Efficient editing DNA regions with high sequence identity in actinomycetal genomes by a CRISPR-Cas9 system

Jingjun Mo\textsuperscript{a}, Shuwen Wang\textsuperscript{a}, Wan Zhang\textsuperscript{a}, Chunyu Li\textsuperscript{a}, Zixin Deng\textsuperscript{a}, Lixin Zhang\textsuperscript{b}, Xudong Qu\textsuperscript{a,\ast}

\textsuperscript{a} Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Ministry of Education, School of Pharmaceutical Sciences, Wuhan University, Wuhan, China
\textsuperscript{b} State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China

A R T I C L E   I N F O

Keywords:
Actinobacteria
Biosynthesis
Polyketide synthase
CRISPR/Cas9
Antimycin
Actinomycetes

A B S T R A C T

Actinobacteria able to produce varieties of bioactive natural products have been long appreciated by the field of drug discovery and development. Recently, a few of CRISPR/Cas9 systems bearing different types of replicons (pSG5 and pLJ101) were developed to efficiently edit their genomes. Despite wide application in gene editing, their utility in editing challenging DNA regions e.g. high sequence identity has not been compared. In this study, we confirmed that the widely used temperature-sensitive pSG5 replicon is indeed not suitable for editing modular polyketide synthase (PKS) genes due to causing unpredicted gene recombination. This problem can be addressed by replacing the pSG5 with the segregationally unstable pLJ101 replicon. By introducing a counter-selection marker CadA, convenient cloning sites in the single guide RNAs (sgRNAs) and homologous template scaffolds, we developed a new CRISPR-Cas9 system pMWCas9. This system was successfully used to delete/replace erythromycin PKS and other biosynthetic genes in Saccharopolyspora erythraea and Streptomyces sp. AL2110. By swapping the promoters of antB and amm with ermE and kasOp, we achieved a deacyl-antimycin hyper producer which produces a 9-fold higher yield than the original Streptomyces sp. AL2110 strain. Our results provide a robust and useful Cas9 tool for genetic studies in Actinobacteria.

1. Introduction

Natural products (NPs) are critical for drug discovery and development [1]. Over the past decades, significant efforts have been devoted to understanding the logic of NP biosynthesis [2]. With this basis, biosynthetic pathways engineering or genome-based NP discovery have been successfully used to identify numerous novel bioactive compounds [3]. However, it was revealed that the majority of microbial metabolites repertoire indeed still have not yet been accessed. More than 90% of biosynthetic pathways are silenced under normal laboratory culturing conditions due to the absence of the essential regulatory signals to trigger biosynthesis [4]. To activate their biosynthesis, gene modification targeting different regulation stages is usually required [5,6].

Actinomycetes are among of the most prolific producers of diverse NPs, which contribute about 73% of total bacterial NPs and 45% of total microbial NPs [7]. The diverse genomic contents and relatively low growth rate make genetic manipulation of their genome much more challenging than other model organisms, such as Escherichia coli. The typical approach for genome editing is based on RecA mediated double-crossover [8]. However, the efficiency of inducing the second-crossover by this approach is low, which often takes weeks to accumulate a significant number of double-crossover mutants for PCR verification [8]. Although the introduction of a double strand break (DSB) at the genomic locus of interest can drastically increase the efficiency of double-crossover, previous approaches, such as using the meganuclease I-SceI, require prior integration of the endonuclease recognition site at the target locus [9]. The overall efficacy of this method is, thus, still very low.

Recently, the more efficient DSB mediated genome editing has been achieved by the type II clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated (Cas) system [10–13]. By co-expression with the customized single guide RNAs (sgRNAs), the Streptococcus pyogenes Cas9 nuclease can mediate a DSB in the sgRNA binding region; efficient double crossover can be achieved when the homologous templates are provided in the same plasmid. Currently, a few CRISPR systems have been developed and applied in editing actinomycetal genomes, among of which pCRISPR-Cas9, pCRISPomycetes, pKCas9D0 and pWHU2653 are the four most widely used systems [10–13]. These systems are mainly different in the replicon type as well as the way of expressing Cas9 nuclease. The pCRISPR-Cas9,
pCRISpomycetes and pKCcas9dO have a temperature sensitive pSG5 replicon, while pWHU2653 bears a segregationally unstable pLI101 replicon; Cas9 protein in pCRISPR-Cas9 and pKCcas9dO is induced by thiostrepton (Tsr) while the nucleases in other two systems are constitutively expressed. Although all these systems have been successfully applied in genome editing, their efficiency in editing challenging DNA regions, e.g. with high sequence identity, has been not comparatively evaluated. Recently, the result shows that pCK1139 derived (with pSG5) plasmids can cause random recombination of the rapamycin and tylosin modular PKS genes when they were used to gene replacement [14]. It was assumed the pSG5 replicon might be the major reason for causing recombination. As three of the four widely used systems pCRISPR-Cas9, pCRISpomycetes and pKCcas9dO are pSG5-based, thus evaluation their utility in editing modular PKS genes is necessary.

