

Supporting Material for

**Cloning and Characterization of the Ravidomycin and Chrysomycin
Biosynthetic Gene Clusters**

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Table S1. ORFs and proposed function of corresponding enzymes

ORFs	Size ^[a]	ID/SM (%) ^[b]	Closest homologue of corresponding enzyme	Protein accession no.	Proposed function
<i>ravX</i>	106	94/100	PaaB, <i>S. clavuligerus</i>	ZP_03184882	Phenylacetic acid degradation protein
<i>ravX1</i>	325	92/94	PaaA, <i>S. clavuligerus</i>	ZP_03184883	Phenylacetate-CoA oxygenase
<i>ravX2</i>	270	65/79	<i>S. pristinaespiralis</i>	YP_002198855	Unknown
<i>ravX3</i>	65	-	-	-	Hypothetical protein
<i>ravF</i>	261	77/87	SimA6, <i>S. antibioticus</i>	AAK06787	Ketoreductase
<i>ravX4</i>	161	66/79	<i>S. griseus</i>	YP_001826336	Regulatory protein
<i>ravV</i>	107	30/48	GilV, <i>S. griseoflavus</i>	ABE03981	Hypothetical protein
<i>ravH</i>	189	55/67	GilH, <i>S. griseoflavus</i>	AAP69587	NADPH-dependent FMN reductase
<i>ravOI</i>	503	57/67	JadH, <i>S. venezuelae</i>	AAV52248	Bifunctional monooxygenase-dehydratase
<i>ravG</i>	108	76/83	schP9, <i>S. sp. SCC 2136</i>	CAH10118	Cyclase
<i>ravC1</i>	82	68/79	KinC, <i>S. murayamaensis</i>	AAO65348	Acyl carrier protein
<i>ravK</i>	315		Ovma, <i>S. antibioticus</i>	CAG14969	Aromatase/cyclase
<i>ravOIV</i>	520	48/58	LndM2, <i>S. globisporus</i>	AAT64432	Monooxygenase
<i>ravA</i>	423	79/86	UrdA, <i>S. fradiae</i>	CAA60569	Ketoacyl synthase α (KS α)
<i>ravB</i>	401	71/84	<i>S. venezuelae</i>	AAB36563	Ketoacyl synthase β (KS β)
<i>ravC</i>	86	62/75	<i>S. venezuelae</i>	AAB36564	Acyl carrier protein
<i>ravOIII</i>	394	70/80	GilOIII, <i>S. griseoflavus</i>	AAP69584	Cytochrome P-450 monooxygenase
<i>ravY</i>	72	43/63	<i>Nocardia farcinica</i> IFM 10152	YP_116742	Ferredoxin
<i>ravP</i>	323	51/64	PgaH, <i>S. sp. PGA64</i>	AAK57533	Malonyl-CoA acyl carrier protein transacylase
<i>ravQ</i>	330	45/55	AknF, <i>S. galilaeus</i>	BAB72049	Acyltransferase
<i>ravJ</i>	289	42/53	SchP11, <i>S. sp. SCC 2136</i>	CAH10120	Unknown

<i>ravX5</i>	202	56/74	<i>S. violaceoruber</i>	CAA09631	Regulatory protein
<i>ravE</i>	328	77/85	<i>S. sviveus</i>	YP_002205670	NDP-glucose 4,6-dehydratase
<i>ravD</i>	355	79/88	<i>S. sviveus</i>	YP_002205669	NDP-glucose synthase
<i>ravX6</i>	55	-	-	-	Hypothetical protein
<i>ravGT</i>	379	46/66	GilGT, <i>S. griseoflavus</i>	AAP69578	C-glycosyltransferase
<i>ravNMT</i>	265	56/71	SnogX, <i>S. nogalater</i>	CAA12020	N,N-dimethyltransferase
<i>ravIM</i>	156	47/69	<i>Burkholderia phymatum</i>	YP_001858530	NDP-6-deoxy-3, 4-keto-hexulose isomerase
<i>ravAMT</i>	369	60/75	<i>Saccharopolyspora erythraea</i>	YP_001103001	Aminotransferase
<i>ravMT</i>	339	51/66	<i>S. tubercidicus</i>	AAT45298	O-methyltransferase
<i>ravOII</i>	231	68/77	GilOII, <i>S. griseoflavus</i>	AAP69583	Anthrone Monooxygenase
<i>ravRM</i>	776				
	N-terminal 535 aa,	54/67;	<i>S. galilaeus</i>	ABI15166	Dehydrogenase
	C-terminal, 241 aa	41/58	GilM, <i>S. griseoflavus</i>	AAP69591	Unknown
<i>ravX7</i>	126	-	-	-	Hypothetical protein
<i>ravW</i>	247	37/46	<i>Arthrobacter aurescens</i> TC1	YP_950015	N-acetyltransferases
<i>ravX8</i>	564	59/74	<i>S. griseus</i>	YP_001827711	Hypothetical protein

