J Ind Microbiol Biotechnol (2010) 37:759–772
DOI 10.1007/s10295-010-0730-9

REVIEW

# *Streptomyces* and *Saccharopolyspora* hosts for heterologous expression of secondary metabolite gene clusters

**Richard H. Baltz**

Received: 7 April 2010 / Accepted: 22 April 2010 / Published online: 14 May 2010


**Abstract** Natural products discovery from actinomycetes has been on the decline in recent years, and has suffered from a lack of innovative ways to discover new secondary metabolites within a background of the thousands of known compounds. Recent advances in whole genome sequencing have revealed that actinomycetes with large genomes encode multiple secondary metabolite pathways, most of which remain cryptic. One approach to address the expression of cryptic pathways is to first identify novel pathways by bioinformatics, then clone and express them in well-characterized hosts with known secondary metabolomes. This process should eliminate the tedious dereplication process that has hampered natural products discovery. Several laboratory and industrial production strains have been used for heterologous production of secondary metabolite pathways. This review discusses the results of these studies, and the pros and cons of using various *Streptomyces* and one *Saccharopolyspora* strain for heterologous expression. This information should provide an experimental basis to help researchers choose hosts for current application and future development to express heterologous secondary metabolite pathways in yields sufficient for rapid scale-up, biological testing, and commercial production.

**Keywords** Antibiotic biosynthesis · BAC vector · *ermEp** promoter · Heterologous expression · Insertion vector · *Saccharopolyspora* · *Streptomyces*

R. H. Baltz (✉)
CognoGen Biotechnology Consulting,
6438 North Olney Street, Indianapolis, IN 46220, USA
e-mail: rbaltz923@aol.com

## Introduction

Secondary metabolites produced by actinomycetes continue to be excellent starting scaffolds for the development of antibiotics, anticancer agents, immunomodulators, anthelminthic agents, and insect control agents. Although the rate of discovery of novel secondary metabolite chemical structures has declined in recent years, there is good reason to believe that many more important new secondary metabolites await discovery [7, 10, 12]. Many secondary metabolites have eluded discovery because their biosynthetic pathways are not widely dispersed among actinomycetes commonly isolated in soils or marine sediments, or they may not be produced in sufficient yields for detection under standard fermentation conditions. With the advent of genome sequencing, it is now apparent that actinomycetes with large genomes have the potential to produce multiple secondary metabolites, and only a fraction of these are produced under standard fermentation conditions [12, 21, 51, 57, 95, 100, 102, 123]. Bioinformatic approaches can predict certain features of the products of cryptic biosynthetic pathways [4, 35, 59, 79, 117, 126], and there are physiological and genetic approaches to stimulate the expression of otherwise cryptic genes [30, 51, 52, 120, 121, 124]. The latter include fermentations in multiple media, genomo-isotopic enrichment labeling of predicted structures, and engineering positive regulation. Another important complementary approach reviewed here is to express cryptic pathways in heterologous hosts suitable for the expression of otherwise silent secondary metabolite gene clusters.

There are a number of potential actinomycete hosts identified from molecular engineering, combinatorial biosynthesis, and gene cluster expression studies. Some of these are widely used laboratory strains that are easy to manipulate genetically, such as *Streptomyces coelicolor*,

*Streptomyces lividans*, and *Streptomyces albus* J1074 [50], some are industrial strains that produce products that have not been commercialized, and some are producers of commercial products. The last two groups include relatively high producing wild-type strains and genetically advanced strains capable of producing elevated and often very large quantities of specific compounds. With the current genomics efforts to identify cryptic secondary metabolite biosynthetic gene clusters, it will be important to develop a suite of streptomycete hosts for high-level heterologous expression of specific types of secondary metabolites, and to facilitate rapid identification and testing of novel products. In this review, I discuss the history, rationale, and examples of *Streptomyces* (and one *Saccharopolyspora*) hosts used for the heterologous expression of secondary metabolites. This information should provide a rationale and experimental basis to help choose hosts for the expression of cryptic secondary metabolite pathways. Some of these hosts have already proven to be valuable for combinatorial biosynthesis to produce novel derivatives of pharmacologically active secondary metabolites. In many cases, genomic sequences of the expression hosts are available.

## General cloning hosts

### *Streptomyces albus* J1074

The establishment of *S. albus G* as a readily transformable cloning host began years before cloning was possible in streptomycetes. Chater and Wilde [31] demonstrated that *S. albus G* expresses restriction endonuclease *Sal*I that restricts the growth of bacteriophage Pal6. They isolated a mutant (*S. albus* J1074) defective in *Sal*I restriction and modification. Subsequent studies showed that *S. albus G* restricts the growth of 8 of 10 broad host range streptomycete bacteriophages, but all 10 formed plaques on J1074 [36]. Bacteriophage FP22, which has no *Sal*I sites in its double-stranded DNA, formed plaques on *S. albus* G, whereas FP4 and FP43, which have more than 30 *Sal*I sites, did not form plaques on *S. albus* G, but formed plaques on J1074. Protoplasts of *S. albus* J1074 regenerated viable colonies efficiently on modified R2 medium [15], a prerequisite for protoplast transformation. These early studies established the potential suitability of *S. albus* J1074 as a host for cloning and expression of streptomycete genes.

*S. albus* J1074 was first used as a cloning host by the Salas group [48, 109]. They used *S. albus* for the heterologous expression of the steffimycin biosynthetic genes, and obtained a yield of purified compounds of about 10 mg/l [53]. In this case, they needed to clone a set of rhamnose biosynthetic genes with the steffimycin gene cluster to obtain steffimycin production, because *S. albus* lacks rhamnose biosynthetic genes. This system was used for combinatorial biosynthesis of molecules related to steffimycin, with yields of novel compounds with antitumor activities of up to 35 % of control [101].

*S. albus* J1074 has been used for the expression of several other secondary metabolite gene clusters (see Table 1). The fredericamycin biosynthetic gene cluster was expressed from both high copy number [127] and single copy number $\phi$C31-integrating vectors [32], yielding 120 and 132 mg/l product, respectively. In contrast, when the fredericamycin pathway was inserted into the *S. lividans* $\phi$C31 *attB* site, no product was detected [32]. In *S. lividans*, a small amount of product (0.5 mg/l) was detected when the *fdmR1* gene encoding a positive regulatory protein belonging to the *Streptomyces* antibiotic regulatory protein (SARP) family [22, 23, 124] was added to the strain on a multicopy plasmid. The yield was further enhanced to 1.4 mg/l when the *fdmR1* gene was expressed from the strong constitutive *ermEp** promoter on a high copy number vector. Transcription studies identified the *fdmC* gene, encoding a putative ketoreductase, as a possible bottleneck. When they expressed both *fdmR1* and *fdmC* genes driven by the *ermEp** promoter, the yield of fredericamycin was increased to 17 mg/l, still only 13% of the yield obtained by expressing the fredericamycin gene cluster from its native promoters in *S. albus* J1074.

Another interesting use of *S. albus* J1074 was the cloning and expression of the complete biosynthetic gene cluster for isomigrastatin from *Streptomyces platensis* NRRL18993 into pStreptoBac V [47]. In this study, the isomigrastatin gene cluster was inserted into the $\phi$C31 *attB* site in *S. albus*, *S. lividans, S. coelicolor*, and *S. avermitilis*, and fermentations were carried out in two media. The best yields were obtained in medium B2. The parental strain produced 58 mg/l, whereas the *S. albus* J1074 recombinant produced 46 mg/l. *S. lividans* K4-114 and *S. coelicolor* M512 produced about twofold lower yields, and *S. avermitilis* SUKA5 produced a tenfold lower yield (Table 1).

A particularly relevant application of *S. albus* J1074 was the expression of the thiocoraline pathway cloned from a marine *Micromonospora* strain [80]. In this study, the native thiocoraline pathway was not expressed in *S. albus* or *S. lividans* unless the *tioA* positive regulatory gene encoding an OmpR-family regulator was transcribed from the *ermEp** promoter. This demonstrated that a non-streptomycete actinomycete secondary metabolite gene cluster can be expressed in *S. albus* and *S. lividans*, and points out the utility of ensuring the expression of all necessary genes by intervening in the expression of a single positive regulatory gene. This approach may be applicable to the heterologous expression of otherwise cryptic secondary metabolite pathways, particularly from non-streptomycete

