Supplementary information

A comparative metabologenomic approach reveals new mechanistic insights into *Streptomyces* antibiotic crypticity

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Keywords: *Streptomyces griseus*, cryptic metabolism, regulation, metabologenomics, tetramic acid

Supplementary methods

Marker-less gene deletion/promoter replacement

All gene deletions and P_{ftdA} mutants were constructed using double homologous recombination as previously described (1). To avoid undesired recombination between the wild-type and the mutant P_{ftdA} sequences, an intermediate ΔP_{ftdA}::*tsr* mutant was constructed, and the steps will be described as an example for all mutants:

Streptomycin-resistant (Str^R) mutants of *Streptomyces sp.* strain JV180 were isolated on ISP2 + Str^{100} agar. The *rpsL* genes were amplified with primers (YQ4-Sg_rpsL_f and YQ5-Sg_rpsL_r) and sequenced. JV307 bearing the *rpsL*_K43R mutation was chosen for subsequent experiments as no phenotypic changes were observed, including PTM production. The upstream and downstream homology regions flanking P_{ftdA} were amplified from JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r). The *tsr* gene was amplified from pJVD53 (primers YQ215-tsr-f and YQ216-tsr-r). The vector pUC19 was linearized by PCR-amplification (primers YQ268-pUC19-us and YQ269-pUC19-ds) and digested by DpnI. PCR products were assembled using the NEBuilder HiFi Assembly kit (NEB). Correct cloning was confirmed by restriction digest and Sanger sequencing. The resulting pUC19-ΔP_{ftdA}::*tsr* and were digested with *Xba*I and *Hind*III and ligated into pJVD52.1 using the same restriction sites. The resulting pJVD52.1-ΔP_{ftdA}::*tsr* was introduced into JV307 by conjugation, and apramycin-resistant (Apr^R) exconjugants were selected. Exconjugants were grown in TSB non-selectively at 37˚C and recombinants were selected for on ISP2 + Apr^{50}. Apr^R colonies were grown TSB non-selectively at 37˚C and double recombinants were selected for on ISP2 + Str^{100}. The resulting ΔP_{ftdA}::*tsr* mutant was confirmed by colony PCR.

Circular Rapid Amplification of cDNA Ends

Streptomyces sp*.* strain JV180 was cultured on solid ATCC172 medium overlaid with a cellophane film. After six days, mycelia were harvested from three plates and pooled in 5 mL RNAlater with 4 mm diameter beads. The mycelia were homogenized by vortexing for about 1 minute and allowed to settle for 5 minutes. The cell suspension was transferred to a clean tube and pelleted by centrifugation at 3,214 × *g* for 10 minutes. The supernatant was discarded the pellet was frozen at −20°C. The following day, approximately 100 mg of the frozen pellet was powdered by grinding with mortar and pestle in liquid nitrogen and resuspended in 450 µL of buffer RLT (QIAGEN). The suspension was lysed with a Fisherbrand

model 120 sonic dismembrator at 30% amplitude with 4 cycles of 15 seconds of sonication followed by 30 second of resting. Total RNA was extracted with the QIAGEN RNeasy kit following the manufacturer's protocol after this point and its integrity was verified by gel electrophoresis.

The cRACE protocol was adapted from that of Bose and Newman (2). Genomic DNA was removed with Turbo DNase (Ambion). Superscript II Reverse Transcriptase (Invitrogen) was used to generate cDNA with random hexamers. Leftover RNA was removed with RNase H and cDNA was purified with Wizard SV Gel and PCR clean-up kit (Promega) and eluted in nuclease-free water. Adaptor ligation was performed in a buffer containing 1X RNA ligase buffer, 25% PEG 8000, 10 µg/mL BSA, 1 mM hexamine cobalt (III) chloride, 100 pmol WNp213, and 25 Units of T4 RNA (Ambion) ligase. The reaction was carried out at room temperature overnight and purified with the Wizard SV Gel and PCR clean-up kit. The eluate was used for PCR amplification with Taq DNA polymerase (NEB) using Failsafe Premix G (Epicentre) with primers WNp210 and YQ186. PCR products were amplified in a second reaction using 1.5 µL of the first reaction as template with nested primers YQ188 and YQ185 as reverse primers. PCR products were cloned into pCR2.1-TOPO using the TOPO-TA cloning kit (Invitrogen) and the inserts were Sanger sequenced by Genewiz to map the transcript start site.

Griseorhodin enrichment

A griseorhodin-enriched fraction was obtained using a modified Kupchan scheme (3, 4) and solid phase extraction column. Strain JV180 was plated on approximately 1 L worth of 8340 agar plates. After 6 days, the agar plates were diced and collected in a large 2 L flask and the agar was submerged with acetonitrile overnight. The acetonitrile was collected and evaporated under low pressure and the agar was extracted with acetonitrile again. The dried extract was resuspended in 9:1 (v/v) methanol/water. The solution was extracted with equal volume of petroleum ether followed by equal volume of ethyl acetate, which extracted the red pigment from the aqueous solution. The ethyl acetate was evaporated under low pressure and resuspended with 1:1 methanol/water. A Bond Elut C18-OH (Agilent) solid phase extraction column was used to further purify the extract. The column was primed with water before the extract was loaded. The column was washed with water, and 1:3 acetonitrile /water. The red pigments were eluted by 1:1 acetonitrile/water. This fraction was dried under low pressure and resuspended in 500 µL 1:1 acetonitrile/water and filtered before LC-MS analysis.

The griseorhodin-enriched fraction was analyzed using a Phenomenex Luna C18 column (75 x 3 mm, 3 µm pore size) installed on an Agilent 1260 Infinity HPLC connected to an Agilent 6420 Triple-Quad

mass spectrometer. For each run, 10 µL sample was injected and the chromatography conditions were as follows: *T* = 0, 5% B; *T* = 2, 5% B; *T* = 16, 100% B, *T* = 20, 100% B; A: water + 0.1% formic acid, B: acetonitrile + 0.1% formic acid; 0.7 mL/min. The diode array detector was set to measure absorbance at 488 nm. The mass spectrometer was set to mass scan mode with *m/z* ranges 100 – 1000 for positive and negative ions and the fragmentor voltage was set to = 70 V.

Table S1. List of strains used in this study

Strain	Relevant characteristics / Genotype	Source or reference
Escherichia coli		
DH5α	cloning host, F⁻ ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17 (rK⁻, mK⁺) *pho*A *sup*E44 λ⁻ *thi*-1 *gyr*A96 *rel*A1	NEB
BL21(DE3) + Rosetta2	plasmid expression host, F⁻ *omp*T *hsd*SB (rB⁻, mB⁻) *gal dcm* (DE3)	Novagen
JV36	conjugal donor strain, *dam*-3 *dcm*-6 *metB1 galK2 galT27 lacY1 tsx*-78 *supE44 thi*-1 *mel*-1 *tonA31 ΔhsdRMS-mrr*::FRT(rK⁻ mK⁻) attHK::pJK202 (*ΔoriR6K-aadA*::FRT *bla*::*pir*) (Tra⁺, Amp^S)	Blodgett *et al*(5)
Streptomyces strains		
Streptomyces griseus subsp. *griseus* IFO13350	model *Streptomyces griseus* strain, aka strain ISP-5235	NRRL
Streptomyces sp. strain JV180	environmental isolate, PTM producer	Blodgett *et al*(6)
JV251	*Streptomyces californicus* strain NRRL B-3320	NRRL
JV252	*Streptomyces floridae* strain NRRL 2423	NRRL
JV253	*Streptomyces puniceus* strain NRRL B-2895	NRRL
JV254	*Streptomyces griseus subsp. griseus* strain NRRL F-5144	NRRL
JV255	*Streptomyces globisporus subsp. globisporus* strain NRRL B-2709	NRRL
JV256	*Streptomyces albus subsp. albus* strain NRRL B-2445	NRRL
JV257	*Streptomyces griseus subsp. griseus* strain NRRL WC-3480	NRRL
JV258	*Streptomyces baarnensis* strain NRRL B-2842	NRRL
Streptomyces sp. strain SP18CM02	environmental isolate, PTM producer	Qi *et al*(7)
JV307	JV180 *rpsL*_K43R	This study
JV352	JV307 Δ*ftdA-F*	This study
JV631	JV307 ΔP_{ftdA}::*tsr*	This study
JV1851	JV307 ΔP_{ftdA}::P_{ermE*}_pHM11a	This study
JV847	JV307 ΔP_{ftdA}::P_{ftdA}_IFO13350	This study
JV1961	JV307 ΔP_{ftdA}::P_{ftdA}_SP18CM02	This study
JV1176	JV307 ΔP_{ftdA}::P_{ftdA}_JV251	This study
JV1178	JV307 ΔP_{ftdA}::P_{ftdA}_JV252	This study
JV1673	JV307 ΔP_{ftdA}::P_{ftdA}_JV253	This study
JV898	JV307 ΔP_{ftdA}::P_{ftdA}_JV254	This study
JV1186	JV307 ΔP_{ftdA}::P_{ftdA}_JV255	This study
JV1182	JV307 ΔP_{ftdA}::P_{ftdA}_JV256	This study
JV1184	JV307 ΔP_{ftdA}::P_{ftdA}_JV257	This study
JV1188	JV307 ΔP_{ftdA}::P_{ftdA}_JV258	This study
JV503	JV180 attBΦC31::pJMD1-P_{ftdA}_IFO13350-*xylE*	This study
JV505	JV180 attBΦC31::pJMD1-P_{ftdA}_JV180-*xylE*	This study
JV527	JV180 attBΦC31::pJMD1-P_{ermE*}_pHM11a	This study
JV1871	JV180 attBΦC31::pJMD1-P_{ftdA}_JV180-*ftdA-xylE*	This study

JV1873	JV180 attBΦC31::pJMD1-P_{ftdA}_IFO13350-*ftdA-xylE*	This study
JV1743	IFO13350 attBΦC31::pJMD1-P_{ftdA}_IFO13350-*xylE*	This study
JV1745	IFO13350 attBΦC31::pJMD1-P_{ftdA}_JV180-*xylE*	This study
JV1747	IFO13350 attBΦC31::pJMD1-P_{ermE*}_pHM11a	This study
JV1875	IFO13350 attBΦC31::pJMD1-P_{ftdA}_JV180-*ftdA-xylE*	This study
JV1877	IFO13350 attBΦC31::pJMD1-P_{ftdA}_IFO13350-*ftdA-xylE*	This study
JV712	JV307 P_{ftdA}Δ−528_−207	This study
JV716	JV307 P_{ftdA}Δ−528_−57	This study
JV1933	JV307 P_{ftdA}Δ−528_−38	This study
JV1836	JV307 P_{ftdA}Δ−528_−31	This study
JV1986	JV307 P_{ftdA}Δ2_28	This study
JV936	JV307 P_{ftdA}Δ29_48	This study
JV1675	JV307 P_{ftdA}Δ49_68	This study
JV1655	JV307 P_{ftdA}Δ69_88	This study
JV1680	JV307 P_{ftdA}Δ89_108	This study
JV1688	JV307 P_{ftdA}Δ109_128	This study
JV1697	JV307 P_{ftdA}Δ129_148	This study
JV1693	JV307 P_{ftdA}Δ149_181	This study
JV2084	JV307 P_{ftdA}Δ−528_−31:: $-35box_{JV254}$	This study
JV2088	JV307 P_{ftdA}Δ−528_−31:: $-35box_{JV255}$	This study
JV2090	JV307 P_{ftdA}Δ−528_−31:: $-35box_{JV256}$	This study
JV2100	JV307 P_{ftdA}Δ−528_−31:: $-35box_{JV257}$	This study
JV556	JV307 Δ*adpA*	This study
JV623	JV556 attBΦC31::pSET152-*adpA*_JV180	This study
JV664	JV556 attBΦC31::pSET152-*adpA*_IFO13350	This study
JV629	JV556 attBΦC31::pJMD3-*adpA*_JV180	This study
JV618	JV556 attBΦC31::pJMD3-*adpA*_IFO13350	This study
JV2111	JV1851 Δ*adpA*	This study
JV938	JV307 P_{ftdA}Δ29_48::IFO13350	This study
JV1553	JV307 P_{ftdA}_35G/T	This study
JV1565	JV307 P_{ftdA}_37C/A	This study
JV1555	JV307 P_{ftdA}_40G/T	This study
JV1567	JV307 P_{ftdA}_42C/A	This study
JV894	JV307 P_{ftdA}ΔAG	This study
JV1673	JV307 P_{ftdA}ΔAG::CT	This study
JV1032	JV307 ΔP_{ftdA}::P_{ftdA}_IFO13350+AG	This study
JV564	JV307 Δ*grhR2-V*	This study
JV1832	JV307 Δ*grhR1-E*	This study
JV1898	JV307 Δ*grhFGH*	This study
JV1698	JV307 Δ*grhGH*	This study
JV1892	JV307 Δ*grhI-P*	This study

JV1706	JV307 Δ*grhQSAB*	This study
JV1854	JV1851 Δ*grhGH*	This study
JV2108	JV1851 Δ*grhR2-V*	This study
JV671	JV564 attBΦC31::pJMD2-*grhR2*	This study
JV669	JV564 attBΦC31::pJMD2-*grhR3*	This study
JV919	JV564 attBΦC31::pJMD2-*grhF*	This study
JV921	JV564 attBΦC31::pJMD2-*grhG*	This study
JV923	JV564 attBΦC31::pJMD2-*grhH*	This study
JV1070	JV564 attBΦC31::pJMD2-*grhGH*	This study
JV1054	JV564 attBΦC31::pJMD2-*accBE*_JV180	This study
JV1060	JV564 attBΦC31::pJMD2-SGR1943-44	This study
JV1066	JV564 attBΦC31::pJMD2-SGR3280-81	This study
JV1018	JV180 attBΦC31::pJMD2-*grhG*	This study
JV978	JV254 attBΦC31::pJMD2-*grhG*	This study
JV982	JV258 attBΦC31::pJMD2-*grhG*	This study
JV986	IFO13350 attBΦC31::pJMD2-*grhG*	This study

Table S2. List of plasmids used in this study.

plasmid	description	source
pUC19	*bla* ori^{pUC} cloning vector	NEB
pCR2.1-TOPO	Amp^R ori^{pBR322} TOPO-TA cloning vector	Invitrogen
pET11a	Amp^R ori^{pBR322} *lacI* T7 promoter protein expression vector	Novagen
pCM130	ori^{pBR322} Tet^R *xylE* reporter gene vector	Marx *et al*(8)
pSET152	*aac(3)IV oriT* ori^{pUC} $int^{\Phi C31}$ $attP^{\Phi C31}$ self-integrating vector	Bierman *et al*(9)
pJMD2	Apr^R *oriT* ori^{colE1} $int^{\Phi C31}$ $attP^{\Phi C31}$ P_{ermE*} sequence from pDA1652	Ko *et al*(10)
pJMD3	pJMD2 variant with P_{ermE*} sequence from pHM11a	Ko *et al*(10)
pJVD52.1	Apr^R *oriT* $ori^{pSG5(ts)}$ rep^{pUC} $rpsL^+$ shuttle vector	Blodgett *et al*(5)
pJVD53	Tsr^R Amp^R *oriT* ori^{pIJ101} ori^{R6K} *nitR* protein expression vector	Blodgett *et al*(5)
pJMD1	ligation of PCR product of pSET152 (primers PXPX2 and PXPF2) digested with BamHI, SpeI and overlap extension PCR product of [pJVD53 (primers Fd-pSET and Fd-xylE) and pCM130 (primers xylE-pSET and xylE-fd)] amplified with primers PXFP2 and PXXP2, digested with BglII and XbaI	This study
pJVD52.1-ΔftdA-F	ligation of pJVD52.1 and overlap extension PCR products of JV180 (primers YQ82-180PTM_us_f and YQ91-180PTM_us_o_v2; YQ84-180PTM_ds_f and YQ85-180PTM_ds_r), both digested with XbaI and HindIII	This study
pUC19-ΔPftdA::tsr	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and pJVD53 (primers YQ215-tsr-f and YQ216-tsr-r)	This study
pUC19-ΔPftdA::PermE*	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and pJMD2 (primers YQ243-DPro-ermEh-r and YQ258-DPro-ermEh-f-v2)	This study
pUC19-ΔPftdA::P13350	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and IFO13350 (primers YQ249-DPro-158-r and YQ259-DPro-158-f-v2)	This study
pUC19-ΔPftdA::P251	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV251 (primers YQ231-DPro-180-r and YQ260-Dpro-25123-f-v2)	This study
pUC19-ΔPftdA::P252	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV252 (primers YQ231-DPro-180-r and YQ260-Dpro-25123-f-v2)	This study
pUC19-ΔPftdA::P253	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV253 (primers YQ231-DPro-180-r and YQ260-Dpro-25123-f-v2)	This study
pUC19-ΔPftdA::P254	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV254 (primers YQ252-DPro-254-r and YQ261-DPro-254-f-v2)	This study
pUC19-ΔPftdA::P255	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV255 (primers YQ254-DPro-255-r and YQ262-DPro-255-f-v2)	This study
pUC19-ΔPftdA::P256	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV256 (primers YQ256-DPro-2568-r and YQ263-DPro-2568-f-v2)	This study
pUC19-ΔPftdA::P257	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV257 (primers YQ249-DPro-158-r and YQ264-DPro-257-f-v2)	This study
pUC19-ΔPftdA::P258	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), JV180 (primers YQ211-180DPro-us-f and YQ212-180DPro-us-r; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and JV258 (primers YQ256-DPro-2568-r and YQ263-DPro-2568-f-v2)	This study

pJVD52.1-ΔPftdA::tsr	ligation of pJVD52.1 and pUC19-ΔPftdA::tsr, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::PermE*	ligation of pJVD52.1 and pUC19-ΔPftdA::PermE*, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P13350	ligation of pJVD52.1 and pUC19-ΔPftdA::P13350, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P251	ligation of pJVD52.1 and pUC19-ΔPftdA::P251, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P252	ligation of pJVD52.1 and pUC19-ΔPftdA::P252, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P253	ligation of pJVD52.1 and pUC19-ΔPftdA::P253, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P254	ligation of pJVD52.1 and pUC19-ΔPftdA::P254, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P255	ligation of pJVD52.1 and pUC19-ΔPftdA::P255, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P256	ligation of pJVD52.1 and pUC19-ΔPftdA::P256, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P257	ligation of pJVD52.1 and pUC19-ΔPftdA::P257, both digested with XbaI and HindIII	This study
pJVD52.1-ΔPftdA::P258	ligation of pJVD52.1 and pUC19-ΔPftdA::P258, both digested with XbaI and HindIII	This study
pJMD1-PermE*_pHM11a-xylE		Qi *et al*(11)
pJMD1-PftdA_JV180-xylE	ligation of pJMD1 and overlap extension PCR product of [pJMD1 (primers YQ67-XylE-fw and xylE-REV) and JV180 (primers YQ50-P180-overlap-r and YQ51-P180-overlap-f)], both digested with AscI and HpaI	This study
pJMD1-PftdA_IFO13350-xylE	ligation of pJMD1 and overlap extension PCR product of [pJMD1 (primers YQ67-XylE-fw and xylE-REV) and JV180 (primers YQ69-P13350-overlap-r and YQ70-P13350-overlap-f)], both digested with AscI and HpaI	This study
pJMD1-PftdA_JV180-ftdA-xylE	gibson assembly of PCR products of pJMD1 (primers YQ67-XylE-f and YQ177-pXylE-r) and JV180 (YQ501-P180-ftdA-xylE-f and YQ502-PftdA-ftdA-xylE-r)	This study
pJMD1-PftdA_IFO13350-ftdA-xylE	gibson assembly of PCR products of pJMD1 (primers YQ67-XylE-f and YQ177-pXylE-r) and pUC19-ΔPftdA::P13350 (YQ503-P13350-ftdA-xylE-f and YQ502-PftdA-ftdA-xylE-r)	This study
pUC19-ΔadpA	ligation of pUC19 and overlap extension PCR product of JV180 (primers YQ180-DadpA-u-f-xbaI and YQ189-DadpA-us-r-2; YQ190-DadpA-d-f-o-3 and YQ169-DadpA-d-r-h-2), both digested with XbaI and HindIII	This study
pJVD52.1-ΔadpA	ligation of pJVD52.1 and pUC19-ΔadpA, both digested with XbaI and HindIII, for the construction of the ΔadpA mutant	This study
pSET152-adpA_13350	ligation of pSET152 and the PCR product of IFO13350 (primers YQ225-adpA-f-xbaI and YQ226-adpA-r-bamHI), both digested with BamHI and XbaI	This study
pSET152-adpA_180	ligation of pSET152 and the PCR product of JV180 (primers YQ225-adpA-f-xbaI and YQ226-adpA-r-bamHI), both digested with BamHI and XbaI	This study
pJMD3-adpA_13350	ligation of pJMD3 and the PCR product of IFO13350 (primers YQ119-AdpA_fw_n and YQ120-AdpA_rv1_x), both digested with NdeI and XbaI	This study
pJMD3-adpA_180	ligation of pJMD3 and the PCR product of JV180 (primers YQ119-AdpA_fw_n and YQ121-AdpA_rv2_x), both digested with NdeI and XbaI	This study
pUC19-PftdA-Δ-528_-307	gibson assembly of PCR product of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), PCR products of JV180 (primers YQ211-180DPro-us-f and YQ265-Dpro-us-r-v2; YQ231-DPro-180-r and YQ229-Dpro-D500-f; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r)	This study
pJVD52.1-PftdA-Δ-528_-307	ligation of pJVD52.1 and pUC19-PftdA-Δ-528_-307, both digested with XbaI and HindIII	This study
pJVD52.1-PftdA-Δ-528_-207	ligation of pJVD52.1-PftdA-Δ-528_-307 and PCR product of JV180 (primers YQ237-Dpro-ds-ncoI-r and YQ232-Dpro-D400-f), both digested with SpeI and NcoI	This study

