Article:
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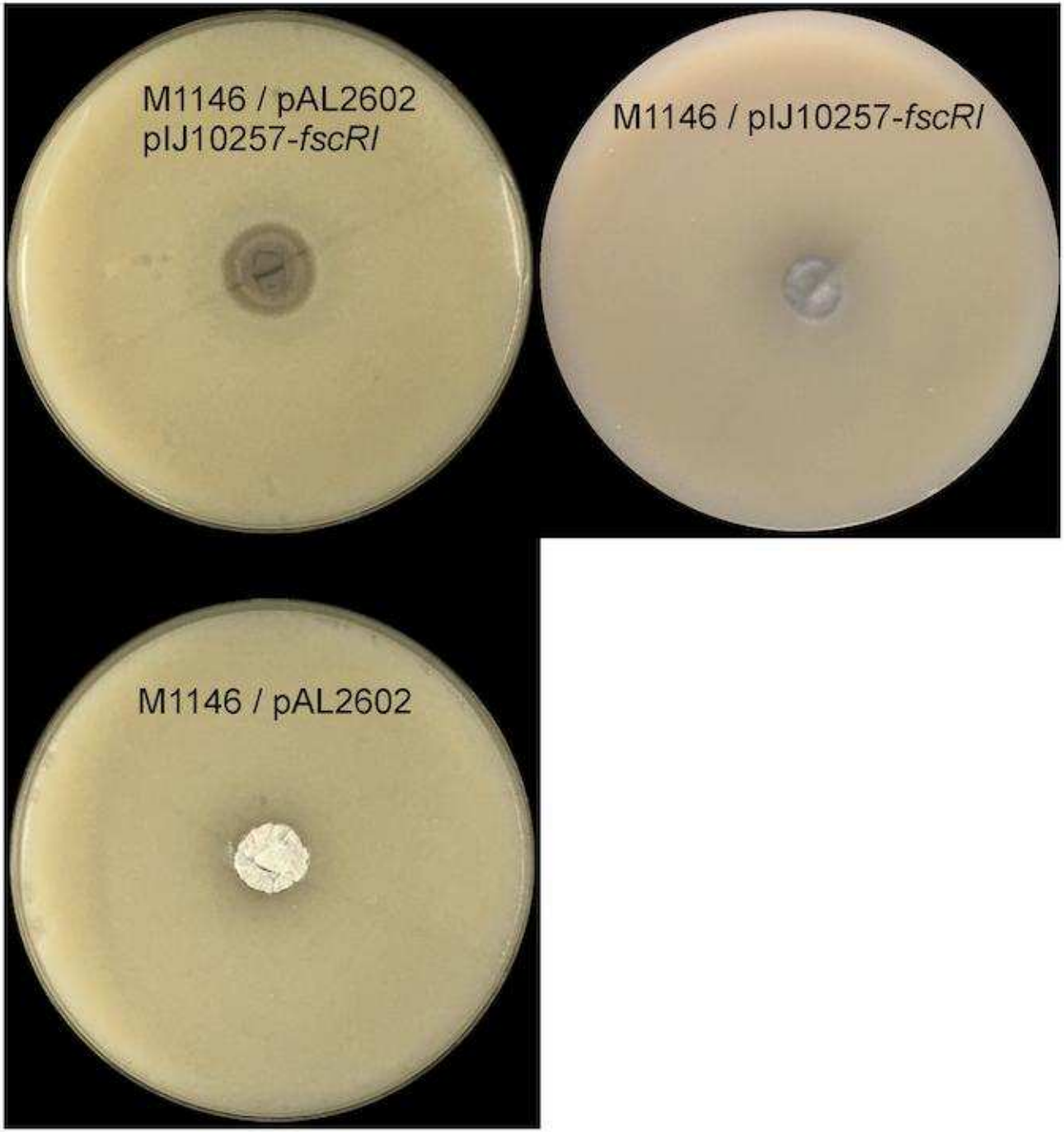
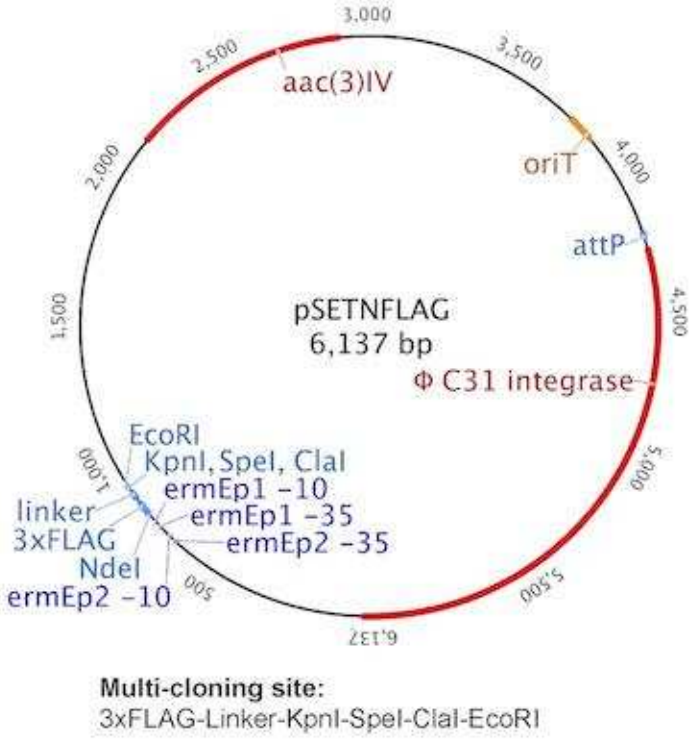