**Table 1** Examples of heterologous expression of complete secondary metabolic biosynthetic pathways in *Streptomyces* hosts

| Native strain | Product | Insert (kb) | Expression plasmid | Expression host | Yield (mg/l) | Reference |
|---|---|---|---|---|---|---|
| *S. griseus* | Fredericamycin | 33 | pWHM3 | *S. albus* J1074 | 120 | [127] |
| | | | pSET152 | *S. albus* J1074 | 132 | [32] |
| | | | | *S. lividans* K4-114 | 0 | [32] |
| *S. platensis* | Iso-migrastatin | 65 | pStreptoBAC V | *S. albus* J1074 | 46 | [47] |
| | | | | *S. lividans* K4-114 | 25 | [47] |
| | | | | *S. coelicolor* M512 | 23 | [47] |
| | | | | *S. avermitilis* SUKA5 | 4.2 | [47] |
| *Streptomyces* sp. | Napyradiomycin | 36 | pOJ446 | *S. albus* J1074 | NR | [129] |
| *Micromonospora* sp. | Thiocoraline | 53 | $\phi$C31 *attP/int*-based | *S. albus* J1074 | NR | [80] |
| *S. fradiae* A54145 | A54145 | >100 | pDA2002 | *S. ambofaciens* BES2074 | 386 | [3] |
| | | | | *S. roseosporus* UA431 | 100 | [3] |
| *S. griseus* | Streptomycin | 41.2 | pKU465cos | *S. avermitilis* SUKA5 | ~180 | [66] |
| *S. clavuligerus* | Cephamycin C | ~40 | pKU465cos | *S. avermitilis* SUKA17 | ~80 | [66] |
| | | | pKU465cos + *rpsJp-ccaR* | *S. avermitilis* SUKA17 | ~130 | [66] |
| *S. spheroides* | Novobiocin | 34.5 | $\phi$C31 *attP/int*-based | *S. coelicolor* M512 | 31 | [44, 45] |
| | | | | *S. lividans* TK24 | <1 | [45] |
| *S. erythraea* | 6dEB | ~30 | pSET152 | *S. coelicolor* CH999 | 40, 47[a] | [110] |
| | | | | *S. lividans* K4-114 | 33, 28[a] | [110] |
| *S. roseochromogenes* | Chlorobiocin | 45 | $\phi$C31 *attP/int*-based | *S. coelicolor* M512 | 26 | [45] |
| *S. alboniger* | Puromycin | 15 | pKC505 | *S. griseofuscus* | 2–4 | [72] |
| | | | | *S. lividans* 66 | 8–15 | [72] |
| *S. rimosus* | Oxytetracycline | 34 | pIJ916 | *S. lividans* 1326 | 20 | [25] |
| *Streptomyces* sp. | Staurosporine | 34 | $\phi$C31 *attP/int*-based | *S. lividans* TK23 | 2.6 | [103] |
| *S. griseus* | Macrotetrolide | 25 | pSET152 | *S. lividans* 1326 | 10 | [71] |
| *S. roseosporus* | Daptomycin | 128 | pStreptoBac V | *S. lividans* TK23 *Δact* | 20, 55[b] | [104] |
| *S. mutabilis* | Capreomycin | ~35 | pOJ436-based | *S. lividans* 1326 | 50 | [46] |
| *S. tendae* | Nikkomycin | ~43 | pKC505 | *S. lividans* TK23 | 400–500 | [29] |

*NR* not reported

[a] Yields for genes cloned from wild-type and high producing strains of *S. erythraea*, respectively

[b] Yields in standard and phosphate-supplemented media, respectively

actinomycetes not amenable to standard molecular genetic and fermentation manipulations that are routinely executed in streptomycetes.

The genome of *S. albus* J1074 has been sequenced at the Broad Institute, and is available at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/).

## *S. coelicolor* and *S. lividans*

*S. coelicolor and S. lividans* are two very closely related laboratory strains that have been used extensively in the development of streptomycete genetic tools [62]. The genomic sequences of both strains are available at NCBI, and they differ from each other primarily by their composition of horizontally acquired genes [58]. *S. coelicolor* has five large (>25 kb) and 18 smaller genomic islands that are not present in *S. lividans*. Much is known about the biosynthesis and regulation of secondary metabolites in these strains [22, 23, 30, 124]. In spite of the very close phylogenetic relationship, *S. coelicolor* and *S. lividans* differ dramatically in expression of their common secondary metabolite biosynthetic pathways. For instance, wild-type *S. lividans* contains essentially silent or cryptic biosynthetic gene clusters for actinorhodin (Act), undecyleprodigiosin (Red), and calcium-dependent antibiotic (CDA), whereas all three of these are normally produced by *S. coelicolor*. There are a number of ways to trigger the expression of one or more of these gene clusters in *S. lividans*. Notably, certain *rpsL* and *rsmG* mutations strongly activate the expression of Act, and certain *rpoB* mutations associated with resistance to rifampin (RifR) activate the expression of Act, Red, and CDA [99]. In addition, it has been shown that some rare, naturally RifR actinomycetes have a second paralogous

*rpoB*(R) gene that has mutations associated with RifR [120]. Introduction of this gene into the chromosome of *S. lividans* triggered abundant expression of Act, Red, and CDA. It is not yet known if the mechanism(s) of activating these three pathways will be generally translatable to the activation of cryptic genes in other actinomycetes, but it is an area that needs further investigation. An advantage of the *S. lividans* system is that Act (blue) and Red pigments are easily observed, so this system can be used to screen genes, fermentation conditions, and chemical compounds for the activation of expression of these antibiotics. These in turn might be further screened to identify potential general activators of cryptic pathways in other actinomycetes.

*S. coelicolor* and *S. lividans* have been used as hosts for the expression of several secondary metabolite biosynthetic gene clusters (Table 1). *S. coelicolor* was used in early studies on the expression the erythronolide PKS genes, with production yields of about 50 mg/l [61]. The heterologous product yields of other compounds have varied from 23 to 47 mg/l in *S. coelicolor* and from 0 to ~450 mg/l in *S. lividans* (Table 1). In some cases, product yields were substantially better in *S. coelicolor* than in *S. lividans*. For instance, when the novobiocin biosynthetic gene cluster was inserted into the $\phi$C31 *attB* sites of *S. coelicolor* M512 and *S. lividans* TK24, the product yields were 31 and less than 1 mg/l, respectively [44]. The fermentation yield in *S. coelicolor* was increased to 54 mg/l by miniaturizing the fermentation and adding an oxygen carrier, siloxylated ethylene oxide/propylene oxide copolymer. The yield was further increased to 163 mg/l by overexpressing the *novG* gene encoding a StrR-like positive regulatory protein [113].

The complete daptomycin gene cluster present in pStreptoBAC V [92] was inserted into the $\phi$C31 *attB* site of a *S. lividans* strain deleted for the *act* gene cluster, and the recombinant produced the natural A21978C lipopeptides at ~20 mg/l [104]. The recombinant also produced the otherwise cryptic CDA lipopeptides, confounding the analysis. CDA production was suppressed, and A21978C production stimulated to 55 mg/l by increasing the phosphate level in the medium. This yield was about 50% of the yield normally obtained with wild-type *S. roseosporus* in an optimized medium, but only a fraction of that produced in mutationally advanced strains.

*S. lividans* 1326 has also been used for the heterologous expression of tuberactinomycin family nonribosomal peptide antibiotics capreomycin [46] and viomycin [19]. Capreomycin is normally produced by *Saccharothrix mutabilis* subsp. *capreolus*, a strain apparently intractable to molecular genetic manipulations. The capreomycin gene cluster was inserted into the $\phi$C31 *attB* site in *S. lividans*, and the recombinant produced 50 mg/l of capreomycin. The viomycin gene cluster was cloned from *Streptomyces* sp. strain ATCC 11861 and inserted in a similar manner into *S. lividans*. The gene cluster was manipulated by $\lambda$-Red-mediated recombination in *E. coli* to generate specific deletions, then inserted into *S. lividans* by conjugation and site-specific integration in the $\phi$C31 *attB* site to study gene functions [19]. These two examples point out the value of *S. lividans* as a host for molecular genetic and biosynthetic studies that otherwise cannot be carried out in the native hosts.

Recently, a strain of *S. coelicolor* deleted for the actinorhodin (*act*), prodiginines (*red*), PKSI (*cpk*), and calcium dependent antibiotic (*cda*) clusters was constructed, and combined with mutations in *rpoB* and *rpsL* that enhance secondary metabolite production (J. P. Gomez-Escribano and M. J. Bibb, Abstracts of the 15th International Symposium on the Biology of Actinomycetes, Shanghai, August 20–25, 2009). This strain has been used to produce enhanced levels of cloned secondary metabolite gene clusters, but product yields were not reported.

### *Streptomyces griseofuscus*

*S. griseofuscus* was identified many years ago as an excellent host for the isolation and propagation of broad host range *Streptomyces* bacteriophages [36]. Of the 30 *Streptomyces* species tested, *S. griseofuscus* was the best strain to determine bacteriophage titers because it displayed no discernable DNA restriction. *S. griseofuscus* was also shown to form protoplasts that regenerated viable colonies at high efficiency [5, 15]. As anticipated from the protoplast regeneration and bacteriophage restriction studies, *S. griseofuscus* was shown to be readily transformable by unmodified plasmid DNA, and was used to generate bifunctional *E. coli–Streptomyces* shuttle plasmids and cosmids [74, 75, 107]. *S. griseofuscus* was also used for the development of bacteriophage FP43-mediated plasmid transduction [88], and transposon technology [54, 88, 89, 114, 115], and served as a host for cloning of tylosin, carbomycin, and spiramycin resistance genes [26, 42, 107], which provided entry points for the cloning and engineering macrolide antibiotic biosynthetic genes at Lilly [37, 43, 70].

*S. griseofuscus* was used as a host to clone and express the complete puromycin biosynthetic gene cluster from *Streptomyces alboniger* ATTC12461 from a low copy number bifunctional cosmid pKC505 [72, 107]. The puromycin yields were 2–4 mg/l from different clones. The same plasmids gave yields of 8–15 mg/l in *S. lividans* 66. *S. griseofuscus* represents a laboratory strain with potential for heterologous whole pathway expression that has been relatively unexplored. Its rapid growth (doubling time ~1 h in TS broth at 34°C [5]) and ease of genetic manipulation make it an attractive strain for further studies.

## Hosts derived from industrial polyketide producers

### *Streptomyces ambofaciens*

*S. ambofaciens* produces the clinically important macrolide antibiotic spiramycin [111]. Early work at Lilly demonstrated that, like *S. griseofuscus*, *S. ambofaciens* was a suitable host for gene cloning because it did not restrict double-stranded DNA from a variety of streptomycete bacteriophages [36], and protoplasts regenerated viable cells efficiently [15] and were readily transformable with plasmid DNA prepared from other hosts [81]. This enabled the use of *S. ambofaciens* as a reliable host for the development of site-specific integration vectors [67–69]. These studies identified the highly efficient chromosomal insertion by non-replicating plasmids containing $\phi$C31 *attP* and *int* functions [69], thus enabling the further development of a suite of very useful plasmid and cosmid vectors with site-specific integration and conjugal transfer functions [24]. Recent studies have reconfirmed that plasmid pSET152 can be introduced into *S. ambofaciens* at high efficiency ($1.4 \times 10^{-2}$ per recipient) by conjugation from *E. coli* and site-specific insertion into the $\phi$C31 *attB* site when selections were done on AS-1 agar [63].

