REVIEW

Recent advances in natural products exploitation in *Streptomyces* via synthetic biology

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Natural products of microbial origin have proven to be the wellspring of clinically useful compounds for human therapeutics. *Streptomyces* species are predominant sources of bioactive compounds, most of which serve as potential drug candidates. While the exploitation of natural products has been severely reduced over the past two decades, the growing crisis of evolution and dissemination of drug resistant pathogens have again attracted great interest in this field. The emerging synthetic biology has been heralded as a new bioengineering platform to discover novel bioactive compounds and expand bioactive natural products diversity and production. Herein, we review recent advances in the natural products exploitation of *Streptomyces* with the applications of synthetic biology from three major aspects, including recently developed synthetic biology tools, natural products biosynthetic pathway engineering strategies as well as chassis host modifications.

KEYWORDS

chassis engineering, heterologous expression, natural products, *Streptomyces*, synthetic biology

1 | INTRODUCTION

Molecules from natural sources have substantially contributed their therapeutic properties since time immemorial. Over the centuries, natural products and compounds derived there from and inspired thereby have been an immense source for new pharmaceuticals, therapeutic agents, and industrially useful compounds [1,2]. Since the late 1940s, *Streptomyces* species have been recognized as prolific producers of specialized bioactive natural products, especially antibiotics. Over two-

thirds of the medically useful antibiotics on the market are sourced from this genus, such as tetracycline, erythromycin, daptomycin, chloramphenicol, streptomycin, and vancomycin [3,4]. Clinically useful natural products originated from *Streptomyces* are not just confined to antibiotics, but also include antifungals (e.g., amphotericin B), antivirals (e.g., boromycin), antiparasitics (e.g., ivermectin), antitumorals (e.g., doxorubicin), anti-hypertensives (e.g., pikromycin), and immunosuppressants (e.g., rapamycin) [5–10].

The bioactive natural products are mainly generated by the rich reservoir of secondary metabolites biosynthetic pathways. One of the unique features of *Streptomyces* genomes is the wealth of secondary metabolites biosynthetic gene clusters (BGCs) that encode the protein machinery responsible for the production of secondary metabolites with a variety of chemotypes, including polyketides, lactams, terpenes, and nonribosomal peptides. In the recent 5–10 years, the advent of fast and inexpensive next-generation genome sequencing techniques has significantly enabled the practical exploration of tremendous secondary metabolites biosynthetic pathways with known or unknown products in *Streptomyces*.

Abbreviations: BAC, bacterial artificial chromosome; BGC, biosynthetic gene cluster; bp, base pair; CATCH, Cas9-assisted targeting of chromosome segments; CCTL, Cpf1-assisted cutting and taq DNA ligase-assisted ligation; CRISPR, clustered regularly interspaced short palindromic repeats; DSB, double-strand break; *E. coli*, *Escherichia coli*; exoCET, exonuclease in vitro assembly combined with RecET recombination; ICE, in vitro CRISPR/Cas9-mediated editing; LLHR, full-length RecET enhanced linear-linear homologous recombination; MASTER Ligation, methylation-assisted tailorable ends rational ligation; PTM, polycyclic tetramate macrolactam; TAR, transformation-associated recombination.

Meanwhile, the development of bioinformatics databases and computational metabolic engineering strategies, such as antiSMASH, kyoto encyclopedia of genes and genomes, genome-scale metabolic models, and StreptomeDB, have strikingly facilitated more effective and customized engineering of *Streptomyces* species for secondary metabolites production [11]. Furthermore, in order to activate the cryptic BGCs, as well as to either optimize or reconstruct the known valuable BGCs, the discovery and engineering of the related BGCs have been intensively studied [12–14] (Figure 1).

Synthetic biology is an engineering discipline with the aspiration to use synthetic circuitry and other engineered components to implement control over dynamic regulatory systems, hence interrogating natural biological phenomena, as well as creating novel cellular functions. In the context of natural products metabolism, synthetic biologists begin to draw on this diversity of sensors and regulatory elements, incorporating them into gene networks and applying their own design criteria to construct and further re-engineer the specific natural products pathways and host cells [1,3,4,15–18] (Figure 1). A multitude of previous studies on the *Streptomyces* species make them more amenable to the constantly updated technology of synthetic biology, leading to their maximal potential of producing bioactive natural products.