FIG S1. Bioactivity of *Streptomyces coelicolor* M1146 harboring pAL2602 is FscRI dependent. *S. coelicolor* M1146 harboring both pAL2602 and pIJ10257-*fscRI* antagonizes the growth of *Candida albicans* while M1146 harboring only pAL2602 or pIJ10257-*fscRI* does not.



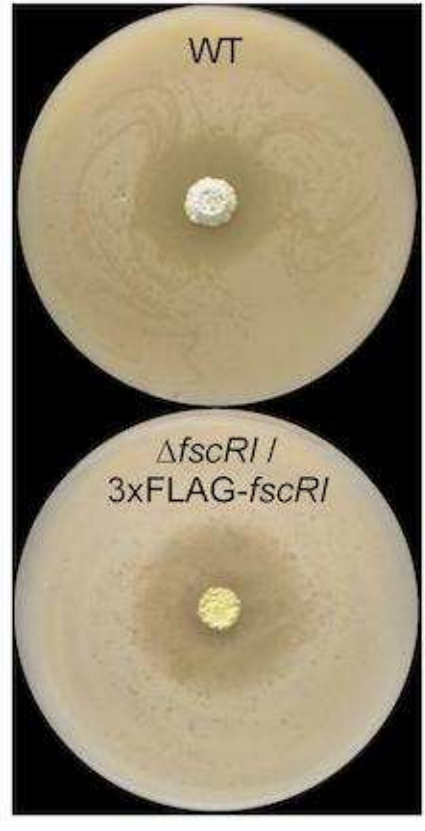


FIG S2. Schematic of the pSETNFLAG-*fscRI* plasmid (left) and antifungal bioactivity of Δ*fscRI* expressing 3xFLAG-FscRI against *Candida albicans*. *aac(3)IV*, apramycin resistance cassette; *oriT*, origin of transfer; *attP*, ΦC31 attachment site. The Genbank files of pSETNFLAG and its parent, pSET152-*ermE*p are available at: http://www.ryanseipkelab.com/tools.html