^[a]: number of amino acids of corresponding enzymes ; ^[b]: identity/similarity; aa: amino acids

Table S2. ORFs and proposed function of corresponding enzymes

ORFs	Size ^[a]	ID/SM (%) ^[b]	Closest homologue of corresponding enzyme	Protein accession no.	Proposed function
<i>chryX</i>	149	84/89	<i>S. griseoflavus</i> Tu 4000	ZP_05536903	Spore-associated protein precursor
<i>chryF</i>	261	71/83	FabG, <i>S. echinatus</i>	ABL09955	Ketoreductase
<i>chryV</i>	110	31/52	GilV, <i>S. griseoflavus</i>	ABE03981	Hypothetical Protein
<i>chryH</i>	189	62/73	<i>Kribbella flavida</i> DSM 17836	ZP_03859638	Oxidoreductase/NADH-dependent FMN Reductase
<i>chryOI</i>	513	59/70	PgaE, <i>Streptomyces</i> sp. PGA64	2QA1_A	FAD-Dependent Monooxygenase
<i>chryG</i>	109	70/83	OvmC, <i>S. antibioticus</i>	CAG14964	Cyclase
<i>chryC1</i>	83	58/71	SimA3, <i>S. antibioticus</i>	AAL15582	Acyl Carrier Protein
<i>chryK</i>	314	61/71	Tfu_1222, <i>Thermobifida fusca</i> YX	YP_289283	Bifunctional Cyclase/Dehydratase
<i>chryOIV</i>	491	55/63	OvmOII, <i>S. antibioticus</i>	CAG14970	FAD-Dependent Monooxygenase
<i>chryA</i>	422	79/87	Pd2A, <i>Streptomyces</i> sp. WP 4669	AAO65362	Ketoacyl Synthase (KS α)
<i>chryB</i>	402	70/82	UrdB, <i>S. fradiae</i>	CAA60570	Chain Length Factor (KS β)
<i>chryC</i>	87	60/70	Acp, <i>S. venezuelae</i>	AAB36564	Acyl Carrier Protein
<i>chryOIII</i>	394	71/80	GilOIII, <i>S. griseoflavus</i>	AAP69584	Cytochrome P-450 Monooxygenase
<i>chryY</i>	62	44/62	FdxD, <i>Mycobacterium tuberculosis</i> H37rv	NP_218020	Ferredoxin
<i>chryP</i>	322	49/63	PgaH, <i>Streptomyces</i> sp. PGA64	AAK57533	Malonyl-CoA Carrier Protein Transacylase
<i>chryQ</i>	327	49/58	AknF, <i>S. galileus</i>	AAF70110	Propionyl-CoA Carrier Protein Transacylase
<i>chryX1</i>	265	38/50	AurIO, <i>S. aureofaciens</i>	AAK59995	Regulator