In this study, we confirmed that the pSG5 replicon is indeed the major reason for causing non-desired gene recombination, and the Cas9 system with pSG5 is not suitable to edit the modular PKS gene. Substitution the replicon by pLI101 can overcome this problem. By integration of inducible expression of Cas9, convenient cloning sites in the sgRNAs region and homologous templates and a counter-selection marker codA, we further upgraded this Cas9 system. This new system was successfully used to efficiently edit the modular PKS and other genes in erythromycin and antimycin biosynthetic pathways. By engineering the promoter region in Streptomyces sp. AL2110, deacetyl antimycins was dramatically improved to a 9-fold compared with the wild-type strain.

2. Material and methods

2.1. Strains, chemicals, and general materials

Strains and plasmids used in this study are listed in Tables S1 and S2. Escherichia coli DH5α was used as cloning host. E. coli ET12567/pUZ8002 was used for intergeneric conjugation between E. coli and Sac. erythraea HL3168 E3 or S. sp. AL2110. Sac. erythraea HL3168 E3 was grown at 30 °C on corn steep liquid agar medium (10 g corn steep liquor, 10 g sucrose, 3 g NaCl, 3 g (NH4)2SO4, 5 g CaCO3, 3 g yeast extract, 3 g tryptone, 20 g agar per liter, pH = 7.0) and S. sp. AL2110 on MS agar medium [8] (20 g soy flour, 20 g D-mannitol, 20 g agar per liter) for sporulation and at the same temperature. Both Sac. erythraea HL3168 E3 and S. sp. AL2110 were grown in tryptone soya broth (TSB) liquid medium [8] (30 g tryptone soya broth powder per liter) for the growth of mycelium and isolation of total DNA. Corn steep liquor or MS agar medium with the addition of 10 mM MgCl2 was used for intergeneric conjugation between E. coli and Sac. erythraea or Streptomyces, respectively. E. coli strains were maintained in LB medium (10 g tryptone, 5 g yeast extract, 10 g sodium chloride per liter) at 37 °C with the appropriate antibiotic selection (50 μg ml⁻¹ ampicillin, 50 μg ml⁻¹ chloramphenicol, and 50 μg ml⁻¹ kanamycin). For Sac. erythraea, tri-methoprIm (50 μg ml⁻¹) and apramycin (50 μg ml⁻¹) were used in corn steep liquid agar medium or TSB while nalidixic acid (30 μg ml⁻¹) and apramycin (50 μg ml⁻¹) were used in MS agar medium or TSB for Streptomyces. Soy bean oil medium (30 g soluble starch, 30 g soy flour, 40 g dextrin, 10 mL soybean oil, (NH4)2SO4 2 g, CaCO3 6 g per liter, pH = 7.0–7.2) was used for erythromycin production in Sac. erythraea HL3168 E3. M3-S (10 g glucose, 50 g soluble starch, 6 g (NH4)2SO4, 1.5 g CaCO3 per liter, pH = 7.0) was used for deacetyl antimycins production in S. sp. AL2110.

Erythromycin and antimycin were purchased from Energy Chemical and Sigma Co. Ltd., respectively. Chromatography reagent ammonium acetate was purchased from Aladdin. Chromatography reagent acetoneitrile and methanol were purchased from Sigma. All chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd or Shanghai Sangon Biotech (China) Co., Ltd unless noted otherwise. HPLC analysis was carried out on a SHIMADZU LC-20A Prominance HPLC system. LC-MS analysis was carried out on a Thermo Instruments HPLC system connected to LCQ Fleet electrospray ionization (ESI) mass spectrometer (ThermoFisher Scientific Inc.). ESI-high resolution MS (ESI-HRMS) analysis was carried out on ESI-LTQ Orbitrap (ThermoFisher Scientific Inc.).