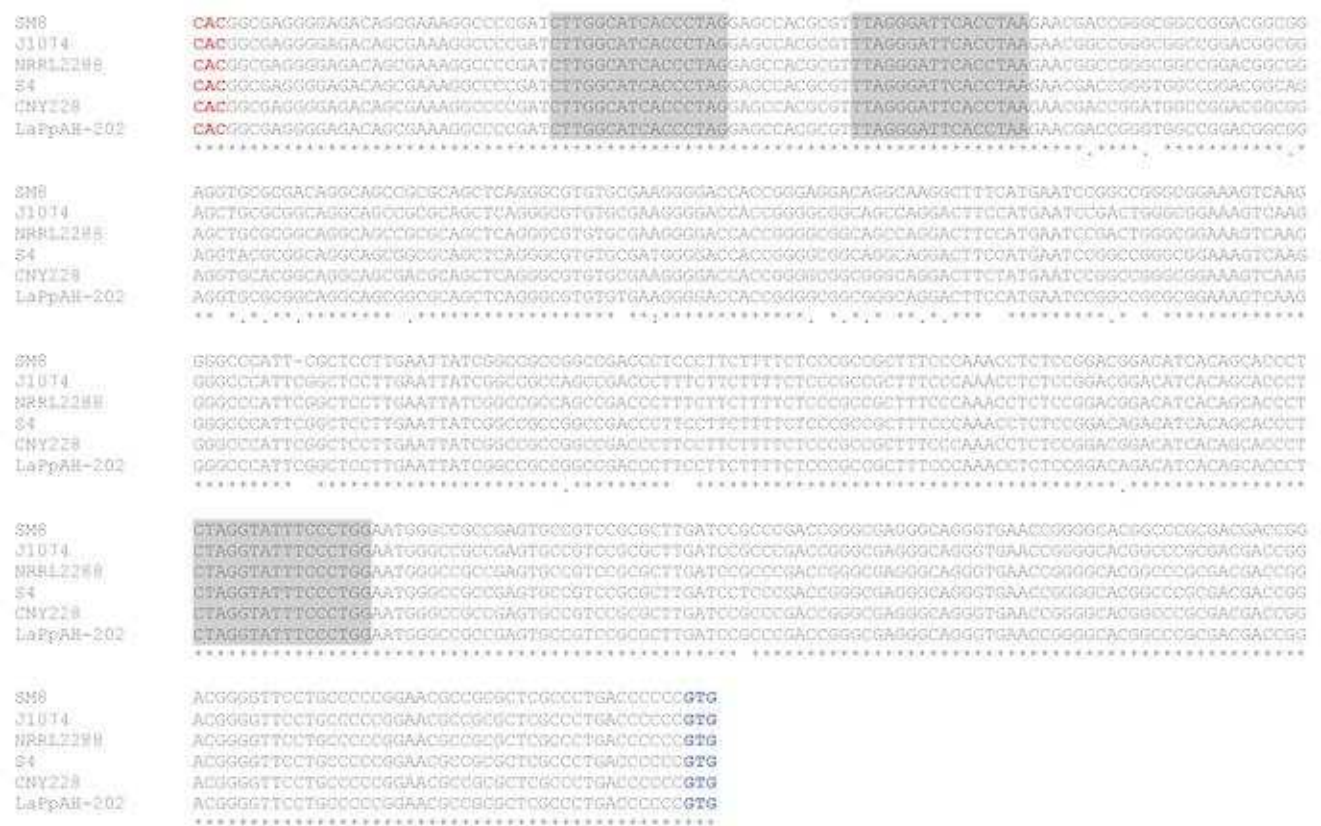


FIG S3. Clustal Ω alignment of the *antB-antC* intergenetic region for S-form *ant* gene clusters. The putative start codons for *antB* (bold, red, reverse orientation) and *antC* (bold, blue forward orientation) and the three conserved FscR1 binding sites are shaded grey.

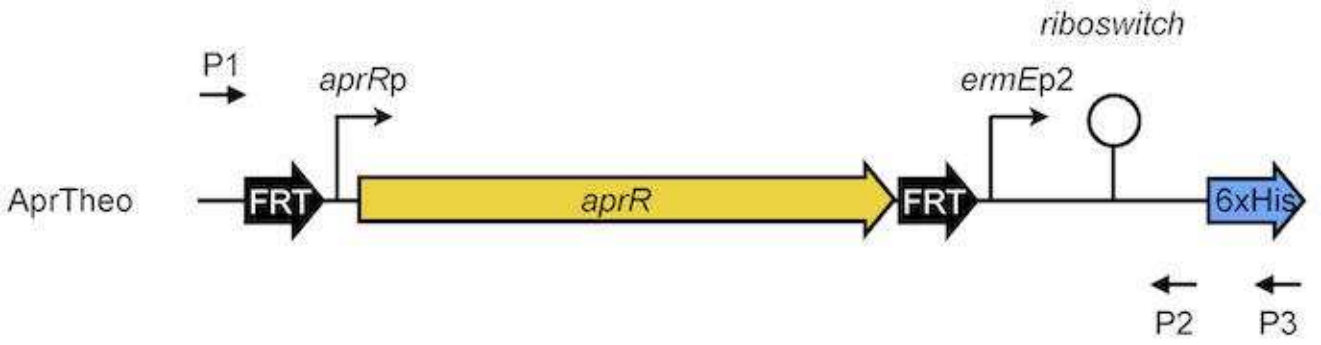


FIG S4. Schematic of the theophylline riboswitch cassette AprTheo. P1, prime site 1; P2, prime site 2; P3, prime site 3; *aprR*, apramycin resistance; 6xHis, hexahistidine affinity purification tag; the riboswitch is represented by a hairpin; FRT sites are for excision of the resistance marker by the Flp recombinase. Genbank file of the plasmid harbouring this cassette is available at: http://www.ryanseipkelab.com/tools.html