<i>chryX2</i>	201	56/72	Gra-orf10, <i>S. violaceoruber</i>	CAA09631	Two-Component Response Regulator
<i>chryE</i>	328	77/84	SSDG_01263, <i>S. pristinaespiralis</i> ATCC 25486	YP_002200633	TDP-glucose-4,6-dehydratase
<i>chryD</i>	355	78/90	SSEG_09374, <i>S. sviveus</i> ATCC 29083	YP_002205669	Glucose-1-phosphate thymidyltransferase
<i>chryJ</i>	570	75/84	SGR_4823, <i>S. griseus</i> subsp. <i>griseus</i> NBRC 13350	YP_001826335	Major facilitator superfamily permease
<i>chryX3</i>	193	71/82	SGR_4824, <i>S. griseus</i> subsp. <i>griseus</i> NBRC 13350	YP_001826336	MarR family transcriptional regulator
<i>chryGT</i>	379	44/62	GilGT, <i>S. griseoflavus</i>	AAP69578	C-glycosyltransferase
<i>chryMT</i>	340	49/63	MetLA1, <i>S. tubercidicus</i>	AAT45282	O-methyltransferase
<i>chryOII</i>	236	65/76	GilOII, <i>S. griseoflavus</i>	AAP69583	Anthrone Monooxygenase
<i>chryRM</i>	761				
	N-terminal 518 aa.	52/67;	ORF 22, <i>S. echinatus</i>	ABL09969/	Oxidoreductase
	C-terminal 243 aa.	41/58	GilM, <i>S. griseoflavus</i>	AAP69591	Thiopurine-S-methyltransferase
<i>chryU</i>	302	46/55	AceI_0416, <i>Acidothermus cellulolyticus</i> 11B	YP_872176	TDP-4-dehydrorhamnose ketoreductase
<i>chryCMT</i>	411	61/72	PCZA361, <i>Amycolatopsis orientalis</i>	CAA11777	C-methyltransferase
<i>chryX4</i>	309	61/75	Tfu_1012, <i>Thermobifida fusca</i> YX	YP_289073	Hexokinase
<i>chryL</i>	210	37/50	GilL, <i>S. Griseoflavus</i>	AAP69590	NAD-dependent epimerase/dehydratase
<i>chryX5</i>	204	70/83	<i>S. griseus griseus</i> NBRC 13350	YP_001824391	TetR Family Regulator
<i>chryX6</i>	135	70/82	SGR_2879, <i>S. griseus</i> subsp. <i>griseus</i> NBRC 13350	YP_001824391	TetR Family transcriptional regulator
<i>chryX7</i>	120	61/74	SC2H2.18, <i>S. coelicolor</i> A3(2)	NP_631661	Hypothetical Protein
<i>chryX8</i>	202	78/83	SGR_429, <i>S. griseus</i>	YP_001821941	TetR Family regulator
<i>chryX9</i>	545	83/89	SSDG_02680, <i>S. pristinaespiralis</i> ATCC 25486	YP_002198950	Acyl-CoA Dehydrogenase

^[a]: number of amino acids in corresponding enzymes; ^[b]: identity/similarity

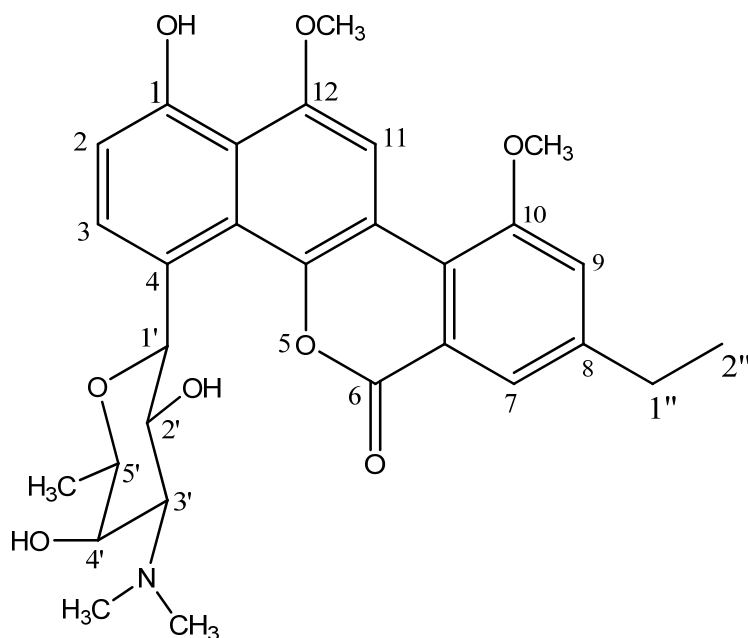
Table S3. Bacterial strains and the plasmids used in this study

