Recent Advances in Synthetic Biology Approaches to Optimize Production of Bioactive Natural Products in Actinobacteria

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Actinobacteria represent one of the most fertile sources for the discovery and development of natural products (NPs) with medicinal and industrial importance. However, production titers of actinobacterial NPs are usually low and require optimization for compound characterization and/or industrial production. In recent years, a wide variety of novel enabling technologies for engineering actinobacteria have been developed, which have greatly facilitated the optimization of NPs biosynthesis. In this review, we summarize the recent advances of synthetic biology approaches for overproducing desired drugs, as well as for the discovery of novel NPs in actinobacteria, including dynamic metabolic regulation based on metabolite-responsive promoters or biosensors, multi-copy chromosomal integration of target biosynthetic gene clusters (BGCs), promoter engineering-mediated rational BGC refactoring, and construction of genome-minimized Streptomyces hosts. Integrated with metabolic engineering strategies developed previously, these novel enabling technologies promise to facilitate industrial strain improvement process and genome mining studies for years to come.

Keywords: actinobacteria, natural product, synthetic biology, dynamic regulation, BGC amplification, pathway refactoring, genome-minimized host

INTRODUCTION

As a kind of Gram-positive bacteria with high GC content, actinobacteria undergo complex morphological differentiation and secondary metabolism processes (Barka et al., 2016). Actinobacteria, particularly the Streptomyces genus, have been recognized as the main sources for microbial bioactive natural products (NPs), such as antibiotics, chemotherapeutics, immunosuppressants and anthelmintics, which make important contributions to health care and crop protection (Jensen et al., 2015; Genilloud, 2017; Nepal and Wang, 2019). With the rapid advances in genome sequencing and genome mining methods (Ziemert et al., 2016; Blin et al., 2019), a wealth of hidden NP biosynthetic gene clusters (BGCs) have been revealed by specialized software (i.e., antiSMASH) and are regarded as an untapped treasure trove for the discovery of novel bioactive compounds (Rutledge and Challis, 2015; Niu, 2018). For instance, a single Streptomyces genome usually harbors around 30 NP BGCs, approximately 10-fold more than...
Dynamic metabolic regulation has proved to be an effective strategy to improve production titers of target compounds by balancing bacterial growth and biosynthesis of specific metabolites (Zhang et al., 2012; Dahl et al., 2013; Gupta et al., 2017). Generally, three different approaches – quorum sensing systems, metabolite-responsive promoters and protein/RNA-based biosensors – are used for autonomous control of metabolic pathway flux (Zhang et al., 2015; Polkade et al., 2016; Tan and Prather, 2017). These new concepts were initially developed in *Escherichia coli* or *Saccharomyces cerevisiae*, and the latter two have been extended to actinobacteria for the optimization of antibiotic biosynthetic pathways.

Metabolite-Responsive Promoters

In the last three decades, a large variety of metabolic engineering strategies have been developed to optimize production of secondary metabolites in microbes. However, few approaches could enable the coordination between bacterial growth and biosynthesis of target compounds. Recently, Li et al. (2018) employed time-course transcriptome analysis to identify a series of antibiotic-responsive promoters with a transcription profile similar to the inducible promoters, when under the optimal conditions (Figure 1A). These dynamic responsive promoters could be used to efficiently optimize the expression of native actinorhodin and heterogeneous oxetetracycline (OTC) BGCs in *Streptomyces coelicolor*, subsequently improving the production titers of ACT and OTC by 1.3- and 9.1-fold, respectively, compared with constitutive promoters (Li et al., 2018). Xu et al. (2012) reported a metabolite-responsive promoter, which controlled the transcription of *actAB* encoding an antibiotic exporter in *S. coelicolor*. They found that the antibiotic ACT and its biosynthetic intermediates [e.g., (S)-DNPA] could relieve the repression of *actAB* by binding the transcriptional regulator ActR. That means that the *actAB* promoter could indirectly respond to intermediates or end-products, thus synergistically regulating ACT biosynthesis and export. The metabolite-responsive promoter based strategy will achieve an autonomous induction of pathway regulation and provide a universal route for titer improvements of desired NPs in actinobacteria.