2.2. DNA manipulation

Primers used in this study are listed in Table S3. Restriction endonucleases and T4 DNA ligase were purchased from NEB. PrimeSTAR HS DNA Polymerase with GC Buffer and In-Fusion HD Cloning kit were purchased from Takara. Oligonucleotide primer synthesis was performed by Genwiz and DNA sequencing was performed by Biosune. Standard procedures were used for DNA purification, PCR, subcloning, and molecular analysis [15]. All kits and enzymes were used according to the manufacturers' recommendations.

2.3. Vector construction for eryAIII knock out in Sac. erythraea

A sgRNA scaffold containing NcoI-XbaI cloning site was introduced to pCRISPR-Cas9 to give pCAS9-1. The spacer inserts including gene specific 20 nt guide sequence were generated by annealing two 34 nt synthesized oligonucleotides, sgerAIII-f and sgerAIII-r. The annealed oligos were cloned into pCAS9-1 at NcoI-XbaI. The 1.5 kb UHA (upper homologous arm) and DNA (down homologous arm) sequences were then amplified by the primer pairs eryAIII-L-f/eryAIII-L-r and eryAIII-R-f/eryAIII-R-r from genomic DNA of Sac. erythraea HL3168 E3, spliced by overlap extension PCR and cloned into the above-mentioned plasmid at Stul by infusion cloning kit, generating plasmid pZW1 for gene knock out. Primers am-ori-f/am-ori-r were used to amplify a fragment containing coliE1 and auxC3IV from pZW-1 and the fragment was cloned into pYH7 at XbaI-KpnI to give pZW-0, containing rep(pLI101). Subsequently, an 8 kb fragment was released from plasmid pZW-1 by BamH-Stul and cloned into pZW-0 to give pZW-2 (Fig. S1). pZW-1 and pZW-2 were then transformed into ET12567/pUZ8002 for subsequent introduction into Sac. erythraea by conjugation, respectively.

2.4. Constructions of the CRISPR-Cas9 vectors

Primers cm-f/cm-r were used to amplify the chloramphenicol resistance gene (cat) from pACYC184. Inducible promoter ptpA and thiostrepton resistance gene (ter) were amplified from pCRISPR-Cas9 using two pairs of primers, tsr-f/tsr-r and ptpA-f/ptpA-r, respectively. Those three fragments were subsequently spliced together by overlap extension PCR, each end flanked by 39-bp homology sequence from pWHU2653. Then, the cat-tptpA-ts-r fragment was inserted into pWHU2653 by PCR-targeting, replacing the constitutive promoter controlling the scas9 gene, the rest of the vector was ligated by T4 ligase to give pMWcas9-1. To construct the double-enzyme digestion sgRNA cloning cassette, primers pmw-l-f/pmw-l-r were used to amplify the upstream fragment from pWHU2653 while another pair of primers, pmw-l/f/pmw-l-r were used for the downstream fragment amplification. The two fragments were spliced together by overlap extension PCR, generates a 0.3 kb fragment. The 0.3 kb sgRNA cloning cassette was then inserted into pMWcas9-1 between XbaI-NheI using infusion cloning kit. Subsequently, the synthesized multiple cloning site (MCS), including Hpal, SpeI, Stul, was cloned into the HindIII site, giving plasmid pMWcas9 (Fig. S2).

2.5. Construction of genome editing vectors

The spacer inserts including gene specific 20 nt guide sequence were generated by annealing two 34 nt synthesized oligonucleotides, sgerAIII-f/sgerAIII-r for eryAIII knock out in Sac. erythraea HL3168 E3 and sgap-l-f/sgap-l-r for promoter antBp and antCp replacement in S. sp AL2110. The annealed oligos were cloned into pMWcas9 at EcoR-
XhoI, respectively. The 2.9 kb HA (homologous arm) was amplified from pZW-2 and cloned into the above-mentioned plasmid at HindIII by infusion cloning kit, generating plasmid pWHU2654 for gene knock out. The 1.5 kb UHA and DHA sequences were amplified from genomic DNA of the S. sp AL2110, using the primer pairs, alpt-l-f/alpt-l-r and alpt-r-f/alpt-r-r. The relevant UHA and DHA were then spliced together by overlap extension PCR, using primer pair alpt-l-f/alpt-r-r to generate approximately 3 kb HA fragments. These fragments were cloned into the above-mentioned plasmid at corresponding cloning site at MCS (shown in Table S3), generates vector pALPT. Promoter permE* and kasOp were amplified using primer pairs erme-l/erme-r and kasop-l/kasop-r from pWHU2653 and pkasop-T, respectively. The two promoters were then spliced together using primer pairs kasop-l/erme-r and introduced to pALPT at Hpal site via in vitro homologous recombination, generates pEKPT. Both pWHU2654 and pEKPT were subsequently used for genome editing.

