J Ind Microbiol Biotechnol (2014) 41:387–402
DOI 10.1007/s10295-013-1352-9

REVIEW

SIMB
Society for Industrial Microbiology and Biotechnology

# Actinomycetes biosynthetic potential: how to bridge in silico and in vivo?

**Yuriy Rebets · Elke Brötz · Bogdan Tokovenko · Andriy Luzhetskyy**

Received: 27 July 2013 / Accepted: 18 September 2013 / Published online: 15 October 2013


**Abstract** Actinomycetes genome sequencing and bioinformatic analyses revealed a large number of "cryptic" gene clusters coding for secondary metabolism. These gene clusters have the potential to increase the chemical diversity of natural products. Indeed, reexamination of well-characterized actinomycetes strains revealed a variety of hidden treasures. Growing information about this metabolic diversity has promoted further development of strategies to discover novel biologically active compounds produced by actinomycetes. This new task for actinomycetes genetics requires the development and use of new approaches and tools. Application of synthetic biology approaches led to the development of a set of strategies and tools to satisfy these new requirements. In this review, we discuss strategies and methods to discover small molecules produced by these fascinating bacteria and also discuss a variety of genetic instruments and regulatory elements used to activate secondary metabolism cryptic genes for the overproduction of these metabolites.

**Keywords** Actinomycetes · Cryptic gene clusters · Synthetic promoters · Heterologous expression · Genome mining

## Introduction

The rapid development of sequencing technologies has led to an avalanche-like increase in the number of sequenced bacterial genomes. With the first report of a complete sequence of *S. coelicolor* genome it became clear that secondary metabolism of Actinobacteria is hiding a huge chemical diversity encrypted in silent (cryptic) biosynthesis gene clusters. Additional actinomycete genome sequencing projects further enhanced this idea. To a large surprise, it was found that well-studied strains with established metabolomic profiles are potentially able to produce a larger variety of chemical structures than were originally determined. Despite years of studies, these compounds remained undiscovered. The community began to develop novel ways to screen for biologically active compounds. Efforts have been redirected from the environmental screening to focus on studying regulation mechanisms of secondary metabolism and to develop strategies to activate silent gene clusters. The years of science devoted to studying actinomycetes genetics accumulated a large number of genetic tools for DNA manipulations and identified a set of elements controlling gene expression. The application of synthetic biology to actinomycetes genetics further expanded this set of tools. Many of the newly developed approaches combine novel genetic techniques and regulatory elements for gene expression to successfully discover novel natural products produced from cryptic gene clusters. In this review we summarize some of the strategies used for silent gene cluster activation as well as discuss the most important tools and instruments used in these approaches. Furthermore, we will describe a set of transcriptional and translational control elements of synthetic biology as well as reporter genes for studying cryptic secondary metabolism. The right choice of strategies and tools for activation of cryptic metabolic pathway will lead to a higher efficiency of discovery of chemical structures hidden in actinomycetes genomes.

Y. Rebets · E. Brötz · B. Tokovenko · A. Luzhetskyy (✉)
Helmholtz-Institute for Pharmaceutical Research Saarland, Campus, Building C2.3, Saarbrücken 66123, Germany
e-mail: andriy.luzhetskyy@helmholtz-hzi.de

## OSMAC: old dog, new tricks

For years it was postulated that *S. coelicolor* produces only four compounds—actinorhodin, undecylprodigiosin, methylenomycin, and calcium-dependent antibiotic (CDA) [59, 94, 116, 128]. Genome analysis of this bacterium showed a much broader chemical potential than was thought [6]. This has led to the isolation of several new compounds. Some of them are discussed below. *S. avermitilis*, another well-studied antibiotic producer, has been found to encode 38 secondary metabolites gene clusters [46, 78]. The number of such examples is growing with the growing number of actinomycetes genomes sequenced. Some of these secondary metabolism genes are non-functional or silent under standard laboratory conditions. However, transcriptome analysis clearly shows that the majority of these genes are transcribed at very low levels. DNA microarrays of *S. coelicolor* grown on solid media showed transcription of 12 out of 22 secondary metabolism gene clusters [131]. Seven of these were considered to be cryptic. Furthermore, during exponential growth of *S. coelicolor* culture RNA sequencing showed transcription of 7,800 individual genes, including those involved in secondary metabolism [31]. Eighteen secondary metabolite gene clusters out of 25 were found to be transcribed in *S. avermitilis*, whereas only two compounds were known to be produced before the genome was sequenced [17, 78]. Furthermore, *S. coelicolor* proteomics revealed the presence of enzymes from three gene clusters with unknown products [41, 49]. These data show that the majority of secondary metabolism gene clusters in streptomycetes are not silent, but expressed at a very low level under laboratory conditions. Often the transcription of the gene clusters under these conditions is not sufficient to produce detectable amounts of secondary metabolites. Several approaches have been used to boost secondary metabolism gene expression in order to reveal novel compounds.

It is known that under certain conditions secondary metabolites may provide selective advantages to the producing strains [15]. Accordingly, production of these secondary metabolites occurs only under certain environmental conditions. This idea has been used to identify new compounds from well-studied producers or multiple compounds from the same strain. This strategy is named “one strain/many compounds” (OSMAC) [12, 77]. Typical screening programs concentrate their efforts on the most abundant or most active compounds produced by a strain. These programs are based on existing knowledge about a particular metabolite production, thus applying standard conditions. It is well known that even insignificant changes in nutritional or environmental factors during cultivation can influence the quantity and the variety of accumulated metabolites. The OSMAC approach combined with well-established chemical screening strategies has led to the discovery of novel secondary metabolites from old sources.

Bioinformatic analysis of the *S. coelicolor* genome uncovered a set of cryptic secondary metabolite gene clusters. Further investigation into these cryptic genes led to the discovery of several new compounds (Fig. 1). Among them, type III polyketide synthases (PKSIII) synthesized germicidins and isogermicidins [107] and PKSI synthesized yellow polyketide compound coelimycin P1 [35]. The later one was identified after deletion of the regulatory gene *scbR2* within the cryptic PKSI *cpk*-cluster [36]. Coelimycin P1 was also reported to be detected using the OSMAC approach by growing *S. coelicolor* on glucose-free-rich media [84]. The carotenoids isorenieratene and β-carotene accumulate only after *S. coelicolor* is illuminated with blue light [114]. Accordingly, the expression of carotegenesis genes was shown to be dependent on the light-inducible sigma factor associated with the cluster. A combination of *S. coelicolor* genome mining and OSMAC approaches led to the identification and isolation of the NRPS-synthesized siderophore coelichelin (Fig. 1) [61]. Here, researchers used in silico analysis to predict the chemical structure and properties of a secondary metabolite encoded by a cryptic gene cluster. Their hypothesis that the metabolite would act as a $Fe^{3+}$-chelating agent was critical for selecting culture conditions used to identify the corresponding compound. *S. coelicolor* was found to accumulate coelichelin when growing on iron-deficient, but not on iron-rich media [61]. This study is a great example of combining several approaches to identify novel metabolites encrypted in silent PKS and NRPS gene clusters in *Streptomycetes* genomes.

Carlson and co-authors isolated an entire group of amphiphilic siderophore compounds produced by *S. coelicolor* using a combination of OSMAC and integrated global metabolomic analyses [104]. When grown at elevated temperatures, *S. coelicolor* was found to accumulate 25 new compounds that are absent when grown under regular conditions. Fragmentation pattern comparison was used to group metabolites and thus to simplify further analysis. This led to the identification of a cluster of 17 similar compounds, one of which, ferrioxamine B (Fig. 1), was previously found to be produced under iron-deficient growth conditions [5]. The 16 remaining compounds, though produced by other strains, are new to the *S. coelicolor* metabolome. Ochi and co-authors found that the addition of the rare earth elements scandium and lanthanum dramatically boosts transcription of genes from nine secondary metabolism biosynthesis gene clusters in *S. coelicolor* including four with unidentified metabolites [115] (for detailed review see [77]).

A great number of successful examples of applying the OSMAC approach to discover new secondary metabolites from different sources including bacteria and fungi are provided by the excellent works of Zeeck et al. [12].

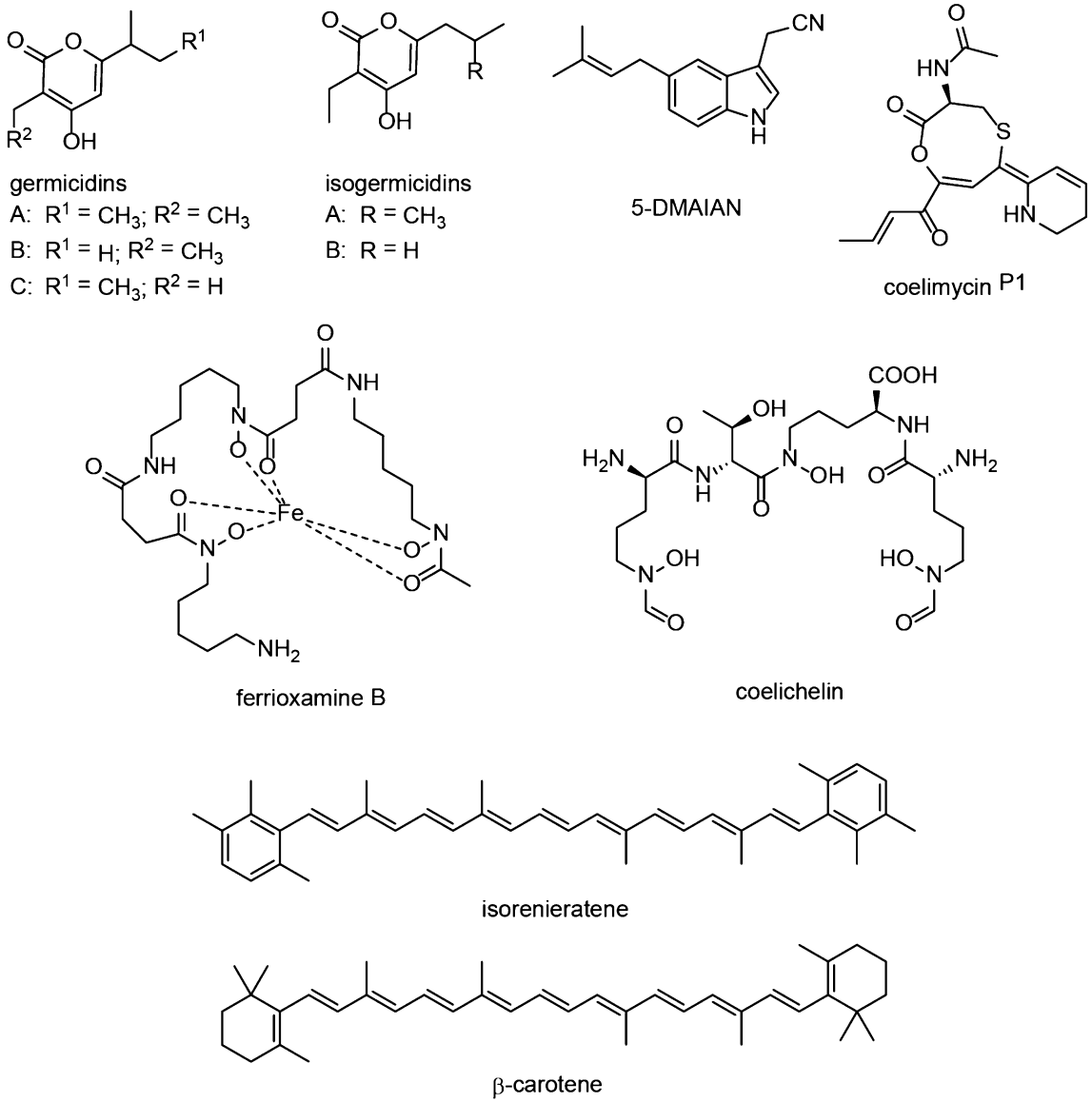


**Fig. 1** Chemical structures of secondary metabolites accumulated by *S. coelicolor* as a result of activation of cryptic gene clusters