pJVD52.1-PftdA-Δ-528_-57	ligation of pJVD52.1-PftdA-Δ-528_-307 and PCR product of JV180 (primers YQ237-Dpro-ds-ncoI-r and YQ234-Dpro-D250-f), both digested with SpeI and NcoI	This study
pUC19-PftdA-ΔAG	gibson assembly of PCR product of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), PCR products of JV180 (primers YQ211-180DPro-us-f and YQ265-Dpro-us-r-v2; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and overlap extension PCR product of JV180 (primers YQ238-Dpro-DAG-r and YQ260-Dpro-25123-f-v2; YQ231-DPro-180-r and YQ229-Dpro-D500-f)	This study
pUC19-PftdA-ΔAG::CT	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds) and JV180 (primers YQ211-180DPro-us-f and YQ390-180PTM-dAG-CT-r; YQ389-180PTM-dAG-CT-f and YQ214-180DPro-ds-r)	This study
pUC19-PftdA-Δ29-48	gibson assembly of PCR product of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), PCR products of JV180 (primers YQ211-180DPro-us-f and YQ265-Dpro-us-r-v2; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and overlap extension PCR product of JV180 (primers YQ239-Dpro-DadpA-r and YQ260-Dpro-25123-f-v2; YQ231-DPro-180-r and YQ229-Dpro-D500-f)	This study
pUC19-PftdA-Δ29-48::IFO13350	gibson assembly of PCR product of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds), PCR products of JV180 (primers YQ211-180DPro-us-f and YQ265-Dpro-us-r-v2; YQ213-180DPro-ds-f and YQ214-180DPro-ds-r), and overlap extension PCR product of JV180 (primers YQ240-Dpro-DadpA158-r and YQ260-Dpro-25123-f-v2; YQ231-DPro-180-r and YQ229-Dpro-D500-f)	This study
pUC19-ΔPftdA::P13350+AG	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds) and pUC19-ΔPftdA::P13350 (primers YQ211-180DPro-us-f and YQ407-P158+AG-us-r; YQ340-P254+AG-ds-f and YQ214-180DPro-ds-r)	This study
pJVD52.1-PftdA-ΔAG	ligation of pJVD52.1 and pUC19-PftdA-ΔAG, both digested with XbaI and HindIII	This study
pJVD52.1-PftdA-ΔAG::CT	ligation of pJVD52.1 and pUC19-PftdA-ΔAG::CT, both digested with XbaI and HindIII	This study
pJVD52.1-PftdA-Δ29_48	ligation of pJVD52.1 and pUC19-PftdA-Δ29-48, both digested with XbaI and HindIII	This study
pJVD52.1-PftdA-Δ29_48::IFO13350	ligation of pJVD52.1 and pUC19-PftdA-Δ29-48::IFO13350, both digested with XbaI and HindIII	This study
pUC19-ΔPftdA::P13350+AG	ligation of pJVD52.1 and pUC19-ΔPftdA::P13350+AG, both digested with XbaI and HindIII	This study
pUC19-ΔgrhR2-V	ligation of pUC19 and overlap extension PCR product of JV180 (primers YQ183-Dgrh-us-r-3 and YQ191-Dgrh-us-f-x-4; YQ184-Dgrh-ds-f-o-2 and YQ163-Dgrh-ds-r-hindIII), both digested with XbaI and HindIII	This study
pJVD52.1-ΔgrhR2-V	ligation of pJVD52.1 and pUC19-ΔgrhR2-V, both digested with XbaI and HindIII	This study
pUC19-ΔgrhGH	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds) and JV180 (primers YQ466-grhGdel-u-f and YQ467-grhGdel-u-r; YQ469-grhGHdel-d-r and YQ473-grhGHdel-d-f)	This study
pUC19-ΔgrhQSAB	gibson assembly of PCR products of pUC19 (primers YQ268-pUC19-us and YQ269-pUC19-ds) and JV180 (primers YQ485-grhQdel-u-f and YQ486-grhQdel-u-r; YQ487-grhBdel-d-f and YQ488-grhBdel-d-r)	This study
pJVD52.1-ΔgrhGH	ligation of pJVD52.1 and pUC19-ΔgrhGH, both digested with XbaI and HindIII	This study
pJVD52.1-ΔgrhQSAB	ligation of pJVD52.1 and pUC19-ΔgrhQSAB, both digested with XbaI and HindIII	This study
pJMD2-grhR2	ligation of pJMD2 and the PCR product of JV180 (primers YQ244-grhR2-f-n and YQ245-grhR2-r-x), both digested with NdeI and XbaI	This study
pJMD2-grhR3	ligation of pJMD2 and the PCR product of JV180 (primers YQ246-grhR3-f-n and YQ247-grhR3-f-r), both digested with NdeI and XbaI	This study
pJMD2-grhF	ligation of pJMD2 and the PCR product of JV180 (primers YQ286-grhF-f-n and YQ287-grhF-r-x), both digested with NdeI and XbaI	This study
pJMD2-grhG	ligation of pJMD2 and the PCR product of JV180 (primers YQ288-grhG-f-n and YQ289-grhG-r-x), both digested with NdeI and XbaI	This study
pJMD2-grhH	ligation of pJMD2 and the PCR product of JV180 (primers YQ294-grhH-f-n and YQ295-grhH-r-x), both digested with NdeI and XbaI	This study
pJMD2-grhGH	ligation of pJMD2 and the PCR product of JV180 (primers YQ288-grhG-f-n and YQ295-grhH-r-x), both digested with NdeI and XbaI	This study

pJMD2-accBE-180	ligation of pJMD2 and the PCR product of JV180 (primers YQ351-180accB-f and YQ354-180accE-r), both digested with NdeI and XbaI	This study
pJMD2-SGR1943-44	ligation of pJMD2 and the PCR product of IFO13350 (primers YQ355-158accB-f and YQ358-158accE-r), both digested with NdeI and XbaI	This study
pJMD2-SGR3281-80	ligation of pJMD2 and the PCR product of IFO13350 (primers YQ359-3281-f and YQ362-3280-r), both digested with NdeI and XbaI	This study
pKN001	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR products of JV180 (primers G35T-us-F and G35T-us-R; G35T-ds-F and G35T-ds-R) for construction of the PftdA-AdpAo::G35T point mutant	This study
pKN002	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers G40T-us-F and G40T-us-R; G40T-ds-F and G40T-ds-R) for construction of the PftdA-AdpAo::G40T point mutant	This study
pKN003	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers C37A-us-F and C37A-us-R; C37A-ds-F and C37A-ds-R) for construction of the PftdA-AdpAo::C37A point mutant	This study
pKN004	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers C42A-us-F and C42A-us-R; C42A-ds-F and C42A-ds-R) for construction of the PftdA-AdpAo::C42A point mutant	This study
pKN012	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers 69-88-us-F and 69-88-us-R; 69-88-ds-F and 69-88-ds-R) for construction of the PftdAΔ(69_88) mutant	This study
pKN015	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers 49-68-us-F and 49-68-us-R; 49-68-ds-F and 49-68-ds-R) for construction of the PftdAΔ(49_68) mutant	This study
pKN016	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers 89-108-us-F and 89-108-us-R; 89-108-ds-F and 89-108-ds-R) for construction of the PftdAΔ(89_108) mutant	This study
pKN017	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers 129-148-us-F and 129-148-us-R; 129-148-ds-F and 129-148-ds-R) for construction of the PftdAΔ(129_148) mutant	This study
pKN018	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers 109-128-us-F and 109-128-us-R; 109-128-ds-F and 109-128-ds-R) for construction of the PftdAΔ(109_128) mutant	This study
pKN019	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers 149-181-us-F and 149-181-us-R; 149-181-ds-F and 149-181-ds-R) for construction of the PftdAΔ(149_181) mutant	This study
pKN027	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers grhR1-grhE-us-F and grhR1-grhE-us-R; grhR1-grhE-ds-F and grhR1-grhE-ds-R) for construction of the ΔgrhR1-E mutant	This study
pKN028	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers -528--31-us-F and -528--31-us-R; -528--31-ds-F and -528--31-ds-R) for construction of the PftdAΔ(−528_−31) mutant	This study
pKN029	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers grhF-grhH-us-F and grhF-grhH-us-R; grhF-grhH-ds-F and grhF-grhH-ds-R) for construction of the ΔgrhFGH mutant	This study
pKN030	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers grhI-grhP-us-F and grhI-grhP-us-R; grhI-grhP-ds-F and grhI-grhP-ds-R) for construction of the ΔgrhI-P mutant	This study
pKN031	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR product of JV180 (primers -528--38-us-F and -528--38-us-R; -528--38-ds-F and -528--38-ds-R) for construction of the PftdAΔ(−528_−38) point mutant	This study
pUC19-JV180-ΔPftdA::PCM02	gibson assembly of the PCR products of pUC19-JV180-ΔPftdA::tsr (primers BE277-PftdA500 bp-F and BE277-PftdA500 bp-R1) and SP18CM02 (primers CM02-PftdA500 bp-F and CM02-PftdA500 bp-R)	This study
pKN038	ligation of pJVD52.1 and pUC19-JV180-ΔPftdA::PCM02, both digested with HindIII and XbaI	This study

pKN040	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR products of JV180 (primers 2-28-us-F and 2-28-us-R; 2-28-ds-F and 2-28-ds-R) for construction of the PftdAΔ(2-28) point mutant	This study
pKN047	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR products of JV180 (primers -35JV254-us-F and-35JV254-us-R; -35JV254-ds-F and-35JV254-ds-R) for insertion of -35 box from JV254 in JV1836	This study
pKN048	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR products of JV180 (primers -35JV255-us-F and-35JV255-us-R; -35JV255-ds-F and-35JV255-ds-R) for insertion of -35 box from JV255 in JV1836	This study
pKN049	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR products of JV180 (primers -35JV256-us-F and-35JV256-us-R; -35JV256-ds-F and-35JV256-ds-R) for insertion of -35 box from JV256 in JV1836	This study
pKN050	gibson assembly of pJVD52.1 digested with BamHI and HindIII and PCR products of JV180 (primers -35JV257-us-F and-35JV257-us-R; -35JV257-ds-F and-35JV257-ds-R) for insertion of -35 box from JV257 in JV1836	This study
pKN052	ligation of pET11a and the PCR product of JV180 (primers pET11a-AdpA-F and pET11a-AdpA-R), both digested with NdeI and NheI	This study

Table S3. List of oligonucleotides used in this study.

name	Sequence (5' to 3')	description
PXPX2	AGATTACAACTAGTGATTACACGCTCACTGCCCGCTTTCC	Construction of pJMD1
PXPF2	AGATTACAGGATCCGATTACATTACCCGCAGGACATATCCACGC	
Fd-pSET	AGATCTAAGCGGCCTTTGACTCCC	
Fd-xylE	CTTTGTTCATGGCGCGCCTCGTCTTTCCAGACGTTAGTAAATGAATTTTCT	
xylE-pSET	TCTAGAGTTAACTCAGGTGAGCACGGTCATGAATC	
xylE-fd	GGAAAGACGAGGCGCGCCATGAACAAAGGTGTAATGCGACCGG	
PXFP2	AGATTACAGGATCCGATTACATTACCCGCAGGACATATCCACGC	
PXXP2	AGATTACAACTAGTGATTACACGCTCACTGCCCGCTTTCC	
YQ4-Sg_rpsL_f	CACGAACGGCACACAGAAAC	sequencing *rpsL* mutation
YQ5-Sg_rpsL_r	GATGATGACCGGGCGCTTC	
YQ82-180PTM_us_f	ATGTTGGCGTCGTGGTCCAGC	PCR upstream homology region (us) for Δ*ftdA-F*
YQ91-180PTM_us_r_v2	TCAACCGGCTGCACGACGGGACCGTGCACGACTGGTGAGACCCCATCGGCCCAGATCATC	
YQ84-180PTM_ds_f	TCACCAGTCGTGCACGGTCCC	PCR downstream homology region (ds) for Δ*ftdA-F*
YQ85-180PTM_ds_r	GTCGCTGTACCGGGGCGCGTA	
YQ38-180PTM_us_f1153	TCTCCGGTCACCGSGTCGAA	verify Δ*ftdA-F* plasmid and mutant
YQ42-180PTM_ds_r1155	GRGARCTGGCSGTCGTCAGC	
YQ113-180PTM_us_f326	ACTAAGGAATGTCCCGCCCAG	
YQ114-180PTM_ds_r203	CACGCCTACACCTTCACCGAC	
YQ268-pUC19-us	TCTAGAGGATCCCCGGGTAC	amplify pUC19 for gibson assembly
YQ269-pUC19-ds	AAGCTTGGCGTAATCATGGTC	
YQ211-180DPro_us_f	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGGGCCGAAGAACTTGTACG	PCR us for JV180 P_{ftdA} replacement
YQ265-Dpro_us_r_v2	CACCCCGCGGTTCCCCGTCGAG	
YQ213-180DPro_ds_f	ATGAACGAGCGCGAACCATC	PCR ds for JV180 P_{ftdA} replacement
YQ214-180DPro_ds_r	TCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTGGTTGTTCACGTCGGCCATG	
YQ215-tsr_f	GGCCGGTGGAGGGCTCCGGCGAAGGGCTCTGGCGAAGCTCATCAAGGCGAATACTTCATATGC	PCR *tsr* for JV180 P_{ftdA} replacement plasmid
YQ216-tsr_r	AATGGTTTTCGCCATCGGCGGATGGTTCGCGCTCGTTCATCGAGGAACAGAGGCGCTTATC	
YQ217-tsr_int_f	TCATTCGGGACAGCGGTATG	verify Δ P_{ftdA}::*tsr* replacement
YQ218-tsr_int_r	GACCTCGATGAACTCCACCC	

YQ219-180DPro_us_out	CCATCATCTCGTAGGGCAGC	verify JV180 P_{ftdA} replacement
YQ36-180PTM_us_f738	GGACGTCGTACGACAAGGRGG	
YQ221-180DPro_ds_out	GAAGCCGTCGATGTATCCGC	
YQ222-180DPro_ds_int	AGCAGAAGAACGGGATAGGC	
YQ229-Dpro_D500_f	GGAACTGTGCGCGCACCGCGCGGACGTCGTACGACAAACTAGTCCGTCGGGCCGGTGGAG	PCR truncated JV180 P_{ftdA}
YQ232-Dpro_D400_f	GAGAGAACTAGTTGCGTGCGCCTCCGCGCTC	
YQ234-Dpro_D250_f	GAGAGAACTAGTCGGATATTTTGATGAGCAAG	
YQ237-Dpro_ds_r	ACCGCTACGAGGAGCTGTG	
YQ260-Dpro_25123_f_v2	GGCGCGGGCCGGGGGCGGCTCGACGGGGAACCGCGGGGTGACTAGTCCGTCGGGCCGGTGGAGGGCTC	PCR JV251-JV253 P_{ftdA} for JV180 P_{ftdA} replacement
YQ231-DPro_25123_r	AATGGTTTTCGCCATCGGCGGATGGTTCGCGCTCGTTCATCGGTGACCCTCCACGAGAGT	
YQ258-DPro_ermEh_f_v2	GGCGCGGGCCGGGGGCGGCTCGACGGGGAACCGCGGGGTGACTAGTAGCCCGACCCGAGCACGC	PCR P_{ermE*} for JV180 P_{ftdA} replacement
YQ243-DPro_ermEh_r	AATGGTTTTCGCCATCGGCGGATGGTTCGCGCTCGTTCATATGGGTCCTCCTGTGGAGTG	
YQ261-DPro_254_f_v2	GGCGCGGGCCGGGGGCGGCTCGACGGGGAACCGCGGGGTGACTAGTGGCGGAAGGAGCCCGGAGTC	PCR JV254 P_{ftdA} for JV180 P_{ftdA} replacement
YQ252-DPro_254_r	ATGGTTTTCGCCATCGGCGGATGGTTCGCGCTCGTTCATAGGTGACCCCCACAAGGGAAC	
YQ262-DPro_255_f_v2	GGCGCGGGCCGGGGGCGGCTCGACGGGGAACCGCGGGGTGACTAGTGGGCGGAGCCCTCCGGCGGAAG	PCR JV255 P_{ftdA} for JV180 P_{ftdA} replacement
YQ254-DPro_255_r	ATGGTTTTCGCCATCGGCGGATGGTTCGCGCTCGTTCATACGTGACCCCCACAAGGGAAC	
YQ263-DPro_2568_f_v2	GGCGCGGGCCGGGGGCGGCTCGACGGGGAACCGCGGGGTGACTAGTAGGCGTGGGGCCGGTCGGG	PCR JV256, JV258 P_{ftdA} for JV180 P_{ftdA} replacement
YQ256-DPro_2568_r	ATGGTTTTCGCCATCGGCGGATGGTTCGCGCTCGTTCATCCGTGACCCTCCGCAAGTGAC	
YQ259-DPro_13350_f_v2	GGCGCGGGCCGGGGGCGGCTCGACGGGGAACCGCGGGGTGACTAGTACTCCCCGGCCCGGGAGGCG	PCR IFO13350 P_{ftdA} for JV180 P_{ftdA} replacement
YQ264-DPro_257_f_v2	GGCGCGGGCCGGGGGCGGCTCGACGGGGAACCGCGGGGTGACTAGTCACTTGCCGGCACGGGAGGC	PCR JV257 P_{ftdA} for JV180 P_{ftdA} replacement
YQ249-DPro_13350_r	AATGGTTTTCGCCATCGGCGGATGGTTCGCGCTCGTTCATCCGTGACCCTCCACAAGTG	PCR IFO13350 and JV257 P_{ftdA} for JV180 P_{ftdA} replacement
YQ238-Dpro_DAG_r	TTCGGATCATTCCGGCCGTGACGTGAACCGATGCCCCCGACCCCATCGGCCCAGATCATC	PCR P_{ftdA} ΔAG for P_{ftdA} editing plasmid
YQ389-180PTM-dAG_CT-f	GGGCATCGGTTCACGTCACG	PCR P_{ftdA} ΔAG::CT for P_{ftdA} editing plasmid
YQ390-180PTM-dAG_CT-r	CTTCGGATCATTCCGGCCGTGACGTGAACCGATGCCCCCAGGACCCCATCGGCCCAGATC	
YQ239-Δ29_48_r	GCACCACACTGACCGATTCCCGACGCCCGGCTTCGGATCCGATGCCCCCCTGACCCCATC	PCR P_{ftdA} Δ29_48 for P_{ftdA} editing plasmid
YQ240-Δ29_48_13350_r	ACTGACCGATTCCCGACGCCCGGCTTCGGATCAATGCGGCCGTGGCGTGAACCGATGCCC	PCR P_{ftdA} Δ29_48::13350 for P_{ftdA} editing plasmid

YQ67-XylE-f	ATGAACAAAGGTGTAATGCG	Amplification of *xylE*, *xylE* reporter plasmid
xylE-REV	CAGTGAGCTGTTAACCAGGTGAGCACGGTCATGAATCG	Amplification of *xylE*
YQ177-pXylE-rv	TCGTCTTTCCAGACGTTAG	Amplification of *xylE* reporter plasmid
YQ123-pXylE-MCS-f	TCTCACTCCGCTGAAACTGT	checking clone into *xylE* reporter plasmid
YQ371-PXS6	GGCCGATTCATTAATGCAGC	
YQ50-P180-overlap-r	ATGGCCCGGTCGCATTACACCTTTGTTCATCGGTGACCCTCCACGAGAGTTC	PCR JV180 P_{ftdA} for *xylE* reporter plasmid
YQ51-P180-overlap-f	GAGGCGCGCCGAGAAGGGTTCTGGCGAAGCTC	
YQ69-P13350-overlap-r	ATGGCCCGGTCGCATTACACCTTTGTTCATCCGTGACCCTCCACAAGTG	PCR IFO13350 P_{ftdA} for *xylE* reporter plasmid
YQ70-P13350-overlap-f	GAGGCGCGCCGAGGCTTCGGGGGCGCTATTC	
YQ501-P180-ftdA-xylE-f	CATACAGAAAATTCATTTACTAACGTCTGGAAAGACGACCGTCGGGCCGGTGGAGGGCTC	PCR JV180 P_{ftdA}-*ftdA* for *xylE* reporter plasmid
YQ503-P13350-ftdA-xylE-f	CATACAGAAAATTCATTTACTAACGTCTGGAAAGACGAACTCCCCGGCCCGGGAGGCG	PCR IFO13350 P_{ftdA}-*ftdA* for *xylE* reporter plasmid
YQ502-PftdA-ftdA-xylE-r	ACGCAGCTGCACATGGCCCGGTCGCATTACACCTTTGTTCATGCGAACTCCTAGATCGTC	PCR P_{ftdA}-*ftdA* for *xylE* reporter plasmid
YQ495-xylE-q-507	CAAGGTGCTCGGTTTCTATCT	qPCR primers for *xylE*
YQ496-xylE-q-625	TTCCGGATGGTGAATGAAGG	
YQ178-WNp213	GTCTCGTTAGCTCGCTGGATCCTA/3InvdT/	cRACE - adapter
YQ179-WNp210	TAGGATCCAGCGAGCTAACGAGAC	cRACE - forward primer
YQ185-180PTM_r_-115	GATAGGCCAGGAAACGGAGG	cRACE - nested reverse primers
YQ186-180PTM_r_-227	AGAGCGAGATAGGAGATGACC	
YQ188-180PTM_r_-46	CGGCCGAATGGTTTTCGCCATCGG	
YQ193-180FtdA_f_754	CAGCACCGATCTGTCCGAAG	*ftdA-B* junction PCR
YQ196-180FtdB_r_234	GAAGCCGTCGATGTATCCGC	
YQ203-180FtdB_f_203	AGGTCACCGTCATCACCGAC	*ftdB-C* junction PCR
YQ204-180FtdC_r_146	TCGAAGATCCGGGTCCTGG	
YQ205-180FtdC_f_134	AGTACCTCTGCCGTGAACTC	*ftdC-D* junction PCR
YQ206-180FtdD_r_254	CAGATCTGGTGCATCTCGTTG	
YQ207-180FtdD_f_244	GGAACTGATCAAGAAGCACGG	*ftdD-E* junction PCR
YQ208-180FtdE_r_218	ACCACCTCCATGATCGAGTC	
YQ209-180FtdE_f_113	AGATCACCTTCGACCACACC	*ftdE-F* junction PCR
YQ210-180FtdF_r_249	CTGGTTGAACATGAGGTCGG	
YQ180-DadpA_u_f_x	ACTAGTGATCTAGAGCGTCTGG	us for JV180 Δ*adpA* plasmid

