Identification of a butenolide signaling system that regulates nikkomycin biosynthesis in *Streptomyces*

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Supporting Information

Supporting Information contains 2 supplementary tables (Table S1 and S2) and 6 supplementary figures (Figure S1-S6).

Supplementary Tables

Table S1. ^{1}H-NMR and ^{13}C-NMR data of SAB1 in $CDCl_3$.
Table S2. Primers used in this study.

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Table S1. ^{1}H-NMR and ^{13}C-NMR data of SAB1 in $CDCl_3$

Position	^{1}H (δ, mult., *J*)	^{13}C (δ)
C(2)		172.085
C(3)		130.389
C(4)		158.126
C(5)	5.861, s	98.885
C(1')	4.504, t, 7	66.412
C(2')	1.8, m/1.64, m	38.233
C(3')	1.42, m/1.3, m	18.786
C(4')	0.937, t, 7.5	13.794
CH3-C(4)	2.083, s	11.637

Chemical shifts δ in ppm relative to TMS. Coupling constants *J* in Hz. s, singlet. t, triplet. m, multiplet.

Table S2. Primers used in this study

Primers	Sequence (5′-3′)	Purpose
sabALF	GGAATTCGCGATGCTGCTGTTGTTGACGA	*sabA* disruption
sabALR	GCTCTAGAGGGTCCGTAGAAGGTGTGCGC	*sabA* disruption
sabARF	GCTCTAGATTCCACCGGTACGCGGAGCTG	*sabA* disruption
sabARR	CCCAAGCTTACCAAGGGCAAGTGGACGCCAT	*sabA* disruption
sabAcF	CGCGGATCCGGATTCATCTCTCCTCCCGCG	*sabA* complement
sabAcR	GCTCTAGATACGTCGTGGTCAGGCCGTG	*sabA* complement
sabR1LF	CGCGGATCCCAGTCAACTCTGCACGCTCCCTT	*sabR1* disruption
sabR1LR	GCTCTAGACCTGTCGAGAATCTGCGCGAT	*sabR1* disruption
sabR1RF	GCTCTAGATCTTCGCCTACACGCTGCCCA	*sabR1* disruption
sabR1RR	CCCAAGCTTATCGAACATCTCCGCCGACGACT	*sabR1* disruption
sabR1cF	CGGAATTCGGGCTGACCCGGACGGAGAT	*sabR1* complement
sabR1cR	CGCGGATCCTCGACGCGGGAGTCTTGGCA	*sabR1* complement
sabR2LF	GCTCTAGACCTTCTCCCACACGGACACGT	*sabR2* disruption
sabR2LR	GGAATTCCATATGCCGTCGCGGTCGAATATCTG	*sabR2* disruption
sabR2RF	GGAATTCCATATGCGGTGTGGAGTCCGTCGTGG	*sabR2* disruption
sabR2RR	CCCAAGCTTCAGCGACTTCGAGCACGGCTAC	*sabR2* disruption
sabR2OF	GGAATTCCATATGAGGGCCGAAGAGACC	*sabR2* overexpression
sabR2OR	GGAATTCGACCGGCTGTCGTTTCAC	*sabR2* overexpression
cprCLF	GCTCTAGATTCAGGTCGTTCTTCTTCGCC	*cprC* disruption
cprCLR	GGAATTCCATATGGCGTAACTGCCTCGCCATC	*cprC* disruption
cprCRF	GGAATTCCATATGGTCTGCTTCTTCGTCGGCA	*cprC* disruption
cprCRR	CCCAAGCTTGCGATCATCGACTTCACCAC	*cprC* disruption
cprCcF	GCTCTAGACCAGGTCACCCGTTCGAAG	*cprC* complement
cprCcR	GGAATTCGGCTTCTGTGACGGACCATGG	*cprC* complement
cprCoF	CCAATTCCATATGGCGAGGCAGTTACGCG	*cprC* overexpression
cprCoR	GGAATTCGGCTTCTGTGACGGACCATGG	*cprC* overexpression
PhrdBF	GTCGACTCTAGACCGCCTTCCGCCG	*hrdB* promoter
PhrdBR	GGAATTCCATATGAACAACCTCTCGGAACGTTGA	*hrdB* promoter
SabR1F	GGAATTCCATATGGCACAGCAGTTGCGCGCGA	SabR1 expression
SabR1R	CCGCTCGAGGCTCGCCGGTTCCGCCACCAG	SabR1 expression
CprCF	CCAATTCCATATGGCGAGGCAGTTACGCG	CprC expression
CprCR	CCGCTCGAGGGCGGCACGGACCTCCCGTT	CprC expression
PsabR1F	AGCCTGGTGAGTCGCGGCGT	P_{sabR1} for EMSA
PsabR1R	CGCAACTGCTGTGCCATGTGTTC	P_{sabR1} for EMSA
PsabR2F	GCCATGGCCCAACTCTATGAGC	P_{sabR2} for EMSA
PsabR2R	TTGCCTCATGCGGCGGAC	P_{sabR2} for EMSA