The initial problem with using *S. ambofaciens* as an expression host for the genetic engineering of macrolide antibiotic biosynthetic pathways was its genetic instability. In early protoplast studies, it was observed that spiramycin production was lost at very high frequencies among colonies derived from regenerated protoplasts [Matsushima and Baltz, unpublished]. Instability in *S. ambofaciens* has been the subject of extensive research (e.g., see [1, 2, 33, 39, 40, 76–78, 128]), and the chromosome ends which harbor regions prone to DNA deletion and amplification have recently been sequenced [33]. In the pre-genomic era of the early 1980s, the pragmatic solution to genetic instability in *S. ambofaciens* was the use of chemical mutagenesis and selection for stability. *S. ambofaciens* was mutagenized with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG), protoplasts were prepared from mutagenized cells and regenerated, colonies were screened for production of elevated levels of spiramycin, and the process was repeated [49]. The starting strain (ATCC 15154) produced 128 mg/l of spiramycin, and the stable mutant strain (*S. ambofaciens* 111–59) produced 1.7 g/l. A derivative of strain 111–59 was used as a cloning host to produce hybrid 16-member macrolides [70].

*S. ambofaciens* BES2074, a derivative of strain 111–59 blocked in spiramycin and netropsin production, has been used as a host for the heterologous production of the cyclic lipopeptide A54145 [3, 18]. A BAC vector containing a larger than 100-kb insert, which included the complete A54145 biosynthetic gene cluster [93], was conjugated into *S. ambofaciens* from *E. coli*, and the genes inserted into the chromosomal $\phi$C31 *attB* site. The recombinant produced an average of 385 mg/l of the A54145 lipopeptides (Table 1). This represented 91% of the yield of a moderately improved strain of *S. fradiae*, and an ~285 % increase over the wild-type *S. fradiae* strain [3, 28]. Now that *S. ambofaciens* BES2074 has shown clear potential to produce products derived from PKS and NRPS pathways, it would be interesting to sequence its genome to see how empirical mutation and selection for stability has influenced the genome size and structure.

### *Streptomyces avermitilis*

*S. avermitilis* is the commercial producer of avermectins [57]. The *S. avermitilis* genome has been sequenced and annotated, and many cryptic secondary metabolite pathways have been identified [57, 60, 65, 95, 122]. Besides avermectins, wild-type *S. avermitilis* normally produces oligomycins and filipins as major secondary metabolites [66]. Tanaka et al. [121] have shown that the oligomycin biosynthetic gene cluster, which is normally very poorly expressed, can be highly expressed (~1 g/l) in an *rpsL* mutant (K43M) deleted for the *aveA1* (PKS) gene. Several strains containing deletions of large segments of the *S. avermitilis* genome have been generated, including a series of strains with genomes reduced from 9.03 to 7.35–7.50 Mb [60, 66]. These include strains deleted for avermectins, oligomycin, filipin, the terpines geosmin and neopentalenolactone, and carotenoid. The large deletion mutation in the left arm of the sub-teleomeric region also removed about 78% of the 111 transposase genes, perhaps improving the overall stability of the genome-minimized strains.

The genome-minimized strains were used to express heterologous secondary metabolite pathways, including the streptomycin gene cluster from *Streptomyces griseus*, and the cephamycin C gene cluster from *Streptomyces clavuligerus*, and the pladienolide pathway from *Streptomyces platensis*. The streptomycin gene cluster was inserted into the $\phi$C31 *attB* site in the wild-type *S. avermitilis* strain and in a deletion mutant, SUKA5. The highest levels of production were obtained in an avermectin production medium. The wild-type *S. avermitilis* recombinant produced ~30 mg/l streptomycin, whereas the SUKA5 recombinant produced ~180 mg/l. The latter compared favorably with wild-type *S. griseus* which produced ~50 mg/l of streptomycin in a streptomycin production medium. They also showed that the positive regulatory gene *strR* could be driven by the *rpsJ* and the *aveR* promoters, and streptomycin yields of ~170 and ~200 mg/l, respectively, were obtained. The use of alternative promoters to drive expression of *strR* decoupled the pathway regulation from a more complex mechanism that involved induction by A-factor.

Cephamycin production was assessed in another deletion mutant, *S. avermitilis* SUKA17, by inserting the pathway into the $\phi$C31 *attB* site. In this case, no cephamycin production was observed in the cephamycin production medium, but cephamycin was produced in the avermectin production medium at ~80 mg/l. A second copy of the positive regulatory gene *ccaR* was introduced into the bacteriophage $\phi$K38-1 *attB* site under the transcriptional control of the *rpsJ* promoter, and the yield was increased to ~130 mg/l. In this case, the use of the *rpsJ* promoter decoupled expression of the cephamycin gene cluster from transcriptional control involving an anti-sigma factor antagonist encoded by *bldG* in *S. clavuligerus*, and which is fortuitously provided by the *rsvV* gene in *S. avermitilis*. Deletion of *rsvV* in *S. avermitilis* SUKA17 abolished cephamycin production, but this was overcome by driving expression of *ccrR* by the *rpsJ* promoter.

A third example was the expression of the *S. platensis* pladienolide biosynthetic gene cluster from a conjugal BAC vector inserted in the $\phi$C31 *attB* site. In this case, no expression was observed in wild-type or genome-minimized *S. avermitilis* strains, and transcription analysis indicated that the pathway transcriptional activator gene *pldR* was not transcribed. However, when a second copy of *pldR* under the control of the *ermEp* promoter was inserted into $\phi$K38-1 *attB* site in recombinants containing the complete pathway, pladienolides were produced in both strains, but at substantially higher yield in the genome-minimized strain. The authors speculated that the genome-minimized strain was able to channel acyl-CoA intermediates common to the avermectin and pladienolide polyketide pathways into pladienolides exclusively, thus accounting for the higher productivity.

*Streptomyces fradiae* (tylosin producer)

Tylosin and a semisynthetic derivative, tilmicosin, are important antibiotics used in animal health [64]. Tylosin is produced by *S. fradiae* T59235, C4 and related strains [112], and the genetics and biosynthesis of tylosin production have been under investigation for many years [6, 16, 17, 38, 112, 119]. *S. fradiae* C4 is a relatively high tylosin producer derived by multiple rounds of mutagenesis starting from the wild-type *S. fradiae* T59235, and many derivatives of C4 have been described [16, 17, 112]. The advantages of developing an advanced tylosin production strain of *S. fradiae* as a cloning host to produce hybrid macrolides were articulated many years ago [6, 17]. To achieve this, early genetic studies on *S. fradiae* focused on the development of protoplast fusion [5, 15], protoplast transformation [81, 84, 85], and tylosin biosynthesis [16, 17]. *S. fradiae* protoplasts are transformable by plasmids modified by passage through *S. fradiae* M1, a non-sporulating mutant that is less restricting than typical tylosin production strains, but poorly transformable by plasmids prepared from other streptomycetes [81]. *S. fradiae* was shown to express multiple restriction/modification systems, and the genes encoding restriction enzymes were systematically inactivated by multiple rounds of MNNG mutagenesis followed by selection for improved transformation by different plasmids, starting with strain JS85, a tylosin non-producing derivative of T59235 [84]. This process yielded *S. fradiae* PM76 that was highly transformable by plasmids prepared from other streptomycetes, and loss of restriction was supported by bacteriophage host range studies [118]. DNA from JS85 was insensitive to cleavage by *Sca*I, *Pst*I, *Xho*I, and *Mst*I, but PM76 was cleaved by all four enzymes, suggesting that the mutations associated with improved transformation disrupted restriction and modification for these systems. Like its parent strain JS85, PM76 lacked a functional tylosin biosynthetic pathway [17, 84], but was an efficient recipient for the conjugal transfer of tylosin genes from other strains [17, 118]. Since *trans*-conjugants retained the non-restricting phenotype of the recipient, this provided a means to reintroduce specific mutations in tylosin biosynthetic genes into a non-restricting background for cloning studies. A recombinant containing the *tyl* gene cluster with a *tylB* mutation was instrumental in identifying cosmids containing spiramycin biosynthetic genes by heterospecific complementation [108].

Since *S. fradiae* PM76 was derived from a wild-type tylosin producer, the problem of DNA restriction remained a barrier to the use of the best tylosin production strains for genetic engineering in the 1980s. This dilemma was solved by introducing DNA into *S. fradiae* by plasmid RP4-mediated conjugation of streptomycete plasmid DNA from *E. coli*. Mazodier et al. [87] demonstrated that plasmids containing *oriT* from RP4 could be transferred from *E. coli* to streptomycetes by conjugation, and Bierman et al. [24] extended this observation by showing that plasmids could be conjugated from *E. coli* into highly restricting *S. fradiae* strains at high frequencies. This indicated that the plasmid DNA introduced by RP4 transfer functions, which was likely transferred as single-stranded concatemers generated by rolling circle replication [73], was able to bypass the potent restriction barriers that normally cut double-stranded DNA. This system has also been used to bypass potent restriction barriers in *Saccharopolyspora spinosa* [82, 86], the producer of the commercial insect control agents spinosad and spinetoram [56], further emphasizing the utility of conjugation to engineer important industrial strains that otherwise might not be amenable to genetic manipulation.

