
Cytoplasmic contractile injection systems mediate cell death in *Streptomyces*

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Supplementary Table 1. Proteins detected by mass spectrometry in samples of purified CIS from WT *S. coelicolor* (ScoWT) and *S. venezuelae* (SvenWT), the corresponding $\Delta cis2$ mutants and the *S. coelicolor* non-contractile CIS^{Sc} mutant CIS–N5. Experiments were performed in biological replicates.

Protein ID	CIS ID	<i>S. coelicolor</i> WT	<i>S. coelicolor</i> $\Delta cis2$	<i>S. coelicolor</i> CIS–N5	<i>S. venezuelae</i> WT	<i>S. venezuelae</i> $\Delta cis2$
Sco4244/Vnz_28875	Cis11	-	-	29% coverage / 10 total unique peptide	-	-
Sco4245/Vnz_28880	Cis9	-	-	21% coverage / 2 total unique peptide	-	-
Vnz_28885	Cis10	-	-	-	-	-
Sco4246/Vnz_28890	Cis8	-	-	46% coverage / 18 total unique peptide	-	-
Sco4247/Vnz_28895	Cis7	-	-	51% coverage / 8 total unique peptide	-	-
Sco4248/Vnz_28900	Cis5	-	-	39% coverage / 4 total unique peptide	-	-
Sco4251/Vnz_28915	-	-	-	-	-	-
Sco4252/Vnz_28920	Cis1	27% coverage / 4 total unique peptide	-	32% coverage / 5 total unique peptide	17% coverage / 3 total unique peptide	-
Sco4253/Vnz_28925	Cis2	48% coverage / 26 total unique peptide	-	51% coverage / 28 total unique peptide	38% coverage / 17 total unique peptide	-
Sco4254	-	-	-	10% coverage / 6 total unique peptide	-	-
Sco4259/Vnz_28930	Cis15	-	-	-	-	-
Sco4260/Vnz_28935	Cis16	-	-	24% coverage / 4 total unique peptide	-	-

7 **Supplementary Table 2. Cryo-EM data statistical analysis**

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	Contracted sheath shell	Extended sheath-tube module
Data collection and processing		
Nominal magnification		81,000
Voltage (kV)		300
Electron exposure (e ⁻ /Å)		60 e ⁻ /dose weighting (K2 Summit camera)
Defocus range (μm)		1.5-3.5
Pixel size (Å/pixel)		1.4
Symmetry imposed	C6 + helical	C6 + helical
	(twist = 26.58°, rise = 17.22 Å)	(twist = 38.50°, rise = 23.10 Å)
Final particles (No.)	4838	18822
Map resolution	3.6	3.9
FSC threshold	0.143	0.143
Refinement		
Map sharpening B factor (Å ²)	-118	-130

Model composition	(Four layers)	(Four layers)
Non-hydrogen atoms	68280	96384
Protein residues	8808	12480
Chains	24	48
R.M.S deviations		
Bond length (Å)	0.004	0.018
Bond angles (°)	0.983	1.818
Validation		
MolProbity score	1.88	1.85
Clashscore	9.74	8.19
Rotamer outlier (%)	0.00	0.50
Ramachandran plot		
Favored (%)	94.61	93.89
Allowed (%)	5.39	5.62
Outlier (%)	0.00	0.50
Masked CC	0.85	0.68

Supplementary Table 3. Experimental approaches to study effects of CIS^{Sc} on interspecies competition.

Target organisms	Functional assay	Procedures
<i>Saccharomyces cerevisiae</i> <i>Escherichia coli</i> <i>Bacillus subtilis</i> <i>Micrococcus luteus</i> <i>S. venezuelae</i>	Killing assay on plate or in liquid	Co-incubation with <i>S. coelicolor</i> wild-type, Δ CIS ^{Sc} and CIS-N5 mutant strains
		Co-incubation with purified CIS ^{Sc} particles
<i>Lactococcus lactis</i> (Nisin producer)	Killing assay on plate	Co-incubation with nisin-treated <i>S. coelicolor</i> wild-type, Δ CIS ^{Sc} and CIS-N5 mutant strains
Wax moth larvae	Injection into larvae hemocoel	Injection of purified CIS ^{Sc} particles from <i>S. coelicolor</i> wild-type, Δ CIS ^{Sc} and CIS-N5 mutant strains