PsabAF	GACCATGCGGAGCTTCTCGAGCT	P_{sabA} for EMSA
PsabAR	GATGGGCGCAAACGTGGTCAT	P_{sabA} for EMSA
PadpAF	GCAAAGAGGCGGACGTCACA	P_{adpA} for EMSA
PadpAR	GGCAGTGGAGTCGTGGCTCAT	P_{adpA} for EMSA
PsanG1F	GTCGTCGTCCGCTTCCACCT	P_{sanG1} for EMSA
PsanG1R	CGGGTCGGCTGTGGTGAGT	P_{sanG1} for EMSA
PsanG2F	ATCGCGAACACCATGAACGCA	P_{sanG2} for EMSA
PsanG2R	AAAGGCCGTCTACTGCCGCCT	P_{sanG2} for EMSA
PsanG3F	AGGCGGCAGTAGACGGCCTTT	P_{sanG3} for EMSA
PsanG3R	ATGCCGGGGGTTCTTGCCTTT	P_{sanG3} for EMSA
PsanFF	GAAGTGCCGGAAGGGGTGGT	P_{sanF} for EMSA
PsanFR	GTCAGCACCACGGTGTCTCCTT	P_{sanF} for EMSA
PsanO-NF	GGGTTGACCACGGATCGCAGT	P_{sanON} for EMSA
PsanO-NR	ACAGCCGTCCAGTTGCTTGCAT	P_{sanON} for EMSA
PcprCF	CCAGGTCACCCGTTCGAAG	P_{cprC} for EMSA
PcprCR	CGTAACTGCCTCGCCATCC	P_{cprC} for EMSA
PcprCMR	ATAAGAATGCGGCCGCCAACCCCCCGCATACGATAA	P_{cprCM} for EMSA
PcprCMF	ATAAGAATGCGGCCGCCCGCATGTCCTGTTCTTTTGA	P_{cprCM} for EMSA
FAM-F	CGGGCTGCAGGAATTCGAT	For DNaseI footprinting
HEX-R	GGTCGACGGTATCGATAAGCTTG	For DNaseI footprinting
qsabR1-F	ACGAACACATGGCACAGCAGTT	*sabR1* qRT-PCR
qsabR1-R	CGATGCTGGCACCGTGGTA	*sabR1* qRT-PCR
qsabR2-F	GTGAGGGCCGAAGAGACC	*sabR2* qRT-PCR
qsabR2-R	GCTGCTGAAGTGGAACGAGA	*sabR2* qRT-PCR
qsabA-F	GTCGGACTGACCACATTGACGA	*sabA* qRT-PCR
qsabA-R	GGTCCGTAGAAGGTGTGCGC	*sabA* qRT-PCR
qadpA-F	GATGCCGCAGCCCGGAAACT	*adpA* qRT-PCR
qadpA-R	GTCGATGCCGAACACCGAAAGC	*adpA* qRT-PCR
qsanG-F	GGAGGTCATCCGTGTCAACT	*sanG* qRT-PCR
qsanG-R	GGAAGTCCATCTGGTCCGAG	*sanG* qRT-PCR
qsanO-F	TCCGCCGTGAACCGCTACTTC	*sanO* qRT-PCR
qsanO-R	GGCACGCTCAGGAAGGTCGT	*sanO* qRT-PCR
qsanN-F	GACCTGACGCCCAGCAGAGT	*sanN* qRT-PCR
qsanN-R	TCGAGGTTCAGTCGTGAGGCG	*sanN* qRT-PCR
qsanF-F	GAACCTCGTGGACCTCATCGT	*sanF* qRT-PCR
qsanF-R	TGGTCGGCTTGTCCTTGTGT	*sanF* qRT-PCR
qcprC-F	GTCTGCTTCTTCGTCGGCA	*cprC* qRT-PCR
qcprC-R	GGACCTCCCGTTCCAGATG	*cprC* qRT-PCR