Natural Product-Specific Biosensors

Genetically encoded biosensors are a kind of important synthetic biology tool for real-time detection of intracellular metabolites for specific readouts (Zhang et al., 2015). A typical biosensor is composed of three modules: (i) a signal input module, such as transcription factors (TFs) or riboswitches; (ii) a regulatory module, such as TF-dependent promoters; (iii) a signal output module, such as reporter genes (Mahr and Frunzke, 2016; Lim et al., 2018). Until now, three major categories of biosensors – fluorescence resonance energy transfer (FRET)-based sensors, TF-based sensors and riboswitches – have been widely used in metabolic engineering of microbial hosts (Zhang et al., 2015; Lim et al., 2018). As a kind of natural sensory protein, a classical TF has evolved to contain a ligand-binding domain for responding to environmental changes and a DNA-binding domain for regulating gene expression. Interestingly, many NP BGCs encode cluster-situated regulators (CSRs) in actinobacteria, such as TetR-like regulators and *Streptomyces* antibiotic regulatory proteins (SARPs; Liu et al., 2013; Romero-Rodriguez et al., 2015). These CSRs can be used to dynamically detect developmental state, population density or other environmental changes, and
Thus determine the onset and production levels of secondary metabolites in actinobacteria. Recently, Rebets et al. (2018) developed a new antibiotic-specific whole-cell biosensor based on a TetR-like repressor for the development of antibiotic-overproducing strains (Figure 1B). Briefly, the highly active macrolide antibiotic pamamycins BGC encodes a transporter PamW and a corresponding transcriptional repressor PamR2, which can be deactivated by binding to pamamycins (Rebets et al., 2015). Generally, PamW expression is controlled by PamR2 at low level of pamamycins. However, at very high levels of pamamycins, the native PamR2-based biosensors will reach the detection limit and cannot effectively regulate PamW expression (Rebets et al., 2018). The \( \text{pamW} \) promoter was directly used to control expression of the kanamycin resistance gene, thus generating the resistance-based G0 whole-cell biosensor system. After UV-induced mutagenesis, strains resistant to a high-concentration of kanamycin showed a significant increase in pamamycins production (up to 15–16 mg/L). Of note is the fact that the native pamamycins-responsive biosensor showed limited operating and dynamic ranges for further applications.
To overcome this obstacle, the G1 pamamycins biosensor was further developed by combining different promoters, varying the number and position of operators, as well as using diverse reporter genes. Using the new biosensor with higher sensitivity, three mutated strains were obtained, which could produce up to 30 mg/L of pamamycins. Furthermore, to overcome the low detection limit of the G1 biosensor, the binding affinity of PamR2-pamamycins was also efficiently decreased by designing a panel of PamR2 mutations. As expected, the G2 biosensor showed a better operating dynamic range than that of the G1 biosensor (Rebets et al., 2018). In fact, at least 17% of NP BGCs encode TetR-like regulators and putative transporters simultaneously, which provides possibilities for the development of diverse antibiotic-responsive biosensors (Rebets et al., 2018).

With the aid of a metabolite-responsive biosensor system, it will become very convenient to assess the functional expression of NP BGCs after random mutagenesis, genetic manipulation or exposure to various cultivation conditions. In the future, TF-based biosensors will hold great promise for accelerating cell factory development for pharmaceutical production and the activation of silent BGCs for the discovery of novel compounds in actinobacteria (Kim et al., 2016; Zarins-Tutt et al., 2016; Sun et al., 2017).

