

In addition to exogenous secondary metabolic gene clusters, *S. lividans* is widely used for expression or overproduction of enzymes derived from diverse microbial and environmental sources. The expression of thermophilic genes (Kieser et al., 2000; Diaz et al., 2008; Li et al., 2013a), secretory enzymes (Noda et al., 2010), superoxide dismutase (SOD) genes (Halliwell and Gutteridge, 1985; Kang et al., 2006; Kang et al., 2007; Kanth et al., 2011), and glycosyltransferases (Quiros et al., 2000; Nakazawa et al., 2011) are some of the most common examples.

4. *Streptomyces albus* as a heterologous host

S. albus J1074, derived from *S. albus* G, is defective in both restriction and modification enzymes of the *SalI* system. The 6,823,670 bp *S. albus* genome (accession number NZ_DS999645.1) is the smallest among all sequenced *Streptomyces* genomes and has the highest G+C content (73.3%) (Table 1) (Olano et al., 2014; Zaburannyi et al., 2014). *S. albus* J1074 has long been known as a suitable host for the heterologous production of NPs, at least partially due to the efficient genetic manipulation systems, well-known genetic background, minimized genome, and easy culture. In addition, *S. albus* contains two highly active *attB* sites, which could also explain its excellence as a heterologous host (Bilyk and Luzhetskyy, 2014). Under normal growth conditions, *S. albus* J1074 produces no bioactive secondary metabolites, although genome analyses predicted 27 secondary metabolic BGCs. The Salas group applied different strategies for the activation of cryptic gene clusters and isolated a few secondary metabolites such as antimycin and 6-*epi*-alteramides (polyketide-NRP hybrid), candicidin (Type I polyketide), indigoidine (NRP), and glycosylated paulomycins (Olano et al., 2014).

The examples mentioned in this section are summarized in Table 4. Moenomycin A (Figure 5) was isolated from its native strain *S. ghanaensis* ATCC 14676 with low yield. In an attempt to express the moenomycin gene cluster in various *Streptomyces* hosts, the moenomycin-resistant host *S. albus* J1074 produced the highest level of moenomycin A; production was further increased by co-expressing the *relA* gene, an important component in the ppGpp signal pathway (Makitrynskyy et al., 2010). The heterologous production of furaquinocin, a polyketide-isoprenoid hybrid originally isolated from *S.* sp. KO-3988 (Kawasaki et al., 2006), in *S. albus* has been demonstrated (Isogai et al., 2012). The heterologous expression of three contiguous genes encoding a type III PKS (*fur1*), a monooxigenase (*fur2*) and an aminotransferase (*fur3*) in *S. albus* produced a novel intermediate 8-amino-2,5,7-trihydroxy naphthalene-1,4 dione. The deletion of *fur3* diminished the production of furaquinocins, which can be restored by supplying 8-amino-2,5,7-trihydroxy naphthalene-1,4 dione (Isogai et al., 2012).

For the overproduction of a type I polyketide iso-migrastatin, the gene cluster was cloned from its producing strain *S. platensis* NRRL18993 and expressed in five different hosts, *S. albus, S. lividans, S. coelicolor,* and two mutants of *S. avermitilis.* Production was improved by 3 to 18 fold via R2YE media optimization: the highest yield of 128.6 mg/L was achieved in *S. albus* (Yang et al., 2011). Secondary metabolic gene clusters can sometimes span over 100-kb. To incorporate large DNA fragments, Bilyk et al. developed a new shuttle vector system which is based on the pi5a and F-factor replicons and can be maintained in *E. coli,* yeast and *Streptomyces.* Using this system, PCR amplified gene products covering several parts of the gene cluster of aromatic polyketides grecocyclines were transferred into yeast together with the newly developed vector; introduction of the assembled construct into *S. albus* led to the production of grecocycline A (Figure 5) and its derivatives (Bilyk et al., 2016). The fredericamycin (FDM) BGC has been expressed in *S. albus* yielding 120~132 mg/L of FDM, which also enabled the study of gene functions. In-frame deletion of *fdmM* or *fdmM1*, responsible for C-6 or C-8 hydroxylation, respectively, diminished the production of FDM A and a key intermediate FDM E (Chen et al., 2009; Baltz, 2010). Steffimycin is an anthracycline antibiotic isolated from *S. steffisburgensis* NRRL 3193 (Kunnari et al., 1997). The expression of the steffimycin BGC in *S. albus* J1074 produced 10 mg/L steffimycin (Gullon et al., 2006). Co-expression with neutral or branched deoxyhexose pathways has generated newly glycosylated steffimycin analogues; 3'-*O*-methy1steffimycin and *D*-digitoxyl-8-demethoxy-10-deoxy-steffimycin showed improved anti-proliferative activity with GI50 values less than 1 μM while steffimycin's GI_{50} values are 2.61 ~ 6.79 μM (Olano et al., 2008). In addition, the *S. albus* host has been used for the *in vivo* characterization/verification of tailoring enzymes in the biosynthesis of specific antibiotics. For example, three methyltransferase genes *elmMI*, *elmMII* and *elmMIII* were expressed individually in *S. albus* and exhibited their consecutive methylation at different hydroxyl groups of the sugar moiety of the polyketide elloramycin (Patallo et al., 2001).

Kinamycins, diazole-containing aromatic polyketide antibiotics, were isolated from *S. galtieri* sgt26. A complete understanding of biosynthetic pathways was hindered by the lack of genetic manipulation systems in the native strain. Expression of a BAC clone containing kinamycin gene cluster in *S. ablus* J1074 restored the heterologous production of kanamycin; which also enabled the identification of boundaries of the cluster (spanning 75-kb) and the characterization of gene functions in the pathway (Liu et al., 2018).

Dithiolopyrrolone-type compounds show diverse activities including antibacterial, antifungal, insecticidal and anticancer (Li et al., 2014). Expression of the dithiolopyrrolone (*aut*) gene cluster, which was isolated from *S. thioluteus* DSM 40027, in *S. albus* revealed the characteristic UV spectrum of dithiolopyrrolone-type compounds; however, the yield was too low and was insufficient for structural determination. The comparison of the *aut* gene cluster with holomycin (*hlm*) gene cluster from *S. clavuligerus* showed the *aut* gene cluster lacks the *hlmK* gene which encodes a distinctive type II thioesterase. The production of dithiolopyrrolone in *S. albus* was greatly improved through the co-expression of *hlmK* (Zhai et al., 2016).

Rebeccamycin and staurosporine (Figure 5) are antitumor compounds which belong to the family of indolocarbazole alkaloids (Sánchez et al., 2002). Over 30 indolocarbazole

derivatives have been identified through the dissection and reconstitution of the rebeccamycin biosynthetic gene cluster and co-expression of the staurosporine gene cluster in *S. albus.* Further, the expression of *pyrH* and *thaI,* halogenase genes from *S. rugosporus* and *S. albogriseolus,* respectively, successfully produced the halogenated derivatives (Sánchez et al., 2005). The biosynthesis of indolocarbazole K252a has remained unclear in the native producer *Nocardiopisis longicantena.* The co-expression of *inkO* and *inkD* genes which are responsible for the earliest steps of K252a biosynthesis in *S. albus* yielded chromopyrrolic acid, which may serve as a good starting point for further understanding the K252a biosynthetic pathway and the development of bioactive improved indolocarbazole analogues (Kim et al., 2007; Chae et al., 2009).

Herbicidins (Figure 5), the adenosine-based nucleoside antibiotics with a rare and unusual tricyclic undecose core decorated with a (5-hydroxy)-tiglyl moiety, have been isolated from *S.* sp. L-9-10 with a yield of 50~100 mg/L. It is notable that this was the first report of a bacterial pathway that uses tiglyl-CoA from the L-isoleucine catabolism to modify a secondary metabolite The completeness of the herbicidin gene cluster was confirmed by heterologous production of herbicidins in *S. albus* J1074 (Jung et al., 2006). Thiocoraline is an antitumor compound produced by two actinomycetes *Micromonospora* sp. ACM2-092 and *Micromonospora* sp. ML1 isolated from marine invertebrates. The thiocoraline gene cluster was cloned but was neither expressed in *S. albus* nor *S. lividans* unless the positive regulatory gene, *tioA,* encoding an OmpR family activator was transcribed from the *ermE** promoter (Lombó et al., 2006).

Carotenoids and terpenoids are common NPs found in all photosynthetic organisms. They play vital roles as food colorants, feed supplements, nutraceuticals and pharmaceuticals. Some *Streptomyces* strains also contain carotenoid-type gene clusters, e.g., *crta,* but most of them remain silent. Mining the *S. argiliaceus* genome showed the presence a *crta* gene cluster but no carotenoids have been isolated from this strain. Becerril et al. attempted several approaches to reveal the final products of the *crta* gene cluster in both native and heterologous hosts. Expression of a cosmid pKC505-C25 containing the entire *crta* gene cluster in *S. albus* produced yellow-pigmented compounds which were further identified as carotenoids: leprotene, β-isorenieratene, 3,3'-dihydroxyleprotene, 3-hydroxyleprotene, and monomethylated derivatives of 3,3'-dihydroxyleprotene and 3-hydroxyleprotene (Becerril et al., 2018).

S. albus has also been very fruitful in expressing environmental DNA (eDNA) and yielding new NPs. Brady and his colleagues propagated DNA extracted directly from the environment, and produced a tetracyclic anti-MRSA antibiotic tetarimycin A , and two new azaquinones, utahmycins A and B, by expressing environmental DNA in *S. albus* (Bauer et al., 2010; Kallifidas et al., 2012). As a continuation of this work, the same group heterologously produced three types of antibiotics, the well-known landomycin E, a new compound with a previously uncharacterized pentacyclic ring scaffold (Figure 5), and a unique KB-3346-5 derivative which shows activity against MRSA and VRE (Feng et al., 2011). Screening for BGCs from eDNA metagenomic clones has been improved by expressing a PPTase gene in *S. albus* (Bitok et al., 2017), which enabled Brady's group to identify clones containing NRPS, PKS and hybrid PKS/NRPS gene clusters and to confirm

that a clone is responsible for the production of myxochelin A (Bitok et al., 2017). More recently, the same group discovered malacidins (Figure 5), a group of calcium-dependent antibiotics which are potently active against multidrug-resistant pathogens and which sterilize MRSA infections in an animal model (Hover et al., 2018). Brady argued that addressing the following points will significantly hasten the discovery of NPs from metagenomics: (1) cloning methods for large DNA fragments (100~150 kb); (2) capability of hosts to accept versatile foreign DNA; and (3) efficient screening methods (Bitok et al., 2017).