They isolated over 100 compounds belonging to 25 different scaffold classes from only six organisms. OSMAC represents a powerful tool for the investigation of a microorganism's secondary metabolome by changing basic growth conditions like media composition, aeration rate, illumination, and temperature. This method does not require any genomic information or genetic manipulations with the strain to achieve the desired results. However, due to a lack of understanding of environmental triggers of secondary metabolism this approach requires a systematic alteration of growth parameters in order to achieve the maximum efficiency of metabolite isolation. This makes it inefficient and time consuming. Furthermore, there is no guarantee that the necessary conditions can be found to facilitate the production of all compounds from a particular strain. Since the OSMAC approach relies on specific conditions for each desired metabolite, it is challenging to use it to develop basic rules that can be used for all producers. However, by combining OSMAC with genome mining, genetic engineering and deep chemical analysis will lead to a greater realization of streptomycetes secondary metabolism potential.

## Post-genomic strategies for cryptic secondary metabolism gene cluster activation

The availability of genomic information provides opportunities for the design of rational approaches for silent gene cluster activation. The number of successful examples of

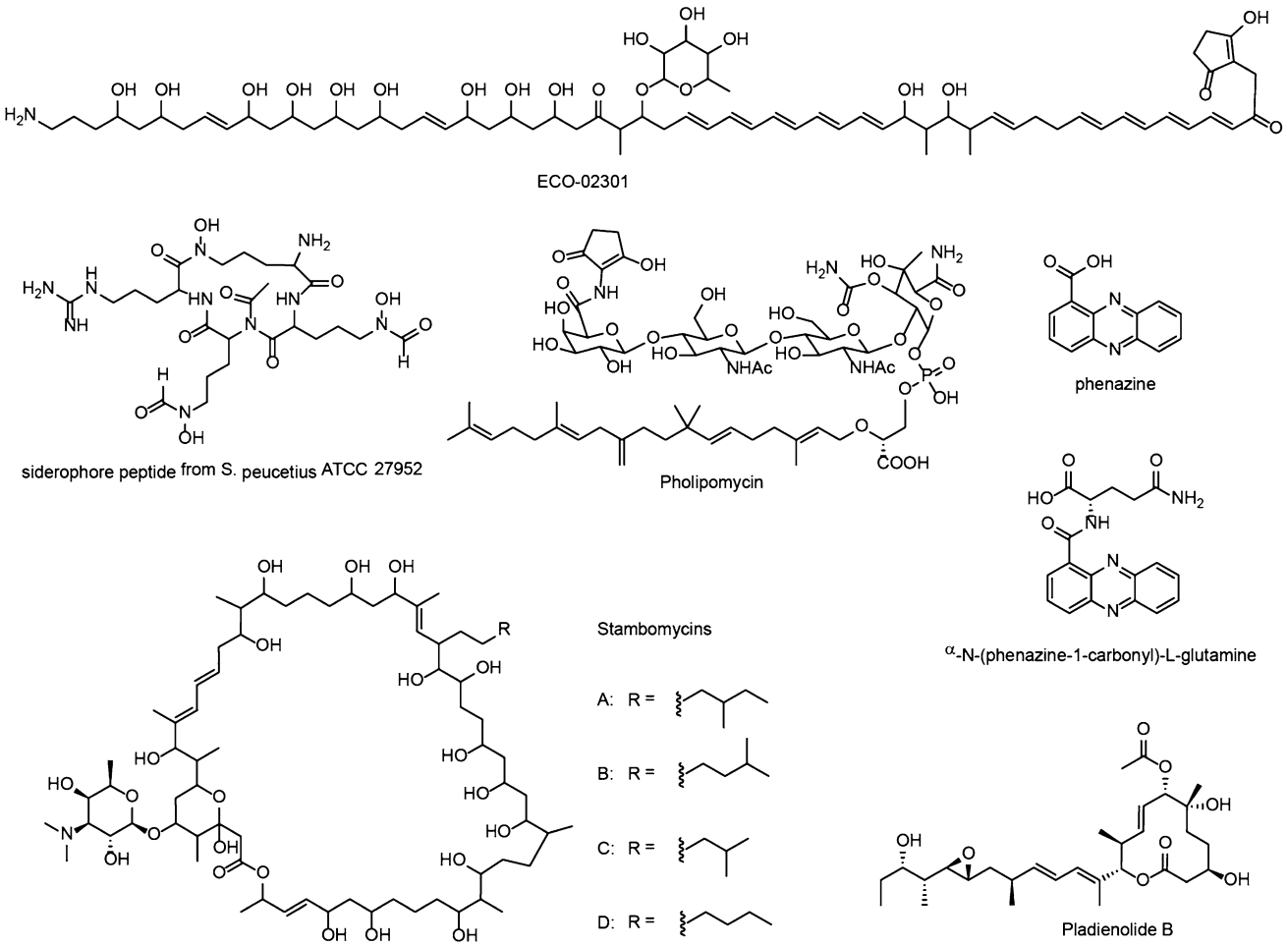


**Fig. 2** Structures of secondary metabolites obtained as a result of different application of strategies for activation of silent clusters in streptomycetes

converting genomic information into secondary metabolites is rapidly growing with the increasing number of sequenced Actinobacteria genomes. MS-guided genome mining strategies work from gene-to-metabolite. These strategies led to the identification of new NRPS siderophore production by *S. peucetius* [83] and PKSI assembled polyketide ECO-02301 by bicyclomycin producer *S. aizunensi* (Fig. 2) [66]. Dorrestein and co-authors used an opposite workflow approach to identify genes encoding peptide natural products from bacteria with sequenced genomes [52, 110]. Their strategy used MS–MS sequencing of peptides, identification of its biosynthetic origin (lantibiotic, cyclic peptide, etc.), followed by identification of the corresponding gene and gene cluster. They were able to identify genes for lantibiotics, lasso-peptides, and NRPs production [62]. A reverse approach allows rapid and fast identification of peptide natural products and corresponding gene clusters. However, reverse procedures (from gene to metabolite) are limited to compounds whose chemical structures are directly determined by gene organization such as lantibiotics, NRPS, and PKSI. Development of in silico tools for predicting secondary metabolite chemical structures from corresponding enzymes will significantly expand the utilization of this approach [10, 130].

Secondary metabolism in bacteria is usually tightly controlled, and the activation of corresponding genes requires certain external and/or internal triggers. Replacement of promoters in biosynthetic or regulatory genes as well as heterologous expression of entire clusters releases these genes from the tight environmental control of the native strain, which can lead to the accumulation of the respective compounds. Expression of biosynthetic genes in heterologous hosts with well-characterized metabolomic profiles allows rapid identification of new compounds. This procedure is also especially useful when working with strains without established gene engineering methodologies. An example of such an approach is the isolation of the new *S. coelicolor* metabolite dimethylallylindole-3-acetonitrile (5-DMAIAN) by expression

of a putative gene cluster containing an indole-prenyltransferase in *S. lividans* (Fig. 1) [80]. The accumulation of the new product was achieved by a simple increase in copy number of the corresponding genes. Pholipomycin biosynthetic genes are usually silent in *S. clavuligerus*. This gene cluster was efficiently expressed in engineered *S. avermitilis* SUKA22 strain [55]. Constitutive *ermEp**-driven expression of the LAL family regulator of the *S. ambofaciens* cryptic PKSI gene cluster led to the accumulation of a new group of glycosylated macrolides stambomycins [60]. Similarly, pladienolide production in *S. avermitilis* was achieved only after the introduction of *pldR* transcriptional regulator gene under control of *ermEp** [56]. The phenazine biosynthetic gene cluster of *S. tendae* was activated by replacement of the natural promoter in the major operon by *ermEp**, resulting in an accumulation of this compound and its derivative α-*N*-(phenazine-1-carbonyl)-L-glutamine by recombinant *S. coelicolor* strain (Fig. 2) [96].

Genome mining approaches require several conditions to efficiently activate silent gene clusters. First, the producing organism should be genetically tractable. This limitation may be overcome by the utilization of a heterologous host. Secondly, the size of many interesting secondary metabolism gene clusters exceeds the current capacities of traditional cloning systems. Thus, shuttle *E. coli-Streptomyces* bacterial artificial chromosomes have been developed as a way to introduce large DNA inserts into *Streptomyces* (for review see [1]). Thirdly, manipulations with gene clusters require that a set of tools for engineering large fragments of DNA be available. Lastly, a pool of transcriptional and translation control elements is required for successful controlled expression of target genes.

## Bio-bricks to build a metabolic factory

Synthetic biology offers new strategies for decrypting actinomycetes secondary metabolism potential [30]. This biological engineering approach suggests breaking the entire biosynthetic cluster into separate blocks in which the native regulatory elements are replaced to allow for fine tuning and coordinated gene expression independent of the host factors. A similar strategy was used in the early 1990s by Khosla and co-authors to study aromatic polyketide biosynthesis [67]. The expression plasmid, pRM5, was constructed to allow simple swapping of the individual genes from the biosynthetic pathway. This system also utilized the first engineered *Streptomyces* host strain *S. coelicolor* CH999, lacking production of actinorhodin and undecylprodigiosin.

Efficient expression of entire biosynthetic gene clusters is often limited by the chosen host strain. High GC content of streptomycetes genes and differences in regulatory elements of gene expression limits utilization of classical microorganisms like *E. coli* or *Saccharomyces cerevisiae* as hosts for secondary metabolism gene expression. In all known cases of antibiotic biosynthetic gene cluster reconstitution in *E. coli*, the respective genes were transcribed from the T7 promoter [11, 86]. Recently, a very interesting attempt at expressing the oxytetracycline gene cluster from *S. rimosus* in *E. coli* was reported by Boddy and co-authors [108]. Overexpression of the *E. coli* alternative sigma factor $\sigma^{54}$ involved in nitrogen assimilation facilitated transcription of the *otc* genes and consequent accumulation of oxytetracycline up to 2 mg/l. However, in many cases streptomycetes are preferable hosts for such experiments. The ideal host should allow for simple and quick genetic manipulations, be suitable for high level production of a diverse group of secondary metabolites, have a high supply of precursors for different metabolic pathways, and have a simple secondary metabolism profile that will allow for rapid identification and purification of produced compounds. The generation of *S. coelicolor* and *S. avermitilis* host strains partially fulfill these requirements [33, 34, 46, 55, 56, 134]. Several other strains used as heterologous expression hosts include *S. lividans TK24* and *S. albus G J1074* (for review see [4]). A recently proposed alternative to these traditional strains is the fast-growing thermophilic streptomycetes. These bacteria are liked because their short cell cycle can lead to higher biomass accumulation as compared to traditional hosts in the same period of growth [18].

