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Supplemental Information

Refactoring the Cryptic Streptophenazine Biosynthetic Gene Cluster Unites Phenazine, Polyketide, and Nonribosomal Peptide Biochemistry

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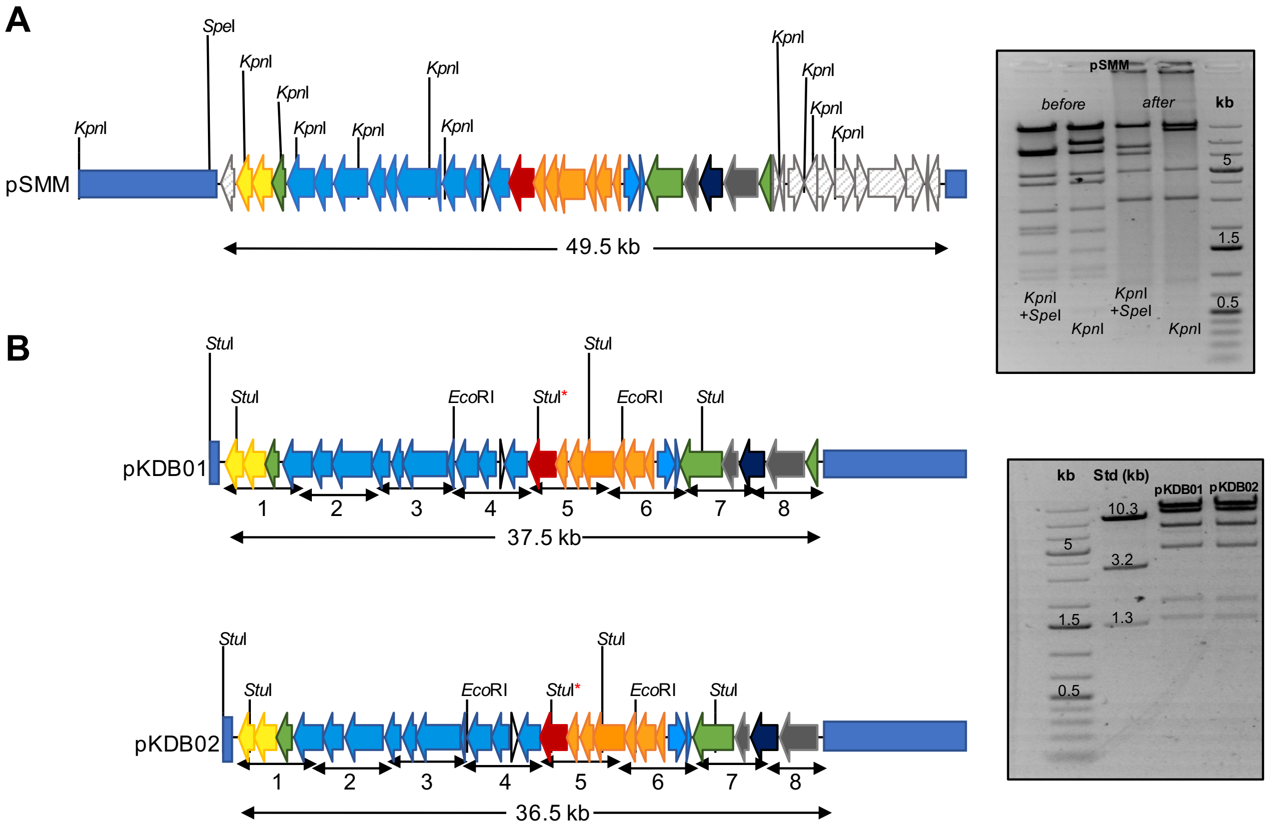


Figure S1. Confirmation of the integrity of the captured *spz* BGC, related to Figures 1 and 2 and Table 1. A. Restriction digest of pSMM before and after transformation in *E. coli* using KpnI and KpnI + SpeI enzymes. KpnI digest, expected sizes (kb) = 23.9, 11.1, 8.2, 4.9, 4.1, 2.7, 1.9, 1.5, 1.1, 1.0, 0.5. KpnI + SpeI digest, expected sizes (kb) = 22.9, 8.9, 8.2, 4.9, 4.1, 2.7, 2.1, 1.9, 1.5, 1.1, 1.0, 0.5. **B.** Restriction digest of pKDB01, expected sizes (kb): 18.0, 13.7, 8.3, 4.9, 2.0, 1.6, and pKDB02, expected sizes (kb): 17.0, 13.3, 8.3, 4.9, 2.0, 1.5, with EcoRI + StuI.* indicates digest is blocked due to overlapping *dcm* methylation. Std – sequenced plasmid digested with EcoRI + StuI, used as a control, expected sizes (kb) = 10.3, 3.2, 1.3. Plasmid backbone represented by blue rectangle.