q23S-F	CTCACCTACTAACCGCTTGGT	23S rRNA qRT-PCR
q23S-R	CAGGGTAAGTCGGGACCTAA	23S rRNA qRT-PCR
sabAF	GGAATTCCATATGACCACGTTTGCGCCCAT	SabAPD expression
sabAR	GCTCTAGATACGTCGTGGTCAGGCCGTG	SabAPD expression
sabPDF	GCTCTAGAGGATCGCGGGAGGAGAGAT	SabAPD expression
sabPDR	GGAATTCTTACCAATCCGTGAAGCCG	SabAPD expression
gusA-F	GGAATTCCATATGACCGGTCTGCGGCCCG	For GusA activity
gusA-R	CCGCTCGAGTCACTGCTTCCCGCCCTGCT	For GusA activity
PGcprCF	GACTAGTCCAGGTCACCCGTTCGAAG	For GusA activity
PGcprCR	GGAATTCCATATGCGGACCCCTCCTCGCACCT	For GusA activity
adpAoF	GGAATTCCATATGAGCCACGACTCCACTGC	*adpA* overexpression
adpAoR	CGGAATTCTCCGTCGTGCTCAGTTACCG	*adpA* overexpression

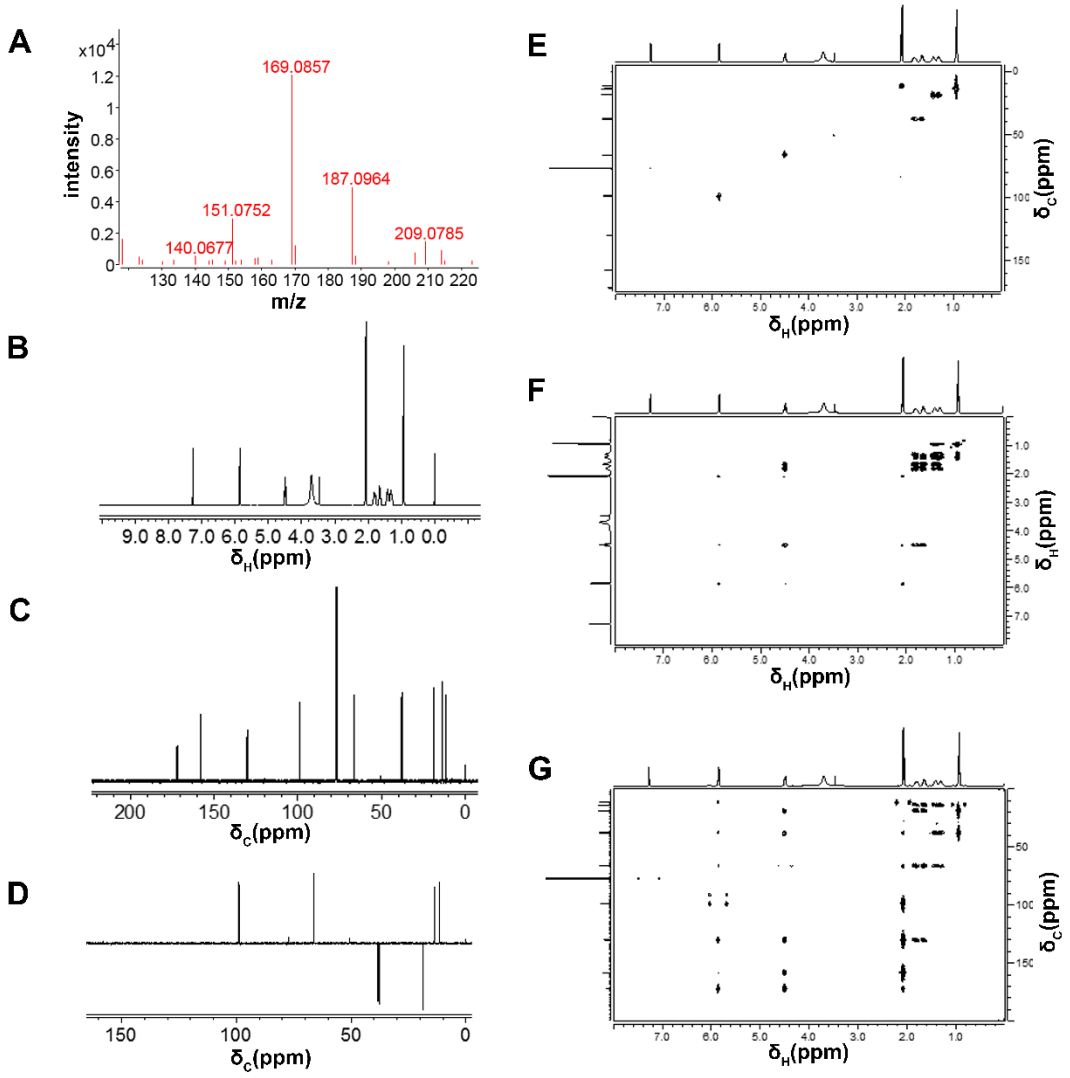

Figure S1. HRMS and NMR analyses of SAB1 from *E. coli* C41 containing *sabAPD*. (A) HRMS of SAB1. (B) ^{1}H-NMR spectrum of SAB1. (C) ^{13}C-NMR spectrum of SAB1. (D) DEPT135 spectrum of SAB1. (E) ^{1}H-^{13}C HSQC spectrum of SAB1. (F) ^{1}H-^{1}H COSY spectrum of SAB1. (G) ^{1}H-^{13}C HMBC spectrum of SAB1.

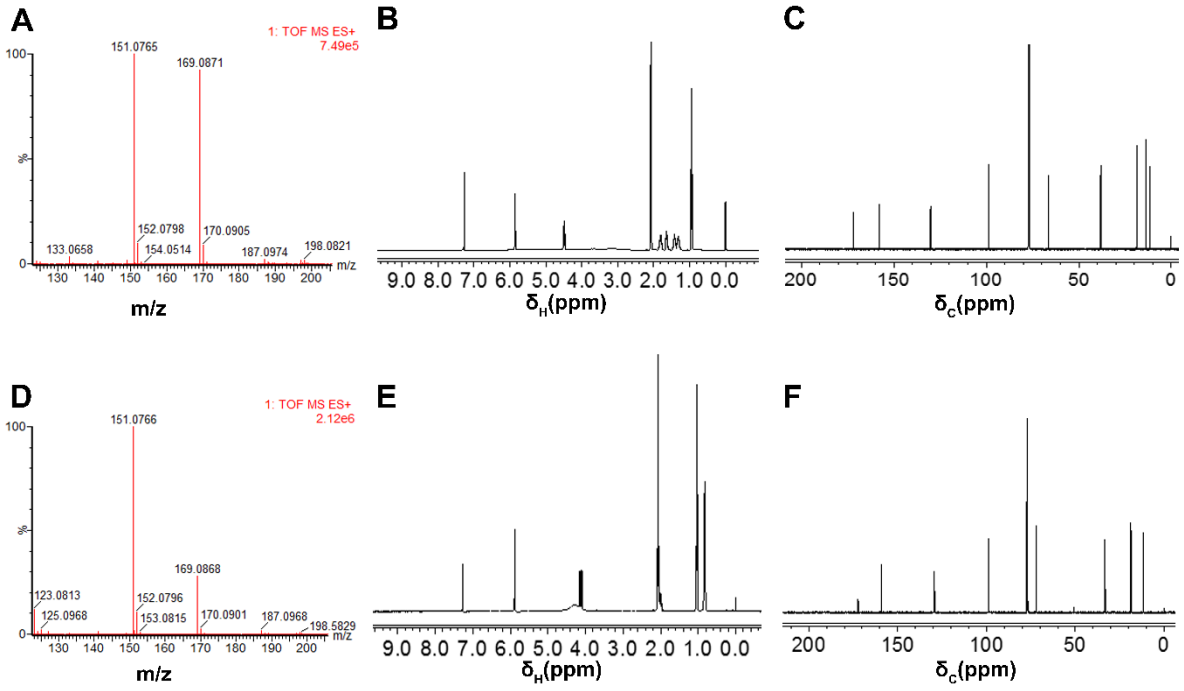

Figure S2. HRMS and NMR analyses of SAB1 and SAB2 from *S. coelicolor* M1146 containing *sabAPD*. (A) HRMS of SAB1. (B) ^{1}H-NMR spectrum of SAB1. (C) ^{13}C-NMR spectrum of SAB1. (D) HRMS of SAB2. (E) ^{1}H-NMR spectrum of SAB2. (F) ^{13}C-NMR spectrum of SAB2.