### Transcriptional control

Important components of genome-mining approaches are elements providing well-tuned and balanced expression of target genes. This can include constitutive and inducible promoters with different strengths of transcription initiation, an RBS site specifically tuned for individual genes, and strong terminators to prevent generation of unstable transcripts. Features of some of the widely used *Streptomyces* promoters and terminators are summarized in Tables 1 and 2. The promoter of the erythromycin resistance gene from *S. erythreae* is one of the most studied and widely used promoters in *Streptomyces* genetics [8]. The original *ermEp* region contains two different promoters, *ermEp1* and *ermEp2*, and is partially inducible in a native context [9]. Deletion of TGG in the −35 region of the *ermEp1* resulted in a higher initiation rate [9]. This new upregulated variant was named as *ermEp** and has been used in numerous applications, including multiple cases of silent gene cluster activation.

Another strong constitutive promoter *SF14p* consisting of two tandemly arranged promoters was cloned from *S. ghanaensis* bacteriophage I19 [58]. It was demonstrated

**Table 1** *Streptomyces* promoters used in expression vectors

| Promoter | Description | References |
|---|---|---|
| *ermEp* | Strong, constitutive promoter from erythromycin resistance gene, partially inducible in *S. erythreae* | [8] |
| *ermEp** | Modification of *ermEp* with increased activity. Strong, constitutive | [9] |
| *SF14p* | Strong constitutive promoter from *S. ghanaensis* bacteriophage. Two fold more active than *ermEp** | [58] |
| *actIp* | Promoter of major actinorhodin biosynthesis operon of *S. coelicolor*. Strong, temporally controlled. Activity of *actIp* requires actII-ORF4 gene product | [67, 126] |
| *kasOp** | Modified promoter of *cpkO* (*kasO*) gene from *S. coelicolor* coelimycin P1 gene cluster. Strong, constitutive. Shown to be more active than *ermEp** and *SF14p* | [122] |
| *A1p-D4p* | Library of synthetic promoters with consensus −10 and −35 sequences recognized by HrdB sigma polymerase. Constitutive promoters of different strengths are available. Activity of the strongest variant does not exceed the activity of *ermEp** | [100] |
| *P21p* | Library of synthetic promoters based on −10 and −35 sequences of *ermEp1*. Constitutive promoters of different strengths are available. Activity of the strongest variant P21 exceeds the activity of *ermEp** by 1.6 fold | [105] |
| *tipAp* | Strong, thiostrepton-inducible promoter from *S. coelicolor*. Induction range up to 200 fold. Requires TipA protein for activation. Leaky | [57] |
| *tcp830p* | Strong, tetracycline-inducible promoters combining *ermEp1* sequence and *tet* operators from *E. coli* Tn5. Induction range up to 270 fold in the case of *tcp830p*. Requires TipA protein for activation. Leaky | [91] |
| *T7p* | T7p/T7 RNA polymerase system adopted from *E. coli*. Strong, thiostrepton inducible. Large range of induction, suitable for transcription of long DNA fragments. Tightly controlled, not leaky. Strain construction required | [63] |
| *nitAp* | Nitrilase gene promoter from *Rhodococcus rhodochrous.* Strong, inducible with ε-caprolactam. Requires repressor gene *nitR*. Tightly controlled and expression level strictly depends on inducer concentration | [39] |
| *gylP* | Glycerol-inducible promoter from *S. coelicolor*. Requires *gylR* regulator for activity | [42] |
| *TREp* | Temperature-induced promoter from *S. nigrifaciens* plasmid pSN22. Requires *traR* gene for activity. Induced by incubation at 37 °C | [51] |

to be twofold more active than *ermEp**, however it has not found much application in streptomycetes genetics. Ikeda and co-authors successfully used the promoter of the *S. avermitilis rpsJ* (*sav4925*) gene, which encodes ribosomal protein S10, to express several regulatory genes and entire biosynthetic gene clusters in heterologous hosts [46, 55, 56]. Utilization of housekeeping gene promoters from the host bacteria may improve expression of the target gene since some of them (e.g., those encoding ribosomal proteins, RNA polymerase, etc.) are usually expressed at very high levels. However, activity of these promoters may be susceptible to changes in growth conditions and other internal and external factors. Studies on actinorhodin biosynthesis regulation led to a deep understanding of factors influencing the activity of *act* promoters. Based on this data, several *actII-ORF4/actIp*-containing vectors were developed for gene expression in different actinomycetes [126]. However, this system was found to be efficient only in *S. coelicolor* but not in other strains. Replacement of the native promoter of *actII-ORF4*gene with *ermEp** resulted in much more efficient expression of the erythromycin PKS module DEBS1-TE and accumulation of the respective triketide lactone in multiple hosts. Several other native promoters were tested to drive transcription of target genes in different *Streptomyces* species, however, none of them have been used extensively [93, 98].

As an alternative to native streptomycetes promoters, synthetic promoters or promoter systems from other bacteria could be used. In such cases, transcription of the target gene is less dependent on host factors. The first fully synthetic promoter for use in streptomycetes was based on the consensus sequences of the *Streptomyces–E. coli* like promoter, however it was found to be inefficient [98]. The first synthetic promoter combining features of consensus promoter sequences from both *E. coli* and *Streptomyces* was constructed by Denis and Brzezinski and is active in both bacteria [25]. Recently, several reports of generating synthetic *Streptomyces* promoters have been published. Yang et al. reported consequent modification of a *cpkO* (*kasO*) gene promoter encoding an SARP-type transcriptional regulator from the coelimycin P1 cluster of *S. coelicolor* [122]. Expression of this gene is known to be negatively regulated by two γ-butyrolactone receptor proteins ScbR and ScbR2 [129]. Sequential truncations of the 400-bp region containing the core sequence of the promoter resulted in the generation of a *cpkOp* variant whose expression is not dependent on ScbR2. Further randomizing of the ScbR binding region led to the generation of a constitutive promoter, *kasOp**. Activity analysis of this synthetic promoter showed that it is stronger than *ermEp** and *SF14p*.

Virolle and co-authors generated a set of 38 synthetic promoters of different strengths by randomizing the spacer

region between the −10 and −35 consensus sequences of *Streptomyces* promoters, which are recognized by the major vegetative sigma factor HrdB [100]. The obtained promoters were grouped into three categories: weak, medium, and strong. Though these promoters were active in several *Streptomyces* species, even the strongest of them did not exceed the level of activity of *ermEp**.

A similar approach was used in our laboratory to generate a library of *Streptomyces* synthetic promoters based on the −10 and −35 sequences of *ermEp1* [105]. The library of 56 synthetic promoters has activity ranging from 2 to 319 % compared to *ermEp1* with the strongest promoter, *P21p*, exceeding the *ermEp** activity up to 1.6 fold. *P21p* was successfully used to overexpress the chalcone synthase *rppA* gene in *S. albus* resulting in higher yields of flaviolin accumulation compared to a construct with *ermEp**. Three promoters of different strengths were shown to be active in multiple Actinobacteria strains. We predict that further screening of randomized promoters will expand our current library. This randomization strategy could also be applied to generate promoter libraries with a broad range of activity using promoter sequences from highly expressed genes as the starting base. Furthermore, described experiments provide the first insights into features influencing the strength of *Streptomyces* promoters. This information can aid the rational design of synthetic promoters with desired characteristics. Well-characterized promoters of different strengths will allow for the controlled expression of different genes or operons to enable preservation of native enzyme stoichiometry in secondary metabolism biosynthetic pathways.

Several inducible promoter systems are also utilized in *Streptomyces* genetics. The *tipAp* promoter was cloned from *S. lividans* after observation of its ability to boost transcription of respective gene *tipA* in the presence of the antibiotic thiostrepton [71]. *tipAp* is a strong promoter that is induced upon addition of thiostrepton, up to 200 fold in *S. lividans* and at least 60–80 fold when expressed in other strains [57, 98]. *tipAp* was used to construct a range of vectors successfully utilized for gene expression in streptomycetes [113, 126]. However, the *tipAL* gene encoding the positive regulator of *tipAp* is not present in all streptomycetes [132]. To avoid this problem, the *tipA* gene was placed together with *tipAp* on plasmid vectors [2]. The drawbacks to the *tipAp* promoter system are that the presence of the thiostrepton resistance gene is necessary in many cases to protect host cells against toxicity from the inducer compound and that *tipAp* is a leaky promoter that cannot be fully repressed due to the nature of its regulation [19]. Despite these disadvantages, *tipAp* remains the most used inducible promoter in *Streptomyces* genetics.

Smith and co-authors reported construction of a set of synthetic inducible promoters based on *ermEp1* and *tetO1* and *tetO2* operator regions from the *E. coli* transposon Tn*10*, which are responsible for binding of the TetR repressor protein [91]. The *tetR* repressor gene variant (tTA-2) developed for use in higher eukaryotes was further adapted for expression in streptomycetes and showed efficient repression and induction in the tested system. The anhydrotetracycline inducer has an insignificant effect on a host's genes expression. One promoter, *tcp830p*, showed the largest change in transcription levels between uninduced and induced states and was among the strongest promoters tested in this work. This system is functional in several commonly used streptomycetes strains and was successfully used to express the novobiocin biosynthetic gene cluster in *S. coelicolor* [22]. The main advantages of this system are the use of non-toxic anhydrotetracycline and tighter induction control compared to *tipAp*. However, *tcp* promoters are not fully repressed, and some level of transcription still can be observed even without the inducer.

Shareck and co-authors generated a T7 expression system suitable for streptomycetes by cloning a TTA-less T7-DNA polymerase gene under control of *tipAp* in *S. lividans* and constructing a complimentary T7 promoter expression plasmid [63]. The system was shown to tightly control transcription and have a great induction range. However, the level of expression is generally lower than available constitutive promoters. Additionally, this system requires construction of an expression host by introducing the T7-polymerase expression plasmid first. Furthermore, the use of the *tipA* promoter that drives the expression of the T7-polymerase creates the same limitations that are found in the *tipAp* system.