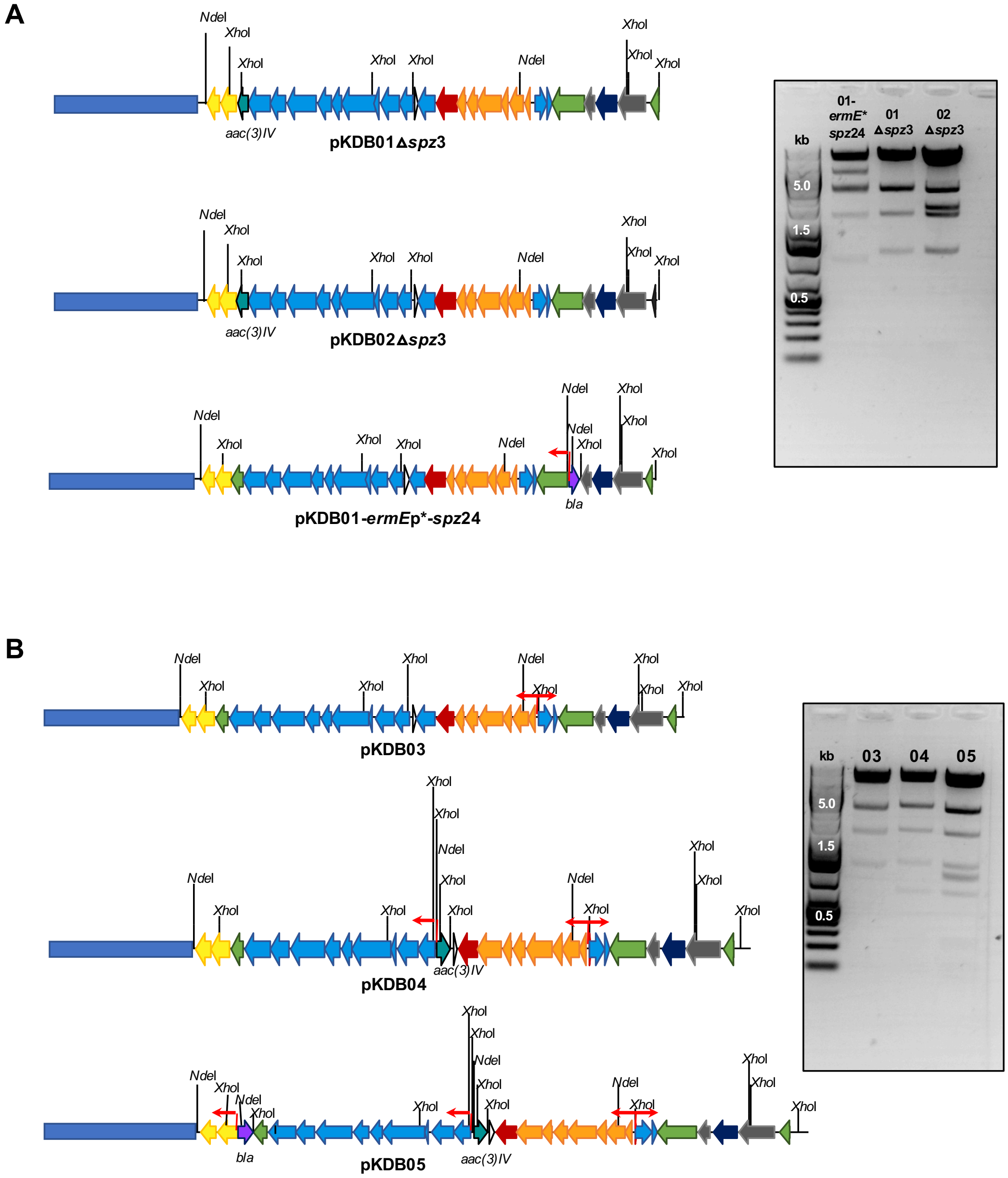


Figure S2. Engineering the *spz* BGC, related to Figures 2 and 3. **A**. Agarose gels of restriction digestions and corresponding maps of regulatory gene mutant plasmids. Enzyme pair XhoI + NdeI used for digestion. pKDB01Δ*spz*3, expected sizes (kb): 10.6, 10.5, 8.7, 8.6, 3.2, 3.1, 1.9, 1.0, 0.1**.** pKDB02Δ*spz*3, expected sizes (kb): 10.6, 10.5, 8.7, 8.6, 3.2, 2.1, 1.9, 1.0, 0.1. pKDB01-*ermE*p*-*spz*24, expected sizes (kb): 11.7, 10.5, 8.7, 5.2, 3.4, 3.2, 3.1, 1.9, 0.9, 0.2, 0.1. **B.** Restriction digest with XhoI + NdeI of refactored plasmids, agarose gel and corresponding plasmid map showing restriction sites: pKDB03, expected sizes (kb): 11.7, 10.5, 8.7, 7.6, 3.2, 3.1, 1.9, 1.1, 0.1; pKDB04, expected sizes (kb): 11.7, 10.5, 8.6, 7.5, 3.2, 3.1, 1.9, 1.1, 0.7, 0.3, 0.2, 0.1; pKDB05, expected sizes (kb): 10.9, 10.5, 8.7, 7.5, 3.2, 3.1, 1.9, 1.1, 0.9, 0.9 0.7, 0.3, 0.3, 0.1. Plasmid backbone represented by blue rectangle.

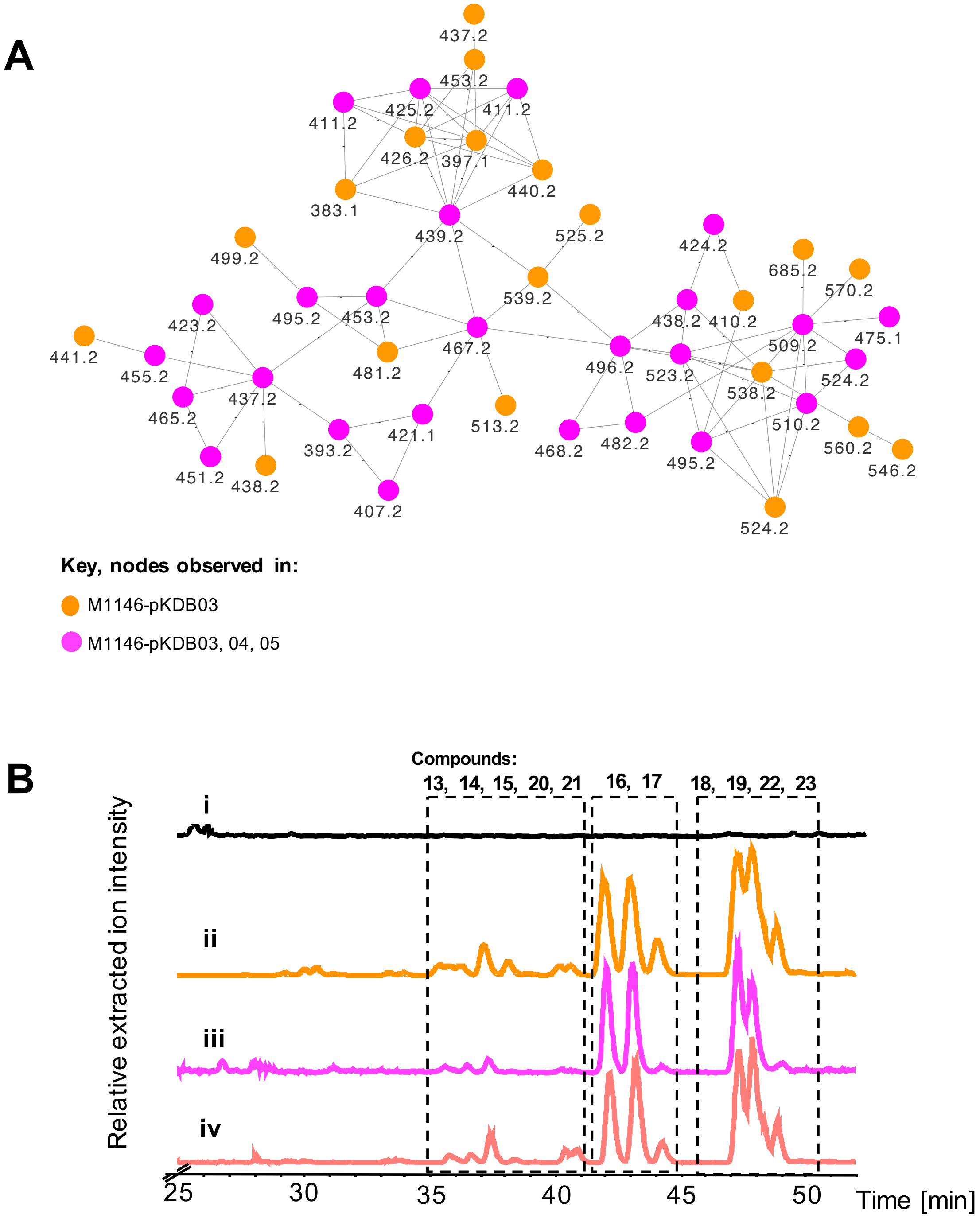


Figure S3. Comparative production of streptophenazines by the refactored *spz* BGC, related to Figures 2, 3, and 4. A. Streptophenazine metabolite cluster of nodes from the molecular network including MS/MS data of extracts of M1146-pKDB03, -pKDB04, and -pKDB05. Nodes are labeled with the corresponding precursor ion mass. **B.** Extracted ion chromatograms (*m/z* 411.2, 425.2, 439.2, 510.2), corresponding to isolated compounds in refactored pathway: (**i**) M1146-pKDB01, (**ii**) M1146-pKDB03, (**iii**) M1146-pKDB04, and (**iv**) M1146-pKDB05.