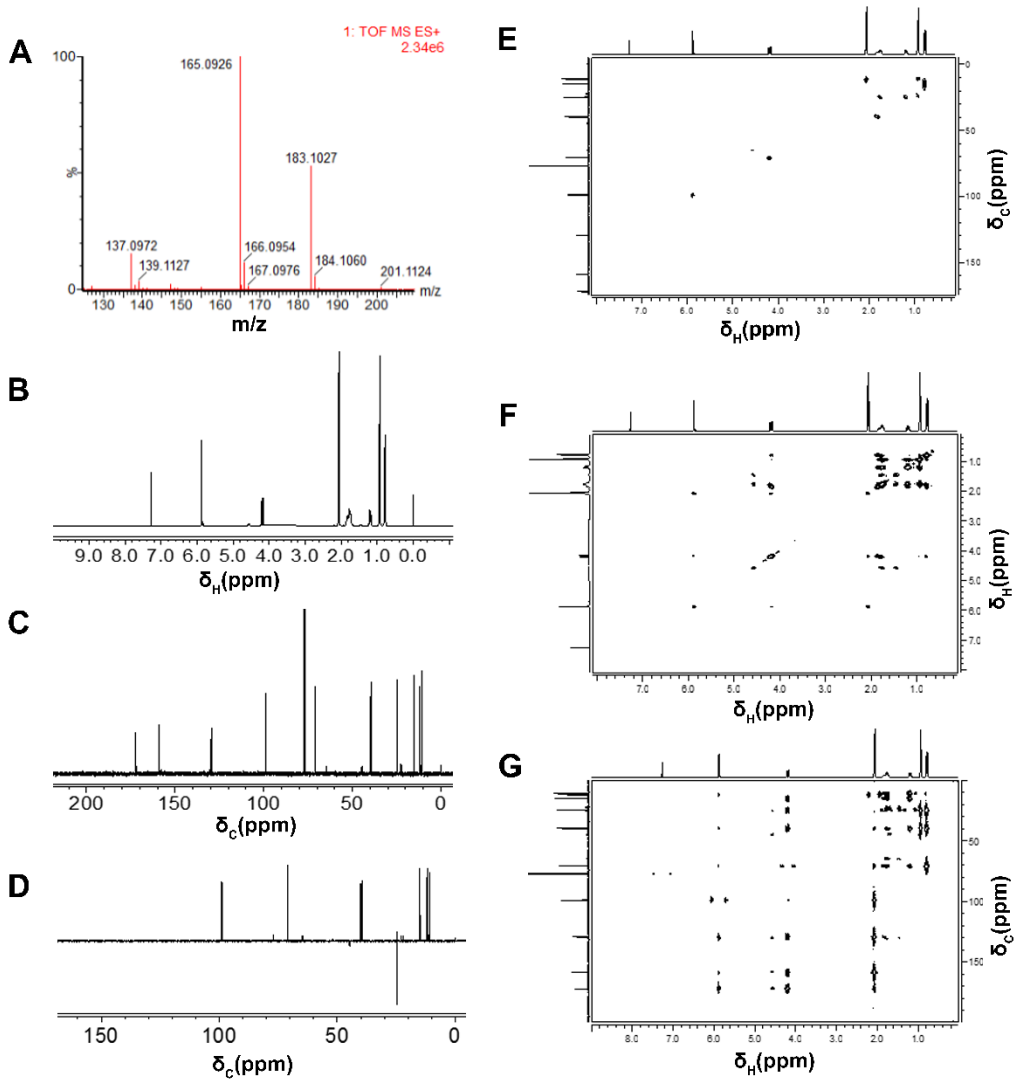

Figure S3. HRMS and NMR analyses of SAB3 from *S. coelicolor* M1146 containing *sabAPD*. (A) HRMS of SAB3. (B) ^{1}H-NMR spectrum of SAB3. (C) ^{13}C-NMR spectrum of SAB3. (D) DEPT135 spectrum of SAB3. (E) ^{1}H-^{13}C HSQC spectrum of SAB3. (F) ^{1}H-^{1}H COSY spectrum of SAB3. (G) ^{1}H-^{13}C HMBC spectrum of SAB3.

