

**Characterization and heterologous expression of the
neoabyssomicin/abyssomicin biosynthetic gene cluster from
Streptomyces koyangensis SCSIO 5802**

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Table S1. Bacteria used in this study.

Strains	Description	Source/ [Ref.]
Streptomyces		
<i>Streptomyces coelicolor</i> M1152	Host strain for heterologous expression of the <i>abm</i> biosynthetic gene cluster	
<i>Streptomyces koyangensis</i> SCSIO 5802	Wild-type producer of neoabyssomicins/abyssomicins (1–4)	This work
$\Delta orf(-1)$	<i>S.koyangensis</i> SCSIO 5802 with a 900 bp fragment of <i>orf(-2)</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmI$	<i>S.koyangensis</i> SCSIO 5802 with a 609 bp fragment of <i>abmI</i> substituted by a <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmF4$	<i>S.koyangensis</i> SCSIO 5802 with a 1005 bp fragment of <i>abmF4</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmF3$	<i>S.koyangensis</i> SCSIO 5802 with a 837 bp fragment of <i>abmF3</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmF2$	<i>S.koyangensis</i> SCSIO 5802 with a 684 bp fragment of <i>abmF2</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmF1$	<i>S.koyangensis</i> SCSIO 5802 with a 1545 bp fragment of <i>abmF1</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmD$	<i>S.koyangensis</i> SCSIO 5802 with a 1173 bp fragment of <i>abmD</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmA1$	<i>S.koyangensis</i> SCSIO 5802 with a 624 bp fragment of <i>abmA1</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmA2$	<i>S.koyangensis</i> SCSIO 5802 with a 1629 bp fragment of <i>abmA2</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmA3$	<i>S.koyangensis</i> SCSIO 5802 with a 138 bp fragment of <i>abmA3</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmA4$	<i>S.koyangensis</i> SCSIO 5802 with a 507 bp fragment of <i>abmA4</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmA5$	<i>S.koyangensis</i> SCSIO 5802 with a 744 bp fragment of <i>abmA5</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmB1$	<i>S.koyangensis</i> SCSIO 5802 with a 2649 bp fragment of <i>abmB1</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta abmH$	<i>S.koyangensis</i> SCSIO 5802 with a 2391 bp fragment of <i>abmH</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta orf(+1)$	<i>S.koyangensis</i> SCSIO 5802 with a 1095 bp fragment of <i>orf(+1)</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta orf(+2)$	<i>S.koyangensis</i> SCSIO 5802 with a 2100 bp fragment of <i>orf(+2)</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
$\Delta orf(+3)$	<i>S.koyangensis</i> SCSIO 5802 with a 738 bp fragment of <i>orf(+3)</i> substituted by <i>aac(3)IV+oriT</i> cassette	This work
5802:: <i>abmI</i>	<i>abmI</i> overexpressed strain, derived from <i>Streptomyces koyangensis</i> SCSIO 5802	This work
5802:: <i>abmH</i>	<i>abmH</i> overexpressed strain, derived from <i>Streptomyces koyangensis</i> SCSIO 5802	This work
E.coli		
DH5 α	Host strain for general clone	
BW25113/pIJ 790	K-12 derivative: <i>araBAD</i> , <i>rhaBAD</i> ; host strain for Red/ET-mediated recombination	[1]
ET12567/pU Z8002	<i>dam</i> , <i>dcm</i> , <i>hsdM</i> , <i>hsdS</i> , <i>hsdR</i> , <i>cat</i> ^R , <i>tef</i> ^R ; donor strain for conjugation between <i>E.coli</i> and <i>Streptomyces</i>	[2]

XL 1-Blue
MR

Host strain for construction of genomic cosmid library

Stratagene

Table S2. Plasmids used in this study.

Plasmids	Description	Source/ [Ref.]
Plasmids		
pIJ773	<i>aac(3)IV</i> (AprR), <i>oriT</i> ; used for amplifying the <i>aac(3)IV-oriT</i> gene cassette for gene inactivation	[3]
pL646	Derived from pSET152, containing <i>ermE</i> *P promoter and the ribosome-binding site of the <i>tufI</i> gene, used for overexpression of <i>abmI</i> and <i>abmH</i> gene	[4]
SuperCos1	Used for construction of genomic cosmid library	Stratagene
cosmid 9-7C	A cosmid which contains partial <i>abm</i> biosynthetic cluster	This work
cosmid 6-7F	A cosmid which contains partial <i>abm</i> biosynthetic cluster	This work
cosmid 21-9A	A cosmid which contains partial <i>abm</i> biosynthetic cluster	This work
pΔ <i>orf(-1)</i>	A 900 bp fragment in <i>orf(-1)</i> in cosmid 6-7F was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmI</i>	A 609 bp fragment in <i>abmI</i> in cosmid 6-7F was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmF4</i>	A 1005 bp fragment in <i>abmF4</i> in cosmid 6-7F was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmF3</i>	A 837 bp fragment in <i>abmF3</i> in cosmid 6-7F was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmF2</i>	A 684 bp fragment in <i>abmF2</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmF1</i>	A 1545 bp fragment in <i>abmF1</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmD</i>	A 1173 bp fragment in <i>abmD</i> in cosmid 9-7C B was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmA1</i>	A 624 bp fragment in <i>abmA1</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmA2</i>	A 1629 bp fragment in <i>abmA2</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmA3</i>	A 138 bp fragment in <i>abmA3</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmA4</i>	A 507 bp fragment in <i>abmA4</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmA5</i>	A 744 bp fragment in <i>abmA5</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmB1</i>	A 2649 bp fragment in <i>abmB1</i> in cosmid 9-7C was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>abmH</i>	A 2391 bp fragment in <i>abmH</i> in cosmid 21-9A was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>orf(+1)</i>	A 1095 bp fragment in <i>orf(+1)</i> in cosmid 21-9A was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>orf(+2)</i>	A 2100 bp fragment in <i>orf(+1)</i> in cosmid 21-9A was substituted by the <i>aac(IV)+oriT</i> cassette	This work
pΔ <i>orf(+3)</i>	A 738 bp fragment in <i>orf(+3)</i> in cosmid 21-9A was substituted by the <i>aac(IV)+oriT</i> cassette	This work

Table S3. Primers used in this study.

Name	Sequence (5'-3')	Purpose
screen- abmB1-F	GCTGCGGAGTTGGTGGAGGA	For screening the <i>S.koyangensis</i> SCSIO 5802 genomic library
screen- abmB1-R	ACTGCGGTGTCGAGGAGGGA	
screen- orf(-2)-F	GCGGAGTTCGAGATCAAGCC	For screening the <i>S.koyangensis</i> SCSIO 5802 genomic library
screen- orf((-2)-R	CTGGAGCACCGTCTGGGAGG	
screen- orf(+3)-F	TGGAGCAGCAGGGGCACA	For screening the <i>S.koyangensis</i> SCSIO 5802 genomic library
screen- orf(+3)-R	GCTGCTCGTCCTCTCTACACC	
orf(-1)-Del-F	GTGCTGCTGATCCTCTCGCTGGCGGGAGCGTCGGCGTACATTCCGGG GATCCGTCGACC	For disrupting <i>orf(-1)</i>
orf(-1)-Del-R	GACCATGACGCCGGGGTGAAGTGGAGGCGCCCCTTGAGTGTAGGC TGGAGCTGCTTC	
abmI-Del-F	GAAGAGGGCGTCGCCGGGGTTCGAGGCCAGTTCTTCGCGATTCCGG GGATCCGTCGACC	For disrupting <i>abmI</i>
abmI-Del-R	TTCCTCGACTGGCGCGAGCCGCGAGCGGCGATCCGGCCTGTAGGC TGGAGCTGCTTC	
abmF3-Del-F	ctcccgcagtcgggggtcgacacccgtagagccctcATTCCGGGGATCCGTCGACC	For disrupting <i>abmF3</i>
abmF3-Del-R	accgccctgtgggtgacacccggctcctcgcggtcTGTAGGCTGGAGCTGCTTC	
abmF2-Del-F	tgctcccgcagtcgggggtcgacacccgtagagcccATTCCGGGGATCCGTCGACC	For disrupting <i>abmF2</i>
abmF2-Del-R	cgcacgaccgccctgtgggtgacacccggctcctcTGTAGGCTGGAGCTGCTTC	
abmF1-Del-F	cccaccaggtcacgtcgtggaacagcgatacgcgtgATTCCGGGGATCCGTCGACC	For disrupting <i>abmF1</i>
abmF1-Del-R	tccccgtcccggcgggcggcagagcgtccgcgcgTGTAGGCTGGAGCTGCTTC	
abmD-Del-F	gtcaacgtcggcatcgactccctgggcaagagtcgacATTCCGGGGATCCGTCGACC	For disrupting <i>abmD</i>
abmD-Del-R	gatgacggccgcgccacgaccaccagaagcgtggtgTGTAGGCTGGAGCTGCTTC	
abmA1-Del-F	atggacgccctgtgggagcagtggtcgacgacctcattcATTCCGGGGATCCGTCGACC	For disrupting <i>abmA1</i>
abmA1-Del-R	caggcctgttcgagctggaagaagctgagccgtgccTGTAGGCTGGAGCTGCTTC	
abmA2-Del-F	ctggccgagccgacgagccgaactcggcgcgggaATTCCGGGGATCCGTCGACC	For disrupting <i>abmA2</i>
abmA2-Del-R	gtccagggcggcaccggtgtcggcggcggaagccgtgTGTAGGCTGGAGCTGCTTC	
abmA3-Del-F	tcccacgacggctcgtcactcctccgcgaggaagtgATTCCGGGGATCCGTCGACC	For disrupting <i>abmA3</i>
abmA3-Del-R	gcccacggccagcgtagaggtgctccaggtcTGTAGGCTGGAGCTGCTTC	
abmA4-Del-F	cacctggacaccgaggtcgacatgaccgctcctcgcATTCCGGGGATCCGTCGACC	For disrupting <i>abmA4</i>
abmA4-Del-R	ctccagggcggccttgatcggcgaggacgtccggcTGTAGGCTGGAGCTGCTTC	