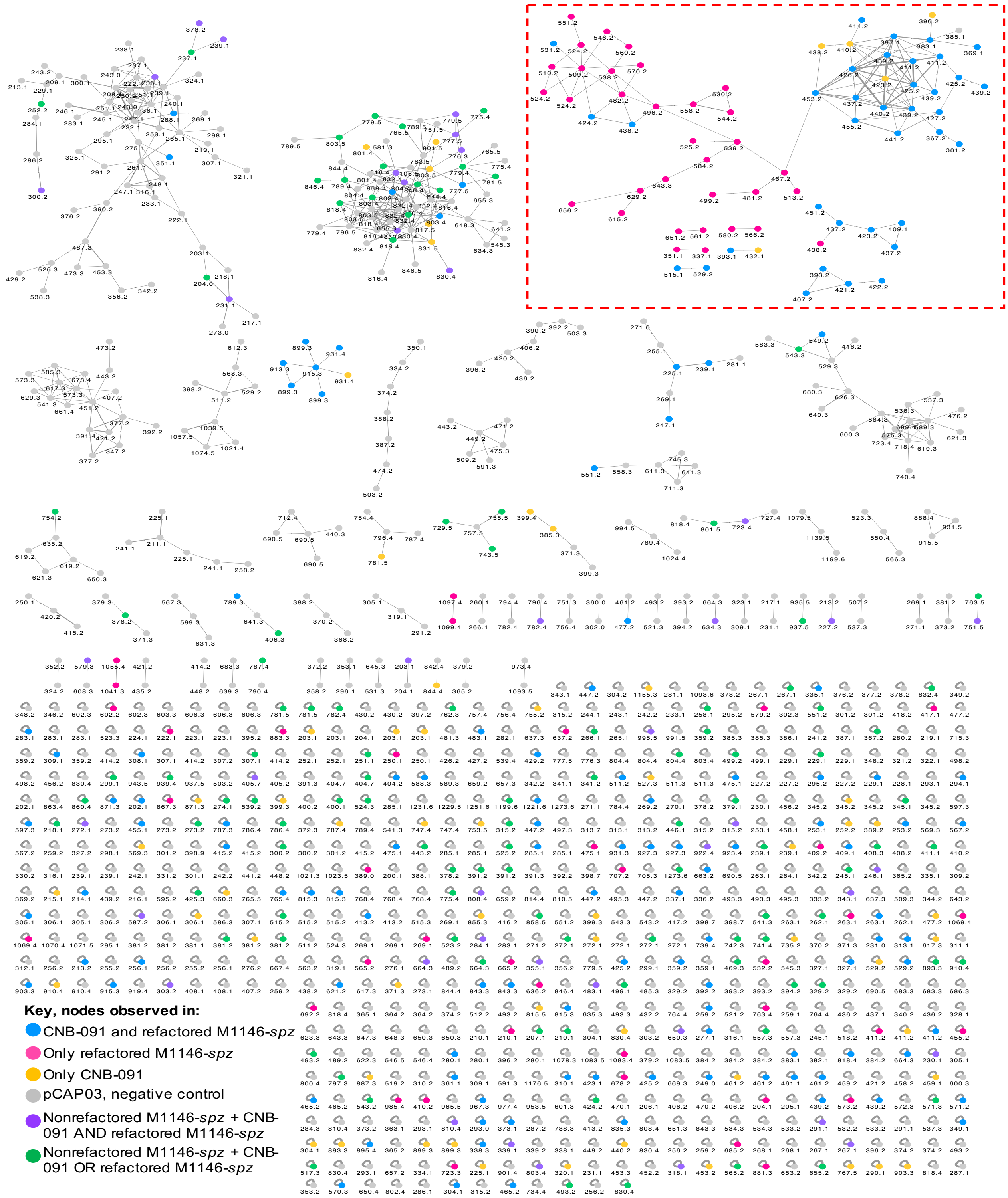
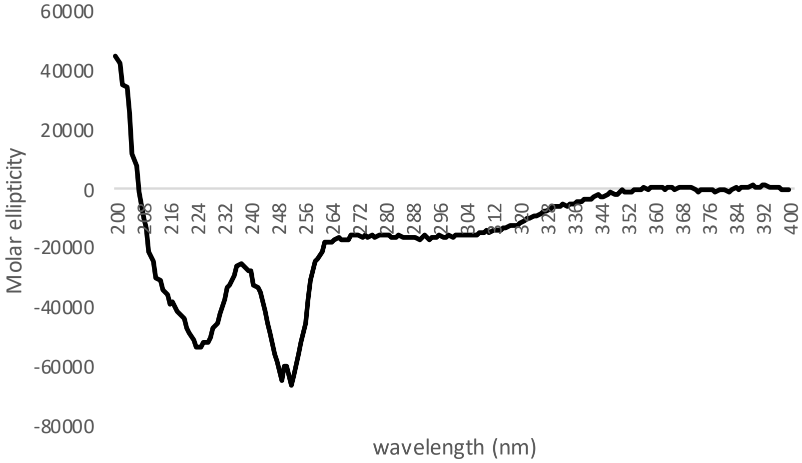


Figure S4. Molecular network showing production of streptophenazines, related to Figure 4. LC-MS/MS data of ethyl acetate extracts from CNB-091, M1146-pCAP03, M1146-pKDB01 (non-refactored *spz* BGC) and M1146-pKDB03 (refactored *spz* BGC) were used for network generation. Nodes corresponding to streptophenazines highlighted in red square.

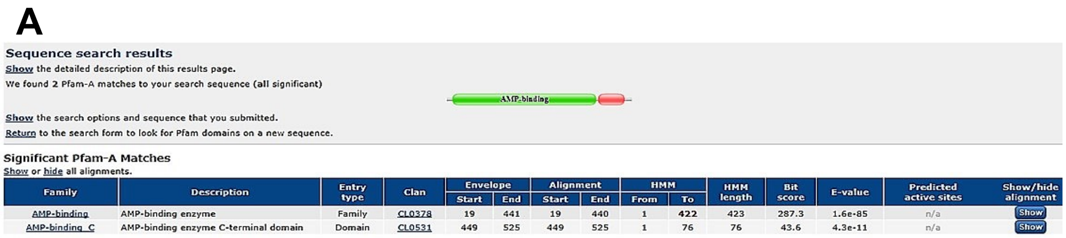
A



B

Spz7_KR	···–DVYWAHLPDT––VTPVEEILAA**LDD**LVRAGKILHAGLSNFPAWRV–···
Ery1_KR	···––––LGGIGDDVPLSAVFHAAAT**LDD**GT–VDTLTGERIERASRAKVL···
SlnA1_KR	···–––LLDRIPEAHPLTGVFHAAGV**LDD**GM–VGALSAERLDAVLRPKTD···
RifA_KR	···LEAVLRAIPAEHPLTAVIHTAGV**LDD**GV–VTELTPDRLATVRRPKVD···
AmphJ_KR	···–––LLASVPAEHPLTAVVHTAGV**LDD**GI–FPSLTPDRLDSVMRPKVD···
TlmH_KR	···–––VLAQIRSRGPIGGVVHAAGL**LDD**SI–LANMTPEQLHRVLRSKVD···

Figure S5. Confirmation of stereochemistry at C-1' position of compound 18, related to Figure 1. **A.** Circular dichroism (CD) spectrum of streptophenazine G (**18**). **B.** Alignment of type I PKS KR domains and Spz7. Amino acid residues (LDD motif) defining Spz7 as type B KR are highlighted. Ery1 = erythromycin, SlnA1 = salinomycin, RifA = rifamycin, AmphJ = amphotericin, TlmH = thiolactomycin.



Sequence search results

Show the detailed description of this results page.

We found 2 Pfam-A matches to your search sequence (all significant)

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Significant Pfam-A Matches

Show or hide all alignments.

