

1 **SUPPLEMENTARY DATA**

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3 **Biosynthesis of Sibiromycin, a potent antitumor antibiotic<sup>†</sup>**

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**Table S1. Strains and plasmids used in this study.**

Strain & vector	Genotype comment	Source or reference
<i>Bacillus subtilis</i>	antibiotic test strain	NRRL354
<b>E coli strains</b>		
GeneHogs	F <sub>-</sub> <i>mcrA</i> <sub>-</sub> ( <i>mrr-hsdRMS-mcrBC</i> ) 80 <i>dlacZ</i> <sub>-</sub> M15/ <i>araD139</i> <sub>-</sub> ( <i>ara-leu</i> )7697 <sub>-</sub> <i>lacX74 galU galK rpsL deoR endA1 nupG recA1 trfA Ampr fhuA::IS2</i>	Invitrogen
XL-1 Blue MR.	( <i>mcrA</i> )183 .( <i>mcrCB-hsdSMR-mrr</i> )173 <i>endA1 supE44 thi-1 recA1 gyrA96 relA1 lac</i> .	Stratagene
ET12567	( <i>dam</i> <sup>-</sup> <i>dcm</i> - <i>hsdM</i> -), Tet <sup>R</sup> , Cm <sup>R</sup>	Dr. B. Gust (University of Tübingen) (27)
<b>Streptosporangium strains</b>		
<i>S. sibiricum</i>	wild-type strain, sibiromycin producer	DSM 44093
<i>S. sibiricum</i> Δ <i>X1</i>	<i>orfX1</i> -disrupted mutant of <i>S. sibiricum</i>	This study
<i>S. sibiricum</i> Δ <i>X2</i>	<i>orfX2</i> -disrupted mutant of <i>S. sibiricum</i>	This study
<i>S. sibiricum</i> Δ <i>X3</i>	<i>orfX3</i> -disrupted mutant of <i>S. sibiricum</i>	This study
<i>S. sibiricum</i> Δ <i>sibA</i>	<i>sibA</i> -disrupted mutant of <i>S. sibiricum</i>	This study
<i>S. sibiricum</i> Δ <i>sibC</i>	<i>sibC</i> -disrupted mutant of <i>S. sibiricum</i>	This study
<i>S. sibiricum</i> Δ <i>sibE</i>	<i>sibE</i> -disrupted mutant of <i>S. sibiricum</i>	This study
<i>S. sibiricum</i> Δ <i>sibG</i>	<i>sibG</i> -disrupted mutant of <i>S. sibiricum</i>	This study
<b>Plasmids</b>		
SuperCos1	Cosmid cloning vector; <i>bla</i> , <i>neo</i> , <i>cos</i>	Stratagene
pUZ8002	non-transmissible helper plasmid; <i>tra</i> , <i>neo</i> RP4	Dr. B. Gust (University of Tübingen) (19)
pGEM-T easy	<i>E. coli</i> subcloning vector	Promega
pIJ790	λ-RED ( <i>gam</i> , <i>bet</i> , <i>exo</i> ), <i>cat</i> , <i>araC</i> , <i>rep101</i> <sup>ts</sup>	Dr. B. Gust (University of Tübingen) (8)
pIJ773	<i>aac(3)IV</i> (Apr <sup>R</sup> ) <sub>-</sub> <i>oriT</i>	Dr. B. Gust (University of Tübingen) (8)
pT-Glu	541bp PCR product of the dTDP-glucose 4,6 dehydratase gene in pGEM-T Easy	This study
pT-A3A7	724 bp PCR product of the NRPS adenylation domain in	This study