Supplementary Table 4. Shown is a list of CIS-positive (presence of a CIS gene cluster in the genome indicated by “+”) and CIS-negative (absence of a CIS gene cluster in the genome indicated by “-“) *Streptomyces* strains based on Chen et al.⁸. All genomes were searched by BLAST for homologues of the *S. coelicolor* effectors (Sco4256-58; “+” indicates at least one homologue was present, “-“ indicates no homologue was present) and of the putative cell-envelope adaptor (Sco4242; “+”, present; “-“, absent).

Organism Name – CIS-positive strains	Effectors (Sco4256-58)	Adaptor (Sco4242)
<i>Streptomyces albireticuli</i> MDJK11	-	+
<i>Streptomyces alboflavus</i> MDJK44	+	+
<i>Streptomyces albulus</i> NK660	-	-
<i>Streptomyces albulus</i> ZPM	-	-
<i>Streptomyces albus</i> DSM 41398	+	+
<i>Streptomyces albus</i> SM254	+	+
<i>Streptomyces albus</i> BK£-25	+	+
<i>Streptomyces albus</i> J1074	+	+
<i>Streptomyces alfalfae</i> ACCC40021	-	+
<i>Streptomyces ambofaciens</i> DSM 40697	+	+
<i>Streptomyces ambofaciens</i> ATCC 23877	+	+
<i>Streptomyces anulatus</i> ATCC 11523	+	+
<i>Streptomyces autolyticus</i> CGMCC0516	-	+

<i>Streptomyces avermitilis</i> MA-4680 = NBRC 14893	-	+
<i>Streptomyces bingchenggensis</i> BCW-1	-	+
<i>Streptomyces cattleya</i> NRRL 8057 = DSM 46488	-	+
<i>Streptomyces cattleya</i> NRRL 8057 = DSM 46488	-	+
<i>Streptomyces chartreusis</i> NRRL 3882	-	-
<i>Streptomyces clavuligerus</i> F613-1	+	+
<i>Streptomyces clavuligerus</i> ATCC 27064	+	+
<i>Streptomyces coelicolor</i> A3(2)	+	+
<i>Streptomyces collinus</i> Tu 365	-	+
<i>Streptomyces cyaneogriseus</i> subsp. <i>Noncyanogenus</i> NMWT 1	-	+
<i>Streptomyces davaonensis</i> JCM 4913	-	-
<i>Streptomyces formicae</i> KY5	+	-
<i>Streptomyces fulvissimus</i> DSM 40593	+	+
<i>Streptomyces glaucescens</i> GLA.O	-	+
<i>Streptomyces globisporus</i> TFH56	+	+
<i>Streptomyces globisporus</i> C-1027	+	+
<i>Streptomyces griseochromogenes</i> ATCC 14511	-	-
<i>Streptomyces griseus</i> subsp. <i>griseus</i> NBRC 13350	+	+
<i>Streptomyces hygroscopicus</i> XM201	-	+

<i>Streptomyces hygroscopicus</i> subsp. <i>jinggangensis</i> 5008	-	+
<i>Streptomyces hygroscopicus</i> subsp. <i>jinggangensis</i> TL01	-	+
<i>Streptomyces hygroscopicus</i> subsp. <i>Limoneus</i> KCTC 1717	-	+
<i>Streptomyces incarnatus</i> NRRL 8089	-	-
<i>Streptomyces laurentii</i> ATCC 31255	-	+
<i>Streptomyces lavendulae</i> subsp. <i>Lavendulae</i> CCM 3239	-	+
<i>Streptomyces leeuwenhoekii</i> C34	-	+
<i>Streptomyces lividans</i> 1326	+	+
<i>Streptomyces lividans</i> TK24	+	+
<i>Streptomyces lunaelactis</i> MM109	-	-
<i>Streptomyces lydicus</i> A02	-	+
<i>Streptomyces lydicus</i> 103	-	+
<i>Streptomyces malaysiensis</i> DSM 4137	-	+
<i>Streptomyces niveus</i> SCSIO 3406	-	+
<i>Streptomyces niveus</i> NCIMB 11891	-	+
<i>Streptomyces nodosus</i> ATCC 14899	-	+
<i>Streptomyces noursei</i> ATCC 11455	-	-
<i>Streptomyces pactum</i> KLBMP 5084	+	+
<i>Streptomyces pactum</i> ACT12	+	+