Family	Description	Entry type	Clan	Envelope		Alignment		HMM		HMM length	Bit score	E-value	Predicted active sites	Show/hide alignment
				Start	End	Start	End	From	To					
AMP-binding	AMP-binding enzyme	Family	CL0378	19	441	19	440	1	**422**	423	287.3	1.6e-85	n/a	Show
AMP-binding_C	AMP-binding enzyme C-terminal domain	Domain	CL0531	449	525	449	525	1	76	76	43.6	4.3e-11	n/a	Show

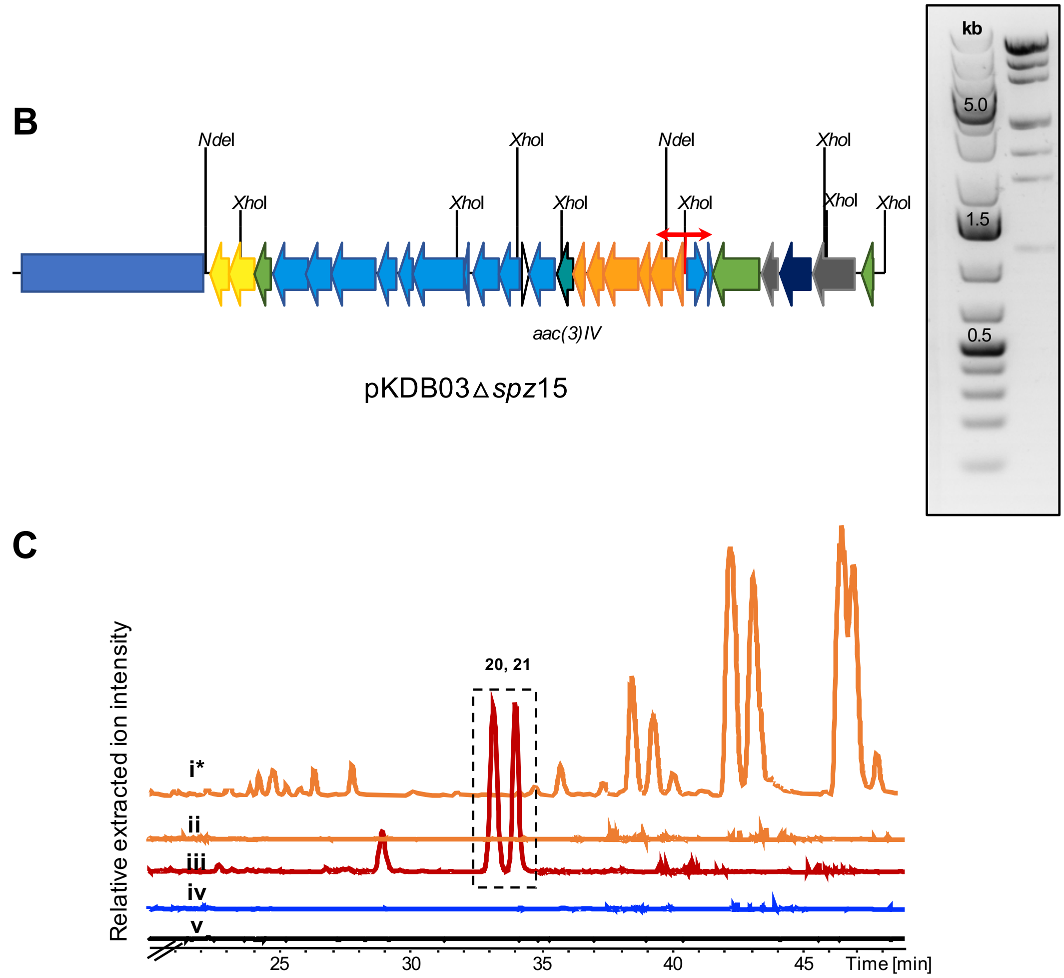

Figure S6. Bioinformatic analysis and gene deletion of *spz*15, related to Figures 5 and 6. A. Protein family (Pfam) analysis of Spz15. Identified CL00378 AMP-binding domain which is characteristic for ANL superfamily of enzymes that includes adenylation domains. **B.** Restriction digest of pKDB03∆*spz*15 with XhoI and NdeI restriction enzymes. Expected sizes (kb): 11.7, 10.5, 7.5, 5.6, 3.2, 3.1, 2.3, 1.9, 1.1, 0.1. **C.** LC-MS chromatograms: (**i**) Base Peak Chromatogram (BPC) of M1146-pKDB03∆*spz*15 **zoomed out 100x,* (**ii**) Extracted Ion Chromatogram (EIC) (*m/z* 510.2, corresponding to compounds **20** and **21**) of M1146-pKDB03∆*spz*15, (**iii**) M1146-pKDB03 EIC (*m/z* 510.2), (**iv**) CNB-091 EIC (*m/z* 510.2), and (**v**) M1146-pCAP03 EIC (*m/z* 510.2).

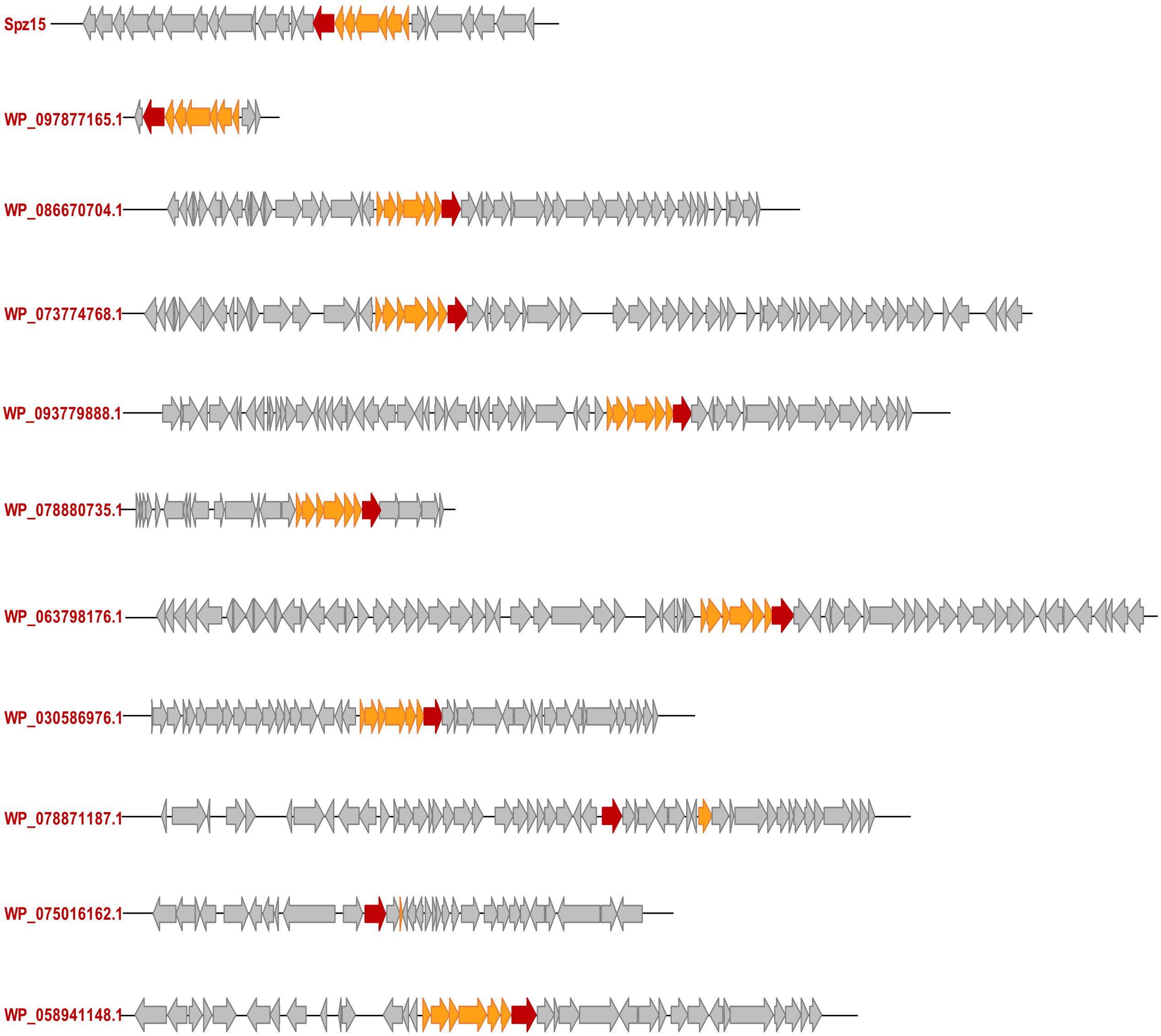

Figure S7. antiSMASH predicted gene neighborhoods of *phz*-associated discrete adenylation proteins, related to Figure 5. Red gene = adenylation enzyme homolog found through blastp, orange genes = phenazine biosynthesis homologs. Spz15 = from *Streptomyces* sp. CNB-091, WP_097877165.1 = from *Streptomyces* sp. ms184, WP_086670704.1 = from *Streptomyces albovinaceus*, WP_073774768.1 = from *Streptomyces* sp. TSRI0445, WP_093779888.1 = from *Streptomyces* sp. yr375, WP_078880735.1 = from *Kitasatospora purpeofusca*, WP_063798176.1 = from *Streptomyces* sp. 150FB, WP_030586976.1 = from *Streptomyces anulatus*, WP_078871187.1 = from *Streptomyces caatingaensis*, WP_075016162.1 = from *Streptomyces rubidus*, WP_058941148.1 = from *Streptomyces kanasensis*.

