

Additional file 1

Identification and characterization of a strong constitutive promoter *stnYp* for activation of biosynthetic genes and production of natural products in *Streptomyces*

Wenli Guo¹, Zhihong Xiao¹, Tingting Huang^{1,3}, Kai Zhang⁴, Hai-Xue Pan⁴, Gong-Li Tang⁴, Zixin Deng^{1,3},
Rubing Liang^{1,3*}, and Shuangjun Lin^{1,2,3*}

¹ State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory on Metabolic & Developmental Sciences, School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

² Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

³ Haihe Laboratory of Synthetic Biology, Tianjin 300308, China

⁴ State Key Laboratory of Bioorganic and Natural Products Chemistry, Center for Excellence in Molecular Synthesis, Shanghai Institute of Organic Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, China

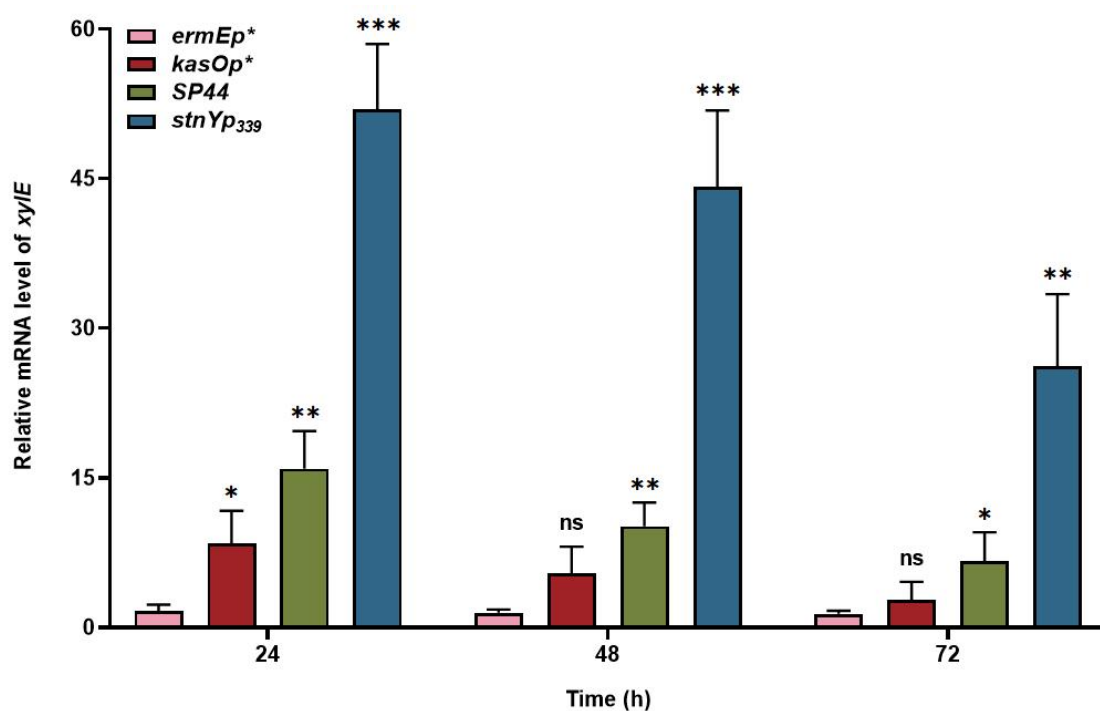
Corresponding author:

Rubing Liang: State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory on Metabolic & Developmental Sciences, School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China. Email: icelike@sjtu.edu.cn

Shuangjun Lin: State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory on Metabolic & Developmental Sciences, School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China; Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China. Email: linsj@sjtu.edu.cn

- 24 **Figure S1** Transcription analyses of *xylE* in the control of different promoters.
- 25 **Figure S2** Nucleotide sequences of promoter *stnYp*₃₃₉.
- 26 **Figure S3** The co-transcription analysis of genes in the *ym* cluster.
- 27 **Figure S4** Applications of promoter *stnYp* for aureonuclemycin overproduction in heterologous hosts.
- 28 **Figure S5** Applications of Promoter *stnYp* for YM-216391 overproduction in heterologous hosts.
- 29 **Figure S6** Transcript assay of YM-216391 biosynthetic gene cluster.
- 30 **Figure S7** Applications of promoter *stnYp* for eliminating by-products of tylosin in industrial strains *S.*
- 31 *fradiae*.
- 32 **Table S1.** Bacterial strains used in this study.
- 33 **Table S2.** Plasmids used in this study.
- 34 **Table S3.** Primers used in this study.
- 35
- 36

37 **Figure S1. Transcription analyses of *xyIE* in the control of different promoters.** Quantitative analysis of
 38 the transcriptional levels of *xyIE* reporter gene driven by different promoters in *S. albus* J1074 after 24 h, 48 h
 39 and 72 h culture in YEME. Transcription of *hrdB* gene was used to normalize the transcription levels in
 40 different samples. The relative transcription level of *ermEp** were set as 1.0.



