Biochemical and Structural Characterization of Germicidin Synthase: Analysis of a Type III Polyketide Synthase that Employs Acyl-ACP as a Starter Unit Donor

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Methods

LC-MS of enzymatic reactions. LC-MS analysis was performed with a Shimadzu LCMS-2010EV mass spectrometer (Columbia, MD) with an Agilent Zorbax Stablebond C18 reverse phase column (4.6 x 150 mm). 10 μ l samples were separated under isocratic conditions of 25% CH₃CN (vol/vol) with 0.1% (vol/vol) formic acid over 20 min at a flow rate of 0.5 mLl/min at room temperature. Samples were desalted online by diverting the flow to waste for five min before switching the divert valve to the MS detector. The absorbance at 290 nm was monitored and profile mode data was gathered from m/z 100-300 utilizing electrospray ionization in scanning ion mode.

Characterization of enzymatically-synthesized germicidin A. The NMR data were recorded at 600 MHz for ¹H on a Varian Performa IV spectrometer with a Protasis CapNMR inverse dual-band probe. Proton chemical shifts are reported relative to the residual proton signal of the deuterated solvent MeOH- d_4 (3.29 ppm).

Germicidin A: ¹H NMR (600 MHz, MeOH-*d*₄) δ 1.01 (t, *J*=7.4 Hz, 3H), 1.20 (d, *J*=7.0 Hz, 6H), 2.37 (q, *J*=7.4 Hz, 2H), 2.68 (spt, *J*=7.0 Hz, 1H), 5.91 (s, 1H).

UV: λ_{max} (202, 290 nm). Low resolution ESI-MS: $[M+H]^+ = 183.00 \text{ m/z}$; expected = 183.10.

a)															
	(1)	1 1	0	20	30		4	0		50		60			75
GCS_S_coelicolor_A3(2)	(1)				MAAYLC	A <mark>PA</mark> Y	/IHGEH	SVET	R <mark>E IV</mark>	EEVF	R <mark>GRHP</mark> H	APWAPF	<mark>XI</mark> DG <mark>I</mark>	AAS	[GIE <mark>S</mark>
GCS_S_lividans	(1)			<mark> </mark>	MAAYLC	AP <mark>A</mark> Y	/IHGEH	SVET	R <mark>EIV</mark>	<mark>e</mark> evf	R <mark>GRHP</mark> H	APWAPF	<mark>{I</mark> DG <mark>I</mark>	AAS	GIE <mark>S</mark>
GCS_S_sviceus	(1)					· <mark>1</mark>	<mark>4IHG</mark> KH	P <mark>V</mark> R <mark>I</mark>	SQIV	DEVF	R <mark>GRHP</mark> N	I <mark>AA</mark> WAPF	<mark>{I</mark> DG <mark>I</mark>	AAS	CGIE <mark>T</mark>
GCS_S_viridochromogenes	(1)				MA <mark>A</mark> YLC	P <mark>PA</mark>	/IHGRH	T <mark>V</mark> KI	SQ <mark>IV</mark>	A <mark>evf</mark>	RE <mark>RHP</mark> H	APWAPF	<mark>{I</mark> DG <mark>I</mark>	AAS	<mark>GIE</mark> N
GCS_S_scabiei	(1)			<mark> </mark>	MAAYLC	PP <mark>S</mark> V	/IHG <mark>K</mark> H	A <mark>V</mark> R <mark>T</mark>	SQ <mark>IV</mark>	DEVF	R <mark>GRHP</mark> N	I <mark>AA</mark> WAPF	<mark>{I</mark> DG <mark>I</mark>	<mark>A</mark> DS#	A <mark>GIE</mark> T
GCS_S_zinciresistens	(1)	MRRAAVVQDT	AVVTSGLA	SRRKVPD	MTAYLC	P <mark>P</mark> A	/IHGEH	A <mark>v</mark> k1	S <mark>EIV</mark>	A <mark>EV</mark> S	SA <mark>RHP</mark> Ç	AAWAPF	<mark>≀I</mark> GS <mark>I</mark>	AAG 1	[GIE <mark>S</mark>
THNS_S_coelicolor_A3(2)	(1)				-M <mark>A</mark> TLC	RPS	/SVP <mark>EH</mark>	V <mark>IT</mark> M	E <mark>E</mark> TL	ELAF	R <mark>RH</mark> TI	H <mark>P</mark> QL <mark>P</mark> I	_ALR <mark>I</mark>	IEN	<mark>ľG</mark> VRT
FabH_E_coli	(1)			<mark> </mark>	MYTK <mark>I</mark> I	GTG	SYLP <mark>e</mark> ç	VR <mark>T</mark> N	ia <mark>dl</mark> e	KM <mark>v</mark> e	TS	-DEWIN	/TR	<mark>1</mark>	<mark>'GI</mark> RE
CHS_M_sativa	(1)	MV	SVSEIRKA	QRAEGPA'	FILA <mark>I</mark> G	TAN	PANCVE	QS <mark>T</mark> Y	'P <mark>D</mark> FY	FK <mark>I</mark> I	NSEHK	TELKE	FQRM	CDK	MI KR
Steely1_D_discoideum	(1)		-GSLSRLS	YKSNNNS	FVLG <mark>I</mark> G	ISVI	PGEPIS	QQ <mark>S</mark> I	KDSI	SNDE	'SDK	AETNE <mark>P</mark>	(VKR I	FEQ <mark>s</mark>	QIKT
PhID_P_fluorescens	(1)				-M <mark>S</mark> TLC	KPS1	LLFPP <mark>H</mark>	K <mark>IT</mark> Ç	QQ <mark>MI</mark>	DHLE	HL <mark>H</mark> DE)H <mark>P</mark> RMAI	lakr <mark>n</mark>	IQN	Q <mark>V</mark> NE
RppA_S_griseus	(1)				-M <mark>A</mark> T <mark>LC</mark>	R <mark>P</mark> A	AVP <mark>eh</mark>	VITM.	IQQT <mark>L</mark>	DLA <mark>F</mark>	ET <mark>H</mark> AG	h <mark>p</mark> qrdi	. <mark>V</mark> LR <mark>I</mark>	IQN	<mark>ľG</mark> VQT
Consensus	(1)			I	M A LC	PA	/IHGEH	VTT	EIV	DEVF	R RHP	APWAPF	≀I I	A 1	CGIET