abmA5-Del-F	ggcgaccggctcggcaggaggacacacctctggcgccATTCCGGGGATCCGTCGACC	For disrupting <i>abmA5</i>
abmA5-Del-R	caggctggctcggtcagggtggcggggtgagccgtggacTGTAGGCTGGAGCTGCTTC	
abmT-Del-F	ctctctgcttccaccacgccggcgccaccgcctcggcATTCCGGGGATCCGTCGACC	For disrupting <i>abmT</i>
abmT-Del-R	ctggcgctccctgatgaacaggtgtccgccgggcacggtTGTAGGCTGGAGCTGCTTC	
abmB1-Del-F	tacaagaccgctgccctaccgctccgaccgggacagcATTCCGGGGATCCGTCGACC	For disrupting <i>abmB1</i>
abmB1-Del-R	cgccgtgtcgtgcccctcgggtcgtggggcagcggTGTAGGCTGGAGCTGCTTC	
abmH-Del-F	gcttccctggactgccattggtgctcggcctccctATTCCGGGGATCCGTCGACC	For disrupting <i>abmH</i>
abmH-Del-R	gaggcaggagaggtcgagccgtcggcggggagcgtTGTAGGCTGGAGCTGCTTC	
orf(+1)-Del-F	cttgaccgggtggcagattggcctgttggcagaataATTCCGGGGATCCGTCGACC	For disrupting <i>orf(+1)</i>
orf(+1)-Del-R	cgggagctgatgagccgactggaagagtcctgtcgtaTGTAGGCTGGAGCTGCTTC	
orf(+2)-Del-F	cgcgcccttctcgggcttccatctcgacaggagctcATTCCGGGGATCCGTCGACC	For disrupting <i>orf(+2)</i>
orf(+2)-Del-R	gacatcacggagacacaccggggtacgtggccgcgctcTGTAGGCTGGAGCTGCTTC	
orf(+3)-Del-F	gctgatgaagaccgacgggtcgaggacgcggaggcgaATTCCGGGGATCCGTCGACC	For disrupting <i>orf(+3)</i>
orf(+3)-Del-R	gtcgctcctgcggctgtggcaccgatctccacatcTGTAGGCTGGAGCTGCTTC	
orf(-1)-TF	TGCCCAAACGGCTCCTCA	For verifying the disruption of <i>orf(-1)</i>
orf(-1)-TR	CCGGTGCCCTTCTTCTCCAC	
abmI-TF	TTTGATCTGTTTCCGATGACGC	For verifying the disruption of <i>abmI</i>
abmI-TR	CGAGAAGTGGTGCTCCGTGA	
abmF3-TF	GCGTCGAAGACGAGGTGGG	For verifying the disruption of <i>abmF3</i>
abmF3-TR	CGGACATCTGCGCTACTGG	
abmF2-TF	ATCACCTTGATCCAGTCGGTGCCGG	For verifying the disruption of <i>abmF2</i>
abmF2-TR	CCGGCACCGACTGGATCAAGGTGAT	
abmF1-TF	GAGGACGACGAGGACCGC	For verifying the disruption of <i>abmF1</i>
abmF1-TR	GCCGAGACTCTGGCCGAG	
abmD-TF	CACCTCCGACTGGTTCACGC	For verifying the disruption of <i>abmD</i>
abmD-TR	AGTTCACCTTGCCGGTCTCG	
abmA1-TF	ACGGTTAGGCGCAGGGTTAGA	For verifying the disruption of <i>abmA1</i>
abmA1-TR	AGCAGGTGCTGGGTGAGGGA	
abmA2-TF	GCCGGATGACCGAACGTATCGT	For verifying the disruption of <i>abmA2</i>
abmA2-TR	AGGTGGACGGGTGCCAAGGA	

abmA3-TF	CGCCGAGTTCTACCCAAGG	For verifying the disruption of <i>abmA3</i>
abmA3-TR	CGGTAGTGGTCGATCCTGTCC	
abmA4-TF	GGCTTCGTCGCACTCCTCC	For verifying the disruption of <i>abmA4</i>
abmA4-TR	CCCCTGTTTCCTTGATGCTGAC	
abmA5-TF	CGTCCTGCGGCTCAACCT	For verifying the disruption of <i>abmA5</i>
abmA5-TR	TCCCAGCCCTTGAGACCG	
abmT-TF	TCATCCCGCTGGAGGACTGG	For verifying the disruption of <i>abmT</i>
abmT-TR	GGAGACGGAGGCGAAGGAG	
abmB1-TF	AGCCAGTACGGCGCATTCC	For verifying the disruption of <i>abmB1</i>
abmB1-TR	TCAGCGAGTCGAAGCCCAGTT	
abmH-TF	ACATTCTGCTGAGGGAACACCG	For verifying the disruption of <i>abmH</i>
abmH-TR	GACCACAGAGCAACGGGAGC	
orf(+1)-TF	CGTCTACCGCAAACCTCAACATC	For verifying the disruption of <i>orf(+1)</i>
orf(+1)-TR	TTCCGCATTGGCACTGGA	
orf(+2)-TF	CGTTTCCAGTGCCAATGCG	For verifying the disruption of <i>orf(+2)</i>
orf(+2)-TR	GCGGGAGAAGACGGACGAT	
orf(+3)-TF	ACAGCCTCCGCGTGATTCTT	For verifying the disruption of <i>orf(+3)</i>
orf(+3)-TR	CGTCCACCTGACCACCTTCGT	
com-abmI-F	AAATTTCCCATATG <u>GTGCCC</u> GCCGCGCCACTCAGTAC (<i>NdeI</i> sites underlined)	For cloning, and overexpression of <i>abmI</i>
com-abmI-R	ATATCGTA <u>ACTAGT</u> TCACACCGCCTGGCGTAGC (<i>SpeI</i> sites underlined)	
com-abmH-F	AAATTTCCCATATG <u>gtggaggctctggcgcgatc</u> (<i>NdeI</i> sites underlined)	For cloning and overexpression of <i>abmH</i>
com-abmH-R	TTATATA <u>CTAGT</u> tcacgccgccgccgggctc (<i>SpeI</i> sites underlined)	

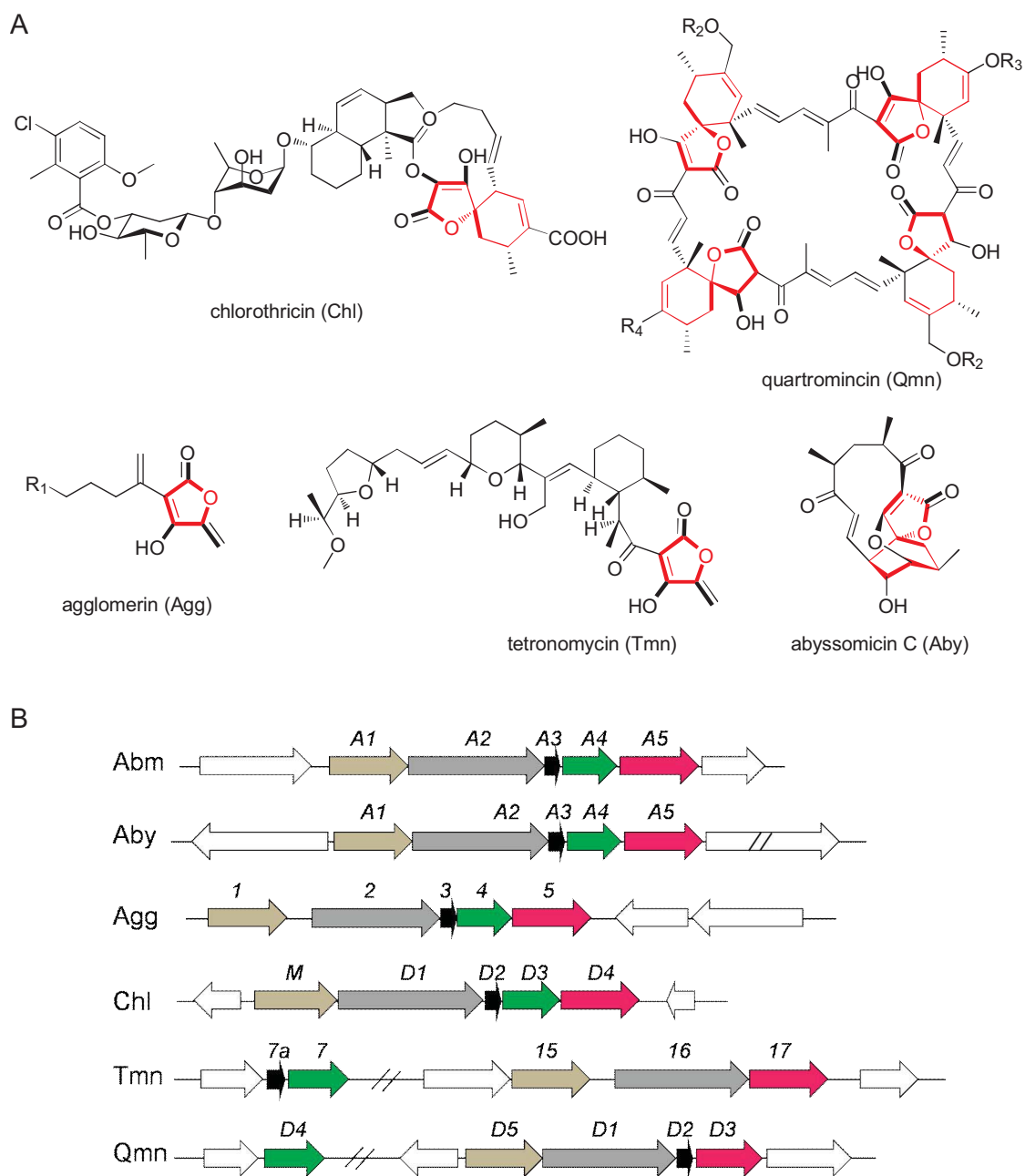


Fig. S1 Chemical structures of tetronate-containing natural products (A) and the unique set of five highly conserved genes responsible for tetronate biosynthesis (B).