PRACTICAL APPLICATIONS

In this Mini-Review, we describe the recent advances in natural products discovery and overproduction in *Streptomyces* with emphasis on the applications of synthetic biology. It will highlight the recently developed synthetic biology techniques that are not only employed for the engineering of natural products biosynthetic pathways in *Streptomyces*, but also for the modification of *Streptomyces* native hosts.

This review aims at providing insights into some of the most recent progresses in natural products exploitation of *Streptomyces* with emphasis on the applications of synthetic biology strategies from three aspects. The first aspect is to summarize the newly developed synthetic biology tools for effective and specific natural products biosynthetic pathways engineering in *Streptomyces*. The second aspect is to epitomize the pathway specific engineering strategies for natural products exploitation in the *Streptomyces* native hosts. The third aspect is to recapitulate the *Streptomyces* host modification that prevails for heterologous expression of natural

FIGURE 1 General strategies for natural products discovery, modification, and overproduction in *Streptomyces* via synthetic biology

FIGURE 2 CRISPR-assisted in vivo genome editing tools for natural products discovery. (A) Using the CRISPR-Cas9/Cpf1 assisted knock-in/knock-out system, either the efficient and precise introduction of promoter cassettes (green-shaded arrows) or the deletion of negative regulators (black) drives the expression of biosynthetic genes (blue); (B) Using the CRISPR-dCas9/ddCpf1 assisted transcriptional activation/repression system, either the effective and strict repression of negative regulators (black) or the activation of cryptic biosynthetic genes (white) enables the expression of biosynthetic genes (blue)

products BGCs of interest. The strategies from all aspects have significantly facilitated overproduction of already known natural products as well as discovery, activation, and reengineering of novel natural products BGCs.

2 | CURRENT SYNTHETIC BIOLOGY TOOLS DEVELOPED FOR APPLICATIONS IN *STREPTOMYCES*

With the development of genetics and molecular biology, a collection of synthetic biology tools can be used for genetic manipulation in *Streptomyces*. Especially, the emerging of CRISPR/Cas systems contribute to the development of fast and efficient tools [19]. Here, we provide the concise descriptions and discussions of recent important tools in two parts: in vivo genome editing tools in *Streptomyces* and in vitro large DNA editing methods.

2.1 | In vivo genome editing tools in *Streptomyces*

Over the past decades, a set of in vivo genome editing methods have been developed to facilitate pathway engineering and modification. However, previous studies for genome editing in *Streptomyces* were mainly based on the traditional double crossover or single crossover [20–22]. Luckily, with the advent of CRISPR systems, tools for site-specific genome

editing in *Streptomyces* were rapidly developed (Figure 2). Those CRISPR-based gene manipulation tools complement traditional approaches and facilitate discovery of new compounds with significant bioactivities [19,23,24]. Ryan Cobb et al. [25] introduced the CRISPR/Cas9 system into *Streptomyces* and achieved fast multiplex genome editing via selected gene knock-in and knock-out. By using this system, the targeted chromosomal deletions of various sizes ranging from 20 bp to 30,000 bp were obtained in three different *Streptomyces* species with efficiency ranging from 70 to 100%. Subsequently, Mingzi Zhang et al. [26] developed an efficient CRISPR/Cas9 knock-in strategy for the efficient and precise insertions of the constitutive promoters upstream of the main biosynthetic operons or the pathway-specific activators, and successfully triggered the production of various novel natural products in different *Streptomyces* species. Moreover, Yawei Zhao et al. [27] developed an integrative CRISPR interference system based on the dCas9 protein, and efficient repression of four genes was achieved. In 2015, a new RNA-guided endonuclease Cpf1 (later known as Cas12a) of the Class 2 CRISPR system was characterized. Subsequent studies demonstrated that it has great potential in eukaryotic cell genome editing with low toxicity and off-target rate [28–30]. Because the toxicity of *SpCas9* in some important industrial *Streptomyces* strains severely limited the applications of the Cas9-based genome editing method, Lei Li et al. [31] developed a high-efficiency CRISPR/Cpf1 system for multiplex genome editing and transcriptional repression