For construction of the pWHU2653 based plasmid. A sgRNA scaffold including gene specific 20 nt guide sequence CTGCGATCAACCTCGA CCA were amplified from pWHU2653 with primer pairs sg2653-eryaIIIfF/sg2653-eryaIII-b and sg2653-eryaIIIcF/sg2653-erya3-d, then spliced by overlap extension PCR and cloned into pWHU2653 at XhoI/NheI by infusion cloning kit. The 2.9 kb HA (homologous arm) was released from pWHU2654 with HindIII and cloned into the above-mentioned plasmid at HindIII by T4 ligase, generating plasmid pWHU2655 for gene knock out.

2.6. Transfer of the plasmids from E. coli to Sac. erythraea or S. sp. AL2110 by conjugation

The relevant plasmids first were transferred into E. coli ET12567/pUZ8002 cells by electroporation. Conjugation between E. coli ET12567/pUZ8002 and Sac. erythraea HL3168 E3 or S. sp. AL2110 was carried out as described previously [16,17]. The plates were incubated for 3–7 days at 30 °C, or until conjugates became visible.

2.7. Genome editing induced by CRISPR-Cas9

After conjugation, apramycin-resistant exconjugants were inoculated into 3 mL TSB medium again containing apramycin and trimethoprim and grown at 30 °C for 2 or 3 days. Subsequently, 2 μg mL⁻¹ Tsr was added to induce the cleavage of their target DNA. The induced culture was then streaked onto MS agar plate to obtain single colonies. Each colony was inoculated into TSB liquid medium for growth of culture was then streaked onto MS agar plate to obtain single colonies. Tsr was added to induce the cleavage of their target DNA. The induced mutant mWHU2484 were inoculated into 50 mL TSB medium and grown at 30 °C for 3 or 4 days as the seed culture. The mycelium grown in the TSB medium was then fermented in 100 mL soy bean oil medium.

For erythromycin, strains Sac. erythraea HL3168 E3 and its derived mutant mWHU2484 were inoculated into 50 mL TSB medium and grown at 30 °C for 3 or 4 days as the seed culture. The mycelium grown in the TSB medium was then fermented in 100 mL soy bean oil medium.
mWHU2486 were deduced by fitting to the standard curve of antimycin sample (Sigma Co., Ltd.).

HPLC and LC-MS analysis of erythromycin were performed on a column of Venusil HPL C18 (2) (5 μm, 250 × 4.6 mm, Agela Technologies Inc.) at a flow rate of 1 mL min⁻¹ and a UV detection at 210 nm over a 40 min gradient program: T = 0 min, 40% B; T = 10 min, 40% B; T = 11 min, 55% B; T = 36 min, 55% B; T = 37 min, 40% B; T = 40 min, 40% B. (A, H₂O + 10 mM CH₃COONH₄ pH = 9.7, and B, CH₃CN). For deacyl antimycins, HPLC and LC-MS analysis were performed on a column of Inertsil ODS-3 (5 μm, 250 × 4.6 mm, GL Sciences B.V.) at a flow rate of 1 mL min⁻¹ and a UV detection at 229 nm and 319 nm over a 30 min gradient program [18]: T = 0 min, 40% B; T = 20 min, 80% B; T = 21 min, 100% B; T = 25 min, 100% B; T = 26 min, 40% B; T = 30 min, 40% B. (A, H₂O + 0.1% formic acid, and B, CH₃CN + 0.1% formic acid).