<i>Streptomyces parvulus</i> 2297	+	+
<i>Streptomyces peucetius</i> subsp. <i>caesius</i> ATCC 27952	-	+
<i>Streptomyces pratensis</i> ATCC 33331	+	-
<i>Streptomyces pristinaespiralis</i> HCCB 10218	-	-
<i>Streptomyces pristinaespiralis</i> ATCC 25486	-	-
<i>Streptomyces rapamycinicus</i> NRRL 5491	-	+
<i>Streptomyces reticuli</i> TUE45	-	-
<i>Streptomyces roseochromogenus</i> subsp. <i>oscitans</i> DS 12.976	-	-
<i>Streptomyces rubrolavendulae</i> MJM4426	-	-
<i>Streptomyces scabiei</i> 87.22	+	-
<i>Streptomyces</i> sp. 2114.2	+	-
<i>Streptomyces</i> sp. 3214.6	+	+
<i>Streptomyces</i> sp. 4F	+	+
<i>Streptomyces</i> sp. 769	-	+
<i>Streptomyces</i> sp. CCM_MD2014	+	+
<i>Streptomyces</i> sp. CFMR 7	+	+
<i>Streptomyces</i> sp. CLI2509	+	+
<i>Streptomyces</i> sp. fd1-xmd	+	-
<i>Streptomyces</i> sp. FR-008	+	+

<i>Streptomyces sp. GBA 94-10</i>	+	+
<i>Streptomyces sp. M56</i>	-	+
<i>Streptomyces sp. NEAU-S7GS2</i>	+	+
<i>Streptomyces sp. PAMC 26508</i>	+	+
<i>Streptomyces sp. PBH53</i>	-	+
<i>Streptomyces sp. PVA 94-07</i>	+	+
<i>Streptomyces sp. S8</i>	+	+
<i>Streptomyces sp. SAT1</i>	+	+
<i>Streptomyces sp. Sge12</i>	-	+
<i>Streptomyces sp. SirexAA-E</i>	-	-
<i>Streptomyces sp. SM17</i>	+	+
<i>Streptomyces sp. SM18</i>	-	+
<i>Streptomyces sp. TLI_053</i>	-	+
<i>Streptomyces sp. TN58</i>	-	+
<i>Streptomyces sp. Tu6071</i>	+	+
<i>Streptomyces sp. Tue 6075</i>	+	+
<i>Streptomyces sp. WAC00288</i>	+	-
<i>Streptomyces venezuelae</i> NRRL B-65442	+	+
<i>Streptomyces venezuelae</i> ATCC 15439	+	+

<i>Streptomyces venezuelae</i> ATCC 15439 chr I	+	+
<i>Streptomyces venezuelae</i> ATCC 10712	+	+
<i>Streptomyces vietnamensis</i> GIM4.0001	-	+
<i>Streptomyces violaceoruber</i> S21	+	+
<i>Streptomyces violaceusniger</i> Tu 4113	-	+
<i>Streptomyces xiamenensis</i> 318	+	-

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Organism Name – CIS-negative strains	Effectors (Sco4256-58)	Adaptor (Sco4242)
<i>Streptomyces actuosus</i> ATCC 25421	+*1	+*2
<i>Streptomyces antibioticus</i> DSM 41481	-	+*2
<i>Streptomyces gilvosporeus</i> F607	-	-
<i>Streptomyces lincolnensis</i> NRRL 2936	-	-
<i>Streptomyces pluripotens</i> MUSC 135	-	+*2
<i>Streptomyces pluripotens</i> MUSC 137	-	-
<i>Streptomyces puniscabiei</i> TWIS1	+*1	-
<i>Streptomyces</i> sp. 2323.1	-	-
<i>Streptomyces</i> sp. 452	-	-
<i>Streptomyces</i> sp. CdTB01	-	-