5. *Streptomyces venezuelae* as a heterologous host

S. venezuelae has been used as a heterologous host because of its relatively fast growth with a doubling time of ~1 h (the shortest time among streptomycetes), genetic manipulation tools, and high transformation efficiency. The genome of *S. venezuleae* ATCC 15439 has been sequenced recently by two independent groups (accession No. LN881739 and CP013129) revealing a genome of 9.03-9.05 Mb (Table 1) (He et al., 2016; Song et al., 2016). Three major mutants of *S. venezuelae* ATCC 15439 have been generated to better express foreign BGCs: DHS2001 which lacks all *pik* genes (Jung et al., 2006), YJ003 which lacks the *des* gene cluster (Hong et al., 2004), and YJ28 which lacks both *pik* and *des* genes (Jung et al., 2007).

The examples mentioned in this section are summarized in Table 4. Aminoglycoside (AMG) antibiotics including amikacin, gentamicin, kanamycin, neomycin, spectinomycin, streptomycin, and tobramycin are important antibacterial drugs, especially for serious Gram-negative infections. Heterologous production of AMGs and their derivatives in *S. venezuelae* have been studied rigorously and shown to be successful. The production of gentamicin A2 (Figure 6A) in *S. venezuelae* YJ003 was the first heterologous expression of a pseudodisaccharide biosynthetic pathway (Park et al., 2008). The pSpc8 cosmid containing the spectinomycin BGC, isolated from its producing strain *S. spectabilis* ATCC27741, was expressed in *S. venezuelae* YJ003 leading to a heterologous production of spectinomycin (Thapa et al., 2008). Interestingly, expression of only five genes *spcA, spcB, spcS2, spcM and spcG* in *S. venezuelae* ATCC 15439 also produced spectinomycin; it has been suggested that the sugar is synthesized from host catalysts (Lamichhane et al., 2014). In the biosynthesis of ribostamycin, it was generally believed that ribose is a sugar donor only for neamine. However, Sohng's group reported the biosynthesis of 6'-deamino-6'-hydroxyribostamycin, pseudoribostamycin (Figure 6A), in *S. venezuelae* YJ003 as a result of ribosylation of paromamine (Kurumbang et al., 2011). Further, pseudoribostamycin was isolated from *S. lividans* TK24 which harbors a ribostamycin cosmid. These results demonstrated that ribosylation is possible at both paromamine and neamine (Subba et al., 2007; Kurumbang et al., 2011).

Due to the difficulty of genetic manipulation in the native kanamycin producer *S. kanamyceticus,* the biosynthesis of kanamycins has been studied heterologously in *S. venezuelae* YJ003, which allows further chemical biology and combinatorial biosynthesis studies (Thapa et al., 2007). Generally, only kanamycin A, B, and C are produced in *S. kanamyceticus*, with kanamycin A as the major (>80%) product. By *in vivo* genetic

engineering and combinatorial biosynthesis in *S. venezuelae* YJ003, i.e., expression of different sets of biosynthetic genes, and *in vitro* enzymatic reconstitutions, Park et al. identified a previously unexpected substrate flexibility of two glycosyltransferases KanF and KanE. Beside the known sugar donor UDP-D-*N*-acetylglucosamine (UDP-GlcNAc), KanF also accepts UDP-D-glucose (UDP-Glc) as the substrate; KanE accepts UDP-Glc in addition to the known donor UDP-D-kanosamine (UDP-Kns). These findings not only revealed the biosynthesis of kanamycin A, but also enabled the identification of five new kanamycins, 3”-deamino-3”-hydroxy-kanamycin B, 3”-deamino-3”-hydroxy-kanamycin C, kanamycin D, kanamycin X, and 3”-deamino-3”-hydroxy-kanamycin X (Figure 6B). Combinatorial biosynthesis further allowed the production of 1-N-AHBA-kanamycin A (also known as amikacin) (Park et al., 2011a), and a hybrid aminoglycoside oxykanamycin C (Figure 6B) through a chimeric fusion of spectinomycin and kanamycin biosynthetic genes (Nepal et al., 2010).

In addition to AMGs, various polyketides have been produced in *S. venezuelae* hosts. The production of oxytetracycline (oct) was increased from 75 to 431 mg/L in only 48 h by the expression of two pathway-specific regulators OctR and OtrR along with precursor supply in *S. venezuelae* WVR2006, a level comparable to 8 days in its native producer *S. rimosus* M4018. (Yin et al., 2016). The 40-kb DNA region containing five PKS genes from the tylosin pathway was cloned under the control of the strong *ermE** promoter in two plasmids and introduced into *S. venezuelae* DHS2001, which led to the production of about 0.5 mg/L of the 16-membered tylactone and 5-*O*-mycaminosyl tylactone (Figure 6A) (Jung et al., 2007). Production was increased to 1.4 mg/L upon exogenous feeding of 10 mM diethyl malonate, and further improved, by 2.7- and 17.1-fold, respectively, by the co-expression of the positive regulatory gene *pikD* (Jung et al., 2006; Jung et al., 2008).

S. venezuelae has also been used successfully for the expression of cryptic BGCs and heterologous detection of NPs. A unique chlorinated lipopeptide 4-*O*-demethylbarbamide, which was originally isolated from a marine cyanobacterium *Moorea praducens* (Engene et al., 2012), was produced at approximately 1 μg/L in *S. venezuelae* DHS2001 (Kim et al., 2012). The Sohng group isolated hopene (Figure 6A) from *S. venezuelae* YJ28, which harbors an *ermE** promoter-controlled 4-gene operon {*hopA, hopB, hopD and hopE*) of the hopene gene cluster from *S. peucetius* (Ghimire et al., 2015).

Plants are one of the richest sources of NPs; structurally diverse plant NPs have been used for drugs, cosmetics, seasonings, dyes, industrial chemicals, and nutraceuticals (Liu et al., 2017). There are major limitations for producing and purifying metabolites in plants: very low yield in plants (Chemler and Koffas, 2008; Liu et al., 2017), long time to produce a mature metabolite, seasonal production, and complex and tedious purification procedures (Chemler and Koffas, 2008). Due to the structural complexity of most plant NPs, total chemical synthesis is often challenging and usually yields large amounts of chemical wastes. Production of plant NPs in microbial hosts is an attractive alternative as it is renewable, environmentally friendly and season-independent, although there are still few challenges: (1) biosynthetic pathways are still mysterious for many plant NPs; (2) genetic background differences; (3) low or poor precursor supply or expression ofplant enzymes (Chemler and Koffas, 2008; Liu et al., 2017). Nonetheless, it is notable that *S. venezuelae* strains have

been used to produce various plant NPs, such as naringenin (4 mg/L), pinocembrin (6.0 mg/L), resveratrol (0.4 mg/L), pinosylvin (0.6 mg/L), apigenin (15.3 mg/L) and chrycin (30.9 mg/L) through the expression of corresponding codon-optimized biosynthetic genes and exogenous feeding of precursors (Park et al., 2009; Park et al., 2010; Park et al., 2011b; Kim et al., 2015b).

6. *Streptomyces avermitilis* as a heterologous host

Streptomyces avermitilis is an important industrial microorganism, which produces the anthelmintic macrocyclic lactones avermectins (AVMs) (Burg et al., 1979; Nett et al., 2009; Thuan et al., 2014). In addition to AVMs, *S. avermitilis* also produces oligomycin and filipins (Ikeda et al., 2003; Komatsu et al., 2010); all of them are polyketides. The 9.03-Mb *S. avermitilis* genome (see more details in Table 1) was the second report of *Streptomyces* genomes (Ōmura et al., 2001; Ikeda et al., 2003). Genomic analysis indicated two unusual chromosomal features of *S. avermitilis*: (1) unlike terminal inverted repeats (TIRs) in other streptomycetes (generally tens to hundreds of kilobases), the TIRs in *S. avermitilis* is only 49-bp, which makes the *S. avermitilis* chromosome much more stable than others; (2) unlike the nearly even distribution of subtelomeric regions (~ 1 Mb) at both ends of a *Streptomyces* chromosome, *S. avermitilis* has 2 Mb and 0.5 Mb subtelomeric regions at the left and right end of its chromosome, respectively (Ikeda et al., 2003). In addition to the aforementioned polyketide secondary metabolites, the *S. avermitilis* genome indicates the presence of 34 extra secondary metabolic BGCs (mainly for NRPs, terpenoids, siderophores and bacteriocins), mostly at the left subtelomeric region (Ōmura et al., 2001; Ikeda et al., 2003; Nett et al., 2009).

Due to its unique chromosomal features, *S. avermitilis* is considered to be a good heterologous host; the Ikeda group systemically deleted the left subtelomeric region together with traditional gene knock-out to generate a series of large DNA deletion mutants SUKA1-SUKA17 and SUKA22 in an aim to engineer *S. avermitilis* as a versatile heterologous host (Komatsu et al., 2010). Of them, SUKA5 is defective in the production of AVMs, filipins and oligomycins by deleting a 1.5-Mb DNA region; SUKA17 was constructed by further deleting the BGCs of terpene compounds carotenoid, geosmin and neopentalenolactone in SUKA5 (Komatsu et al., 2010). The genome size of SUKA5 and SUKA17 is 83.2% and 81.46% of the wild-type *S. avermitilis,* respectively (more details listed in Table 3) (Komatsu et al., 2010). SUKA22 is isogenic to SUKA17, except the right side of the deletion region uses a mutant *loxP* in order to prevent unexpected recombination. Notably, all large-deletion mutants grew well on various media indicating that no essential genes were deleted (Komatsu et al., 2013). Since the production of all major endogenous metabolites, such as AVMs, filipin and oligomycin, were removed in the genome-minimized hosts, these large DNA deletions not only release metabolic precursors for exogenous biosynthetic pathways, but also facilitate the downstream purification of final products (Komatsu et al., 2013).

In his 2010 and 2013 reports, Komatsu et al. expressed a total of 25 foreign BGCs in the genome-minimized hosts (Komatsu et al., 2010; Komatsu et al., 2013). Based on the origin and generation of precursors, these pathways were grouped into 5 categories: sugar pathway, polyketide pathway, amino acid pathway, shikimate pathway, and mevalonate (MVA) or

methylerythritol phosphate (MEP) pathway (Komatsu et al., 2013). Notably, most BGCs were expressed well in *S. avermilitis* hosts with yields better than in each gene cluster's native strain. In this review, we will present one or two examples in each category. For a unified illustration in this section, foreign pathways reported in other references were also grouped into these categories. A Hill list of examples mentioned in this section is shown in Table 4.