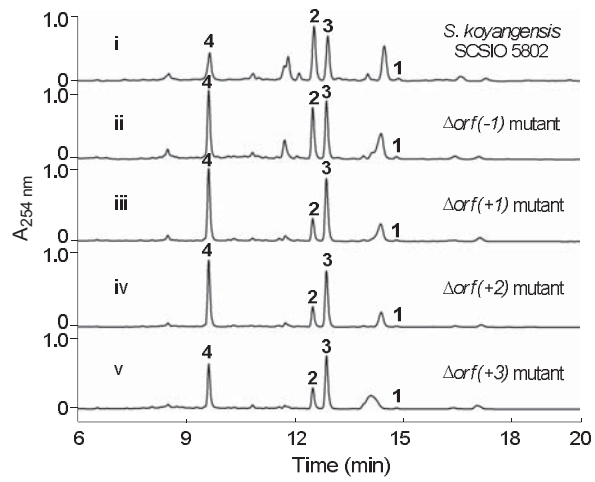


Fig. S2 HPLC analyses of fermentation extracts. i: wild-type; ii: $\Delta orf(-1)$ mutant; iii: $\Delta orf(+1)$ mutant; iv: $\Delta orf(+2)$ mutant; v: $\Delta orf(+3)$ mutant.

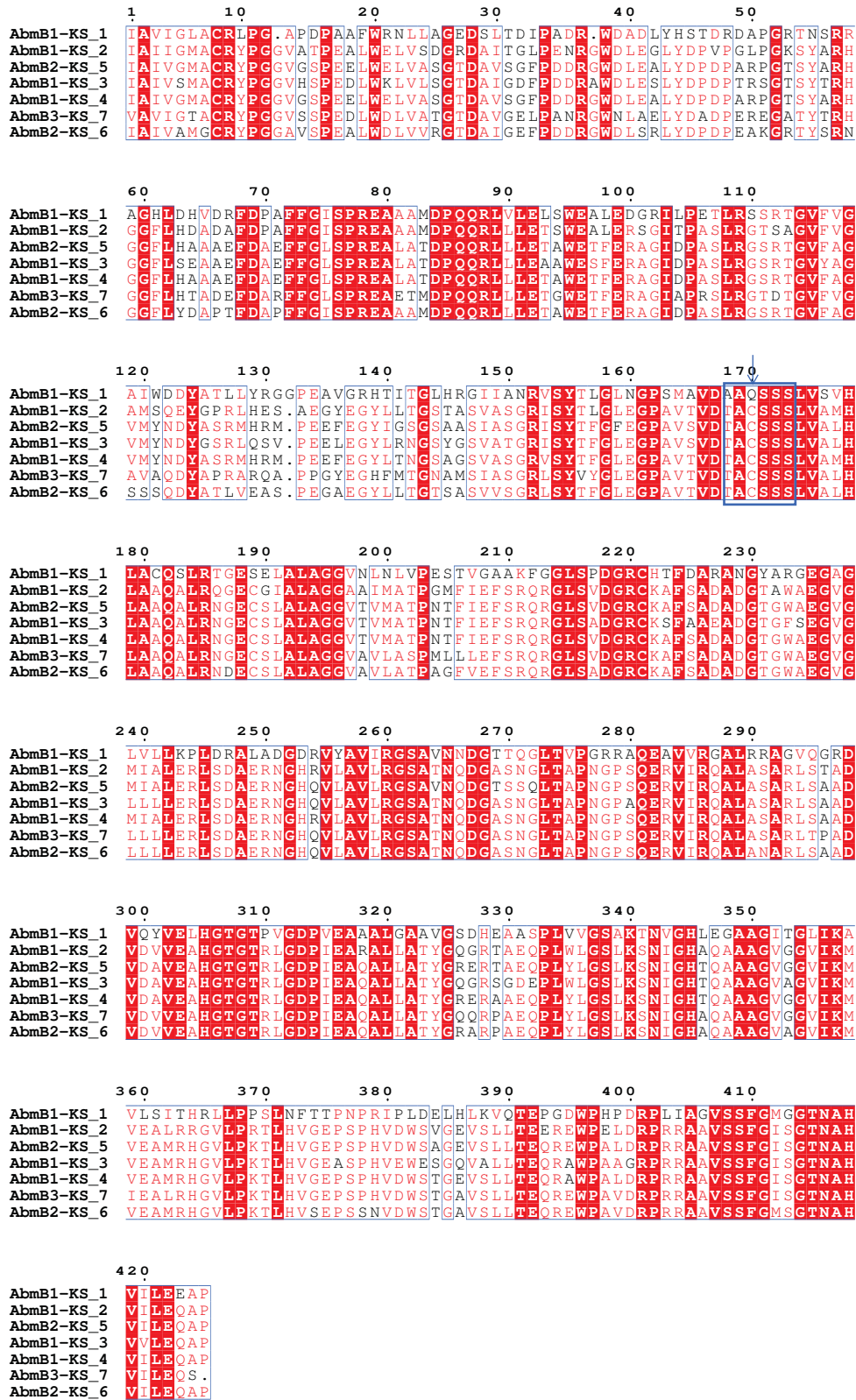


Fig. S3 Alignments of seven KS domains of AbmB1-B3. The conserved motif of KS domains is indicated by red box. The vertical arrow indicates the replacement of the transthiosterification site Cys (C) of AbmB1-KS_1 with Gln (Q).

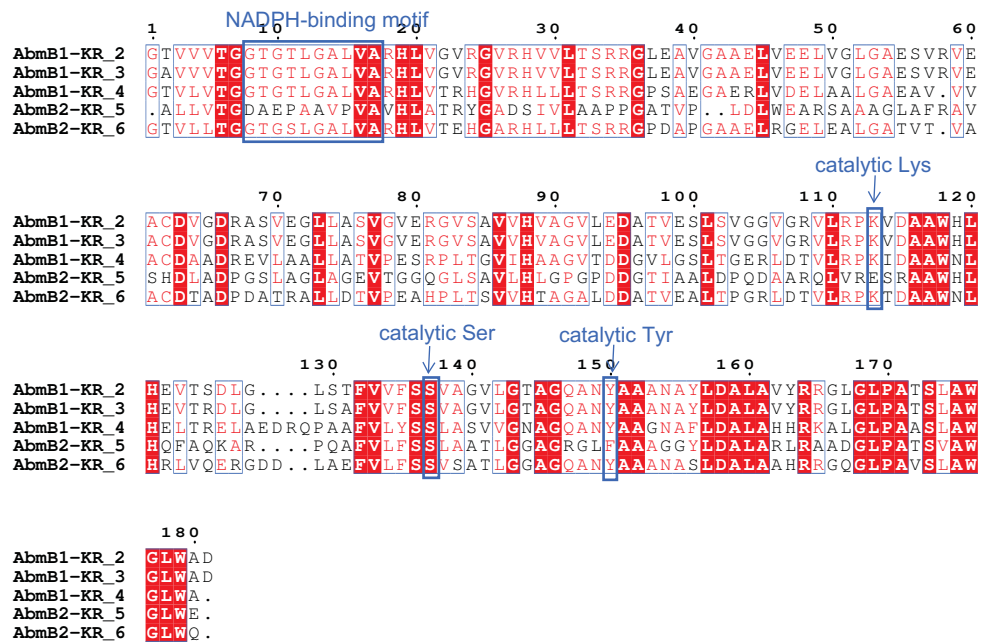


Fig. S4 Alignments of five KR domains of AbmB1-B2. The NADPH-binding motif of KR domains is indicated by blue box. The vertical arrow indicates the active catalytic amino acids.

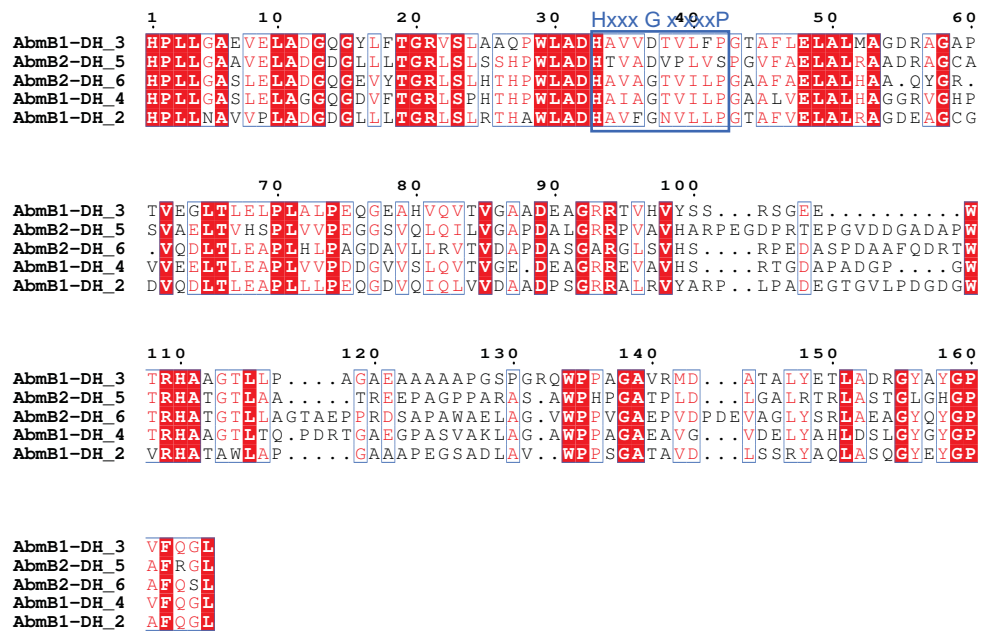


Fig. S5 Alignments of five DH domains of AbmB1-B2. The conserved mitif HxxxGxxxxP motif is indicated by blue box.