Strain/ plasmid no.	Strain/ plasmid	Characteristics and relevance	References
1	<i>E. coli</i> XL1-Blue-MRF	Host for routine cloning works and for the construction of genomic libraries	Stratagene
2	<i>E. coli</i> ET12567/pUZ8002	Host for conjugal transfer of plasmid into <i>Streptomyces</i>	[1]
<i>Streptomyces</i> species			
3	<i>S. ravidus</i> C23201	Ravidomycin producer-wild strain	Wyeth
4	<i>S. albaduncus</i> AD819	Chrysomycin producer-wild strain	Wyeth
5	<i>S. lividans</i> TK24		
6	<i>S. lividans</i> TK24/cosG9B3-GT ⁻	Produces defucoGE and defucoGM-the strain harbors the GilGT deleted mutant cosmid cosG9B3-GilGT ⁻	[2]
7	<i>S. lividans</i> TK24/cosG9B3-OIII ⁻	Produces defucoGE and defucoGM-the strain harbors the GilOIII deleted mutant cosmid cosG9B3-OIII ⁻	[2]
8	<i>S. lividans</i> TK24/cosG9B3-OII ⁻	Produces dehydrorabelomycin, dehydro-homo-rabelomycin and -the strain harbors the GilOII deleted mutant cosmid cosG9B3-OII ⁻	[3]
9	<i>S. lividans</i> TK24/cosG9B3-OIV ⁻	Produces rabelomycin and homorabelomycin-the strain harbors the GilOIV deleted mutant cosmid cosG9B3-OIV ⁻	[4]
10	<i>S. lividans</i> TK24/cosRav32	Produces 2,3-dehydroUWM6, 2,3-dehydro-HomoUWM6, preGM-O-quinone, preGV-O-quinone, 4'-O-deacetylraavidomycin M, 4'-O-deacetylraavidomycin E -the strain harbors the cosRav32	This work
11	<i>S. lividans</i> TK24/cosG9B3-GT ⁻ /pRavGT	Produces GE and GM-pRavGT was introduced into strain no. 6	This work
12	<i>S. lividans</i> TK24/cosG9B3-OIII ⁻ /pRavOIII	Produces GV and GM-pRavOIII was introduced into strain no. 7	This work
13	<i>S. lividans</i> TK24/cosG9B3-OIII ⁻ /pChryOIII	Produces GV, GE and GM-pChryOIII was introduced into strain no. 7	This work
14	<i>S. lividans</i> TK24/cosG9B3-OII ⁻ /pChryOII	Produces GV, GE and GM -pChryOII was introduced into strain no. 8	This work
15	<i>S. lividans</i> TK24/cosG9B3-OIV ⁻ /pChryOIV	Produces GV and GM-pChryOIV was introduced into strain no. 9	This work
Plasmids/cosmids			
16	pOJ446	Vector for cosmid library construction	[5]
17	cosRav32	Entire ravidomycin gene cluster cloned into pOJ446	This work
18	cosChry1-1	Partial chrysomycin gene cluster cloned into pOJ446	This work
19	cosChryF2	Partial chrysomycin gene cluster cloned into pOJ446.	This work

20	pEM4		[6]
21	pUWL201PW	<i>E. coli-Streptomyces</i> shuttle plasmid that contains ermEp*for the expression of genes in <i>Streptomyces</i>	[7]
22	pRavGT	<i>ravGT</i> cloned into pUWL201PW	This work
23	pRavOIII	<i>ravOIII</i> cloned into pEM4	This work
24	pChryOIV	<i>chryOIV</i> cloned into pEM4	This work
25	pChryOIII	<i>chryOIII</i> cloned into pEM4	This work
26	pChryOII	<i>chryOII</i> cloned into pEM4	This work
27	PCR-Blunt-II-TOPO	To clone PCR products	Invitrogen