The genome-minimized strains were initially used for the production of streptomycin (Figure 7) which was originally isolated in *S. griseus* IFO13350 (Ohnishi et al., 2008). The productivity of streptomycin in SUKA5 (~180 mg/L) is much higher than that in the *S. avermilitis* wild-type host (~30 mg/L) and the native streptomycin producer (~50 mg/L) (Komatsu et al., 2010). It is notable that the yield was always higher in the avermectin production medium than in the streptomycin production medium that was originally used with the native strain (Komatsu et al., 2010). In addition, 3 other pathways in the "sugar" category, ribostamycin, kasugamycin, and pholipomycin (structurally similar to moenomycin A in Figure 5), were successfully expressed in SKUA17 or 22 (Table 4) (Komatsu et al., 2013).

The ~35-kb cephamycin gene cluster derived from *S. clavuligerus* ATCC 27064 (Alexander and Jensen, 1998) was introduced into SUKA17 to check the efficacy of large deletion mutants for the expression of "amino acid" pathways. The production of cephamycin C (Figure 7) in the avermectin producing medium was ~80 mg/L, higher than that in the normally used starch-asparagine medium; further, expression of an extra copy of *ccaR* encoding the cephamycin pathway-specific activator further enhanced cephamycin C production to ~130 mg/L (Komatsu et al., 2010). Interestingly, adjacent to the cephamycin gene cluster, there is the gene cluster (25-kb) for the β-lactam compound clavulanic acid (Figure 7), a strong inhibitor of bacterial β-lactamases, and its expression is also controlled by CcaR (Bignell et al., 2005). Expression of the clavulanic acid gene cluster together with *ccaR* produced 16 mg/L clavulanic acid, whereas, if the *ccaR* gene is absent, no clavulanic acid was produced (Komatsu et al., 2013). In addition, holomycin and lactacystin in the "amino acid" category were also produced efficiently in SUKA22 (Table 4) (Komatsu et al., 2013).

Expression of polyketides has been attempted most frequently (8 compounds) in *S. avermilitis* hosts (see details in Table 4) (Komatsu et al., 2010; Komatsu et al., 2013). Pladienolides, a group of antitumor macrocyclic polyketides, were isolated in *S. platensis* Mer-11107 (Machida et al., 2008). Since major metabolites in *S. avermitilis* are polyketides (AVMs, filipins and oligomycin), the deletion of these BGCs assumes to release rich precusors for the production of foreign polyketides. However, an initial expression of the 75-kb pladienolide gene cluster in both *S. avermitilis* wild-type and the SUKA5 mutant hosts showed no production of pladienolides (Komatsu et al., 2010). It was demonstrated that the pathway-specific activator PldR was not expressed; the use of the constitutive *ermE** promoter led to the production of pladienolide B (Figure 7) (Komatsu et al., 2010). As expected, the production of pladienolide B in SUKA5 was higher (20-fold) than that in the wild-type host. In the 2013 report, either SUKA17 or SUKA22 was used for the heterologous production of aureothin (116 mg/L), bafilomycin B1 (16 mg/L), erythromycin

(4 mg/L), leptomycin (5 mg/L), oxytetracycline (20 mg/L) and resistomycin (360 mg/L) (Table 4) (Komatsu et al., 2013). The production of modular PKS-derived polyketides such as bafilomycin B1 and erythromycin indicated that the large-deletion mutants can express large gene clusters in BAC (bacterial artificial chromosome) clones with a total size of ~100-kb without detectable deletions.

Since no genes related to the shikimate pathway have been found in the *S. avermitilis* genome, the production of compounds using precursors or substrates, such as tryptophan and chorismite, derived from the shikimate pathway is intriguing. The indolocarbazole antibiotic rebeccamycin (Figure 5) was synthesized from two molecules of L-tryptophan in *Lechevalieria aerocolonigenes* ATCC 39243 (Onaka et al., 2003). The expression of the 16-kb rebeccamycin gene cluster in SUKA17 yielded 7 mg/L of rebeccamycin (Komatsu et al., 2013). Chloramphenicol and novobiocin, both derived from the important shikimate pathway intermediate chorismate, are additional examples of the “shikimate” category to be produced in SUKA22 with a titer of 250 and 1 mg/L, respectively (Table 4) (Komatsu et al., 2013).

Terpenoids, a large group of diverse natural products with more than 55,000 members, are all constructed from the common precursor isopentenyl diphosphate (IPP) (Christianson, 2017). IPP can be synthesized via the MVA pathway, also known as the 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase pathway (Buhaescu and Izzedine, 2007), or the MEP pathway (Banerjee and Sharkey, 2014). Both microbial and plant terpenoids were successfully produced in the *S. avermitilis* host SUKA17, in which all terpene gene clusters were deleted (Table 4) (Komatsu et al., 2010; Komatsu et al., 2013). Pentalenolactone (Figure 7) is a sesquiterpnoid antibiotic that is produced by various *Streptomyces* strains including *S. exfoliatus* UC5319 and *S. arenae* TÜ469 (Takeuchi et al., 1969; Martin et al., 1970; Cane et al., 1990). Interestingly, *S. avermitilis* prodcued a structurally-distinct new pentalenolactone, neopentalenolactone D (Figure 7) (Jiang et al., 2009). Comparing the *ptl* gene cluster (*sav2990*-sav3002, 13.4-kb) in *S. avermitilis* with *pen* (*S. exfoliatus* UC5319) and *pnt* (*S. arenae* TÜ469) gene clusters revealed 8 conserved and undirectionally transcribed genes (Tetzlaff et al., 2006; Jiang et al., 2009; Seo et al., 2011); a *penM* or *pntM* gene which encodes a P450 monooxygenase only exists in the *pen* and *pnt* gene clusters. It has been demonstrated that early steps are identical in all three pathways to yield a common intermediate 1-deoxy-11-oxopentalenic acid (Figure 7). In the *ptl* pathway (*S. avermitilis*), a regio-specific Baeyer-Villiger oxygenase PtlE oxidizes the intermediate to the final product neopentalenolactone D (Jiang et al., 2009); whereas in the *pen* or *pnt* pathway, PenE or PntE oxidizes the intermediate into pentalenolactone D (Figure 7), which is further catalyzed by PenD or PntD (a non-heme iron-depend dehydrogenase/oxygenase) and the cytochrome P450 PenM or PntM (for an unprecedented oxidative ring rearrangement) to yield the final product pentalenolactone (Seo et al., 2011; Zhu et al., 2011). Based on these observations, SUKA17 was transformed first with the *ptl* gene cluster minus *ptlE/ptlD*, and then stepwise with *pntE/pntD* genes under the control of the *ermE* promoter, and with *penM* gene together with *fdxD* (*sav3129*, encoding a ferredoxin) and *fprD* (*sav5675*, encoding a ferredoxin reductase) as an operon also under the *ermE* promoter, the final SUKA17 recombinant strain produced pentalenolactone, but not the *S. avermitilis* native product neopentalenolactone D (Komatsu et al., 2013).

A codon-optimized *ads* gene that encodes a plant sesquiterpene synthase, amorpha-4,11-diene synthase (ADS) in *Artemisia annua* and the native *S. avermitilis ptlB* gene (*sav2997*) encoding a farnesyl diphosphate synthase in the *ptl* pathway were co-expressed in SUKA17, yielding abundant amorp ha-1,4-diene (Figure 7) (Komatsu et al., 2010). In the 2013 report, co-expression of synthetic plant diterpene synthase genes, *tds* or *lps* gene involved in the production of taxa-4,11-diene or levopimaradine in the taxol or ginkgolide A pathway, respectively, with the *S. avermitilis crtE* gene (*sav1022*) encoding a geranylgeranyl diphosphate synthase yielded taxa-1,4-diene (Figure 7), and levopimaradine (Figure 7) and its analogue abietatriene, respectively (Komatsu et al., 2013). Notably, plant terpenoids were heterologously produced in a synthetic medium without the addition of precursors.

Cyslabdans, consisting of a unique labdan-type diterpene and an *N*-acetylcysteine residue, were identified in a search for compounds that could restore the anti-MRSA activity of imipenem from *S.* sp. K04–0144, later renamed as *S. cyslabdanicus* K04–0144 (Fukumoto et al., 2008a; Fukumoto et al., 2008b; Ikeda et al., 2016). Cyslabdan enhances the anti-MRSA activity by over 1,000-fold for imipenem and other β-lactams (100~1,000-fold), by inhibiting the formation of pentaglycine interpeptide bridge (Koyama et al., 2012). Genome mining of *S. cyslabdanicus* K04-0144 revealed the presence of four genes, *cldA-cldD*, putatively involved in the biosynthesis of cyslabdans. The heterologous expression of the four genes in SUKA22 (lacking all endogenous terpene pathways) produced cyslabdan A (Figure 7), and two new analogues, 2-hydroxycyslabdan A and 17-hydroxycyslabdan A (Ikeda et al., 2016). Another labdane-type diterpene raimonol, which was originally reported in the plant *Nicotiana raimondii* (Noma et al., 1982), was produced in SUKA22 harboring a *cld*-like gene cluster (*rmn*) isolated from *S. anulatus* GM95 (Ikeda et al., 2016).

The macrocyclic peptide telomestatin (Figure 7) was identified in *S. anulatus* 3533-SV4 as a strong telomerase inhibitor (Shin-ya et al., 2001). Low yield and difficulties in chemical synthesis of telomestatin are major bottlenecks impeding its wider applications (Linder et al., 2011; Amagai et al., 2017). The telomestatin gene cluster was identified in the native producer as a RiPP pathway and was heterologously expressed in SUKA17 under the control of a xylose-inducible promoter of a *S. avermitilis* gene *sav*7182, however, no telomestatin was produced (Amagai et al., 2017). The use of the promoter of *sav2902* gene, encoding a LuxR-family transcriptional activator in oligomycin biosynthesis (Ikeda et al., 2003), produced trace amounts of telomestatin; whereas the use of *sav2794* promoter efficiently yielded telomestatin at more than 5 mg/L (Amagai et al., 2017). Sav2794 is a secreted neutral metalloprotease, possibly expressed during the late logarithmic growth phase (Ikeda et al., 2003). With this system, authors generated telomestatin derivatives, 6-desmethyltelomestatin, 9-desmethyltelomestatin and 6,9-didesmethyltelomestatin by amino acid substitution in the core peptide TlsC (Amagai et al., 2017).