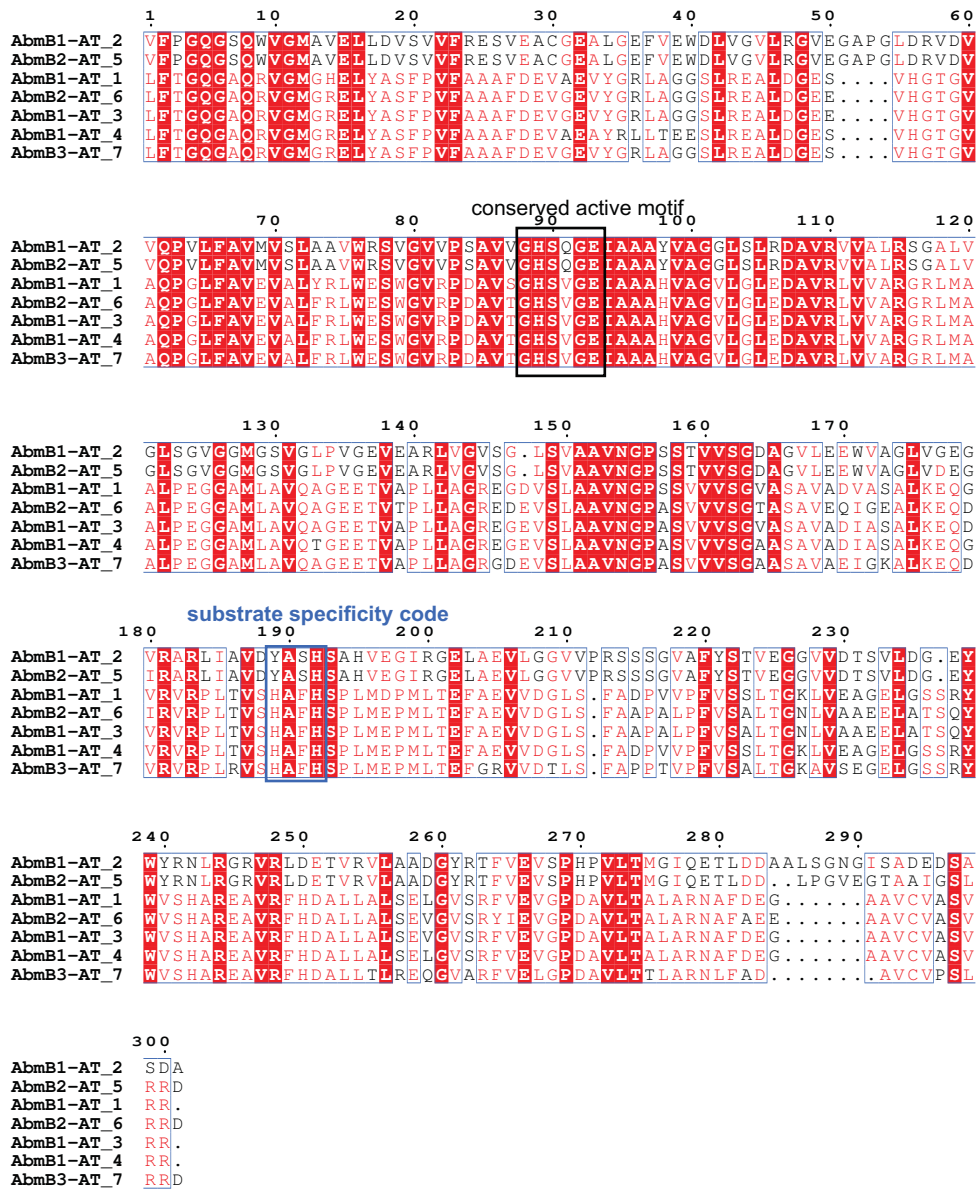


Fig. S6 Alignments of seven AT domains of AbmB1-B3. The conserved active motif of AT domains is indicated by black box. The red box indicates the motif for the substrate specificity.

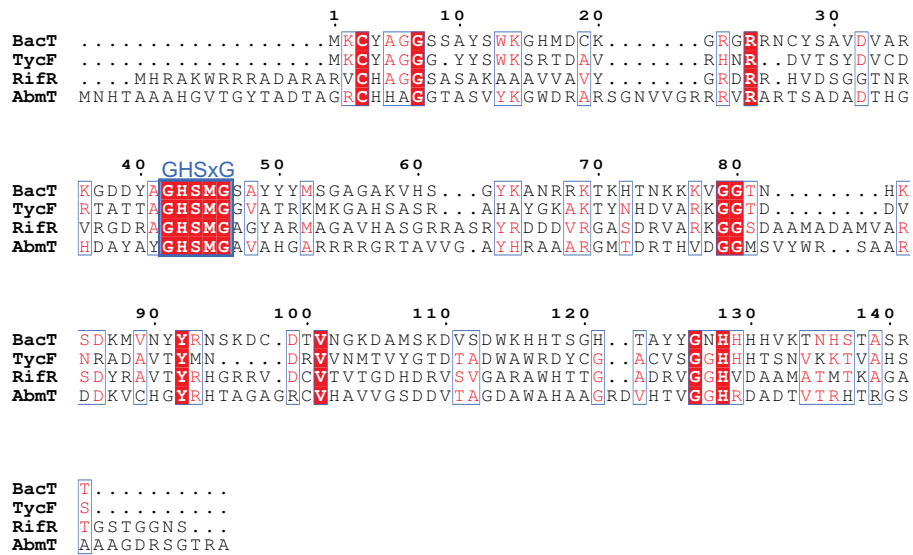


Fig. S7 Alignments of AbmT with the typical type II TEs. The following type II TEs are used for amino acid alignments: BacT from *Bacillus licheniformis* (AF007865.2); TycF from *Brevibacillus brevis* (AF004835); RifR from *Amycolatopsis mediterranei* S699 (AF040570). The conserved GHSxG motif of type II TEs is indicated by blue box.

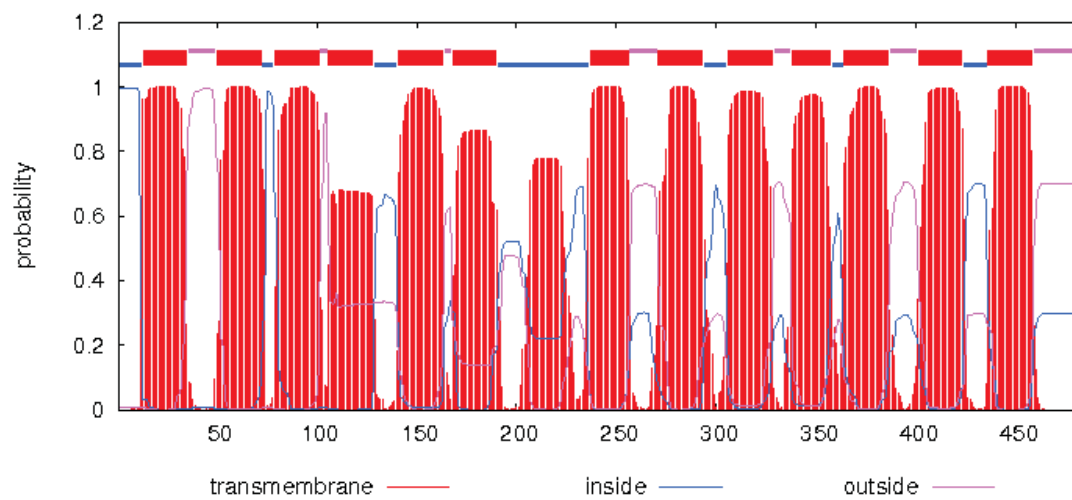


Fig. S8 The 14 transmembranes helixes of AbmD predicted by HMMTOP pogram.

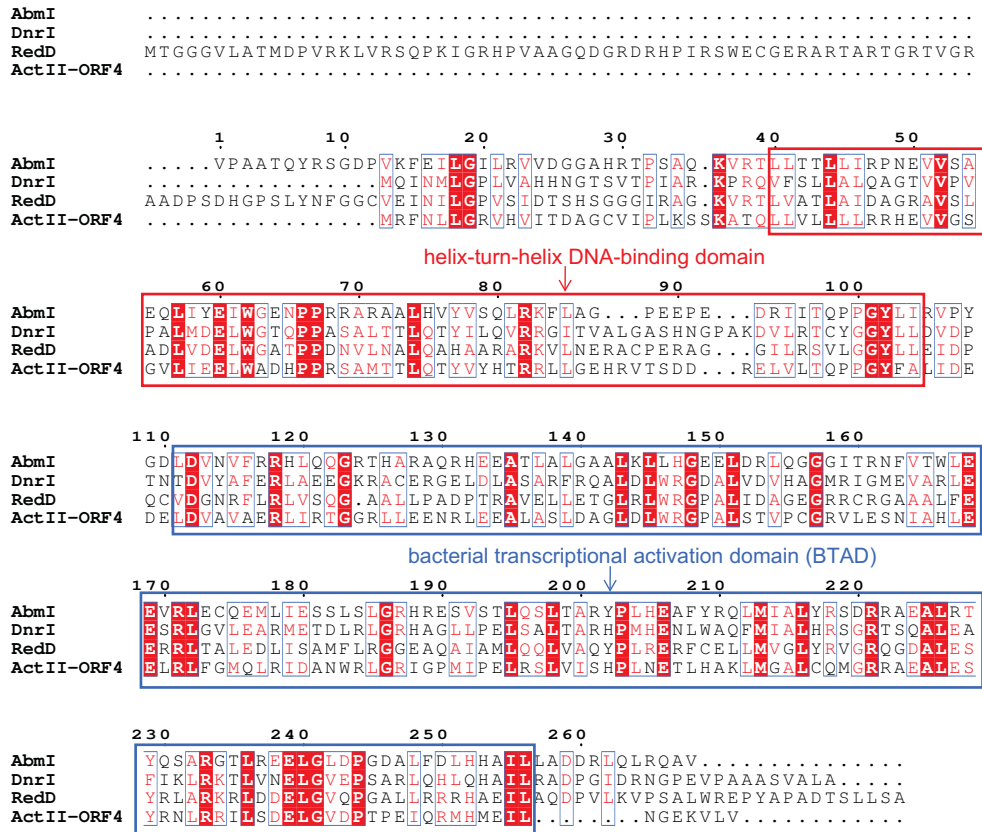


Fig. S9 Alignments of AbmI with the characterized SARP regulators. The following regulators are used for amino acid alignments: DnrI from *Streptomyces peuceitii* (AAA26736.1); RedD from *Streptomyces coelicolor* A3(2) (AAA88556.1); ActII-ORF4 from *synthetic construct* (AAK32147.1). The conserved HTH DNA-binding domain is indicated by red box, and the conserved BTAD is indicated with blue box.