<i>Streptomyces sp. CMB-StM0423</i>	-	-
<i>Streptomyces sp. CNQ-509</i>	-	-
<i>Streptomyces sp. HNM0039</i>	-	-
<i>Streptomyces sp. Mgl</i>	-	-
<i>Streptomyces sp. MOE7</i>	-	-
<i>Streptomyces sp. P3</i>	-	-
<i>Streptomyces sp. S10(2016)</i>	-	-
<i>Streptomyces sp. SCSIO 03032</i>	-	-
<i>Streptomyces sp. XZHG99</i>	-	-
<i>Streptomyces spongiicola HNM0071</i>	-	-
<i>Streptomyces sviveus ATCC 29083</i>	-	-

*1: only N-terminal part detected (up to 270 amino acids)

*2: only C-terminal part detected (from 200 amino acids)

25 **Supplementary Table 5:** Bacterial strains, plasmids and cosmids used in this study.

Strain	Description	Construction	Source
<i>Escherichia coli</i> strains			
TOP10	<i>F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara leu) 7697 galU galK rpsL (Str^R) endA1 nupG</i>	Cloning	Invitrogen
ET12567/pUZ8002	<i>F⁻ dam13::Tn9 dcm6 hsdM hsdR recF143:: Tn10 galK2 galT22 ara-14 lacY1 xyl-5 leuB6 thi-1 tonA31 rpsL hisG4 tsx-78 mtl-1 glnV44</i>	ET12567 with helper plasmid pUZ8002	¹
BW25113/pIJ790	<i>Δ(araD-araB)567 ΔlacZ4787(::rrnB-4) lacIp-4000(lacIQ), λ-rpoS369(Am) rph-1 Δ(rhaD-rhaB)568 hsdR514</i>	BW25113 containing λ RED recombination plasmid pIJ790	²
Rosetta (DE3)	<i>F⁻ ompT hsdS_B(r_B⁻ m_B⁻) gal dcm (DE3) pRARE (Cam^R)</i>	Host strain for protein overexpression	Merck
<i>Streptomyces</i> strains			
<i>S. venezuelae</i> NRRL B-65442	Wild Type (Sv-WT)		³
<i>S. coelicolor</i> M145	Wild Type (Sc-WT)		⁴

	SCP1 ⁻ SCP2 ⁻ derivative from <i>S. coelicolor</i> A3(2)		
SS381	<i>Sv-WT Δvnz_28920::apr</i>	chromosomal <i>vnz_28920</i> (<i>cis2</i>) locus deleted using pSS489	This study
SS383	<i>Sc-WT Δsco4253::apr</i>	chromosomal <i>sco4253</i> (<i>cis2</i>) locus deleted using pSS480	This study
SS387	<i>Sc-WT Δsco4253-4251::apr</i>	chromosomal <i>sco4253-4251</i> locus deleted using pSS480	This study
SS389	<i>Sc-WT Δsco4253::apr attB ΦBT1 Sco4253-I274-ypet_Sco4252-51, hyg^R</i>	pSS501 integrated at ϕ BT1 attachment site of SS383	This study
SS392	<i>Sc-WT Δsco4253-51::apr attB ΦBT1 sco4253-N3-sco4252-51, hyg^R</i>	pSS503 integrated at ϕ BT1 attachment site of SS387	This study
SS393	<i>Sc-WT Δsco4253-51::apr attB ΦBT1 sco4253-N5-sco4252-51, hyg^R</i>	pSS504 integrated at ϕ BT1 attachment site of SS387	This study
SS394	<i>Sc-WT Δsco4253-51::apr attB ΦBT1 sco4253-N2-sco4252-51, hyg^R</i>	pSS505 integrated at ϕ BT1 attachment site of SS387	This study
SS395	<i>Sc-WT Δsco4253-51::apr attB ΦBT1 sco4253-51, hyg^R</i>	pSS500 integrated at ϕ BT1 attachment site of SS387	This study