Table S1, related to Figures 2, 3 and Method Details. Promoter cassette sequences used for refactoring. Bold = restriction site, italic = added for scar, blue = FRT site, red = antibiotic resistance, green = promoter, purple = *actII-ORF4*.

Cassette	Sequence
sp44–p21	**CTCGAG**GGTGAACCGATCTCCTCGTTAGGGTCACACCAGACTTTACAACACCGCACAGCATGTTGTCAAAGCAGAGACGGTTCGAATGTGAACAGCCACTAT**CATATG***TGCAGTTC*GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGGTTCATGTGCAGCTCCATCAGCAAAAGGGGATGATAAGTTTATCACCACCGACTATTTGCAACAGTGCCGTTGATCGTGCTATGATCGACTGATGTCATCAGCGGTGGAGTGCAATGTCGTGCAATACGAATGGCGAAAAGCCGAGCTCATCGGTCAGCTTCTCAACCTTGGGGTTACCCCCGGCGGTGTGCTGCTGGTCCACAGCTCCTTCCGTAGCGTCCGGCCCCTCGAAGATGGGCCACTTGGACTGATCGAGGCCCTGCGTGCTGCGCTGGGTCCGGGAGGGACGCTCGTCATGCCCTCGTGGTCAGGTCTGGACGACGAGCCGTTCGATCCTGCCACGTCGCCCGTTACACCGGACCTTGGAGTTGTCTCTGACACATTCTGGCGCCTGCCAAATGTAAAGCGCAGCGCCCATCCATTTGCCTTTGCGGCAGCGGGGCCACAGGCAGAGCAGATCATCTCTGATCCATTGCCCCTGCCACCTCACTCGCCTGCAAGCCCGGTCGCCCGTGTCCATGAACTCGATGGGCAGGTACTTCTCCTCGGCGTGGGACACGATGCCAACACGACGCTGCATCTTGCCGAGTTGATGGCAAAGGTTCCCTATGGGGTGCCGAGACACTGCACCATTCTTCAGGATGGCAAGTTGGTACGCGTCGATTATCTCGAGAATGACCACTGCTGTGAGCGCTTTGCCTTGGCGGACAGGTGGCTCAAGGAGAAGAGCCTTCAGAAGGAAGGTCCAGTCGGTCATGCCTTTGCTCGGTTGATCCGCTCCCGCGACATTGTGGCGACAGCCCTGGGTCAACTGGGCCGAGATCCGTTGATCTTCCTGCATCCGCCAGAGGCGGGATGCGAAGAATGCGATGCCGCTCGCCAGTCGATTGGCTGAGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC**AAGCTT**TG**CTCGAG**TGTGCGGGCTCTAACACGTCCTAGTATGGTAGGATGAGCAATCTAGTCGAGCAACGGAGGTACGGAC**CATATG**
actIp	**CTCGAG**GGTTCATGTGCAGCTCCATCAGCAAAAGGGGATGATAAGTTTATCACCACCGACTATTTGCAACAGTGCCGTTGATCGTGCTATGATCGACTGATGTCATCAGCGGTGGAGTGCAATGTCGTGCAATACGAATGGCGAAAAGCCGAGCTCATCGGTCAGCTTCTCAACCTTGGGGTTACCCCCGGCGGTGTGCTGCTGGTCCACAGCTCCTTCCGTAGCGTCCGGCCCCTCGAAGATGGGCCACTTGGACTGATCGAGGCCCTGCGTGCTGCGCTGGGTCCGGGAGGGACGCTCGTCATGCCCTCGTGGTCAGGTCTGGACGACGAGCCGTTCGATCCTGCCACGTCGCCCGTTACACCGGACCTTGGAGTTGTCTCTGACACATTCTGGCGCCTGCCAAATGTAAAGCGCAGCGCCCATCCATTTGCCTTTGCGGCAGCGGGGCCACAGGCAGAGCAGATCATCTCTGATCCATTGCCCCTGCCACCTCACTCGCCTGCAAGCCCGGTCGCCCGTGTCCATGAACTCGATGGGCAGGTACTTCTCCTCGGCGTGGGACACGATGCCAACACGACGCTGCATCTTGCCGAGTTGATGGCAAAGGTTCCCTATGGGGTGCCGAGACACTGCACCATTCTTCAGGATGGCAAGTTGGTACGCGTCGATTATCTCGAGAATGACCACTGCTGTGAGCGCTTTGCCTTGGCGGACAGGTGGCTCAAGGAGAAGAGCCTTCAGAAGGAAGGTCCAGTCGGTCATGCCTTTGCTCGGTTGATCCGCTCCCGCGACATTGTGGCGACAGCCCTGGGTCAACTGGGCCGAGATCCGTTGATCTTCCTGCATCCGCCAGAGGCGGGATGCGAAGAATGCGATGCCGCTCGCCAGTCGATTGGCTGA**CATATG**CCACTGCCTCTCGGTAAAATCCAGCAAAAATTAATCAGTGCAGCTCGCTGCACTGATTAATTTTTGATCAATAGGAGATCGCTTGTGACGGCAAGCACATTGAAATCTGTTGAGTAGGCCTGTTATTGTCGCCCCCAGGAGACGGAGAATCTCGACGGGGGCGCAGATGAGATTCAACTTATTGGGACGTGTCCATGTAATCACCGATGCGGGATGTGTAATTCCGCTTAAATCCTCGAAGGCGACCCAGCTCCTGGTGCTGCTGCTCCTCAGGCGGCACGAGGTGGTGGGATCGGGGGTGCTCATCGAGGAGTTGTGGGCGGACCACCCGCCCCGCAGCGCCATGACGACGCTGCAGACGTACGTGTACCACACCCGCCGGCTGCTGGGGGAGCACCGGGTGACGAGCGACGACCGGGAATTGGTCCTGACCCAGCCGCCCGGCTACTTCGCCCTGATCGACGAGGACGAACTCGACGTCGCGGTCGCCGAGCGTCTGATCCGCACCGGCGGCCGGCTGCTCGAGGAGAACCGGCTCGAGGAGGCGCTCGCCTCGTTGGACGCGGGACTGGATCTCTGGCGAGGCCCGGCGCTGTCCACCGTACCGTGCGGCCGGGTGCTCGAAAGCAATATCGCGCACCTGGAAGAGCTGCGGCTTTTTGGAATGCAGCTCCGTATCGACGCGAATTGGCGGCTGGGCAGAATAGGGCCGATGATTCCGGAACTCCGGTCCCTGGTAATTTCGCATCCGCTGAACGAGACCCTGCACGCCAAACTGATGGGCGCGCTCTGTCAGATGGGCAGGCGCGCCGAGGCGCTGGAATCGTATCGGAATCTCCGGCGGATACTGTCCGACGAACTGGGGGTGGATCCGACGCCGGAAATCCAGCGTATGCACATGGAAATTCTCAACGGTGAGAAGGTGCTCGTGTAGCACCGGTCCGTGAACGCGGTGGAGCCCTATGTCTCTTAAGTGTTCCCCTCCCTGCCTCGTGGTCCCTCACGCGCTCAGCTTTGGGCGCCCGGCTCGAGCGGCGGTCGAAGGGAGATGGGGTGCCGCTGGACGCGGCGCCGGTGGATCCGGCATCGAGGGGTCCCGTATCGGCCTTCGAGCCTCCTTCGAGCCACGGGGCCGACGATGACGACGACCACCGGACGAACGCATC

*ermE*p*	ATGGGGACCTCCTGGGGTGCGTTGGACCGCTGGATCCTACCAACCGGCACGATTGTGCCCACAACAGCATCGCGGTGCCACGTGTGGACCGCGTCGGTCAGATCCTCCCCGCACCTCTCGCCAGCCGTCAAGATCGACCGCGTGCACCA**CATATG**TTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATCATGATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATG**CTCGAG**

Table S3, related to STAR Methods section "Bioactivity testing of streptophenazines and MIC determination." Minimum Inhibitory Concentrations (MICs) (μg/mL) of oxo-streptophenazine A (**9**), streptophenazine C (**13**), streptophenazine A (**16**), and streptophenazine Q (**20**).