### MULTIPLEX SITE-SPECIFIC GENOME ENGINEERING FOR NPs OVERPRODUCTION

In the last three decades, due to their broad host specificities, SSR systems have been widely applied to strain improvements, combinatorial biosynthesis, and heterologous expression of the entire BGCs in *Streptomyces*, *Actinoplanes* and other industrial actinobacteria (Baltz, 2012; Stark, 2017; Merrick et al., 2018). To date, at least ten SSR systems have been identified in actinobacteria, which are derived from bacteriophage CBG73463, R4, SV1, TGI, VWB, ΦBT1, ΦC31, ΦJoe, ΦK38-1 or Φ1/6 (Baltz, 2012; Fogg et al., 2017; Li et al., 2017a; Kormanec et al., 2019). In particular, ΦBT1, ΦC31 and TGI systems have been widely used for cell factory development via multi-copy amplification of target genes or BGCs (Baltz, 2012). For instance, production titers of the antibiotic goadsporin were increased by 2.3-fold by a step-by-step introduction of two extra copies of the goadsporin BGC based on ΦC31 and TGI integration systems (Haginaka et al., 2014). However, this strategy requires repeated rounds of conjugal transfer and is limited by the number of selection markers. In 2011, Murakami and coworkers demonstrated that the ZouA-RsA/B-mediated recombination system could be used to achieve tandem amplification of the ACT gene cluster with 4–12 copies, resulting in a 20-fold increase in ACT production (Murakami et al., 2011). However, the engineered strains may be genetically unstable in the absence of antibiotic selection due to the presence of tandem amplification. In addition, the method requires the introduction of the ZouA-RsA/B system into both flanks of target BGCs in advance via two-round conjugal transfer before BGC amplification, which is time-consuming and labor-intensive.

To address these limitations, our group has recently developed two novel enabling technologies for multi-locus chromosomal integration of target genes or BGCs, including MSGE and aMSGE (Li et al., 2017b, 2019b).

### MSGE: Multiplex Site-Specific Genome Engineering Based on the “One Integrate-Multiple attB Sites” Concept

In the actinomycetal genome, there is typically no, or only one, native attB site for each SSR system (Baltz, 2012). In theory, one-step, multi-copy integration of target genes or BGCs could be achieved by introducing multiple artificial attB sites into the host chromosome in advance. Based on this “one integrate-multiple attB sites” concept, an innovative approach MSGE was developed for discrete BGC amplification (Figure 2A; Li et al., 2017b). Using the high-efficiency CRISPR/Cas9 genome editing method (Huang et al., 2015), we sequentially introduced three ΦC31 and two ΦBT1 attB sites into the genomic loci of deleted, non-target secondary metabolite BGCs in the industrial strain *Streptomyces pristinaeaspiralis*. Then, five extra copies of the pristinamycin II BGC were integrated into the modified chromosome in two steps using the ΦC31 and ΦBT1 compatible integration systems, which led to significantly improved PII titers (Li et al., 2015, 2017b). Importantly, the novel strategy was also extended to develop a series of powerful *Streptomyces coelicolor* heterologous expression hosts. Up to four copies of the chloramphenicol or YM-216391 BGCs were simultaneously integrated into these new chassis strains, thus resulting in increased production titers (Li et al., 2017b).

Using the highly effective heterologous expression system, YM-216391 BGC was engineered to generate aurantizolicin and a hybrid compound 3, which exhibits significantly increased antitumor activity (Pei et al., 2018). Similarly, Myronovskyi et al. (2018) also constructed a panel of cluster-free, powerful *Streptomyces albus* chassis strains based on the “one integrate-multiple attB sites” concept. The production titers of a variety of bioactive compounds, including aloesaponarin II, cinnamycin, didemethylmensacarin and griseorhodin A, were significantly enhanced in these heterologous expression superhosts (Myronovskyi et al., 2018). It is worth noting that compared with the ZouA-RsA/B recombination system, MSGE-based engineered strains would be genetically stable in the absence of antibiotic selection due to discrete, site-specific integration of target BGCs (Li et al., 2017b). However, when the MSGE method is used to amplify target BGCs with multiple copies in a single step, there is an upper limit to the number of integrated BGCs. For example, we found that the chloramphenicol or YM-216391 BGCs could be simultaneously inserted into *S. coelicolor* with up to 4 copies. The possible reason is that the high-order amplification of BGCs or the accumulation of target products places an excess burden on bacterial growth (Li et al., 2017b). In the near future, we believe that these versatile *Streptomyces* hosts will greatly accelerate (meta)genomic mining and combinatorial biosynthesis studies for novel bioactive NP discovery (Li et al., 2017a; Liu et al., 2018; Zhang et al., 2019).
aMSGE: Advanced Multiplex Site-Specific Genome Engineering With Orthogonal Modular Recombinases