	pGEM-T Easy	
pT-S2	455 bp PCR product of probe S2 in pGEM-T Easy	This study
pSuperSib1	Cosmid clone from <i>S. sibiricum</i> DSM 44093 genomic library, 38.7-kb chromosomal fragment in SuperCos1; Ap <sup>R</sup> , Km <sup>R</sup>	This study
pSuperSib2	Cosmid clone from <i>S. sibiricum</i> DSM 44093 genomic library, 45-kb chromosomal fragment in SuperCos1; Ap <sup>R</sup> , Km <sup>R</sup>	This study
pSuperSib1Δ <i>X1</i>	<i>orfX1</i> replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib1	This study
pSuperSib1Δ <i>X2</i>	<i>orfX2</i> replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib1	This study
pSuperSib1Δ <i>X3</i>	<i>orfX3</i> replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib2	This study
pSuperSib1Δ <i>sibA</i>	<i>sibA</i> gene replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib1	This study
pSuperSib1Δ <i>sibC</i>	<i>sibC</i> gene replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib1	This study
pSuperSib1Δ <i>sibE</i>	<i>sibE</i> gene replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib1	This study
pSuperSib1Δ <i>sibG</i>	<i>sibG</i> gene replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib1	This study
pSuperSib1Δ <i>CTRL</i>	<i>bla</i> gene on SuperCos1 replaced with pIJ773 Apr <sup>R</sup> - <i>oriT</i> cassette in pSuperSib1	This study

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**Table S2. Oligonucleotides PCR primers used in this study.**

Primer	Sequence (5'—3')
<b>For library screening</b>	
Glu1	CSGGSGSSGCSGGSTTCATSGG
Glu2	GGGWRCTGGYRSGGSCCGTAGTTG
A3	GCSTACSYSATSTACACSTCSGG
A7R	SASGTCVCCSGTSCGGTAS
S2_F	CGGGACGGTGTTGTTTCG
S2_R	GTGGTCAGCTACCTGATG
<b>For gene inactivation<sup>a</sup></b>	
<i>orfX1_F</i>	<u>AACCCGCCACCACCCTTCACCGAAGAGGAGACGCCATGCCTAGGgggatccgtcgac</u> c

<i>orfX1_R</i>	CCACGATCGTCGGCGAGGCGGGAGACATGCCGGCCCTCACTAGTggctggagctgcttc
<i>orfX2_F</i>	GCCGCCCCGACGACGGCGGCCACCGGAAAGGACAAGGATGCCTAGGgggatccgtcgacc
<i>orfX2_R</i>	GGCGTCTCCTCTTCGGTGAAGGGTGGTGGGCGGGTTTCACTAGTggctggagctgcttc
<i>orfX3_F</i>	GGGGCCGCGACGAATCGACCCGCCAGGAAGAAGCGAATGCCTAGGgggatccgtcgacc
<i>orfX3_R</i>	ACCGCTTCCGTGGACGCCACCGCTTCCGGGGACGCCTCACTAGTggctggagctgcttc
<i>sibA_F</i>	AGACGATCCGGAGATCCATTGACATGGGGGTAATGAATGCCTAGGgggatccgtcgacc
<i>sibA_R</i>	TCCCCGGACGGTCTTCGCCTTTCGTAAGGACATCGTCACTAGTggctggagctgcttc
<i>sibC_F</i>	TACCTCGGCGCCATGACCTCAGGAAGGGCTCCCTCCATGATTCCGgggatccgtcgacc
<i>sibC_R</i>	GAGGGTCCACGGTGACCTCGTACGGGGTGTCCGGACATCATGTAaggctggagctgcttc
<i>sibE_F</i>	TCCGCCCTTCCCTCTCTCCGAGTCCCCACCCTTCCGGTGTCTAGAgggatccgtcgacc
<i>sibE_R</i>	GCCAGCGGTCGCGGACCACAGTGAGCAGCACGTCGCTCAACTAGTctggagctgcttc
<i>sibG_F</i>	GATCAACCGGATCAGCCAGTTGATCCAGGACAGTCGATGTCTAGAgggatccgtcgacc
<i>sibG_R</i>	TCCTCCCAATCATGTTGCTGTTCGATCGATGTGCGTTCAACTAGTggctggagctgcttc
Control-F	AATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAgttaggctggagctgcttc
Control-R	TGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGattccggggatccgtcgacc