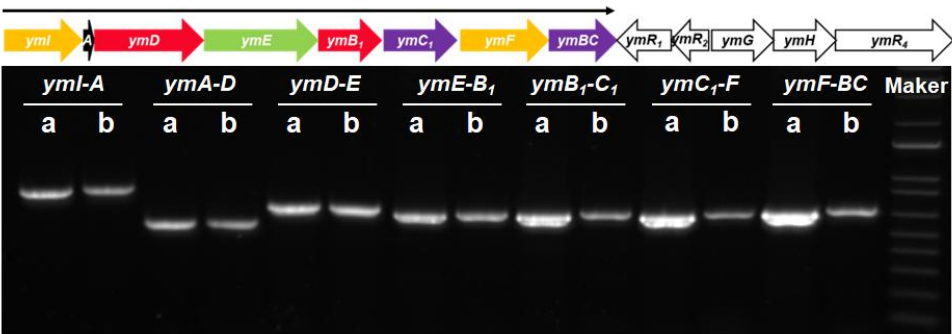
43 **Figure S2. Nucleotide sequences of promoter *stnYp₃₃₉*.** The sequence is numbered on the left. The TSS is
 44 indicated by a bent arrow and bigger letters. The TSS was determined by rapid amplification of 5'-cDNA ends
 45 (5'RACE). The putative -10 and -35 motifs of *stnYp₃₃₉* are marked by dashed frames. Promoter elements of
 46 the *stnYp₃₃₉* were deduced based on promoter prediction database SAPPHERE. The translation start codon of
 47 *stnY* gene is marked in gray letters and the translated amino acids are given below the nucleotide sequence.
 48 TSS, Transcription Start Site.

```

1  TGTGCGAGCATAACCTCTGCCGCCGGGTCGGGGTAACTCACTGCGGTGCC
51  GACTCGCCTACGGCATAACGGTGGTTGTACGCGCTATTACGGCGCCTTCG
101 CATTCTCGCGCAGCACACCCATCACGCCCATGGTGAATGCCGGTGGCGGG
151 CCGAGGCGGCGAATACGGGGCGGTGCCGCCGGCCGCCGGCCGTGGCGGG
201 GCAGGGAGCGGCGGGGGACGGCATCGTCCGCATCCGGTCCGCGAAGGATG
251 GCCGGAACCTTCTCCATGAGGTCGCCGCGGCGGGCATGCATGGCGTGCGA
301 CGGCTAGCCTGCTAGCATGCTCATGACTGCTGAAGAGGTGAGGCAG
      -10 motif      TSS      -35 motif
                        [StnY]: Val Arg Gln
  
```

49

50 **Figure S3. The co-transcription analysis of genes in the *ym* cluster.** Horizontal black arrows above genes
 51 represent the putative transcripts, namely, *ymI-BC*. The present products of the intergenic regions showed that
 52 the two genes are co-transcribed, otherwise their transcripts were independent. a: the cosmid pTG1104 was
 53 used as a template. b: the cDNA of *S. albus*-pTG1104 was used as a template.



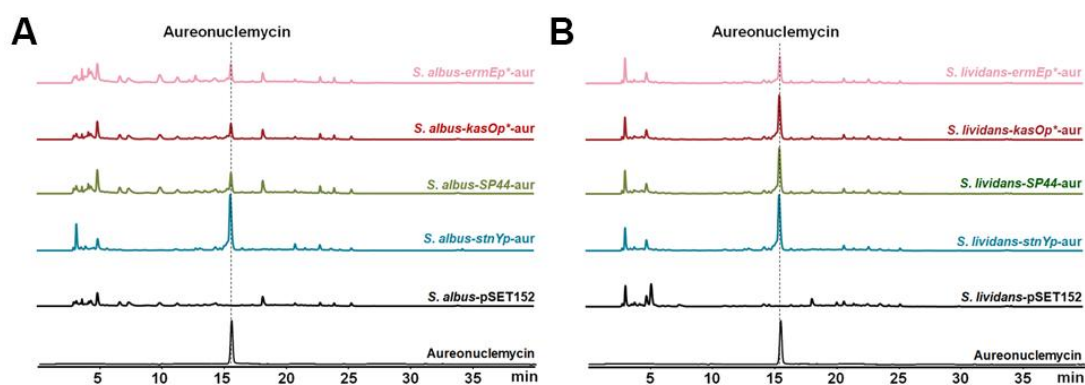
55 **Figure S4. Applications of promoter *stnYp* for aureonuclemycin overproduction in heterologous hosts.**

56 (A) Chromatograms of aureonuclemycin production in five recombinant strains (*S. albus-ermEp**-aur, *S.*

57 *albus-kasOp**-aur, *S. albus-SP44*-aur, *S. albus-stnYp*-aur and *S. albus*-pSET152). (B) Chromatograms of

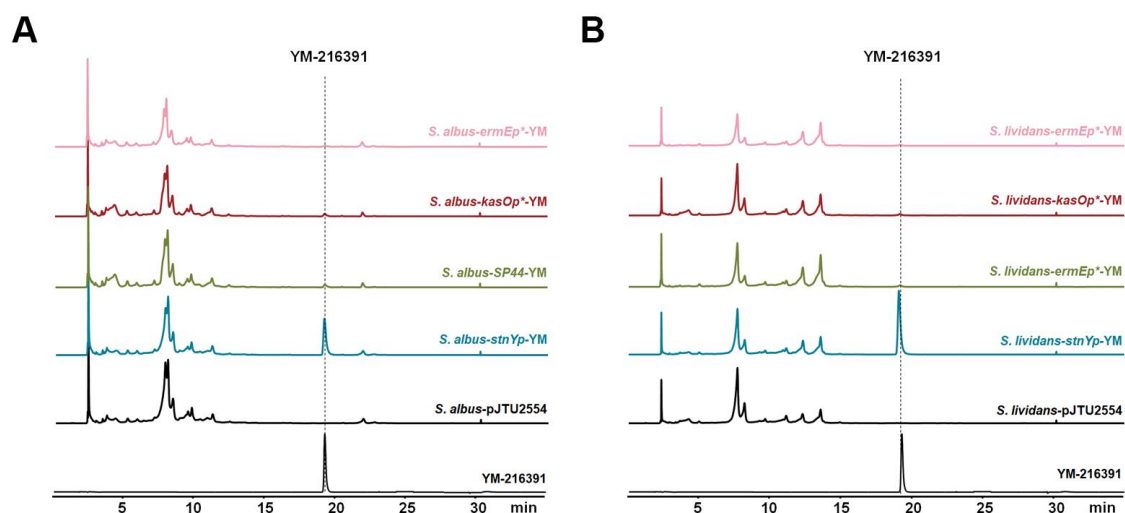
58 aureonuclemycin production in five recombinant strains (*S. lividans-ermEp**-aur, *S. lividans-kasOp**-aur, *S.*

59 *lividans-SP44*-aur, *S. lividans-stnYp*-aur and *S. lividans*-pSET152).



60

61 **Figure S5. Applications of Promoter *stnYp* for YM-216391 overproduction in heterologous hosts. (A)**
 62 Chromatograms of YM-216391 production in five recombinant strains (*S. albus-ermEp**-YM, *S.*
 63 *albus-kasOp**-YM, *S. albus-SP44*-YM, *S. albus-stnYp*-YM and *S. albus-pJTU2554*). (B) Chromatograms of
 64 YM-216391 production in in five recombinant strains (*S. lividans-ermEp**-YM, *S. lividans-kasOp**-YM, *S.*
 65 *lividans-SP44*-YM, *S. lividans-stnYp*-YM and *S. lividans-pJTU2554*).