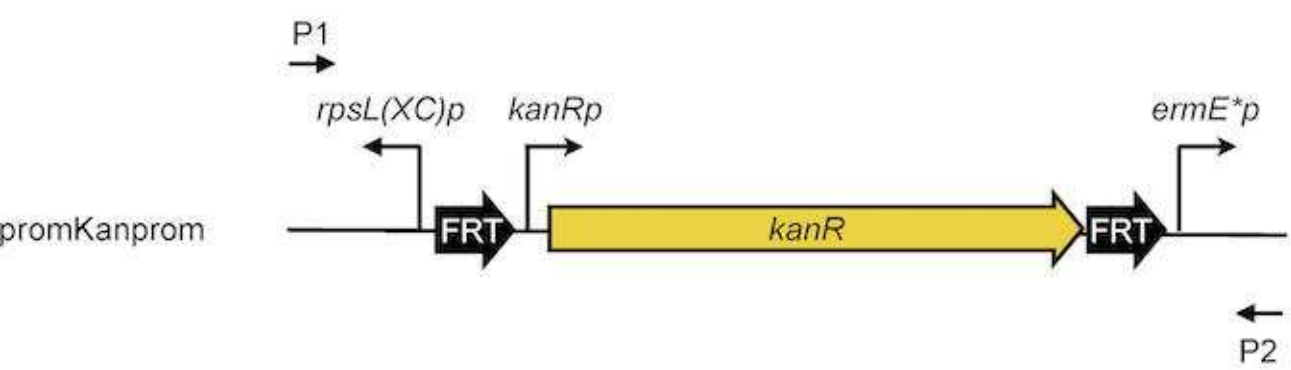


FIG S5. Schematic of the promKanprom cassette. P1, prime site (tacgtctccgtcgtctactc) 1; P2, prime site (catatggggcctcctgttct); *kanR*, kanamycin resistance; FRT sites for excision of the resistance marker by the Flp recombinase. The Genbank file of the plasmid harbouring this cassette is available at: http://www.ryanseipkelab.com/tools.html

Table S1. Bacterial strains, cosmids, fosmids and plasmids used in this study

Strain/cosmid/plasmid	Description [a]	Reference
Streptomyces		
S4	Wild type Streptomyces albus S4	(1)
ΔfscRI	S4 fscRI null mutant	This study
ΔfscRI attB ΦBT1::pIJ10257	fscRI null mutant harboring the empty pIJ10257 vector; Hyg^R	This study
ΔfscRI attB ΦBT1::pIJ10257-fscRI	fscRI null mutant complemented with fscRI expressed from the ermE* promoter; Hyg^R	This study
ΔfscRI attB ΦC31::pSETNFLAG	fscRI null mutant harboring the empty pSETNFLAG vector; Apr^R	This study
ΔfscRI attB ΦC31::pSETNFLAG-fscRI	fscRI null mutant complemented with 3xFLAG-fscRI expressed from the ermE* promoter; Apr^R	This study
M1146	S. coelicolor M145 harboring mutations in the biosynthetic pathways for: actinorhodin, undecylprodigiosin, calcium-dependent antibiotic and coelimycin	(2)
M1146 attB ΦC31::Cosmid213	M1146 derivative harboring Cosmid 213; $Carb^R$, Apr^R	This study
M1146 attB ΦC31::Cosmid213-BC-prom	M1146 derivative harboring Cosmid 213-BC-prom; $Carb^R$, Kan^R, Apr^R	This study
M1146 attB ΦC31::Cosmid213-ABribo-FLP	M1146 derivative harboring Cosmid 213-ABribo-FLP; $Carb^R$, Apr^R	This study
M1146 attB ΦC31::Cosmid213-CDEribo-FLP	M1146 derivative harboring Cosmid 213-CDEribo-FLP; $Carb^R$, Apr^R	This study
M1146 attB ΦC31::Cosmid213 attB ΦBT1::pIJ10257	M1146 derivative harboring Cosmid 213 and pIJ10257; $Carb^R$, Apr^R, Hyg^R	This study
M1146 attB ΦC31::Cosmid213 attB ΦBT1::pIJ10257-fscRI	M1146 derivative harboring Cosmid 213 and pIJ10257-fscRI; $Carb^R$, Apr^R, Hyg^R	This study
M1146 attB ΦC31::Cosmid213-ABribo-FLP attB ΦBT1::pIJ10257-fscRI	M1146 derivative harboring Cosmid 213-ABribo-FLP and pIJ10257-fscRI; $Carb^R$, Apr^R, Hyg^R	This study
M1146 attB ΦC31::Cosmid213-CDEribo-FLP ΦBT1::pIJ10257-fscRI	M1146 derivative harboring Cosmid 213-CDEribo-FLP and pIJ10257-fscRI; $Carb^R$, Apr^R, Hyg^R	This study
M1146 attB ΦC31::Cosmid213-ABCDEribo-FLP	M1146 derivative harboring Cosmid 213-CDEribo-FLP and pIJ10257-fscRI; $Carb^R$, Apr^R, Hyg^R	This study
Escherichia coli		
BL21	Host for heterologous protein expression	Novagen
BW25113	Host for REDIRECT PCR targeting system	(3)
TOP10	General cloning host	Invitrogen
ET12567	Non-methylating host for transfer of DNA into Streptomyces spp. (dam, dcm, hsdM); Cam^R	(4)
XL1-Blue	General cloning host	Agilent Technologies
GB0R-red	Host for RecET recombination	(5)
Cosmids and fosmids		
Supercos1	Cosmid backbone for S. albus S4 Cosmid 213; $Carb^R$, Kan^R	Stratagene
Cosmid 213	Supercos1 derivative containing the entire antimycin gene cluster; $Carb^R$, Kan^R	This study