SS430	<i>Sc-WT</i> $\Phi BT1$ P_{ermE^*} - <i>sfgfp</i> , <i>hyg^R</i>	pSS150 integrated at $\Phi BT1$ attachment site of Sc-WT	This study
SS431	<i>Sc-WT</i> $\Delta sco4253::apr$ <i>attB</i> $\Phi BT1$ P_{ermE^*} - <i>sfgfp</i> , <i>hyg^R</i>	pSS150 integrated at $\Phi BT1$ attachment site of SS383	This study
SS459	<i>Sc-WT</i> $\Delta sco4253-51::apr$ <i>attB</i> $\Phi BT1$ <i>sco4253-N5-</i> <i>sco4252-51</i> , P_{ermE^*} - <i>sfgfp</i> , <i>hyg^R</i>	pSS610 integrated at $\Phi BT1$ attachment site of SS387	This study
SS484	<i>Sc-WT</i> $\Phi BT1$ P_{cis2} - <i>ypet</i> , <i>hyg^R</i>	pSS619 integrated at $\Phi BT1$ attachment site of Sc-WT	This study
SS540	<i>Sc-WT</i> $\Delta sco4256-58::apr$	chromosomal <i>sco4256-58</i> locus deleted using pSS703	This study
SS550	<i>Sc-WT</i> $\Delta sco4256-58::apr$ <i>attB</i> $\Phi BT1$ P_{ermE^*} - <i>sfgfp</i> , <i>hyg^R</i>	pSS150 integrated at the $\Phi BT1$ attachment site of SS540	This study
Plasmids			
pIJ773	pBluescript KS (+) containing the apramycin resistance gene <i>apr</i> and <i>oriT</i> of plasmid RP4, flanked by FRT sites (<i>Apr^R</i>). Used as template for the amplification of the <i>apr-oriT</i> cassette for		5

	'REDIRECT' PCR targeting, Apr ^R		
pIJ10257	Cloning vector for the conjugal transfer of DNA (under control of the <i>ermE</i> * constitutive promoter). Integrates at the <i>ΦBT1</i> attachment site, Hyg ^R		6
pIJ10770	Cloning vector for the conjugal transfer of DNA from <i>E. coli</i> to <i>Streptomyces</i> spp. Integrates at the <i>ΦBT1</i> attachment site, Hyg ^R		4
pIJ10772	Modified pIJ10770, carries <i>mcherry</i> for construction of C-terminal fluorescent gene fusion. Integrates at the <i>ΦBT1</i> attachment site, Hyg ^R		4
pUC19	<i>E. coli</i> multicopy cloning vector, Carb ^R		7
pET21b	<i>E. coli</i> expression vector with C-terminal 6xHis tag, Carb ^R		EMD Biosciences
pIJ12738	Derivative of pGM1190, an intermediate copy number, conjugative plasmid containing the temperature-	Used as intermediated cloning vector	8

	sensitive replication origin of pSG5, Apr ^R		
pIJ10773	Modified pIJ10770, carries <i>ypet</i> for construction of C-terminal fluorescent gene fusion. Integrates at the Φ BT1 attachment site, Hyg ^R	Codon-optimised <i>ypet</i> was PCR amplified with primer 34/4b followed by restriction digestion with XhoI/KpnI and ligation into pIJ10770 cut with XhoI/KpnI	This study
pSS150	pIJ10257 carrying <i>P_{ermE}*</i> - <i>sfgfp</i> , Hyg ^R	Codon-optimised <i>sfgfp</i> was PCR amplified with primer 268/269 followed by restriction digestion with NdeI/XhoI and ligation into pIJ10257 cut with NdeI/XhoI	This study
pSS480	Mutated cosmid StD-49 for REDIRECT containing Δ <i>sco4253::apr</i> , Km ^R , Carb ^R , Apr ^R	The <i>sco4253</i> coding sequence on the cosmid vector StD-49 was replaced by an oriT-containing apramycin resistance cassette, which was amplified from pIJ773 using primer 1037/1038.	This study
pSS481	Mutated cosmid StD-49 for REDIRECT containing Δ <i>sco4253-4251::apr</i> , Km ^R , Carb ^R , Apr ^R	The <i>sco4253-51</i> coding sequence on the cosmid vector StD-49 was replaced by an oriT-containing apramycin	This study