YQ189-DadpA_us_r	CCAGCTCAATGTCGATAAGGG	
YQ190-DadpA_d_f_o	GAACGCTTCACGCCACGTCCCCTTATCGACATTGAGCTGGGAGGCGTTCGTTCCCGGAC	ds for JV180 Δ*adpA* plasmid
YQ169-DadpA_d_r_h	ACTAGTGAAAGCTTGTTCTACGC	
YQ117-adpA_seq_f_int	TCGAACTCACCGCGCCGTACG	verify Δ*adpA* plasmid and mutant
YQ118-adpA_seq_r_int	TCGACGTGCACCGACGGATAG	
YQ164-DadpA_out_f	ATGGACGATGATCAGACCGG	
YQ165-DadpA_out_r	TTCCGGTACTACGTCACCAAG	
YQ119-AdpA_fw_n	ACTAGTGACATATGAGCCAGGACTCCGCC	clone *adpA* into pJMD3
YQ120-AdpA_rv1_x	ACTAGTGATCTAGACTACGGGGCGCTCCGCTGTC	clone *adpA*_JV180 into pJMD3
YQ121-AdpA_rv2_x	ACTAGTGATCTAGACTACGGGGCACTCCGCTGTC	clone *adpA*_IFO13350 into pJMD3
YQ225-adpA_f_x	ACTAGTGATCTAGAGTGACCGGATTCAGCACAC	clone *adpA* into pSET152
YQ226-adpA_r_b	ACTAGTGAGGATCCAGTCGATCCACACCATGCG	
YQ191-Dgrh_us_f_x_4	ACTAGTGATCTAGAGTGGACATCGAAATACTGGG	PCR us for JV180 Δ*grhR2-V* plasmid
YQ183-Dgrh_us_r_3	GTTCGGACGTCATGCGCAC	
YQ184-Dgrh_ds_f_o_2	GATCACCGCAGGTGGGAAGGGGTGCGCATGACGTCCGAACCCCCGGAGAGAAGGAGCAAG	PCR ds for JV180 Δ*grhR2-V* plasmid
YQ163-Dgrh_ds_r_h	ACTAGTGAAAGCTTCGAAGATCATCCAGAACGCG	
YQ146-grh_ds_seq_f	GTTCGAGATCTACCGGGTGC	verify Δ*grhR2-V* plasmid and mutant
YQ147-grh_ds_seq_r	CATCCCGAAGAAGCTGAACC	
YQ152-grh_ds_seq_f_2	GAGAACACCATGGCCAAGAG	
YQ153-grh_ds_seq_r_2	CCTGGCACCTCGGCTTCG	
YQ166-Dgrh_int_f	CCAGTTCATCATCGACCACC	
YQ167-Dgrh_out_f	TTCGGACCAGTCGATGATGC	
YQ168-Dgrh_out_r	CATGACGACGAAGCTCAGCC	
YQ244-grhR2-f-n	ACTAGTGACATATGAGGATCCGGGTTCTGGG	clone JV180 *grhR2*
YQ245-grhR2-r-x	ACTAGTGATCTAGAGGGACGCACGTTCATGGCAC	
YQ246-grhR3-f-n	ACTAGTGACATATGGACCCGCTCGACGCGGTG	clone JV180 *grhR3*
YQ247-grhR3-r-x	ACTAGTGATCTAGAGGTCAGGCGTCGAGGGCGCC	
YQ286-grhF-f-n	ACTAGTGACATATGCCGCGCCTCGCGCCGC	clone JV180 *grhF*
YQ287-grhF-r-x	ACTAGTGATCTAGATCAAGGGCGGTCGGAAGAAG	
YQ288-grhG-f-n	ACTAGTGACATATGACCACCGAGACCGCCG	clone JV180 *grhG*
YQ289-grhG-r-x	ACTAGTGATCTAGATCGTCGTCGGTGTCATCGTC	
YQ294-grhH-f-n	ACTAGTGACATATGACACCGACGACGACCGG	clone JV180 *grhH*

YQ295-grhH-r-x	ACTAGTGATCTAGATCATCTGCCGCGCCACGTAC	
YQ298-grhG-r-int	TACGGGCGTTTGTTCTCCAG	verify JV180 *grhG* clone
YQ351-180accB-f	ACTAGTGACATATGACCGTTGTGGACGAAAC	clone JV180 *accBE*
YQ354-180accE-r	ACTAGTGATCTAGATCTCAGCCCTGCCAGCTGTG	
YQ355-13350accB-f	ACTAGTGACATATGACCGTTGTGGACGAAAC	clone SGR1943-44 (IFO13350 *accB*)
YQ358-13350accE-r	ACTAGTGATCTAGATGGTCAGCCCTGCCAGCTGTG	
YQ359-3281-f	ACTAGTGACATATGCATGACCGGGTGGGCGAAC	clone SGR3280-3281
YQ362-3280-r	ACTAGTGATCTAGACTAGCTGCCGGGCACGGGCTC	
YQ363-180accB-int	CAGATGTTCATCACCGGCCC	verify JV180 *accBE* clone
YQ364-13350accB-int	CCAGAAGGGTGATGATCGGG	verify SGR1943-44 clone
YQ365-13350grhG-int	AGCGGAATGTTGAAGGCGTC	verify SGR3280-3281 clone
YQ372-grihrdBF	CACCAAGGGCTACAAGTTCT	qPCR primers for hrdB, from Claesen and Bibb(12)
YQ373-grihrdBR	CGAGCTTGTTGATGACCTC	
YQ376-180ftdB1153	ATCAACCTGGAGAACCTCAAC	qPCR primers for *ftdB*
YQ377-180ftdB1278	GAAGCCGAAGGAGTTGACC	
YQ466-grhGHdel-u-f	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGAGCTG GACCTCGCCTACC	PCR us for JV180 Δ*grhGH* plasmid
YQ467-grhGHdel-u-r	GTCGGATGCGGCGGCGGTCTC	
YQ469-grhGHdel-d-r	TCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTCGGGCGT CTACTTGTCGATG	PCR ds for JV180 Δ*grhGH* plasmid
YQ473-grhGHdel-d-f	AGGAGATCGCGTGACCACCGAGACCGCCGCCGCATCCGACCCCTCCC CCGGTACGTGGCG	
YQ477-grhGHdel-f-i	TTCTTCCGACCGCCCTTGAG	verify JV180 Δ*grhGH* plasmid and mutant
YQ478-grhGHdel-f-o	GAACCAGCTCCACCGGCAG	
YQ480-grhGHdel-r-o	GCGGAGGCGTGGTTCATCC	
YQ483-grhGHdel-r-i	CCGATGTTCTTGGCGTTGAC	
YQ485-grhQdel-u-f	GCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGATCTCCCTCC TGCGGTTCTTC	PCR us for JV180 Δ*grhQSAB* plasmid
YQ486-grhQdel-u-r	CTCGGGCGGCTCAGGCGGTGTGGTCGGTTCGGTCGCCCCCCATCCTG GCCACGATCAGGG	
YQ487-grhBdel-d-f	GGGGGCGACCGAACCGACCAC	PCR ds for JV180 Δ*grhQSAB* plasmid
YQ488-grhBdel-d-r	TCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTTGGAGCC GAGGTTGACGATC	
YQ489-grhQdel-f-i	AGACAGCTTTGAGAACCGCC	verify JV180 Δ*grhQSAB* plasmid and mutant
YQ490-grhQdel-f-o	TTCTCGGTCAACTTCACGGG	
YQ491-grhBdel-r-i	CATCAGAATCGGGCCTCCTC	
YQ492-grhBdel-r-o	GATGTAGGAGGACTGGTCGC	

CM02-PftdA500bp-F	CGGCTCGACGGGGAACCGCGGGGTGCGTCGTGCCGGTGGAGGGCTCCGGCGA	clone P_{ftdA} from strain SP18CM02 to construct pKN038
CM02-PftdA500bp-R	TCGGCGGATGGTTCGCGCTCGTTCATCGGTGACCCTCCACGAGAGTTCCTG	
2-28-us-F	GTTGTAAAACGACGGCCAGTGCCAGTCGAAGTCGGCCATGCGCACGT	PCR us for pKN040
2-28-us-F	CGGATCATTCCGGCCGTGACGTGAACGATCATCTATTTCCCGATCGCCAGGCT	
2-28-ds-F	AGCCTGGCGATCGGGAAATAGATGATCGTTCACGTCACGGCCGGAATGATCCG	PCR ds for pKN040
2-28-ds-F	CGATATCGCGCGCGGCCGCGGATCAGGTGAAGCTGCCGAAGAGGT	
49_68-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN015
49_68-us-R	GGTCAGGGGCACCACACTGACCGATTCATTCCGGCCGTGACGTGAACCGAT	
49_68-ds-F	GCATCGGTTCACGTCACGGCCGGAATGAATCGGTCAGTGTGGTGCCCCTGACCG	PCR ds for pKN015
49_68-ds-R	ATATCGCGCGCGGCCGCGGATCCGTGATGCAGTCCTTGCCCGCCACCAG	
69_88-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN012
69_88-us-R	AGACGATGTCCGGCACGGCGGTCAGGCCGACGCCCGGCTTCGGATCATTCCGG	
69_88-ds-F	CCGGAATGATCCGAAGCCGGGCGTCGGCCTGACCGCCGTGCCGGACATCGTCTCT	PCR ds for pKN012
69_88-ds-R	ATATCGCGCGCGGCCGCGGATCCGTGATGCAGTCCTTGCCCGCCACCAG	
89_108-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN016
89_108-us-R	GGTCATACCTGTTGGTGAGAGACGAGGCACCACACTGACCGATTCCCGA	
89_108-ds-F	GTCGGGAATCGGTCAGTGTGGTGCCTCGTCTCTCACCAACAGGTATGACCT	PCR ds for pKN016
89_108-ds-R	ATATCGCGCGCGGCCGCGGATCCGTGATGCAGTCCTTGCCCGCCACCAG	
109_128-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN018
109_128-us-R	CCGCCGCATCGCGTCACCGCAGGTCATGTCCGGCACGGCGGTCAGGGGCA	
109_128-ds-F	TGCCCCTGACCGCCGTGCCGGACATGACCTGCGGTGACGCGATGCGGC	PCR ds for pKN018
109_128-ds-R	ATATCGCGCGCGGCCGCGGATCCGTGATGCAGTCCTTGCCCGCCACCAG	
129_148-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN017
129_148-us-R	TCCTGCCTGGGGGAGAGAGGCCGCCGTACCTGTTGGTGAGAGACGATGTCCGG	
129_148-ds-F	GACATCGTCTCTCACCAACAGGTACGGCGGCCTCTCTCCCCCAGGCAGGA	PCR ds for pKN017
129_148-ds-R	ATATCGCGCGCGGCCGCGGATCCGTGATGCAGTCCTTGCCCGCCACCAG	
149_181-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN019
149_181-us-R	CGCTCGTTCATCGGTGACCCTCCACATCGCGTCACCGCAGGTCATACCTGT	
149_181-ds-F	AGGTATGACCTGCGGTGACGCGATGTGGAGGGTCACCGATGAACGAGCGCG	PCR ds for pKN019
149_181-ds-R	ATATCGCGCGCGGCCGCGGATCCGTGATGCAGTCCTTGCCCGCCACCAG	
-528_-31-us-F	GTTGTAAAACGACGGCCAGTGCCAACGCGGACAGTCCCACGCGCA	PCR us for pKN028

-528_-31-us-R	TATTTCCCGATCGCCAGGCTTTAATTGTCGTACGACGTCCGCGCGGTG	
-528_-31-ds-F	CACCGCGCGGACGTCGTACGACAATTAAAGCCTGGCGATCGGGAAATA	PCR ds for pKN028
-528_-31-ds-F	CGATATCGCGCGCGGCCGCGGATCTCATTCCGGGCGGGTGGTGCCCGGT	
-528_-38-us-F	GTTGTAAAACGACGGCCAGTGCCAACGCTCACCCCTTCGAGGCCGCCCA	PCR us for pKN031
-528_-38-us-R	ATTTCCCGATCGCCAGGCTTTAACCACCACTTGTCGTACGACGTCCGCGCGGTGCGC	
-528_-38-ds-F	GCGCACCGCGCGGACGTCGTACGACAAGTGGTGGTTAAAGCCTGGCGATCGGGAAAT	PCR ds for pKN031
-528_-38-ds-F	CGATATCGCGCGCGGCCGCGGATCGCCGGTGCTGCTCGGGACTGGCCA	
-528_-31seq-us-F	AAAATACCGCATCAGGCGCCATTC	Sequencing primers for pKN028 and pKN038
-528_-31seq-us-R	CAACAGGTATGACCTGCGGT	
-528_-31seq-ds-F	ATCTCCTCCGCGTTGACCTC	
-528_-31seq-ds-R	AAAGCGGGCAGTGAGCGCAAC	
-528_-31mut-F	TGCGCGAGAAGTCGAAGGTGAG	verify P_{ftdA} Δ(−528_−31) and (−528_−38) mutants
-528_-31mut-R	ATCCCATCCCAGGCGTAATG	
-35JV254-us-F	GTTGTAAAACGACGGCCAGTGCCAACGCGGACAGTCCCACGCGCA	PCR us for pKN047
-35JV254-us-R	TATTTCCCGATCGCCAGGCTTTAATCACGCCTTGTCGTACGACGTCCGCGCGGTG	
-35JV254-ds-F	CACCGCGCGGACGTCGTACGACAAGGCGTGATTAAAGCCTGGCGATCGGGAAATA	PCR ds for pKN047
-35JV254-ds-R	CGATATCGCGCGCGGCCGCGGATCTCATTCCGGGCGGGTGGTGCCCGGT	
-35JV255-us-F	GTTGTAAAACGACGGCCAGTGCCAACGCGGACAGTCCCACGCGCA	PCR us for pKN048
-35JV255-us-R	TATTTCCCGATCGCCAGGCTTTAACCACGCCTTGTCGTACGACGTCCGCGCGGTG	
-35JV255-ds-F	CACCGCGCGGACGTCGTACGACAAGGCGTGGTTAAAGCCTGGCGATCGGGAAATA	PCR ds for pKN048
-35JV255-ds-R	CGATATCGCGCGCGGCCGCGGATCTCATTCCGGGCGGGTGGTGCCCGGT	
-35JV256-us-F	GTTGTAAAACGACGGCCAGTGCCAACGCGGACAGTCCCACGCGCA	PCR us for pKN049
-35JV256-us-R	TATTTCCCGATCGCCAGGCTTTAATCACGACTTGTCGTACGACGTCCGCGCGGTG	
-35JV256-ds-F	CACCGCGCGGACGTCGTACGACAAGTCGTGATTAAAGCCTGGCGATCGGGAAATA	PCR ds for pKN049
-35JV256-ds-R	CGATATCGCGCGCGGCCGCGGATCTCATTCCGGGCGGGTGGTGCCCGGT	
-35JV257-us-F	GTTGTAAAACGACGGCCAGTGCCAACGCGGACAGTCCCACGCGCA	PCR us for pKN050
-35JV257-us-R	TATTTCCCGATCGCCAGGCTTTAATCACGGCTTGTCGTACGACGTCCGCGCGGTG	
-35JV257-ds-F	CACCGCGCGGACGTCGTACGACAAGCCGTGATTAAAGCCTGGCGATCGGGAAATA	PCR ds for pKN050
-35JV257-ds-R	CGATATCGCGCGCGGCCGCGGATCTCATTCCGGGCGGGTGGTGCCCGGT	
Seq-35box_JV1836-F	AGGACGATCTCCTCCGCG	verify double crossover for −35 box mutants
Seq-35box_JV1836-F	GAGGAGGCGAACCGATTGT	
G35T-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN001

G35T-us-R	TCATTCCGGCCGTGAAGTGAACCGATGCCCCCCTGACCC	
G35T-ds-F	GGGCATCGGTTCACTTCACGGCCGGAATGATCCGAAG	PCR ds for pKN001
G35T-ds-R	ATATCGCGCGCGGCCGCGGATCGGAGGCCGACGGCGGTGGGCT	
C37A-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN003
C37A-us-R	GATCATTCCGGCCGTTACGTGAACCGATGCCCCCCTGACC	
C37A-ds-F	GCATCGGTTCACGTAACGGCCGGAATGATCCGAAGCCGG	PCR ds for pKN003
C37A-ds-R	ATATCGCGCGCGGCCGCGGATCGGAGGCCGACGGCGGTGGGCT	
G40T-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN002
G40T-us-R	TCGGATCATTCCGGCAGTGACGTGAACCGATGCCCCCCT	
G40T-ds-F	TCGGTTCACGTCACTGCCGGAATGATCCGAAGCCGGGCG	PCR ds for pKN002
G40T-ds-R	ATATCGCGCGCGGCCGCGGATCGGAGGCCGACGGCGGTGGGCT	
C42A-us-F	TAAAACGACGGCCAGTGCCATCACCGCGACCACCCGGGTCCG	PCR us for pKN004
C42A-us-R	CTTCGGATCATTCCGTCCGTGACGTGAACCGATGCCCCC	
C42A-ds-F	GGTTCACGTCACGGACGGAATGATCCGAAGCCGGGCGTC	PCR ds for pKN004
C42A-ds-F	ATATCGCGCGCGGCCGCGGATCGGAGGCCGACGGCGGTGGGCT	
Seq-AdpA-us-F	AAAATACCGCATCAGGCGCCATTC	Sequencing primers for pKN001-004
Seq-AdpA-us-R	ACTGACCCAGGCCGGATCCCAT	
Seq-AdpA-ds-F	TAATCCTACTAAGGAATGTCC	
Seq-AdpA-ds-R	AAAGCGGGCAGTGAGCGCAAC	
AdpAMT-R	ATGCTTCCGGCTCGTATGTTG	verify double cross-over for AdpA binding site mutants
AdpAMT-R1	CAGGACAGGTCGGAGCCGTA	
AdpAMT35-F	GTTCACTTCACGGCCGGAAT	verify double cross-over for G35T mutant
AdpAMT37-F	GTTCACGTAACGGCCGGAAT	verify double cross-over for C37A mutant
AdpAMT40-F	GTTCACGTCACTGCCGGAAT	verify double cross-over for G40T mutant
AdpAMT42-F	GTTCACGTCACGGACGGAAT	verify double cross-over for C42A mutant
AdpAop-seq-F1	CTCGACGGGGAACCGCGGGGT	amplify AdpA binding site to sequence binding site mutations
AdpAop-seq-R1	ACCCAGGCCGGATCCCATCCCAG	
grhR1-grhE-us-F	GTTGTAAAACGACGGCCAGTGCCAAAGGGGGTGCGGCCCGTCGAGCGGGA	PCR us for pKN027
grhR1-grhE-us-R	GAAGGGGCGCACGGGCGTCCGCCGTCTGCGCTCCACCCGCGGTCGATACGGGT	
grhR1-grhE-ds-F	ACCCGTATCGACCGCGGGTGGAGCGCAGACGGCGGACGCCCGTGCGCCCCTTC	PCR ds for pKN027
grhR1-grhE-ds-R	CGATATCGCGCGCGGCCGCGGATCAGCGCCCGGTGGGCGCCGAAGGTCCA	
grhR1-grhE-seq-us-F	AAAATACCGCATCAGGCGCCATTC	Sequencing primers for pKN027
grhR1-grhE-seq-us-R	TCGGAGGGTGCTGGGATGG	
grhR1-grhE-seq-ds-F	CCATCCCAGCACCCTCCGA	
grhR1-grhE-seq-ds-R	AAAGCGGGCAGTGAGCGCAAC	
grhR1-E-mut-F1	TGCACGGCCGCTTACCGGAAG	Primers PCR used to verify Δ*grhR1-E*
grhR1-E-mut-R1	CTGCGTGTACGACCTCCAGCAT	
grhR1-E-mut-F2	TCGTCTACGGACTGGTCGCG	

grhR1-E-mut-R2	CTCCGTACGCGTTTCCGTGT	
grhR1-E-mut-F3	ACTCGGTGTTCCACCGGTG	
grhR1-E-mut-R3	CGGCGGTCTCAGGGATGT	
grhR1-E-mut-F4	TGCTGTGGGCGCTGACCG	
grhR1-E-mut-R4	TTCGGCGTCCACCCCGATC	
grhF-grhH-us-F	GTTGTAAAACGACGGCCAGTGCCAACGGCCTTCCACCACACGTCCCACCGT	PCR us for pKN029
grhF-grhH-us-R	TGGCGCGGCGGCCCGGCCGGGCGGGGGCGTCCGCCGTTCAGGGCTTCG	
grhF-grhH-ds-F	CGAAGCCCTGAACGGCGGACGCCCCCGCCCGGCCGGGCCGCCGCGCCA	PCR ds for pKN029
grhF-grhH-ds-R	CGATATCGCGCGCGGCCGCGGATCTCCGGTCGCTCGCCACCCCGAACT	
grhF-grhH-seq-us-F	AAAATACCGCATCAGGCGCCATTC	Sequencing primer for pKN029
grhF-grhH-seq-us-R	ACGCGTACGGAGTCGGGCAC	
grhF-grhH-seq-ds-F	GTGCCCGACTCCGTACGCGT	
grhF-grhH-seq-ds-R	AAAGCGGGCAGTGAGCGCAAC	
grhF-H-mut-F1	AGACCGACCCGGCGGAGC	Primers used to verify Δ*grhF-H*
grhF-H-mut-R1	TCGTGGCGGGCCACGCCG	
grhF-H-mut-F2	GTCTCGCTGGCCTGGCCGA	
grhF-H-mut-R2	TGCCAGCCGGTGCCGGTG	
grhF-H-mut-F3	TACGCGCAGAGCGCCGTG	
grhF-H-mut-R3	CGGTCGTCGGAGACGAACT	
grhI-grhP-us-F	GTTGTAAAACGACGGCCAGTGCCATCGATCCCGCTGGTGACCCTGGTCGA	PCR us for pKN030
grhI-grhP-us-R	ACCGGCCTGGACCGGTCTCCGGCGGCCGGTGGGTCTCTCCTGACGTGGGTCCGAG	
grhI-grhP-ds-F	CTCGGACCCACGTCAGGAGAGACCCACCGGCCGCCGGAGACCGGTCCAGGCCGGT	PCR ds for pKN030
grhI-grhP-ds-R	CGATATCGCGCGCGGCCGCGGATCGGCGACCTGCGAGCGGAACGCCGACGCGT	
grhI-grhP-seq-us-F	AAAATACCGCATCAGGCGCCATTC	Sequencing primers for pKN030
grhI-grhP-seq-us-R	AGATGACCGCCCGGCCGGGC	
grhI-grhP-seq-ds-F	GCCCGGCCGGGCGGTCATCT	
grhI-grhP-seq-ds-R	AAAGCGGGCAGTGAGCGCAAC	
grhI-P-mut-F1	ACCGAGCTGGAGGACGAC	Primers used to verify Δ*grhI-P*
grhI-P-mut-R1	AGGGTCTCGAAGGCGGTCA	
grhI-P-mut-F2	ACCTCTTCGCCTGGGTGACC	
grhI-P-mut-R2	GCCGAAGACCACCATGTAGA	
grhI-P-mut-F3	TCCGCGCCGAACGGGACG	
grhI-P-mut-R3	GATCGGGACCAGCAGCGAGT	
pET11a-AdpA-F	TATCATATGAGCCAGGACTCCGCCACC	clone *adpA* for pKN052

pET11a-AdpA-R	AATGCTAGCTCAGTGGTGGTGGTGGTGGTGCTCGAGCGGGGCGCTCCGCTGTCCCGG	
JV180EMSADS_sense	TTCGGATCATTCCGGCCGTGACGTGAACCGATGCC	JV180 P_{ftdA} AdpA binding site for EMSA
JV180EMSADS_anti	GGCATCGGTTCACGTCACGGCCGGAATGATCCGAA	
IFO13350EMSADS_sense	TTCGGATCAATGCGGCCGTGGCGTGAACCGATGCC	IFO13350 P_{ftdA} AdpA binding site for EMSA
IFO13350EMSADS_anti	GGCATCGGTTCACGCCACGGCCGCATTGATCCGAA	
JV180G35TEMSADSsense	GGCATCGGTTCACTTCACGGCCGGAATGATCCGAA	JV180 P_{ftdA} AdpA binding site G35T mutant for EMSA
JV180G35TEMSADSanti	TTCGGATCATTCCGGCCGTGAAGTGAACCGATGCC	
JV180C37AEMSADSsense	GGCATCGGTTCACGTAACGGCCGGAATGATCCGAA	JV180 P_{ftdA} AdpA binding site C37A mutant for EMSA
JV180C37AEMSADSanti	TTCGGATCATTCCGGCCGTTACGTGAACCGATGCC	
JV180G40TEMSADSsense	GGCATCGGTTCACGTCACTGCCGGAATGATCCGAA	JV180 P_{ftdA} AdpA binding site G40T mutant for EMSA
JV180G40TEMSADSanti	TTCGGATCATTCCGGCAGTGACGTGAACCGATGCC	
JV180C42AEMSADSsense	GGCATCGGTTCACGTCACGGACGGAATGATCCGAA	JV180 P_{ftdA} AdpA binding site C42A mutant for EMSA
JV180C42AEMSADSanti	TTCGGATCATTCCGTCCGTGACGTGAACCGATGCC	

Table S4. % Identity of 16S, concatenated MLST genes, and PTM enzyme amino acid sequences of the strains used in this study, relative to *Streptomyces* sp. strain JV180. Average Nucleotide Identity (ANI) values were calculated using the OrthoANIu algorithm (13).