	(76)	76	9	0	100	110		120	13	0 1	40 150
GCS_S_coelicolor_A3(2)	(51)	<mark>R</mark> GWMLPL	EAAVAPGG	<mark>G</mark> GD	lgaare <mark>a</mark>	LVR <mark>DGF</mark> I	FEQ <mark>D</mark> AN <mark>F</mark>	RA <mark>IA<mark>A</mark>LKA</mark>	<mark>V</mark> PAS <mark>QT</mark>	VQERTAPAN	IE <mark>AV<mark>QAYGE</mark>RA</mark>
GCS_S_lividans	(51)	<mark>r</mark> gwmlpi	EAAVAPGG	<mark>G</mark> GD	lgaare <mark>a</mark>	LVR <mark>DGF</mark> I	FEQ <mark>D</mark> AN <mark>F</mark>	RA <mark>IA</mark> ALKA	<mark>VP</mark> AS <mark>QT</mark>	VQERTAPAN	IE <mark>AV<mark>QAYGE</mark>RA</mark>
GCS_S_sviceus	(42)	<mark>r</mark> gwmlpi	EAAVAPGS	<mark>G</mark> SGLPAAG	IRPTRE <mark>A</mark>	LAG <mark>DGF</mark> I	req <mark>d</mark> vd <mark>f</mark>	RVIA <mark>A</mark> LEA	<mark>I P</mark> AP <mark>QT</mark>	VQERTAPAN	IS <mark>AV<mark>QSYGE</mark>RA</mark>
GCS_S_viridochromogenes	(51)	<mark>r</mark> gwmlpi	ETAVAPGE	GS-GLAAG	ARAARE <mark>A</mark>	LAR <mark>DGF</mark> A	AEP <mark>D</mark> VD <mark>F</mark>	RVIA <mark>S</mark> LEA	<mark>I P</mark> VP <mark>QT</mark>	VQERTAPAN	IA <mark>AV</mark> R <mark>SYGE</mark> QA
GCS_S_scabiei	(51)	<mark>r</mark> gwmlpi	EAAVAPGS	<mark>G</mark> SGLLAVG	IGPARE <mark>A</mark>	LAG <mark>DGF</mark> I	req <mark>d</mark> vd <mark>f</mark>	RVIA <mark>A</mark> LEA	<mark>I P</mark> AP <mark>QT</mark>	VQERTAPAW	IS <mark>AVQ</mark> SYGERA
GCS_S_zinciresistens	(76)	<mark>r</mark> gwmlpl	ESAVAPGR	<mark>G</mark> GALRGAG	TGPAQQ <mark>A</mark>	llr <mark>egf</mark> s	sqq <mark>d</mark> vd <mark>f</mark>	RVIA <mark>S</mark> LEA	LPTAQT	VQERTAPAW	IE <mark>AVQ<mark>A</mark>YGERA</mark>
THNS_S_coelicolor_A3(2)	(50)	RHI <mark>VQP</mark> I	EDT <mark>L</mark> EHPG							•FE <mark>Dr</mark> nkvye	REAK <mark>S</mark> RVPAV
FabH_E_coli	(42)	RHIAAPN	ET								<mark>v</mark> stm <mark>g</mark> fea
CHS_M_sativa	(68)	RYMY <mark>I</mark> TE	E ILKENPN					VC	EYMAP <mark>S</mark>	LDA <mark>R</mark> QDMVV	VE <mark>V</mark> PRL <mark>G</mark> KEA
Steely1_D_discoideum	(63)	RHL <mark>V</mark> RDY	TKPENSIK	FR					HLET	ITDVNNQFF	kv <mark>v</mark> pdl <mark>a</mark> qqa
PhID_P_fluorescens	(50)	RHL <mark>VLP</mark> I	DELAVHTG							FTH <mark>RS</mark> IVYE	REARRMSSLA
RppA S griseus	(50)	RHLVOPI	EKT <mark>LA</mark> HPG							FEV <mark>R</mark> NOVYE	AEAKTRVPEV
Consensu	ıs (76)	RGWMLPL	e avapg	G	A	L DGF	DF	R IAAL A	IP QT	VQERTAPAW	AV SYGE A
	(151)	151	160	170	18	30	190	2	00	210	225