Walker A
↓

1 10 20 30 40 GXXXRQK[T/S]

AbmHVEALGAIRDGRRVVELVGDPTGKTRLLTQLAAEARSRGLVVAQGRATEGDQRVML
AveRMQGVSKLHPPRKPEELTLVDRETQFRALRLALTECAAGTVKLLVAEGGMGC GKSTFLG
GdmRI MTAEINSSLRNPPQRLGMSMGLTDYQSRRLRDVLGTAARNGGLLVVQVGGPGV GKATLLS
NysRIMRKQSGSSGLLTLVGRDDELRTLARHAAAARDGRAGLVLLHGPAGMGKTSLLR
PikDMNLVERDGEIAHLRAVLDAASAAGDGTLLLVSGPAGSGKTELLR
LuxR

60 70 80 90 100 110

AbmH K S I T A S L D C R L V L A A S R D L P N D A A A T I R A I W S Q S P V R F G E A F G D G D H E S V P G V A G R E P .
AveR E A L H T A A A S G F A V L R A A G L P A D H R Q P L G V L Q Q L L N D P A P E D T A R T A V R P M V Q H V R G A . .
GdmRI S L E E Q A A E S D A V C L T A S G F A E D T A I P F N I V E Q L I R S S A A M G E L D V V A R W R T A G E R Y S E A E
NysRI S F T A S D V C R G M T V L Y G T C G E T V A G A G Y G G V R E L L G G L G L S G G D A R R S P L L E G L A A R A L P A
PikD S L R R L A A E R E T P V W S V R A L P G D R D I P L G V L C Q L L R S A E Q H G A D T S A V R D L L D A A S R R A G N
LuxR

Walker B
↓

120 130 140 150 160

AbmH R D L V L S A M R G L L E R L A G N S P T V V I L D D S H W A D P Y T T D I L D Q L V R Y P L
AveR L E R L A A G A P L A I G I D D V Q D A D P E S L H C L M R L L T R H S P
GdmRI H G M L A M L V R E I S D V L H R I A G G K Q L V I A V D D V E H V D Y P S L M C L L H I A R H A S
NysRI L T A D P A G P D A A T G A Y P V L H G L Y W L A A R L M A Q R P L V L V L D D V H W C D E R S L A W I D F L L R R A E
PikD L T S P A D A P L R V D E T H R L H D W L L S V S R R T P F L V A V D D L T H A D T A S L R F L L Y C A A H H D
LuxR

170 180 190 200 210

AbmH N A P L L I V L A H R P R Q T G S T L R S T L A Q G V V S G V V E R T Q T N E I T L G Q A A D L I S V E A R S R
AveR T S R I L L C T A L A C S P . A A D P V L E A E L M R Q T A F E R I T L D C L S L D G V T G L V S D R C A R P T A P P
GdmRI G T R T L V A M S S . G R T H H L C A R V Q G F H N L Y K V E I G T L S E S G V V R L L E R H A D A D L A D R
NysRI D L P L L V V L A W R S E A E . P V A P A V L A D I A A Q R R P T V L G L H P L G P D D I G E M V R R V F R T T A A P S
PikD Q G G I G F V M T E R A S Q R . A G Y R V F R A E L L R Q P H C R N M W L S G L P P S G V R Q L L A H Y Y G P E A A E R
LuxR

220 230 240

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AveR P A D Y C L T V T G G N P L L L R A L L E E H S E A D A P S A P R P A E P S A L H S P P Q A A P P R P V V G G R F Y Q S
GdmRI I G A S C H A I S G G N P R L V R A L L H D H L M S A P D G S E R E V T V G A E F R D
NysRI F V S R V A A V S G G N P L A L A R L L D E L R A E G V R P D A A G E R R A E V G S H D L A R S
PikD R A P A Y H A T I G G N P L L L R A L T Q D R Q A S H T T L G A A G G D E P V H G D A F A Q A
LuxR

250 260 270 280 290 300

AbmH V Q T E A A L L G D D E R R V L E A A A V I D G C F D V T A L A S V A G L E R A R V C V A T R L L N R D L F R H A T P
AveR V L A C L S R T E T A I R Q T A G A L A V L G G R A R A D L L P Q L L G A S P A S V T R G L R A L E A T G L T T S . . .
GdmRI S Y R W S L L C H P S L L Q M A Q I L A V L G P Y G S P R R V A G L L E C G E E R A A R A I D V L D S A G L L E D . . .
NysRI V R C L L E R R P P W V R G V A R A I A V L G . P E C T E L L A A L A G V P A A T V D E A L L V L R R A G I L A A D R .
PikD V L D C L H R S A E G T L E T A R W L A V L E . Q S D P L L V E R L T G T T A A A V E R H I Q E L A A I G L L D E D . .
LuxR

310 320 330 340 350 360

AbmH A S V M V L R H P L V R D A V Y A A T D G C W R R E A H R R A I D Y L R D C G A P P S E L A H H V A A P D S S D S D D
AveR G R F R H P V A E A A A L D A L D P S R R A H L H R R A A A L Q H H D G A A P R D V A R H L L A A R H A A G P W A
GdmRI G C F R H P A A R S V A L E T L A V E E R A R L G A K A A E L L Y A D G A D P I A V A E L L V T A G K T P D H K G
NysRI V D F V H D V V R S A V L D D V A P P T L A E L R T N A A L L L S D A G R P S E E L A G Q L M L L P V L D Q P N M
PikD G T L G Q P A I R E A A L Q D L P A G E R T E L H R R A A E Q L H R D G A D E D T V A R H L L V G G A P D A P W A
LuxR

370 380 390 400 410 420

AbmH L D T I L R A A E E P L P . G N P E R A I T W L E A A L R L S L P R R Q R G L A L I A L S R A L C V A E R F A D A M P H
AveR V S V L R D A A E Q S L A Q D D V A S A V S C L E L A Y G A C V R E R E R P E I R I R L A A A F G R T N . I A V A E E H
GdmRI V A V L W H A A Q K N L D E G R T E E A V A G L R L A R A D L G R R E R L D I R M A L V G A L W A G N . P A A A E P E
NysRI A A V L R D A A A Q A E S R G A P E A G V R C L Y R V L E V E P D N V A V R I Q M A R A L A E I N . P P E A M R L
PikD L P L L E R G A Q Q A L F D D R L D D A F R I L E F A V R S S T D N T Q L A R L A P H L V A A S W R M N . P H M T T R A
LuxR

430 440 450 460 470 480

AbmH L R E A V A M G A A L P V E I W A K A V S Q S A A I D T L F G R Y S E A N A T L D G A L A M L R G R P S L E E V E L
AveR L A D L V A T L R E G . E L T G H Q T A L L V P L L V N H G R L G E A R E A M D R L N A A D D A R G L C A D G G F
GdmRI L D R L M A A I R T E . S P A D I P E Q Y L C F L L F M V L W F G R F S D V E E A F T W L S G S G S G S N A S M A A L
NysRI L K E A L S L A G D V R T R A Q V A V Q Y G F T C L A V Q E S P S G V R M L E D A L A E L T A E L G P E P G P V D R E L
PikD L A . L F D R L L S G . E L P P S H P V M A L I R C L V W Y G R L P E A D A D A L S R L R P S S D N D A L E L S . . .
LuxR

490 500 510 520 530

AbmH L V R R G V I G L Y D G G A L D T G T V D L A A R R A Q E F A C H T S T A A A M A L R A L K S T S I G
AveR P M A A P W P S T A H L A A R R D P A A R R D P G T R R D P A D K P F L P R Q S G A P Q P R P E D G R G Q O P T A A L W
GdmRI R V T R Q W V T F L K P T L I H H F P D Q D P S D R E V D G L W A A N L E H G L Q L S V D E L G M E I G
NysRI R T L V E S V L L I V G A D E K V T I G A V R D R A A R L T M P P G D T P A Q R Q M L A M T V L
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LuxR