67 **Figure S6. Transcript assay of YM-216391 biosynthetic gene cluster.** The transcription of representative
 68 *ym* genes in the four recombination strains were detected at 24 h and 72 h. Transcription of *hrdB* gene was
 69 used to normalize the transcription levels in different samples. The relative transcription level of *ym* genes
 70 under the control of promoter *ermEp** was valued to 1.0.

71

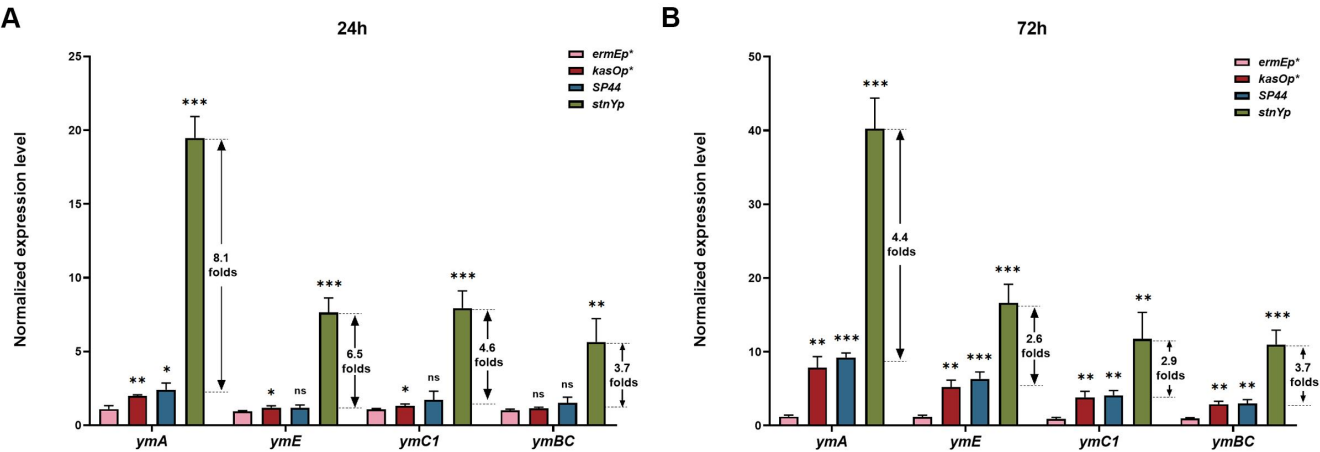


Figure S7 Applications of promoter *stnYp* for eliminating by-products of tylosin in industrial strains *S. fradiae*. (A) Chromatograms of fermentation culture of the recombinant strains with *tylF* driven by promoters *ermEp**, *kasOp**, *SP44* or *stnYp*. (B) Chromatograms of fermentation culture of the recombinant strains with *tylI* driven by promoters *ermEp**, *kasOp**, *SP44* or *stnYp*. (C) Chromatograms of fermentation culture of the recombinant strains with *tylF* and *tylI* driven by promoter *stnYp*.

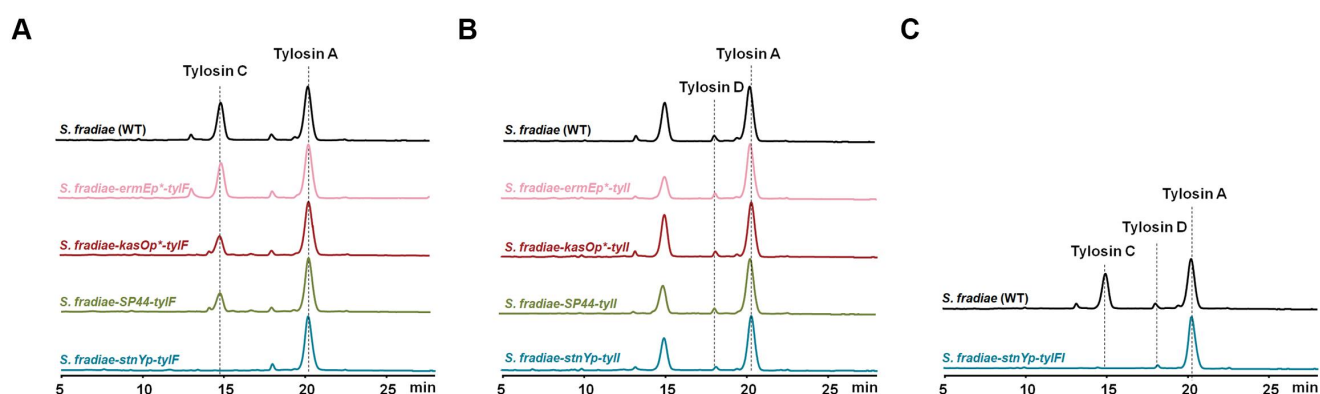


Table S1. Bacterial strains used in this study.