Mycosporines and mycosporine-like amino acids (MAAs) with an absorption maxima ranging from 310 to 360 nm are widely produced by fungi, marine bacteria, cyanobacteria, and other marine organisms to protect themselves from UV-radiation (Sinha et al., 2007; Bhatia et al., 2011). Genome mining revealed that Gram-positive bacteria *Actinosynnema mirum* DSM 43827 and *Pseudonocardia* sp. strain P1 possess a gene cluster (*mysA-mysD*) highly homologous to BGCs identified in cyanobacteria. However, the gene cluster was

either poorly expressed in *P.* sp. P1 yielding a very small amount of MAA-like compounds or not transcribed at all in *A. mirum* DSM 43827 (Miyamoto et al., 2014). Heterologous expression of the *mys* gene cluster in SUKA22 using the constitutive *rpsJ* promoter produced shinorine (mycosporine-glycine-serine) (Figure 7), indicating clearly that the *mys* gene cluster is responsible for shinorine biosynthesis in *A. mirum* DSM 43827 and *P.* sp. P1, which is the first demonstration of MAA production in Gram-positive bacteria (Miyamoto et al., 2014). In addition to shinorine, porphyra-334 (mycosporine-glycine-threonine) (Figure 7) and a novel MAA compound mycosporine-glycine-alanine (Figure 7) were detected in the extracts of SUKA22 expressing the *A. mirum mys* gene cluster. The yield of mycosporine-glycine-alanine was increased 9.6-fold by supplementation with 400 mM NH_4Cl (Miyamoto et al., 2014). It is notable that heterologous expression of the *A. mirum* gene cluster in SUKA22 yielded total MAAs (shinorine, porphyra-334, mycosporine-glycine and mycosporine-glycine-alanine) at 188 mg/L, equivalent to 13.9 mg MAA/g dry cell weight, which is much higher than the productivity of MAAs in native producers such as cyanobacterium (0.03 to 0.98 mg MAA/g dry cell weight) or red algae (0.2 to 7.8 mg MAA/g dry cell weight) (Karsten et al., 2009; Komatsu et al., 2008), and in heterologous producers such as *E. coli* expressing shinorine biosynthetic genes from cyanobacterium *Anabaena variabilis* ATCC 29413 under the T7 promoter (0.15 to 0.65 mg/L) (Balskus and Walsh, 2010).

7. Summary

This review shows the importance of *Streptomyces* hosts for the determination of boundaries or minimal gene sets for BGCs (typically seen in early studies), yield improvement, heterologous production of NPs and discovery of novel compounds via genetic engineering, or expression of cryptic BGCs and those derived from metagenome DNA. Since the report of *S. coelicolor* A3(2) genome in 2002, the past one and a half decades has witnessed an exponential advancement in actinomycete genomic studies, which unexpectedly revealed that secondary metabolisms in actinomycetes have been largely underestimated. Although a single digit number of NPs is usually produced by a microbe, dozens of BGCs are encoded in its genome. Equally significant advances have been made in metagenomic studies of uncultured microorganisms derived directly from environmental samples. In parallel with the advance in genomics/metagenomics, a huge number of BGCs has been predicted by bioinformatic analysis. As of this writing, there are more than 22,000 BGCs deposited in the antiSMASH platform, one of the most widely used tools for genome/gene cluster analysis (Blin et al., 2017b; Medema et al., 2011), and this number is increasing daily. How to efficiently mine the genomes, express BGCs, and detect final products are becoming the core disciplines in the study of natural products and drug discovery (Baltz, 2016; Nah et al., 2017; Walsh and Tang, 2017; Xu et al., 2017a; Ziemert et al., 2016). With this background, there is no doubt that heterologous expression will play a greater role in accessing the vast untapped chemical potential and in harnessing the unprecedented opportunity of NPs in drug discovery.

New methods such as cloning techniques for large DNA fragments as well as traditional host engineering strategies such as the removal of endogenous BGCs and the use of strong promoters, make heterologous expression an even more powerful tool for the production of

new NPs (Malcolmson et al., 2013; Ochi, 2017; Sun et al., 2015; Xu et al., 2016; Walsh and Tang, 2017; Tu et al., 2018). Omics-guided pathway reconstitution and host engineering have become a very effective strategy for optimized production of targeted products in heterologous hosts. A nearly 1,000-fold increase in the yield of the insecticide spinosyn (Figure 3) in *S. albus* is a recent example of this approach (Tan et al., 2017). Introduction of mutations with resistance to drugs such as rifampin and streptomycin has proven an efficient strategy to activate secondary metabolic potential (Ochi, 2017). Using *S. coelicolor* A3(2) as a model organism, the introduction of multiple drug resistance mutations led to 1.63 g/L production of actinorhodin, i.e. ~180 fold higher than that of the wild-type strain (Wang et al., 2008). This approach has been used in engineering both native producers and heterologous hosts, such as *S. coelicolor* M1154 which contains a single nucleotide mutation in *rpoB* (rifampicin-resistance) and *rpsL* (streptomycin resistance), respectively (Gomez-Escribano and Bibb, 2011).

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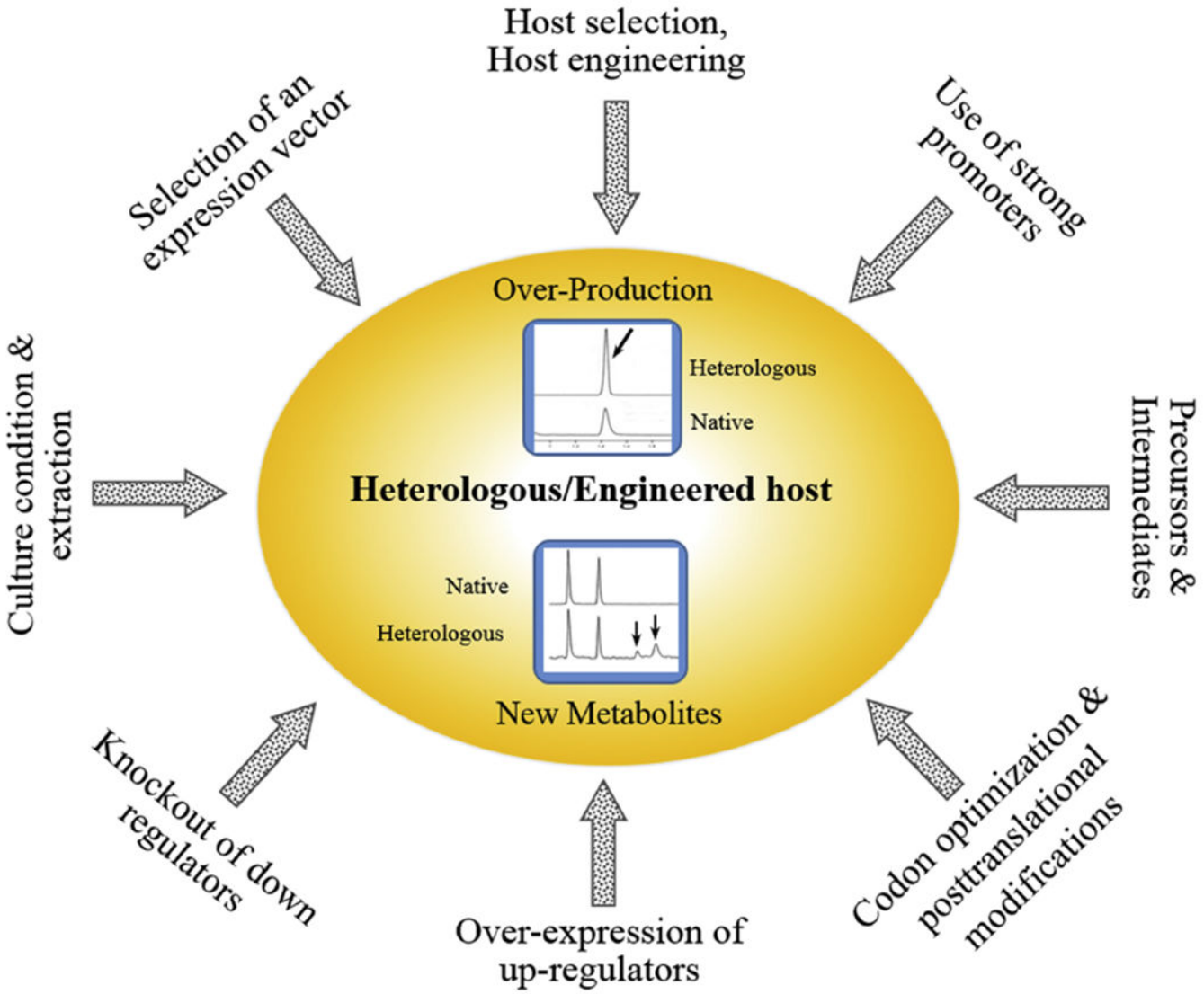

Figure 1.

Major strategies and factors to be considered for better heterologous production of natural products or unnatural natural products via combinatorial biosynthesis.

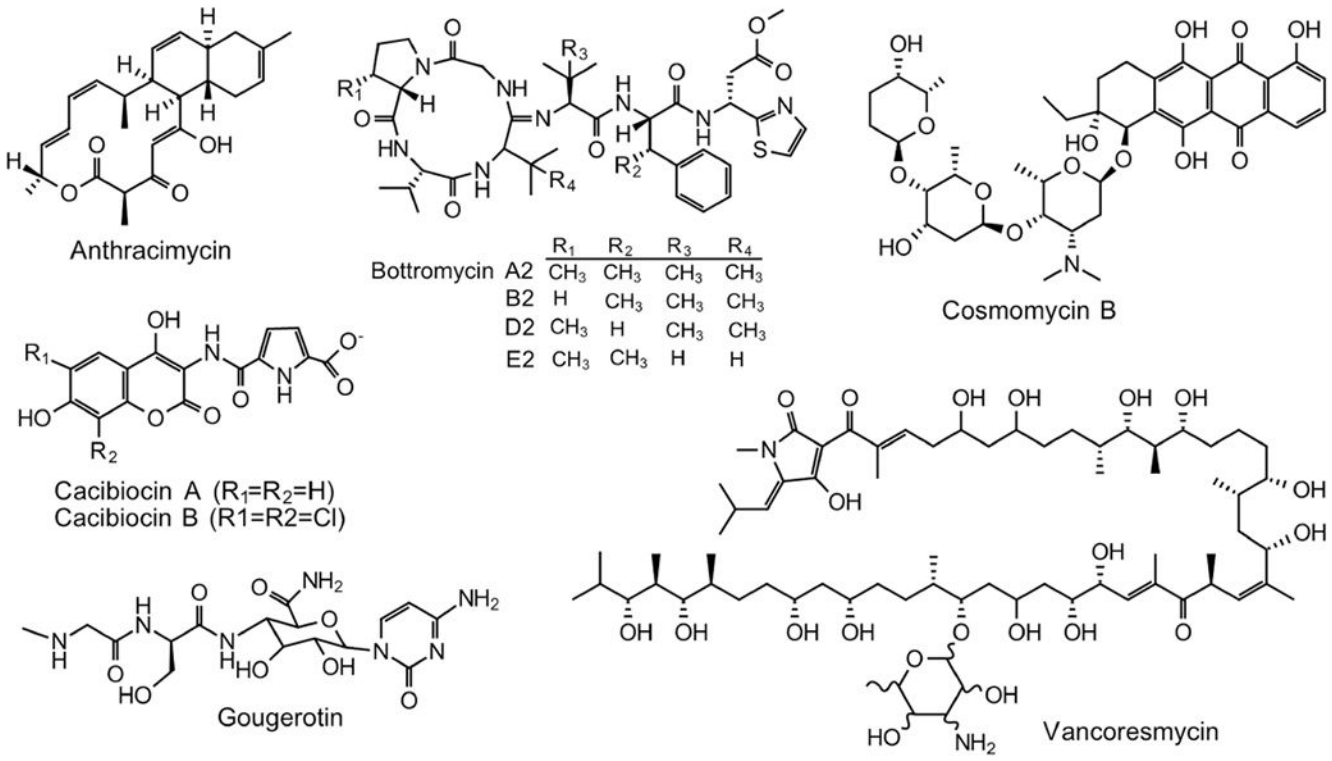

Figure 2.