in *Streptomyces*. This system was then successfully applied in an HDR-mediated gene deletion in the 5-oxomilbemycin-producing strain *Streptomyces hygroscopicus* SIPI-KF, in which *SpCas9* could not perform very well. Therefore, this Cpf1-based genome editing method would serve as an indispensable addition for the Cas9-based technologies and accelerate discovery and development of bioactive natural products. All in all, the development of new tools or genome editing platforms could provide more convenient, fast, and alternative solutions for genome editing in *Streptomyces* and thus boost the discovery of novel bioactive natural products.

2.2 | In vitro large DNA editing methods

To achieve the goal of heterologous BGC expression in designated chassis, approaches for manipulating and reconstructing BGCs were developed to exploit and characterize natural products. As the size of nature products BGCs usually ranges from 20 kb to 200 kb [32,33], the bacterial artificial chromosome (BAC) library construction is the most common tool applied for heterologous expression and screening of target BGCs in *Streptomyces* [34]. However, BAC library construction is usually laborious and time consuming. Therefore, the development of large DNA cloning methods is something indeed in need. In recent years, two major groups of cloning methods have been developed in *Streptomyces* that can bypass library construction (Table 1), including (1) direct cloning methods and (2) multiple DNA fragment assembly tools.

2.2.1 | Direct cloning methods

The yeast-based transformation-associated recombination (TAR) cloning was reported to perform the selective cloning of an entire genomic loci with sizes up to 250 kb from complex genomes [35]. This method relies on the powerful in vivo homologous recombination mechanism in *S. cerevisiae* and has been reported to successfully assemble the entire *Mycoplasma genitalium* genome [36]. In 2015, Nicholas Lee et al. [37] introduced the CRISPR/Cas9 system to generate double-strand breaks (DSBs) near the targeted genomic region, resulting in a dramatic increase of positive colonies (up to 32%). In the same year, Wenjun Jiang et al. [38] developed a method termed as Cas9-Assisted Targeting of CHromosome segments (CATCH) relying on the Cas9-assisted in vitro site-specific cleavage to clone large genomic regions into BACs. In this study, a 100 kb DNA fragment from *E. coli* genome was cloned with a 21.6% efficiency, and a 36 kb jadomycin-producing gene cluster from *Streptomyces venezuelae* was cloned with an efficiency of 90.5%. Although careful operation is required for CATCH manipulation, this method could serve as an effective molecular tool for the cloning of large gene clusters. Meanwhile, Li Lei et al. reported that the accept vector with high A+T% homologous arms can increase the assembly efficiency in cloning high

G+C% DNA fragments via Gibson assembly [39]. Therefore, the application of CATCH in BGC cloning was proven to be feasible. In 2016, another platform termed as “RedET” was developed on the basis of RecET direct cloning and Reda β recombineering systems [40]. This system has been successfully applied in LLHR (full-length RecET enhanced linear-linear homologous recombination) for a 52 kb *plu2670* clone from *Photobacterium luminescens* with a correction of 28.6% [41], and for a 106 kb salinomycin BGC from *Streptomyces albus* DSM41398 with a 16.6% correction [42]. In 2017, Hailong Wang et al. developed a new method termed as exoCET by coupling LLHR with the Cas9-assisted genome DNA digestion [43]. In this study, a 106 kb salinomycin BGC was obtained with a 4.2% correction and a 38 kb gene cluster cloning from *Photobacterium luminescens* was accomplished with a 100% correction. By using homologous recombination in *E. coli*, the RedET system usually saves 1–2 days to achieve large DNA cloning compared with yeast mediated assembly method, therefore it has been recognized as an ideal tool for direct cloning.

By incorporating with the Cas9-assisted DSB, TAR, CATCH, and exoCET can be used to directly clone a user-defined target in a more effective and flexible way compared with the restriction enzyme mediated direct cloning like LLHR. Besides, the development of large DNA modification methods leads to the further derived applications of BGCs cloning, such as the in vitro CRISPR/Cas9-mediated editing (ICE) method [44] and Cpf1-assisted cutting and taq DNA ligase-assisted ligation (CCTL) method [45]. These methods enable us to reassemble the BGCs constructs in a user-designed way, in terms of BGCs refactoring.