Strain	16S	MLST[#]	FtdA	FtdB	FtdC	FtdD	FtdE	FtdF	ANI
Streptomyces sp. strain JV180	100	100	100	100	100	100	100	100	100
S. sp. strain SP18CM02	100	99.91	99.36	99.4	100	99.83	100	98.96	98.97
S. californicus strain NRRL B-3320 (JV251)	99.93	99.75	99.36	98.52	99.64	99.30	99.72	99.74	98.36
S. floridae strain NRRL 2423 (JV252)	99.79	99.72	99.68	98.74	99.82	99.65	99.15	99.48	98.63
S. puniceus strain NRRL B-2895 (JV253)	99.11	99.80	99.36	98.89	99.82	99.83	99.43	98.96	98.64
S. griseus subsp. griseus strain NRRL F-5144 (JV254)	99.79	97.37	83.44	87.74	91.34	91.30	91.17	87.21	89.03
S. globisporus subsp. globisporus strain NRRL B-2709 (JV255)	99.59	97.49	81.21	87.11	87.95	92.00	89.74	86.68	89.27
S. albus subsp. albus strain NRRL B-2445 (JV256)	99.59	97.19	81.37	88.64	93.04	92.32	91.74	87.21	88.86
S. griseus subsp. griseus strain NRRL WC-3480 (JV257)	99.73	97.74	82.48	88.01	91.96	92.86	91.45	87.99	88.53
S. baarnensis strain NRRL B-2842 (JV258)	99.59	97.14	81.37	88.55	93.04	92.32	91.74	87.21	88.87
S. griseus subsp. *griseus* strain IFO13350	99.73	97.70	83.44	88.11	92.14	93.03	91.17	87.99	88.62

- MLST: concatenated partial sequences of *atpD*, *gyrB*, *recA*, *rpoB*, and *trpB* corresponding to the sequences used in references (14, 15)

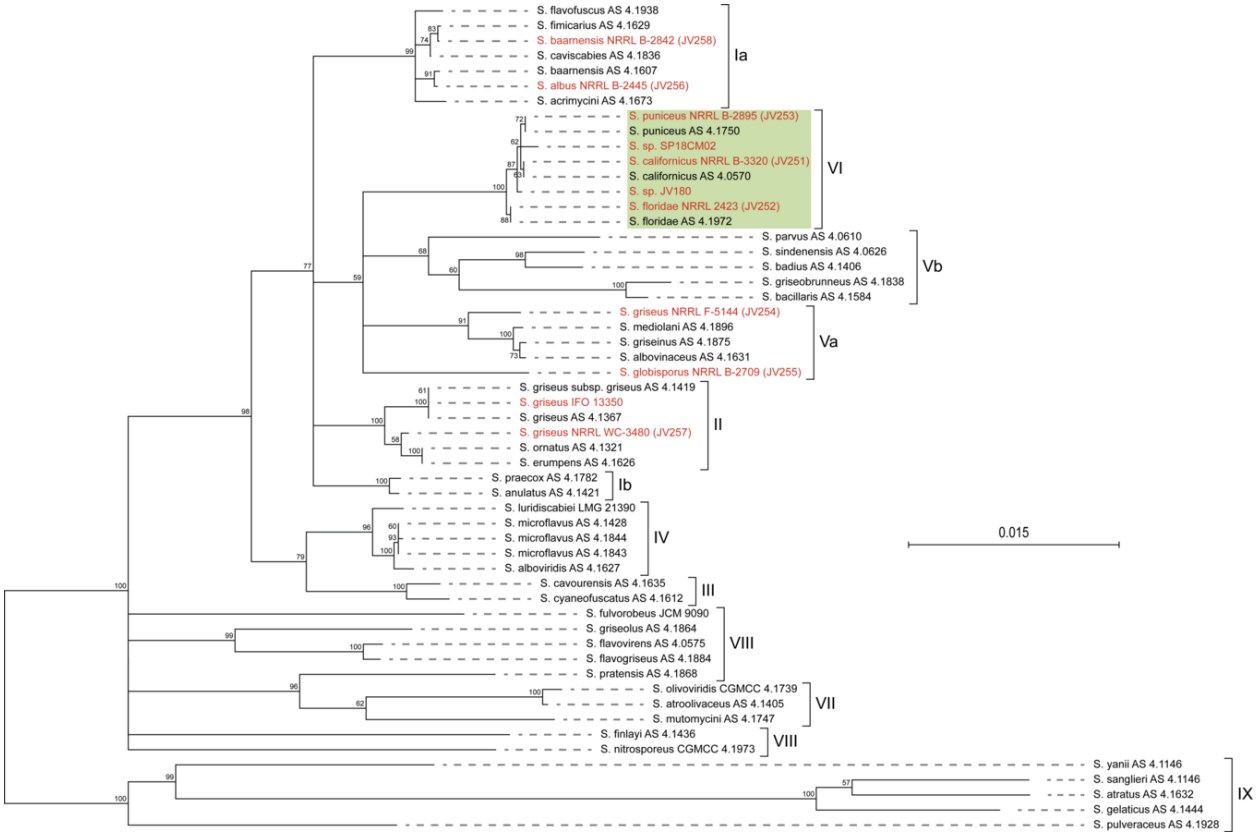


Figure S1. Maximum likelihood phylogenetic tree of selected *S. gris*eus clade strains. Strains used in this study are highlighted in red for clarity and all other strains are previously published members of the *S. griseus* clade (black). The strain JV180-like group is highlighted in green, which includes several apparent members of cluster VI as defined by Rong *et al* (14, 15). This MLST phylogeny was created from *atpD*, *gyrB*, *recA*, *rpoB*, and *trpB* sequences obtained from published lists (strains in black (14, 15)), in-house sequencing (JV180 and SP18CM02 (7)) or from GenBank (other strains in red). Sequences were aligned with MUSCLE and the alignments were concatenated. The maximum likelihood tree was built using the GTR+I+G model using CLC Main Workbench. Branches supported by less than 50% of 500 bootstrap simulations were collapsed.

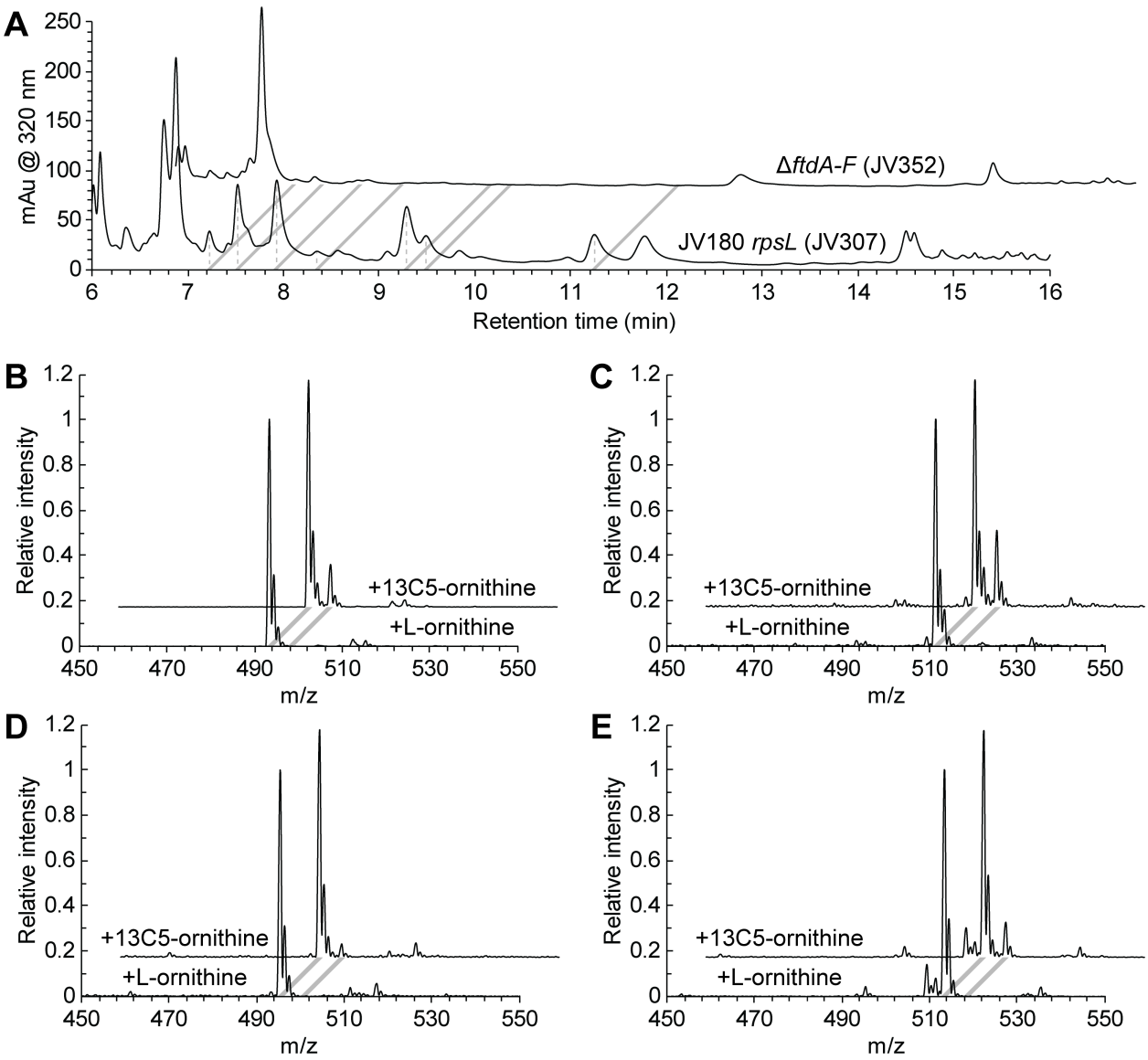


Figure S2. Genetic and metabolomic identification of PTMs produced by $^{13}C_5$-ornithine feeding. (**A**) LC-MS chromatograms of extracts from JV180 *rpsL* and Δ*ftdA-F* mutants. The retention times of major PTM peaks were marked in gray. (**B**) Mass spectra of clifednamide A produced by the known clifednamide producer *S. torulosus* strain NRRL B-3889(11) on media supplemented with 50 mg/L of L-ornithine or $^{13}C_5$-labeled L-ornithine. Mass spectra of selected PTM peaks produced by JV180 on media supplemented with 50 mg/L of L-ornithine or $^{13}C_5$-labeled L-ornithine, which eluted at (**C**) 8.4, (**D**) 11.2, and (**E**) 7.9 minutes.

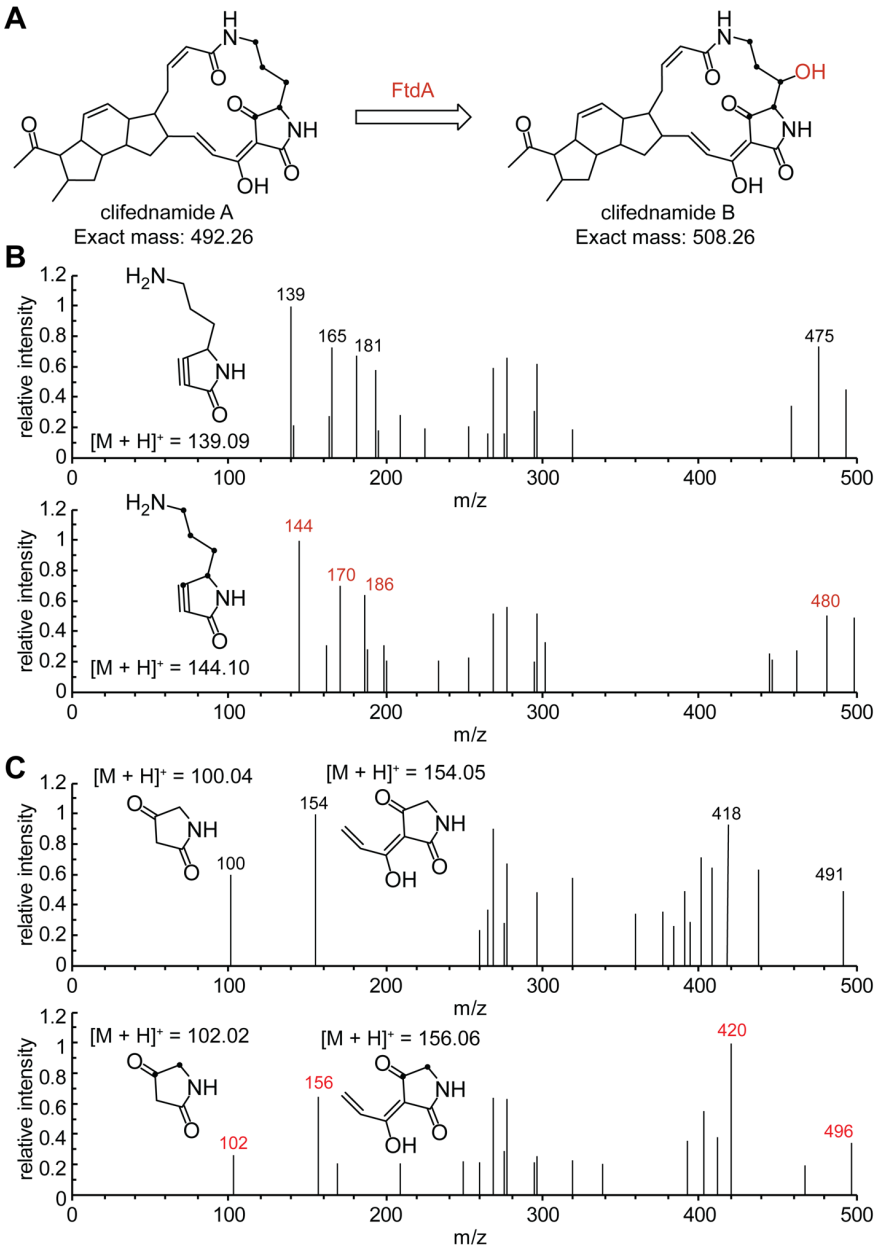


Figure S3. Tandem mass spectrometry-based identification and quantification of PTMs. (**A**) The structures of known clifednamides A and B, which differ by an FtdA-installed hydroxylation (shown in red). The product ion spectra of (**B**) clifednamide A and (**C**) clifednamide B produced by collision-induced dissociation at 20 V. Mass spectra were converted to centroids for visual clarity. *S. torulosus* strain NRRL B-3889 was grown on media supplemented with (top) L-ornithine or (bottom) $^{13}C_5$-labeled L-ornithine. Inset: proposed structures of ornithine-derived fragments, with $^{13}C_5$ atoms indicated by heavy dots, which would result in the heavier daughter ions observed (red).

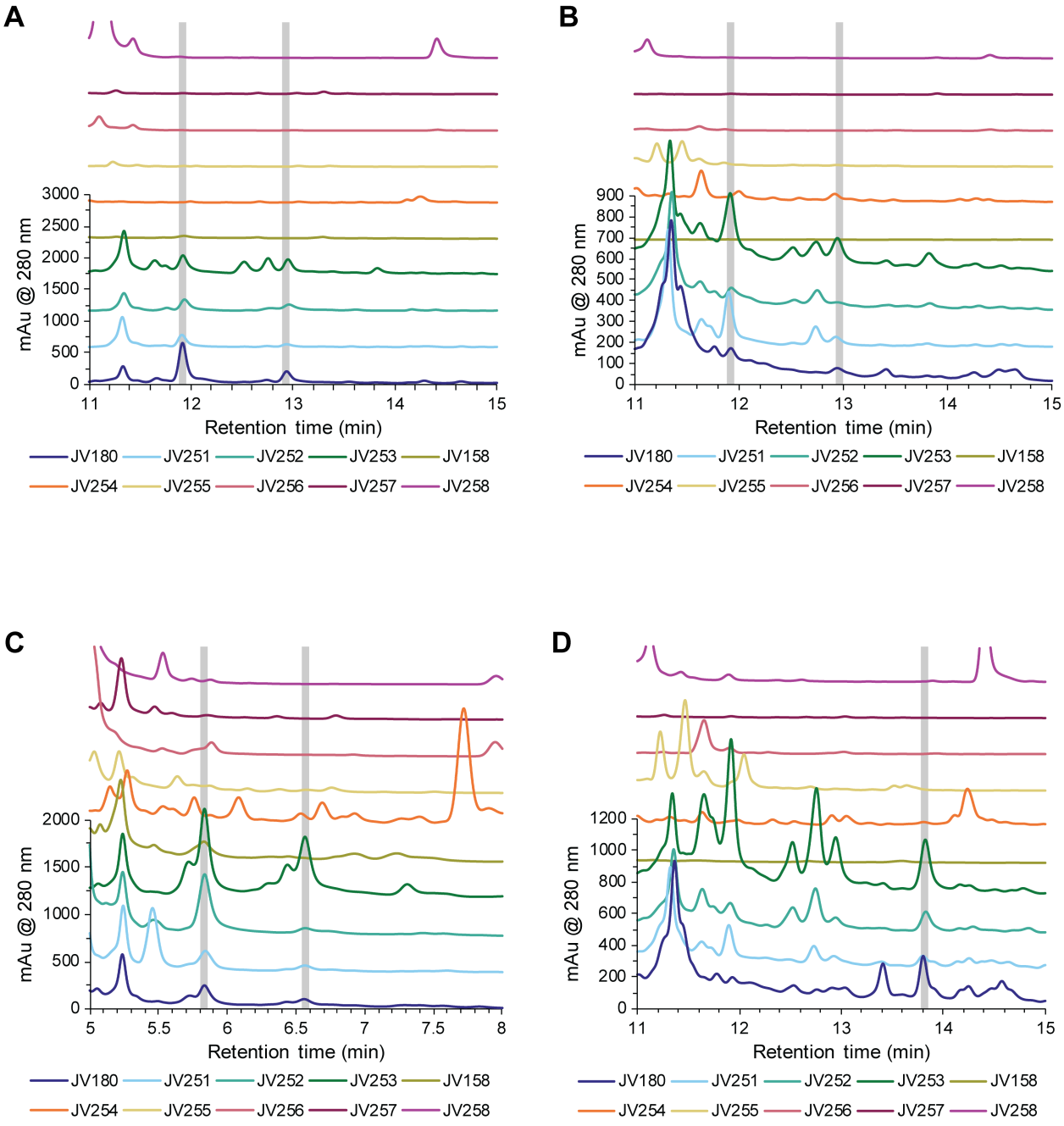


Figure S4. PTM production by *S. griseus* clade strains on various media: (**A**) ISP2 (Difco), (**B**) ISP4 (Difco), (**C**) ISP-S (see methods), and (**D**) YMS8 (0.4% yeast extract; 1% malt extract; 1.2% soluble starch; 2% agar; pH 7.4). Retention times for PTM peaks are highlighted in gray.