Rodriquez et al. [110] used the approach of engineering an *S. fradiae* strain that had undergone mutagenesis and selection for elevated tylosin production. The strain produced 2 g/l tylosin in shake flask cultures. They deleted the *tylGI-V* PKS genes, then conjugated and inserted pSET152

containing the PKS genes from the wild-type *S. fradiae* strain into the $\phi$C31 *attB* site under the control of its natural *tylGIp* promoter, and the recombinant produced 1.3 g/l of tylosin. They concluded that mutations associated with high productivity resided outside of the main PKS cluster. This is consistent with the finding of the Stratigopoulos and Cundliffe [119] that strains derived from *S. fradiae* C4, which had undergone at least seven cycles of mutation and selection at Lilly [112], and produced more than 3 g/l of tylosin in shake flasks [Seno and Baltz, unpublished], had only one mutation in the tylosin gene cluster, mapping to a negative regulatory gene, so the other mutations must reside outside of the tylosin gene cluster. This observation is important in that the Kosan strain and the Lilly C4 strain may be genetically well suited to produce high levels of other heterologous type I polyketides.

The Kosan group used the *S. fradiae* expression system, driven by the *tylGIp* promoter, to replace the *tylGI-V* PKS genes with the comparable genes (>40 kb) from the chalcomycin pathway to produce hybrid macrolide antibiotics [125]. In a separate study, Reeves et al. [106] used this system to generate a hybrid 16-member macrolide derived from the first two chalcomycin PKS genes (*chmGI–II*) and the last three spiramycin PKS genes (*srmIII–V*), and the recombinant produced novel macrolide antibiotics at nearly 2 g/l.

The success of the Kosan group suggests that even higher yields of heterologous polyketides might be obtained by using more advanced tylosin production strains. For instance, a number of mutants derived from strain C4 are generally available [17]. Some of these (e.g., GS3 and GS22) are deleted for segments of the PKS genes [16, 17, 20], and might be useful hosts for the production of type I polyketides. Preferably, strains further developed along the production strain lineage could be used as cloning hosts after deleting the *tylGI–V* PKS genes, as demonstrated by the Kosan group, or after deleting the complete *tyl* gene cluster to eliminate the sugar biosynthetic and other tailoring functions as well.

### *Saccharopolyspora erythraea*

*S. erythraea* is the commercial producer of erythromycin, and its genome has been sequenced [102]. Although *S. erythraea* is not a member of the genus *Streptomyces*, it is included in this review because it is as an example of how molecular genetic tools developed mainly in *Streptomyces* species can be adapted for use in other actinomycetes. *S. erythraea* does not have a high efficiency $\phi$C31 *attB* site for insertional cloning. In order to exploit the streptomycete vector pSET152, which has *oriT* for conjugation and $\phi$C31 *att/int* functions [24], Rodriquez et al. [110] generated a *S. erythraea* cloning host by deleting the PKS genes *eryAI–III*, and replacing them with the $\phi$C31 *attB* site from *S. lividans*. They also inserted the *ermEp** promoter to drive expression of downstream *ery* genes. This expanded the utility of the $\phi$C31-integrating vectors to a genus lacking an efficient *attB* for integration, and provided a means for precise insertion of cloned PKS genes into the vacant *eryAI–III* PKS locus. They reintroduced the *eryAI–III* genes under the control of the natural *eryAIp* promoter from the high producer, which normally produces 1–2 g/l erythromycin in shake flasks, and from the wild-type strain, which normally produces about 200 mg/l erythromycin. Both recombinants produced about 1.3 g/l erythromycins, indicating that mutations in the high producer accounting for elevated erythromycin production must reside outside of the *eryAI–III* genes and the *eryAIp* promoter. Therefore, as demonstrated with the *S. fradiae* system, the *S. erythraea* system may also be suitable for the expression of cryptic PKS gene clusters from other actinomycetes. As with *S. fradiae* tylosin producers, there are *S. erythraea* industrial strains capable of producing much higher levels of erythromycin that might be manipulated as discussed above to produce heterologous macrolide biosynthetic pathways.

## Hosts derived from nonribosomal peptide producers

### *Streptomyces roseosporus*

*S. roseosporus* is used for the commercial production of the therapeutically important lipopeptide antibiotic daptomycin [41], and its genome has been sequenced by the Broad Institute. *S. roseosporus* is a facile host for genetic manipulation by conjugation, plasmid transduction, transposition, and gene replacement [55, 90, 91], and it has been developed as a cloning host for genetic engineering and combinatorial biosynthesis of novel lipopeptide antibiotics at Cubist Pharmaceuticals [13, 14, 18, 34, 94, 96, 97]. A variety of deletion mutants have been generated in a strain that normally produces about 300 mg/l of daptomycin in shake flasks, including strain UA431 which is deleted for the complete daptomycin biosynthetic gene cluster [34, 96, 97]. Many novel lipopeptides have been generated by combinatorial biosynthesis with yields ranging from 1 to 100 mg/l. The complete A54145 lipopeptide biosynthetic gene cluster cloned in a BAC vector was conjugated from *E. coli* into an *S. roseosporus* UA431, inserted into the $\phi$C31 *attB* site, and the recombinant produced 100 mg/l of A54145 factors [3]. *S. roseosporus* UA431 may be suitable host for cloning and expression of cryptic NRPS gene clusters.

### *Streptomyces fradiae* A54145

*S. fradiae* A54145 is one of several streptomycete strains isolated at the Lilly Research Laboratories that produce a family

of the cyclic lipopeptide antibiotics called A54145 factors [28]. *S. fradiae* A54145 was chosen for preclinical structure–activity relationship studies because it was the most robust strain, producing about 100 mg/l of A54145 factors without any strain improvement. A strain that has been through minimal mutagenesis and selection and produces more than 400 mg/l of A54145 factors has been developed at Cubist Pharmaceuticals as a host for the genetic engineering of lipopeptide biosynthesis [3, 98]. *S. fradiae* DA1187 is deleted for the complete A54145 biosynthetic gene cluster [3], and readily accepts BAC vectors containing more than 100-kb inserts via conjugation from *E. coli*, and site-specific insertion into the $\phi$C31 or $\phi$BT1 *attB* sites. Exconjugants containing the complete A54145 gene cluster inserted into either of these sites produced more than 400 mg/l of A54145 factors [3]. *S. fradiae* DA1187 may be another useful host for the expression of cryptic NRPS pathways.

### *Streptomyces toyocaensis* A47934

*S. toyocaensis* A47934 produces the non-glycosylated "glycopeptide" A47934 which comprises the sulfated core heptapeptide of teicoplanin [27, 130]. Because A47934 contains no sugar residues, it is a convenient starting scaffold for the addition of sugar residues to generate novel glycopeptides. *S. toyocaensis* protoplasts are transformable by some plasmids prepared from *S. lividans*, and it is a good recipient for bacteriophage FP43-mediated plasmid transduction [83]. Although it appears to express some DNA restriction, non-replicating plasmids, including one with a 37-kb insert of heterologous DNA, could be introduced into *S. toyocaensis* by conjugation from *E. coli* and inserted into the $\phi$C31 *attB* site at high efficiencies. Recombinants produced A47934 at yields ranging from about 300 to 600 mg/l [83], which represented only minor reductions from control A47934 yields. The conjugation/insertion system was used to generate the first recombinant to produce a hybrid glycopeptide antibiotic, glucosyl-A47934, by introducing the *gtfE* gene from the vancomycin-producing *Amycolatopsis orientalis* [116]. Importantly, the product yield was about 500 mg/l. This system may be suitable for the heterologous expression of cryptic NRPS pathways. The genes for the biosynthesis of A47934 have been cloned and sequenced [105], so the nucleotide sequence information is available to delete A47934 biosynthetic genes to generate expression hosts.

## Discussion

### Model organism vs. a suite of production hosts

In academia, there is a tendency to focus on model systems to solve problems that may be relevant in the long term to commercial enterprise. In industry, there is a tendency to work on short-term problems that can be solved with existing technology. This dual approach worked reasonably well in the past, but it is probably not sufficiently robust for the discovery of novel secondary metabolites from cryptic pathways in actinomycetes. The concept of having a "model organism" for carrying out molecular engineering manipulations in bacteria has been well served by *E. coli*. The focused work on *E. coli* has paid off over the years, and many of its advantages have been exploited for the genetic engineering of actinomycetes, as witnessed by the facile recombineering of actinomycete secondary metabolite biosynthetic pathways in *E. coli* using $\lambda$-Red-mediated recombination, followed by RP4-mediated conjugation into actinomycetes and site-specific integration into different bacteriophage or IS element *attB* sites [14, 18]. The notion that a single model organism can be developed for antibiotic-producing actinomycetes for the heterologous expression of secondary metabolite pathways is not realistic, and does not reflect the current state-of-the-art of facile genetic manipulations or the current knowledge about the potential for high-level production of specific types of secondary metabolites in different *Streptomyces* species. Most streptomycetes, and many other actinomycetes, can now be manipulated genetically. The main driver for this is the ability to conjugate between *E. coli* and actinomycetes [24, 86, 87], and in so doing to bypass host restriction barriers, which are common in actinomycetes [36]. Another important driver is the ability to insert large blocks of genes cloned in cosmid or BAC vectors very efficiently into $\phi$C31, $\phi$BT1 or other bacteriophage *attB* sites for stable expression of the cloned genes [3, 14, 18, 66]. Having broadly applicable genetic tools has opened the possibility to have a "suite of production hosts" for whole pathway expression in *Streptomyces*, and very probably in other actinomycetes, as witnessed by the successes in *S. erythraea* [110].