		resistance cassette, which was amplified from pIJ773 using primer 1037/1039.	
pSS489	Mutated cosmid P11-F14 for REDIRECT containing Δ <i>vnz28920::apr</i> , Km ^R , Carb ^R , Apr ^R	The <i>vnz28920</i> coding sequence on the cosmid vector P11-F14 was replaced by an oriT-containing apramycin resistance cassette, which was amplified from pIJ773 using primer 1048/1049	This study
pSS494	pIJ12738 carrying <i>sco4253::21IE</i> (CIS ^{Sc} -N2), Apr ^R	Insertion of "IE" at amino acid position 21 in Sco4253. Plasmid was generated via Gibson Assembly from PCR fragments generated using genomic DNA and primer 1057/1058 and 1059/1060 and pIJ12738 cut with HindIII	This study
pSS495	pIJ12738 carrying <i>sco4253::21IEG</i> (CIS ^{Sc} -N3), Apr ^R	Insertion of "IEG" at amino acid position 21 in Sco4253 Plasmid was generated via Gibson Assembly from PCR fragments generated using genomic DNA primer 1061/1057 and	This study

		1059/1060 and pIJ12738 cut with HindIII	
pSS496	pIJ12738 carrying <i>sco4253::2IEGVG</i> (CIS ^{Sc} -N5), Carb ^R	Insertion of " IEGVG " at amino acid position 21 in Sco4253. Plasmid was generated via Gibson Assembly from PCR fragments generated using genomic DNA primer 1057/1062 and 1063/1060 and pIJ12738 cut with HindIII	This study
pSS497	pUC19 carrying <i>sco4253-51</i> , Carb ^R	Amplification of <i>sco4253-4251</i> from genomic DNA with primer 1091/1092 followed by Gibson Assembly into pUC19 cut with HindIII/EcoRI	This study
pSS498	pSS497 carrying <i>sco4253::ypet(I274)-Sco4252-51</i> , Carb ^R	Insertion of <i>ypet</i> with linker after AA I274 in <i>sco4253</i> . pSS497 was amplified with primer 1075/1078, <i>ypet with 7AA linker</i> was amplified with primer 1076/1077, both fragments were combined using Gibson Assembly	This study
pSS500	pIJ10770 carrying <i>sco4253-51</i> , Hyg ^R	Sco4253-4251 was PCR amplified with primer	This study

		1042/1101, digested with HindIII/NdeI and ligated into pIJ10770 cut with HindIII/NdeI	
pSS501	<i>pIJ10770</i> carrying <i>sco4253::ypet(I274)-sco4252-51</i> , Hyg ^R	<i>sco4253::ypet(I274)-Sco4252-51</i> was PCR amplified with primers 1042/1101 from pSS498, digested with HindIII/NdeI and ligated into pIJ10770 cut with HindIII/NdeI	This study
pSS503	<i>pIJ10770</i> carrying <i>CIS^{Sc}-N3 (sco4253::2HIEG-sco4252-51)</i> , Hyg ^R	Fragment 1: <i>sco4253-N3-4251</i> from pSS495 was PCR amplified with primer 1042/1043 and digested with HindIII/NruI; Fragment 2: <i>sco4251-53</i> was PCR amplified from pSS494 with primer 1042/1102 and digested with NruI/AvrII; Triple ligation of both fragments with pIJ10770 cut with HindIII/AvrII	This study
pSS504	<i>pIJ10770</i> carrying <i>CIS^{Sc}-N5 (sco4253::2HIEGVG-sco4252-51)</i> , Hyg ^R	fragment 1: <i>sco4253-N5-4251</i> from pSS496 was PCR amplified with primer 1042/1043 and digested with	This study

		HindIII/NruI; Fragment 2: <i>sco4251-53</i> was PCR amplified from pSS494 with primer 1042/1102 and digested with NruI/AvrII; Triple ligation of both fragments with pIJ10770 cut with HindIII/AvrII	
pSS505	<i>pIJ10770</i> carrying <i>CIS^{Sc}-N2</i> (<i>sco4253::21IE-sco4252-51</i>), Hyg ^R	Fragment 1: <i>sco4253-N2-4251</i> from pSS494 was PCR amplified with primer 1042/1043 and cut with HindIII/NruI; Fragment 2: <i>sco4251-53</i> was PCR amplified from pSS494 with primer 1042/1102 and cut with NruI/AvrII; Triple ligation of both fragments into pIJ10770 cut with HindIII/AvrII	This study
pSS610	pSS504 carrying <i>P_{ermE*}-sfgfp</i> , Hyg ^R	<i>P_{ermE*}-sfgfp</i> fragment was isolated from pSS150 by restriction digestion with Bsu361/AvrII and ligated between the Bsu361/AvrII site of pSS504	This study