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**For gene disruption confirm**

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<i>orfX2_C_F</i>	CGGAAAGGACAAGGATG
<i>orfX2_C_R</i>	GTGGTGGGCGGGTTTC
<i>orfX3_C_F</i>	GCCAGGAAGAAGCGAATG
<i>orfX3_C_R</i>	CCGAGGTTGTGCTTCTAC
<i>sibA_C_F</i>	CATGGGGGTAATGAATG
<i>sibA_C_R</i>	CCGTAAGGACATCGTC
<i>sibC_C_F</i>	GAAAGGCGAAGACCGTCC
<i>sibC_C_R</i>	CGCTGGGGCTCGAGATTC
<i>sibE_C_F</i>	GGTGGTGAATTCCCCACCCTTCCG

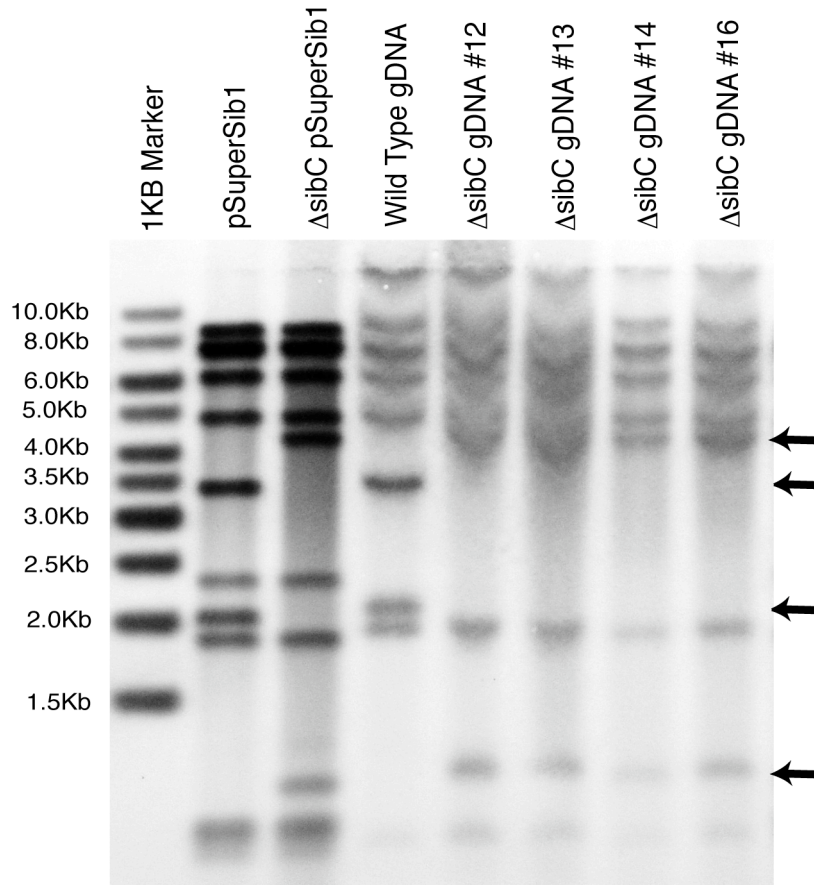
*sibE\_C\_R* GGTGGTGGATCCACGCCCGAGGATG

*sibG\_C\_F* TGCCTTTGAGGTTGTTGG

*sibG\_C\_R* TTCCCCAGGTGACGCT

<sup>a</sup>Homologous region of the apramycin cassette are in lowercase.

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22 **Fig. S1** Southern blot analysis of genomic and cosmid DNA of wild-type and  $\Delta$ sibC strains.

23 Digestion with Pst1 of wild-type DNA yields two fragments of 3.3 and 2 Kb. The absence of

24 these fragments and the presence of two fragments new at 4.3 and 1 Kb in  $\Delta$ sibC strain are

25 consistent with a successful gene replacement.