Although the MSGE method could be widely used to construct a variety of suitable chassis organisms for NP discovery and overproduction, two major limitations need to be addressed. On the one hand, it will be difficult or even impossible to initially insert foreign attB sites into the chromosomes of genetically intractable industrial actinobacteria due to the lack of available replicative plasmids and also their low homologous recombination capability, such as spinoasad-producing Saccharopolyspora spinosa and epoxomicin-producing Goodfellowiella coeruleoviolacea. On the other hand, repeated introduction of multiple artificial attB sites is still time-consuming and labor-intensive, especially for the slow-growing actinobacteria. To overcome these bottlenecks, our group recently developed an advanced MSGE method (aMSGE) based on the “multiple integrases-multiple attB sites” concept (Figure 2B; Li et al., 2019b). In this improved method, native attB sites of different orthogonal SSR systems in the actinomycetal genome are simultaneously applied to multiplex isolation of target genes or BGCs, rather than introducing foreign attB sites into the host chromosome. Accordingly, a plug-and-play amplification toolkit was designed and constructed, which contains 27 modular recombination plasmids with single or multiple SSR systems. Using this innovative technique, we achieved a high-efficiency introduction of the 5-oxomilbemycin A3/A4 BGC into the parental strain Streptomyces hygroscopicus with up to four extra copies, thus resulting in a significant increase in the production titers of 5-oxomilbemycin A3/A4 (Li et al., 2019b). Compared with previously developed metabolic engineering tools, the aMSGE method doesn’t require the introduction of any genetic modifications before target gene or BGC amplification, which will considerably simplify and accelerate efforts to facilitate NP discovery and overproduction. The whole process for BGC amplification takes only 18 days for the construction of high-yield engineered strains (e.g., growth period is ∼6 days). More importantly, the aMSGE method could be applicable to genetically intractable actinobacteria for strain improvements. Given that SSR systems are widely distributed in a variety of microorganisms (Fogg et al., 2014; Stark, 2017), our newly developed methodology should be widely extended to establish more efficient industrial platforms for overproducing valuable chemicals and drugs. However, the aMSGE method could not be used for BGC amplification in actinobacteria without native attB sites.

RATIONAL PATHWAY REFACTOING OF NP BIOSYNTHESIS

Generally, NP biosynthesis is under the control of highly complicated transcriptional, translational and metabolic regulation, which hampers the ability of systematic BGC engineering to maximize biosynthetic efficiency (Liu et al., 2013). Pathway refactoring provides an effective synthetic biological approach to decouple gene expression from complex native regulation and to achieve precise control of metabolite production by redesigning target BGCs in versatile surrogate hosts (Tan and Liu, 2017; Niu and Li, 2019). The key steps of BGC refactoring are to develop a set of well-characterized genetic control elements and high-efficiency DNA assembly methods (Figure 3).

In the last ten years, a series of synthetic regulatory elements have been identified to precisely regulate gene expression with wide dynamic ranges in actinobacteria, including constitutive or inducible promoters, ribosomal binding sites (RBSs), terminators and protein degradation tags (Myronovskyi and Luzhetskyy, 2016; Horball et al., 2018; Ji et al., 2019). For instance, the expression levels of 200 native or synthetic promoters and 200 artificial RBSs were systematically quantitated, which provides a universal toolbox of synthetic modular regulatory elements for the scalable and cost-effective optimization of NP biosynthetic pathway in different Streptomyces (Bai et al., 2015).