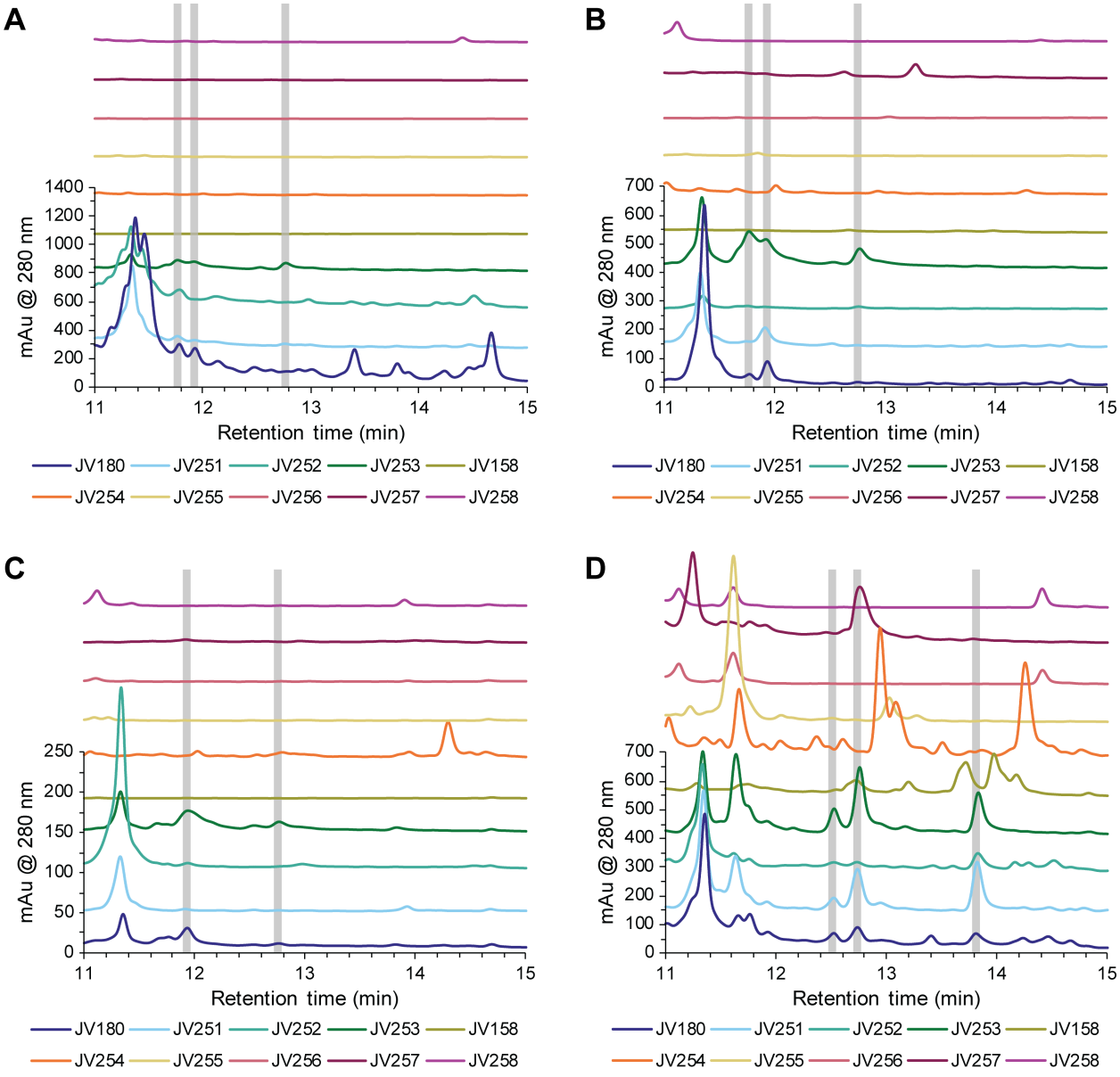


Figure S5. PTM production by *S. griseus* clade strains on various media: (**A**) supplemented minimal medium (16), (**B**) minimal medium (16), (**C**) low tryptone-yeast extract (16), and (**D**) medium 2 (17). Retention times for PTM peaks are highlighted in gray.

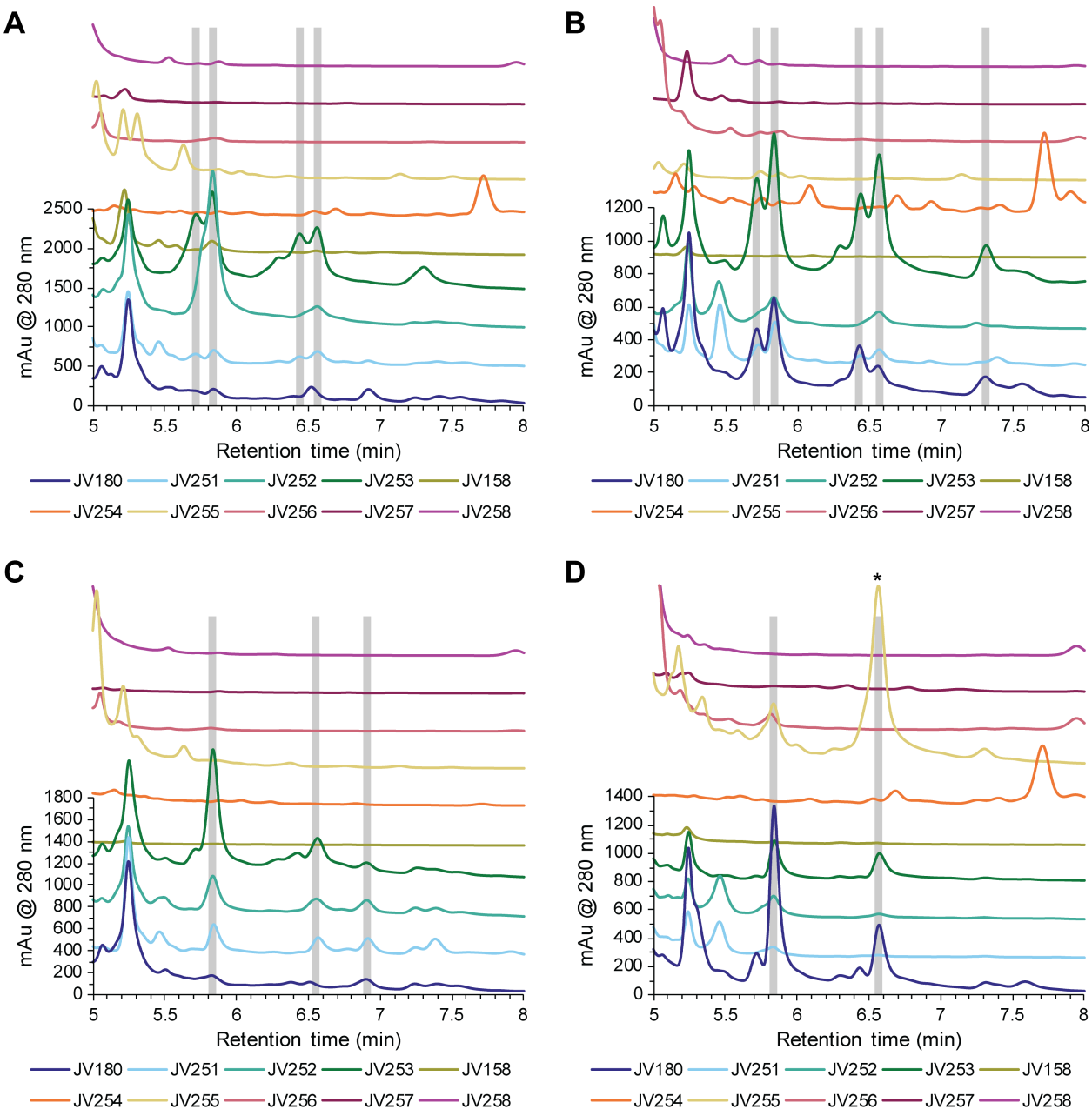


Figure S6. PTM production by *S. griseus* clade strains on various media: (**A**) YMS (16), (**B**) ATCC172, (**C**) Hickey-Tresner (18), and (**D**) SFM (16). Retention times for PTM peaks are highlighted in gray. A peak marked with an asterisk in the extract of JV255 from SFM had a similar retention time as PTMs but did not have the correct absorbance spectrum.

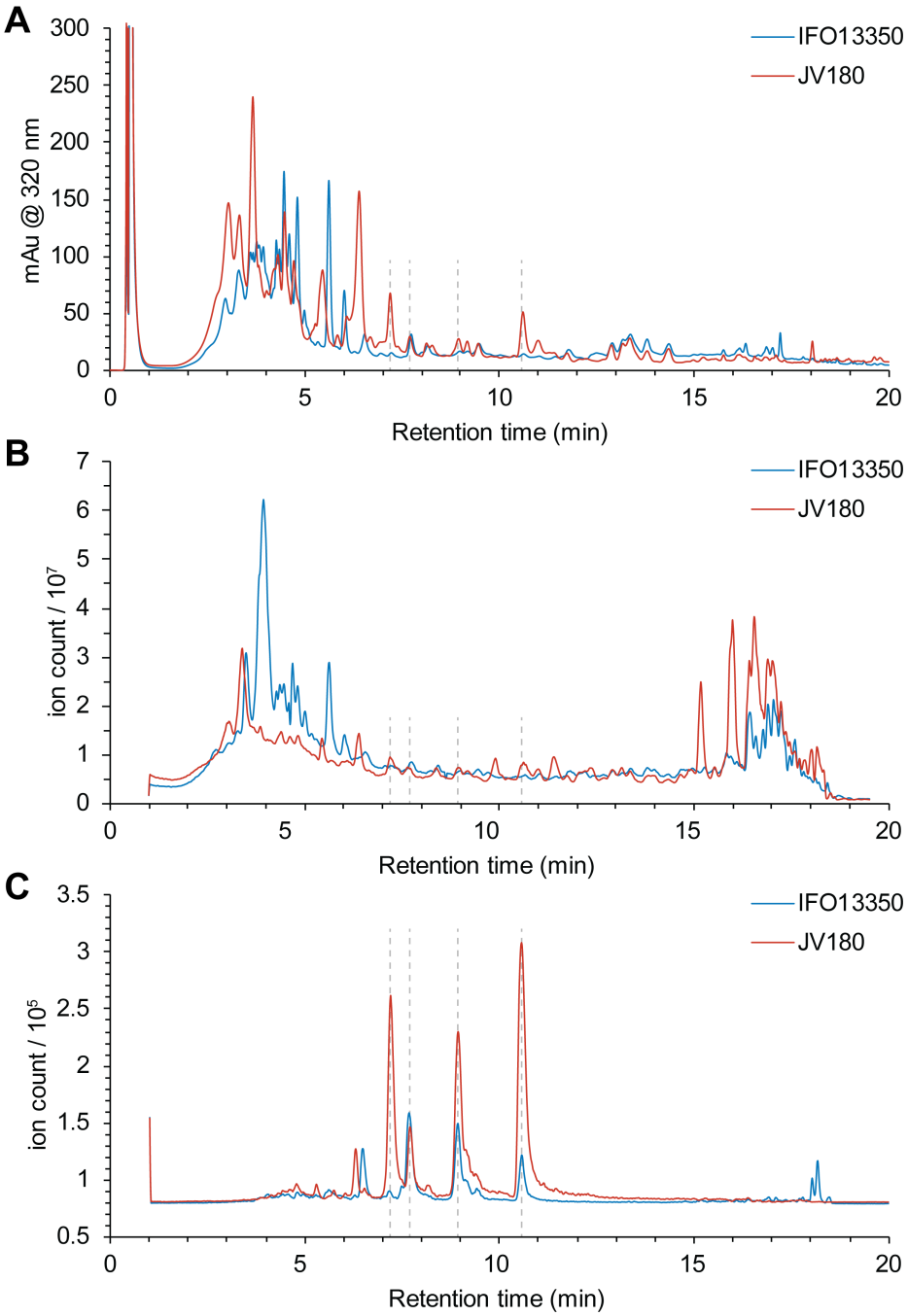


Figure S7. Improved detection for PTMs via LC-MS/MS. Chromatograms of extracts of (Red) *Streptomyces* sp. strain JV180 and (blue) *Streptomyces griseus* subsp. *griseus* strain IFO13350 monitored using (**A**) UV absorbance at 280 nm, (**B**) MS scan mode in positive mode for *m/z* from 100-1000, and (**C**) precursor ion scan mode for ions fragmenting into *m/z* 139 or 154 daughter ions. Retention times for prominent PTM peaks are marked with dotted grey lines.

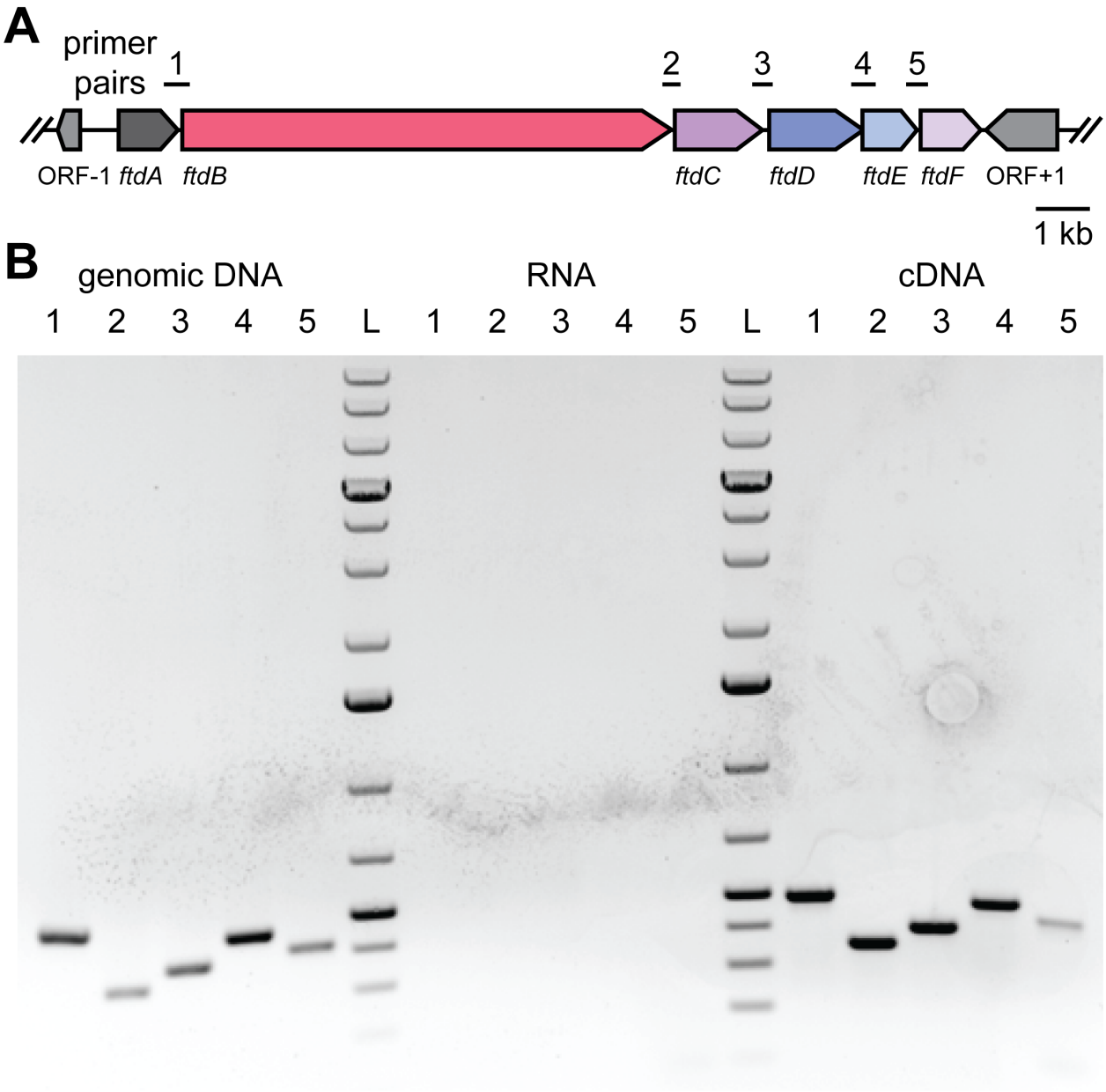


Figure S8. *ftdA-F* are transcribed on one mRNA molecule. (**A**) Diagram of the PTM biosynthetic gene cluster, and PCR probes for intergenic regions. (**B**) PCR amplification of PTM intergenic regions using genomic DNA (positive control), RNA (negative control), and cDNA templates. Ladder: GeneRuler 1kb Plus DNA marker (Thermo Fisher)

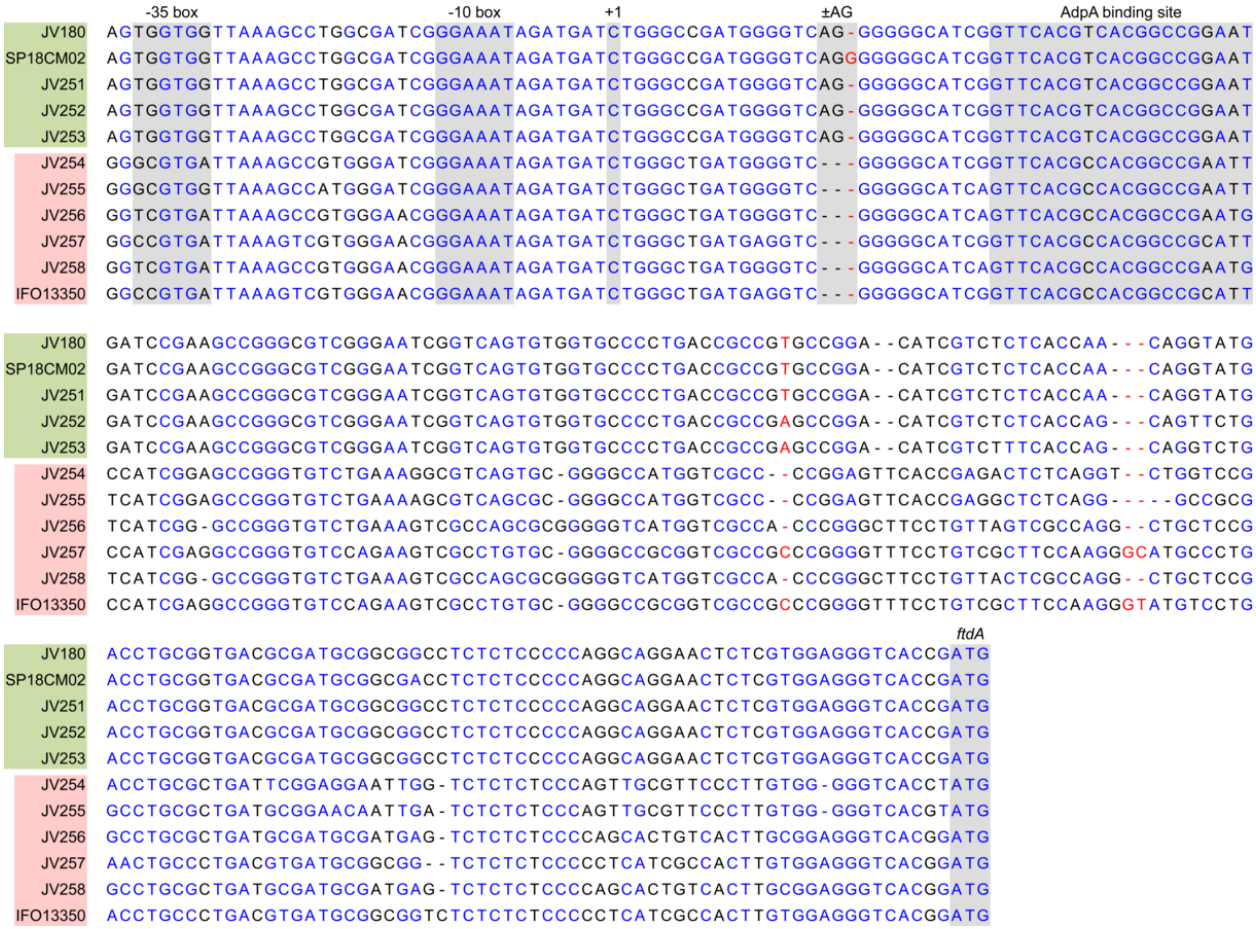


Figure S9. Nucleotide alignment of PTM promoters and 5' UTRs belonging to *S. griseus* clade strains used in this study. Nucleotides in blue have >70% conservation, while nucleotides in red have <30% conservation. Many nucleotides in black (between 30-70% conservation) are still conserved within the JV180-like strain (green) and IFO13350-like strain groups (red). Nucleotides were aligned using MUSCLE with 500 bootstrap simulations.

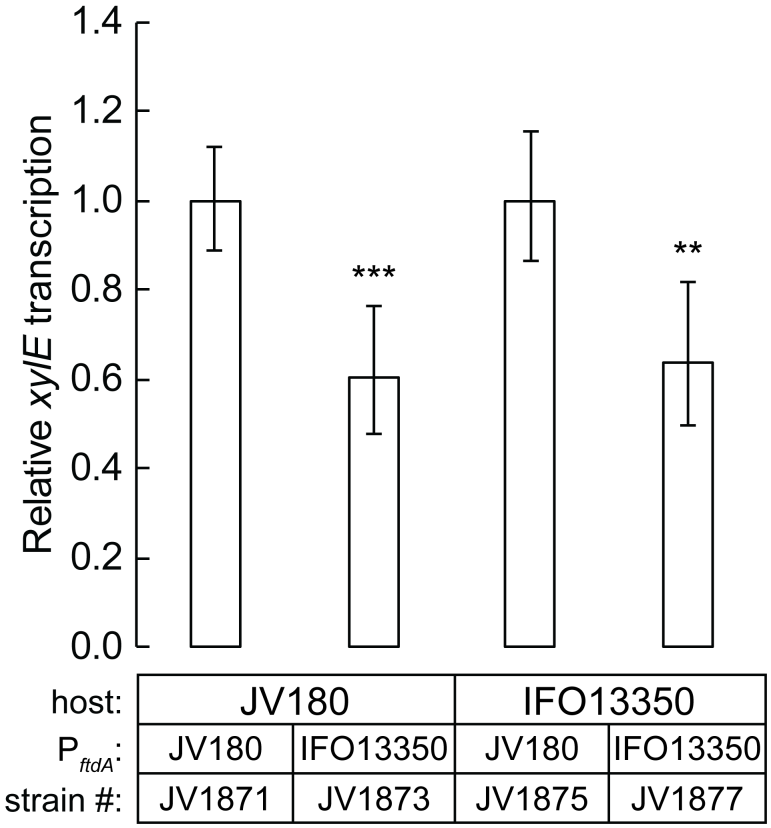


Figure S10. P_{ftdA_JV180} drives stronger *xylE* transcription than $P_{ftdA\_IFO13350}$. RT-qPCR was used to compare *xylE* transcript abundance from different P_{ftdA}-*ftdA-xylE* constructs in both JV180 and IFO13350 hosts. We used the P_{ftdA}-*ftdA-xylE* constructs in case proper expression required mRNA secondary structures forming from the 5' UTR and the *ftdA* coding sequences. Relative transcription and statistical significance were calculated by Student's T-test relative to the JV180 P_{ftdA} in each respective host strain (n = 6; *p < 0.05, **p < 0.01, ***p < 0.001).