Table S4. Oligo primers used in this study

Oligo's name	Oligonucleotide sequence
ChryOII_for	5'-ATTCTAGACCACCCGTACCGAGCCAC-3'
ChryOII_rev	5'-ATGAAATCCCCGTCGTCCGCCGCGCG-3'
ChryOIII_for	5'-ATTCTAGAGACCCGTTCCCGCAGCTC-3'
ChryOIII_rev	5'-ATGAAATTCGAACACGAAGGCTGTCGT-3'
ChryOIV_for	5'-GCGGTTCAGACCGCGCGCACCGGCTGCCCGGG-3'
ChryOIV_rev	5'-CCGGTGAATTCACAGGAATCCGATGGCGGT-3'
CM_cons_F1	5'-GCCCTVGTSAACSGGNGNACCAGY-3'
CM_cons_R1	5'-GTTGCCVAGNCCGCCGCASACGTT-3'
RavOIII_for	5'-AAGTCTAGAAGGAGACGGAGCACCATGGCG-3'
RavOIII_rev	5'-CGGAATTCCTCTGCTCGTCGGCCGTGGTCACGT-3'
RavGT_for	5'-ATCATATGAAAGTCCTGTTTCATCGCAGCGGGA-3'
RavGT_rev	5'-ATGAAATTCATCCGCGCACCCAGTCCTTCCAG-3'
KS_probe_for	5'-GTSTCSACSGGSTGYACSTCSGGS-3'
KS_probe_rev	5'-SCCGATSGCSCCSAGSGARTGSCC-3'
DH_probe_for	5'-CSGGSGSSGCSGGSTTCATSGG-3'
DH_probe_rev	5'-CAGTGGTCSACGTGSAGCCACTCSCG-3'



^1H and ^{13}C NMR data of deacetylravidomycin E (31): ^1H NMR (CDCl_3 , 300 MHz) δ 9.83 (br, s, 1H, -OH1, D_2O exchangeable) 8.51 (s, 1H, H11), 7.97 (d, $J = 9.0$ Hz, 1H, H3), 7.95 (d, $J = 3.3$ Hz, 1H, H7), 7.25 (d, $J = 3.3$ Hz, 1H, H9), 7.08 (d, $J = 9.0$ Hz, 1H, H2), 6.01 (d, $J = 9.0$ Hz, 1H, H1'), 4.44 (t, $J = 9.0$ Hz, 1H, H2'), 4.16 - 4.26 (m, 2H, H4', H5'), 4.15 (s, 3H, 12-OMe), 4.12 (s, 3H, 10-OMe), 3.35 (dd, $J = 9.0, 3.0$ Hz, 1H, H3'), 2.95 (s, 6H, 3'-N(Me) $_2$), 2.83 (q, $J = 7.5$ Hz, 2H, H1''), 1.32 (t, $J = 7.5$ Hz, 3H, H2'') 1.31 (d, $J = 6.0$ Hz, 3H, 6'-Me); ^{13}C NMR (CDCl_3 , 75 MHz) δ 170.2, 161.0, 157.5, 155.0, 152.5, 146.8, 129.8, 125.3, 124.7, 122.4, 122.4, 121.3, 117.7, 116.0, 114.5, 113.0, 102.8, 79.8, 78.5, 71.6, 69.6, 68.8, 68.4, 56.6, 43.1, 29.2, 16.95, 15.37.