3. Results and discussion

3.1. pCRISPR-cas9 causes unpredicted recombination of modular PKS genes

Constitutive expression of Cas9 will induce DSB immediately after the plasmid is transformed into the cell. As the rate of successful reparation of DSB by gene replacement is low, these kinds of Cas9 systems e.g. pWHU2653 often require a high plasmid transformation efficiency. This could be challenging for bacteria system recalcitrant to be transformed, for instance, many industrial strains. Thus, for getting higher transformation efficiency, the inducible expression is more advantageous over constitutive expression of the Cas9 nuclease. Based on this consideration, we chose pCRISPR-Cas9 for this study.

Erythromycin is an important family of antibiotics. Currently, its natural form and semi-synthetic derivatives, such as azithromycin, clarithromycin, dirithromycin, and roxithromycin have been widely used for treating the second recombination event to lose the plasmid. This pSG5-mediated replication fork collides with the natural fork leading to the temporary arrest and subsequent genome instability and rearrangements. To repair this catastrophic event, flanking PKS genes are forced to exert homologous recombination for removing the pSG5 encoding region, which leads to many unpredicted recombination events. However, pCRISPR-Cas9 system is different from pKC1139 integrated into genome, which exerts double crossover in plasmid form. Thus, the mechanism for causing random recombination should be different as assumed before.

To verify the essential role of pSG5 in this event, the pSG5 replicon was replaced by the segregational instability plJ101 replicon together with a Tsr resistance gene from the plasmid pYH7 [22]. The generated plasmid pZW-2 (Fig. S1) was introduced into Sac. erythraea HL3168 E3 through the same procedure. Identical to pZW-1, efficient gene replacement was observed when 2 μg mL⁻¹ Tsr was added into the culture (Fig. 1A). This strain was inoculated onto the MS agar plate to lose the delivery plasmid. Unlike the aforementioned pSG5-derived Cas9 system, all the tested colonies (80) from five-round culture have successfully lost the delivery plasmid. The deletion of eryAIII was further verified by both PCR and fermentation analysis (Fig. 1B), which confirms the plJ101 is effective for editing modular PKS gene. The mechanism for pSG5-induced recombination is currently not clear. We assumed that the elevated temperature (39°C) to prevent the replication of pSG5 may cause the emergency reaction of bacteria. As such reactions, for instance, SOS response can extensively induce DNA repair systems; and genes with high sequence identity can probably undergo homogeneous recombination. Taken together these results confirmed that the pSG5 replicon indeed is the major factor for causing the recombination even and using plJ101 replicon can overcome this problem.

3.3. Construction of a new Cas9 system and used to edit biosynthetic genes of erythromycin in Sac. erythraea

As the pZW-2 is not convenient for gene cloning, we further set out to construct a new plJ101-based Cas9 vector (Fig. 2). The tar and tipA cassette from pCRISPR-CAS was introduced into the pWHU2653 via PCR-Targeting to replace the original constitutive promoter. The resulted plasmid was then infused with a sgRNA cassette containing an EcoRI-XbaI cloning site via in vitro recombination. In this cassette, two nucleotides A and G in the original sgRNA scaffold were mutated into C and T for introducing a unique XbaI site. For installation of the target sequence, two short primers (each is 34 nt in a form of 5’-AATTCCN20 GTTTTAGG-3’ and 5’-CTAGCTTAAANCN20G-3’ (N20 is the target sequence and N20 is its complementary sequence) were mixed and annealed to form a double stranded DNA. This DNA fragment contains the correct nucleotides AG and has terminal cohesive ends complementary to the Ncol and XbaI sticky sites. Through ligation, both target sequences and correct nucleotides AG can be efficiently introduced into sgRNA scaffold to constitute the functional sgRNA (Fig. 2A). Finally, a 44 bp synthetic oligonucleotide was inserted into the restriction site of HindIII for introducing a multi-cloning site to insert homologous template. This new plasmid pMWCas9 has a plJ101 replicon, a cloning site in the sgRNA region, a multi-cloning site for the homologous template and a codA counter-selectable marker, thus could be more robust and convenient than its parent Cas9 systems.