The *snpAp* promoter of the metalloproteinase gene from *Streptomyces* sp. strain C5 whose transcription is controlled by the SnpR transcriptional regulator was used to construct expression vectors [26, 76]. Expression from this promoter does not require adding an inducer. Rather, its expression is modulated by physiological changes of the host strain during transition into the stationary phase of growth. The *nitAp* promoter of the nitrilase gene and the corresponding regulator NitR of actinomycetes *Rhodococcus rhodochrous* are widely used for gene expression in streptomycetes [39]. Expression from *nitAp* is induced by ε-caprolactam and is highly dose-dependent. This is extremely useful for fine-tuning of target gene expression. *nitAp/nitR*-based vectors are used mostly for protein overproduction in streptomycetes. *S. coelicolor* possesses a glycerol-inducible system consisting of a *gylR* regulator gene and a *gylP1/P2* glycerol-inducible promoter [42]. However, utilization of this system is limited because the addition of glycerol to the media may affect the production of secondary metabolites. Yoshida and co-authors developed a temperature-inducible expression system by mutating the *traR/TREp* region from *S. nigrifaciens* plasmid pSN22 [51]. Growth of *S. lividans*

at elevated temperatures induces transcription. This system is tightly controlled, unfortunately, however, not all *Streptomyces* species can be successfully cultivated at such conditions.

Several other constitutive and inducible streptomycetes promoters are well studied and some of them are used for gene expression. These include the galactose-inducible, *galP1p*, and constitutive, *galP2p*, promoters from the galactose operon of *S. lividans* [29], the metalloendopeptidase (SSMP) gene promoter from *S. cinnamoneus* TH-2 [38], the principal sigma factor *hrdBp* gene promoter of *S. coelicolor* [27], the cellobiose-inducible system from plant pathogenic *S. scabies* [50], the γ-butyrolactone receptor system from *S. coelicolor* [112], and the mitomycin C-inducible promoter of mitomicin resistance locus *mrc* of *S. lavendulae* [3]. Among all tested promoters, the *hrdBp* was shown to be the strongest, exceeding the activity of *ermEp** and others in practical applications [27]. In summary, a set of well-characterized promoters with different strengths for gene expression in streptomycetes are available. If strong and constitutive expression is required, promoters of major housekeeping genes like those encoding ribosomal proteins or RNA polymerase components or well-studied promoters from secondary metabolism genes would be a preferable choice. However, if inducible gene expression or tuned expression of several independent genes or operons is desired, several promoters are available, but all have limitations. The streptomycetes community would greatly benefit from enhanced tools for tuned expression of genes. There exists a need for a broader library of promoters with different transcription initiation rates and a need for tightly controlled inducible systems. Synthetic biology approaches may expand the number of well-characterized native and synthetic promoters with desired features for use in streptomycetes gene expression.

Other important components of transcriptional regulation systems are terminators. Efficient termination of transcripts influences their stability (for review see [90]). The use of terminators in cloning vectors ensures controlled expression of the target gene from the desired promoter and prevents their expression from promoters of vector components. Several *rho*-independent terminators have been characterized and used in streptomycetes (Table 2). Typically, *rho*-independent terminators consist of a GC-rich hairpin loop followed by a T-rich stretch [97]. This structure is important for both efficient termination of transcription and mRNA stability. Cloning of the *S. fradiae aph* gene terminator downstream of the human interferon gene significantly improved production of this protein in *S. lividans* presumably by preventing generation of long unstable transcripts [87]. This terminator was found to provide 90 % termination efficiency. Terminators $t_{a,b,c}$ from øC31 phage were also tested and showed up to 75 % termination efficiency for $t_a$ [47]. Interestingly, both the $t_{aph}$ terminator and the $t_{a,b,c}$ terminators lack the T-rich stretch. A bidirectional terminator was cloned from *Streptomyces* plasmid pIJ101 and shown to be active both in *E. coli* and *S. lividans* [24]. Several terminators from other organisms have also been used successfully in *Streptomyces* expression systems. The most commonly used are the major terminator of *E. coli* fd phage [123] and $t_0$ terminator of phage λ [99]. The $t_{fd}$ terminator is a bidirectional terminator used in a set of reporter and expression vectors. S1 nuclease mapping experiments demonstrated its high efficiency in terminating transcription ranging from 97 to 99 % [123]. $t_0$ terminator is the end of a short λ phage antisense RNA (*oop* RNA), which is initiated at promoter $p_0$ and was described as one of the most efficient terminators [68]. *TD1* terminator from *B. subtilis* bacteriophage ø29 was shown to terminate transcription in *S. lividans* with 80–85 % efficiency [88]. The high efficiency of preventing transcription by studied heterologous terminators suggests that most of the *rho*-independent transcription terminators would be active in streptomycetes. Furthermore, highly efficient transcription termination of a target gene may be achieved by using a combination of a several terminators. The availability of genomic information made possible the global identification and analysis of 3′-untranslated region from different bacteria and provided tools for generation of terminator sequences with specific features [69, 74].

## Translational control

A high rate of gene expression requires efficient translation and translation initiation. Translation initiation depends on numerous factors, among which the appropriate start codon usage and the ribosome binding site (RBS) are the most important. The implication of rare codons in regulation of translation and gene expression in streptomycetes is a well-known fact (for review see [16]). Many *Streptomyces* genes

**Table 2** Terminators used in *Streptomyces* gene expression

| Terminator | Description | References |
|---|---|---|
| $t_{fd}$ | *E. coli* phage fd terminator. Provides high-efficiency transcriptional termination (97–99 %). Bidirectional | [123] |
| $t_0$ | *E. coli* phage λ terminator. Provides high-efficiency transcriptional termination (93–98 %). Unidirectional | [68, 99] |
| $t_{aph}$ | *S. fradiae aph* gene terminator. Provides high-efficiency transcriptional termination (90 %) | [87] |

utilize alternative start codons GTG (32 %) and TTG (3 %) instead of ATG. Recently, we demonstrated that among all possible start codons, TTG is the most efficient in initiating translation of *gusA* β-glucuronidase reporter gene in *S. albus* whereas ATG and GTG showed a 2–3 times lower level of translation initiation [72]. CTG was the least efficient and expressed only 5 % of activity compared to the TTG variant.

The RBS is usually a sequence upstream from the start codon complementary to the 3′ region of 16S rRNA. Analysis of a large number of *Streptomyces* genes showed that often the Shine-Dalgarno (SD) sequence ranged from 5 to 12 bp from the translational start codon [109]. However, the analysis also showed that many streptomycetes genes do not contain a 5′-untranslated region (5′UTR). Rather transcription initiates directly at the translation start point [119]. Manipulations with RBSs and their numbers can significantly affect efficiency of gene expression in streptomycetes [81]. Several natural and synthetic RBSs have been used in streptomycetes expression vectors. Human interferon was successfully expressed in *S. lividans* using the RBS of the *E. coli* lipoprotein encoding gene [89]. A synthetic RBS complementary to *B. subtilis* 16S rRNA was used to express the streptothricin acetyltransferase gene from *S. lavendulae* in both *B. subtilis* and *S. lividans* [44]. A set of vectors based on the *actII-ORF4/actIp* system containing an *actI* RBS site upstream from the MCS were used to overexpress erythromycin biosynthesis genes [93]. The ribosome binding site of the *tipA* gene is used in many *tipAp*-based vectors [113]. Two different synthetic RBSs (RBSa and RBSb) were utilized in a set of integration expression vectors with *ermEp** promoter [70]. Both RBSs include the consensus hexamer AGGAGG, in two different sequence contexts. RBS-a was derived from the highly expressed *S. ramocissimus* gene encoding elongation factor EF-Tu whereas RBS-b is adapted from the consensus RBS sequence for *E. coli*. In most cases, however, the native RBS and native transcriptional terminator of a target gene are used in expression constructs.

It is well known that elements of a translation initiation region (TIR) other than SD also affect gene expression. The nucleotides directly upstream and downstream of the start codon as well as the distance of a SD sequence from the start codon have been shown to influence the translation initiation rate in bacteria [32]. This means that any manipulation of the native promoter sequence and coding region of the target gene will affect the translation initiation efficiency. Therefore, design of a new RBS is often required. This could be done experimentally by cloning a library of randomly synthesized RBS sequences between the promoter of choice and the first few nucleotides of the target gene. This construct when fused to a reporter gene will allow selection a vast array of RBSs with different rates of translation initiation of a particular target gene. This strategy was used to study features of the *E. coli* RBSs and the collected data was used in generating RBS prediction software such as the RBS Calculator [32], RBS Designer [73], or UTR Designer [102]. RBSs with desired translation initiation efficiency for each particular gene can be predicted by using this software, however, due to general biology differences between *E. coli* and *Streptomyces*, the designed RBSs should be still verified experimentally using reporter genes.

RNA silencing is another type of translational control that is becoming widely used to regulate gene expression in prokaryotes (for review see [92]). It is well documented that bacteria use small non-coding RNA (ncRNA) to regulate diverse cellular processes. Silencing ncRNA act by binding to the target mRNA, which inhibits ribosome access or promotes RNA degradation. A combination of RNA sequencing and bioinformatics led to the identification of putative small RNAs in the genomes of *S. coelicolor* [119]. Stach and co-authors recently reported the use of synthetic RNA silencing to regulate actinorhodin production in *S. coelicolor* [117]. Peptide nucleic acid (PNA), a DNA with non-natural linkages between bases, fused to a cell wall permeating peptide complementary to the *actI-ORF1* transcript was found to nearly abolish actinorhodin production when supplemented to the media. The use of PNA-peptide prevents degradation by the host cell enzymes and allows simple introduction of silencing molecules into the cell without genetic manipulations. This approach is particularly interesting for use in streptomycetes, since genetic transformation is not required. However, due to the high cost of synthesis, PNA utilization is limited. In another approach, a 155-bp fragment complementary to the 5′UTR of *actI-ORF1* was expressed in *S. coelicolor* from *nitAp* and *tipAp* resulting in the production of *trans*-acting antisense RNA and a consequent decrease in antibiotic accumulation in the strain. This approach requires genetic manipulations with the strain so it cannot be applied to the many species lacking protocols for genetic transformation. However, it is still a useful tool for studying lethal gene function or for controlling toxic proteins or secondary metabolite production.

Another approach involving small RNA to control gene expression in streptomycetes was developed by Suess and co-authors [95]. They tested the ability of six different synthetic theophylline-dependent riboswitches to silence the expression of the *gusA* reporter gene from *ermEp1*. A typical riboswitch consists of an aptamer region that binds an inducer molecule and an expressional part that modulates the expression of a target gene [13]. Supplementing the host strain with the inducer leads to conformational changes of the RNA which allows ribosomes to access the RBS. In *S. coelicolor* harboring the respective constructs, the addition

of theophilin to the growth media induced expression of the *gusA* transcript. The maximum level of induction achieved by one of the riboswitches resulted in a 32-fold increase in reporter activity compared to the uninduced culture. However, in all cases, the full induction of *gusA* expression was not achieved. Riboswitches were found to be compatible with three different promoters tested, showed strictly dose-dependent induction of target gene expression, and good induction-repression kinetics. Thus riboswitches are alternatives to inducible promoter systems. The disadvantages of this system are that in high concentrations theophyline is toxic to *Streptomyces* cells and full repression of gene expression was also not observed with any construct. Despite these problems, this approach represents an interesting tool for controlling *Streptomyces* gene expression on a translational level.