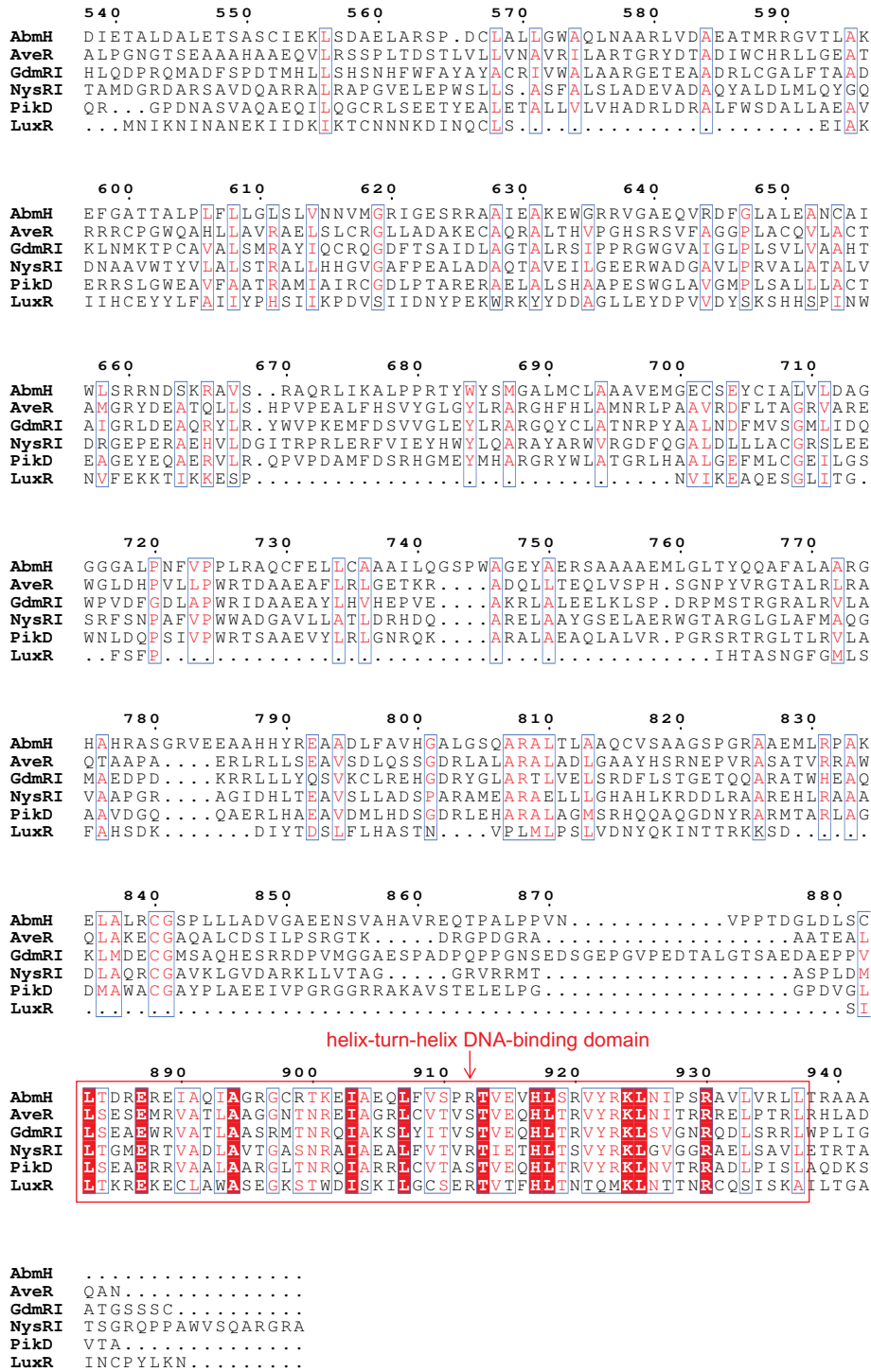


Fig. S10 Alignments of AbmH with the characterized LuxR-regulators from *Streptomyces avermitilis* (AveR, BAA84600.1), *Streptomyces hygroscopicus* (GdmRI, ABI93791.1), *Streptomyces noursei* ATCC 11455 (NysRI, AAF71778.1), *Streptomyces venezuelae* (PikD, AAC68887.1) and *Vibrio fischeri* ES114 (LuxR, YP_206883.1). The conserved Walker A motif (GxxxxGK[T/S]) and Walker B motif (hhhhD) (where h is a hydrophobic residue) in the NTP-binding domain is indicated by blue box; the conserved HTH DNA-binding domain is indicated with red box.

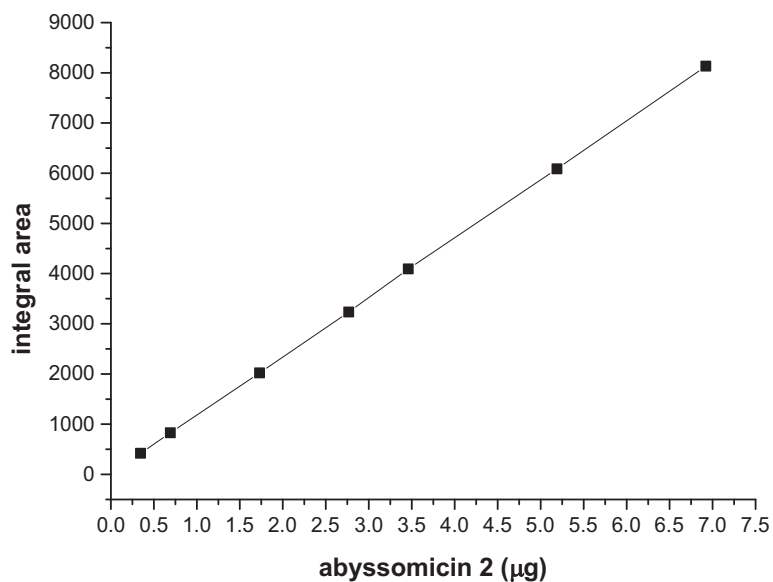


Fig. S11 The quantitative HPLC standard curve for abyssomicin 2 (**3**). The curve was generated via analysis of a concentration gradient of 0.346 µg, 0.692 µg, 1.730 µg, 2.768 µg, 3.461 µg, 5.191 µg, 6.922 µg of **3**. UV absorptions were maintained below 1 A unit to ensure appropriate confidence of the generated standard curve and avoid deviation from linearity.

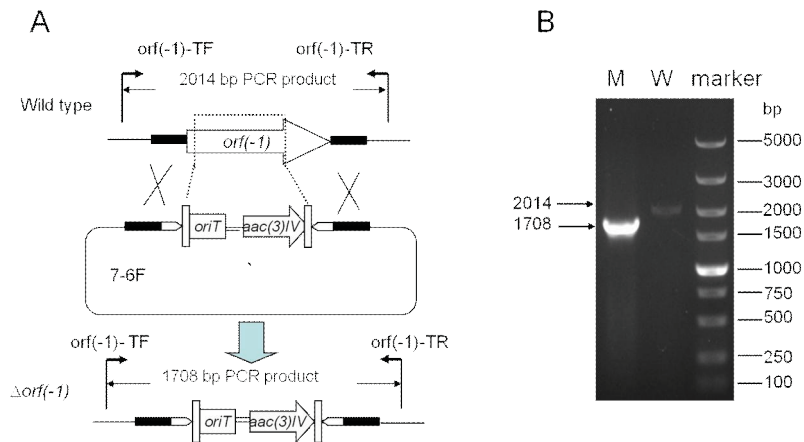


Fig. S12 Disruption of *orf(-1)* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *orf(-1)*. (B) PCR analyses of the wild-type strain and the of *orf(-1)* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *orf(-1)* mutant as template.

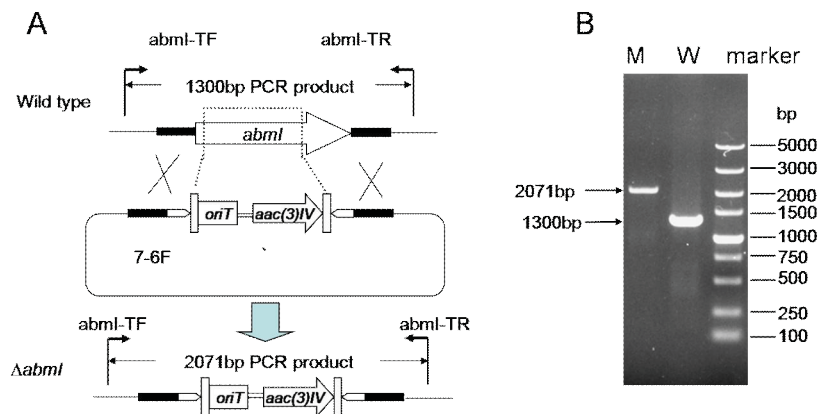


Fig. S13 Disruption of *abmI* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmI*. (B) PCR analyses of the wild-type strain and the of *abmI* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmI* mutant as template.

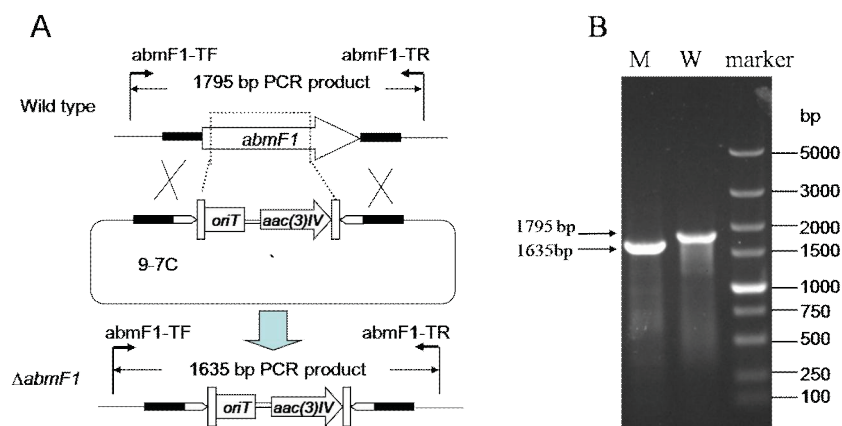


Fig. S14 Disruption of *abmF1* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmF1*. (B) PCR analyses of the wild-type strain and the of *abmF1* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmF1* mutant as template.

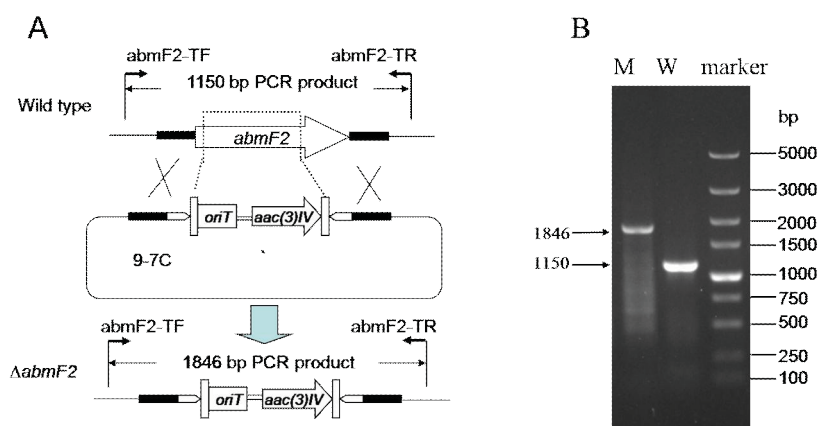


Fig. S15 Disruption of *abmF2* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmF2*. (B) PCR analyses of the wild-type strain and the of *abmF2* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmF2* mutant as template.

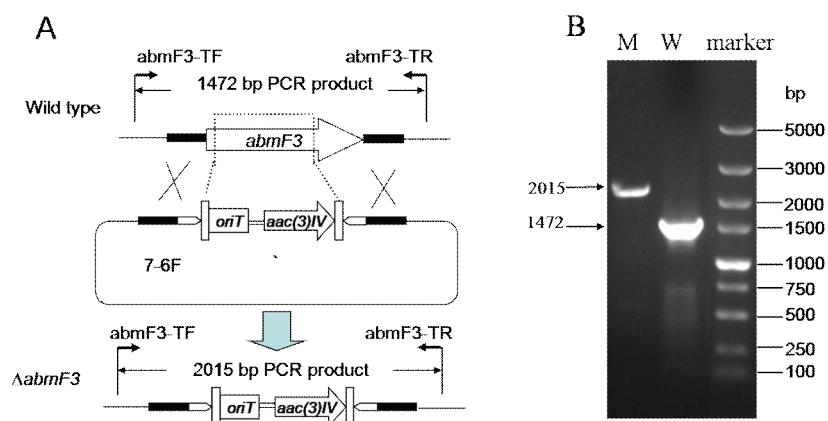


Fig. S16 Disruption of *abmF3* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmF3*. (B) PCR analyses of the wild-type strain and the of *abmF3* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmF3* mutant as template.