In summary, the advantages of the abovementioned direct cloning methods are time saving, easy to use and broadly feasible across a wide range of fragment sizes and genome complexities. It can be combined with other modification methods to facilitate the heterologous expression of various BGCs constructs.

2.2.2 | Multiple DNA fragments assembly tools

Apart from direct cloning methods, the tremendous developments of multiple DNA fragments assembly tools enable the DNA constructs cloning up to several hundred kilobases. Therefore, this type of methods has been widely recognized as an alternative way to clone BGCs.

A yeast mediated homologous recombination method, known as DNA assembler, can assemble an entire biochemical pathway containing multiple DNA fragments in a single step via in vivo transformation in *Saccharomyces cerevisiae* [46]. As a user-defined system, DNA assembler can be performed in accordance with the specific criterion of BGCs construction to achieve the BGCs refactoring in one step. For example, an optimized 45 kb spectinabilin BGC was

TABLE 1 Summary of recently developed cloning methods for BGCs construction and refactoring in *Streptomyces*

CLASS	METHOD	PRINCIPLE	CLONE TIME COST	EXAMPLE	EFFICIENCY	REFERENCE
DIRECT CLONING	TAR (Transformation-associated recombination)	In vivo homologous recombination of <i>Saccharomyces cerevisiae</i>	4-6 days	64.2 kb amorphadiene cluster	N/A	[79]
	Cas9-assisted TAR (Transformation-associated recombination)	Cas9-assisted site-specific cleavage and <i>in vivo</i> homologous recombination of <i>Saccharomyces cerevisiae</i>	5-7 days	55 kb human <i>NBS1</i> gene	N/A	[37]
	CATCH (Cas9-Assisted Targeting of CHromosome segments)	Cas9-assisted site-specific cleavage and Gibson assembly	3-4 days	150 kb <i>E. coli lacZ</i> gene contained genome fragment	1.90%	[38]
				36 kb jadmycin BGC from <i>Streptomyces venezuelae</i>	90.50%	[38]
				32 kb chlortetracycline BGC from <i>Streptomyces aureofaciens</i>	90.80%	[38]
	RedET (RecET direct cloning and Red α recombineering)	RecET-mediated linear-plus-linear homologous recombination (LLHR)	3 days	52 kb <i>plu2670</i> clone from <i>Photobacterium luminescens</i>	28.57%	[40]
				106 kb salinomycin BGC from <i>Streptomyces albus</i> DSM41398	16.66%	[42]
	exoCET (exonuclease <i>in vitro</i> assembly combined with RecET recombination)	T4 polymerase (T4pol) for <i>in vitro</i> annealing and RecET-mediated linear-plus-linear homologous recombination in <i>E. coli</i>	N/A	38 kb <i>plu3535-3532</i> gene cluster from <i>Photobacterium luminescens</i>	100%	[43]
LARGE DNA MODIFICATION METHOD		With the assist of Cas9	N/A	106 kb salinomycin BGC from <i>Streptomyces albus</i> DSM41398	4.16%	[43]
	ICE (in vitro CRISPR/Cas9-mediated editing)	Cas9-assisted site-specific cleavage and T4 polymerase annealing	3 days	specific insertion of the ampicillin resistance gene (bla, 0.7 kb) into pYH285(20 kb)	N/A	[44]
	CCTL (Cpf1-assisted Cutting and Taq DNA ligase-assisted Ligation)	Cpf1-assisted site-specific cleavage and Taq DNA ligase-assisted Ligation	2 days	substitution of the <i>actII-orf4</i> promoter in the actinorhodin BGC construct (32 kb) with <i>ermP</i> (300 bp).	78%	[45]
MULTIPLE DNA FRAGMENTS ASSEMBLY TOOLS	DNA assembler	Based on yeast <i>in vivo</i> HR on mechanism	4 days	assembly of 45 kb refactored spectinabilin BGC	N/A	[47]
	MASTER Ligation	Using MspII, which specifically recognizes methylated 4 bp sites and generates a 4 bp arbitrary overhang	2 days	assembly of 29 kb actinorhodin BGC from <i>Streptomyces coelicolor</i>	N/A	[49]

successfully assembled using this method with an efficiency of 30% [47].