Further development of new genetic tools as well as their adaptation from other bacteria will lead to a large number of available transcriptional and translational control elements for *Streptomycetes* genetics. Implementation of such technologies like aptamers, synthetic riboswitches, and self-processing ribozymes will further expand the number of expression control elements for use in streptomycetes.

## Tools to build a metabolic factory

Most secondary metabolism gene clusters span large regions of the chromosome (a typical type II PKS gene cluster is around 35 kb in size). Manipulations with large fragments of DNA require utilization of specific tools and non-traditional approaches for cloning, assembly, rearrangement, and delivery into the expression hosts. Shuttle BAC-based genomic libraries could be a partial solution. However, even in such cases, additional assembly of large fragments of DNA from several clones might be required. Implementation of cloning methods with the use of restriction endonucleases for modification of secondary metabolites gene clusters is almost impossible. The development of recombination cloning methods overcame the difficulties of manipulating large fragments of DNA. The use of a homologous recombination approach to assemble two overlapping fragments of the *red* cluster in *S. coelicolor* was first reported by Hopwood and co-authors [65]. Discovery of Red/ET cloning based on λ phage RedA/RedB recombinase became a breakthrough technology in gene engineering [23, 133]. With further adaptation to streptomycetes applications it became an indispensable tool for engineering secondary metabolism gene clusters [37]. Recently, this technique was successfully applied to assemble two clones containing the myxochromide S biosynthetic gene cluster from *Stigmatella aurantiaca* [125]. Several other approaches for large DNA fragment assembly exist and are reviewed elsewhere [135]. Combined with long-range amplification PCR, these methods allow direct cloning of entire gene clusters from streptomycetes. Homologous recombination in yeast was used to clone and express the aureothin and spectinabilin biosynthesis gene clusters from *S. thioluteus* and *S. spectabilis* in yeast [103]. These methods are far superior to the time-consuming and expensive construction and screening of gene libraries. The big advantage of this technique is that site-directed modifications of biosynthetic genes can be generated by simply introducing desired mutations into the corresponding primers. With second-generation sequencing technologies allowing fast verification of assembled constructs, this approach will become a powerful tool for rapid cloning and modification of secondary metabolite gene clusters.

The Red/ET system is often used to further modify gene clusters that were assembled or cloned in conventional ways. However, in such cases, resistance markers are usually introduced that may cause a polar effect on downstream gene expression. The number of resistance markers that can be efficiently used in streptomycetes is limited. Thus, the generation of multiple genetic mutations in the same cosmid or the same strain is also restricted. Site-specific recombination has become an indispensable tool for marker re-utilization. Several recombinases have been successfully adopted for use in *Streptomyces*. The *Cre/loxP* system has been used for marker excision in different actinomycetes with efficiencies ranging from 60 to 100 % [28, 53]. Recently, two other site-specific recombinases *Flp* and *Dre* have been adopted for use in actinomycetes [28, 40]. These systems have been used in different applications ranging from simple marker removal to more complex genome minimization projects. Several markerless expression vectors have been developed using Cre and Dre recombinases as well.

With the significant cost decrease in genome sequencing and the development of rapid screening approaches, conventional mutagenesis techniques acquired a new life. UV and chemical mutagenesis can be used as ways to increase the production of known metabolites as well as to awaken silent gene clusters. Combined with whole-genome sequencing, it will provide new information about the regulation of secondary metabolism in streptomycetes. Development of reporter systems with simple and reliable methods of transcriptional and translational activity detection will facilitate the design of screening strategies for activation of silent clusters. This can be achieved by transcriptional or translational fusion of the target gene promoter to the reporter. A similar strategy was used in the reporter guided screen for daptomycin overproducing strains [121]. The daptomycin biosynthetic gene *dptE* promoter was fused to a *neo* reporter gene and strains harboring this construct were mutagenized. Activation of the *dptE* promoter produced colonies that were able to grow on

high concentrations of neomycin and 20 % of them were characterized by increased antibiotic production. Reporter genes can also be used for direct detection of metabolite accumulation. A large number of secondary metabolism gene clusters contain *tetR* family regulator and transporter gene pairs. The regulatory protein controls expression of the transporter gene in response to the accumulation of the produced compound. Binding of the compound to TetR releases this repressor from the transport gene promoter resulting in active expression of the reporter gene. Using the appropriate reporter, the system can be tuned to detect the compound quantitatively. This approach was used in the generation of a biosensor system for detection of new landomycins by cloning the *lanK* regulatory gene and fusing the *lanJ* gene target promoter to a *neo* reporter [79].

Several reporter systems have been successfully used in streptomycetes genetics (Table 3). The *E. coli lacZ* gene encoding β-galactosidase is one of the most widely used reporters. However, this gene cannot be used in *Streptomyces* because most strains possess extracellular enzymes with β-galactosidase activity which interferes with reporter detection. Special mutant strains or growth conditions are required for utilization of *lacZ* [54]. Two reporter systems based on either neomycin/kanamycin resistance (*neo* encoding aminoglycoside phosphotransferase) or chloramphenicol resistance (*cat* encoding chloramphenicol acetyltransferase) have been widely used for cloning and characterization of streptomycetes promoters [7, 123]. In vitro assays exist for simple detection of Cat and Neo activity, however, direct in vivo quantification is limited.

Several vectors utilizing enhanced GFP variants have been constructed and successfully used to study the subcellular localization of proteins in *Streptomyces* [111]. However, eGFP utilization is limited due to the high levels of auto-fluorescence of mycelia as well as the low applicability for quantitative analysis [45]. To overcome at least one of these two issues, an *S. coelicolor* strain with decreased levels of autofluorescence was selected and used in several studies [127]. Recently, monomeric red fluorescent protein (mRFP1) was reported for detection of protein localization in streptomycetes [75]. This system is compatible with eGFP providing a useful tool for simultaneous detection of two proteins. However, it has the same limitations as the eGFP system.

Luciferase assays provide rapid and sensitive methods of gene expression analysis due to the simplicity of light emission detection and its linearity in a long range of measurements. Furthermore, streptomycetes typically do not show any autoluminescence resulting in a low signal-to-noise ratio. The activity of luciferase can be easily measured quantitatively in 96-well-plate formats and on solid media. Several different luciferase systems have been applied in

**Table 3** Reporter genes used in *Streptomyces* research

| Reporter | Description | References |
|---|---|---|
| *lacZ* | *E. coli* β-galactosidase. Low activity in streptomycetes, requires dedicated host strains. Activity can be detected in vivo and in vitro | [54] |
| *neo* | Aminoglycoside phosphotransferase from Tn5. Neomycin/kanamycin resistance. Activity can be detected in vivo and in vitro. Limited use for in vivo quantification | [123] |
| *cat* | Chloramphenicol acetyltransferase from Tn9. Chloramphenicol resistance. Activity can be detected in vivo and in vitro. Limited use for in vivo quantification | [7] |
| *eGFP* | Enhanced Green Fluorescent Protein from jelly fish *Aequorea Victoria*. Fluorescence emission peak at 509 nm in green visible spectra. Can be detected in vivo and in vitro. In vivo quantification is not possible | [111, 127] |
| *mRFP1* | Monomeric Red Fluorescent Protein. Fluorescence emission peak at 607 nm in green visible spectra. Can be detected in vivo and in vitro. In vivo quantification is not possible | [14, 75] |
| *LuxAB* | Luciferase from *Vibrio harveyii.* Chemiluminescent detection. Requires supply of substrate *n*-aldehyde decanal. Can be detected in vivo and in vitro. Allows in vivo quantification in 96-well plates and to some extent on solid media | [106] |
| *luxCDABE* | Luciferase and substrate biosynthesis operon from *Photorhabdus luminescens.* Chemiluminescent detection. Does not require external substrate supply. Can be detected in vivo and in vitro. Allows in vivo quantification in 96-well plates and to some extent on solid media | [21] |
| *melC* | Tyrosinase from *S. glaucescens.* Chromogenic detection. Requires external supply of substrate tyrosine. Can be detected in vivo and in vitro. Not suitable for quantitative analysis | [82] |
| *xylE* | Catechol 2,3-dioxygenase from *Pseudomonas putida.* Chromogenic detection. Requires supply of substrate catechol. Can be detected in vivo and in vitro. Suitable for quantitative analysis in liquid and solid media | [48] |
| *rppA* | Chalcone synthases from *S. erythraea*. Chromogenic detection. Does not require external substrate supply. Can be detected in vivo and in vitro. Suitable for quantitative analysis in liquid and solid media | [64] |
| *gusA* | β-Glucuronidase from *E. coli.* Requires substrate supply. Different glucuronide derivatives are available allowing chromogenic, spectrophotometric, fluorimetric, and chemiluminescent detection. Can be detected in vivo and in vitro. Suitable for quantitative analysis in liquid and solid media | [72] |

streptomycetes research including *luxAB* from *Vibrio harveyii* and firefly *luc*-gene [85, 106]. However, the use of the original *luxAB* operon is limited due to high AT codon usage and a complex enzymatic reaction that requires multiple substrates for activity detection. The *luxAB* operon lacking TTA codons was constructed to overcome one of these issues [91, 124]. Firefly *luc* encoded luciferase also strictly depends on the externally supplied substrate luciferin. Recently, a synthetic *luxCDABE* operon of the bioluminescent bacterium *Photorhabdus luminescens* has been reported to be used as a transcriptional reporter for streptomycetes [21]. It encodes a luciferase (LuxA and LuxB) and substrate producing enzymes (LuxC, LuxD, and LuxE) such that cells expressing the operon emit light spontaneously. There is no need to externally supply substrates, which makes this *luxCDABE* system more reliable for quantitative analysis.

The *melC* operon of *S. glaucescens* is the most used chromogenic reporter for gene expression analysis in streptomycetes [82]. It consists of two ORFs: *melC1*, responsible for transport of copper to tyrosinase, and *melC2*—encoding tyrosinase. MelC2 is a monooxygenase that oxidizes L-tyrosine to form the black pigment melanin. Several reporter vectors based on this system have been constructed. *melC* is well suited for the selection of mutants due to chromogenic activity detection that allows for simple screening of a large number of colonies. However, it cannot be used for quantitative analysis because tyrosinase is secreted from the cells.

The catechol 2,3-dioxygenase *xylE* gene of *Pseudomonas putida* has been used successfully as an alternative chromogenic reporter in *Streptomyces* [48]. The simplicity of detection based on conversion of colorless catechol to the yellow compound 2-hydroxy muconic semialdehyde and the absence of *xylE* in the majority of organisms has led to wide utilization of this reporter. In addition, *xylE* activity in cell extracts can be measured spectrophotometrically. Multiple reporter vectors have been created utilizing *xylE* that allow simple and rapid identification of mutant colonies during growth on agar media as well as further quantitative analysis in vitro [20, 101].