Chemical structures of representative natural products generated in the heterologous host *S. coelicolor.* Anthracimycin; Bottromycins; Cacibiocins; Cosmomycin B; Gougerotin; and Vancoresmycin.

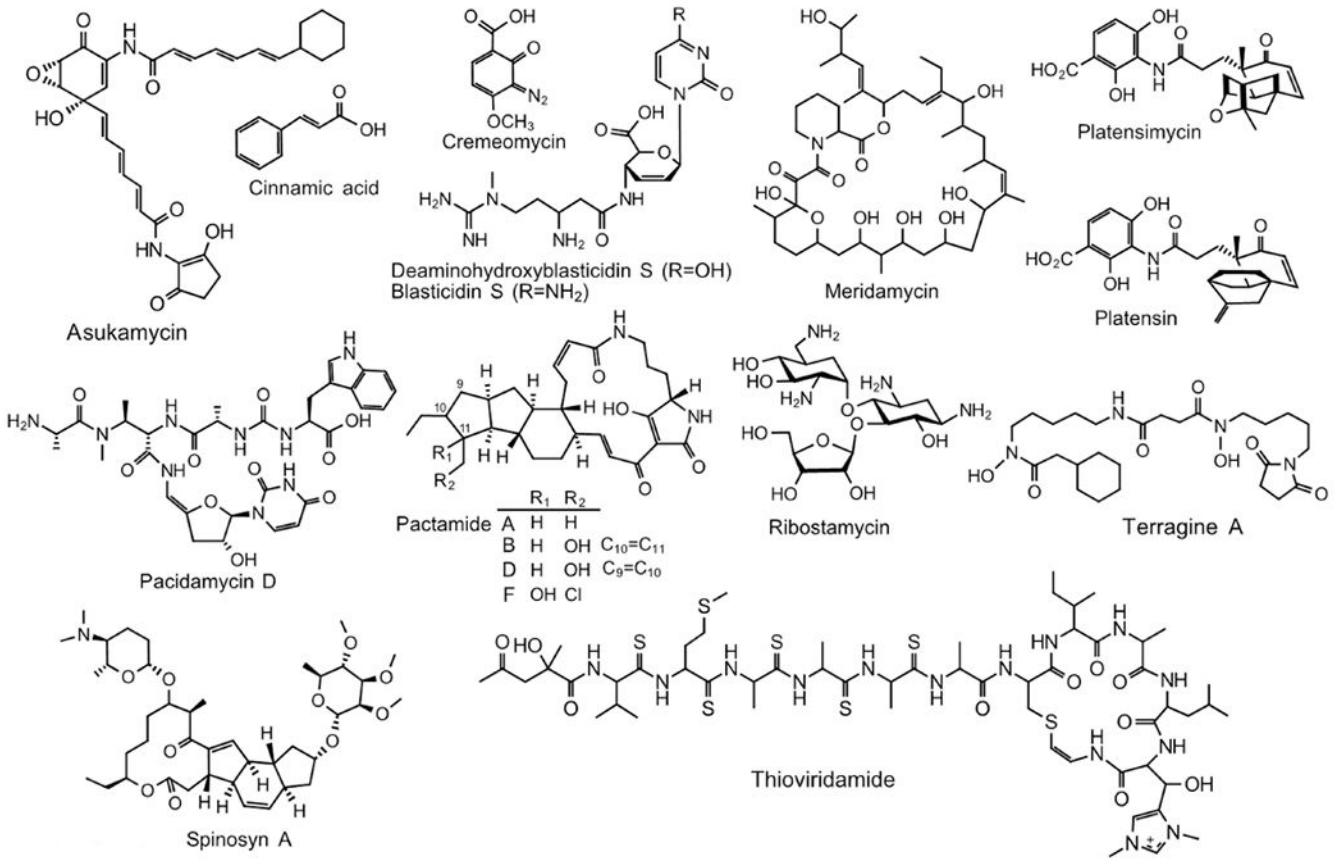

Figure 3.

Chemical structures of representative natural products generated in the heterologous host *S. lividans.* Asukamycin; Cinnamic acid; Cremeomycin; Deaminohydroxyblasticidin S (blasticidin S is shown as the native compound); Meridamycin; Pacidamycin D; Pactamides; Platensimycin and Platensin; Ribostamycin; Spinosyn A; Terragine A; and Thioviridamide. C_9=C_{10}, C_{10}=C_{11}: double bond between the carbons.

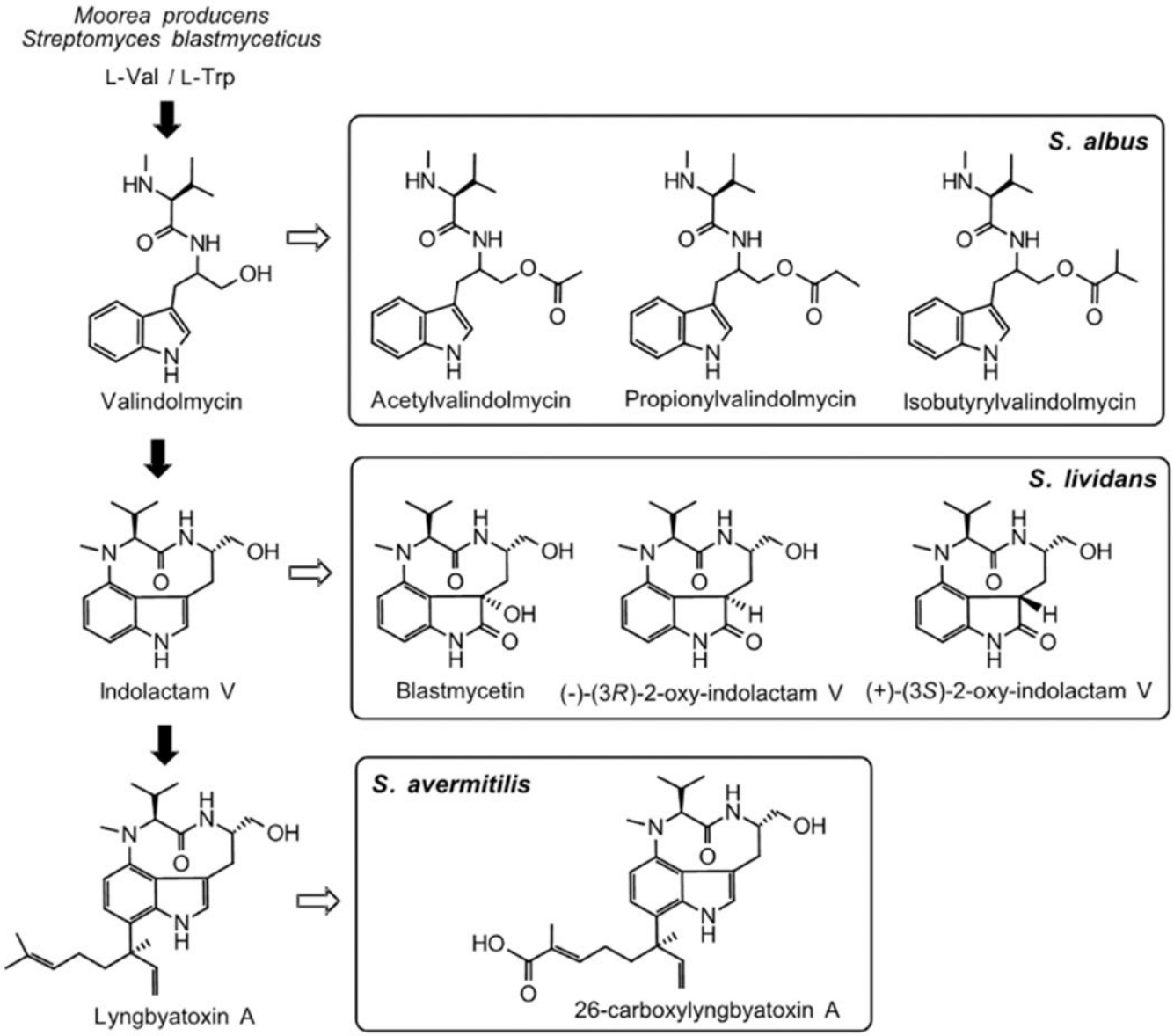

Figure 4.

Heterologous generation of lyngbyatoxin A and its derivatives via host-specific interactions. The figure is adapted from Zhang et al., 2016a.

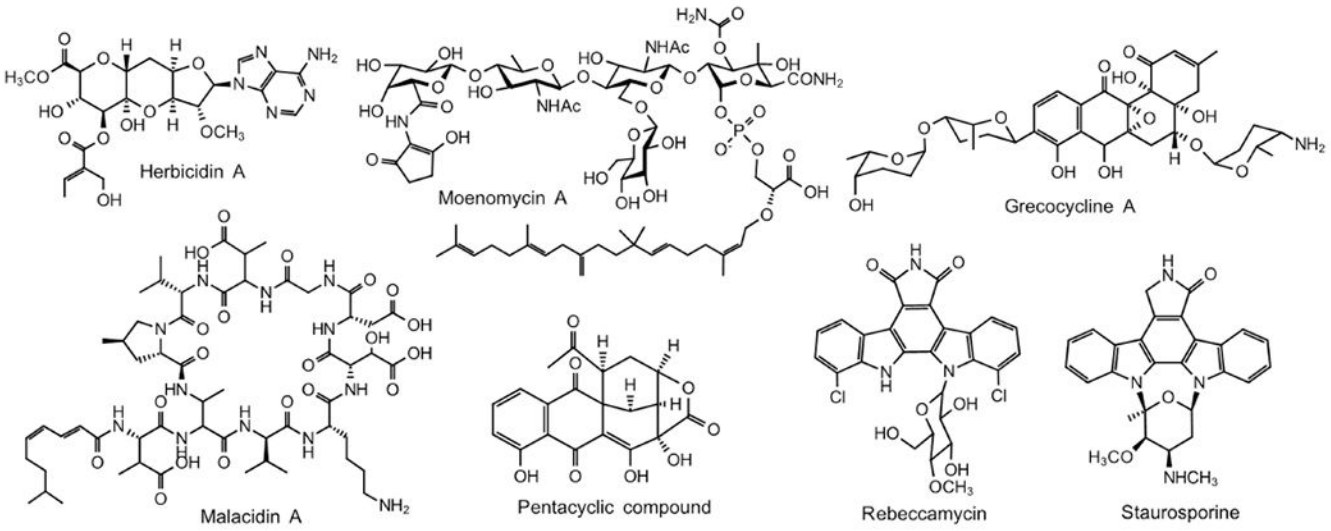

Figure 5.