Cosmid 213-ABribo-FLP	Cosmid 213 derivative with antBA expression controlled by a theophylline inducible riboswitch; $Carb^R$, Kan^R	This study
Cosmid 213-CDEribo-FLP	Cosmid 213 derivative with antCDE expression controlled by a theophylline inducible riboswitch; $Carb^R$, Kan^R	This study
Cosmid 213-ΦC31	Cosmid 213 derivative engineered to integrate into the ΦC31 attB site; $Carb^R$, Apr^R	This study
Cosmid 213-ΦC31-BC-prom	Cosmid 213-ΦC31derivative with antBC and antCDE expression controlled by rpsL(XC) and ermE* promoters, respectively; $Carb^R$, Kan^R, Apr^R	This study
Cosmid 213-ABribo-FLP-ΦC31	Cosmid 213-ABribo-FLP derivative engineered to integrate into the ΦC31 attB site; $Carb^R$, Apr^R	This study
Cosmid 213-CDEribo-FLP- ΦC31	Cosmid 213-CDEribo-FLP derivative engineered to integrate into the ΦC31 attB site; $Carb^R$, Apr^R	This study
pAL2602	ΦC31 integrative fosmid clone harboring the antimycin gene cluster from Streptomyces sp. NRRL 2288; Apr^R	(6)
Plasmids		
pCRISPomyces-2	pGM1190 derivative harboring the CRISPR/Cas9 machinery; Apr^R	(7)
pCRISPomyces-2-fscRI	Derivative of pCRISPomyces-2 derivative containing the fscRI targeting protospacer cloned into the BbsI site and homology-directed repair arms cloned into the XbaI site; Apr^R	This study
pET28a	Commercial protein expression vector; Kan^R	Novagen
pET30a	Commercial protein expression vector; Kan^R	Novagen
pET28a-fscRI	pET28a derivative containing fscRI cloned into the NdeI-HindIII sites; Kan^R	This study
pET30a-fscRI	pET30a derivative containing the fscRI without a stop codon cloned into the NdeI-HindIII sites; Kan^R	This study
pSET152	E. coli – Streptomyces shuttle vector, integrates into the ΦC31 attB site in actinomycetes; Apr^R	(8)
pSET152ermEp	pSET152 derivative containing ermE*p cloned into the EcoRV-EcoRI sites; Apr^R	This study
pSETNFLAG	pSET152ermE* derivative with an N-terminal 3xFLAG tag and multi-cloning site cloned into the NdeI-KpnI sites; Apr^R	This study
pSETNFLAG-fscRI	pSETNFLAG derivative harboring fscRI cloned into KpnI-EcoRI sites; Apr^R	This study
pIJ773	ReDirect PCR template plasmid harboring an apramycin resistance cassette; $Carb^R$, Apr^R	(3)
pIJ773KnFRT	pIJ773 derivative where the apramycin resistance gene has been replaced by the neomycin/kanamycin resistance gene from Supecos1; $Carb^R$, Kan^R	This study
pIJ10257	pMS81 derivative containing ermE*p, integrates into the ΦBT1 attB site in Streptomyces; Hyg^R	(9)
pIJ10257-fscRI	pIJ10257 derivative containing the fscRI coding sequence cloned into the NdeI-HindIII sites	This study
pIJ10702	Supercos1 derivative harboring the ΦC31 integrase, attP, oriT and apramycin resistance gene from pSET152; $Carb^R$, Apr^R	(10)
pIJ12738	Conjugative vector, non-replicative in Streptomyces; Apr^R	(11)
pIJ12738-fscRI-UPDN	pIJ12738 derivative harboring the downstream fscRI homology-directed repair arms cloned into the KpnI-HindIII sites; Apr^R	This study
pUC19	General cloning plasmid; $Carb^R$	New England Biolabs
pUC19-promKanprom	pUC19 derivative harboring a kanamycin resistance gene flanked by divergently firing rpsL(XC) and ermE* promoters; $Carb^R$, Kan^R	This study
pUC57-AprTheo	pUC57 derivative harboring the commercially synthesised apramycin theophylline riboswitch cassette; $Carb^R$, Apr^R	This study