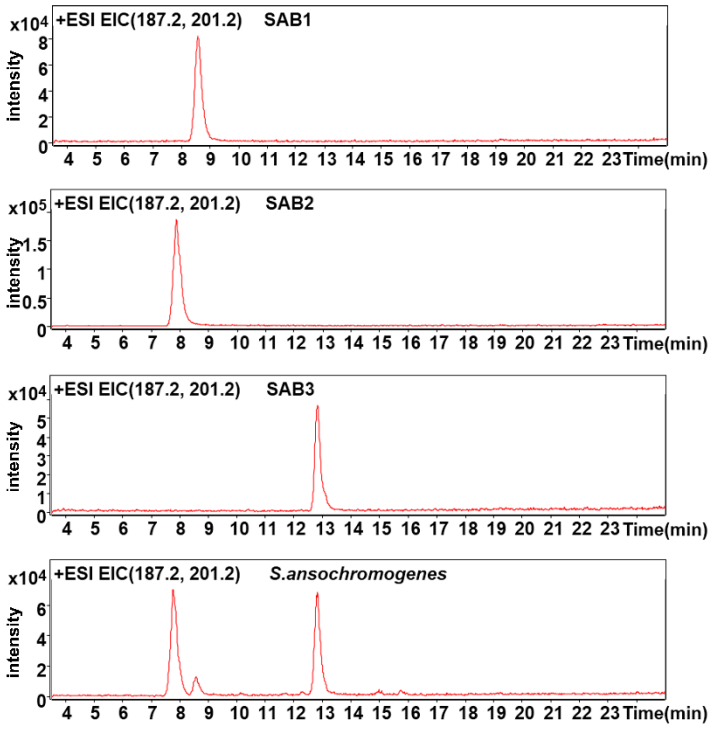

Figure S4. LC-MS analysis of SABs in *S. ansochromogenes* 7100. Extracted ion chromatograms (EIC) of *m/z* = 187 (SAB1 and SAB2) and 201 (SAB3).

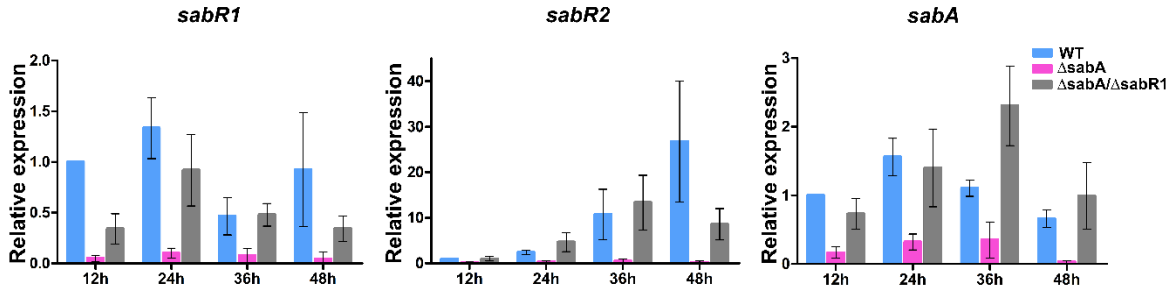

Figure S5. qRT-PCR transcriptional analyses of *sabR1*, *sabR2* and *sabA* in WT, ΔsabA and ΔsabA/ΔsabR1. WT, *S. ansochromogenes* 7100. ΔsabA, *sabA* disruption mutant. ΔsabA/ΔsabR1, disruption mutant of *sabA* and *sabR1*. Error bars were calculated from three independent experiments and show standard deviations.

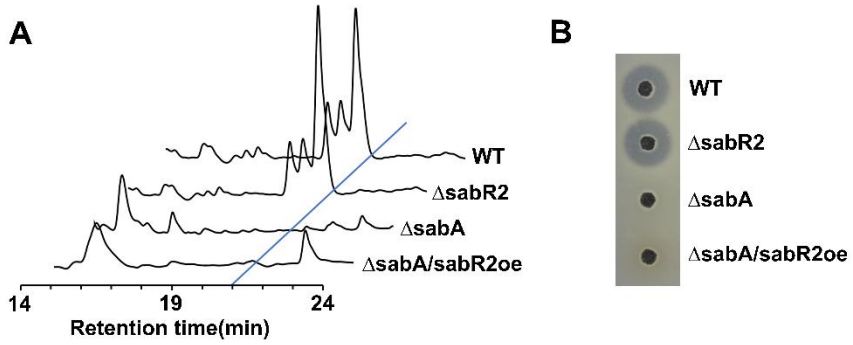

Figure S6. Effect of *sabR2* disruption and overexpression on nikkomycin production. (A) HPLC analysis of nikkomycin production in different strains. (B) Nikkomycin bioassays with fermentation filtrates of different strains. WT, *S. ansochromogenes* 7100 wild-type strains. ΔsabR2, *sabR2* disruption strain. ΔsabA, *sabA* disruption mutant. ΔsabA/sabR2oe, *sabR2* overexpression strain in ΔsabA.