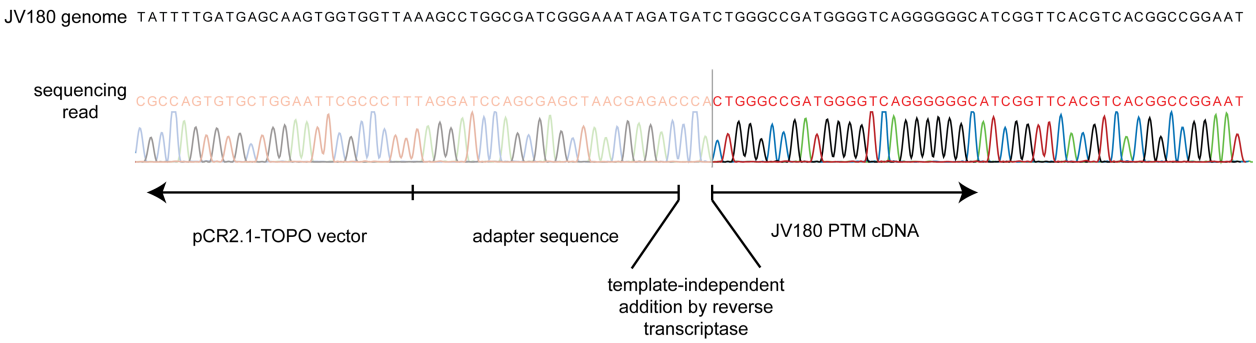


Figure S11. Transcript start site mapping of the JV180 P_{ftdA} by cRACE. The sequencing read was aligned with the JV180 genome sequence.

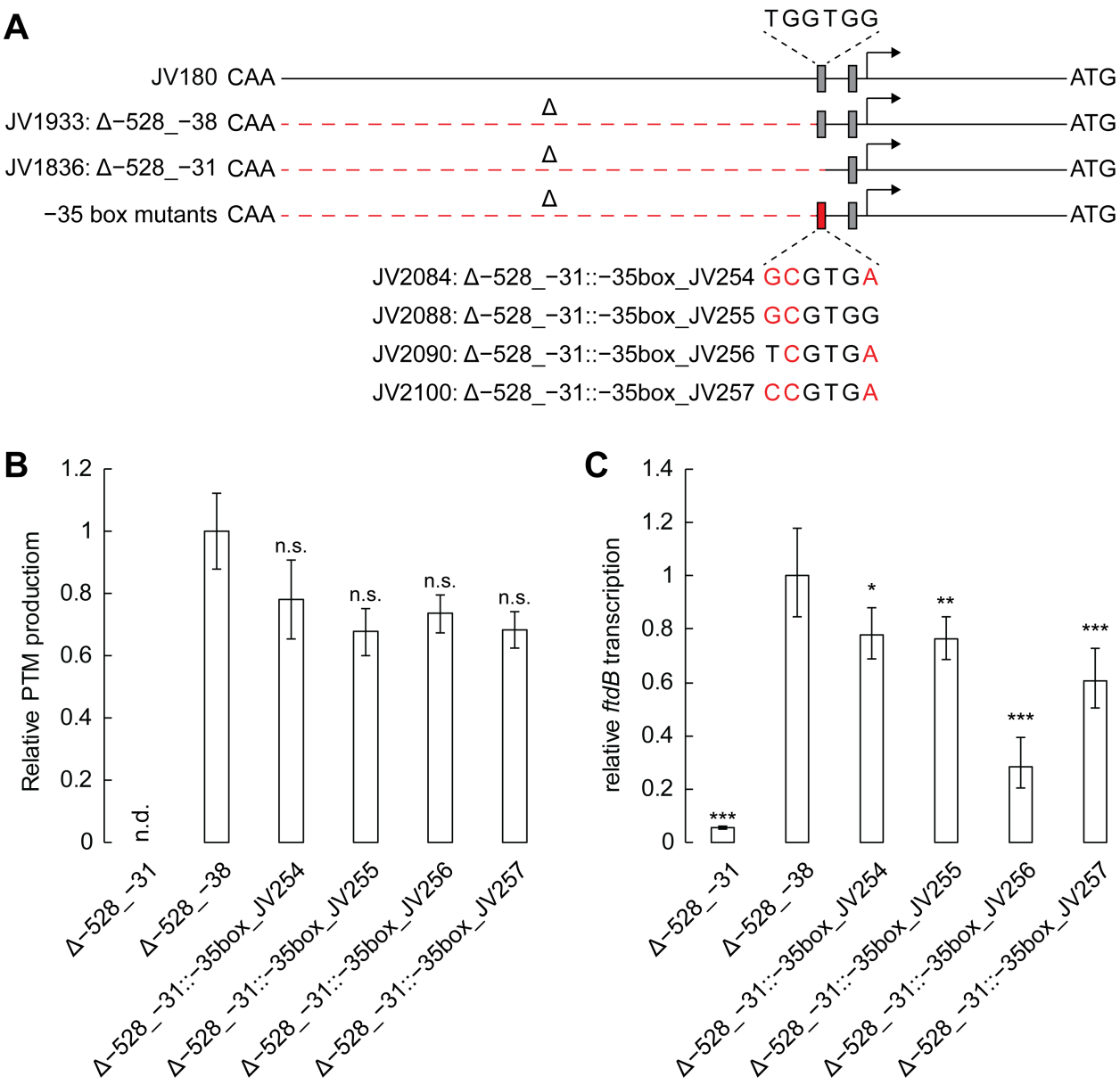


Figure S12. Mutations in the –35 box cause slight decreases in PTM BGC expression. (**A**) A diagram of the P_{ftdA} region in –35 box mutants. Due to difficulties in cloning, IFO13350-type –35 boxes were introduced into the Δ–528_–31 mutant background. (**B**) Relative PTM production from –35 box mutants (n = 3). (**C**) Relative *ftdB* transcript abundance from –35 box mutants (n = 6). Statistical significance was calculated by Student's T-test relative to the Δ–528_–38 strain (*p < 0.05, **p < 0.01, ***p < 0.001). Bars represent standard deviation.

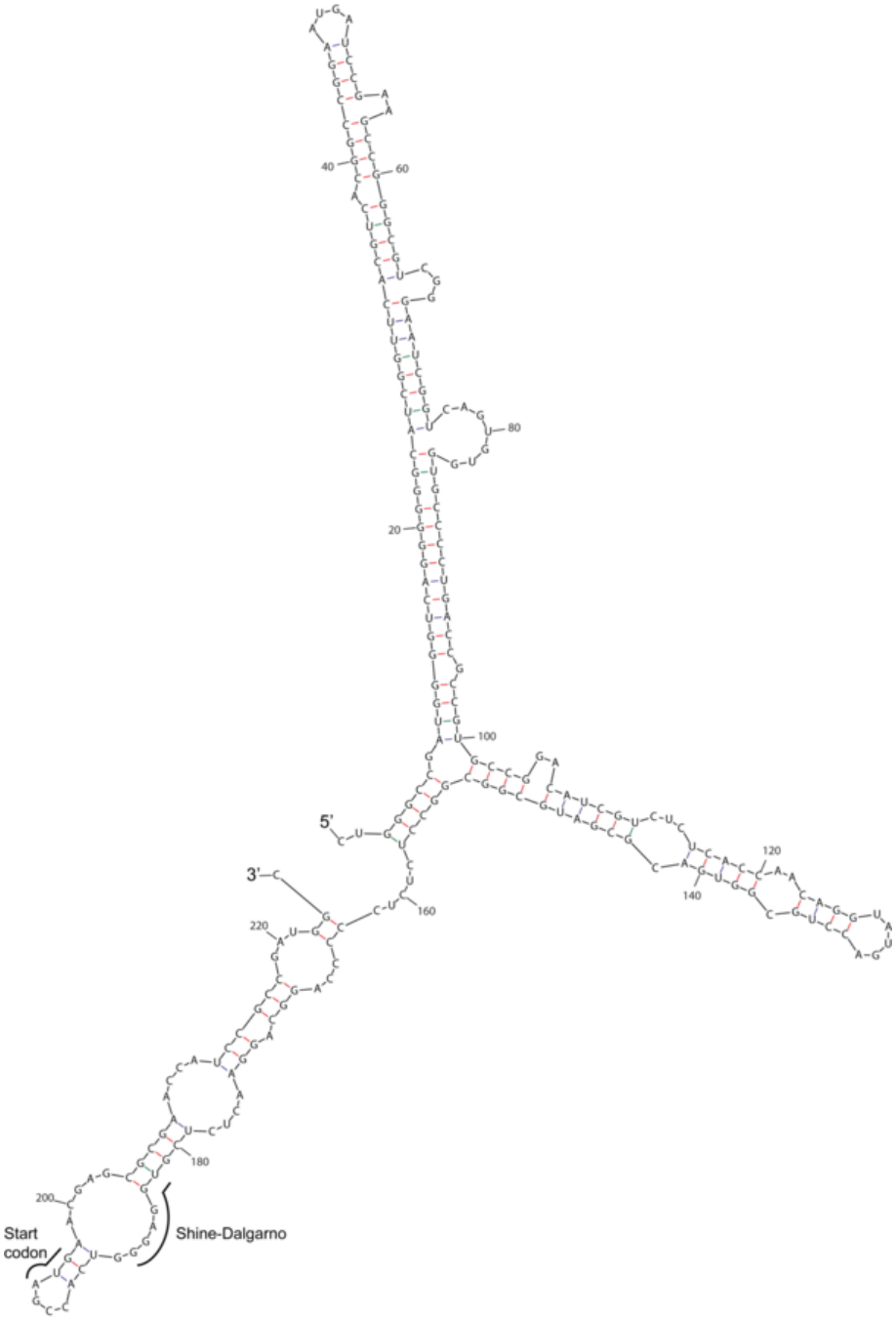


Figure S13. Predicted secondary structure of the JV180 P_{ftdA} UTR. The structure was predicted with mFold using the RNA folding form for the UTR sequence and the first 30 nucleotides of *ftdA*. The 5' end, 3' end, Shine-Dalgarno sequence, and the start codon are labeled.

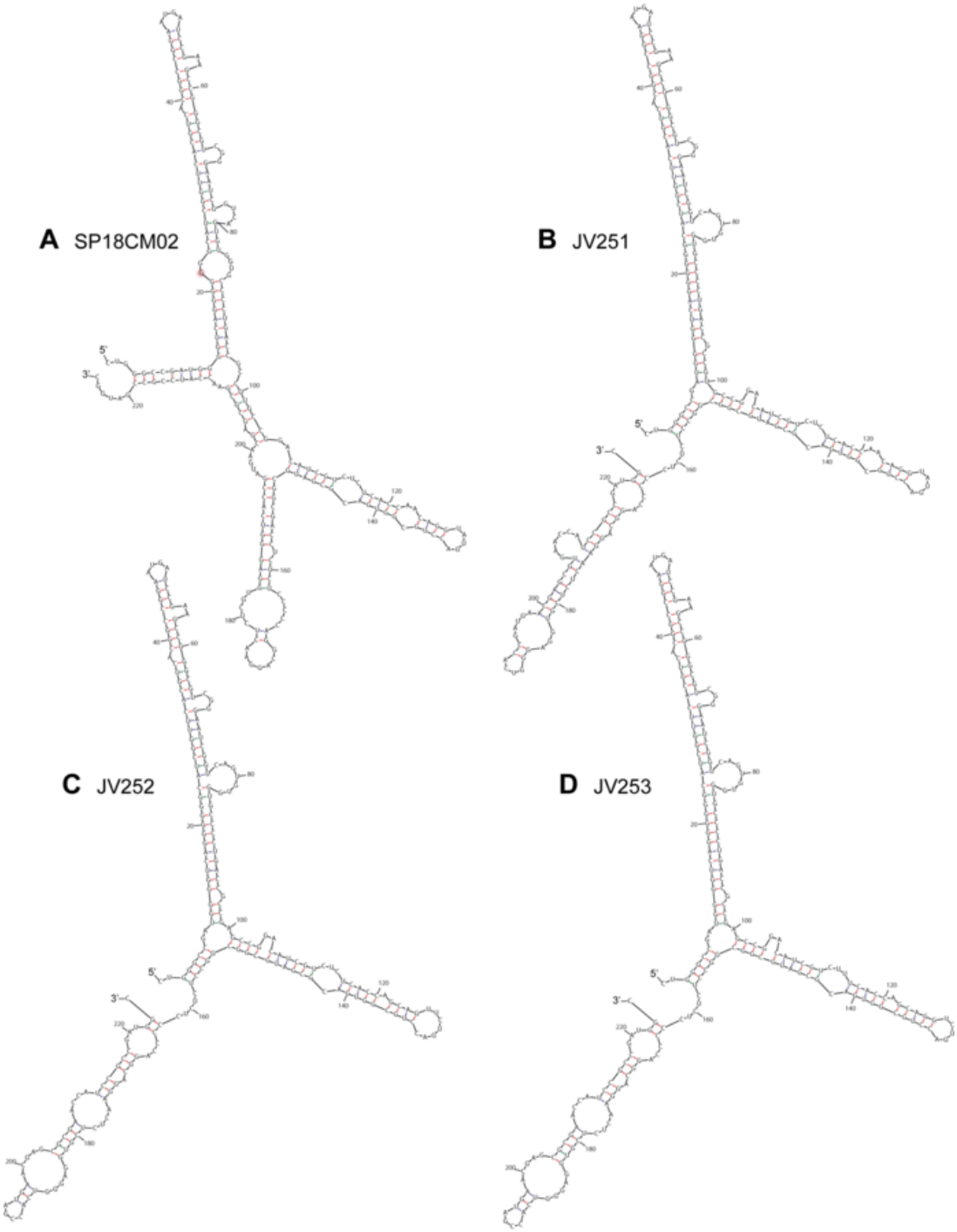


Figure S14. Predicted secondary structure of the P_{ftdA} UTR from JV180-like P_{ftdA}'s. The structures were predicted with mFold using the RNA folding form for the UTR sequence and the first 30 nucleotides of *ftdA* for (**A**) SP18CM02, (**B**) JV251, (**C**) JV252, and (**D**) JV253. The SP18CM02 has an additional nucleotide (indicated with a red circle) that greatly alters its structure.

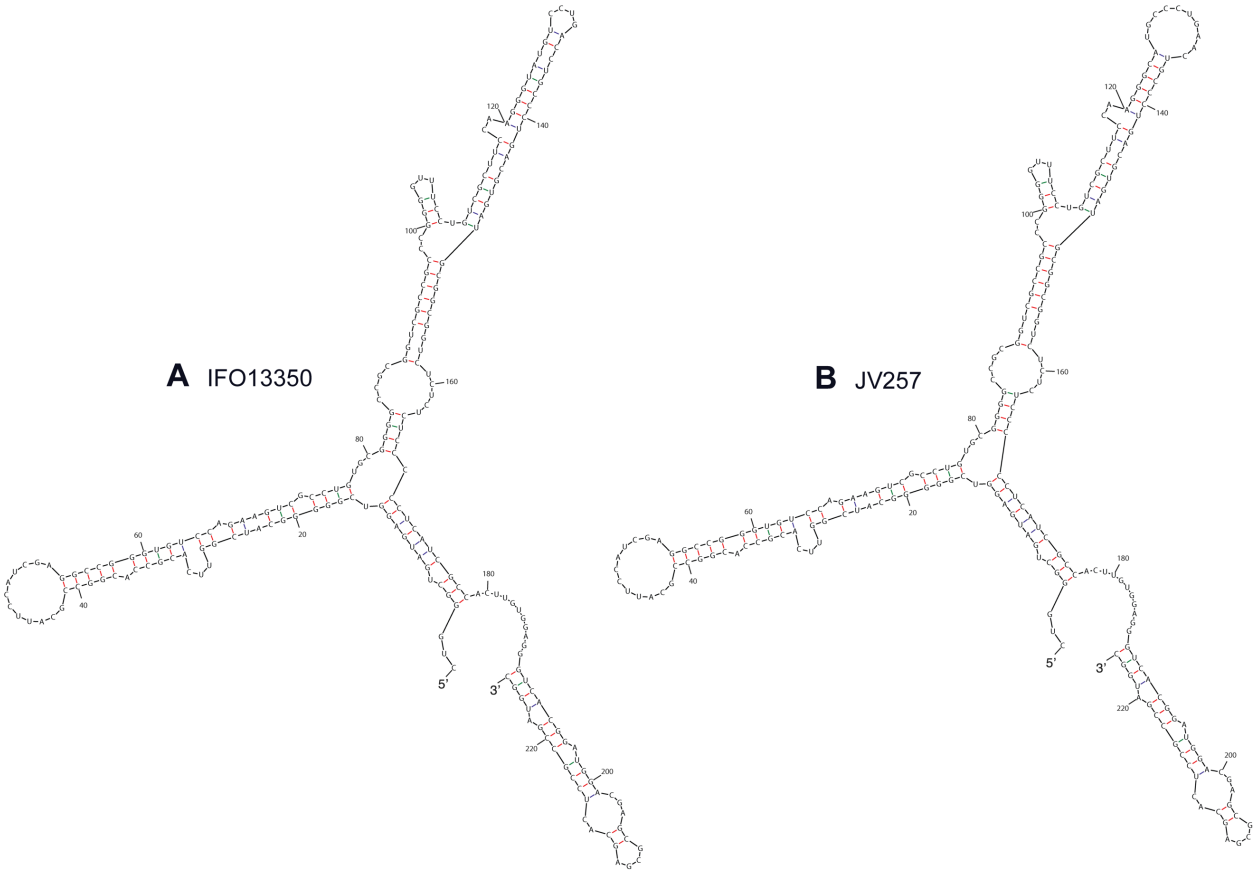


Figure S15. Predicted secondary structure of the P_{ftdA} UTR from strains (A) IFO13350 and (B) JV257. The structures were predicted with mFold using the RNA folding form for the UTR sequence and the first 30 nucleotides of *ftdA*.

Figure S16. Predicted secondary structure of the P_{ftdA} UTR from strains (A) JV254 and (B) JV255. The structures were predicted with mFold using the RNA folding form for the UTR sequence and the first 30 nucleotides of *ftdA*.

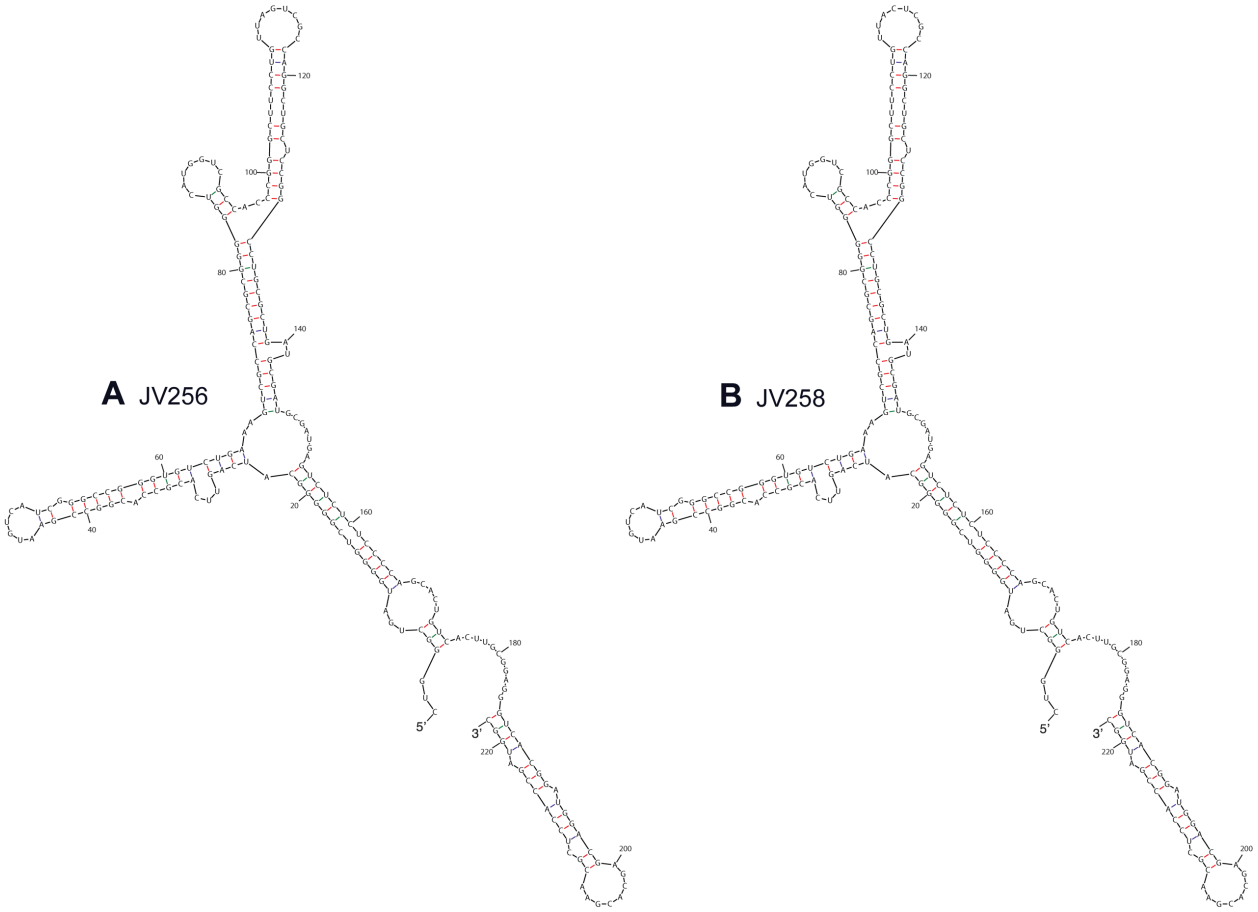


Figure S17. Predicted secondary structure of the P_{ftdA} UTR from strains (A) JV256 and (B) JV258. The structures were predicted with mFold using the RNA folding form for the UTR sequence and the first 30 nucleotides of *ftdA*.