Compound	**9**	**13**	**16**	**20**
Strain				
Group A *Streptococcus*	>50	>50	>50	2.5
Acinetobacter baumannii 5075	>50	>50	>50	40
Klebsiella pneumoniae 1100	>50	>50	>50	>40
MRSA TCH1516	>50	>50	>50	40

Table S4, related to STAR methods. Plasmids and strains used in this work.

Plasmids	Description	Sources
pCAP03	TAR cloning and broad-host-range heterologous expression vector; *CEN6-ARS*4, *oriT, traJ, pUC ori, Kan*^{r}*, Apra*^{r}*, pADH1, URA3, TRP1*	Tang et al., 2015
pSMM	Derivative of pCAP03 with 48 kb captured *spz* cluster	This work
pKDB01	Derivative of pCAP03 with 37.5 kb captured *spz* cluster	This work
pKDB02	Derivative of pKDB01 without TetR regulatory gene (*spz*28)	This work
pKDB01△*spz*3	Derivative of pKDB01 without *spz*3 (LysR type regulatory gene)	This work
pKDB02△*spz*3	Derivative of pKDB02 without *spz*3 (LysR type regulatory gene)	This work
pKDB01-*ermE*p*-*spz*24	Derivative of pKDB01 with *ermE** promoter in front of *spz*24 (LuxR-type regulatory gene), *Kan*^{r}, *Amp*^{r}	This work
pKDB03	Derivative of pKDB01 with *sp44-p21* cassette	This work
pKDB04	Derivative of pKDB03 with *actIp* cassette	This work
pKDB05	Derivative of pKDB04 with *ermE*p* cassette	This work
pKDB03△*spz*15	Derivative of pKDB03 with *spz*15 (putative adenylation protein-encoding gene) deleted	This work
pCAP03-*ermE*p*	Derivative of pCAP03 containing *ermE*p* cassette between XhoI and NdeI, *Kan*^{r}*, Amp*^{r}	This work
pCAP03-*actIp*	Derivative of pCAP03 containing *actIp* cassette between XhoI and NdeI. *Kan*^{r}*, Apra*^{r}	This work
pCAP03-*sp44-p21*	Derivative of pCAP03 containing *sp44-p21* cassette between XhoI, NdeI. *Kan*^{r}*, Apra*^{r}	This work
pIJ790	*λ*-RED (*gam, bet, exo*), *cat*, *ara*C, rep101^{ts}, oriR101, P araBAD	Gust, 2003
pUB307	Self-transmissible plasmid that mobilizes other plasmids *in trans* for DNA transfer into hosts: RP4, *neo*	Flett, 1997
Strains	**Description**	
Streptomyces		
Streptomyces sp. CNB-091	Native producer of streptophenazines	Trischman et al., 1994
Streptomyces coelicolor M1146	Host strain for heterologous expression derived from *S. coelicolor* M145: Δ*act*, Δ*red*, Δ*cpk*, Δ*cda*.	Gomez-Escribano and Bibb, 2011
S. coelicolor M1146-pCAP03	Heterologous host containing empty pCAP03 as a control	This work
S. coelicolor M1146-pSMM	Heterologous host containing pSMM (*spz* BGC captured in 48 kb DNA fragment)	This work
S. coelicolor M1146-pKDB01	Heterologous host with integrated pKDB01 (37.5 kb captured *spz* cluster)	This work
S. coelicolor M1146-pKDB02	Heterologous host with integrated pKDB02 (△*spz*28)	This work
S. coelicolor M1146-pKDB01△*spz*3	Heterologous host with integrated pKDB01△*spz*3	This work
S. coelicolor M1146-pKDB02△*spz*3	Heterologous host with integrated pKDB02△*spz*3	This work
S. coelicolor M1146-pKDB01-*ermE*p*-*spz*24	Heterologous host with integrated pKDB01-*ermE*p*-*spz*24	This work

S. coelicolor M1146-pKDB03	Heterologous host with integrated pKDB03	This work
S. coelicolor M1146-pKDB04	Heterologous host with integrated pKDB04	This work
S. coelicolor M1146-pKDB05	Heterologous host with integrated pKDB05	This work
S. coelicolor M1146-pKDB03Δ*spz*15	Heterologous host with integrated pKDB03Δ*spz*15	This work
Escherichia coli		
DH10B	F– *mcr*A Δ(*mrr*-*hsd*RMS-*mcr*BC), Φ80*lac*ZΔM15, Δ *lac*X74 *rec*A1 *end*A1 *ara*D139 Δ (*ara leu*)7697 *gal*U *gal*K *rps*L *nup*G *λ*–. Storage and maintenance	
BW25113	K-12 derivative: Δ*ara*BAD, Δ*rha*BAD,	Datsenko and Wanner, 2000
BT340	DH5α/pCP20, containing FLP recombinase	Cherepanov and Wackernagel, 1995
ET12567	F- *dam*13::Tn9, *dcm*6, *hsd*M, *hsd*R, *rec*F,143 *zjj*-202::Tn10, *gal*K2, *gal*T22, *ara*-14, *pac*Y1, *xyl*-5, *leu*B6, *thi*-1, *ton*A31, *rps*L136, *his*G4, *tsx*-78, *mtl*-1, *gln*V44. Donor strain for conjugation between *E. coli* and *Streptomyces* in triparental mating	MacNeil et al., 1992
Other		
Group A *Streptococcus*	Clinical isolate used for bioactivity assays	Nizet lab, UCSD
Acetinobacter baumannii 5075	Clinical isolate used for bioactivity assays	Nizet lab, UCSD
Klebsiella pneumoniae 1100	Clinical isolate used for bioactivity assays	Nizet lab, UCSD
MRSA TCH1516	Clinical isolate used for bioactivity assays	Nizet lab, UCSD
S. cerevisiae VL6-48N	MATα *trp*1-Δ1 *ura*3-Δ1 *ade*2-101 *his*3-Δ200 *lys*2 *met*14 cir°, TAR cloning	Kouprina and Larinova, 2016

Table S5, related to STAR methods. Primers used in this work.