*S. coelicolor* and *S. lividans* have served as model organisms for many years, and they have helped progress the field of *Streptomyces* genetics enormously. The sequencing of the *S. coelicolor* genome [21] has served as a catalyst for others to sequence more actinomycete genomes (e.g., see [57, 95, 102]), and as a harbinger of the observation that actinomycetes with large genomes encoded multiple secondary metabolite functions, most of which are silent under standard fermentation conditions. This observation has been expanded dramatically with the sequencing of multiple streptomycete and other actinomycete genomes by the Broad Institute and the US Department of Energy.

Not withstanding the important impact of *S. coelicolor* and *S. lividans* as model organisms in the past, the cumulative results of heterologous secondary metabolite expression levels in *S. coelicolor* and *S. lividans* do not support

them as first choices for heterologous expression of cryptic pathways. In fact, another laboratory strain, *S. albus* J1074, which has been recently sequenced by the Broad Institute, has proven to be a more robust producer of a wide variety of heterologously expressed secondary metabolites (Table 1). Many industrial strains also have clear advantages for high-level production of heterologous secondary metabolites.

### Industrial strains selected for high-level production of specific compounds

In principle, a suite of ideal expression hosts would include several species that are capable of producing very high levels of specific types of molecules (e.g., those derived from PKS or NRPS pathways). Since the speed of discovery and evaluation of new natural products is intimately linked to fermentation production yields, having high-level production strains will be important determinants of the success or failure of genomics efforts to identify novel secondary metabolites. The yields obtained for heterologous expression of complete secondary metabolite biosynthetic pathways reviewed here ranged from about 4 to 400 mg/l, and expression of hybrid pathways ranged from 1 to 2 g/l. It is not surprising that the highest yields for the production of hybrid PKS pathways were obtained in strains of *S. fradiae* and *S. erythraea* deleted for the production of the commercial antibiotics tylosin and erythromycin, respectively. These expression hosts were derived from mutationally improved strains that produce about 2 g/l of product in shake flask fermentations. It is noteworthy that most of the mutations that influence polyketide yields in *S. fradiae* and *S. erythraea* are located outside of the tylosin and erythromycin biosynthetic gene clusters. These strains have not been used for heterologous expression of complete secondary metabolite pathways, but the location of most mutations influencing product yields outside of the main polyketide biosynthetic clusters bodes well for the channeling of precursors into heterologously expressed PKS pathways in these hosts.

### Silencing of major secondary metabolite pathways

Mutational silencing of major secondary metabolite biosynthetic pathways has two key advantages: (1) it simplifies the identification of the products of cloned heterologous gene clusters; and (2) it eliminates the channeling of key precursors into competing product pathways, thus improving the yields of the desired product. Two key examples are *S. avermitilis* and *S. ambofaciens*. In *S. avermitilis*, disruption of the avermectin pathway caused a large increase in the production of oligomycin, a product normally produced in low levels in *S. avermitilis*. Elimination of multiple secondary metabolite pathways in the genome-minimized strains provided hosts that produced several other heterologously expressed secondary metabolites at fairly good yields. There are undoubtedly strains of *S. avermitilis* in industry that produce much higher yields of avermectins than the starter strain used by Ōmura, Ikeda, and colleagues [66]. It would be interesting to see if minimizing the genome of such a strain would give even higher yields.

In *S. ambofaciens* BES2074 which was initially selected for stable, elevated production of the 16-membered macrolide spiramycin, then subsequently blocked in biosynthesis of spiramycin and netropsin, the heterologous expression of the lipopeptide antibiotic A54145 was surprisingly high (385 mg/l) without any modification of a medium optimized for A54145 production by *S. fradiae*. It would be instructive to sequence the genome of BES2074 to see how it differs from the unstable, low spiramycin-producing wild-type strain.

### Engineering the regulation of secondary metabolite biosynthesis

The biosynthesis of secondary metabolites can have multiple levels of regulation [22, 23] that may not be predictable a priori. However, many secondary metabolite biosynthetic pathways have pathway-specific positive regulation that can be identified by the inclusion of the regulatory gene(s) within the biosynthetic gene clusters. There are several examples discussed in this review where the simple replacement of a promoter for a key positive regulatory gene can uncouple the expression from a more complicated regulatory cascade. For example, the use of the *rpsJ* or *aveR* promoters decoupled heterologous streptomycin production in *S. avermitilis* from a more complicated regulatory cascade requiring A-factor. Likewise, cephamycin C production in *S. avermitilis* was improved by adding a second copy of the positive regulatory gene *ccaR* under the transcriptional control of *ermEp**. In a third case, the pladienolide biosynthetic gene cluster was not expressed in the genome-minimized *S. avermitilis* unless a second copy of the positive regulatory gene *pldR* was expressed from the *ermEp** promoter. In these three cases, the genetic manipulations were facilitated by having vectors for the insertion of the original gene clusters into the $\phi$C31 *attB* site, and the second engineered regulatory gene into the $\phi$K38-1 *attB* site. The $\phi$BT1 *attB* site could potentially provide a third site for insertion of additional genes [3].

The yield of novobiocin was improved ~3-fold in *S. coelicolor* by overexpression of the positive regulatory gene *novG* [113]. In *S. lividans*, the fredericamycin biosynthetic gene cluster was not expressed unless the positive regulatory gene *fdmR1* was expressed from *ermEp** [127]. However, in the same study, the fredericamycin pathway

was expressed at much higher levels without additional engineering in *S. albus* J1074. This is a good example of the desirability of testing multiple expression hosts. All of these examples (and one below) lend credence to the notion that expression of heterologous secondary metabolite biosynthetic pathways can often be assured by simply expressing a single positive regulatory gene from a strong constitutive promoter, and that *ermEp** is a highly successful promoter of choice.

## Expression of secondary metabolite biosynthetic pathways from non-streptomycete actinomycetes

There are well over 200 genera of actinobacteria described, and many more yet to be discovered [7]. Many grow slowly relative to their *Streptomyces* relatives, and are not easily scaled in fermentation. However, genome sequencing can be used to explore the potential of rare and poorly studied actinomycetes as sources for novel secondary metabolites, followed by cloning and expression of the novel pathways in more robust streptomycete hosts. In this regard, the two examples reviewed here are particularly compelling. Capreomycin is produced by a species of *Saccharothrix* which is apparently not tractable for genetic studies. The complete pathway was expressed in *S. lividans* at 50 mg/l without modifying its promoters or expression of regulatory elements [46]. On the other hand, the gene cluster encoding thiocoraline biosynthesis from a marine *Micromonospora* species was not initially expressed in *S. albus* J1074, but was expressed after the *ermEp** promoter was inserted to drive the expression of the *tioA* positive regulatory gene [80]. These two examples suggest that streptomycete expression hosts may be widely applicable for the expression of cryptic pathways from non-streptomycete actinomycetes.

## Enhancing production by manipulating fermentation conditions

Although fermentation optimization was not a major focus of this review, it is worth mentioning several observations. First, product yields for daptomycin production in *S. lividans* were improved substantially (from 20 to 55 mg/l) by simply increasing the phosphate level in a production medium that had been optimized previously for daptomycin production in *S. roseosporus* [104]. Similarly, novobiocin yields in *S. coelicolor* were improved ~2-fold by miniaturizing the fermentation vessels and increasing the oxygen transfer [113]. Therefore, medium development can improve productivity in laboratory strains. Secondly, in *S. avermitilis*, three structurally unrelated antibiotics, streptomycin, cephamycin C, and pladienolide, were produced in highest yield in a medium optimized for avermectin production. This may be a second general advantage of industrial production strains which have undergone both strain improvement and medium optimization during the course of scale-up studies. Finally, as observed in many industrial fermentations, optimum yields are ultimately obtained in large-scale fermentation vessels with continuous or batch feeds of carbon and nitrogen sources (e.g., glucose and ammonium), pH control, etc. This is a third advantage of applying highly productive industrial strains, which have undergone extensive large-scale fermentation optimization, as hosts for the expression of cryptic secondary metabolites.

## In search of the "dream team" of actinomycete expression hosts

Although two hosts for high-level production of polyketides have been reported, much higher producing strains are used in industrial fermentations. In many cases, industrial fermentations yield more than 10 g/l of product. Many of these highly productive strains are proprietary in large pharmaceutical companies, and held as trade secrets. These same companies have witnessed the demise of their own programs in natural products discovery in recent years, including the very successful Eli Lilly and Company. In earlier years, Lilly was very open to sharing strains, plasmids, and gene clones, and this facilitated academic research on glycopeptide and macrolide biosynthesis. The "Lilly plasmids", including the commonly used pSET152 [24], have become part of the repertoire of molecular genetic tools exploiting conjugation and site-specific integration that have helped progress the molecular genetic manipulation of actinomycetes. For rapid progress on the exploitation of actinomycete genome sequences to identify and express the products of novel secondary metabolite biosynthetic pathways in sufficient yields for robust discovery and development, it would be very useful if the very high producing "dream team" industrial strains were made available as hosts for heterologous expression. This could help revitalize natural products discovery and development.

Finally, a suite of strains suitable for heterologous expression of secondary metabolite biosynthesis to discover novel secondary metabolites will also be useful for the further development of combinatorial biosynthesis [8, 9], another technology that can help revitalize natural products discovery and development [11, 12]. We currently have a number of relatively poor to average strains to work with, and it might be advisable to pick four or five strains, including some laboratory strains and some industrial strains as hosts for heterologous expression experiments. Perhaps over time, the "dream team" strains will become available for exploitation. In the meantime, the funding agencies should be encouraged to expand genome sequencing

initiatives directed at a broad range of actinomycete genera. This will undoubtedly help drive the renaissance of natural products discovery.