Next, a variety of in vitro or in vivo DNA assembly methods have been established to reconstruct target BGCs for diverse applications (Li et al., 2017a; Zhang et al., 2019). On the one hand, a panel of in vivo DNA assembly methods for single-marker or marker-free multiplexed promoter engineering of large BGCs have been developed on the basis of powerful homologous recombination capacity in S. cerevisiae, including DNA assembler (Shao et al., 2011), mCRISTAR (multiplexed Cas9-transformation-associated recombination) (Kang et al., 2016) and miCRISTAR (multiplexed in vitro Cas9-transformation-associated recombination) (Kim et al., 2019). For example, using the miCRISTAR strategy, the activation of a silent BGC led to the characterization of two bacterial cyclic sesterterpenes atolyptene A and B (Kim et al., 2019). In another study, yeast-mediated construction of a riboswitch-controlled pathway achieved a 120-fold increase in bottromycin productivity (Eyles et al., 2018). Intriguingly, multiple new bottromycin-related metabolites were also generated by using high-efficiency, flexible BGC modifications. On the other hand, a range of in vitro DNA assembly methods suitable for pathway refactoring have been developed, including modified Gibson assembly (Li et al., 2015), MASTER (methyltransfer-assisted tailorable ends rational) ligation (Chen et al., 2013) and SLIC (Sequence- and Ligation-Independent Cloning) (D’Agostino and Gulder, 2018). Recently, an innovative DNA assembly method, ExoCET (Exonuclease Combined with RecET recombination), was also developed for large-size, multi-operon BGC refactoring (Song et al., 2018; Wang et al., 2018). The artificial 79-kb spinosad BGC with 7 artificial operons under the control of strong constitutive promoters achieved a 328-fold enhanced spinosad production compared to the native BGC (Song et al., 2018). As a simple and robust genetic platform, BGC refactoring will have potentially broad applications in combinatorial biosynthesis and antibiotic overproduction, as well as high-throughput activation of silent BGCs from either metagenomes or uncultured actinobacteria.
CONSTRUCTION OF GENOME-REDUCED _Streptomyces_ HOSTS FOR NPs DISCOVERY AND OVERPRODUCTION

_Streptomyces_ species are capable of producing a wide range of secondary metabolites, such as polyketides, non-ribosomal peptides and terpenes, and possess the unique metabolic background needed for heterologous expression of NP BGCs from actinobacteria or other bacteria with high-GC content (Liu et al., 2018; Nepal and Wang, 2019). Within the streptomycetes, _S. coelicolor_, _S. albus_ J1074 and _Streptomyces lividans_ have been widely used as surrogate hosts for NPs discovery and overproduction (Beites and Mendes, 2015; Liu et al., 2018). With the continuous advances of novel bioinformatics tools and genetic manipulation techniques (Zhang et al., 2016, 2019; Ziemert et al., 2016), a series of versatile _Streptomyces_ chassis have been developed by deleting non-essential genomic regions, such as redundant BGCs, genome islands and insertion sequences (Liu et al., 2018; Huo et al., 2019; Table 1). Particularly, deletion of redundant BGCs are predicted to increase the supply of primary metabolite precursors, remove competing carbon and nitrogen sinks, and also facilitate the biosynthesis of heterologous BGCs. In addition, engineered strains without redundant BGCs will possess simple extracellular metabolite profiles for convenient structural characterization of novel bioactive compounds.

Along with the emergence of genome mining as a robust approach to discover novel drug leads, the development of reliable _Streptomyces_ chassis for heterologous expression of cloned BGCs is becoming increasingly important (Huo et al., 2019; Nepal and Wang, 2019). As a model strain, _S. coelicolor_ has been widely used to study the molecular regulation of antibiotic biosynthesis and morphological differentiation (Liu et al., 2013;
Barka et al., 2016). In the last 10 years, a series of advanced genome editing tools and diverse synthetic regulatory elements have been developed for the optimization of heterologous pathways in *S. coelicolor* (Myronovskyi and Luzhetskyy, 2016; Alberti and Corre, 2019). In 2011, on the basis of the wild-type strain *S. coelicolor* M145, two versatile engineered hosts M1146 and M1152 were developed, which contained deletions in four BGCs, responsible for the biosynthesis of ACT, calcium-dependent antibiotic (CDA), cryptic type I polyketides (CPK) and prodiginine (RED), or in combination with a point mutation in the regulatory gene *afsRS* (Myronovskyi and Luzhetskyy, 2016; Thanapipatsiri et al., 2015). As another important chassis, *S. albus* strain has the deletions of both oligomycin BGC and the left subtelomeric region, and the SUKA17 strain is a derivative of SUKA5 with the additional deletions of three terpene BGCs (Komatsua et al., 2010). Herein, the genome-reduced strategy has been already optimized to efficiently supply primary precursors. To construct a versatile industrial chassis for heterologous expression of secondary metabolite BGCs, a series of genome-reduced *S. avermitilis* mutants were obtained by deleting the left subtelomeric region (~2 Mb) that corresponds to the more variable genome regions (Komatsua et al., 2010). Herein, the SUKA5 and SUKA17 strains were highlighted, which had genome reductions of 17.9 and 18.5%, respectively. *S. avermitilis* SUKA5 strain has the deletions of both oligomycin BGC and the left subtelomeric region, and the SUKA17 strain is a derivative of SUKA5 with the additional deletions of three terpene BGCs (Komatsua et al., 2010). The feasibility and superiority of these two engineered hosts has been widely confirmed by the efficient production of more than 20 exogenous secondary metabolite BGCs (Komatsua et al., 2013; Ikeda et al., 2014). Similarly, 1.3-Mb and 0.7-Mb possible non-essential genomic regions were deleted in the natamycin-producing industrial strain