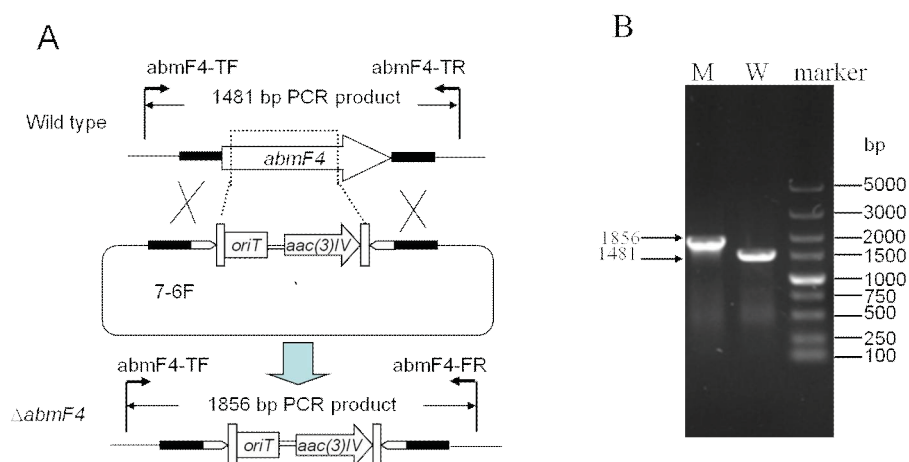


Fig. S17 Disruption of *abmF4* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmF4*. (B) PCR analyses of the wild-type strain and the of *abmF4* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmF4* mutant as template.

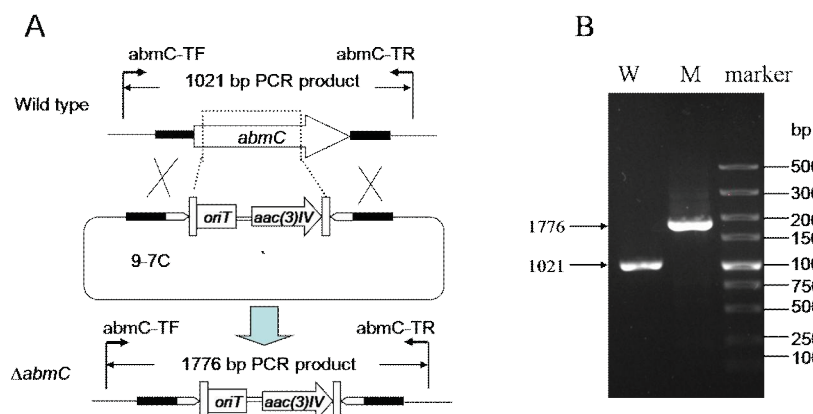


Fig. S18 Disruption of *abmC* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmC*. (B) PCR analyses of the wild-type strain and the of *abmC* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmC* mutant as template.

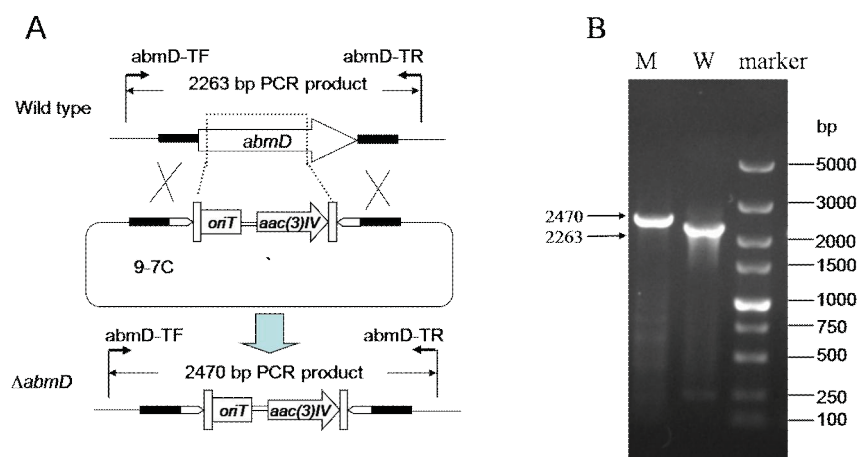


Fig. S19 Disruption of *abmD* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmD*. (B) PCR analyses of the wild-type strain and the of *abmD* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmD* mutant as template.

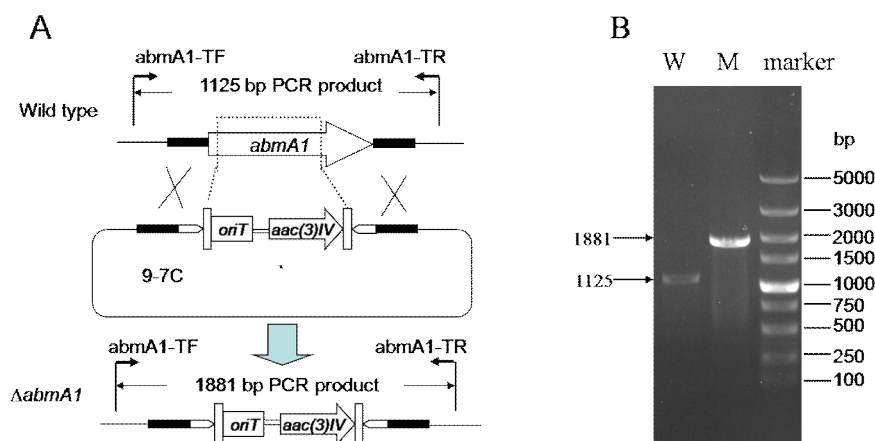


Fig. S20 Disruption of *abmA1* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmA1*. (B) PCR analyses of the wild-type strain and the of *abmA1* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmA1* mutant as template.

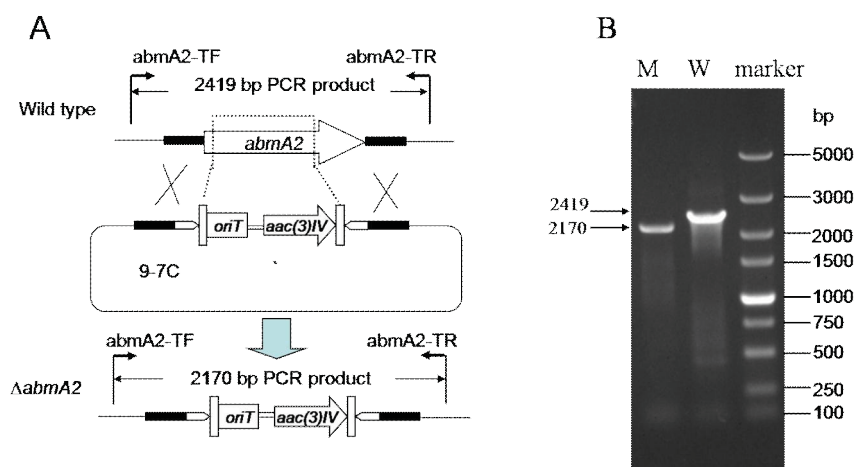


Fig. S21 Disruption of *abmA2* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmA2*. (B) PCR analyses of the wild-type strain and the of *abmA2* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmA2* mutant as template.

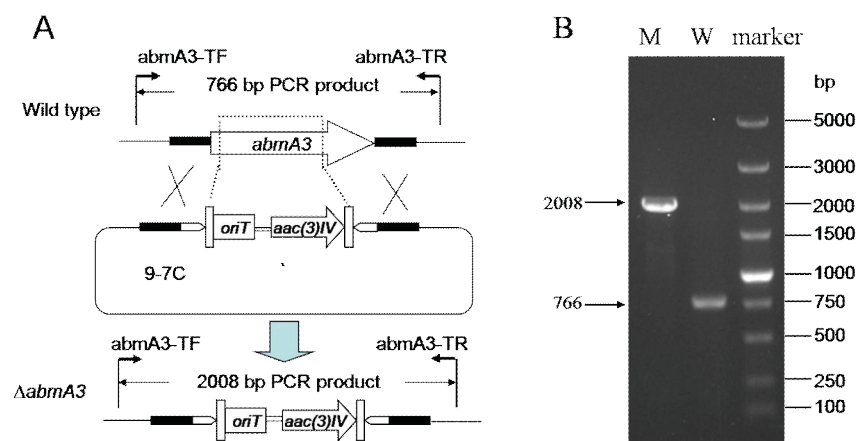


Fig. S22 Disruption of *abmA3* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmA3*. (B) PCR analyses of the wild-type strain and the of *abmA3* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmA3* mutant as template.

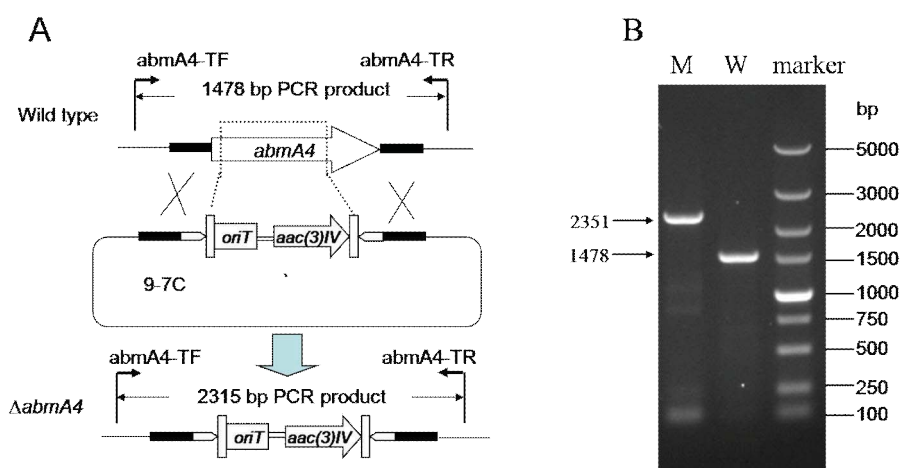


Fig. S23 Disruption of *abmA4* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmA4*. (B) PCR analyses of the wild-type strain and the of *abmA4* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmA4* mutant as template.