Bacteria strains	Relevant characteristics	Ref/Source
<i>Streptomyces</i> strains		
<i>S. flocculus</i> CGMCC 4.1223	Streptonigrin wild type producing strain	[1]
<i>S. venezuela</i> ISP5230	Wild type, <i>Streptomyces</i> host strain for heterologous expression	[2]
<i>S. venezuela-xylE</i>	<i>S. venezuela</i> ISP5230 containing pDR3, Apr ^R	This study
<i>S. venezuela-ermEp*-xylE</i>	<i>S. venezuela</i> ISP5230 containing pDR3- <i>ermEp*</i> , Apr ^R	This study
<i>S. venezuela-kasOp*-xylE</i>	<i>S. venezuela</i> ISP5230 containing pDR3- <i>kasOp*</i> , Apr ^R	This study
<i>S. venezuela-SP44-xylE</i>	<i>S. venezuela</i> ISP5230 containing pDR3- <i>SP44</i> , Apr ^R	This study
<i>S. venezuela-stnYp-xylE</i>	<i>S. venezuela</i> ISP5230 containing pDR3- <i>stnYp</i> , Apr ^R	This study
<i>S. coelicolor</i> M1154	A derivative of the wild-type strain A3(2) lacking plasmids SCP1 and SCP2	[3]
<i>S. coelicolor-xylE</i>	<i>S. coelicolor</i> M1154 containing pDR3, Apr ^R	This study
<i>S. coelicolor-ermEp*-xylE</i>	<i>S. coelicolor</i> M1154 containing pDR3- <i>ermEp*</i> , Apr ^R	This study
<i>S. coelicolor-kasOp*-xylE</i>	<i>S. coelicolor</i> M1154 containing pDR3- <i>kasOp*</i> , Apr ^R	This study
<i>S. coelicolor-SP44-xylE</i>	<i>S. coelicolor</i> M1154 containing pDR3- <i>SP44</i> , Apr ^R	This study
<i>S. coelicolor-stnYp-xylE</i>	<i>S. coelicolor</i> M1154 containing pDR3- <i>stnYp</i> , Apr ^R	This study
<i>S. lividans</i> TK24	A plasmid-free derivative of <i>S. lividans</i> 66	[4]
<i>S. lividans-xylE</i>	<i>S. lividans</i> TK24 containing pDR3, Apr ^R	This study
<i>S. lividans-ermEp*-xylE</i>	<i>S. lividans</i> TK24 containing pDR3- <i>ermEp*</i> , Apr ^R	This study
<i>S. lividans-kasOp*-xylE</i>	<i>S. lividans</i> TK24 containing pDR3- <i>kasOp*</i> , Apr ^R	This study

<i>S. lividans</i> -SP44- <i>xylE</i>	<i>S. lividans</i> TK24 containing pDR3-SP44, Apr ^R	This study
<i>S. lividans</i> - <i>stnYp</i> - <i>xylE</i>	<i>S. lividans</i> TK24 containing pDR3- <i>stnYp</i> , Apr ^R	This study
<i>S. lividans</i> -pSET152	<i>S. lividans</i> TK24 containing pSET152, Apr ^R	This study
<i>S. lividans</i> - <i>ermEp</i> *-anm	<i>S. lividans</i> TK24 containing pSET- <i>ermEp</i> *-anm, Apr ^R	This study
<i>S. lividans</i> - <i>kasOp</i> *-anm	<i>S. lividans</i> TK24 containing pSET- <i>kasOp</i> *-anm, Apr ^R	This study
<i>S. lividans</i> -SP44-anm	<i>S. lividans</i> TK24 containing pSET-SP44-anm, Apr ^R	This study
<i>S. lividans</i> - <i>stnYp</i> -anm	<i>S. lividans</i> TK24 containing pSET- <i>stnYp</i> -anm, Apr ^R	This study
<i>S. lividans</i> -pJTU2554	<i>S. lividans</i> TK24 containing pJTU2554, Apr ^R	This study
<i>S. lividans</i> - <i>ermEp</i> *-YM	<i>S. lividans</i> TK24 containing YM- <i>ermEp</i> *, Apr ^R	This study
<i>S. lividans</i> - <i>kasOp</i> *-YM	<i>S. lividans</i> TK24 containing YM- <i>kasOp</i> *, Apr ^R	This study
<i>S. lividans</i> -SP44-YM	<i>S. lividans</i> TK24 containing YM-SP44, Apr ^R	This study
<i>S. lividans</i> - <i>stnYp</i> -YM	<i>S. lividans</i> TK24 containing YM- <i>stnYp</i> , Apr ^R	This study
<i>S. albidoflavus</i> J1074	A derivative of <i>S. albidoflavus</i> G1 defective in the [5] <i>Sal</i> IG1 restriction–modification system.	
<i>S. albus</i> -pDR3	<i>S. albus</i> J1074 containing pDR3, Apr ^R	This study
<i>S. albus</i> - <i>ermEp</i> *- <i>xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>ermEp</i> *, Apr ^R	This study
<i>S. albus</i> - <i>kasOp</i> *- <i>xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>kasOp</i> *, Apr ^R	This study
<i>S. albus</i> -SP44- <i>xylE</i>	<i>S. albus</i> J1074 containing pDR3-SP44, Apr ^R	This study
<i>S. albus</i> - <i>stnYp</i> ₃₃₉ - <i>xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>stnYp</i> ₃₃₉ , Apr ^R	This study
<i>S. albus</i> - <i>stnYp</i> ₁₅₀ - <i>xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>stnYp</i> ₁₅₀ , Apr ^R	This study
<i>S. albus</i> - <i>stnYp</i> ₁₀₀ - <i>xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>stnYp</i> ₁₀₀ , Apr ^R	This study
<i>S. albus</i> - <i>stnYp</i> ₈₀ - <i>xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>stnYp</i> ₈₀ , Apr ^R	This study

<i>S. albus-stnYpup₆₀- xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>stnYp₆₀</i> , Apr ^R	This study
<i>S. albus-stnYpup₃₀- xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>stnYp₃₀</i> , Apr ^R	This study
<i>S. albus-stnYpup₁₀- xylE</i>	<i>S. albus</i> J1074 containing pDR3- <i>stnYp₁₀</i> , Apr ^R	This study
<i>S. albus-indC</i>	<i>S. albus</i> J1074 containing pSET- <i>indC</i> , Apr ^R	This study
<i>S. albus-ermEp*-indC</i>	<i>S. albus</i> J1074 containing pSET- <i>ermEp*-indC</i> , Apr ^R	This study
<i>S. albus-kasOp*-indC</i>	<i>S. albus</i> J1074 containing pSET- <i>kasOp*-indC</i> , Apr ^R	This study
<i>S. albus-SP44-indC</i>	<i>S. albus</i> J1074 containing pSET- <i>SP44-indC</i> , Apr ^R	This study
<i>S. albus-stnYp-indC</i>	<i>S. albus</i> J1074 containing pSET- <i>stnYp-indC</i> , Apr ^R	This study
<i>S. albus-pSET152</i>	<i>S. albus</i> J1074 containing pSET152, Apr ^R	This study
<i>S. albus-ermEp*-anm</i>	<i>S. albus</i> J1074 containing pSET- <i>ermEp*-anm</i> , Apr ^R	This study
<i>S. albus-kasOp*-anm</i>	<i>S. albus</i> J1074 containing pSET- <i>kasOp*-anm</i> , Apr ^R	This study
<i>S. albus-SP44-anm</i>	<i>S. albus</i> J1074 containing pSET- <i>SP44-anm</i> , Apr ^R	This study
<i>S. albus-stnYp-anm</i>	<i>S. albus</i> J1074 containing pSET- <i>stnYp-anm</i> , Apr ^R	This study
<i>S. albus-pJTU2554</i>	<i>S. albus</i> J1074 containing pJTU2554, Apr ^R	This study
<i>S. albus-ermEp*-YM</i>	<i>S. albus</i> J1074 containing YM- <i>ermEp*</i> , Apr ^R	This study
<i>S. albus-kasOp*-YM</i>	<i>S. albus</i> J1074 containing YM- <i>kasOp*</i> , Apr ^R	This study
<i>S. albus-SP44-YM</i>	<i>S. albus</i> J1074 containing YM- <i>SP44</i> , Apr ^R	This study
<i>S. albus-stnYp-YM</i>	<i>S. albus</i> J1074 containing YM- <i>stnYp</i> , Apr ^R	This study
<i>Streptomyces fradiae</i>	Industrial strain	
<i>S. fradiae-ermEp*-tylF</i>	<i>S. fradiae</i> containing pSET- <i>ermEp*-tylF</i> , Apr ^R	This study
<i>S. fradiae-kasOp*-tylF</i>	<i>S. fradiae</i> J1074 containing pSET- <i>kasOp*- tylF</i> , Apr ^R	This study
<i>S. fradiae-SP44-tylF</i>	<i>S. fradiae</i> J1074 containing pSET- <i>SP44- tylF</i> , Apr ^R	This study