pUZ8002	Encodes conjugation machinery for mobilization of plasmids from E. coli to Streptomyces; Kan^R	(4)

[a] Carb, carbenicillin; Apr, apramycin; Hyg, hygromycin, Kan, kanamycin; Cam, chloramphenicol; oriT, origin of conjugal transfer

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Table S2. Oligonucleotide primers and other synthetic DNAs used in this study

Primer alias	Sequence (5'-3')*	Description
RFS406	tgtaggctggagctgcttc	PCR: KnFRT cassette
RFS407	attccggggatccgtcgac	PCR: KnFRT cassette
RFS413	tagagggtgctgtgatgtctgtccggagaggtttgggaatgtaggctggagctgcttc	PCR: antB promoter riboswitch recombineering
RFS414	ggttctccacattgagtgcggagcccctgtcacgcctcatcttgttgcccctctcagg	PCR: antB promoter riboswitch recombineering
RFS415	ttcccaaacctctccggacagacatcacagcaccctctatgtaggctggagctgcttc	PCR: antC promoter riboswitch recombineering
RFS416	acatgacaccaaccctcggttgcgagcaggtacttctcatcttgttgcccctctcagg	PCR: antC promoter riboswitch recombineering
RFS424	ata**catatg**gatcccgcgccggcggc	PCR: fscRI coding sequence
RFS425	tat**aagctt**tcacttgatgaagtcct	PCR: fscRI coding sequence
RFS444	ggaataggaacttatgagctcagccaatcgactggcgagcgaagccctgcaaagtaaact	PCR: KnFRT recombineering template
RFS445	cagttcgaagttcctattctctagaaagtataggaacttctcagaagaactcgtcaagaa	PCR: KnFRT recombineering cassette
RFS521	ata**aagctt**gccctggtccacatcgag	PCR: fscRI homology-directed repair arm
RFS522	ata**actagt**gatccatgagcgtgctgtg	PCR: fscRI homology-directed repair arm
RFS523	ataactagtcctcaaggtcggcacctg	PCR: fscRI homology-directed repair arm
RFS524	ataggtaccggtggcgtcctggagtg	PCR: fscRI homology-directed repair arm
RFS572	ataata**tctaga**gccctggtccacatcgag	PCR: fscRI homology-directed repair arm
RFS573	tatata**tctaga**ggtggcgtcctggagtg	PCR: fscRI homology-directed repair arm
RFS574	aggccggaggacgagccgccgaag	CRISPR protospacer targeting fscRI
RFS575	aaaccttcggcggctcgtcctccg	CRISPR protospacer targeting fscRI
RFS582	atatat**gatatc**agcccgacccgagcacg	PCR: construction of pSET152ermEp
RFS583	tatata**gaattcatcgatactagtggtacc**atgcaggactctagtta	PCR: construction of pSET152ermEp
RFS594	tatata**catatg**gactacaa	PCR: construction of pSET152NFLAG
RFS595	atatat**ggtacc**actaccgc	PCR: construction of pSET152NFLAG
RFS598	gggctaccacagtattg	PCR: confirmation of ΔfscRI mutant strain
RFS599	gtcgaagacggtgactc	PCR: confirmation of ΔfscRI mutant strain
RFS600	tatata**aagctt**cttgatgaagtcctcga	PCR: fscRI coding sequence without stop codon
RFS601	tatata**atcgat**cttgatgaagtcctcga	PCR: fscRI coding sequence without stop codon
RFS602	atatat**ggtacc**gatcccgcgccggcggc	PCR: fscRI coding sequence
RFS603	tatata**gaattc**tcacttgatgaagtcct	PCR: fscRI coding sequence
RFS654	tctccacattgagtgcggagcccctgtcacgcctcactacgtctccgtcgtctactc	PCR: antB-antC rpsL(XC)-Kan-ermE* recombineering
RFS657	cagacatgacaccaaccctcggttgcgagcaggtacttctcatatggggcctcctgttct	PCR: antB-antC rpsL(XC)-Kan-ermE* recombineering
RFS663	actggccgtcgttttacaac	PCR: pUC19
RFS664	gaattcgagctcggtacccg	PCR: pUC19
RFS665	gttgtaaaacgacggccagtcattacgtctccgtcgtcta	PCR: rpsL(XC) promoter
RFS666	gaagcagctccagcctacagccctgcaggcggaagtcag	PCR: rpsL(XC) promoter
RFS667	ggtcgacggatccccggaatagcccgacccgagcacgcgc	PCR: ermE* promoter
RFS668	cgggtaccgagctcgaattccatatggggcctcctgttct	PCR: ermE* promoter