### Table 1: Characteristics of synthetic model Streptomyces chassis.

| Model strains | Streptomyces coelicolor M145 | Streptomyces albus J1074 | Streptomyces lividans TK24 |
|--------------|----------------------------|--------------------------|---------------------------|
| Engineered hosts | M1146/M1152 | Derived from M1146 or M1152 with 1-3 artificial ΦC31 attB sites | Derived from M1146 or M1152 by deleting all three of type III polyketide genes | Derived from M1146 with 1-2 artificial ΦC31 attB sites |
| Characteristics | Deletion of BGCs for ACT, CDA, CPK and RED | Derived from M1146 and M1152 by deleting all three of type III polyketide genes | Derived from M1146 with 1-2 artificial ΦC31 attB sites | Deletion of BGCs for ACT, RED and CDA and insertion of ΦΦΦΦattB |
| Deletion sizes | 173 kb (2%) | 176 kb (2%) | 500 kb (7.3%) | 500 kb (7.3%) |
| Deletion methods | Resistance gene-assisted recombination | RedET-mediated recombination | RedET-mediated recombination | Resistance gene-assisted recombination |
| Compounds | Chloramphenicol and YM-216391 | Flavioin and Germicin | Cinnamycin, Griseorhodine A, Tunicamycin B2 et al. | Cinnamycin, Griseorhodine A, Tunicamycin B2 et al. |
| Reference | Gomez-Escribano and Bibb, 2011 | Li et al., 2017b | Thanapipatsiri et al., 2015 | Myronovskyi et al., 2018 |
| Derived from M1146 | | | Myronovskyi et al., 2018 | Bai et al., 2014 |
| Derived from M1152 | | | | |
| Deletion of BGCs for ACT, CDA, CPK and RED | Derived from M1146 or M1152 with 1-3 artificial ΦC31 attB sites | Derived from M1146 or M1152 by deleting all three of type III polyketide genes | Derived from M1146 with 1-2 artificial ΦC31 attB sites | Deletion of BGCs for ACT, RED and CDA and insertion of ΦΦΦΦattB |
| Deletion of 15 endogenous BGCs | | | | |
| Deletion of BGCs for ACT, RED and CDA | | | | |

*ACT*, actinorhodin; *CDA*, calcium-dependent antibiotic; *CPK*, cryptic type I polyketide; *RED*, prodiginine; *BGC*, biosynthetic gene cluster.
**TABLE 2** | Characteristics of synthetic non-model Streptomyces chassis.