To verify the function of this plasmid, this was then subjected to deleting the PKS genes eryAIII. As similar to the above mentioned, sgRNA and homologous template were individually cloned into the EcoRI-XbaI site and HindIII site. Identical sgRNA and homologous template were also introduced into the pWHU2653. Both constructed plasmids (pWHU2654 and pWHU2655) were then introduced into the Sac.
erythraea HL3168 E3. Like assumed, the transformation of the pWHU2653 system (pWHU2655) was not successful, while pMWCas9 system (pWHU2654) can be efficiently introduced into Sac. erythraea with the efficiency of 21–82 cfu/μL (Fig. S5), confirming inducible expression is more advantageous over constitutive expression of the Cas9 nuclease. Like the pZW-2, both of efficient double crossover and loss of the delivery plasmid were achieved by the pMWCas9 system, which resulted in rapid deletion of eryAIII (Fig. 1C). This result confirms the robustness and usefulness of the new pIJ101-based Cas9 system for editing highly repetitive modular PKS genes.

3.4. Editing the promotor region in S. sp. AL2110 to increase the production yield of deacyl-antimycin

Finally, to demonstrate broad utility of this Cas9 system in other Actinomycetes species, it was used to edit the regulatory regions between antB and antC in the antimycin gene clusters from S. sp. NRRL AL2110 [17]. Antimycin is a group of industrial useful NRPS-PKS hybrid metabolites. Their biosynthesis has been elucidated by us and other groups [23–27]. It was revealed that binding of FscRI in the DNA region between antB and antC is essential for transcription of antimycin biosynthetic genes [27]. In order to remove the regulatory effect and boost the transcription of the ant genes, the promotor regions between antB and antC were replaced by a cassette containing two constitutive strong promoters perm* and kasO (Fig. 3A) [8,28] through Cas9 mediated double crossover. Like in Sac. erythraea, both efficient double crossover and loss of the delivery plasmid was achieved. This recombinant strain was further sent for fermentation analysis. To our gratification, the production yield of deacyl-antimycins is significantly improved in this strain (62 mg/L at D5) which is 9-fold higher than the wild type strain AL2110 (6.4 mg/L at D5) (Fig. 3B and C). This strain provides an excellent basis for further engineering the biosynthesis of antimycin to produce novel bioactive structural varieties. Taken together, these results demonstrated that this new cas9 system is very robust and useful to effectively edit Actinomycetal genomes.

4. Conclusion

In summary, we confirmed that the pSG5 replicon is the major reason for causing unpredicted recombination and not suitable for editing DNA region with high sequence identity e.g. modular PKS genes. This problem can be overcome using the pIJ101 replicon. Our newly developed Cas9 system with pIJ101 replicon, inducible Cas9 cassette, codA counter selection marker and convenient cloning sites in the sgRNA scaffolds and homologous template can largely facilitate plasmid construction, and offer robust and efficient genome editing, which is highly important for study Actinobacteria. Finally, both eryAIII deleted Sac. erythraea and ANTs yield-boosted Streptomycetes.sp. AL2110 strains provide a convenient platform for further engineering their structural diversity through pathway engineering.

Author contributions

JM, SW, WZ and CL conducted the experiments. XQ, LZ and ZD planned and supervised the study. XQ and JM wrote the paper. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing financial interest(s).

Acknowledgements

The authors are grateful to Prof. Wen Liu (Shanghai Institute of Organic Chemistry) for赐予的strain of Sac. erythraea HL3168 E3, Dr. Ming Jiang (Shanghai Jiao Tong University) for helping to develop the HPLC condition of erythromycin and Prof. Yuhui Sun (Wuhan University) for kindly providing the pWHU2653 and pYH7. This work...
was supported by the National Nature Science Foundation of China Grants (Nos. 31570057, 31430002, 31320103911 and 31770063), Taishan Scholarship and “the Fundamental Research Funds for the Central Universities 22221818014.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2019.02.004.