## References


1. Aigle B, Schneider D, Morilhat C, Vandewiele D, Dary A, Holl A-C, Simonet J-M, Decaris B (1996) An amplifiable and deletable locus of *Streptomyces ambofaciens* RP181110 contains a very large gene homologous to polyketide synthase genes
2. Aigle B, Pang X, Decaris B, Leblond P (2005) Involvement of AlpV, a new member of the *Streptomyces* antibiotic regulatory protein family, in regulation of the duplicated type II polyketide synthase *alp* gene cluster in *Streptomyces ambofaciens*. J Bacteriol 187:2491–2500
3. Alexander D, Rock J, He X, Brian P, Miao V, and Baltz RH (2010) Development of genetic system for lipopeptide combinatorial biosynthesis in *Streptomyces fradiae* and heterologous expression of the A54145 biosynthetic gene cluster. Appl Environ Microbiol (submitted)
4. Bachmann BO, Ravel J (2010) Methods for *in silico* prediction of microbial polyketide and nonribosomal peptide biosynthetic pathways from DNA sequence data. Methods Enzymol 458:181–217
5. Baltz RH (1978) Genetic recombination in *Streptomyces fradiae* by protoplast fusion and cell regeneration. J Gen Microbiol 107:93–102
6. Baltz RH (1982) Genetics and biochemistry of tylosin production: a model for genetic engineering in antibiotic-producing *Streptomyces*. Basic Life Sci 19:431–444
7. Baltz RH (2005) Antibiotic discovery from actinomycetes: will a renaissance follow the decline and fall? SIM News 55:186–196
8. Baltz RH (2006) Combinatorial biosynthesis of novel antibiotics and other secondary metabolites in actinomycetes. SIM News 56:148–160
9. Baltz RH (2006) Molecular engineering approaches to peptide, polyketide and other antibiotics. Nat Biotechnol 24:1533–1540
10. Baltz RH (2006) Marcel Faber roundtable: is our antibiotic pipeline unproductive because of starvation, constipation, or lack of inspiration? J Ind Microbiol Biotechnol 33:507–513
11. Baltz RH (2007) Natural products from actinomycetes: back to the future. Microbe 2:125–131
12. Baltz RH (2008) Renaissance in antibacterial discovery from actinomycetes. Curr Opin Pharmacol 8:1–7
13. Baltz RH (2008) Biosynthesis and genetic engineering of lipopeptide antibiotics related to daptomycin. Curr Top Med Chem 8:618–638
14. Baltz RH (2009) Biosynthesis and genetic engineering of lipopeptides in *Streptomyces rosesporus*. Methods Enzymol 458:511–531
15. Baltz RH, Matsushima P (1981) Protoplast fusion in *Streptomyces*: conditions for efficient genetic recombination and cell regeneration. J Gen Microbiol 127:137–146
16. Baltz RH, Seno ET (1981) Properties of *Streptomyces fradiae* mutants blocked in biosynthesis of the macrolide antibiotic tylosin. Antimicrob Agents Chemother 20:214–225
17. Baltz RH, Seno ET (1988) Genetics of *Streptomyces fradiae* and tylosin biosynthesis. Ann Rev Microbiol 42:547–574
18. Baltz RH, Nguyen KT, Alexander DC (2010) Genetic engineering of acidic lipopeptide antibiotics. In: Baltz RH, Davies JE, Demain AL (eds) Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, DC, pp 391–410
19. Barkei JJ, Kevany BM, Felnagle EA, Thomas MG (2009) Investigations into viomycin biosynthesis using heterologous production in *Streptomyces lividans*. ChemBioChem 10:366–376
20. Beckman RJ, Cox K, Seno ET (1989) A cluster of tylosin biosynthetic genes is interrupted by a structurally unstable segment containing four repeated sequences. In: Hershberger CL, Queener SW, Hegeman G (eds) Genetics and molecular biology of industrial microorganisms. American Society for Microbiology, Washington, DC, pp 176–186
21. Bentley SD, Chater KF, Cerdeño-Tárraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabbinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141–147
22. Bibb MJ (2005) Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol 8:208–215
23. Bibb MJ, Hesketh A (2009) Analyzing the regulation of antibiotic production in streptomycetes. Methods Enzymol 458:93–116
24. Bierman M, Logan R, O'Brien K, Seno ET, Rao RN, Schoner BE (1992) Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. Gene 116:43–49
25. Binnie C, Warren M, Butler MJ (1989) Cloning and heterologous expression in *Streptomyces lividans* of *Streptomyces rimosus* genes involved in oxytetracycline biosynthesis. J Bacteriol 171:887–895
26. Birmingham VA, Cox KL, Larson JL, Fishman SE, Hershberger CL, Seno ET (1986) Cloning and expression of a tylosin resistance gene from a tylosin-producing strain of *Streptomyces fradiae*. Mol Gen Genet 204:532–539
27. Boeck LD, Mertz FP (1986) A47934, a novel glycopeptide-aglycone antibiotic produced by a strain of *Streptomyces toyocaensis*: taxonomy and fermentation studies. J Antibiot 39:1533–1540
28. Boeck LD, Papiska HR, Wetzel RW, Mynderse JS, Fukuda DS, Mertz FP, Berry DM (1990) A54145, a new lipopeptide antibiotic complex: discovery, taxonomy, fermentation and HPLC. J Antibiot 43:587–593
29. Bormann C, Möhrle V, Bruntner C (1996) Cloning and heterologous expression of the entire set of structural genes for nikkomycin synthesis from *Streptomyces tendae* Tü901 in *Streptomyces lividans*. J Bacteriol 178:1216–1218
30. Challis GL (2008) Mining microbial genomes for new natural products and biosynthetic pathways. Microbiology 154:1555–1569
31. Chater KF, Wilde LC (1976) Restriction of a bacteriophage of *Streptomyces albus* G involving endonuclease *Sal*I. J Bacteriol 128:644–650
32. Chen Y, Wendt-Pienkowski E, Shen B (2008) Identification and utility of FdmR1 as a *Streptomyces* antibiotic regulatory protein activator for fredericamycin production in *Streptomyces griseus* ATCC 49344 and heterologous hosts. J Bacteriol 190:5587–5596
33. Choulet F, Aigle B, Gallois A, Mangenot S, Gerbaud C, Truong C, Francou F-X, Fourrier C, Guérineau M, Decaris B, Barge V, Pernodet J-L, Leblond P (2006) Evolution of the terminal regions of the *Streptomyces* linear chromosome. Mol Biol Evol 23:2361–2369
34. Coëffet-Le Gal M-F, Thurson L, Rich P, Miao V, Baltz RH (2006) Complementation of daptomycin *dptA* and *dptD* deletion mutations *in-trans* and production of hybrid lipopeptide antibiotics. Microbiology 152:2993–3001