<i>S. fradiae</i> - <i>stnY</i> - <i>tylF</i>	<i>S. fradiae</i> J1074 containing pSET- <i>stnYp</i> - <i>tylF</i> , Apr ^R	This study
<i>S. fradiae</i> - <i>ermEp</i> *- <i>tylI</i>	<i>S. fradiae</i> containing pSET- <i>ermEp</i> *- <i>tylI</i> , Apr ^R	This study
<i>S. fradiae</i> - <i>kasOp</i> *- <i>tylI</i>	<i>S. fradiae</i> J1074 containing pSET- <i>kasOp</i> *- <i>tylI</i> , Apr ^R	This study
<i>S. fradiae</i> - <i>SP44</i> - <i>tylI</i>	<i>S. fradiae</i> J1074 containing pSET- <i>SP44</i> - <i>tylI</i> , Apr ^R	This study
<i>S. fradiae</i> - <i>stnY</i> - <i>tylI</i>	<i>S. fradiae</i> J1074 containing pSET- <i>stnYp</i> - <i>tylI</i> , Apr ^R	This study
<i>S. fradiae</i> - <i>stnY</i> - <i>tylFI</i>	<i>S. fradiae</i> J1074 containing pSET- <i>stnYp</i> - <i>tylFI</i> , Apr ^R	This study

***E. coli* strains**

<i>E. coli</i> DH5 α	F ⁻ ϕ 80 <i>lacZ</i> Δ M15 Δ (<i>lacZYA</i> - <i>argF</i>)	ThermoFisher
	U169 <i>recA1 endA1 hsdR17</i> (r _K ⁻ ,m _K ⁺) <i>phoA supE44</i>	Scientific
	λ^- <i>thi-1 gyrA96 relA1</i>	
<i>E. coli</i> ET12567/pUZ8002	<i>dam dcm hsdS cat tet</i> /pUZ8002	[6]
<i>E. coli</i> BW25113/pIJ790	K-12 derivative; Δ <i>araBAD</i> Δ <i>rhaBAD</i>	[7]

Table S2. Plasmids used in this study.

Plasmid	Relevant characteristics	Ref/source
Plasmids		
pDR3	Double-reporter vector containing a promoterless <i>xylE-neo</i> cassette, Apr ^R	[8]
pDR3- <i>ermEp</i> *	A derivative of pDR3 containing the promoter <i>ermEp</i> *, Apr ^R	This study
pDR3- <i>kasOp</i> *	A derivative of pDR3 containing the promoter <i>kasOp</i> *, Apr ^R	This study
pDR3- <i>SP44</i>	A derivative of pDR3 containing the promoter <i>SP44</i> , Apr ^R	This study
pDR3- <i>stnYp₃₃₉</i>	A derivative of pDR3 containing the promoter <i>stnYp₃₃₉</i> , Apr ^R	This study
pDR3- <i>stnYpup₁₅₀</i>	A derivative of pDR3 containing the promoter <i>stnYpup₁₅₀</i> , Apr ^R	This study
pDR3- <i>stnYpup₁₀₀</i>	A derivative of pDR3 containing the promoter <i>stnYpup₁₀₀</i> , Apr ^R	This study
pDR3- <i>stnYpup₈₀</i>	A derivative of pDR3 containing the promoter <i>stnYpup₈₀</i> , Apr ^R	This study
pDR3- <i>stnYpup₆₀</i>	A derivative of pDR3 containing the promoter <i>stnYpup₆₀</i> (<i>stnYp</i>), Apr ^R	This study
(pDR3- <i>stnYp</i>)		
pDR3- <i>stnYpup₃₀</i>	A derivative of pDR3 containing the promoter <i>stnYpup₃₀</i> , Apr ^R	This study

pDR3- <i>stnYpup₁₀</i>	A derivative of pDR3 containing the promoter <i>stnYpup₁₀</i> , Apr ^R	This study
pSET152	Integrative vector, Apr ^R	[9]
pSET- <i>ermEp</i> *- <i>indC</i>	A derivative of pSET- <i>indC</i> containing the promoter <i>ermEp</i> *, Apr ^R	This study
pSET- <i>kasOp</i> *- <i>indC</i>	A derivative of pSET- <i>indC</i> containing the promoter <i>kasOp</i> *, Apr ^R	This study
pSET- <i>SP44</i> - <i>indC</i>	A derivative of pSET- <i>indC</i> containing the promoter <i>SP44</i> , Apr ^R	This study
pSET- <i>stnYp</i> - <i>indC</i>	A derivative of pSET- <i>indC</i> containing the promoter <i>stnYp</i> , Apr ^R	This study
pSET-anmBCDE	A derivative of pSET152 containing the entire Aureonuclemycin gene cluster, Apr ^R	[10]
pSET- <i>ermEp</i> *-anm	A derivative of pSET-anmBCDE with replacement of the native <i>anmB</i> promoter by <i>ermEp</i> *, Apr ^R	This study
pSET- <i>kasOp</i> *-anm	A derivative of pSET-anmBCDE with replacement of the native <i>anmB</i> promoter by <i>kasOp</i> *, Apr ^R	This study
pSET- <i>SP44</i> -anm	A derivative of pSET-anmBCDE with replacement of the native <i>anmB</i> promoter by <i>SP44</i> , Apr ^R	This study
pSET- <i>stnYp</i> -anm	A derivative of pSET-anmBCDE with replacement of the native <i>anmB</i> promoter by <i>stnYp</i> , Apr ^R	This study
pTG1104	Conjugative and Φ C31-integrative cosmid containing the YM-216391 gene cluster, Apr ^R	[11]