pSS619	pIJ10773 carrying <i>P_{str2}-ypet</i> , Hyg ^R	<i>sco4253</i> promoter region (<i>P_{str2}</i>) was PCR amplified from genomic DNA with primer 1403/1404 and cloned into pIJ10773 cut with NdeI/XhoI using Gibson Assembly	This study
pSS703	Mutated cosmid StD8A for REDIRECT containing Δ <i>sco4256-4258::apr</i> , Km ^R , Carb ^R , Apr ^R	The <i>sco4256-58</i> coding sequence on the cosmid vector StD8A was replaced by an oriT-containing apramycin resistance cassette, which was amplified from pIJ773 using primer 1713/1717.	This study
pSS704	pET21b carrying <i>FLAG-yfp-gypA</i> , Carb ^R	<i>FLAG-yfp</i> with synthetic membrane anchor <i>gypA</i> was amplified with primer 1736/1737 and inserted into pET21b by restriction ligation using NdeI and HindIII.	This study
pSS709	pET21b carrying <i>sco4256</i> , Carb ^R	<i>sco4256</i> was PCR amplified with primer 1738/42, pET21b was amplified with primer 1743/1744. Fragments were assembled using Gibson Assembly.	This study

pSS712	pET21b carrying <i>sco4257</i> , Carb ^R	<i>sco4257</i> was PCR amplified with primer 1746/50, pET21b was amplified with primer 1743/1744. Fragments were assembled using Gibson Assembly.	This study
pSS716	pET21b carrying <i>sco4258</i> , Carb ^R	<i>sco4258</i> was PCR amplified with primer 1751/55, pET21b was amplified with primer 1743/1744. Fragments were assembled using Gibson Assembly.	This study
Cosmids			
StD-49 and StD8A	Cosmid vector containing coding sequence for <i>S. coelicolor CIS gene cluster</i> , Km ^R , Carb ^R		http://strepdb.streptomyces.org.uk
Pl1-F14	Cosmid vector containing coding sequence for <i>S. venezuelae CIS gene cluster</i> , Km ^R , Carb ^R		http://strepdb.streptomyces.org.uk

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Supplementary Table 5 References

1. Paget, M. S. B., Chamberlin, L., Atrih, A., Foster, S. J. & Buttner, M. J. Evidence that the Extracytoplasmic Function Sigma Factor σ^E Is Required for Normal Cell Wall Structure in *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **181**, 204–211 (1999).
2. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci.* **97**, 6640–6645 (2000).
3. Gomez-Escribano, J. P. *et al.* *Streptomyces venezuelae* NRRL B-65442: genome sequence of a model strain used to study morphological differentiation in filamentous actinobacteria. *J. Ind. Microbiol. Biotechnol.* **48**, kuab035 (2021).
4. T. Kieser M. J. Bibb M. J. Buttner K. F. Chater and D. A. Hopwood. *Practical Streptomyces Genetics*. (John Innes Foundation, 2000).
5. Gust, B., Challis, G. L., Fowler, K., Kieser, T. & Chater, K. F. 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.* **100**, 1541–1546 (2003).
6. Hong, H.-J., Hutchings, M. I., Hill, L. M. & Buttner, M. J. The role of the novel Fem protein VanK in vancomycin resistance in *Streptomyces coelicolor*. *J. Biol. Chem.* **280**, 13055–13061 (2005).
7. Yanisch-Perron, C., Vieira, J. & Messing, J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**, 103–119 (1985).
8. Fernández-Martínez, L. T. & Bibb, M. J. Use of the meganuclease I-SceI of *Saccharomyces cerevisiae* to select for gene deletions in actinomycetes. *Sci. Rep.* **4**, 7100 (2014).

55 **Supplementary Table 6:** Oligonucleotides used in this study.