Recently, Petković and co-authors reported characterization of chalcone synthase gene *rppA* from *S. erythraea* as a reporter for gene expression analysis [64]. RppA activity leads to accumulation of the dark-red compound flaviolin. RppA does not require external substrate addition, is simple in detection, flaviolin is not toxic to the host strains, and is stable for a long time, allowing measurements to be performed after collection of sample in time-point experiments. However, many *Streptomyces* strains may produce compounds with similar spectral characteristics interfering with flaviolin detection. Broad starter substrate specificity of RppA may cause variations in absorption spectra as well as the availability of malonyl-CoA in different strains and under different growth conditions might influence the yield of compound production. The latter could be used advantageously to probe substrate pools in different strains selected for heterologous expression of PKS genes.

Luzhetskyy and co-authors reported the adaptation of the *gusA* gene encoding β-glucuronidase from *E. coli* for expression in streptomycetes [72]. Heterologous genes as reporters are always preferable since most actinomycetes strains lack β-glucuronidase activity. The *gusA* system was found to be extremely sensitive and simple in both in vivo and in vitro detection. The availability of different glucuronide derivatives including X-gluc, MUG, and *p*-nitrophenyl-β-D-glucuronide makes visual, spectrophotometric, fluorimetric, and chemiluminescent detection of GusA activity possible. Additionally, several variants of *gusA* with different start codons have been produced that allow tuning of translational expression of the reporter gene. When used in a low copy number or an integrative vector, *gusA* did not show high variability of expression from the same promoter in different colonies.

Several other native and heterologous genes have been utilized as reporter systems in streptomycetes as well, including the *redD* regulator for undecylprodigiosin production [118], the *whiE* spore pigment operon [43], and the thermostable malate dehydrogenase of *Thermus flavus* [120]. However, all of them have numerous limitations for usage or are dedicated to specific host strains. In summary, the large number and diversity of reporter systems available provide a large choice of tools for studying different aspects of gene expression in streptomycetes, including activation of silent secondary metabolism gene clusters. These systems, combined with other synthetic biology tools and expression systems, represent a powerful pool of instruments for genetic manipulations in this prominent group of bacteria.

**Acknowledgments** The work in the AL group was supported through funding from the ERC starting grant EXPLOGEN No. 281623 and the DFG grant (Lu1524/2-1). We thank Dr. Stephanie Brown (JIC, Norwich, UK) for help in manuscript preparation.

## References

1. Alduina R, Gallo G (2012) Artificial chromosomes to explore and to exploit biosynthetic capabilities of actinomycetes. J Biomed Biotechnol 2012:462049
2. Ali N, Herron PR, Evans MC, Dyson PJ (2002) Osmotic regulation of the *Streptomyces lividans* thiostrepton-inducible promoter, ptipA. Microbiology 148:381–390
3. August PR, Rahn JA, Flickinger MC, Sherman DH (1996) Inducible synthesis of the mitomycin C resistance gene product (MCRA) from *Streptomyces lavendulae*. Gene 175:261–267
4. Baltz RH (2010) *Streptomyces* and *Saccharopolyspora* hosts for heterologous expression of secondary metabolite gene clusters. J Ind Microbiol Biotechnol 37:759–772

5. Barona-Gomez F, Wong U, Giannakopulos AE, Derrick PJ, Challis GL (2004) Identification of a cluster of genes that directs desferrioxamine biosynthesis in *Streptomyces coelicolor* M145. J Am Chem Soc 126:16282–16283
6. Bentley SD, Chater KF, Cerdeno-Tarraga 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
7. Bibb MJ, Cohen SN (1982) Gene expression in *Streptomyces*: construction and application of promoter-probe plasmid vectors in *Streptomyces lividans*. Mol Gen Genet 187:265–277
8. Bibb MJ, Janssen GR, Ward JM (1985) Cloning and analysis of the promoter region of the erythromycin resistance gene (*ermE*) of *Streptomyces erythraeus*. Gene 38:215–226
9. Bibb MJ, White J, Ward JM, Janssen GR (1994) The mRNA for the 23S rRNA methylase encoded by the *ermE* gene of *Saccharopolyspora erythraea* is translated in the absence of a conventional ribosome-binding site. Mol Microbiol 14:533–545
10. Blin K, Medema MH, Kazempour D, Fischbach MA, Breitling R, Takano E, Weber T (2013) antiSMASH 2.0—a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res 41:204–212
11. Boddy CN, Hotta K, Tse ML, Watts RE, Khosla C (2004) Precursor-directed biosynthesis of epothilone in *Escherichia coli*. J Am Chem Soc 126:7436–7437
12. Bode HB, Bethe B, Hofs R, Zeeck A (2002) Big effects from small changes: possible ways to explore nature's chemical diversity. ChemBioChem 3:619–627
13. Breaker RR (2012) Riboswitches and the RNA world. Cold Spring Harb Perspect Biol. doi:10.1101/cshperspect.a003566
14. Campbell RE, Tour O, Palmer AE, Steinbach PA, Baird GS, Zacharias DA, Tsien RY (2002) A monomeric red fluorescent protein. Proc Natl Acad Sci USA 99:7877–7882
15. Challis GL, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. Proc Natl Acad Sci USA 100:14555–14561
16. Chater KF, Chandra G (2008) The use of the rare UUA codon to define "expression space" for genes involved in secondary metabolism, development and environmental adaptation in *Streptomyces*. J Microbiol 46:1–11
17. Chen L, Chen J, Jiang Y, Zhang W, Jiang W, Lu Y (2009) Transcriptomics analyses reveal global roles of the regulator AveI in *Streptomyces avermitilis*. FEMS Microbiol Lett 298:199–207
18. Chen W, Qin Z (2011) Development of a gene cloning system in a fast-growing and moderately thermophilic *Streptomyces* species and heterologous expression of *Streptomyces* antibiotic biosynthetic gene clusters. BMC Microbiol 11:243. doi:10.1186/1471-2180-11-243
19. Chiu ML, Folcher M, Katoh T, Puglia AM, Vohradsky J, Yun BS, Seto H, Thompson CJ (1999) Broad-spectrum thiopeptide recognition specificity of the *Streptomyces lividans* TipAL protein and its role in regulating gene expression. J Biol Chem 274:20578–20586
20. Clayton TM, Bibb MJ (1990) *Streptomyces* promoter-probe plasmids that utilize the *xylE* gene of *Pseudomonas putida*. Nucleic Acids Res 18:1077–1077
21. Craney A, Hohenauer T, Xu Y, Navani NK, Li YF, Nodwell J (2007) A synthetic *luxCDABE* gene cluster optimized for expression in high-GC bacteria. Nucleic Acids Res 35:e46. doi:10.1093/nar/gkm086
22. Dangel V, Westrich L, Smith MCM, Heide L, Gust B (2010) Use of an inducible promoter for antibiotic production in a heterologous host. Appl Microbiol Biot 87:261–269
23. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci USA 97:6640–6645
24. Deng ZX, Kieser T, Hopwood DA (1987) Activity of a *Streptomyces* transcriptional terminator in *Escherichia coli*. Nucleic Acids Res 15:2665–2675
25. Denis F, Brzezinski R (1991) An improved aminoglycoside resistance gene cassette for use in Gram-negative bacteria and *Streptomyces*. FEMS Microbiol Lett 81:261–264
26. DeSanti CL, Strohl WR (2003) Characterization of the *Streptomyces sp* strain C5 *snp* locus and development of *snp*-derived expression vectors. Appl Environ Microb 69:1647–1654
27. Du D, Zhu Y, Wei J, Tian Y, Niu G, Tan H (2013) Improvement of gougerotin and nikkomycin production by engineering their biosynthetic gene clusters. Appl Microbiol Biotechnol 97:6383–6396
28. Fedoryshyn M, Welle E, Bechthold A, Luzhetskyy A (2008) Functional expression of the Cre recombinase in actinomycetes. Appl Microbiol Biot 78:1065–1070
29. Fornwald JA, Schmidt FJ, Adams CW, Rosenberg M, Brawner ME (1987) Two promoters, one inducible and one constitutive, control transcription of the *Streptomyces lividans* galactose operon. Proc Natl Acad Sci USA 84:2130–2134
30. Frasch HJ, Medema MH, Takano E, Breitling R (2013) Design-based re-engineering of biosynthetic gene clusters: plug-and-play in practice. Curr Opin Biotechnol. doi:10.1016/j.copbio.2013.03.006
31. Gatewood ML, Bralley P, Weil MR, Jones GH (2012) RNA-Seq and RNA immunoprecipitation analyses of the transcriptome of *Streptomyces coelicolor* identify substrates for RNase III. J Bacteriol 194:2228–2237
32. Gingold H, Pilpel Y (2011) Determinants of translation efficiency and accuracy. Mol Syst Biol 7:481. doi:10.1038/msb.2011.14
33. Gomez-Escribano JP, Bibb MJ (2011) Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters. Microb Biotechnol 4:207–215
34. Gomez-Escribano JP BM (2013) Heterologous expression of natural product biosynthetic gene clusters in *Streptomyces coelicolor*: from genome mining to manipulation of biosynthetic pathways. J Indust Microbiol Biotechnol. doi:10.1007/s10295-013-1348-5
35. Gomez-Escribano JP, Song LJ, Fox DJ, Yeo V, Bibb MJ, Challis GL (2012) Structure and biosynthesis of the unusual polyketide alkaloid coelimycin P1, a metabolic product of the *cpk* gene cluster of *Streptomyces coelicolor* M145. Chem Sci 3:2716–2720
36. Gottelt M, Kol S, Gomez-Escribano JP, Bibb M, Takano E (2010) Deletion of a regulatory gene within the *cpk* gene cluster reveals novel antibacterial activity in *Streptomyces coelicolor* A3(2). Microbiol-Sgm 156:2343–2353
37. Gust B, Chandra G, Jakimowicz D, Tian YQ, Bruton CJ, Chater KF (2004) Lambda red-mediated genetic manipulation of antibiotic-producing *Streptomyces*. Adv Appl Microbiol 54:107–128
38. Hatanaka T, Onaka H, Arima J, Uraji M, Uesugi Y, Usuki H, Nishimoto Y, Iwabuchi M (2008) pTONA5: a hyperexpression vector in streptomycetes. Protein Expres Purif 62:244–248
39. Herai S, Hashimoto Y, Higashibata H, Maseda H, Ikeda H, Omura S, Kobayashi M (2004) Hyper-inducible expression system for streptomycetes. Proc Natl Acad Sci USA 101:14031–14035