YM- <i>ermEp</i> *	A derivative of pTG1104 with replacement of the native <i>ymI</i> promoter by <i>emEp</i> *, Apr ^R , Kan ^R	This study
YM- <i>kasOp</i> *	A derivative of pTG1104 with replacement of the native <i>ymI</i> promoter by <i>kasOp</i> *, Apr ^R , Kan ^R	This study
YM- <i>SP44</i>	A derivative of pTG1104 with replacement of the native <i>ymI</i> promoter by <i>SP44</i> , Apr ^R , Kan ^R	This study
YM- <i>stnYp</i>	A derivative of pTG1104 with replacement of the native <i>ymI</i> promoter by <i>stnYp</i> , Apr ^R , Kan ^R	This study
pSET- <i>ermEp</i> *- <i>tylF</i>	A derivative of pSET152 with overexpression of <i>tylF</i> by <i>ermEp</i> *, Apr ^R	This study
pSET- <i>kasOp</i> *- <i>tylF</i>	A derivative of pSET152 with overexpression of <i>tylF</i> by <i>kasOp</i> *, Apr ^R	This study
pSET- <i>SP44</i> - <i>tylF</i>	A derivative of pSET152 with overexpression of <i>tylF</i> by <i>SP44</i> , Apr ^R	This study
pSET- <i>stnYp</i> - <i>tylF</i>	A derivative of pSET152 with overexpression of <i>tylF</i> by <i>stnYp</i> , Apr ^R	This study
pSET- <i>ermEp</i> *- <i>tylI</i>	A derivative of pSET152 with overexpression of <i>tylI</i> by <i>ermEp</i> *, Apr ^R	This study
pSET- <i>kasOp</i> *- <i>tylI</i>	A derivative of pSET152 with overexpression of <i>tylI</i> by <i>kasOp</i> *, Apr ^R	This study
pSET- <i>SP44</i> - <i>tylI</i>	A derivative of pSET152 with overexpression of <i>tylI</i> by <i>SP44</i> , Apr ^R	This study
pSET- <i>stnYp</i> - <i>tylI</i>	A derivative of pSET152 with overexpression of <i>tylI</i> by <i>stnYp</i> , Apr ^R	This study

pSET- <i>stnYp-tylFI</i>	A derivative of pSET152 with overexpression of <i>tylFI</i> by <i>stnYp</i> , Apr ^R	This study
pIJ790	λ-RED (<i>gam</i> , <i>bet</i> , <i>exo</i>), <i>cat</i> , <i>araC</i> , <i>rep101</i> ^{ts}	[12]
pJTU4659	containing the <i>neo</i> cassette used for replace the gene using PCR targeting and λ-Red-mediated recombination, Kan ^R	This lab
pJTU2554	3.9-kb <i>XbaI/XhoI</i> fragment from pSET152 that was ligated with 5.5-kb <i>XbaI/XhoI</i> fragment, Apr ^R	[13]

82 Apr^R apramycin resistance, Kan^R kanamycin resistance, Amp^R ampicillin resistance.

Table S3. Primers used in this study

Primer	Sequence (5'-3')^{a, b}	Purpose
stnYp ₃₉₉ F	aggaggaactatatccgcgggatcctgtgcgagcataacctctgc	For construction of plasmids pDR3- <i>stnYp</i> ₃₃₉ , pDR3- <i>stnYpup</i> ₁₅₀ , pDR3- <i>stnYpup</i> ₁₀₀ , pDR3- <i>stnYpup</i> ₈₀ , pDR3- <i>stnYpup</i> ₆₀ , pDR3- <i>stnYpup</i> ₃₀ , pDR3- <i>stnYpup</i> ₁₀ , pDR3- <i>ermEp</i> *, pDR3- <i>kasOp</i> *, and pDR3- <i>SP44</i> .
stnYpup ₁₅₀ F	aggaggaactatatccgcgggatccccgggtggcgggcccaggcg	
stnYpup ₁₀₀ F	aggaggaactatatccgcgggatccgccgtggcggggcaggag	
stnYpup ₈₀ F	aggaggaactatatccgcgggatccggcggggacggcatcgtc	
stnYpup ₆₀ F	aggaggaactatatccgcgggatccgcacccggtcgcgaaggat	
stnYpup ₃₀ F	aggaggaactatatccgcgggatccttctccatgaggtcgccgcg	
stnYpup ₁₀ F	aggaggaactatatccgcgggatccgcggcatgcttggcgtgcg	
stnYpR	tcaccttcaactcagatactagtctcttcagcagtcgtgag	
ExylEF	aggaggaactatatccgcgggatccgcgagtgctccgttcgagtg	
ExylER	tcaccttcaactcagatactagtgcgtggatcctaccaaccgg	
OxylEF	aggaggaactatatccgcgggatcctgttcacattcgaacggtct	
OxylER	tcaccttcaactcagatactagtaactccccagtcctgcacg	
PxylEF	aggaggaactatatccgcgggatcctgttcacattcgaacggtct	For construction of plasmids pSET- <i>indC</i> , pSET- <i>ermEp</i> *- <i>indC</i> , pSET- <i>kasOp</i> *- <i>indC</i> , pSET- <i>SP44</i> - <i>indC</i> , and pSET- <i>stnYp</i> - <i>indC</i> .
PxylER	tcaccttcaactcagatactagtaactccccagtcctgcacg	
indCF	gcatactactagtaattaattaagccgtaccatcgcatca	
indCR	cgggctttttctgtgttccgcttaggcgaagaggtccaagg	
EindCF	tagccatggatgcatactactagtgcgagtgctcgttcgagtg	
EindCR	ggtacggcttaattaattaactagtcgctggatcctaccaaccgg	
OindCF	tagccatggatgcatactactagtgttcacattcgaacggtct	
OindCR	ggtacggcttaattaattaactagtaactccccagtcctgcacg	
PindCF	tagccatggatgcatactactagtgttcacattcgaacggtct	
PindCR	ggtacggcttaattaattaactagtaactccccagtcctgcacg	
YindCF	tagccatggatgcatactactagtgcacccggtcgcgaaggat	