35. Corre C, Challis GL (2009) New natural product biosynthetic chemistry discovered by genome mining. Nat Prod Rep 26:977–986
36. Cox KL, Baltz RH (1984) Restriction of bacteriophage plaque formation in *Streptomyces* spp. J Bacteriol 159:499–504
37. Cox KL, Fishman SE, Larson JL, Stanzak R, Reynolds PA, Yeh WK, van Frank RM, Birmingham VA, Hershberger CL, Seno ET (1986) The use of recombinant DNA techniques to study tylosin biosynthesis and resistance in *Streptomyces fradiae*. J Nat Prod 49:971–980
38. Cundliffe E (2008) Control of tylosin biosynthesis in *Streptomyces fradiae*. J Microbiol Biotechnol 18:1485–1491
39. Dary A, Kaiser P, Bourget N, Thompson CJ, Simonet J-M, Decaris B (1993) Large genomic rearrangements of the unstable region in *Streptomyces ambofaciens* are associated with major changes in global gene expression. Mol Microbiol 10:759–769
40. Dary A, Martin P, Wenner T, Decaris B, Leblond P (2000) DNA rearrangements at the extremities of the *Streptomyces ambofaciens* linear chromosome: evidence for developmental control. Biochimie 82:29–34
41. Eisenstein BI, Oleson FB Jr, Baltz RH (2010) Daptomycin: from the mountain to the clinic, with the essential help of Francis Tally, MD. Clin Inf Dis 50:S10–S15
42. Epp JK, Burgett SG, Schoner BE (1987) Cloning and nucleotide sequence of a carbomycin-resistance gene from *Streptomyces thermotolerans*. Gene 53:73–83
43. Epp JK, Huber ML, Turner JR, Goodson T, Schoner BE (1989) Production of a hybrid macrolide antibiotic in *Streptomyces ambofaciens* and *Streptomyces lividans* by introduction of a cloned carbomycin biosynthetic gene from *Streptomyces thermotolerans*. Gene 85:293–301
44. Eustáquio AS, Gust B, Li S-M, Pelzer S, Wohlleben W, Chater KF, Heide L (2004) Production of 8′-halogenated and 8′-unsubstituted novobiocin derivatives in genetically engineered *Streptomyces coelicolor* strains. Chem Biol 11:1561–1572
45. Eustáquio AS, Gust B, Galm U, Li S-M, Chater KF, Heide L (2005) Heterologous expression of novobiocin and clorobiocin biosynthetic gene clusters. Appl Environ Microbiol 71:2452–2459
46. Felnagle EA, Rondon MR, Berti AD, Crosby HA, Thomas MG (2007) Identification of the biosynthetic gene cluster and an additional gene for resistance to the antituberculosis drug capreomycin. Appl Environ Microbiol 73:4162–4170
47. Feng Z, Wang L, Rajski SR, Xu Z, Coëffet-LeGal MF, Shen B (2009) Engineered production of iso-migrastatin in heterologous *Streptomyce* hosts. Bioorg Med Chem 17:2147–2153
48. Fernández E, Lombó F, Méndez C, Salas JA (1996) An ABC transporter is essential for resistance to the antitumor agent mithramycin in the producer *Streptomyces argillaceus*. Mol Gen Genet 251:692–698
49. Ford LM, Eaton TE, Godfrey OW (1990) Selection for *Streptomyces ambofaciens* mutants that produce large quantities of spiramycin and determination of optimal conditions for spiramycin production. Appl Environ Microbiol 56:3511–3514
50. Galm U, Shen B (2006) Expression of biosynthetic gene clusters in heterologous hosts for natural product production and combinatorial biosynthesis. Expert Opin Drug Discov 1:409–437
51. Gross H (2007) Strategies to unravel the function of orphan biosynthesis pathways: recent examples and future prospects. Appl Microbiol Biotechnol 75:267–277
52. Gross H (2009) Genomic-mining—a concept for the discovery of new bioactive natural products. Curr Opin Drug Discov Devel 12:207–219
53. Gullón S, Olano C, Abdelfattah MS, Braña AF, Rohr J, Méndez C, Salas JA (2006) Isolation, characterization, and heterologous expression of the biosynthesis gene cluster for the antitumor anthracycline steffimycin. Appl Environ Microbiol 72:4172–4183
54. Hahn DR, Solenberg PJ, Baltz RH (1991) Tn*5099*, a *xylE* promoter probe transposon for *Streptomyces* spp. J Bacteriol 173:5573–5577
55. Hosted TJ, Baltz RH (1996) Use of *rpsL* for dominance selection and gene replacement in *Streptomyces roseosporus*. J Bacteriol 179:180–186
56. Huang KX, Xia L, Zhang Y, Ding X, Zahn JA (2009) Recent advances in the biochemistry of spinosyns. Appl Microbiol Biotechnol 82:13–23
57. Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori M, Ōmura S (2003) Complete genome sequence of and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. Nat Biotechnol 21:526–531
58. Jayapal KP, Lian W, Glod F, Sherman DH, Hu W (2007) Comparative genomic hybridizations reveal absence of large *Streptomyces coelicolor* genomic islands in *Streptomyces lividans*. BMC Genomics 8:229
59. Jenke-Kodama H, Dittmann E (2009) Bioinformatic perspectives on NRPS/PKS megasynthases: advances and challenges. Nat Prod Rep 26:874–883
60. Jiang J, Tetzlaff CN, Takamatsu S, Iwatsuki M, Komatsu M, Ikeda H, Cane DE (2009) Genome mining in *Streptomyces avermitilis*: a biochemical Baeyer-Villiger reaction and discovery of a new branch of the pentalenolactone family tree. Biochemistry 48:6431–6440
61. Kao CM, Katz L, Khosla C (1994) Engineered biosynthesis of a complete macrolactone in a heterologous host. Science 265:509–512
62. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical *Streptomyces* genetics. The John Innes Foundation, Norwich
63. Kim M-K, Ha H-S, Choi S-U (2008) Conjugal transfer using the bacteriophage $\phi$C31 *att/int* system and properties of the *attB* site in *Streptomyces ambofaciens*. Biotechnol Lett 30:695–699
64. Kirst HA (1997) Macrolide antibiotics in food-animal health. Expert Opin Investig Drugs 6:103–118
65. Komatsu M, Tsuda M, Ōmura S, Oikawa H, Ikeda H (2008) Identification and functional analysis of genes controlling biosynthesis of 2-methylisoborneol. Proc Nat Acad Sci USA 105:7422–7427
66. Komatsu M, Uchiyama T, Ōmura S, Cane DE, Ikeda H (2010) Genome-minimized *Streptomyces* host for the heterologous expression of secondary metabolism. Proc Nat Acad Sci USA 107:2646–2651
67. Kuhstoss S, Richardson MA, Rao RN (1989) Site-specific integration in *Streptomyces ambofaciens*: localization of integration functions in *S. ambofaciens* plasmid pSAM2. J Bacteriol 171:16–23
68. Kustoss S, Rao RN (1991) Analysis of the integration function of the streptomycete bacteriophage $\phi$C31. J Mol Biol 222:897–908
69. Kuhstoss S, Richardson MA, Rao RN (1991) Plasmid cloning vectors that integrate site-specifically in *Streptomyces* spp. Gene 97:143–146
70. Kuhstoss S, Huber M, Turner JR, Paschal JW, Rao RN (1996) Production of a novel polyketide through the construction of a hybrid polyketide synthase. Gene 183:231–236
71. Kwon H-J, Smith WC, Xiang L, Shen B (2001) Cloning and heterologous expression of the macrotetrolide biosynthetic gene cluster revealed a novel polyketide synthase that lacks an acyl carrier protein. J Am Chem Soc 123:3385–3386
72. Lacalle RA, Tercera JA, Jimenez J (1992) Cloning of the complete biosynthetic gene cluster for an aminonucleoside antibiotic, puromycin, and its regulated expression in heterologous hosts. EMBO J 11:785–792

73. Lanka E, Wilkins BM (1995) DNA processing reactions in bacterial conjugation. Annu Rev Biochem 64:141–169
74. Larson JL, Hershberger CL (1984) Shuttle vectors for cloning recombinant DNA in *Escherichia coli* and *Streptomyces griseofuscus* C581. J Bacteriol 157:314–317
75. Larson JL, Hershberger CL (1986) The minimal replicon of a streptomycete plasmid produces an ultrahigh level of plasmid DNA. Plasmid 15:199–209
76. Leblond P, Demuyter P, Moutier L, Laakel M, Decaris B, Simonet J-M (1989) Hypervariability, a new phenomenon of genetic instability, related to DNA amplification in *Streptomyces ambofaciens*. J Bacteriol 171:419–423
77. Leblond P, Demuyter P, Simonet J-M, Decaris B (1990) Genetic instability and hypervariability in *Streptomyces ambofaciens*: towards an understanding of a mechanism of genome plasticity. Mol Microbiol 4:707–714
78. Leblond P, Demuyter P, Simonet J-M, Decaris B (1991) Genetic instability and associated genome plasticity in *Streptomyces ambofaciens*: pulsed-field gel electrophoresis evidence for large DNA alterations in a limited genomic region. J Bacteriol 173:4229–4233
79. Li MHT, Ung PMU, Zajkowski J, Garneau-Tsodikova S, Sherman DH (2009) Automated genome mining for natural products. BMC Bioinformatics 10:185
80. Lombo F, Velasco A, Castro A, de la Calle F, Brana AF, Sanchez-Puelles JM, Mendez C, Salas JA (2006) Deciphering the biosynthesis pathway of the antitumor thiocoraline from a marine actinomycete and its expression in two *Streptomyces* species. ChemBioChem 7:366–376
81. Matsushima P, Baltz RH (1985) Efficient plasmid transformation of *Streptomyces ambofaciens* and *Streptomyces fradiae* protoplasts. J Bacteriol 163:180–185
82. Matsushima P, Baltz RH (1994) Transformation of *Saccharopolyspora spinosa* protoplasts with plasmid DNA modified *in vitro* to avoid host restriction. Microbiology 140:139–143
83. Matsushima P, Baltz RH (1996) A gene cloning system for '*Streptomyces toyocaensis*'. Microbiology 142:261–267
84. Matsushima P, Cox KL, Baltz RH (1987) Highly transformable mutants of *Streptomyces fradiae* defective in several restriction systems. Mol Gen Genet 206:393–400
85. Matsushima P, McHenney MA, Baltz RH (1989) Transduction and transformation of plasmid DNA in *Streptomyces fradiae* strains that express different levels of restriction. J Bacteriol 171:3080–3084
86. Matsushima P, Broughton MC, Turner JR, Baltz RH (1994) Conjugal transfer of cosmid DNA from *Escherichia coli* to *Saccharopolyspora spinosa*: effects of chromosomal insertions on macrolide A83543 production. Gene 146:39–45
87. Mazodier P, Petter R, Thompson C (1989) Intergeneric conjugation between *Escherichia coli* and *Streptomyces* species. J Bacteriol 171:3583–3585
88. McHenney MA, Baltz RH (1988) Transduction of plasmid DNA in *Streptomyces* spp. and related genera by bacteriophage FP43. J Bacteriol 170:2276–2282
89. McHenney MA, Baltz RH (1991) Transposition of Tn*5096* from a temperature-sensitive transducible plasmid in *Streptomyces* spp. J Bacteriol 173:5578–5581
90. McHenney MA, Baltz RH (1996) Gene transfer and transposition mutagenesis in *Streptomyces roseosporus*: mapping of insertions that influence daptomycin or pigment production. Microbiology 142:2363–2373
91. McHenney MA, Hosted TJ, Dehoff BS, Rosteck PR Jr, Baltz RH (1998) Molecular cloning and physical mapping of the daptomycin gene cluster from *Streptomyces roseosporus*. J Bacteriol 180:143–151
92. Miao V, Coëffet-Le Gal M-F, Brian P, Brost R, Penn J, Whiting A, Martin S, Ford R, Parr I, Bouchard M, Silva CJ, Wrigley SK, Baltz RH (2005) Daptomycin biosynthesis in *Streptomyces roseosporus*: cloning and analysis of the gene cluster and revision of peptide stereochemistry. Microbiology 151:1507–1523
93. Miao V, Brost R, Chapple J, She K, Coëffet-Le Gal M-F, Baltz RH (2006) The lipopeptide antibiotic A54145 biosynthetic gene cluster from *Streptomyces roseosporus*. J Ind Microbiol Biotechnol 33:129–140
94. Miao V, Coëffet-Le Gal M-F, Nguyen K, Brian P, Penn J, Whiting A, Steele J, Kau D, Martin S, Ford R, Gibson T, Bouchard M, Wrigley SK, Baltz RH (2006) Genetic engineering in *Streptomyces roseoporus* to produce hybrid lipopeptide antibiotics. Chem Biol 13:269–276
95. Nett M, Ikeda H, Moore BS (2009) Genomic basis for natural product biosynthetic diversity in the actinomycetes. Nat Prod Rep 26:1362–1384
96. Nguyen K, Kau D, Gu J-Q, Brian P, Wrigley SK, Baltz RH, Miao V (2006) A glutamic acid 3-methyltransferase encoded by an accessory gene locus important for daptomycin biosynthesis in *Streptomyces roseosporus*. Mol Microbiol 61:1294–1307
97. Nguyen K, Ritz D, Gu J-Q, Alexander D, Chu M, Miao V, Brian P, Baltz RH (2006) Combinatorial biosynthesis of lipopeptide antibiotics related to daptomycin. Proc Natl Acad Sci USA 103:17462–17467
98. Nguyen K, He X, Alexander DC, Li C, Gu J-Q, Mascio C, Van Praagh A, Morton L, Chu M, Silverman JA, Brian P, Baltz RH (2010) Genetically engineered lipopeptide antibiotics related to A54145 and daptomycin with improved properties. Antimicrob Agents Chemother 54:1404–1413
99. Ochi K (2007) From microbial differentiation to ribosome engineering. Biosci Biotechnol Biochem 71:1373–1386
100. Ohnishi Y, Ishikawa J, Hara H, Suzuki H, Ikenoya M, Ikeda H, Yamashita A, Hattori M, Horinouchi S (2008) Genome sequence of the streptomycin-producing microorganism *Streptomyces griseus* IFO 13350. J Bacteriol 190:4050–4060
101. Olano C, Abdelfattah MS, Gullón S, Braña AF, Rohr J, Méndez C, Salas JA (2008) Glycosylated derivatives of steffimycin: insights into the role of sugar moieties for the biological activity. ChemBioChem 9:624–633
102. Oliynyk M, Samborskyy M, Lester JB, Mironenko T, Scott N, Dickens S, Haydock SF, Leadlay PF (2007) Complete genome sequence of the erythromycin-producing bacterium *Saccharopolyspora erythraea* NRRL23338. Nat Biotechnol 25:447–453
103. Onaka H, Taniguchi S-I, Igarashi Y, Furamai T (2002) Cloning the staurosporine biosynthetic gene cluster from *Streptomyces* sp. TP-A0274 and its heterologous expression on *Streptomyces lividans*. J Antibiotics 55:1063–1071
104. Penn J, Li X, Whiting A, Latif M, Gibson T, Silva CJ, Brian P, Davies J, Miao V, Wrigley SK, Baltz RH (2006) Heterologous production of daptomycin in *Streptomyces lividans*. J Ind Microbiol Biotechnol 33:121–128
105. Pootoolal J, Thomas MG, Marshall CG, Neu JM, Hubbard BK, Walsh CT, Wright GD (2002) Assembling the glycopeptide antibiotic scaffold: the biosynthesis of A47034 from *Streptomyces toyocaensis* NRRL 15009. Proc Nat Acad Sci USA 99:8962–8967
106. Reeves CD, Ward SL, Revill WP, Suzuki H, Marcus M, Petrakovsky OV, Marquez S, Fu H, Dong SD, Katz L (2004) Production of hybrid 16-membered macrolides by expressing combinations of polyketide synthase genes in engineered *Streptomyces fradiae* hosts. Chem Biol 11:1465–1472
107. Richardson MA, Kuhstoss S, Solenberg P, Schaus NA, Rao RN (1987) A new shuttle cosmid vector, pKC505, for streptomycetes: its use in the cloning of three different spiramycin-resistance genes from a *Streptomyces ambofaciens* library. Gene 61:231–241