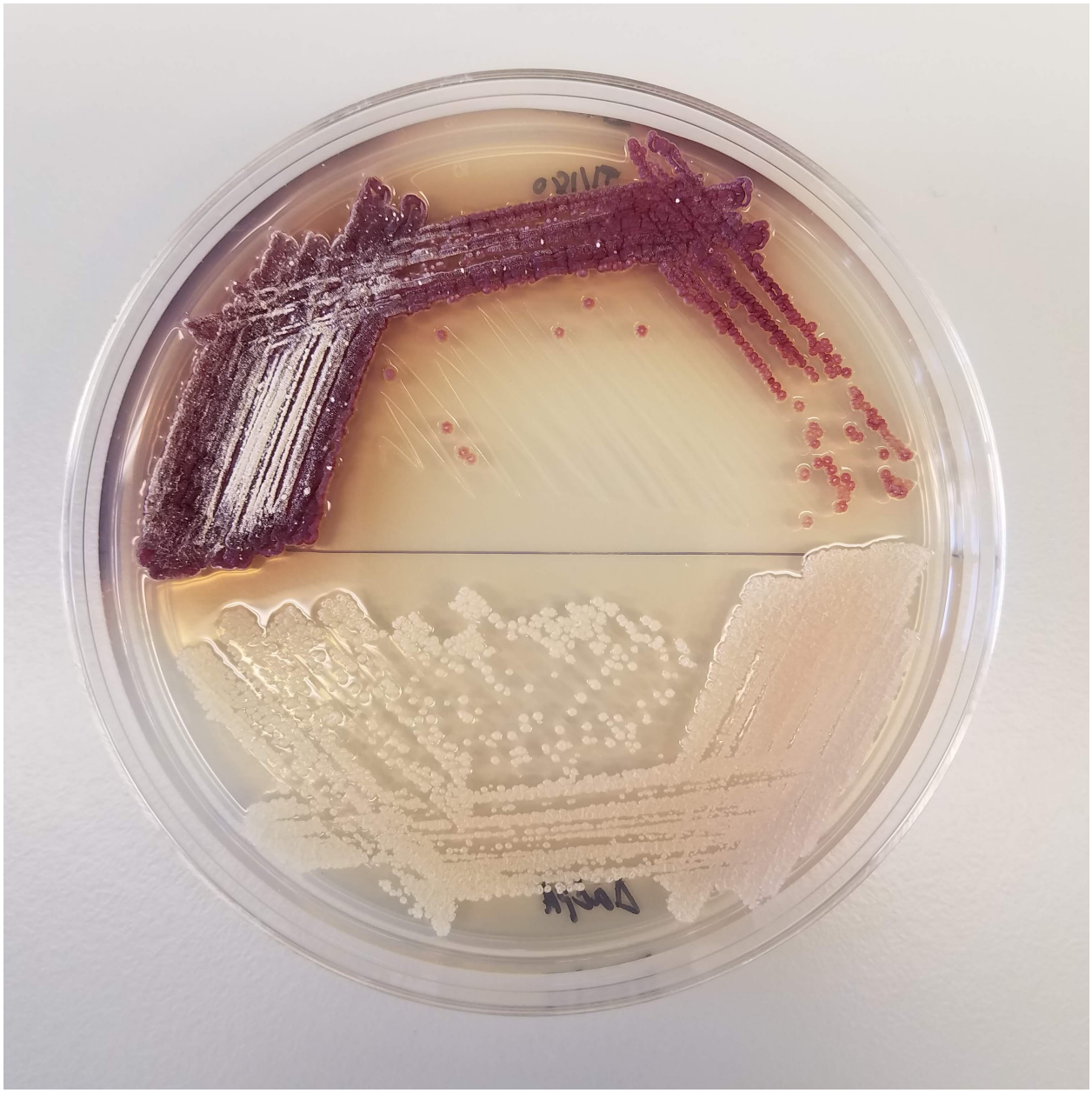


Figure S18. *adpA* is required for morphological development and secondary metabolite expression. (**top**) Wild-type JV180 and (**bottom**) its Δ*adpA* mutant (JV556) were streaked on ISP2 agar and incubated for 2 days at 28 °C. The Δ*adpA* mutant is deficient in secondary metabolite production (red pigment) and morphological differentiation (white aerial mycelia), which are exhibited by the parent strain.

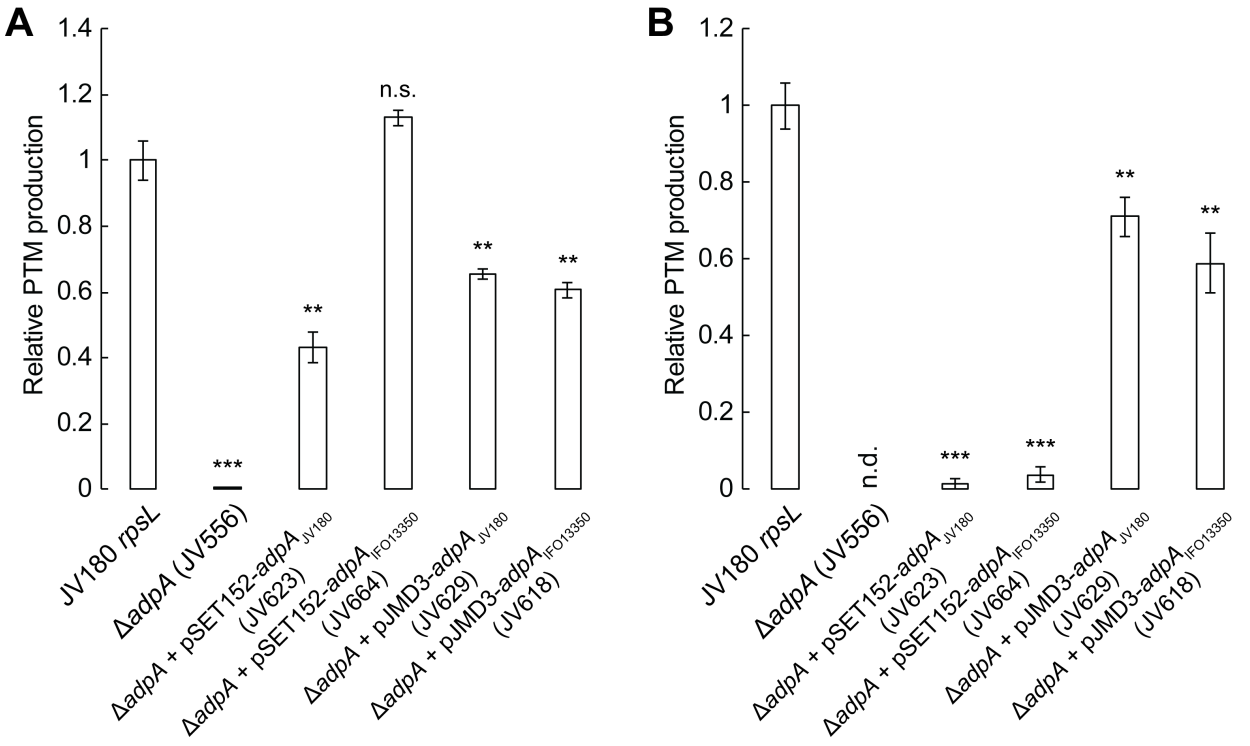


Figure S19. Additional complementation data for Δ*adpA*. Relative PTM production (**A**) on solid agar media (n = 3) and (**B**) in shake flasks (n = 4, except for JV180 *rpsL* where n = 3). The pSET152-*adpA* constructs were designed to express *adpA* under its native promoter and failed to express properly in flask cultures, whereas the pJMD3 vector uses the constitutive P_{ermE*}. We speculate the pSET152-*adpA* construct failed to express properly in flask cultures due to differences in culture conditions, which is in line with the previous report that AdpA only appeared to be a transcriptional activator for the PTM BGC in strain IFO13350 when grown on solid media(19). Additionally, the actual *adpA* promoter could contain more regulatory binding regions upstream which were not included in the construct. Statistical significance was calculated by Student's T-test relative to the JV180 *rpsL* parent strain (*p < 0.05, **p < 0.01, ***p < 0.001). Bars represent standard deviation.

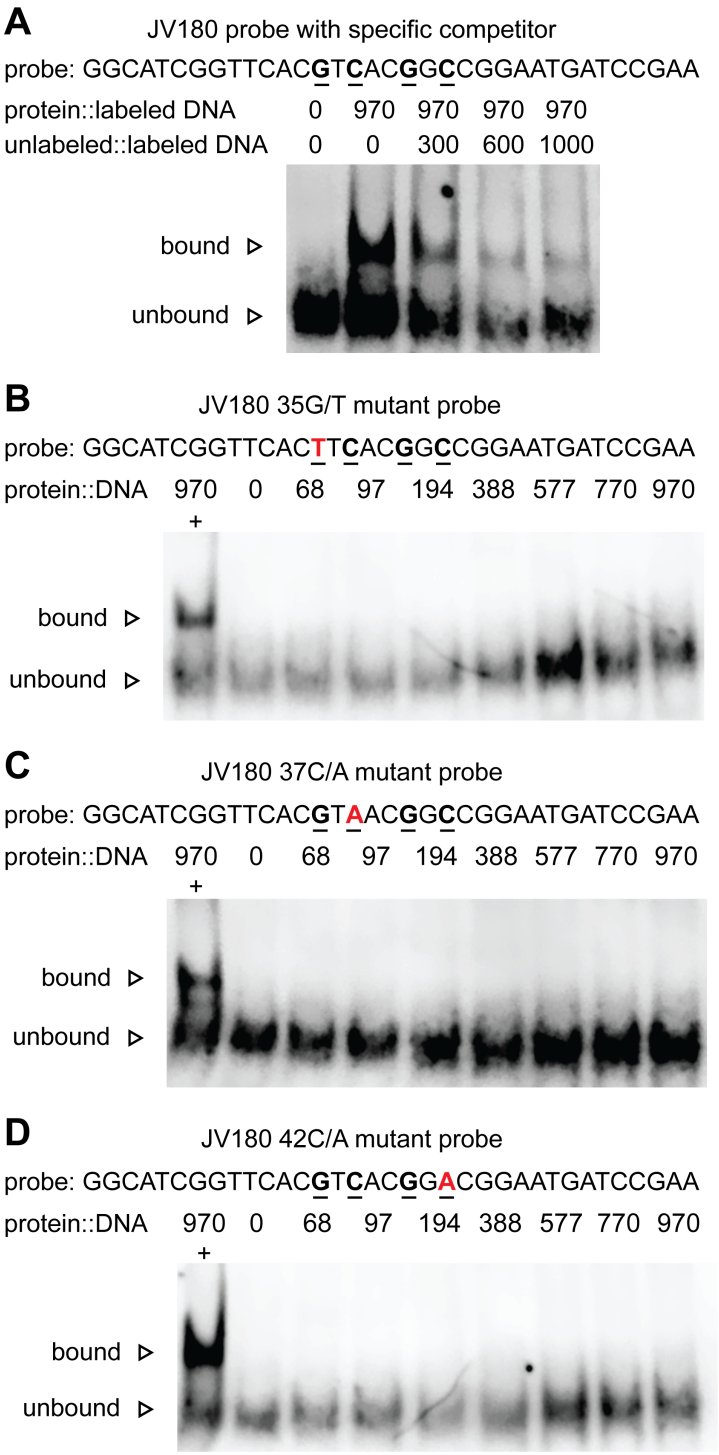


Figure S20. Additional electrophoretic mobility shift assays with AdpA-P*ftdA*. (**A**) Competition assay with unlabeled DNA probe. (**B**-**D**) assays with JV180 probes containing transversion mutations in nucleotides reported to directly interact with AdpA. The lane marked by the (+) sign is a positive control, using wild-type probe sequence.

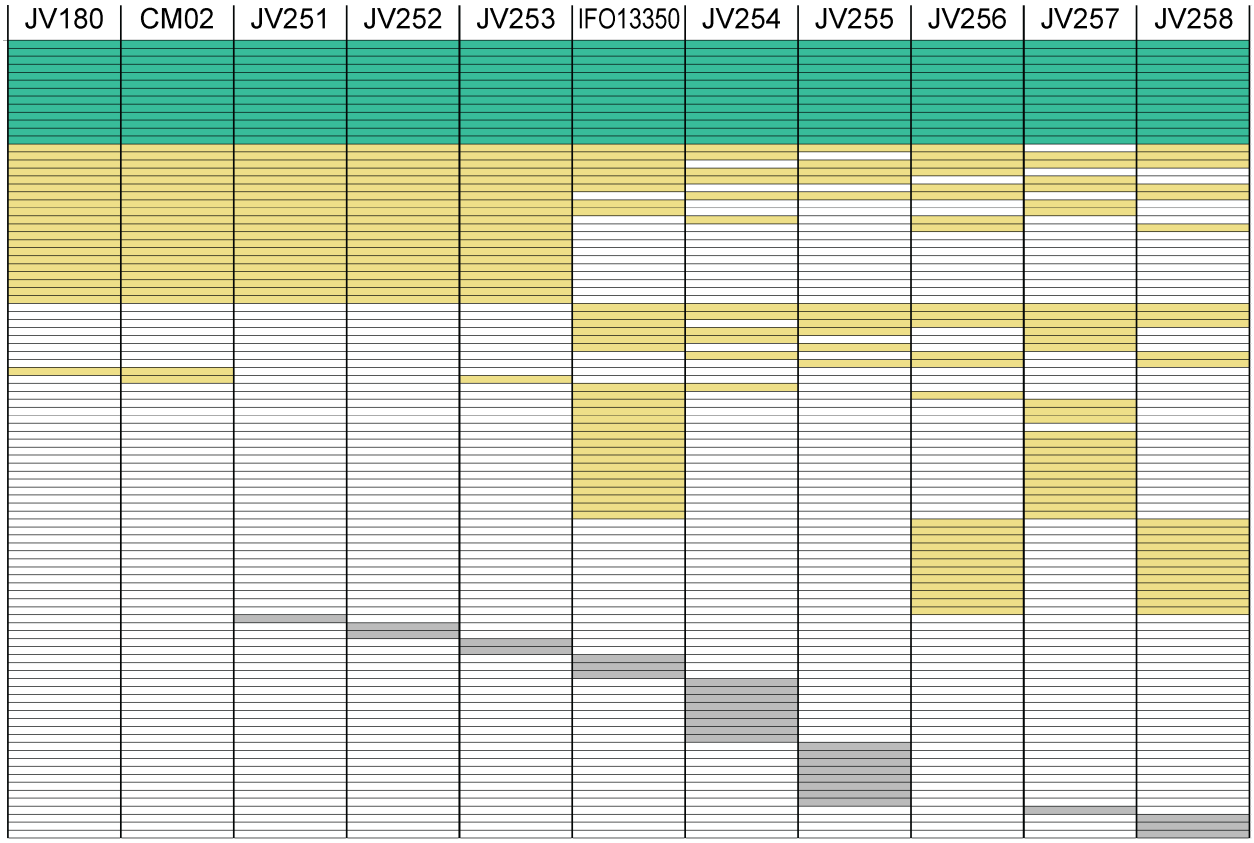


Figure S21. *S. griseus* clade strains contain different secondary metabolite BGCs. Each row represents a unique BGC. BGCs were identified using AntiSMASH (20). BGCs predicted to produce the same compound were individually compared for synteny. In a few cases, AntiSMASH would count two neighboring BGCs as a singular BGC, such as the PTM BGC and an adjacent viomycin BGC in the genome of strain JV180. These BGCs were manually split and counted as two BGCs if the two BGCs were highly similar to previously characterized BGCs. A few PKS- and NRPS-type BGCs that were located on small contigs due to poor genome assembly were collectively counted as single unique BGCs; future sequence data may eventually collapse some of these fragments into single contiguous BGCs. Green boxes indicate BGCs shared by all 11 strains (n = 13). Yellow boxes indicate BGCs shared by two or more strains (n = 59). Gray boxes indicate BGCs that are unique to one strain (n = 28).

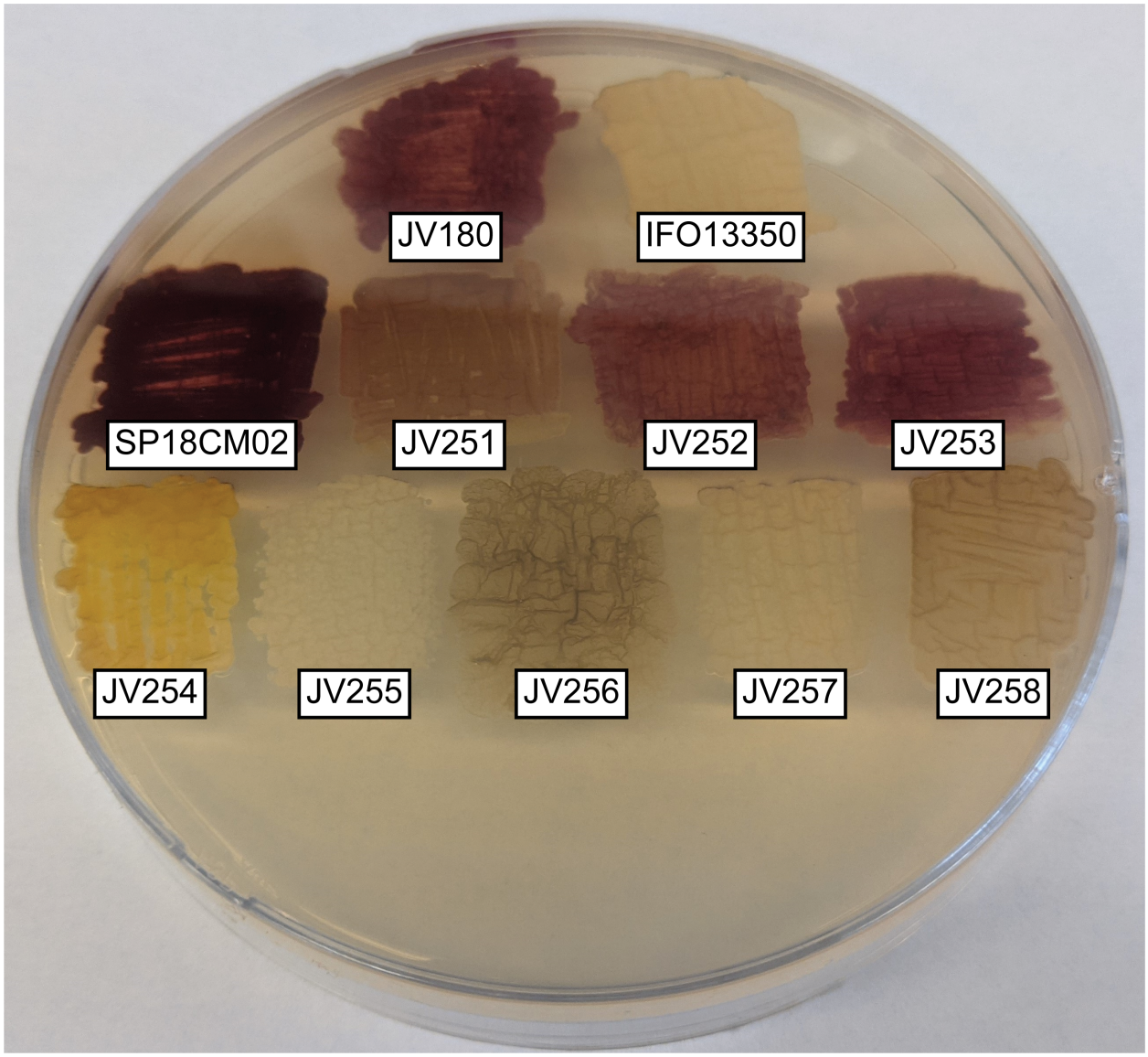
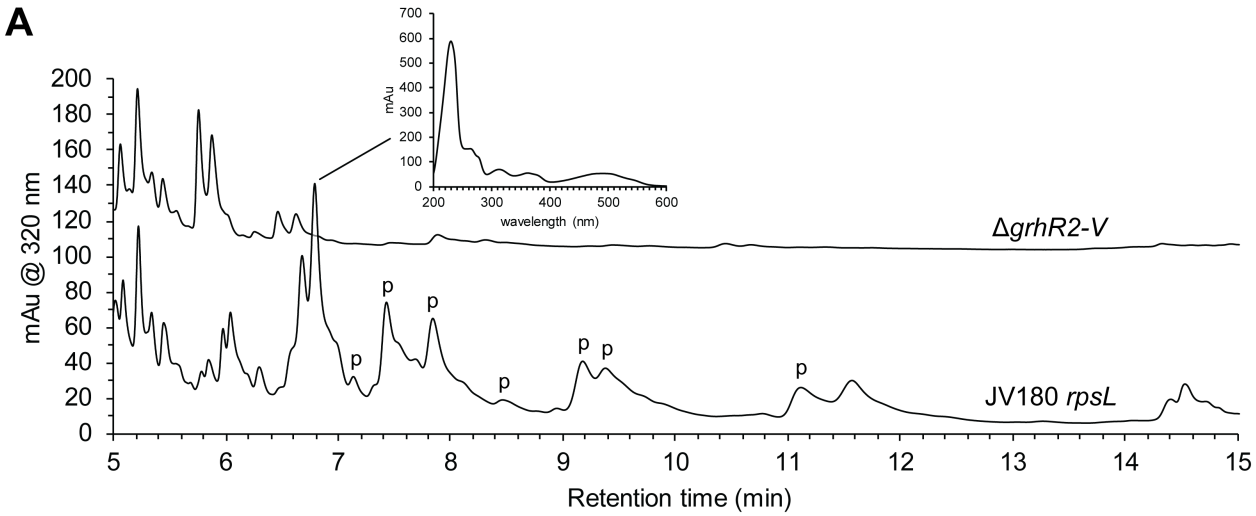


Figure S22. JV180-subclade strains produce a red pigment absent in other *S. griseus* clade strains. Strains JV180, IFO13350, SP18CM02, and JV251-JV258 were streaked on ISP2 and incubated for 2 days at 28°C.



strain	griseorhodin
JV180 *rpsL* (JV307)	yes
Δ*grhR2-V* (JV564)	no
Δ*grhR2-V* + pJMD2-*grhGH* (JV1070)	no
Δ*grhR1-E* (JV1832)	no
Δ*grhFGH* (JV1898)	no
Δ*grhGH* (JV1698)	less
Δ*grhI-P* (JV1892)	no
Δ*grhQSAB* (JV1706)	no

Figure S23. Identification of griseorhodin in JV180 culture extracts. (**A**) UV chromatogram of JV180 *rpsL* and Δ*grhR2-V* mutant mutants. (Inset) absorbance spectrum of the putative griseorhodin A peak, which closely matches the absorbance characteristics reported previously(21). PTM peaks (marked with p) were also missing in the Δ*grhR2-V* mutant. (**B**) The structure of griseorhodin A and the expected and observed *m/z*. (**C**) Image of JV180 *rpsL* (top) and the Δ*grhR2-V* mutant (bottom) streaked on ISP2 agar and incubated for 2 days at 28°C. The Δ*grhR2-V* mutant lacks griseorhodin pigmentation. (**D**) griseorhodin production observed from various *grh* cluster mutants.