YindCR	cgggtggcggcgtgctggtgctcatcggtgacgatccccgagtg	
152EF	gcttgggctgcaggtcgactctagagcgagtgccgttcgagtg	For construction of plasmids pSET- <i>ermEp</i> *-aur, pSET- <i>kasOp</i> *-aur, pSET- <i>SP44</i> -aur, and pSET- <i>stnYp</i> -aur.
aurER	gtccgggcacacggaatcgactagtcgctggatcctaccaaccgg	
EaurF	cggttggtaggatccagcgcactagtcgattccgtgtgcccggacG	
152aurR	cgatatcgcgcgccggcgatcctacacggcgccatcgcg	
152OF	gcttgggctgcaggtcgactctagatgttcacattcgaacggtct	
aurOR	gtccgggcacacggaatcgactagtaactccccagtcctgcacg	
OaurF	gtgcaggactgggggagttactagtcgattccgtgtgcccggacg	
152aurR	cgatatcgcgcgccggcgatcctacacggcgccatcgcg	
152PF	gcttgggctgcaggtcgactctagatgttcacattcgaacggtct	
aurPR	gtccgggcacacggaatcgactagtaactccccagtcctgcacg	
PaurF	gtgcaggactgggggagttactagtcgattccgtgtgcccggacg	
152aurR	cgatatcgcgcgccggcgatcctacacggcgccatcgcg	
152YF	gcttgggctgcaggtcgactctagagcatccggtccgcgaaggat	
aurYR	ggcacacggaatcgactagtccttcagcagtcattgtgagcatgc	
YaurF	tcacatgactgctgaagagactagtcgattccgtgtgcccggacg	
152aurR	cgatatcgcgcgccggcgatcctacacggcgccatcgcg	
ymkanF	tccccgccgcaccgtacggcaggctattccgggatccgtcgacc	For construction of plasmids YM- <i>ermEp</i> *, YM- <i>kasOp</i> *, YM- <i>SP44</i> , and YM- <i>stnYp</i> .
EkanR	agccgccactcgaacggacactcgctgtaggctggagctgcttcg	
kanEF	aactcgaagcagctccagcctacagcgagtgccgttcgagtg	
ymER	acagcttctccggtatctgctaaggcgctggatcctaccaaccgg	
ymkanF	tccccgccgcaccgtacggcaggctattccgggatccgtcgacc	
OkanR	agcagagaccgttcgaatgtgaacatgtaggctggagctgcttcg	
kanOF	aactcgaagcagctccagcctacatgttcacattcgaacggtct	
ymOR	acagcttctccggtatctgctaaggaactccccagtcctgcacg	
ymkanF	tccccgccgcaccgtacggcaggctattccgggatccgtcgacc	
PkanR-	agcagagaccgttcgaatgtgaacatgtaggctggagctgcttcg	
kanPF	aactcgaagcagctccagcctacatgttcacattcgaacggtct	
ymPR	acagcttctccggtatctgctaaggaactccccagtcctgcacg	

ymkanF	tccccgccgcaccgtacggcaggctattccggggatccgtcgacc	
YkanR	cggccatccttcgcggaccggatgctgtaggctggagctgcttcg	
kanY-F	aacttcgaagcagctccagcctacagcatccggtccgcgaaggat	
ymYR	acagcttctccggtatctgctaaggctcttcagcagtcattgtgag	
EtylFF	gtaggatccaccacctctcccagccccgcg	For construction of
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	
152EF	aggaggaactatatccgcgggatccgtcgactctagtagcatgc	plasmids
tylFER	ggagaggtggtggatcctaccaaccggcacg	pSET- <i>ermEp</i> *- <i>tylF</i> ,
OtylFF	actgggggagttccacctctcccagccccgcg	pSET- <i>kasOp</i> *- <i>tylF</i> ,
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	pSET- <i>SP44</i> - <i>tylF</i> ,
152OF	aggaggaactatatccgcgggatcctgttcacattcgaacgggtctc	
tylFOR	agaggtggaactccccagtcctgcacgc	pSET- <i>stnYp</i> - <i>tylF</i> ,
PtylFF	tggtgtaccacctctcccagccccgcgag	pSET- <i>ermEp</i> *- <i>tylI</i> ,
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	
152PF	aggaggaactatatccgcgggatcctgttcacattcgaacgggtctc	pSET- <i>kasOp</i> *- <i>tylI</i> ,
tylFPR	tcgggagaggtggtacaccagactttacaacaccgc	pSET- <i>SP44</i> - <i>tylI</i> ,
YtylFF	tgctgaagagccacctctcccagccccgcg	pSET- <i>stnYp</i> - <i>tylI</i> , and
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	
152YF	aggaggaactatatccgcgggatccgcacccggtccgcgaaggat	pSET- <i>stnYp</i> - <i>tylFI</i> .
tylFYR	ggagaggtggctcttcagcagtcattgtgagcatgctag	
RBStylIF	atcgacccgctactggaggacccatgacgacacagacgctcgaag	
152tylIR	acatgattacactagtgaattctcagtgccgggtgacggtgaccgg	
tylI152F	gaattcactagtgtaatcatgtcatagctg	
tylIRBSR	tcctccagtagcgggtcgatcg	
tylItylFF	tcaccgtcaccggccactgacatatgccacctctcccagccccgcg	
152tylFR	ggaaacagctatgacatgattacactagtgaattctcagccgctgtg	
EtylFF	gtaggatccaccacctctcccagccccgcg	
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	
152EF	aggaggaactatatccgcgggatccgtcgactctagtagcatgc	
tylFER	ggagaggtggtggatcctaccaaccggcacg	