NFLAG Gblock	tatata**catatg**gactacaaggaccacgacggcgactacaaggaccacg acatcgactacaaggacgatgacgacaagggtggaggcggttcaggcg gaggtggctctggcggtggcggtagt**ggtacc**atatat	Gblock for construction of pSETNFLAG

* non-homologous sequences are underlined and engineered restriction endonuclease sites are bolded

Table S3. FscRI[S4] and putative orthologs encoded by antimycin producers

Streptomyces species	Genome accession number	Class of ant gene cluster	Amino acid sequence	Shared amino acid identity (%)
S. albus S4	CADY00000000.1	S-form	MDPAPAADPAGSPERA AVLADGFDRAGAYVAC LDPSLTIQQVNQEFER RFGGSSSELCGSRFCD LVHPSVQQPLMHQFAR MLDGKRHRFATEVIAV DQERTASTLPLNALAV RGGRTPDVAAILVVMN AAEEEAGDADVMAPRK KLLSEIDARILEGIAA GVSTIPLASRLYLSRQ GVEYHVTGLLRKLKVP NRAALVSRAYSMGVLK VGTWPPKVVEDFIK	100
S. albus J1074	NC_020990	S-form	MDPAPAADPAGSPERA AVLADGFDRAGAYVAC LDPSLTIQQVNQEFER RFGGSSSELCGSRFCD LVHPSVQQPLMHQFAR MLDGKRHRFATEVIAV DQERTASTLPLNALAV RGGRTPDVAAILVVMN AAEEEAGDADVMAPRK KLLSEIDARILEGIAA GVSTIPLASRLYLSRQ GVEYHVTGLLRKLKVP NRAALVSRAYSMGVLK VGTWPPKVVEDFIK	100
S. sp. CNY228	ARIN00000000.1	S-form	MDPAPAADPAGSPERA AVLADGFDRAGAYVAC LDPSLTIQQVNQEFER RFGGSSSELCGSRFCD LVHPSVQQPLMHQFAR MLDGKRHRFATEVIAV DQERTASTLPLNALAV RGGRTPDVAAILVVMN AAEEEAGDADVMAPRK KLLSEIDARILEGIAA GVSTIPLASRLYLSRQ GVEYHVTGLLRKLKVP NRAALVSRAYSMGVLK VGTWPPKVVEDFIK	100
S. sp. SM8	AMPN00000000.1	S-form	MDPAPAADPAGSPERA AVLADGFDRAGAYVAC LDPSLTIQQVNQEFER RFGGSSSELCGSRFCD LVHPSVQQPLMHQFAR MLDGKRHRFATEVIAV DQERTASTLPLNALAV RGGRTPDVAAILVVMN AAEEEAGDADVMAPRK KLLSEIDARILEGIAA GVSTIPLASRLYLSRQ GVEYHVTGLLRKLKVP NRAALVSRAYSMGVLK VGTWPPKVVEDFIK	100
S. sp. LaPpAH-202	ARDM00000000.1	S-form	MDPAPAADPAGSPERA AVLADGFDRAGAYVAC LDPSLTIQQVNQEFER	99