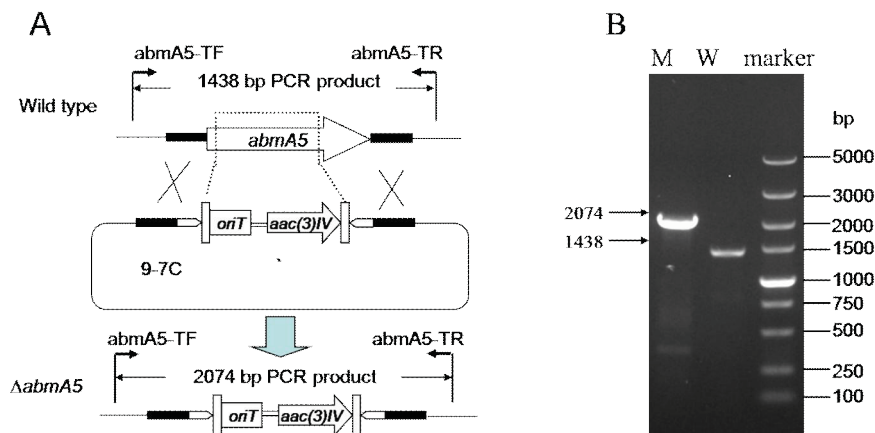


Fig. S24 Disruption of *abmA5* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmA5*. (B) PCR analyses of the wild-type strain and the of *abmA5* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmA5* mutant as template.

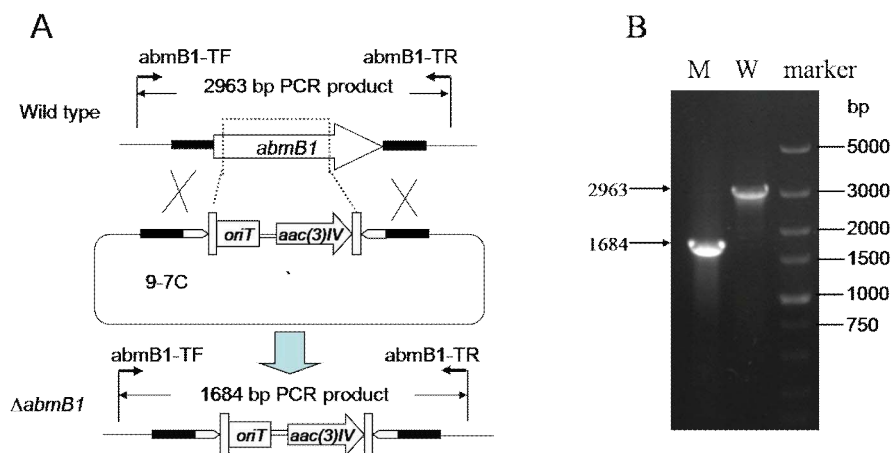


Fig. S25 Disruption of *abmB1* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmB1*. (B) PCR analyses of the wild-type strain and the of *abmB1* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmB1* mutant as template.

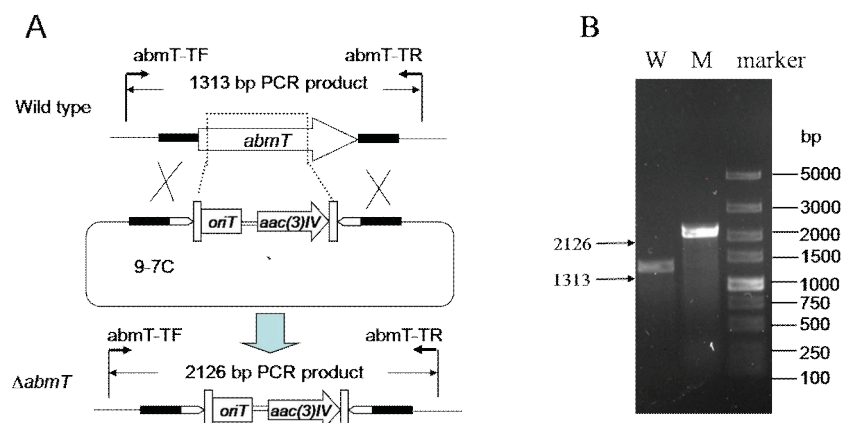


Fig. S26 Disruption of *abmT* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmT*. (B) PCR analyses of the wild-type strain and the of *abmT* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmT* mutant as template.

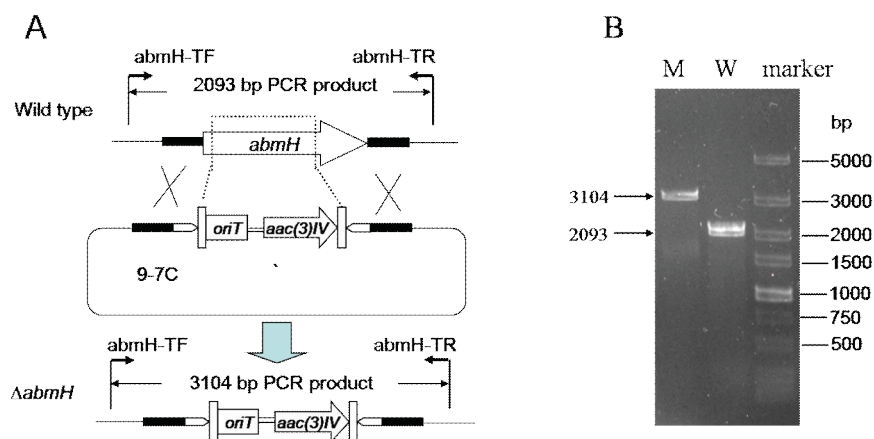


Fig. S27 Disruption of *abmH* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *abmH*. (B) PCR analyses of the wild-type strain and the of *abmH* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *abmH* mutant as template.

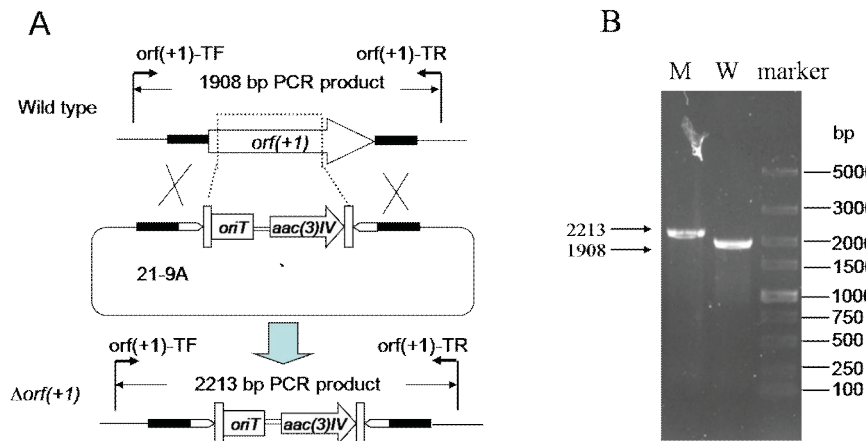


Fig. S28 Disruption of *orf(+1)* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *orf(+1)*. (B) PCR analyses of the wild-type strain and the of *orf(+1)* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *orf(+1)* mutant as template.

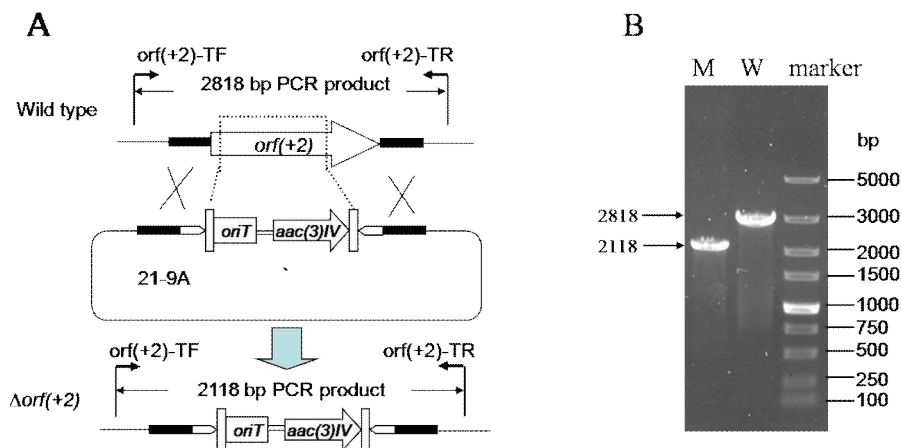


Fig. S29 Disruption of *orf(+2)* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *orf(+2)*. (B) PCR analyses of the wild-type strain and the of *orf(+2)* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *orf(+2)* mutant as template.

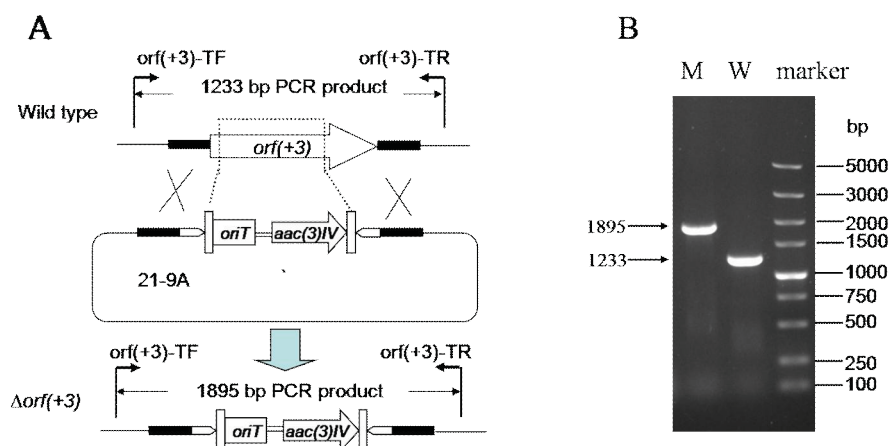


Fig. S30 Disruption of *orf(+3)* in wild-type *S. koyangensis* SCSIO 5802 via PCR-targeting. (A) Schematic representation for disruption of *orf(+3)*. (B) PCR analyses of the wild-type strain and the of *orf(+3)* double-cross mutant carried out using the primers listed in Table S2. Marker: DNA molecular ladder; W: using the genomic DNA of *S. koyangensis* SCSIO 5802 as template; M: using the genomic DNA of *orf(+3)* mutant as template.

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3. Gust B, Challis GL, Fowler K, Kieser T, Chater KF. PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci U S A*. 2003;100:1541-6.
4. Hong B, Phornphisutthimas S, Tilley E, Baumberg S, McDowall KJ. Streptomycin production by *Streptomyces griseus* can be modulated by a mechanism not associated with change in the *adpA* component of the A-factor cascade. *Biotechnol Lett*. 2007;29:57-64.