References

[1] Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016;79:629–61.
[2] Walsh CT, Tang Y. Natural product biosynthesis: chemical logic and enzymatic machinery. Royal Society of Chemistry. 2017.
[3] Katz L, Balza RH. Natural product discovery: past, present, and future. J Ind Microbiol Biotechnol 2016;43:155–76.
[4] Net M, Ikeda H, Moore BS. Genomic basis for natural product biosynthetic diversity in the Actinomycetes. Nat Prod Rep 2009;26:1362–84.
[5] Rutledge PJ, Challis GL. Discovery of microbial natural products by activation of silent biosynthetic gene clusters. Nat Rev Microbiol 2015;13:509–23.
[6] Zhang B, Tian W, Wang S, Yan X, Jia X, et al. Activation of natural products biosynthetic pathways via a protein modification level regulation. ACS Chem Biol 2017;12:1732–6.
[7] Hopwood DA. Streptomyces in nature and medicine: the antibiotic makers. Oxford University Press, Inc.; 2007.
[8] Kieser T, Bibb M, Butter M, Chater KF, Hopwood DA. Practical streptomyces genetics. The John Innes Foundation; 2001.
[9] Fernández-Martínez LT, Bibb MJ. Use of the meganuclease I-SceI of Saccharomyces cerevisiae to select for gene deletions in Actinomycetes. Sci Rep 2014;4:7100.
[10] Tong Y, Charusanti P, Zhang L, Weber T, Lee SY. CRISPR-Cas9 based engineering of Actinomycetal genomes. ACS Synth Biol 2015;4:1020–9.
[11] Cobb RE, Wang Y, Zhao H. High-efficiency CRISPR-Cas9-mediated genome editing in Streptomyces. Acta Biochim Biophys Sin 2015;47:231–43.
[12] Wang Y, Wang Y, Chu J, Zhuang Y, Zhang I, et al. Improved production of erythromycin A by expression of a heterologous gene encoding S-adenosylmethionine synthetase. Appl Microbiol Biotechnol 2007;75:837–42.
[13] Wang Y, Chen J, Zhang L, Zheng Q, Han Y, et al. Multiplexing of combinatorial chemistry in antmicin biosynthesis expansion of molecular diversity and utility. Angew Chem Int Ed 2013;52:12308–12.
[14] Zhang L, Mori T, Zhang Q, Awakawa T, Yan Y, et al. Rational control of polyketide extender units by structure-based engineering of a crotonyl-CoA carboxylase/reductase in antmicin biosynthesis. Angew Chem Int Ed Engl 2015;54:13462–5.
[15] Katz L, Ashley GW. Translation and protein synthesis: Macrolides. Chem Rev 2005;105:499–528.
[16] Dunn BJ, Khosla C. Engineering the acyltransferase substrate specificity of assembly line polyketide synthases. J R Soc Interface 2013;10. 20130297.
[17] Li Y, Zhang W, Zhang H, Tian W, Wu L, et al. Structural basis of a broadly selective acyltransferase from the polyketide synthase of splicinolin. Angew Chem Int Ed 2018;57:5823–7.
[18] Sun Y, He X, Liang J, Zhou X, Deng Z. Analysis of functions in plasmid pHZ1358 influencing its genetic and structural stability in Streptomyces lividans 1326. Appl Microbiol Biotechnol 2009;82:303–10.
[19] Yan Y, Zhang L, Ito T, Qu X, Asakawa Y, et al. Biosynthetic pathway for high structural diversity of a common dilactone core in antmicin production. Org Lett 2012;14:4142–5.
[20] Sandy M, Kui Z, Gallagher J, Zhang W. Enzymatic synthesis of dilactone scaffold of antmicins. ACS Chem Biol 2012;7:1956–61.
[21] Ilka S, Christian P, DJ S, Bertrand A, Pierre L, et al. An unprecedented 1,2-shift in the biosynthesis of the 3-aminoalicyclate moiety of antmicins. Chembiochem 2012;13:769–73.
[22] Chang C, Huang R, Yan Y, Ma H, Dai Z, et al. Uncovering the formation and selection of benzylmalonyl-CoA from the biosynthesis of splicinolin and enterocin reveals a versatile way to introduce amino acids into polyketide carbon scaffolds. J Am Chem Soc 2015;137:4183–90.
[23] Sandy M, Kui Z, Gallagher J, Zhang W. Enzymatic synthesis of dilactone scaffold of antmicins. ACS Chem Biol 2012;7:1956–61.
[24] Sandy M, Kui Z, Gallagher J, Zhang W. Enzymatic synthesis of dilactone scaffold of antmicins. ACS Chem Biol 2012;7:1956–61.
[25] McLean TC, Horkinson PA, Seipke RF. Coordinate regulation of antmicin and candidicidin biosynthesis. mSphere 2016;1:e00305–16.
[26] Wang W, Li X, Wang J, Xiang S, Feng X, et al. An engineered strong promoter for Streptomyces. Appl Environ Microbiol 2013;79:4484–92.