40. Herrmann S, Siegl T, Luzhetska M, Petzke L, Jilg C, Welle E, Erb A, Leadlay PF, Bechthold A, Luzhetskyy A (2012) Site-specific recombination strategies for engineering actinomycete genomes. Appl Environ Microb 78:1804–1812
41. Hesketh AR, Chandra G, Shaw AD, Rowland JJ, Kell DB, Bibb MJ, Chater KF (2002) Primary and secondary metabolism, and post-translational protein modifications, as portrayed by proteomic analysis of *Streptomyces coelicolor*. Mol Microbiol 46:917–932
42. Hindle Z, Smith CP (1994) Substrate induction and catabolite repression of the *Streptomyces coelicolor* glycerol operon are mediated through the GylR protein. Mol Microbiol 12:737–745
43. Horinouchi S, Beppu T (1985) Construction and application of a promoter-probe plasmid that allows chromogenic identification in *Streptomyces lividans*. J Bacteriol 162:406–412
44. Horinouchi S, Furuya K, Nishiyama M, Suzuki H, Beppu T (1987) Nucleotide sequence of the streptothricin acetyltransferase gene from *Streptomyces lavendulae* and its expression in heterologous hosts. J Bacteriol 169:1929–1937
45. Hutter H (2006) Fluorescent reporter methods. Methods Mol Biol 351:155–173
46. Ikeda H, Shin-Ya K, Omura S (2013) Genome mining of the *Streptomyces avermitilis* genome and development of genome-minimized hosts for heterologous expression of biosynthetic gene clusters. J Ind Microbiol Biotechnol. doi:10.1007/s10295-013-1327-x
47. Ingham CJ, Hunter IS, Smith MCM (1995) Rho-independent terminators without 3' poly-U tails from the early region of actinophage øC31. Nucleic Acids Res 23:370–376
48. Ingram C, Brawner M, Youngman P, Westpheling J (1989) *xylE* functions as an efficient reporter gene in *Streptomyces* spp.: use for the study of *galP1*, a catabolite-controlled promoter. J Bacteriol 171:6617–6624
49. Jayapal KP, Philp RJ, Kok YJ, Yap MG, Sherman DH, Griffin TJ, Hu WS (2008) Uncovering genes with divergent mRNA-protein dynamics in *Streptomyces coelicolor*. PLoS ONE 3:e2097. doi:10.1371/journal.pone.0002097
50. Joshi MV, Bignell DR, Johnson EG, Sparks JP, Gibson DM, Loria R (2007) The AraC/XylS regulator TxtR modulates thaxtomin biosynthesis and virulence in *Streptomyces scabies*. Mol Microbiol 66:633–642
51. Kataoka M, Tatsuta T, Suzuki I, Kosono S, Seki T, Yoshida T (1996) Development of a temperature-inducible expression system for *Streptomyces* spp. J Bacteriol 178:5540–5542
52. Kersten RD, Yang YL, Xu Y, Cimermancic P, Nam SJ, Fenical W, Fischbach MA, Moore BS, Dorrestein PC (2011) A mass spectrometry-guided genome mining approach for natural product peptidogenomics. Nat Chem Biol 7:794–802
53. Khodakaramian G, Lissenden S, Gust B, Moir L, Hoskisson PA, Chater KF, Smith MCM (2006) Expression of Cre recombinase during transient phage infection permits efficient marker removal in *Streptomyces*. Nucleic Acids Res 34:e20. doi:10.1093/nar/gnj019
54. King AA, Chater KF (1986) The expression of the *Escherichia coli lacZ* gene in *Streptomyces*. J Gen Microbiol 132:1739–1752
55. Komatsu M, Komatsu K, Koiwai H, Yamada Y, Kozone I, Izumikawa M, Hashimoto J, Takagi M, Omura S, Shin-Ya K, Cane DE, Ikeda H (2013) Engineered *Streptomyces avermitilis* host for heterologous expression of biosynthetic gene cluster for secondary metabolites. ACS Synth Biol 2:384–396
56. Komatsu M, Uchiyama T, Omura S, Cane DE, Ikeda H (2010) Genome-minimized *Streptomyces* host for the heterologous expression of secondary metabolism. Proc Natl Acad Sci USA 107:2646–2651
57. Kuhstoss S, Rao RN (1991) A thiostrepton-inducible expression vector for use in *Streptomyces* spp. Gene 103:97–99
58. Labes G, Bibb M, Wohlleben W (1997) Isolation and characterization of a strong promoter element from the *Streptomyces ghanaensis* phage I19 using the gentamicin resistance gene (*aacC1*) of Tn*1696* as reporter. Microbiology 143:1503–1512
59. Lakey JH, Lea EJ, Rudd BA, Wright HM, Hopwood DA (1983) A new channel-forming antibiotic from *Streptomyces coelicolor* A3(2) which requires calcium for its activity. J Gen Microbiol 129:3565–3573
60. Laureti L, Song L, Huang S, Corre C, Leblond P, Challis GL, Aigle B (2011) Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in *Streptomyces ambofaciens*. Proc Natl Acad Sci USA 108:6258–6263
61. Lautru S, Deeth RJ, Bailey LM, Challis GL (2005) Discovery of a new peptide natural product by *Streptomyces coelicolor* genome mining. Nat Chem Biol 1:265–269
62. Liu WT, Kersten RD, Yang YL, Moore BS, Dorrestein PC (2011) Imaging mass spectrometry and genome mining via short sequence tagging identified the anti-infective agent arylomycin in *Streptomyces roseosporus*. J Am Chem Soc 133:18010–18013
63. Lussier FX, Denis F, Shareck F (2010) Adaptation of the highly productive T7 expression system to *Streptomyces lividans*. Appl Environ Microbiol 76:967–970
64. Magdevska V, Gaber R, Goranovic D, Kuscer E, Boakes S, Duran Alonso MB, Santamaria RI, Raspor P, Leadlay PF, Fujs S, Petkovic H (2010) Robust reporter system based on chalcone synthase *rppA* gene from *Saccharopolyspora erythraea*. J Microbiol Methods 83:111–119
65. Malpartida F, Niemi J, Navarrete R, Hopwood DA (1990) Cloning and expression in a heterologous host of the complete set of genes for biosynthesis of the *Streptomyces coelicolor* antibiotic undecylprodigiosin. Gene 93:91–99
66. McAlpine JB, Bachmann BO, Piraee M, Tremblay S, Alarco AM, Zazopoulos E, Farnet CM (2005) Microbial genomics as a guide to drug discovery and structural elucidation: ECO-02301, a novel antifungal agent, as an example. J Nat Prod 68:493–496
67. McDaniel R, Ebert-Khosla S, Hopwood DA, Khosla C (1993) Engineered biosynthesis of novel polyketides. Science 262:1546–1550
68. McDowell JC, Roberts JW, Jin DJ, Gross C (1994) Determination of intrinsic transcription termination efficiency by RNA polymerase elongation rate. Science 266:822–825
69. Mitra A, Kesarwani AK, Pal D, Nagaraja V (2011) WebGeSTer DB—a transcription terminator database. Nucleic Acids Res 39:129–135
70. Motamedi H, Shafiee A, Cai SJ (1995) Integrative vectors for heterologous gene expression in *Streptomyces* spp. Gene 160:25–31
71. Murakami T, Holt TG, Thompson CJ (1989) Thiostrepton-induced gene expression in *Streptomyces lividans*. J Bacteriol 171:1459–1466
72. Myronovskyi M, Welle E, Fedorenko V, Luzhetskyy A (2011) β-glucuronidase as a sensitive and versatile reporter in actinomycetes. Appl Environ Microb 77:5370–5383
73. Na D, Lee D (2010) RBSDesigner: software for designing synthetic ribosome binding sites that yields a desired level of protein expression. Bioinformatics 26:2633–2634
74. Naville M, Ghuillot-Gaudeffroy A, Marchais A, Gautheret D (2011) ARNold: a web tool for the prediction of Rho-independent transcription terminators. RNA Biol 8:11–13
75. Nguyen KD, Au-Young SH, Nodwell JR (2007) Monomeric red fluorescent protein as a reporter for macromolecular localization in *Streptomyces coelicolor*. Plasmid 58:167–173
76. Nikodinovic J, Priestley ND (2006) A second generation *snp*-derived *Escherichia coli-Streptomyces* shuttle expression