Primer name	Sequence (5' to 3')	Purpose
Frag1_F	GAGTAGCAGCACGTTCCTTATATGTAGCTTTCGACATATGCATGAGCTGTCTCCTGGTGCTGGTGGGCAGG	TAR cloning *spz* BGC
Frag1_R	CGACCTGCCCGAACTCGACGGGCTGGAACTG	TAR cloning *spz* BGC
Frag2_F	CACGATGCCCAGCAGCAGTCCCATGTCGTGG	TAR cloning *spz* BGC
Frag2_R	GCATCCAGTGCGAGTACAGCCTCGCCGAGCG	TAR cloning *spz* BGC
Frag3_F	CCAGGTACTCCTCCAACTGCTCGCCGGTCCG	TAR cloning *spz* BGC
Frag3_R	CTCATAAGGATGCCTTCTGCGGGTGTGGAGACC	TAR cloning *spz* BGC
Frag4_F	CGATCGCCGCCTGGACCGACTGGAGCAGG	TAR cloning *spz* BGC
Frag4_R	GCTGCCGCTGAACGCCAACGGGAAGGTGGAC	TAR cloning *spz* BGC
Frag5_F	GAGGGCGGTCAGGGCAGGATTCACGGAATGC	TAR cloning *spz* BGC
Frag5_R	GGAGGCAGGCGCGTACTGGACGTTCCTCATCC	TAR cloning *spz* BGC
Frag6_F	CCATCATCTTGAGCTCTTCGTCGACGACCATG	TAR cloning *spz* BGC
Frag6_R	GAACCAGGAGATCGCCGACCGGCTCGTCCT	TAR cloning *spz* BGC
Frag7_F	CTCGGCCTGGAATCACTTCAGATGATGCGCCTG	TAR cloning *spz* BGC
Frag7_R	GTTCCTGATCGCCCAGTTCCTGCAGAGCGTG	TAR cloning *spz* BGC
Frag8_F	GTCACTCCGACGAACATCAGGATGGAGGCCG	TAR cloning *spz* BGC
Frag8_R+TetR	CTCGGTTTGACGCCTCCCATGGTATAAATAGTGGCTCGAGGCACAGGACGGCATCGCCCGCAGCTGAGC	TAR cloning *spz* BGC
Frag8_R-TetR	CTCGGTTTGACGCCTCCCATGGTATAAATAGTGGCTCGAGGGTCCGTCCGGTCCCAGGAGAACCAGCTGATC	TAR cloning *spz* BGC
∆*spz*3_F	GGCTTGCGGAGAGACCGGGCGGGGATCAGCCGAGGAGGGCGCGGGCGTGTCAGCCAATCGACTGGCGAGCGGCAT	Delete *spz*3 (LysR)
∆*spz*3_R	GCTCGTCGCACCGTCGCCCCGCTCCTGAACCGGCCGCGGGGAAGGACCACGGTTCATGTGCAGCTCCATCAGCAAAAG	Delete *spz*3 (LysR)
∆*spz*3_seq_F	GTGAAGGCGACGAACAGGAAGTG	Confirm *spz*3 deletion
∆*spz*3_seq_R	GATCGAGCCCGCCGACGTG	Confirm *spz*3 deletion
Upreg-*spz*24_F	GTCGGGAGCGTGCGGCACAGTGCGCACGTCGGGCAGGACTGGCACGGTCATATGGGGACCTCCTGGGGTGCGTTGG	Insert *ermE** promoter in front of *spz*24 (LuxR)
Upreg-*spz*24_R	CCCGATCGTGTACGTGCGCGCCGTCGCGCCCTAGGCCCTCCGGGCGGACGCTCGAGCATTCAAATATGTATCCGCTCATG	Insert *ermE** promoter in front of *spz*24 (LuxR)
Upreg-*spz*24_seq_F	CAGCAGGCTGCTCTTGCCGACAC	Sequencing to confirm insertion
Upreg-*spz*24_seq_R	GATGCGCGGTATGTGGGAGCGC	Sequencing to confirm insertion
***aac(3)IV* +FRT_F**	ATTATACATATGTGCAGTTCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGGTTCATGTGCAGCTCCATCAGCAAAAG	Amplify *aac(3)IV* gene with FRT sites for cloning between pET28a NdeI and HindIII sites
***aac(3)IV* +FRT_R**	ATTATAAAGCTTGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCTCAGCCAATCGACTGGCGAGCGGCATCG	Amplify *aac(3)IV* gene with FRT sites for cloning between pET28a NdeI and HindIII sites
***sp44*_F**	GACGCCTCCCATGGTATAAATAGTGGCTCGAGGGTGAACCGATCTCCTCGTTAGGGTC	Amplify *sp44* promoter with homology to pCAP03 and *aac(3)IV*
***sp44*_R**	TCTAGAGAATAGGAACTTCGAACTGCACATATGATAGTGGCTGTTCACATTCGAACCGTCTCTG	Amplify *sp44* promoter with homology to pCAP03 and *aac(3)IV*

***p21*_F**	CTATTCTCTAGAAAGTATAGGAACTTCAAGCTTTGCTCGAGTGTGCGGGCTCTAACACGTC	Amplify *p21* promoter with homology to pCAP03 and *aac(3)IV*
***p21*_R**	AGCACGTTCCTTATATGTAGCTTTCGACATATGGTCCGTACCTCCGTTGCTCGACTAGAT	Amplify *p21* promoter with homology to pCAP03 and *aac(3)IV*
***aac(3)IV*+FRT_*sp44*_pCAP03_F**	CAGAGACGGTTCGAATGTGAACAGCCACTATCATATGTGCAGTTCGAAGTTCCTATTCTCTAGA	Amplify *aac(3)IV* +FRT with homology to *sp*44 promoters
***aac(3)IV*+FRT_*p21*_pCAP03_R**	ACGTGTTAGAGCCCGCACACTCGAGCAAAGCTTGAAGTTCCTATACTTTCTAGAGAATA	Amplify *aac(3)IV* +FRT with homology to *p*21 promoter
***sp44*+*p21*_cas_insert_F**	TGGTCGGCGTCCTCGTTGCTGAAACTGATGCTCTGGGCGACGTCGGGCATCTCGAGGGTGAACCGATCTCCTCGTTAGG	Amplify *sp44-p21* cassette with homology sequences for targeted insertion into *spz* BGC
***sp44*+*p21*__cas_insert_R**	CCGGGGACGTGGGTGCCGAGTCCGGCGAGGTAGGTGTTCTCGAACTTCATCATATGGTCCGTACCTCCGTTGCTCGAC	Amplify *sp44-p21* cassette with homology sequences for targeted insertion into *spz* BGC
***sp44*+*p21*_cas_insert_seq_F**	GTGAACAGGAGATGCCGGGTC	Confirm *sp44-p21* cassette insertion
***sp44*+*p21*_cas_insert_seq_R**	GCCTCGTACCAGCCTTCCTG	Confirm *sp44-p21* cassette insertion
***actIp*_F**	CCGGTCCGTGAACGCGGTGGAGCCCTATGTCTCTTAAGTGTTCCCCTCCCTGCC	Amplify *actI* promoter with homology to *act*II-ORF4 and pCAP03
***actIp*_R**	CACGTTCCTTATATGTAGCTTTCGAGATGCGTTCGTCCGGTGGTCGTCGTCA	Amplify *actI* promoter with homology to *act*II-ORF4 and pCAP03
***actII*-ORF4_F**	CCGCTCGCCAGTCGATTGGCTGACATATGCCACTGCCTCTCGGTAAAATCC	Amplify *act*II-ORF4 with homology arms for assembly with *aac(3)IV* and *act1p*
***actII*-ORF4_R**	GGCAGGGAGGGGAACACTTAAGAGACATAGGGCTCCACCGCGTTCACGGACCGG	Amplify *act*II-ORF4 with homology arms for assembly with *aac(3)IV* and *act1*p
***aac(3)IV* +*actIp*_F**	GACGCCTCCCATGGTATAAATAGTGGCTCGAGGGTTCATGTGCAGCTCCATCAGCAAAAG	Amplify *aac(3)IV* with homology to *act*II-ORF4 and pCAP03
***aac(3)IV* +*actIp*_R**	GGATTTTACCGAGAGGCAGTGGCATATGTCAGCCAATCGACTGGCGAGCGG	Amplify *aac(3)IV* with homology to *act*II-ORF4 and pCAP03
***actIp*_cas_insert_F**	GGGTCCGCGTCGTACGGAAGGTCAAGAATCTTCGGGTCGGCGGAAGCCACGATGCGTTCGTCCGGTGGTCGTCGTC	Amplify *actIp* cassette with homology sequences for targeted insertion into *spz* BGC
***actIp* _cas_insert_R**	GTGCTCGAATGTCCCATACACCCAAGACGTAGAAGTTCTCTGGAGGAACGACTCGAGGGTTCATGTGCAGCTCCATCAG	Amplify of *actIp* cassette with homology sequences for targeted insertion into *spz* BGC
***actIp*_cas_insert_seq_F**	CTCGAGCCAGTAGCGGGATC	Confirm *actIp* cassette insertion within *spz* BGC
***actIp*_cas_insert_seq_R**	CATTGTGCACGGTCCACCG	Confirm *actIp* cassette insertion within *spz* BGC
***ermE**p*_F**	GTGCCTCACTGATTAAGCATTGGTAACATATGTGGTGCACGCGGTCGATCTTGACGGCTG	Amplify *ermE** promoter with homology arms for assembly with *bla* and pCAP03
***ermE**p*_R**	CAGCACGTTCCTTATATGTAGCTTTCGAATGGGGACCTCCTGGGGTGCGTTGGACC	Amplify *ermE** promoter with homology arms for assembly with *bla* and pCAP03
***bla*+*ermE*p*_F**	TGACGCCTCCCATGGTATAAATAGTGGCTCGAGCATTCAAATATGTATCCGCTCATGAGA	Amplify *bla* with homology to *ermE*p* and pCAP03 for assembly
***bla*+*ermE*p*_R**	CAGCCGTCAAGATCGACCGCGTGCACCACATATGTTACCAATGCTTAATCAGTGAGGCAC	Amplify *bla* with homology to *ermE*p* and pCAP03 for assembly
***ermE*p*_cas _insert_F**	CAGGGTCGCCGTGACGGCGAACTGGAGGCGGGCGAGGTCGACGACGTCCACATGGGGACCTCCTGGGGTGCGTTGG	Amplify *ermE*p* cassette with homology sequences for targeted insertion into *spz* BGC
***ermE*p*_cas_insert_R**	CTTCCACAGCCACGCCCGCGCCCTCCTCGGCTGATCCCCGCCCGGTCTCTCTCGAGCATTCAAATATGTATCCGCTCATG	Amplify *ermE*p* cassette with homology sequences for targeted insertion into *spz* BGC
***ermE*p*_cas_insert_seq_F**	GTGAGGTCGCTCGTGCGCAG	Confirm *ermE*p* cassette insertion within *spz* BGC
***ermE*p*_cas_insert_seq_R**	GTCCTGCCCGAGCAGTATGTCC	Confirm *ermE*p* cassette insertion within *spz* BGC
∆*spz*15_F	ACGGGTTCCAGGCGGCCGGAGACGACGTCCTCGCGCAGCGCCCCCCGGTCTCAGCCAATCGACTGGCGAGCGGCAT	Delete *spz*15 (adenylation protein)
∆*spz*15_R	GGCGGGTCGTCCGGCTCCAGCCCTGAGCCCGGGGCACTCCTCCGCCTCCGGTTCATGTGCAGCTCCATCAGCAAAAG	Delete *spz*15 (adenylation protein)
∆*spz*15_seq_F	GTCAGGGCAGGATTCACGGAATG	Confirmation of *spz*15 deletion
∆*spz*15_seq_R	GTACGAACTCCGTTTCGACAGGGTG	Confirmation of *spz*15 deletion