Chemical structures of representative natural products generated in the heterologous host *S. albus.* Grecocycline A; Herbicidin A; Malacidin A; Moenomycin A; Rebeccamycin and Staurosporine; and the pentacyclic ring compound with a new scaffold.

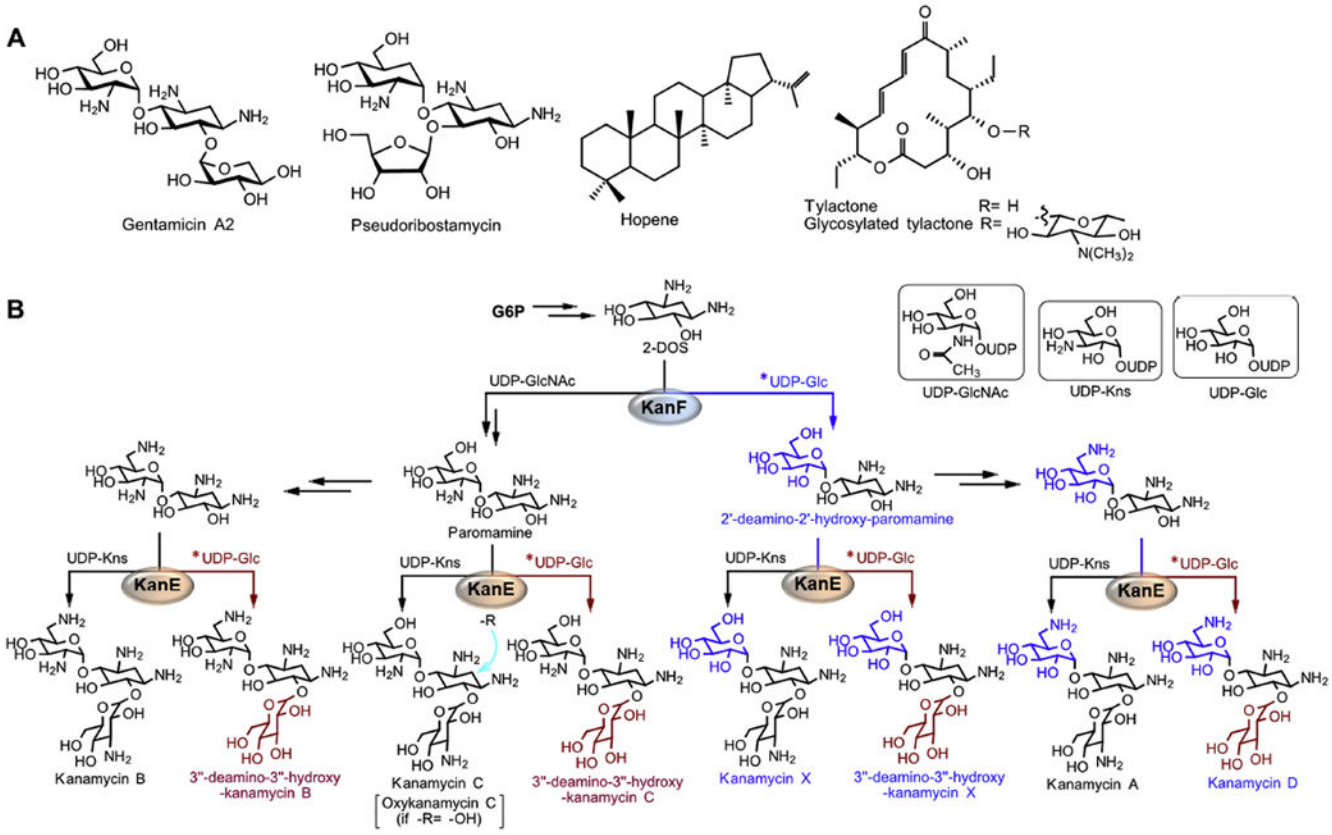

Figure 6.

Heterologous production of natural products in the host *S. venezuelae.* (**A**) Chemical structures of representative natural products. Gentamicin A2; Hopene; Pseudoribostamycin; and Tylactone and its glycosylated derivative 5-*O*-mycaminosyl tylactone. (**B**) New kanamycins generated by the substrate flexibilities of the glycosyltranseferases KanF and KanE. New catalytic route of KanF or KanE is highlighted in blue or orange, respectively; and the corresponding new sugar donor is marked by an asterisk. 2-DOS, 2-deoxystreptamine; G6P, glucose-6-phosphate; UDP-Glc, UDP-D-glucose; UDP-GlcNAc, UDP-*N*-acetylglucosamine; UDP-Kns, UDP-D-kanosamine.

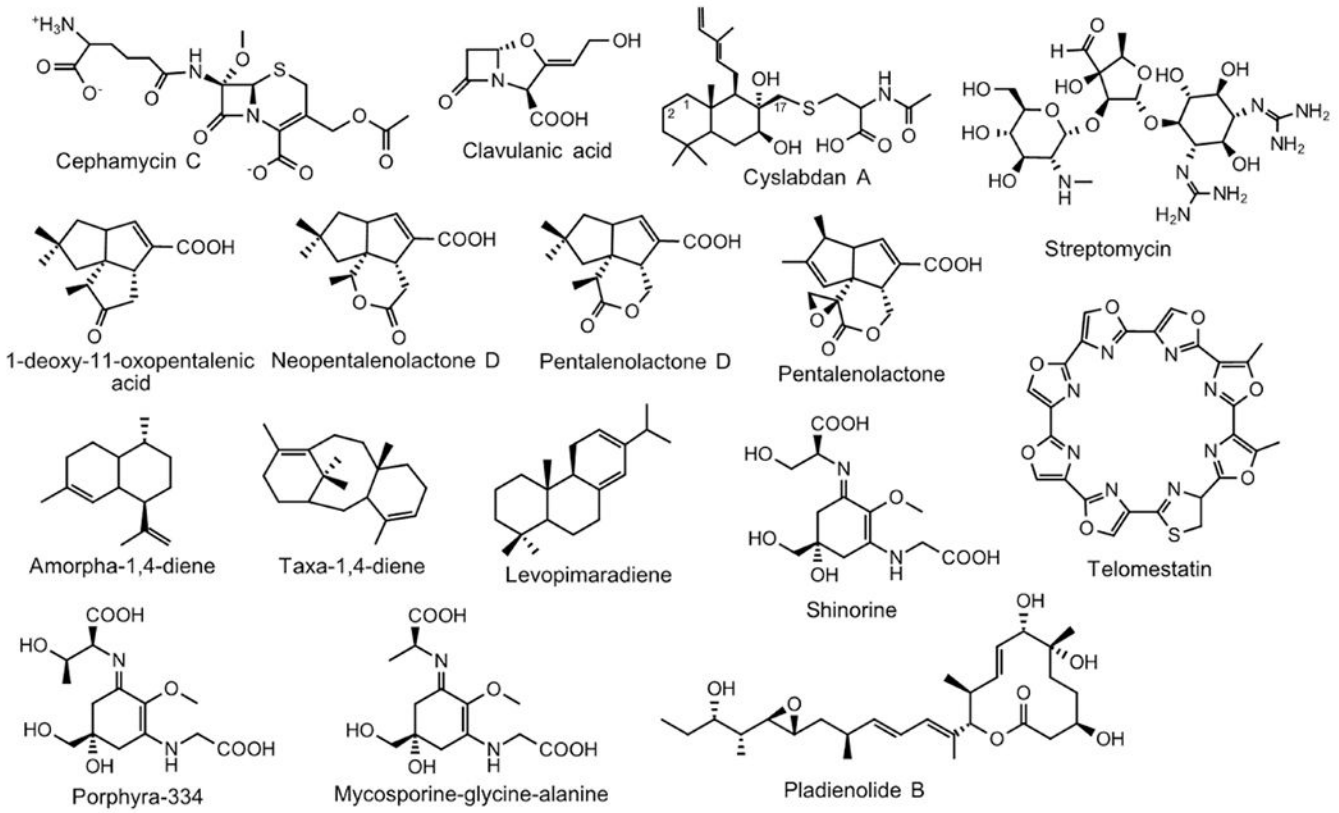

Figure 7.

Chemical structures of representative natural products generated in the heterologous host *S. avermitilis.* 1-deoxy-11-oxopentalenic acid; Amorpha-1,4-diene; Cephamycin C; Clavulanic acid; Cyslabdan A; Levopimaradine; Mycosporine-glycine-alanine; Mycosporine-glycine-serine (Shinorine); Mycosporine-glycine-threonine (Porphyra-334); Neopentalenolactone D; Pentalenolactone D; Pentalenolactone; Pladienolide B; Streptomycin; Taxa-1,4-diene; and Telomestatin.

Table 1.

General chromosomal features of *S. coelicolor* A3(2) (accession No: AL645882), *S. lividans* TK24 (accession No: NZ_CP009124), *S. albus* J1074 (accession No: NC_020990), *S. venezuelae* ATCC 15439 (accession No: NZ_CP013129), and *S. avermitilis* MA-4680 (accession No: AP002021-AP005050, BA000030 and AP005645).

SN	Component	***S. coelicolor*** **A3(2)**	***S. lividans*** **TK24**	***S. albus*** **J1074**	***S. venezuelae*** **ATCC15439**	***S.avermitilis*** **MA-4680**
1	Total Size (bp)	8,667,507	8,345,283	6,841,649	9,054831	9,025,608
2	G+C content (%)	72.12	72.24	73.3	71.74	70.7
3	Average gene length (bp)	991	-	1011	-	1034
4	Ribosomal RNAs (16S-23S-5S)	6×	6×	7×	7×	6×
5	Transfer RNAs	63	64	66	72	68
6	Protein coding sequences	7825	7360	5832	8080	7346
7	Coding Density (%)	88.9	-	86.8	-	-
8	Terminal inverted repeats (TIRs)	21,653 bp	31,000 bp	30,000 bp		49 bp
9	Doubling time	~ 2.2 h (*S. coelicolor* M145)	~ 4.2 h (*S. lividans* TK21)	-	~ 1 h	-
10	Method of gene introduction	Conjugation/PEG-mediated transformation	Mostly PEG-mediated transformation	Conjugation/PEG-mediated transformation	PEG-mediated transformation	Conjugation/PEG-mediated transformation
11	Restriction barriers	Methyl-specific restriction	No methyl-specific restriction	Methyl-specific restriction	Methyl-specific restriction	Methyl-specific restriction

-, information not available.