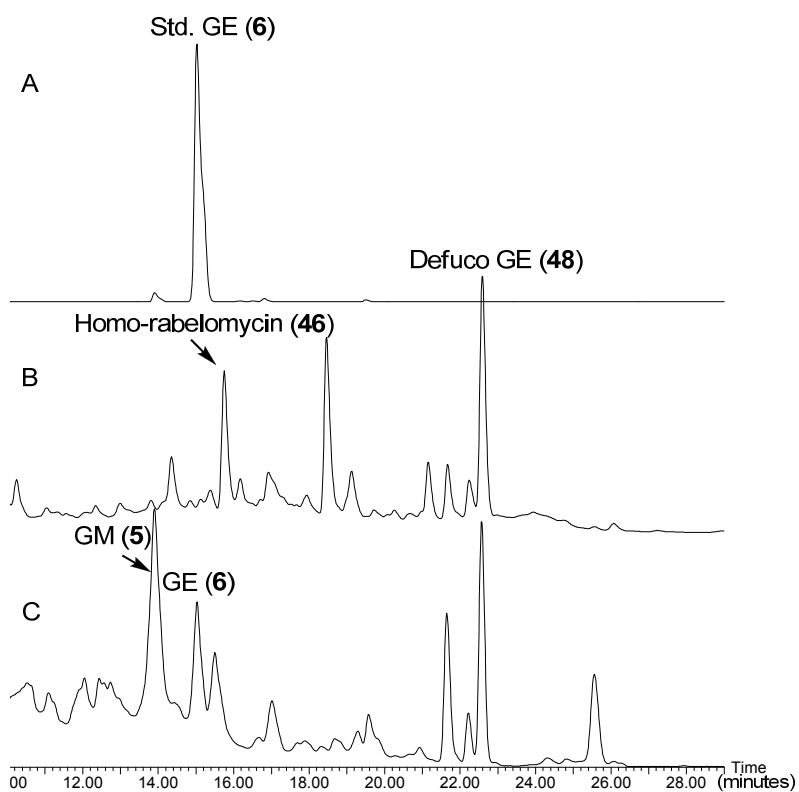
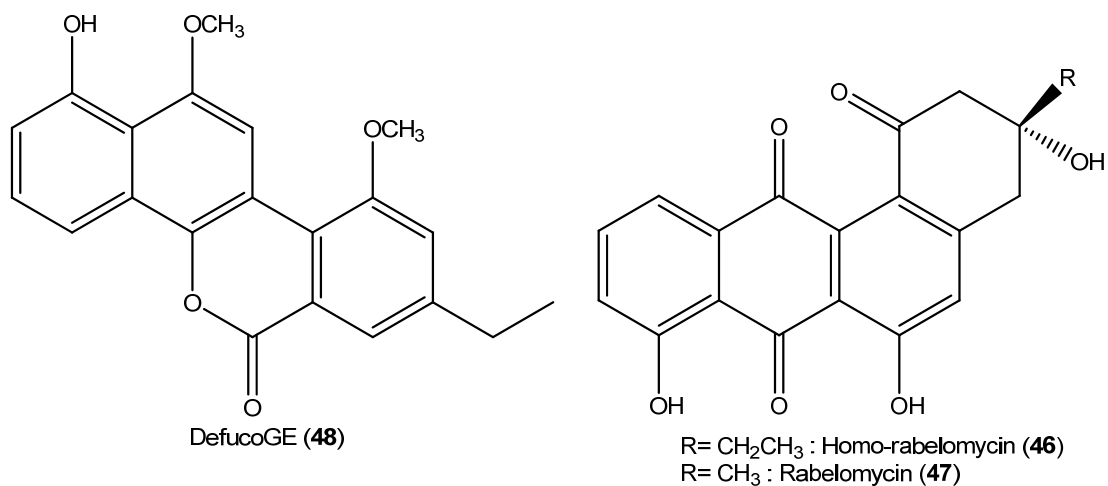


Figure S1. HPLC analyses of the compounds: A, standard GE; B, metabolites from the *S. lividans* TK24(cosG9B3-GilGT⁻) mutant; C, metabolites from the *S. lividans* TK24(cosG9B3-GilGT⁻/pRavGT).