In addition, a type of methods based on the restriction enzyme digestion has also been developed for BGCs construction. In 2013, the methylation-assisted tailorable ends rational (MASTER) ligation method was developed basing on a special type of restriction enzymes like MspII, which possesses both type IIM and type IIS properties and only recognizes the methylation-specific 4 bp sites, mCNR ($R = A \text{ or } G$) [48,49]. This method was used to successfully construct a 29 kb actinorhodin BGC in *Streptomyces coelicolor* [49].

As above described, the restriction enzyme-mediated methods are suitable for both multiple DNA fragment assembly and one step construction and refactoring of BGCs. However, with the limitations of restriction enzymes, these methods have several disadvantages in original genome editing. First of all, they generate undesirable “scar” sequences in almost every ligation site; secondly, the fidelities of PCR amplified DNA fragments for assembly could be compromised by the DNA polymerases, therefore, the assembled construct may contain nonspecific mutations. Thirdly, the molar ratio of DNA fragments is essential for the assembly efficiency, when the more DNA fragments are needed for assembly, the harder these methods to operate. Last, due to the low availability of the restriction enzymes, their scalability and feasibility are still circumscribed. However, there have been great efforts to overcome these drawbacks. For instance, recent studies reported that the CRISPR/Cpf1 system can cleave the 18th to 23rd bases distal from the PAM site, thereby generating a 5 bp user-defined sticky end [50]. Subsequent work further demonstrated its specific activity could integrate both advantages of long recognition sites and short scars when applied in the C-brick standard [50]. Therefore, the high cleavage similarity and compatibility between Cpf1 and type IIS restriction enzymes could confer great application potential and possibilities to develop an integrated high throughput one-pot DNA assembly method for the effective ligation of the multiple DNA fragments, hence resulting in the specific construction and refactoring of natural products BGCs.

In summary, (1) the direct cloning methods can assemble the target BGCs rapidly and accurately, but these constructs usually need postmodifications for the natural products BGCs activation; (2) in comparison to the direct cloning methods, the restriction enzyme assisted multiple DNA fragments assembly is more flexible in the biosynthetic gene rearrangement and the introduction of the bio-functional elements. This type of methods is more suitable for the user-designed and chemically synthesized DNA parts assembly to avoid the “scars” generated by the direct cloning methods; (3) in contrary to the above two types of methods, the yeast in vivo recombination mediated DNA assembler is a scarless gene editing method, but takes 1–2 more days to assemble the target construct.

3 | PATHWAY-SPECIFIC ENGINEERING TO EXPLOIT NATURAL PRODUCTS BIOSYNTHESIS IN *STREPTOMYCES*

The significance of studying *Streptomyces* as a chassis organism is that they are gifted with multitudinous secondary metabolic pathways to produce a variety of natural products. However, the low-titer or nondetectable production of natural products in fermentation is one of the major challenges. In secondary metabolisms, the transcriptional regulation system in *Streptomyces* is in charge of activating or inhibiting the transcription of specific natural products biosynthetic pathways by utilization of different raw materials, according to the extracellular environment and intracellular physiological status [51,52]. Currently, many pathway-specific engineering strategies have been well developed to facilitate natural products exploitations and can be divided into three major groups: (1) overexpressing or disrupting pleiotropic/pathway-specific regulators; (2) modifying transcription/translation elements; and (3) duplicating or higher order amplifying rate-limiting enzyme encoding genes or complete BGCs.