108. Richardson MA, Kuhstoss S, Huber MLB, Ford L, Godfrey O, Turner JR, Rao NR (1990) Cloning of spiramycin biosynthetic genes and their use in constructing *Streptomyces ambofaciens* mutants defective in spiramycin biosynthesis. J Bacteriol 172:3790–3798
109. Rodríquez AM, Olano C, Vilches C, Méndez C, Salas JA (1993) *Streptomyces antibioticus* contains at least three oleandomycin-resistance determinants, one of which shows similarity with proteins of the ABC-transporter superfamily. Mol Microbiol 8:571–582
110. Rodriquez E, Hu Z, Ou S, Volchegursky Y, Hutchinson CR, McDaniel R (2003) Rapid engineering of polyketide overproduction by gene transfer to industrially optimized strains. J Ind Microbiol Biotechnol 30:480–488
111. Rubinstein E, Keller N (1998) Spiramycin renaissance. J Antimicrob Chemother 42:572–576
112. Seno ET, Baltz RH (1982) *S*-adenosyl-L-methionine: macrocin *O*-methyltransferase activities in a series of *Streptomyces fradiae* mutants that produce different levels of the macrolide antibiotic tylosin. Antimicrob Agents Chemother 21:758–763
113. Siebenberg S, Bapat PM, Lantz AE, Gust B, Heide L (2009) Reducing the variability of antibiotic production in *Streptomyces* by cultivation in 24-square deepwell plates. J Biosci Bioeng 109:230–234
114. Solenberg PJ, Baltz RH (1991) Transposition of Tn*5096* and other IS*493* derivatives in *Streptomyces griseofuscus*. J Bacteriol 173:1096–1104
115. Solenberg PJ, Baltz RH (1994) Hypertransposing derivatives of the streptomycete insertion sequence IS*493*. Gene 147:47–54
116. Solenberg PJ, Matshushima P, Stack DR, Wilkie SC, Thompson RC, Baltz RH (1997) Production of hybrid glycopeptide antibiotics *in vitro* and in *Streptomyces toyocaensis*. Chem Biol 4:195–202
117. Starcevic A, Zucko J, Simunkovic J, Long PF, Cullum J, Hranueli D (2008) *ClustScan*: and integrated program package for the semi-automatic annotation of modular biosynthetic gene clusters and *in silico* prediction of novel chemical structures. Nucl Acids Res 36:6882–6892
118. Stonesifer J, Matsushima P, Baltz RH (1986) High frequency conjugal transfer of tylosin genes and amplifiable DNA in *Streptomyces fradiae*. Mol Gen Genet 202:348–355
119. Stratigopoulos G, Cundliffe E (2002) Inactivation of a transcriptional repressor during empirical improvement of the tylosin producer, *Streptomyces fradiae*. J Ind Microbiol Biotechnol 28:219–224
120. Talà A, Wang G, Zemanova M, Okamoto S, Ochi K, Alifano P (2009) Activation of dormant bacterial genes by *Nonomuraea* sp. strain ATCC 39727 mutant-type RNA polymerase. J Bacteriol 191:805–814
121. Tanaka Y, Komatsu M, Okamoto S, Tokuyama S, Kaji A, Ikeda H, Ochi K (2009) Antibiotic overproduction by *rpsL* and *rsmG* mutants of various actinomycetes. Appl Environ Microbiol 75:4919–4922
122. Tetzlaff CN, You Z, Cane DE, Takamatzu S, Ōmura S, Ikeda H (2006) A gene cluster for biosynthesis of the sequiterpine antibiotic pentalenolactone in *Streptomyces avermitilis*. Biochemistry 45:6179–6186
123. Van Lanen S, Shen B (2006) Microbial genomics for the improvement of natural product discovery. Curr Opin Microbiol 9:1–9
124. van Wezel GP, McKenzie NL, Nodwell JR (2009) Applying the genetics of secondary metabolism in model actinomycetes to the discovery of new antibiotics. Methods Enzymol 458:117–141
125. Ward SL, Hu Z, Schirmer A, Reid R, Revill P, Reeves CD, Petrakowsky OV, Dong SD, Katz L (2004) Chalcomycin biosynthesis gene cluster from *Streptomyces bikiniensis*: novel features of an unusual ketolide produced through expression of the chm polyketide synthase in *Streptomyces fradiae*. Antimicrob Agents Chemother 8:4703–4712
126. Weber T, Rausch C, Lopez P, Hoof I, Gaykova V, Huson DH, Wohlleben W (2009) CLUSTEAN: a computer-based framework for the automated analysis of bacterial secondary metabolite biosynthetic gene clusters. J Biotechnol 140:13–17
127. Wendt-Pienkowski E, Huang Y, Zhang J, Li B, Jiang H, Kwon H, Hutchinson CR, Shen B (2005) Cloning, sequencing, analysis, and heterologous expression of the fredericamycin biosynthetic gene cluster from *Streptomyces griseus*. J Am Chem Soc 127:16442–16452
128. Wenner T, Roth V, Fischer G, Fourrier C, Aigle B, Decaris B, Leblond P (2003) End-to-end fusion of linear deleted chromosomes initiates a cycle of genome instability in *Streptomyces ambofaciens*. Mol Microbiol 50:411–425
129. Winter JM, Moffitt MC, Zazopoulos E, McAlpine JB, Dorrestein PC, Moore BS (2007) Molecular basis for chloronium-mediated meroterpene cyclization: cloning, sequencing, and heterologous expression of the napyradiomycin biosynthetic gene cluster. J Biol Chem 282:16362–16368
130. Zmijeweski MJ, Briggs B, Logan R, Boeck LD (1987) Biosynthetic studies on antibiotic A47934. Antimicrob Agents Chemother 31:1497–1501