Streptomyces sp. TOR3209	ARTR00000000.1	I-form	RFGGSSSELCGSRFCD LVHPSVQQPLMHQFAR MLDGKRHRFATEVIAV DQERTASTLPLNALAV RGGRTPNVAAILVVMN AAEEEAGDADVMAPRK KLLSEIDARILEGIAA GVSTIPLASRLYLSRQ GVEYHVTGLLRKLKVP NRAALVSRAYSMGVLK VGTWPPKVVEDFIK MDRTPVAGPAGTAVPA AGHTDGFDRADAYIAC LDPALTIQQVNQEFDR RFGGPASSLCGRNFCD LIHPSVRPPLMQQFSR LLEGKRRRFLTDVIAV DQESTASALPLRAMAV QGGHTPDVAAILVVMS GADERTEDAEEMAPRK KLLSEIDARILEGIAA GVSTIPLASRLFLSRQ GVEYHVTGLLRALKVP NRAALVSRAYSMGVLK VGTWPPKVVEDYIK	79
S. gancidicus BKS 13-15	AOHP00000000.1	L-form	VTGAPHNRDRRSPSLH AAAHRNAPESRTPAPG NRRFYTAHIDPDIQIV AAEPDFSRQFGRTSAD TCGRSLYELLHPSAPS VLNRHFTRLSEGRSAR FAERMVGLGNAGRVFS GELTGIAVQNTTGRLA GIVVQVRPDTEADTTD GKDVIGPPRERLLSKL DAQVLEGIAAGASTVQ LAARLYLSRQGVEYHV GLMLRKLKAPNRAALV ARAHSMGMLTVGQWPP RVLPEFIK	46
,S. hygroscopicus subsp. jinggangensis 5008	NC_017765	L-form	VAAEHRTTERFSDICW SVFVQSGFCIAHLDPR LRISAANGPFCSHIGS SPADVLGRDILDYLHP GVREKVRREFARLADG RSARFADDVIVVDAEG KSFQAELTGVAVHGSA SARVEGIVVLLRPSGS RSPGVAPARQKLFSPV HARVLEGVAAGESTVQ LASRLFLSRGGVEYHV ASLLRKMKVANRPALI SKGYALGVFAVGEWPP RVQPEFIAS	44
S. hygroscopicus subsp. jinggangensis TL01	NC_020895	L-form	VAAEHRTTERFSDICW SVFVQSGFCIAHLDPR LRISAANGPFCSHIGS SPADVLGRDILDYLHP GVREKVRREFARLADG RSARFADDVIVVDAEG KSFQAELTGVAVHGSA SARVEGIVVLLRPSGS RSPGVAPARQKLFSPV	44

Streptomyces sp. 303MFCol5.2	ARTR00000000.1	I-form	HARVLEGVAAGESTVQ LASRLFLSRGGVEYHV ASLLRKMKVANRPALI SKGYALGVFAVGEWPP RVQPEFIAS MSVVTTSITASSTTVV KAGAGVNRRRTYTAHV CPKGMTITAAEADFAA QFGASPGQICDRTLSD LLRAGTPEVLRHRFTD LSEGRTSWFTERVAGR HDSGRVFAADLTGIAV TGATGPAGLVLLLSPL GAAGEPYPRELTLSEL DVQVLEGVAGGASTVQ LAGRLYLSRQGVEYRV RLLLRRFDAPNRPALV ARAHALGLFAPGQWPP RVLPELIE	37
S. ambofaciens ATCC 23877	AM238663	L-form	VATTSFSDASPGQQRN AAAPAAPAHRVPTGGG AHRGAASADAWTAHVS PGDPVVTAAEPEFARQ FGLSADEIRGRRLLDL LRSPVPARLREQFTFL SSGRCRRFTETVTYRD GTGRDFPAELTGVAVR KPSGDVFGVVILLRRA GAAHRAAEMRRAGDRR PPQKGTLAEAAGRPVL SALDAQVLEGVARGES TAQLASRLYLSRQGIE YRVGQMLRRFEAPNRP ALVARAHALGMFAPGQ WPPRVLPERVK	36
S. griseoflavus Tü4000	ACFA00000000.1	L-form	VATKSYPDASPSKKRT AATAVPARRHLITAQD HVTPAATCTAHLSPQD LVVTAAEPEFARQFGL SADEICGRGLLELLRS RTPGHLREQFAALSSG PGRRFKQKVTGRDGDG RSFHADITAIAVRQPS GEMAGVVVLLRRTAEA VTGGPVLSALDAQVLE GVASGESTVQLASRLY LSRQGIEYRVGQMLRR FDAPNRPALVARAHAL GMFAAGQWPPRVLPEC VR	36