Table 2.

Major mutants of *S. coelicolor* used as heterologous expression hosts.

SN	Strain	Features	References
1	*S. coelicolor* A3(2)	Wild-type strain	Gomez-Escribano and Bibb, 2012; 2014
2	*S. coelicolor* M512	Deletion of *redD* and *actII-ORF4* and without plasmids SCP1 and SCP2.	Floriano and Bibb, 1996
3	*S. coelicolor* CH999	Deletion of ACT gene cluster and *redE* gene.	McDaniel et al., 1993
4	*S. coelicolor* M1146	Deletion of BGCs for ACT, RED, coelimycin and CDA	Gomez-Escribano and Bibb, 2011
5	*S. coelicolor* M1152	Derived from M1146 with a mutated *ropB* gene (C1298T).	Gomez-Escribano and Bibb, 2011
6	S. *coelicolor* M1154	Derived from M1152 with a mutated *rpsL* gene (A262G).	Gomez-Escribano and Bibb, 2011
7	*S. coelicolor* M1317	Derived from M1152 by deleting type III polyketides genes and operons (*gcs, srs*A, *rpp*A).	Thanapipatsiri et al., 2015

Table 3.

Major *S. avermitilis* mutants used as heterologous expression hosts.

SN	Strain	Features	Genome size	References
1	*S. avermitilis*	Wild-type strain producing avermictins, oligomycins and filipins as major secondary metabolites.	100%	Ikeda et al., 2003
2	SUKA2	Deletion of ~ 1.4 Mb chromosomal region (using general homologous recombination) from the left subtelomeric region of wild *S. avermitilis ΔolmA* (deletion of 1,487,159 bp spanning the region from *sav6* to *sav1205)*. It does not produce avermictins and filipins.	83.12%	Komatsu et al., 2010
3	SUKA3	Deletion of ≥1.5 Mb chromosomal region (using Cre-*loxP* site specific recombination) from the left subtelomeric region of wild *S. avermitilis* (deletion of 1,516,020 bp spanning the region from *sav17* to *sav1287*. It does not produce avermictins and filipins.	83.2%	Komatsu et al., 2010
4	SUKA4	Deletion of oligomycin gene cluster from SUKA2 using Cre-*loxP* site specific recbombination. It does not produce avermictins, filipins and oligomycins.	82.44%	Komatsu et al., 2010
5	SUKA5	Deletion of oligomycin gene cluster from SUKA3 using Cre-*loxP* site specific recbombination. It does not produce avermictins, filipins and oligomycins.	82.11%	Komatsu et al., 2010
6	SUKA17	Additional deletion of gene clusters encoding the biosynthesis of the terpene compounds geosmin, neopentalenolactone, and carotenoid from SUKA5.	81.46%	Komatsu et al., 2010
7	SUKA22	SUKA22 differs from SUKA17 only by carrying a mutant type *loxP* sequence at the right side of deletion region to prevent undesired recombination.	81.46%	Komatsu et al., 2010

Table 4.

Trials and examples of heterologous expression or production of natural products in *Streptomyces* hosts. CA, Cryptic pathway Activation/awakening; GC, Gene Characterization (individual gene function); GI, Gene cluster Identification; MG, MetaGenomics; NM, New Metabolite; OP, Over-Production; and P, Plant-derived NPs. * stands for genetically intractable organisms.

SN	Compound	Activity	Purpose	Producing strains/Gene cluster	Heterologous host	References
1	Abyssomicins 2 and 4	Anti-HIV	GI/OP	*Streptomyces koyangensis* SCSIO5802	*S. coelicolor*	Tu et al., 2018
2	Albusnodin	-	CA	*S. albus* DSM 41398	*S. coelicolor*	Zong et al., 2018
3	Ammosamides A-C	-	GI/CA	*S.* sp. CNR-698	*S. coelicolor*	Jordan and Moore, 2016
4	Anthracimycin	Anti-MRSA	OP	*S.* sp. CNH365, *S.* sp. T676	*S. coelicolor*	Alt and Wilkinson, 2015
5	Bottromycins	Antibacterial	GC/NM/OP	*S.* sp. BC16019, *S. scabies*	*S. coelicolor*	Hu et al., 2012; Eyles et al., 2018
6	Cacibiocins A and B	Antibacterial	NM/OP	*Catenulispora acidiphila**	*S. coelicolor*	Zettler et al., 2014
7	Caprazamycin analogues	Antibiotic	OP	*S.* sp. MK730-62F2	*S. coelicolor*	Flinspach et al., 2010
8	Clorobiocin	Gyrase B inhibitor	OP	*S. roseochromogenes* subsp. *oscitans*	*S. coelicolor*	Flinspach et al., 2010
9	Cosmomycin	Antitumor	GI/CA	*Streptomyces* sp. CNT-302	*S. coelicolor*	Larson et al., 2017
10	Coumermycin A1	Gyrase B inhibitor	OP	*S. rishiriensis* DSM 40489	*S. coelicolor*	Wolpert et al., 2008; Flinspach et al., 2010
11	Desotamides A and B	Antibacterial	OP/GI/NM	*S. scopuliridis* SCSIO ZJ46	*S. coelicolor*	Song et al., 2014; Li et al., 2015
12	Erythreapeptins	Antibacterial	OP	*Saccharopolyspora erythraea* NRRL 2338*	*S. coelicolor*	Völler et al., 2012
13	FK506	Immunosuppressive	OP	*S. tsukubaensis* NRRL18444	*S. coelicolor*	Jones et al., 2013
14	Fluostatin derivatives	-	NM/CA	*Micromonospora rosaria* SCSION160	*S. coelicolor*	Yang et al., 2015
15	GE2270	Anti-MRSA		*Planobispora rosea* ATCC 53773*	*S. coelicolor*	Flinspach et al., 2014
16	Gougerotin	Antibiotic	GC/NM/OP	*S. graminearus**	*S. coelicolor*	Du et al., 2013; Niu et al., 2013; Wei et al., 2016
17	Intermediates of SPMs, IDMs and LNMs	Antifungal, Anticancer	NM	*S.* sp. SCSIOI 03032	*S. coelicolor*	Ma et al., 2017
18	Kocurin	Anti-MRSA	GI	*Kocuria rosea**	*S. coelicolor*	Linares-Otoya et al., 2017
19	Neoabyssomicins A and B	Anti-HIV	GI/OP	*S. koyangensis* SCSIO5802	*S. coelicolor*	Tu et al., 2018
20	Oviedomycin	Antitumor	CA	*S. antibiotics* ATCC 11891, *S. ansochromogenes*	*S. coelicolor*	Rico et al. 2014; Xu et al., 2017
21	Salinomycin	Antitumor	CA/OP	*S. albus* DSM41398	*S. coelicolor*	Yin et al., 2015
22	Streptocollin	tyrosin phosphatase 1B inhibitor	NM/OP	*S. collinus* Tü 365	*S. coelicolor*	Iftime et al., 2015

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23	Streptothricin	Antibiotic	GI/OP	*S.* sp. fd1-xmd	*S. coelicolor*	Yu et al., 2018
24	Vancoresmycin	Anti-MRSA, Anti-VRE	OP/GC	*Amycolatopsis* sp. ST101170	*S. coelicolor*	Kepplinger et al., 2018
25	2-DOS and Paromamine	-	GC	*S. kanamyceticus*	*S. lividans*	Nepal et al., 2009
26	2,4-HMBA	-	GI	*S. cremeus* NRRL 3241	*S. lividans*	Waldman et al., 2015
27	8-methyl-tetracenomycin C	-	OP		*S. lividans*	Diaz et al., 2013
28	Asukamycin	Antitumor	GI/GC	*S. nodosus* subsp. *asukanensis*	*S. lividans*	Rui et al., 2010
29	Avermectins-A2A, B1a and A1a	Anthelmintics	NM	*S. avermitillis* ATCC 31267	*S. lividans*	Deng et al., 2017
30	Blasticidin S derivatives	Fungicides	NM	*S. griseochromogenes**	*S. lividans*	Li et al., 2013b
31	Capreomycin	Antitubercolosis	OP	*Saccharothrix mutabilis**	*S. lividans*	Felnagle et al., 2007; Chaudhary et al., 2013
32	Cinnamic acid	Important precursor	OP	Various plants and microorganisms	*S. lividans*	Xiang and Moore, 2002
33	Cremeomycin	Antitumor	GI/GC	*S. cremeus* NRRL 3241	*S. lividans*	Waldman et al., 2015
34	D-cycloserine	Antituberculosis/anti-anxiety	GI	*S. lavendulae* ATCC 11924	*S. lividans*	Kumagai et al., 2010
35	Dehydrophos	Antibacterial	NM/GI	*S. luridus*	*S. lividans*	Circello et al., 2010
36	Hatomarubigin	Antitumor	GI/GC/NM	*S.* sp. 2238-SVT4	*S. lividans*	Izawa et al., 2014
37	Labyrinthopeptins A1 and A2	Antiviral, antiallodynic	GC/GI/NM	*Actinomedura namibiensis**	*S. lividans*	Krawczyk et al., 2013
38	Leptomycins	Antifungal, antitumor	GI	*S.* sp. ATCC 39366	*S. lividans*	Hu et al., 2005
39	Lipopeptides 8D1-1 and 8D1-2, and streptothricin and borrelidin	Antibacterial	CA/NM	*S. rochei*	*S. lividans*	Xu et al., 2016
40	Lyngbyatoxin A and derivatives	Cyanotoxin	NM	*Moorea producens**	*S. lividans*	Zhang et al., 2016a
41	Mediomycin A	Antifungal	GI	*S. mediocidicus* ATCC 23936	*S. lividans*	Sun et al., 2018
42	Meridamycin	Neuroprotective	GI/OP	*S.* sp. NRRL 30748	*S. lividans*	Liu et al., 2009
43	Mithramycin A	Anticancer	OP	*S. argillaceus* ATCC12956	*S. lividans*	Novakova et al., 2018
44	Oleandomycin intermediate	Antibiotic	GI/OP	*S. antibioticus**	*S. lividans*	Shah et al., 2000
45	Pacidamycins D and S	Antimicrobial	OP/NM	*S. coeruleorubidus*	*S. lividans*	Rackham et al., 2010
46	Pactamides A-F	Antitumor	CA	*S. pactum* SCSIO 02999	*S. lividans*	Saha et al., 2017
47	Platensismycin/Platensin	Antibacterial	GC/GI/NM	*S. platensis**	*S. lividans*	Smanski et al., 2012
48	Ravidomycin	Antitumor	GI	*S. ravidus*	*S. lividans*	Kharel et al., 2010
49	Ribostamycin	Anti-immuno deficiency	GI	*S. ribosidificus*	*S. lividans*	Subba et al., 2007
50	Terragines A-E	-	NM/MG	Environmental DNA	*S. lividans*	Wang et al., 2000