vector that is generally transferable by conjugation. Plasmid 56:223–227
77. Ochi K, Tanaka Y, Tojo S (2013) Activating the expression of bacterial cryptic genes by *rpoB* mutations in RNA polymerase or by rare earth elements. J Indust Microbiol Biotechnol. doi:10.1007/s10295-013-1349-4
78. Omura S, Ikeda H, Ishikawa J, Hanamoto A, Takahashi C, Shinose M, Takahashi Y, Horikawa H, Nakazawa H, Osonoe T, Kikuchi H, Shiba T, Sakaki Y, Hattori M (2001) Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. Proc Natl Acad Sci USA 98:12215–12220
79. Ostash B, Ostash I, Zhu L, Kharel MK, Luzhetskyy A, Bechthold A, Walker S, Rohr J, Fedorenko V (2010) Properties of *lanK*-based regulatory circuit involved in landomycin biosynthesis in *Streptomyces cyanogenus* S136. Russ J Genet 46:530–535
80. Ozaki T, Nishiyama M, Kuzuyama T (2013) Novel tryptophan metabolism by a potential gene cluster that is widely distributed among actinomycetes. J Biol Chem 288:9946–9956
81. Page N, Kluepfel D, Shareck F, Morosoli R (1996) Increased xylanase yield in *Streptomyces lividans*: dependence on number of ribosome-binding sites. Nat Biotechnol 14:756–759
82. Paget MSB, Hintermann G, Smith CP (1994) Construction and application of streptomycete promoter probe vectors which employ the *Streptomyces glaucescens* tyrosinase-encoding gene as reporter. Gene 146:105–110
83. Park HM, Kim BG, Chang D, Malla S, Joo HS, Kim EJ, Park SJ, Sohng JK, Kim PI (2013) Genome-based cryptic gene discovery and functional identification of NRPS siderophore peptide in *Streptomyces peucetius*. Appl Microbiol Biotechnol 97:1213–1222
84. Pawlik K, Kotowska M, Kolesinski P (2010) *Streptomyces coelicolor* A3(2) produces a new yellow pigment associated with the polyketide synthase Cpk. J Mol Microbial Biotechnol 19:147–151
85. Pedrolli DB, Matern A, Wang J, Ester M, Siedler K, Breaker R, Mack M (2012) A highly specialized flavin mononucleotide riboswitch responds differently to similar ligands and confers roseoflavin resistance to *Streptomyces davawensis*. Nucleic Acids Res 40:8662–8673
86. Pfeifer BA, Admiraal SJ, Gramajo H, Cane DE, Khosla C (2001) Biosynthesis of complex polyketides in a metabolically engineered strain of *Escherichia coli*. Science 291:1790–1792
87. Pulido D, Jimenez A (1987) Optimization of gene-expression in *Streptomyces lividans* by a transcription terminator. Nucleic Acids Res 15:4227–4240
88. Pulido D, Jimenez A, Salas M, Mellado RP (1987) A *Bacillus subtilis* Phage Phi29 transcription terminator is efficiently recognized in *Streptomyces lividans*. Gene 56:277–282
89. Pulido D, Vara JA, Jimenez A (1986) Cloning and expression in biologically active form of the gene for human interferon alpha-2 in *Streptomyces lividans*. Gene 45:167–174
90. Rauhut R, Klug G (1999) mRNA degradation in bacteria. FEMS Microbiol Rev 23:353–370
91. Rodriguez-Garcia A, Combes P, Perez-Redondo R, Smith MCA, Smith MCM (2005) Natural and synthetic tetracycline-inducible promoters for use in the antibiotic-producing bacteria *Streptomyces*. Nucleic Acids Res 33:e87. doi:10.1093/nar/gni086
92. Romby P, Charpentier E (2010) An overview of RNAs with regulatory functions in Gram-positive bacteria. Cell Mol Life Sci 67:217–237
93. Rowe CJ, Cortes J, Gaisser S, Staunton J, Leadley PF (1998) Construction of new vectors for high-level expression in actinomycetes. Gene 216:215–223
94. Rudd BA, Hopwood DA (1980) A pigmented mycelial antibiotic in *Streptomyces coelicolor*: control by a chromosomal gene cluster. J Gen Microbiol 119:333–340
95. Rudolph MM, Vockenhuber MP, Suess B (2013) Synthetic riboswitches for the conditional control of gene expression in *Streptomyces coelicolor*. Microbiology 159:1416–1422
96. Saleh O, Bonitz T, Flinspach K, Kulik A, Burkard N, Muhlenweg A, Vente A, Polnick S, Lammerhofer M, Gust B, Fiedler HP, Heide L (2012) Activation of a silent phenazine biosynthetic gene cluster reveals a novel natural product and a new resistance mechanism against phenazines. Medchemcomm 3:1009–1019
97. Santangelo TJ, Artsimovitch I (2011) Termination and antitermination: RNA polymerase runs a stop sign. Nat Rev Microbiol 9:319–329
98. Schmitt-John T, Engels JW (1992) Promoter constructions for efficient secretion expression in *Streptomyces lividans*. Appl Microbiol Biotechnol 36:493–498
99. Scholtissek S, Grosse F (1987) A cloning cartridge of lambda t(o) terminator. Nucleic Acids Res 15:3185
100. Seghezzi N, Amar P, Koebmann B, Jensen PR, Virolle MJ (2011) The construction of a library of synthetic promoters revealed some specific features of strong *Streptomyces* promoters. Appl Microbiol Biot 90:615–623
101. Sekurova ON, Brautaset T, Sletta H, Borgos SEF, Jakobsen OM, Ellingsen TE, Strom AR, Valla S, Zotchev SB (2004) *In vivo* analysis of the regulatory genes in the nystatin biosynthetic gene cluster of *Streptomyces noursei* ATCC 11455 reveals their differential control over antibiotic biosynthesis. J Bacteriol 186:1345–1354
102. Seo SW, Yang JS, Kim I, Yang J, Min BE, Kim S, Jung GY (2013) Predictive design of mRNA translation initiation region to control prokaryotic translation efficiency. Metab Eng 15:67–74
103. Shao ZY, Luo YZ, Zhao HM (2011) Rapid characterization and engineering of natural product biosynthetic pathways via DNA assembler. Mol Biosyst 7:1056–1059
104. Sidebottom AM, Johnson AR, Karty JA, Trader DJ, Carlson EE (2013) Integrated metabolomics approach facilitates discovery of an unpredicted natural product suite from *Streptomyces coelicolor* M145. ACS Chem Biol. doi:10.1021/cb4002798
105. Siegl T, Tokovenko B, Myronovskyi M, Luzhetskyy A (2013) Design, construction and characterisation of a synthetic promoter library for fine-tuned gene expression in actinomycetes. Metab Eng. doi:10.1016/j.ymben.2013.07.006
106. Sohaskey CD, Im H, Nelson AD, Schauer AT (1992) Tn*4556* and luciferase—synergistic tools for visualizing transcription in *Streptomyces*. Gene 115:67–71
107. Song L, Barona-Gomez F, Corre C, Xiang L, Udwary DW, Austin MB, Noel JP, Moore BS, Challis GL (2006) Type III polyketide synthase β-ketoacyl-ACP starter unit and ethylmalonyl-CoA extender unit selectivity discovered by *Streptomyces coelicolor* genome mining. J Am Chem Soc 128:14754–14755
108. Stevens DC, Conway KR, Pearce N, Villegas-Penaranda LR, Garza AG, Boddy CN (2013) Alternative sigma factor overexpression enables heterologous expression of a type II polyketide biosynthetic pathway in *Escherichia coli*. PLoS ONE 8:e64858. doi:10.1371/journal.pone.0064858
109. Strohl WR (1992) Compilation and analysis of DNA sequences associated with apparent streptomycete promoters. Nucleic Acids Res 20:961–974
110. Sudek S, Haygood MG, Youssef DT, Schmidt EW (2006) Structure of trichamide, a cyclic peptide from the bloom-forming cyanobacterium *Trichodesmium erythraeum*, predicted from the genome sequence. Appl Environ Microbiol 72:4382–4387
111. Sun JH, Kelemen GH, Fernandez-Abalos JM, Bibb MJ (1999) Green fluorescent protein as a reporter for spatial and temporal

gene expression in *Streptomyces coelicolor* A3(2). Microbiology 145:2221–2227
112. Takano E, Kinoshita H, Mersinias V, Bucca G, Hotchkiss G, Nihira T, Smith CP, Bibb M, Wohlleben W, Chater K (2005) A bacterial hormone (the SCB1) directly controls the expression of a pathway-specific regulatory gene in the cryptic type I polyketide biosynthetic gene cluster of *Streptomyces coelicolor*. Mol Microbiol 56:465–479
113. Takano E, White J, Thompson CJ, Bibb MJ (1995) Construction of thiostrepton-inducible, high-copy-number expression vectors for use in *Streptomyces* spp. Gene 166:133–137
114. Takano H, Obitsu S, Beppu T, Ueda K (2005) Light-induced carotenogenesis in *Streptomyces coelicolor* A3(2): identification of an extracytoplasmic function sigma factor that directs photodependent transcription of the carotenoid biosynthesis gene cluster. J Bacteriol 187:1825–1832
115. Tanaka Y, Hosaka T, Ochi K (2010) Rare earth elements activate the secondary metabolite-biosynthetic gene clusters in *Streptomyces coelicolor* A3(2). J Antibiot 63:477–481
116. Tsao SW, Rudd BA, He XG, Chang CJ, Floss HG (1985) Identification of a red pigment from *Streptomyces coelicolor* A3(2) as a mixture of prodigiosin derivatives. J Antibiot 38:128–131
117. Uguru GC, Mondhe M, Goh S, Hesketh A, Bibb MJ, Good L, Stach JE (2013) Synthetic RNA silencing of actinorhodin biosynthesis in *Streptomyces coelicolor* A3(2). PLoS One 8:e67509. doi:10.1371/journal.pone.0067509
118. van Wezel GP, White J, Hoogvliet G, Bibb MJ (2000) Application of *redD*, the transcriptional activator gene of the undecylprodigiosin biosynthetic pathway, as a reporter for transcriptional activity in *Streptomyces coelicolor* A3(2) and *Streptomyces lividans*. J Mol Microb Biotech 2:551–556
119. Vockenhuber MP, Sharma CM, Statt MG, Schmidt D, Xu Z, Dietrich S, Liesegang H, Mathews DH, Suess B (2011) Deep sequencing-based identification of small non-coding RNAs in *Streptomyces coelicolor*. RNA Biol 8:468–477
120. Vujaklija D, Ueda K, Hong SK, Beppu T, Horinouchi S (1991) Identification of an A-factor-dependent promoter in the streptomycin biosynthetic gene cluster of *Streptomyces griseus*. Mol Gen Genet 229:119–128
121. Wang L, Zhao Y, Liu Q, Huang Y, Hu C, Liao G (2012) Improvement of A21978C production in *Streptomyces roseosporus* by reporter-guided *rpsL* mutation selection. J Appl Microbiol 112:1095–1101
122. Wang W, Li X, Wang J, Xiang S, Feng X, Yang K (2013) An engineered strong promoter for streptomycetes. Appl Environ Microbiol 79:4484–4492
123. Ward JM, Janssen GR, Kieser T, Bibb MJ, Buttner MJ, Bibb MJ (1986) Construction and characterization of a series of multi-copy promoter-probe plasmid vectors for *Streptomyces* using the aminoglycoside phosphotransferase gene from Tn*5* as indicator. Mol Gen Genet 203:468–478
124. Weiser J, Buriankova K, Kalachova L, Branny P, Pernodet JL (2006) Construction and testing of a bacterial luciferase reporter gene system for in vivo measurement of nonsense suppression in *Streptomyces*. Folia Microbiol 51:62–64
125. Wenzel SC, Gross F, Zhang YM, Fu J, Stewart AF, Muller R (2005) Heterologous expression of a myxobacterial natural products assembly line in pseudomonads via Red/ET recombineering. Chem Biol 12:349–356
126. Wilkinson CJ, Hughes-Thomas ZA, Martin CJ, Bohm I, Mironenko T, Deacon M, Wheatcroft M, Wirtz G, Staunton J, Leadlay PF (2002) Increasing the efficiency of heterologous promoters in actinomycetes. J Mol Microbiol Biotechnol 4:417–426
127. Willemse J, van Wezel GP (2009) Imaging of *Streptomyces coelicolor* A3(2) with reduced autofluorescence reveals a novel stage of FtsZ localization. PLoS ONE 4:e4242. doi:10.1371/journal.pone.0004242
128. Wright LF, Hopwood DA (1976) Identification of the antibiotic determined by the SCP1 plasmid of *Streptomyces coelicolor* A3(2). J Gen Microbiol 95:96–106
129. Xu G, Wang J, Wang L, Tian X, Yang H, Fan K, Yang K, Tan H (2010) "Pseudo" γ-butyrolactone receptors respond to antibiotic signals to coordinate antibiotic biosynthesis. J Biol Chem 285:27440–27448
130. Yadav G, Gokhale RS, Mohanty D (2009) Towards prediction of metabolic products of polyketide synthases: an in silico analysis. PLoS Comput Biol 5:e1000351. doi:10.1371/journal.pcbi.1000351
131. Yague P, Rodriguez-Garcia A, Lopez-Garcia MT, Martin JF, Rioseras B, Sanchez J, Manteca A (2013) Transcriptomic analysis of *Streptomyces coelicolor* differentiation in solid sporulating cultures: first compartmentalized and second multinucleated mycelia have different and distinctive transcriptomes. PLoS ONE 8:e60665. doi:10.1371/journal.pone.0060665
132. Yun BS, Hidaka T, Kuzuyama T, Seto H (2001) Thiopeptide non-producing *Streptomyces* species carry the *tipA* gene: a clue to its function. J Antibiot 54:375–378
133. Zhang YM, Buchholz F, Muyrers JPP, Stewart AF (1998) A new logic for DNA engineering using recombination in *Escherichia coli*. Nat Genet 20:123–128
134. Zhou M, Jing XY, Xie PF, Chen WH, Wang T, Xia HY, Qin ZJ (2012) Sequential deletion of all the polyketide synthase and nonribosomal peptide synthetase biosynthetic gene clusters and a 900-kb subtelomeric sequence of the linear chromosome of *Streptomyces coelicolor*. FEMS Microbiol Lett 333:169–179
135. Zotchev SB, Sekurova ON, Katz L (2012) Genome-based bioprospecting of microbes for new therapeutics. Curr Opin Biotechnol 23:941–947