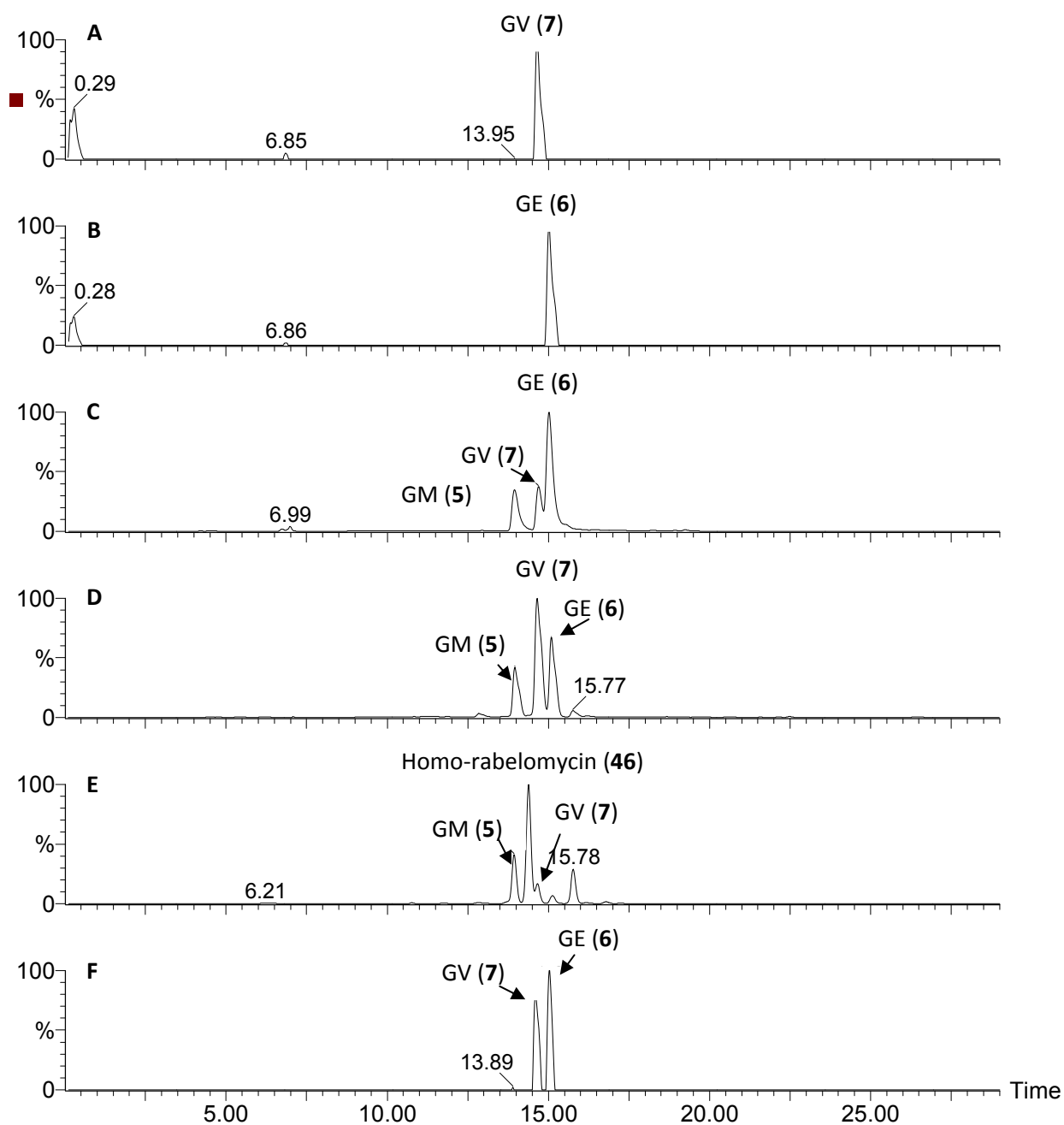


Figure S2. HPLC analyses of the metabolites: trace A, gilvocarcin V (Rt. 14.65 min) isolated from *S. lividans* TK24(cosG9B3); trace B, gilvocarcin E (Rt. 15.03 min) isolated from the *S. lividans* TK24(cosG9B3-GilOIII⁻) mutant; trace C, metabolites isolated from the *S. lividans* TK24(cosG9B3-GilOIII⁻/pRavOIII) mutant; trace D, metabolites isolated from the *S. lividans* TK24(cosG9B3-GilOIII⁻/pChryOIII) mutant; trace E, metabolites isolated from the *S. lividans* TK24(cosG9B3-GilOIV⁻/pChryOIV) mutant; trace F, metabolites isolated from the *S. lividans* TK24(cosG9B3-GilOII⁻/pChryOII) mutant. Arrows indicate the important metabolites. Y and X axes represent relative absorbance and retention time (in minutes), respectively.