OtylFF	actgggggagttccacctctcccgaccccgcg	
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	
152OF	aggaggaactatatccgcgggatcctgttcacattcgaacggtctc	
tylFOR	agaggtggaactccccagtcctgcacgc	
PtylFF	tggtgtaccacctctcccgaccccgcgag	
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	
152PF	aggaggaactatatccgcgggatcctgttcacattcgaacggtctc	
tylFPR	tcgggagaggtggtacaccagactttacaacaccgc	
YtylFF	tgctgaagagccacctctcccgaccccgcg	
152tylFR	cagctatgacatgattacactagtgaattctcagccgctgtgccgcca	
152YF	aggaggaactatatccgcgggatccgcacccggtccgcgaaggat	
tylFYR	ggagaggtggctcttcagcagtcacgtgagcatgctag	
StnYF	gtgaggcagttcaacgtctac	5' RACE verification
StnYR	tcagctcttgagcggcgct	of transcription start
StnR1 (463)	gaccatccgctgggtgccgtag	sites for promoter <i>stnY</i> .
StnR2 (196)	gccttcgacaccctgggtcgtca	
StnR3 (145)	tgtctcgttcttgccgctggtgc	
StnR4 (89)	agcgccgacagcgacagttcg	
ymIAF	ccggcagggaggcgccttct	RT-PCR
ymIAR	gcgacgtccaggctgcctcttcc	co-transcription
ymADF	tgaccgctgagatcgaggaagtgc	analyses of genes in the
ymADR	tgtacctgatggcgtagtag	<i>ym</i> cluster
ymDEF	gcggggcgatgcggtcaccg	
ymDER	cgtggcgtcgtgatccgtgaggac	
ymEB1F	ccgcggcctgacgagttcctcc	
ymEB1R	tttcggtcgttcgcccggg	
ymB1C1F	gttcacctcgccgctgtccttgg	
ymB1C1R	ggaggcgtccggcgagcg	
ymC1FF	cgcggtgctgttcgcgttcg	
ymC1FR	cgagaccggccccggccg	

84	ymFBCF	gccgcatcggtacggagtcttgg	
	ymFBCR	ggaccgagggtacgccgtcca	
	xylEF	caggtgctggacgaaaatgg	qRT-PCR analyses
	xylER	gtcatggagatcaggtcggc	of gene <i>xylE</i> , <i>ymA</i> ,
	hrdBF	cgggatctggtcagcttcg	<i>ymE</i> , <i>ymC1</i> , <i>ymBC</i> , and
	hrdBR	ccgagtgccagtcgtgatg	<i>hrdB</i> .
	ymAF	cggagaacatcatgaccgct	
	ymAR	tccaggtcgtcctcttcag	
	ymEF	cccgaatccccgacattca	
	ymER	atgcactccatcaccgtctg	
	ymC1F	gaggcgtggttctacgagat	
	ymC1R	catcgtccaggagtacgtcg	
	ymBCF	gtatctggagcgcgggtatc	
	ymBCR	agcagatacagagcctggga	

85

86 **References**

- 87 1. Xu F, Kong D, He X, Zhang Z, Han M, Xie X, et al. Characterization of streptonigrin biosynthesis reveals a
88 cryptic carboxyl methylation and an unusual oxidative cleavage of a N-C bond. J Am Chem Soc.
89 2013;135(5):1739-48.
- 90 2. Yang K HL, Vining LC. Regulation of jadomycin B production in *Streptomyces venezuelae* ISP5230:
91 involvement of a repressor gene, jadR2. J Bacteriol. 1995;177(21):6111-7.
- 92 3. Gomez-Escribano JP, Bibb MJ. Engineering *Streptomyces coelicolor* for heterologous expression of secondary
93 metabolite gene clusters. Microb Biotechnol. 2011;4(2):207-15.
- 94 4. Hopwood DA, Kieser T, Wright HM, Bibb MJ. Plasmids, recombination and chromosome mapping in
95 *Streptomyces lividans* 66. J Gen Microbiol. 1983;129(7):2257-69.
- 96 5. Chater KF, Wilde LC. *Streptomyces albus* G mutants defective in the SalGI restriction-modification system. J
97 Gen Microbiol. 1980;116(2):323-34.
- 98 6. Paget MS, Chamberlin L, Atrih A, Foster SJ, Buttner MJ. Evidence that the extracytoplasmic function sigma
99 factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). J Bacteriol.
100 1999;181(1):204-11.
- 101 7. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR
102 products. Proc Natl Acad Sci. 2000;97(12):6640-5.
- 103 8. Wang W, Li X, Wang J, Xiang S, Feng X, Yang K. An engineered strong promoter for *streptomyces*. Appl
104 Environ Microbiol. 2013;79(14):4484-92.
- 105 9. Bierman M, Logan R, Brien K, Seno ET, Rao RN, Schonher BE. Plasmid cloning vectors for the conjugal
106 transfer of DNA from *Escherichia coli* to *Streptomyces* spp. Gene. 1992;116:43-9.
- 107 10. Pan HX, Chen Z, Zeng T, Jin WB, Geng Y, Lin GM, et al. Elucidation of the Herbicidin Tailoring Pathway

108 Offers Insights into Its Structural Diversity. *Org Lett.* 2019;21(5):1374-8.

109 11. Jian XH, Pan HX, Ning TT, Shi YY, Chen YS, Li Y, et al. Analysis of YM-216391 biosynthetic gene cluster
110 and improvement of the cyclopeptide production in a heterologous host. *ACS Chem Biol.* 2012;7:646-51.

111 12. Gust B, Challis GL, Fowler K, Kieser T, Chater KF. PCR-targeted *Streptomyces* gene replacement identifies a
112 protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci.*
113 2003;100(4):1541-6.

114 13. Li L, Xu Z, Xu X, Wu J, Zhang Y, He X, et al. The mildiomycin biosynthesis: initial steps for sequential
115 generation of 5-hydroxymethylcytidine 5'-monophosphate and 5-hydroxymethylcytosine in *Streptoverticillium*
116 *rimofaciens* ZJU5119. *Chembiochem.* 2008;9:1286-94.