3.1 | Overexpressing or disrupting pleiotropic/pathway-specific regulators

Transcriptional regulation of natural products biosynthesis is pivotal for production improvement or new compounds activation. As we know, both global pleiotropic and cluster-situated regulators exist in the transcriptional regulation of secondary metabolism in *Streptomyces* [53]. According to the presence of unique regulatory mechanisms that govern the expression of individual natural products, a widely applicable synthetic biology approach based on the promoter profiling has been developed [54]. Either introduction of strong promoters upstream of main biosynthetic operon and pathway-specific active regulators, or deletion of negative regulators has been reported to improve the natural products production significantly [55–57]. A useful platform named plug-and-play has been developed and used in many cryptic gene clusters activations and BGCs overexpression [58,59]. For example, a giant cryptic BGC (150 kb) responsible for the macrolides stambomycins production in *Streptomyces ambofaciens* has been activated by constitutively expressing a pathway-specific activator within the cluster, which encodes a protein similar to the Large ATP binding of the LuxR (LAL) family proteins [33]. The same strategy has also been successfully applied in the actinorhodin production enhancement by expressing the cluster-specific activator *actII-orf4* [45]. Promoter introduction has been proved to be a generally applicable strategy and widely used in the cryptic gene cluster activation and natural products overproduction [54].

However, insertion of a single promoter in front of the selected genes does not work well in all *Streptomyces* species [60].

Another strategy for activating secondary metabolites expression is to regulate the correlation between *cis*-regulatory elements and transcription factors. Previous work demonstrated that *cebR* is the cellobiose-sensing key, which locks the expression of *txtR*, while *txtR* is the key necessary to unlock the production of phytotoxin [20]. With the understanding of the regulation relationship between the regulators *cebR* and *txtR*, the constitutive thaxtomin A production and hypervirulence of *Streptomyces scabies* were obtained by the *cebR* deletion [20,61]. In addition, the increase of milbemycin production was attributed to the deletion of *SbbA* in the *SbbR/SbbA* pair (an important *ArpA/AfsA*-like system) in a BGC from *Streptomyces bingchengensis* BC04 [21].

Previous works have demonstrated that the disruption of negative regulatory genes expression can improve the production of natural products [62]. For example, Young Ji Yoo et al. [55] deleted the *rapY* (a TetR-family repressor) and *rapRS* (a two-component response regulator and histidine kinase) in the rapamycin BGC in *Streptomyces rapamycinicus*, resulting in the rapamycin titer enhancement. Dongmei Xu et al. [63] identified a novel negative regulator *Mur34* responsible for the biosynthesis of muraymycin in *Streptomyces* sp. NRRL30471. By disrupting *Mur34*, a 10-fold increase in muraymycins C1 and D1 were detected. Disruption of common existed negative regulator genes would provide us a direct way for the titer enhancements of target compounds. For example, the disruption of *wblA* (a widespread negative regulator in polyketide BGCs) improved the yields of actinorhodin in *Streptomyces coelicolor*, doxorubicin, and daunorubicin in *Streptomyces peucetius*, and tautomycin in *Streptomyces* sp. CK4412 [64].

3.2 | Modification of transcription/translation elements

Modification of RNA polymerases (RNAP s), which initiate RNA transcription and regulate enzymes expression, has also attracted a great deal of interest for the activation of cryptic BGCs and the overproduction of natural products. For example, the *rpoB* gene, which encodes the β subunit of RNA polymerase, was well studied for nature products engineering. Kozo Ochi et al. [65] proved that some certain *rpoB* mutations were effective for activating the cryptic secondary metabolite biosynthetic genes, leading to the discovery of novel antibiotics, such as actinorhodin (Act), undecylprodigiosin (Red), and calcium-dependent antibiotic (CDA) from *Streptomyces lividans*. Another subunit of RNA polymerase HrdB has also been well studied. Haiyong Wang et al. [66] employed the exogenous principal sigma factor (σ^{HrdB}) with rational selection and engineering in *Actinoplanes teichomyceticus* L-27, and improved the teicoplanin production by over two-fold.

Similarly, by incorporating with the HrdB mutants, the transcription level of *aveR* (a BGC from *Streptomyces avermitilis*) was significantly increased and the production of avermectin B1a was enhanced by 1.5-fold than the wild strain [67].