		1	50
CosH	(1)	-MNLV TCAGFIGSHF VRTL LSGGY PGHEDDR VT VLD KLTYAC TLN NLP	
RhoH	(1)	-MNI PVTCAGFIGSHF VRTL LSGGY PGHEDDR VT V VD KLTYAC TLN NLP	
Gra-ORF17	(1)	-MRL LVTCAGFIGSHY VEI LAGS Y PESDD VH VT V VR KLTYAC RRD NLP	
LanHI4	(1)	MK LIITCAGFIGSHY VRTL LN DG YEDW KGAH VT VLD KLTYAC N RD NLP	
SchS5	(1)	-MRL LVTCAGFIGSHF VRTL LV EGSY SGWED MQ VT AL KLTYAC N RD NLP	
		51	100
CosH	(50)	RA HPRLTFVHGDI CBTT LL DKVF PCHE AVV HFAE S HVDR SVAGAE FVR	
RhoH	(50)	PR HPRLTFVHGDI CBTP LL GKVF PCHE AVV HFAE S HVDR SVAGAE FVR	
Gra-ORF17	(50)	EA HPRLDFVHGDI CD RD LL DRVL PG HD AVV HFAE S HVDR SLTG PGF FVR	
LanHI4	(51)	EA HPRLTFVHGDI CBFE LL LELL PGH AVV HFAE S HVDR SLES AEF VH	
SchS5	(50)	AS HERLVEVRGD VCD RKLL REL VP GH DA AVV HFAE T HVDR SLE GAGD E FR	
		101	150
CosH	(100)	T NVLG TQ ALL EA LR HGIC VEFV Q VST DE TYG SI AE CS WTE DE PL LPNS PFY	
RhoH	(100)	T NVLG TQ ALL EA LR HGIC VEFV Q VST DE TYG SI AE CS WTE DE PL LPNS PFY	
Gra-ORF17	(100)	T NMG TQ QLD RA LH AG VDR V LHV ST DEV Y G SL DS CT WTE DS PL LPNS PFY	
LanHI4	(101)	T NVT G TQ RL DA V L AT R V KRV V HV ST DEV Y G SI DEC SW TE EW PL APNS PFY	
SchS5	(100)	T NVLG TQ TL DA V L D SG VER V VH ST DEV Y G SI DEC SW TE EW PL APNS PFY	
		151	200
CosH	(150)	RA SKA SAD LI ARS YVRT H GLD V RIT RC ANN Y GP Q HPE K LIP LE FV RL LD	
RhoH	(150)	RA SKA SAD LI ARS YVRT H GLD V RVT RC ANN Y GP Q HPE K LIP LE FV RL LD	
Gra-ORF17	(150)	RA SKA ST TWS RA PTT V R H GLD V RIT RC SNN Y GP Q HPE K LIP LE FV RL LD	
LanHI4	(151)	SA SKA AS DL ARS YVRT H GLD L SIT RC SNN Y GP Q HPE K LIP LE FV RL LD	
SchS5	(150)	RA SKA GS DL V RAY V RS H CV D L SIT RC SNN Y GP Q H PE K LIP LE FV RL LD	
		201	250
CosH	(200)	G QP V PLY CD EN L RE WL HVDD H CA VR L V L D GR PC GE V YN V GGC TH L T NK	
RhoH	(200)	G QP V PLY CD EN L RE WL HVDD H CA VR L V L D GR PC GE V YN V GGC TH L T NK	
Gra-ORF17	(200)	G RQ V PLY CD GR N V RE WL H VDD H CR AL Q L V L T K R AG ET Y N I GGC SG M S NR	
LanHI4	(201)	G EQ V PLY CD GN I RE WL HVDD H CR AI D L V L N K R AG ET Y N I GGC NE Q T NR	
SchS5	(200)	G RR V PLY CD GN V RE WL HVDD H CR GI D L V L N Q R AG ET Y N I GGC NE R T NL	
		251	300
CosH	(250)	EM TGR L LAL C GR D W DL VRR V ADR NC HD FR Y AV DD S KIR REL GY AP W S L E	
RhoH	(250)	EM TGR L LAL C GR D W DL VQR V ADR NC HD FR Y AV DD S KIR REL GY AP W S L E	
Gra-ORF17	(250)	EM TAR L LDL L CAD W D V R H Q E DR L C HD FR Y AI DD S K IR E L GY AP W S L E	
LanHI4	(251)	RI TER L LAL E T Q D W S K VR H VP DR KA HD FR Y SL DE S K IR E L GY AP RI TFE	
SchS5	(250)	RI TE Q L LE IT C AGA E RI Q R V PR DR KA HD FR Y S IE T K IR E L GY AP RI GF E	
		301	329
CosH	(300)	D GL RET VE W Y AA HR DH W D ARE EG GA DE GC Y -	
RhoH	(300)	D GL RET VE W Y AA HR DH W D ARE EG GD GC Y -	
Gra-ORF17	(300)	S GL GA V W Y RD HP DE WR AP S -	
LanHI4	(301)	Q GL AD T V W Y HD N PG W K CT K KN Q C ---	
SchS5	(300)	Q GL A E T V W Y RD NP D W K AA K H GT D RA V A	

Figure S3. Multiple sequence alignment of 4,6-dehydratases used for designing degenerate primers (DH_prob_for and DH_prob_rev): solid arrows indicate the amino acid residues used in this study; the dotted arrow represents the original amino acid sequence region used by Bechthold et al. for the reverse primer^[8]

References

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