Table S6, related to STAR methods. Primers used for RT-PCR.

Primer name	**Sequence (5' to 3')**	**Primer name**	**Sequence (5' to 3')**
*hrd*B_F	CCTCCGCCTGGTGGTCTCG	*hrd*B_R	AACTTGTAGCCCTTGGTGTAGTCGAAC
*spz1*_F	CGTCACCGAGAGCAGCCACAGCG	*spz1*_R	GTTGCTCCAAGCACTGCGACTGCC
*spz2*_F	CGATCAGGGAGAAGTTCACGACG	*spz2*_R	CTGCACTTCCTGTTCGTCGCCTT
*spz3*_F	GGACATACTGCTCGGGCAGGAC	*spz3*_R	CAGTTGGAGTACTTCCTCGCGG
*spz4*_F	GCCTGATCAGCACCACGTCGG	*spz4*_R	GAACTTCGCGTACGACCTGTGCG
*spz5*_F	CGATCTGCTCGAAGTCGAACAGCC	*spz5*_R	GACACCATGTACGCGTCACTCATCC
*spz6*_F	GTGCAGAGTCTCCAGAACGTCGTGG	*spz6*_R	CACACCGCAGGTCGTGTTCACCTTC
*spz7*_F	GCGAGGCTGTACTCGCACTGGATG	*spz7*_R	GTCTCCGAATACGCGCTGGGCAC
*spz8*_F	GAACGTGGTCACCGGAATGTCCAG	*spz8*_R	GACCGAGCTCGACCTGTACTGCTTC
*spz9*_F	CTCGAAGAAGCTCTCGTCCGGTTCG	*spz9*_R	CTTGAGGACTGGAAGCCGTACGGG
*spz10*_F	GTGGTCGAACAGGTCGATCAGCTC	*spz10*_R	GAGAAGGATTCCCTGACCCTCGTCG
*spz11*_F	GTCTTGCTCCGCATCAGGAATTCC	*spz11*_R	GATCACCTCGACATGATCGTCGAC
*spz12*_F	CTCACGTGGTGCATGACCACCTGG	*spz12*_R	GACATCGTCGGCGTCTTCGAGAG
*spz13*_F	CGATGATCGCCTGGTGGGATCTC	*spz13*_R	GTGGTGGCGACGACGTCGAAC
*spz14*_F	CGAGATGACGTACGTCCACCACGC	*spz14*_R	GCTTCCAGACGGCCTACCGGCATC
*spz15*_F	CACGTCCGCCAGGTGGTAGGTGAG	*spz15*_R	GAGGTGACGGTGTGGTTCTCGGTG
*spz16*_F	CTCCACCCTGTCGAAACGGAGTTC	*spz16*_R	GACCGCCGATATCACCGTCCCGTTTC
*spz17*_F	GCGAGAACATCCTGCTGCGCCAC	*spz17*_R	CTCCTATGTGGTCGTCGACGCCTTC
*spz18*_F	CGGTTGATCTCGACGACACCGAGC	*spz18*_R	CTGGACTCCGCGATCCTCATCCGC
*spz19*_F	CACCAGGAAGGTGCGGATGTCGTG	*spz19*_R	CTGGAATTCCCCGCATCCACCCG
*spz20*_F	CAGGTACTTGTCTCCGACGCCCGAC	*spz20*_R	CTCGACTACGAGCTGCCGATGCTG
*spz21*_F	CTGGTCGAACGGGTTCATGAACTCG	*spz21*_R	GCTGACCCGGCATCTCCTGTTCAC
*spz122*_F	CTGGTCACCACGGAGAAGGCCGTC	*spz22*_R	GATTCCAGATCGAAGCCGATCGGATC
*spz23*_F	CTAGTCGCCGGAGAGGAGCTGCGC	*spz23*_R	GTATCGCGCTCCTGCTCCTTCAGC
*spz24*_F	CAACTCCTCGTAGGCGTGCGACAC	*spz24*_R	CTACCGTCTCTCCCTGCGGCTGGATC
*spz25*_F	GGTGGTGTAGGTGAAGTCCACCCAC	*spz25*_R	GAAGTCGTACGACCAGAGCGGTGTG
*spz26*_F	CGAACATCAGGATGGAGGCCGTCAG	*spz26*_R	GCTGGAGTACTTCTGGTGGGGCTCG
*spz27*_F	CGTCGAGCCGGAGGTCGAAGAAG	*spz27*_R	CAAGGACATCTGGCCGCAGTGGCTG
*spz28*_F	CGATCAGCTGGTTCTCCTGGGACCG	*spz28*_R	GACTAGGGCCCAGGCCAAGGAGCG