| Non-model strain | Streptomyces sp. FR-008 | Streptomyces avermitilis wide-type | Streptomyces albus BK3-25 | Streptomyces chattanoogensis L10 |
|------------------|--------------------------|----------------------------------|--------------------------|---------------------------------|
| Engineered host  | LQ3                      | SUKAS                            | SUKA17                   | ZXJ-6                           |
| Characteristic   | Deletion of BGCs for three endogenous polyketide genes | Deletion of left subtelomeric region and oligomycin BGC | Derived from SUKAS and deletion of BGCs for three terpene compounds | Introduction of the ethylmalonyl-CoA biosynthetic pathway and deletion of the salinomycin BGC |
| Deletion size    | 150 kb (2.1%)            | 1.62 Mb (17.9%)                  | 1.67 Mb (18.5%)          | 77 kb (0.9%)                    |
| Deletion method  | Resistance gene-assisted recombination | Cre-loxP recombination | Cre-loxP recombination | Resistance gene-assisted recombination |
| Compound         | NA                       | Cephamycin C, Padienolide, Streptomyces sp. | Kasugamycin, Oxytetracycline, Rebeccamycin et al. | ACT |
| Reference        | Liu et al., 2016         | Komatsusa et al., 2010          | Komatsusa et al., 2010   | Zhang et al., 2017              |
| Reference        | Liu et al., 2016         | Komatsusa et al., 2010          | Komatsusa et al., 2010   | Bu et al., 2019                  |

ACT, actinorhodin; BGC, biosynthetic gene cluster; PKS, polyketide synthase gene; NRPS, non-ribosomal peptide synthase gene; NA, not available.

**Streptomyces chattanoogensis** L10 by Cre-loxP recombination system, respectively, thus generating two efficient chassis L320 and L321 for the production of valuable polyketides (Bu et al., 2019). In another study, a newly engineered host ZXJ-6 was developed based on a salinomycin-producing industrial strain, *Streptomyces albus* BK3-25, by deleting the salinomycin BGC and chromosomal integration of a three-gene cassette for the biosynthesis of ethylmalonyl-CoA. It was successfully used for the heterologous expression of polyketide BGCs, such as the ACT BGC from *S. coelicolor* (Zhang et al., 2017). In the future, the genome-reduced strategy could be widely implemented in a variety of industrial actinobacteria for the improved production of various bioactive compounds.

**CONCLUDING REMARKS**

Actinobacteria, especially bacteria from the genus *Streptomyces*, have long been employed as an important source of a wide range of novel bioactive small molecules (Barka et al., 2016; Newman and Cragg, 2016; Genilloud, 2017). To harness the production potential of actinobacteria, a variety of innovative metabolic engineering and synthetic biology strategies have been developed in the last 10 years, including dynamic metabolic regulation, BGC amplification, pathway refactoring and genome-minimized *Streptomyces* chassis (Beites and Mendes, 2015; Tan and Liu, 2017; Tan and Prather, 2017; Li et al., 2019a). We envision that NP discovery and development will be rapidly accelerated by the refactoring and amplification of whole biosynthetic pathways in combination with powerful heterologous expression platforms. Considering that a large number of BGCs are generally silent or expressed at very low levels, it is still important to develop diverse heterologous hosts and universal refactoring approaches to activate silent BGCs or boost production of secondary metabolites. Next, quorum sensing systems are widely distributed in actinobacteria (Polkade et al., 2016), which provide opportunities for bacterial growth-mediated dynamic regulation of metabolic pathways for enhanced production of target compounds. Furthermore, due to the complex regulation between primary and secondary metabolism, iterative application of the design-build-test-learn cycle will be necessary to overproduce different secondary metabolites (Liu et al., 2013; Kim et al., 2016; van der Heul et al., 2018). Finally, the advancements in the fields of genome sequencing, multi-omics and genome editing techniques are paving the way for systems metabolic engineering of industrial actinobacteria, including pathway engineering, regulatory circuit rewiring, host modification and enzyme engineering (Kim et al., 2016; Zhang et al., 2019). Given the requirement for high titers in commercial production, an integrated approach involving traditional mutagenesis screening and rational host/pathway engineering is required to systematically optimize the biosynthesis of target compounds. It is expected that these synthetic biology tools and metabolic engineering strategies presented in this review and future developments will play increasingly important roles in the discovery of novel drug leads, as well as yield improvement for large-scale manufacturing in actinobacteria.

**AUTHOR CONTRIBUTIONS**

LL and XL wrote the manuscript. LL, WJ, and YL reviewed, edited, and approved its final version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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