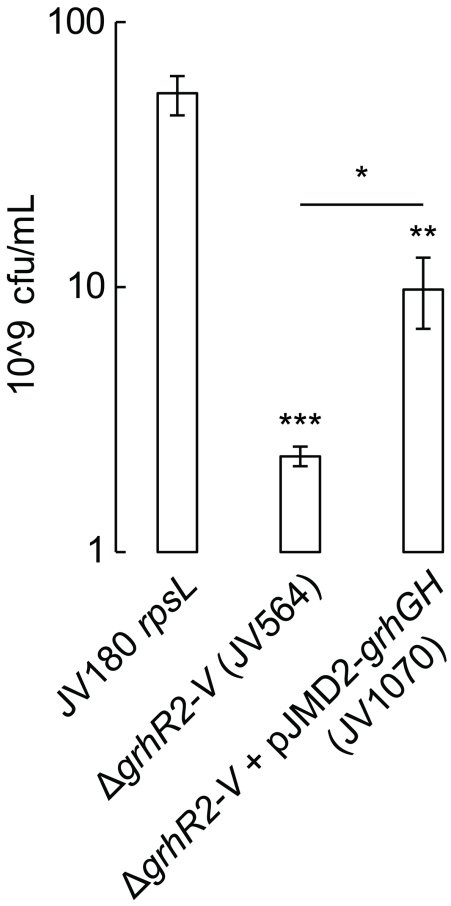


Figure S24. Sporulation is reduced in the Δ*grhR2-V* mutant and partially complemented by ectopically expressing *grhGH*. Statistical significance was calculated by Student's T-test relative to the JV180 *rpsL* parent strain or otherwise indicated (n = 3; *p < 0.05, **p < 0.01, ***p < 0.001). Bars represent standard deviation.

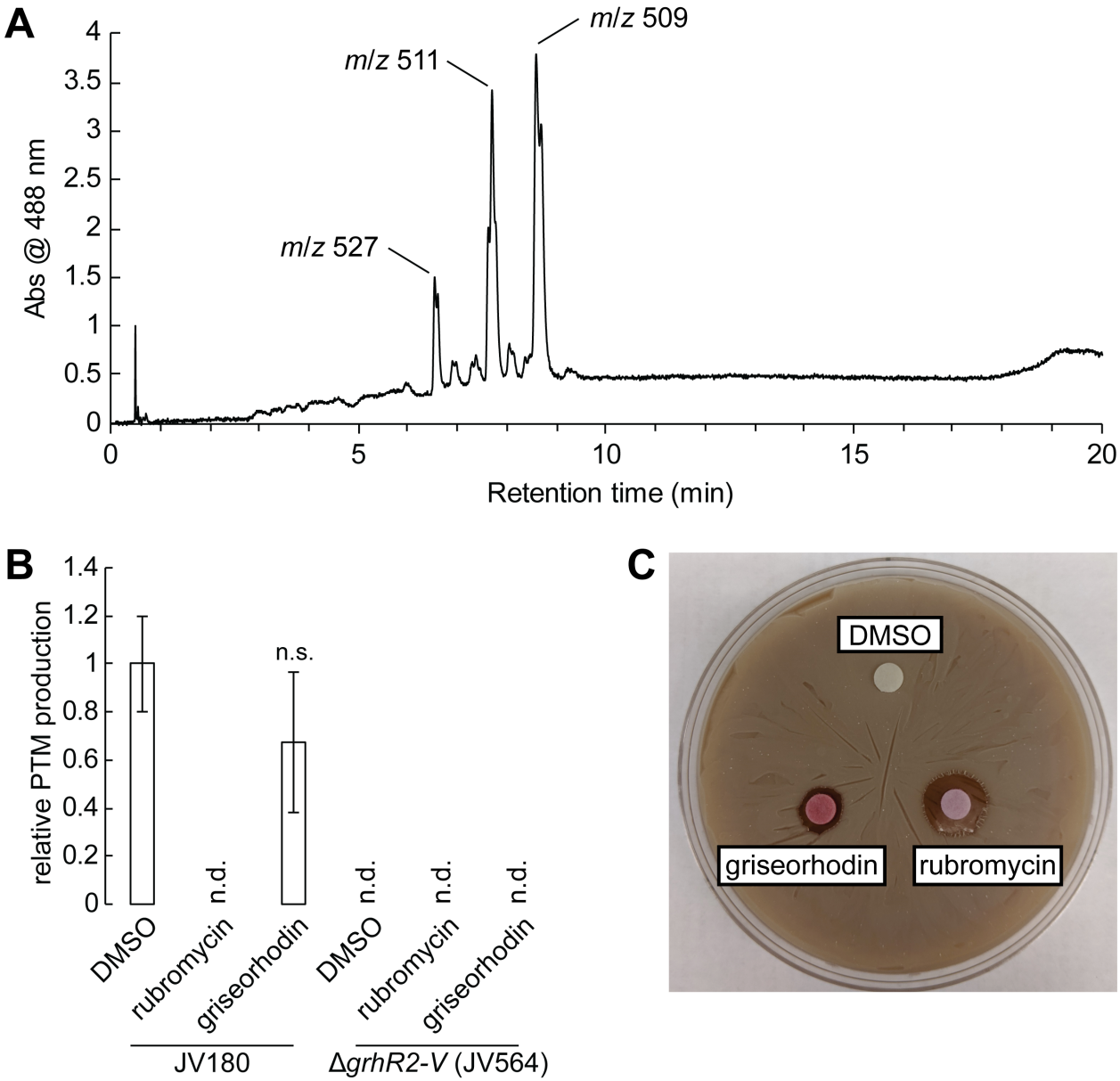


Figure S25. Griseorhodin does not act as a signaling compound in *Streptomyces* sp. strain JV180. (**A**) UV chromatogram of the griseorhodin-enriched fraction. The *m*/*z* 509, 511, and 527 peaks have the same masses as griseorhodin A (22), griseorhodin G (21), and griseorhodin C (23). (**B**) PTM production by JV180 and its Δ*grhR2-V* mutant in flasks with added DMSO (negative control), the griseorhodin- enriched fraction, or γ-rubromycin (n = 3). (**C**) Disc diffusion assay of Δ*grhR2-V* mutant with DMSO (negative control), the griseorhodin- enriched fraction, and γ-rubromycin. PTM production and sporulation were not restored by a chemical signaling mechanism. Instead, production and growth inhibition were observed. Statistical significance was calculated by Student's T-test relative to the DMSO control (*p < 0.05, **p < 0.01, ***p < 0.001).

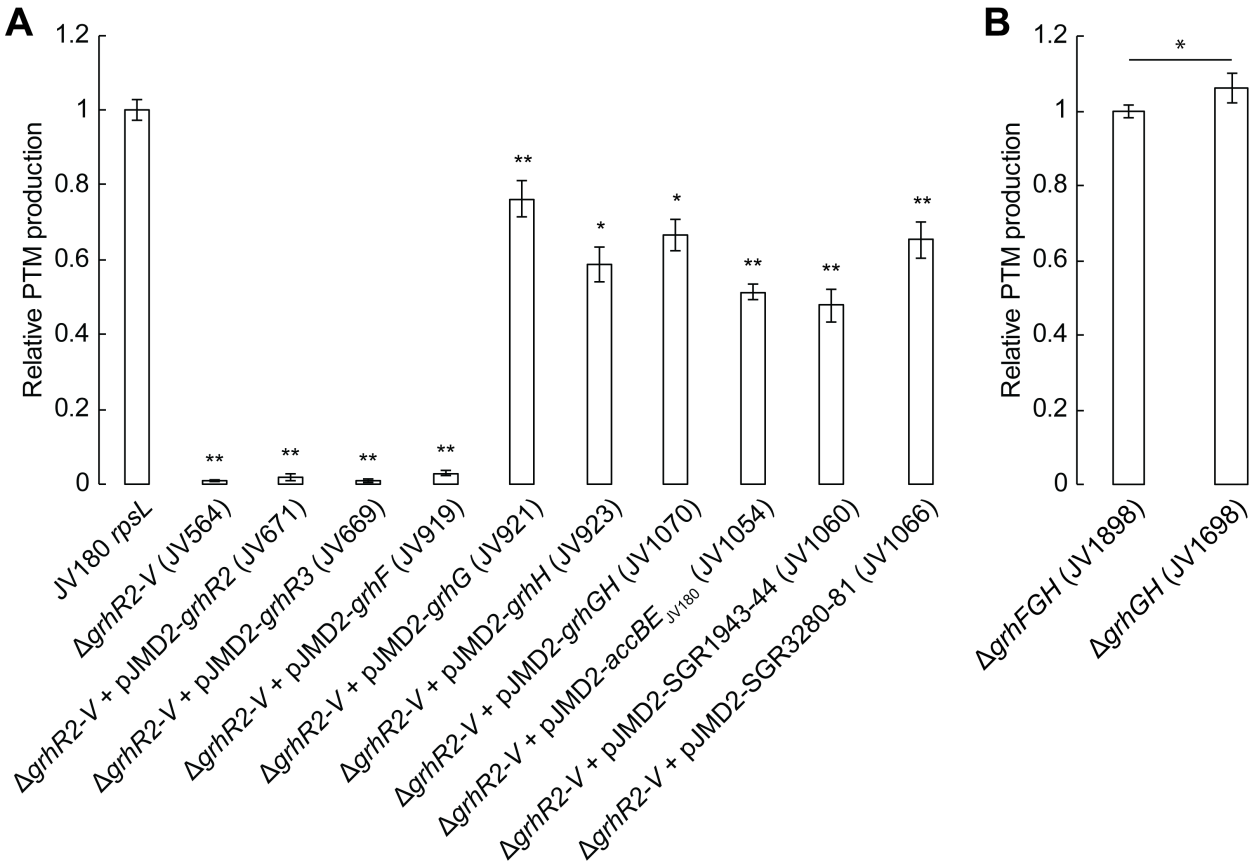


Figure S26. Two acetyl-CoA carboxylase genes play a key role in the *grh*-PTM BGC interaction. (**A**) PTM production by the Δ*grhR2-V* mutant (JV564) on solid media and complementation by various constructs. *grhR2*, *grhR3*, and *grhF* were not able to restore PTM production, while *grhG* and/or *grhH* were able to restore some PTM production. Additionally, *accBE* and their homologs from strain IFO13350 were also able to restore some PTM production (n = 3 for JV180 *rpsL* and JV564, n = 2 for complementation strains). (**B**) PTM production by the Δ*grhFGH* and Δ*grhGH* mutants on solid media barely differs (n = 3). Statistical significance was calculated by Student's T-test relative to the JV180 *rpsL* parent strain (*p < 0.05, **p < 0.01, ***p < 0.001). Bars represent standard deviation.

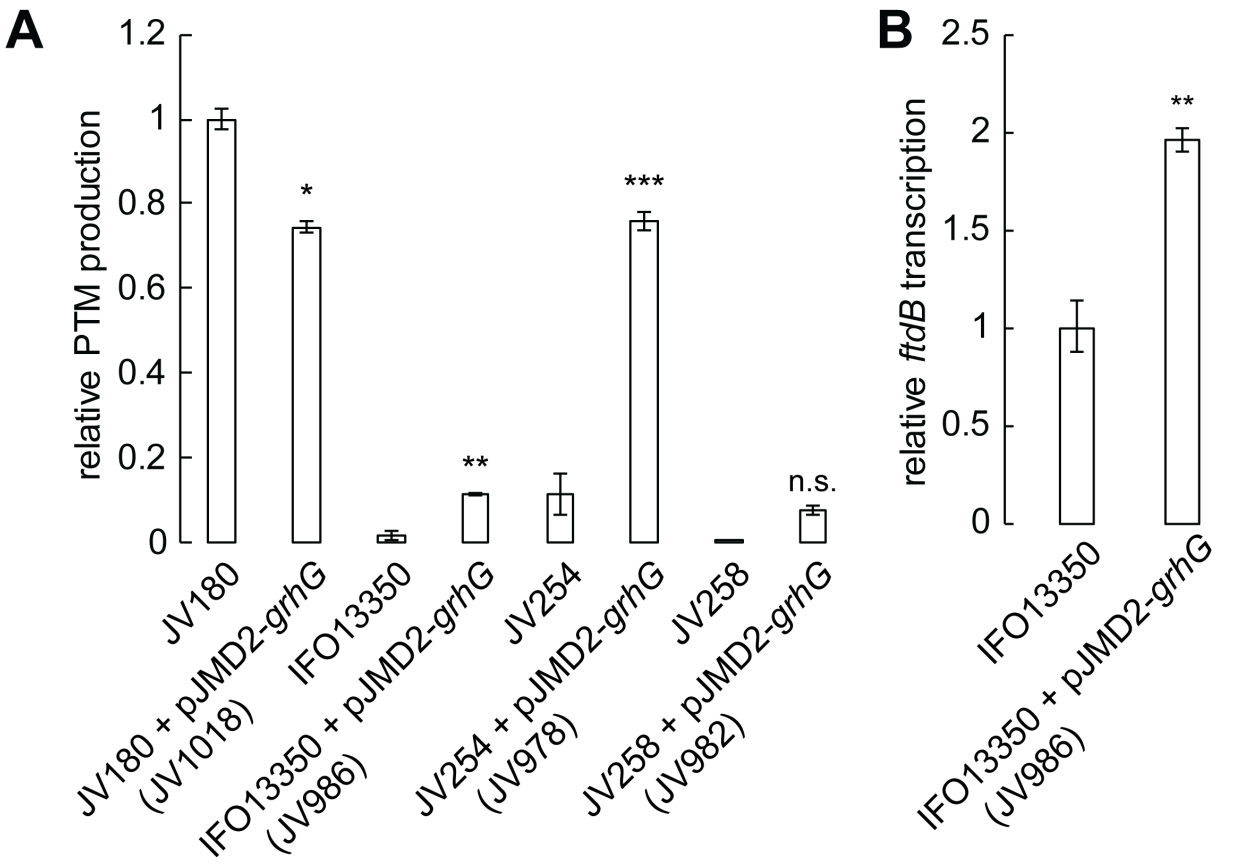


Figure S27. Heterologous expression of *grhG* in IFO13350-like strains increases PTM production and transcription. (**A**) Relative PTM production by strains JV180, IFO13350, JV254, and JV258 heterologously expressing *grhG* on solid agar (for IFO13350, JV254, and JV258, n = 3; otherwise, n = 2). (**B**) Relative *ftdB* transcript abundance in strain IFO13350 heterologously expressing *grhG*. Methods for culturing and extracting RNA were largely the same as for JV180-derived strains, except the samples were harvested after 2 days of culturing for RT-qPCR (n = 3). Statistical significance was calculated by Student's T-test relative to the wild-type strains (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Bars represent standard deviation.

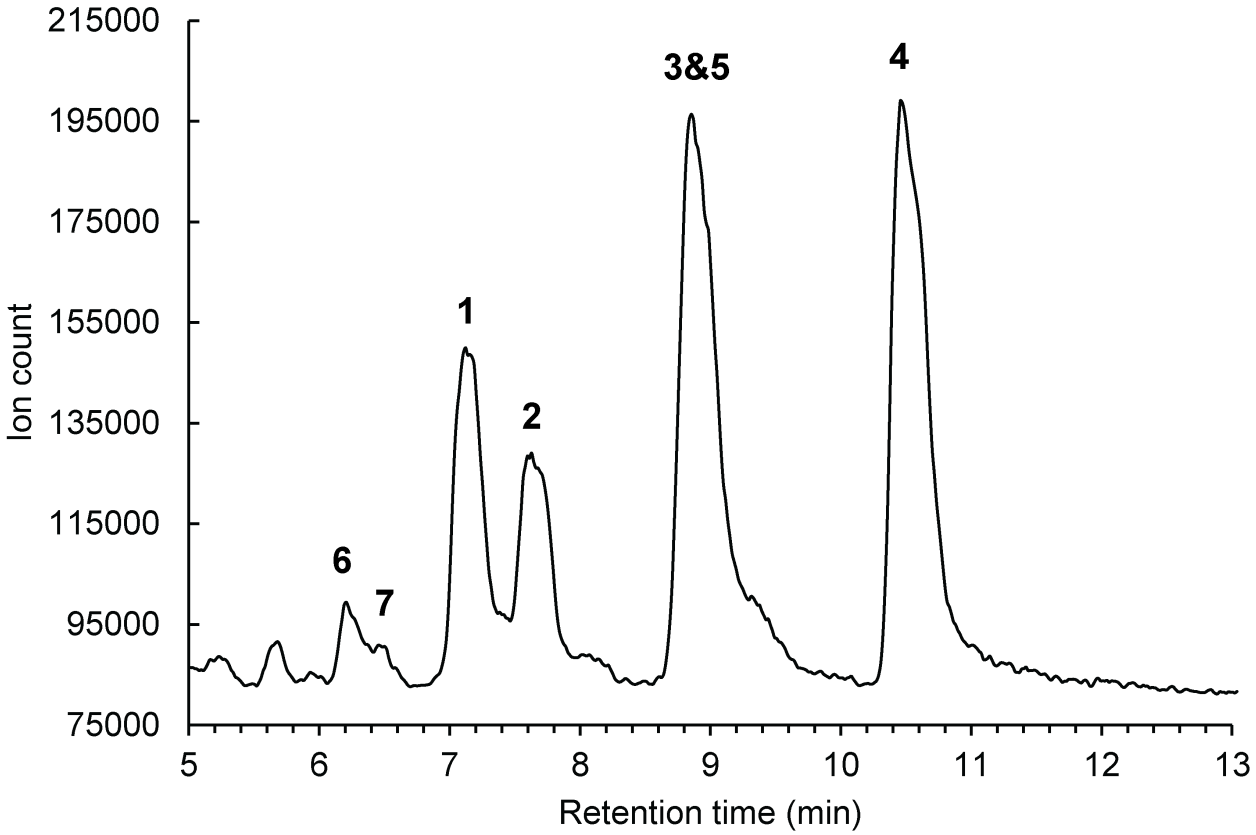


Figure S28. Major PTM congeners produced by *S. griseus* clade strains quantified for comparison of PTM production. A representative LC-MS/MS chromatogram of an extract from *Streptomyces* sp. strain JV180. The mass transitions observed for each peak are: **1**) 511 → 139; **2**) 513 → 154; **3**) 497 → 139; **4**) 495 → 139; **5**) 511 → 154; **6**) 509 → 139; and **7**) 511 → 154.

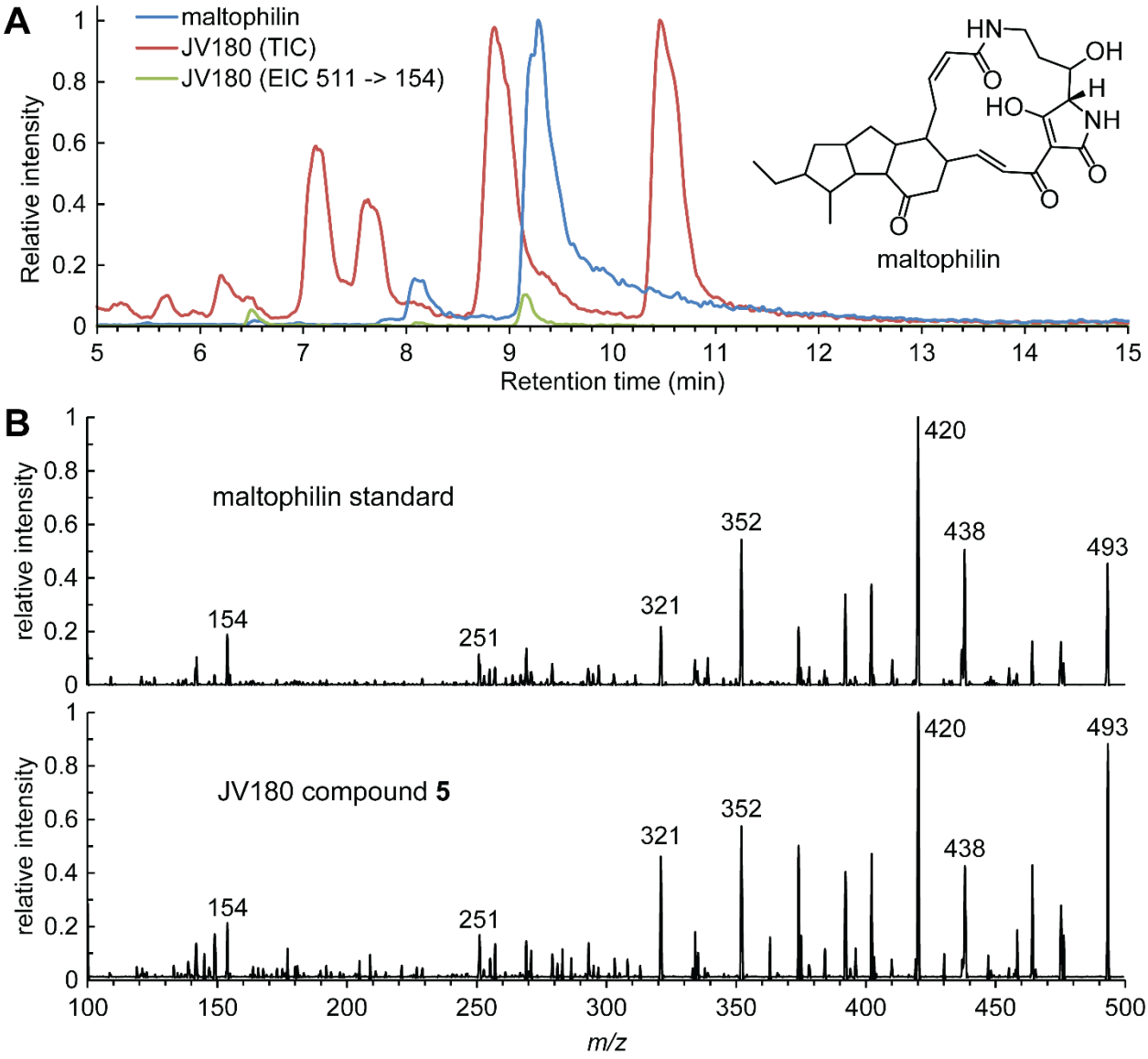


Figure S29. *S. griseus* clade strains produce maltophilin-like PTMs. (**A**) representative LC-MS/MS chromatograms of an extract from *Streptomyces* sp. strain JV180 and a maltophilin standard (structure shown in **inset** without stereocenters). (**B**) Product ion spectra produced by collision-induced dissociation (collision energy = 20 V) of the maltophilin standard and compound **5** produced by strain JV180.

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