Previous studies demonstrated that the ribosome engineering can improve natural products yields. Yuanyuan Pan et al. [68] demonstrated that the disruption of *rimP*, which encodes a 30S ribosome subunit assembly cofactor, resulted in higher titers of actinorhodin and CDA in *Streptomyces coelicolor* and jadomycin in *Streptomyces venezuelae*. Engineering of the transcription/translation elements would attract more attentions because of its great potential in cryptic BGCs activation and natural products yield enhancement.

3.3 | Duplication or higher order amplification of rate-limiting enzyme encoding genes or complete BGCs

The higher order amplification or duplication of individual genes, gene cassettes, or complete BGCs is a well-characterized strategy to improve natural products yields [69]. For example, the independent insertions of a single *ermEp** promoter in front of the *ftdA* and *ftdB* homologous genes from *Streptomyces albus* gave rise to two novel members of the polycyclic tetramate macrolactam (PTM)-family: 6-epi-alteramides A and B [60]. The insertion of *ermEp** in front of the indigoidine synthetase gene led to the discovery of the blue pigment indigoidine [60]. Yunzi Luo et al. [70] cloned and refactored a PTM BGC from *Streptomyces griseus* and discovered several new PTM compounds by introducing six different strong constructive promoters in front of all six genes in the characterized BGC. On the other hand, Lei Li et al. integrated five additional copies of the pristinamycin II (PII) BGC in *Streptomyces pristinaespiralis*, and generated the highest reported PII titers in both flasks and batch fermentations (216% higher than starting strain) [71].

It is concluded that the gene amplification could either relieve the rate limitation or enhance the competitiveness of target biosynthetic pathway, so this approach could be an alternative for natural products discovery, modification, and overproduction.

4 | HOST MODIFICATION FOR HETEROLOGOUS EXPRESSION OF NATURAL PRODUCTS BGCS IN STREPTOMYCES

Because genetic editing tools are more difficult to use in some *Streptomyces* species, the heterologous BGCs expression in well-characterized *Streptomyces* hosts could provide a good solution for natural products BGCs study when the genetic manipulations are not possible in the original host

cell [72]. *Streptomyces* species are broadly considered as the suitable chassis organisms for natural products production because they have a variety of essential elements for natural products overexpression, such as adequate substrates and similar sophisticated postmodification enzymes. Ideal *Streptomyces* chassis should have the following properties: relatively easy genetic manipulations for BGCs introduction, suitable growth rate for fermentation efficiency, and rational secondary metabolite products (“clean background”) for target BGCs detection.

At present, the most widely used *Streptomyces* chassis includes *Streptomyces coelicolor* [73], *Streptomyces lividans* [70], *Streptomyces avermitilis* [74], *Streptomyces albus* [75], and *Streptomyces venezuelae* [72]. To optimize *Streptomyces* chassis, the rational genome minimizing has been performed successfully in both *Streptomyces coelicolor* and *Streptomyces avermitilis* [76]. To engineer *Streptomyces coelicolor*, Min Zhou et al. [73] deleted a total of 1.6 Mb gene fragments and finally obtained a circularized genome in the *Streptomyces coelicolor* strain ZM12. The 14-membered macrolide antibiotic galbonolide B, which is originated from the marine actinomycete *Streptomyces* sp. LZ35, was produced with significantly boosted yield in the heterologous host *Streptomyces coelicolor* ZM12 [77]. The same strategy has also been used to modify *Streptomyces avermitilis*. Mamoru Komatsu et al. [76] deleted a region of more than 1.4 Mb from the 9.02 Mb *Streptomyces avermitilis* linear chromosome to generate a series of defined deletion mutants. Especially the SUKA17 and SUKA22, which exhibited no productivity of the major endogenous secondary metabolites found in the parent strain, were validated for the high efficiency heterologous expression of foreign metabolites like the streptomycin from *Streptomyces griseus*, cephamycin C from *Streptomyces clavuligerus*, and pladienolide from *Streptomyces platensis*. Subsequent studies indicated that the engineered hosts SUKA17 and SUKA22 are versatile for expressing the cryptic BGCs in the original producer [74].