Name	Sequence (5' → 3')
4b	GGGGTACCTCACTTGTACAGCTCGTTCATG
34	ATACTCGAGATGGTCTCCAAGGGCGAGG
268	TTAATTAACATATGTCCCGCCTGAAGCCGCGCTGCACCTCCCTGGAGATGTCCAAGGGCGAGGAGCTGTTC
269	ATATACTCGAGCTCCGGGCCCCGGCAGCTCCGGGCCCCGGCAGCTTGTACAGCTCGTCCATGCCGTG
1037	GAGCAGAGCATGCCGTCTACCTGTCGCCCCGGCGTCTACATTCCGGGGATCCGTCGACC
1038	GCGATCCGCCTACTCGTCCAGTTCGCCGCCGCCGCTGGATGTAGGCTGGAGCTGCTTC
1039	TCGCGTACGTCACGATTCCCCCAGGCGGCTCCCGCCGAGTGTAGGCTGGAGCTGCTTC
1042	ATTAAAGCTTCCCTCCTGACACGCCGTCACC
1043	AATTAATTCATATGCTCGTCCAGTTCGCCGCCGC
1048	GGAGCGAGCATGCCGACGTACCTACCCCCGGGCGTGTACATTCCGGGGATCCGTCGACC
1049	ACGTACGCAGTTCACGCCGATCGGGTTGAGCAGGTCCTGTGTAGGCTGGAGCTGCTTC
1050	CGGCGTCCGTCAGCCGCGCTGGAAGAACTCCACCTCCGCTGTAGGCTGGAGCTGCTTC
1057	CACGACGTTGTAAAACGACGGCCAGTGCCAAGTGTCGTGCGCCGTCCCGTGGTC
1058	GGCCGCCACCGACGTGCCCACTCCCTCGATCTCGATCGGGCGCGAGCCGCTGGCCA
1059	ATCGAGGGAGTGGGCACGTC
1060	GCGGATCCTCTAGAGTCGACCTGCAGCCCAAGTTCTTCGAACACGATGGTGATGG
1061	GGCCGCCACCGACGTGCCCACTCCCTCGATGCCCTCGATCGGGCGCGAGCCGCTGGCCA
1062	GCCCACTCCCTCGATGCCGACGCCCTCGATCGGGCGCGAGCCGCTGGCCA
1063	ATCGAGGGCGTCGGCATCGAGGGAGTGGGCACGTC

1075	CCGAGCCTTCGAGGATCGCGCCGCGCTGGTAGG
1076	CGCGGCGCGATCCTCGAAGGCTCGGGGCAGGGG
1077	GGCCTCCAGGTCGCTCCCCTGGCCGGAGCCCG
1078	CGGCCAGGGGAGCGACCTGGAGGCCGTCAAAGC
1091	TGACCATGATTACGCCAAGCTTCCTCCTGACACGCCGTCAC
1092	AAACGACGGCCAGTGAATCCGCGATCTCCTCGTGCAGCC
1101	AATTAATTCATATGCGCGATCTCCTCGTGCAGCC
1403	TGATAAGTTTATCAAGCTTAGATTCTCTCATATGGTTCAAGCGGTCCGACACG
1404	GTGAACAGCTCCTCGCCCTTGGAGACCATCTCGAGCTCTCCTCGGGGTACGAGACAG
1713	TCTGCCCCGCCAGGTCCTGACGAAGTGCCGGACCGGCCGATTCCGGGGATCCGTCGACC
1717	GGCTGGTGACGGCCGCCGGGCCCCCTCCGGCCGTCAGCCTGTAGGCTGGAGCTGCTTC
1736	ATTAATTCATATGGACTACAAGGACGACGACGAC
1737	ATTAAAGCTTGCAGATCAGGCGGCGGATG
1743	AAGCTTGCGGCCGCACTCG
1744	ATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGGGA
1746	TTTGTTTAACTTTAAGAAGGAGATATACATATGAGCCTTTGGACCTCCC
1750	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTGTCTTCGACGGGTG
1751	TTTGTTTAACTTTAAGAAGGAGATATACATATGAGCCTGTGGACTTCCC
1755	GTGGTGGTGCTCGAGTGCGGCCGCAAGCTTGCCCTTCTTCGGGTG