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51	Thioviridamide	Anticancer	GI	*S. olivoviridis* NA005001*	*S. lividans*	Izawa et al., 2013
52	YM-216391	Antitumor	GI/OP	*S. nobilis*	*S. lividans*	Jian et al., 2012
53	A pentacyclic compound	-	MG/NM	Environmental DNA	*S. albus*	Feng et al., 2011
54	Dithiolopyrrolone	Antibacterial, Antifungal, Insecticidal, Anticancer	OP	*S. thioluteus* DSM 40027	*S. albus*	Zhai et al., 2016
55	Elloramycins	Antitumor	GC/NM	*S. olivaceus*	*S. albus*	Patallo et al., 2001
56	Fredericamycin	Antitumor	OP	*S. griseus* ATCC 49344	*S. albus*	Chen et al., 2009
57	Furaquinocins	Antitumor	GI/GC/NM	*S.* sp. KO-3988	*S. albus*	Isogai, et al., 2012
58	Grecocyclines	-	OP	*S.* sp. Acta 1362	*S. albus*	Bilyk et al., 2016
59	Herbicidins	Antibacterial	GI	*S.* sp. L-9-10	*S. albus*	Jung et al., 2006
60	Iso-migrastatin	Anticancer	OP	*S. platensis* NRRL18993*	*S. albus*	Yang et al., 2011
61	K252a	GI/GC		*Nocardiopisis longicantena* JCM 11136	*S. albus*	Kim et al., 2007; Chae et al., 2009
62	KB-346-5 derivative	Anti-MRSA, Anti-VRE	MG	Environmental DNA	*S. albus*	Feng et al., 2011
63	Kinamycin	Antibiotic	GI/GC	*S. galtieri* sgt26	*S. albus*	Liu et al., 2018
64	Landomycin E	Antibiotic	MG	Environmental DNA	*S. albus*	Feng et al., 2011
65	Leprotene and derivatives	-	NM/CA	*S. argillaceus*	*S. albus*	Becerril et al., 2018
66	Lyngbyatoxin A and derivatives	Neurotoxin	OP	*Moorea producens* *	*S. albus*	Zhang et al., 2016a
67	Malacidins	Antibacterial, Anti-MRSA	GI/NM/MG	Environmental DNA	*S. albus*	Hover et al., 2018
68	Moenomycin A	Antibacterial	OP	*S. ghanaensis* ATCC 14676	*S. albus*	Makitrynskyy et al., 2010
69	Myxochelin A	Iron-chelating	NM/MG	Environmental DNA	*S. albus*	Bitok et al., 2017
70	Pseudoribostamycin	-	NM	*S. ribosidificus*	*S. albus*	Subba et al., 2007; Kurumbang et al., 2011
71	Rebeccamycin	Antitumor	OP/NM	*Saccharothrix aerocolonigenes* ATCC39243	*S. albus*	Sánchez et al., 2005
72	Spinosyn	Insecticides	OP	*Saccharopolyspora spinose*	*S. albus*	Tan et al., 2017
73	Staurosporine	Antitumor	OP/NM	*S. staurosporeus*	*S. albus*	Sánchez et al., 2005
74	Steffimycin and derivatives	Antitumor	OP/NM	*S. steffisburgensis* NRRL 3193	*S. albus*	Gullon et al., 2006; Olano et al., 2008
75	Tetarimycin A	Anti-MRSA	NM	Environmental DNA	*S. albus*	Bauer et al., 2010; Kallifidas et al., 2012
76	Thiocoraline	Antitumor	NM/OP	*Micromonospora* sp. ACM2 and *M.* sp. ML1	*S. albus*	Lombó et al., 2006

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77	Utahmycins A and B	Anti-MRSA	NM/MG	Environmental DNA	*S. albus*	Bauer et al., 2010; Kallifidas et al., 2012
78	4-*O*-dimethylbarbamide	-	NM/GI	*Moorea producens**	*S. venezuelae*	Kim et al., 2012
79	Gentamicin A2	Antibiotic	GC	*Micromonospora echinospora*	*S. venezuelae*	Park et al., 2008
80	Apigenin	Flavonoid	P	Various plants	*S. venezuelae*	Park et al., 2010
81	Chrycin	Flavonoid	P	Plant passionflower	*S. venezuelae*	Park et al., 2010
82	Hopene	-	NM/CA	*S. peucetius*	*S. venezuelae*	Ghimire et al., 2015
83	Kanamycins A-D and derivatives	Antibiotic	GI/GC/NM	*S. kanamyceticus**	*S. venezuelae*	Park et al., 2011a
84	Naringenin	Flavanone	P	Grapefuit	*S. venezuelae*	Park et al., 2011b
85	Oxykanamycin C	-	NM	*S. kanamyceticus/S. spectabilis*	*S. venezuelae*	Nepal et al., 2010
86	Oxytetracycline	Antibacterial	OP	*S. rimous* M4018	*S. venezuelae*	Yin et al., 2016
87	Pinocembrin	Flavanone (antioxidant)	P	Various plants,	*S. venezuelae*	Park et al., 2011b
88	Pinosylvin	Stilbenoid toxin	P	*Pinaceae* trees	*S. venezuelae*	Park et al., 2009
89	Pseudoribostamycin	-	NM	*S. ribosidificus*	*S. venezuelae*	Kurumbang et al., 2011
90	Resveratrol	Stilbenoid (dietary supplement)	P	Grape and various berries	*S. venezuelae*	Park et al., 2009
91	Spectinomycin	Antibiotic	GI/GC	*S. spectabilis*	*S. venezuelae*	Thapa et al., 2008; Lamichhane et al., 2014
92	Tylosin derivatives	-	NM/OP	*S. fradiae* ATCC19609	*S. venezuelae*	Jung et al., 2007; Jung et al., 2008
93	Abietatriene	-	P	*Ginkgo biloba*	*S. avermitilis*	Komatsu et al., 2013
94	Amorpha-1,4-diene	-	P	*Artemisia annua*	*S. avermitilis*	Komatsu et al., 2010
95	Aureothin	Antifungal/antibacterial	OP	*S.* sp. MM3	*S. avermitilis*	Komatsu et al., 2013
96	Bafilomycin B1	Antifungal/anti bacterial	OP	*Kitasatospora setae* KM-6054	*S. avermitilis*	Komatsu et al., 2013
97	Cephamycin C	β-lactam antibiotics	GI/OP	*S. clavuligerus*	*S. avermitilis*	Komatsu et al., 2010
98	Chloramphenicol	Antibacterial	OP	*S. venezuelae* ATCC10712	*S. avermitilis*	Komatsu et al., 2013
99	Clavulanic acid	β-lactam antibiotics	GC/OP	*S. clavuligerus*	*S. avermitilis*	Komatsu et al., 2013
100	Cyslabdan A and derivatives	Anti-MRSA	GI/NM	*S. cyslabdanicus* K04-0144	*S. avermitilis*	Ikeda et al., 2016
101	Erythromycin	Antibacterial	OP	*Sacchropolyspora erythraea* NRRL2338	*S. avermitilis*	Komatsu et al., 2013
102	Holomycin	Antitumor	OP	*S. clavuligerus*	*S. avermitilis*	Komatsu et al., 2013
103	Kasugamycin	Antitumor	OP	*S. kasugaensis* MB273	*S. avermitilis*	Komatsu et al., 2013

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104	Lactacystin	-	OP	*S. lactacvstinaeus* OM-6519	*S. avermitilis*	Komatsu et al., 2013
105	Leptomycin	Antifungal	OP	*S.* sp. EM52	*S. avermitilis*	Komatsu et al., 2013
106	Levopimaradine	-	P	*Ginkgo biloba*	*S. avermitilis*	Komatsu et al., 2013
107	Lyngbyatoxin A and derivatives	Cyanotoxin	OP/NM	*Moorea producens**	*S. avermitilis*	Zhang et al., 2016a
108	Mycosporine-glycineal-anine	Anti-UV	OP	*Actinosynnema mirum* DSM 43827	*S. avermitilis*	Miyamoto et al., 2014
109	Novobiocin	Antibacterial	OP	*S. caeruleus* NCBI11891	*S. avermitilis*	Komatsu et al., 2013
110	Oxytetracycline	Antibacterial	OP	*S. rimous*	*S. avermitilis*	Komatsu et al., 2013
111	Pentalenolactone	Antibiotic	GC	*S. exfoliatus* UC5319, *S. arenae* TÜ469	*S. avermitilis*	Komatsu et al., 2013
112	Pholipomycin	-	OP/GI	*S. clavuligerus*	*S. avermitilis*	Komatsu et al., 2013
113	Pladienolide B	Antitumor	GI/OP	*S. platensis* Mer-11107	*S. avermitilis*	Komatsu et al., 2010
114	Porphyra-334	Anti-UV	OP	*Actinosynnema mirum* DSM 43827	*S. avermitilis*	Miyamoto et al., 2014
115	Raimonol	-	GI	*S. anulatus* GM95	*S. avermitilis*	Ikeda et al., 2016
116	Rebeccamycin	Antitumor	OP	*Lechevalieria aerocolonigenes* ATCC 39243	*S. avermitilis*	Komatsu et al., 2013
117	Resistomycin	Antibiotic	OP/GI	*S.* sp. NA 97	*S. avermitilis*	Komatsu et al., 2013
118	Ribostamycin	Antitumor	OP	*S. ribosidificus* ATCC21294	*S. avermitilis*	Komatsu et al., 2013
119	Shinorine	Anti-UV	OP	*Actinosynnema mirum* DSM 43827, *Pseudonocarida* sp. strain P1	*S. avermitilis*	Miyamoto et al., 2014
120	Streptomycin	Antibiotic	GI/OP	*S. griseus* IFO13350	*S. avermitilis*	Komatsu et al., 2010
121	Taxa-1,4-diene	-	P	*Taxus brevifolia*	*S. avermitilis*	Komatsu et al., 2013
122	Telomestatin and derivatives	Telomerase inhibitor	OP/NM	*S. anulatus* 3533-SV4	*S. avermitilis*	Amagai et al., 2017

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