Besides background elimination, Dimitris Kallifdas et al. [75] rationally engineered the host *Streptomyces albus* also by (1) manipulating the cluster-situated regulatory genes, for example, the deletion of *pfk* gene contributed to the increase of NADPH generation and hence improved the biomass containing NADPH-dependent enzymatic steps, (2) modifying the precursor flux, for instance, the overexpression of *crpSC* gene could boost the malonyl-CoA precursor accumulation, (3) regulating cell growth, and (4) activating the biosynthetic gene transcription to generate a series of mutants that possess enhanced the expression levels of the native and heterologous secondary metabolite BGCs.

The genome minimizing approaches described above were developed on the basis of traditional double crossover method and Cre-*loxP* mediated site-specific recombination. On the other hand, the latest development of CRISPR-assisted

genome deletion strategies has been identified to be capable of eliminating host “background” with site-specific manners, relatively high efficiencies and less time [26]. Undeniably, with the discovery and modification of new *Streptomyces* chassis, the heterologous expression of BGCs would play a more essential role in discovery and overexpression of natural products.

5 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Natural products and their derivatives have been historically splendid as a source of therapeutic agents and continue to endow the immense importance for a substantial fraction of human therapeutics. Microorganisms produce a vast diversity of fascinating natural products not available in any other system. Among them, *Streptomyces* species have been recognized and utilized as an ideal chassis organism for natural products discovery and overexpression. However, they currently still have lots of genetic and biochemical mysteries to be elucidated. The current period is widely anticipated to be a new renaissance or golden age for natural products discovery. Many of the systems presented in this review, such as CRISPR/Cas system and type IIS enzymes based multi-fragments assembly systems, indicate that the advent of synthetic biology provides great potential and possibilities for flexibly and precisely controlled natural products overproduction, structural diversification, and heterologous reproducibility.

The CRISPR/Cas system is the most representative and state-of-the-art technology among the implications of synthetic biology. Due to its preeminent features, including higher sequence specificity, artificial guided targeting, unique adaptive nature, and superior editing efficiency, CRISPR/Cas systems have been proven to be the most versatile genomic engineering technology in the history of molecular biology to date. The diverse CRISPR/Cas systems, of which one was discovered from *Streptomyces* [78], have been developed rapidly over past 5 years and transitioned from exciting new academic research to ubiquitous synthetic biology tools with unprecedented ease to use. The introduction of these techniques for various high-efficiency genome editing in *Streptomyces* has significantly flourished in natural product discovery field, including genomics-driven discovery combined with genetic manipulations in heterologous hosts. However, in contrary to other model organisms, such as *E. coli* and *S. cerevisiae*, CRISPR/Cas system-based genome editing or natural products BGCs refactoring for *Streptomyces* are still relatively narrower in range. Therefore, looking forward, it seems plausible that existing CRISPR systems are being engineered to possess innovative capabilities, and excitingly new CRISPR systems with novel functions are still being exploited. Furthermore,

with the rapid development of synthetic biology, various types of *Streptomyces*-derived elements, especially sensors, regulators, promoters, and well-characterized signal cascade systems, could be explored and characterized for creating novel genetic circuits, hence, rewiring the natural products biosynthetic pathways.

Apart from genome editing technologies suitable for small-scale re-engineering on the limited genome loci, the implementation success of synthetic biology in *Streptomyces* species also relies on de novo genome synthesis technologies to artificially generate genome-minimized heterologous hosts with clean background. The exponentially decreased cost and dramatically enhanced productivity and fidelity of genome synthesis, combined with a growing understanding of natural products BGCs expression criteria as well as biosynthetic biochemistry mechanism expand our capabilities in heterologous host modification of *Streptomyces* underlying large scales and high complexities.

In the future, the combination of leading-edge technologies including advanced genome editing, assembly of sophisticated gene networks, high-throughput genome synthesis, and in silico natural products biosynthesis modeling will drive synthetic biology to the next level. Consequently, the embodiment of traditional metabolic engineering and vast natural products BGCs bioinformatics packages in collaborative synthetic biology would help manipulation of both BGCs and heterologous hosts. We can envision that in *Streptomyces*, overproduction of known natural products would be accomplished; novel natural products with medical or industrial importance could be discovered or constructed, and a better understanding of the promiscuous natural products metabolism machinery would be undertaken.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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