CCS S coolicolor $A3(2)$	(131)			DOT THONO							
	(121)	ARGALQI		DCLITSNS			NDT DT E			AGINSLAI	
	(121)	ARGALQI					NDT DT E			AGINSLAI	
GCS_S_SVICEUS	(11/)		AGLDAADV	DCLITSHS					ATQWAC		
GCS_S_VINUOCITOTIOgenes	(125)	ARGALQI	AGLDPADV	DCLITSNS	TT-PALP	JLDVALA	ADRLPLF		ATQWAC		ADLVAADPD
	(120)	ARGALQ	AGLDAAHV	DOLITSHS	TT-PALP	STDART	MRLPLF	KDVLLLP	ATQWAC	LAGTRSLAI	ADLVAADPD
GCS_S_zinciresistens	(151)	ARGALQA	AGLDASDV	DCLITSHS		GLDV'I'LA	ADRLPLF		ATQWAC	LAGTRSLAI	ADLVAADPD
I HNS_S_COEIICOIOr_A3(2)	(84)		AELLATDI	DVIIYVSC	IG-FMMP		NEMGFL	STTRQLE		AAGGAAINF	AHDFCTAYPE
	(59)	ATRALEM	AGIERDQI	GL <mark>IV</mark> VATT	SATH <mark>A</mark> FP	SAACQIÇ	2SMLG1K	GCPAF	DVAAAC	AGFTYALSV	ADQY <mark>V</mark> K <mark>S</mark> GAV
CHS_M_sativa	(110)	AVKAIKE	WGQPK <mark>S</mark> KI	THLIVCTT	SG-VDMP	GADYQL1	'KL <mark>LGLF</mark>	PYVKRYM	MY <mark>Q</mark> QGC	FAGGTVLRI	AKDLAENNKG
Steely1_D_discoideum	(103)	CLR <mark>AL</mark> KD	WGGDKGDI	TH <mark>IVS</mark> VT <mark>S</mark>	IG-II <mark>I</mark> P	D <mark>V</mark> NFKLI	IDL <mark>LGL</mark> N	IK <mark>DV</mark> ER <mark>V</mark> S	INLMC	LAGLS <mark>SL</mark> RT	'AASLAKASPR
PhID_P_fluorescens	(84)	AREATEN	AGLTTDDI	RMVAVTPC	IG-FMMP	SLTAHLI	INDLGLF	RTST <mark>VQLP</mark>	IAQL <mark>C</mark> C	M <mark>AG</mark> AAAINF	ANDFA <mark>S</mark> LSPQ
RppA_S_griseus	(84)	V <mark>R</mark> R <mark>AL</mark> AN	AETEPSEI	DLIVYVSC	IG-FM <mark>MP</mark>	S <mark>l</mark> taw <mark>i</mark> i	I <mark>NSM</mark> GF <mark>F</mark>	P <mark>E</mark> TRQ <mark>LP</mark>	IAQL <mark>Q</mark> C	A <mark>AG</mark> GA <mark>AI</mark> NF	AHDFCVAYPD
Consensu	us(151)	ARGALQI	AGLD ADI	DCLITS S	TT PALP	GLDV LA	ANRLPLF	R DVLLLP	ATQWAC	IAGTRSLAI	AADLVAADPD
	(226)	226	2	40	250	260		270	28	0 _2	90 300
GCS_S_coelicolor_A3(2)	(226) (195)	226 <mark>RVV</mark> LV <mark>V</mark> I	2. Sealstty	40 <mark>Q-</mark> PADDTL	_250 ESLIVRL	260 L <mark>FAD</mark> TAN	/ <mark>A</mark> AVVTO	270	,28 E <mark>SVLRL</mark>	0 _2 .D <mark>A</mark> AWHHTLE	90 300 <mark>GTR</mark> DLHRL <mark>E</mark> T
GCS_S_coelicolor_A3(2) GCS_S_lividans	(226) (195) (195)	226 RVVLVVI RVVLVVI	2 SEALSTTY SEALSTTY	40 Q-PADDTL Q-PADDTL	_250 ESLIVRL: ESLIVRL:	260_ L <mark>FADTAX</mark> L <mark>FADTAX</mark>	/ <mark>a</mark> avvto / <mark>a</mark> avvto	270 GRPRP GRPRP	28 E <mark>SVLRL</mark> E <mark>SVLRL</mark>	0 ,2 .D <mark>A</mark> AWHHTLE .D <mark>A</mark> AWHHTLE	90 300 GTRDLHRLET GTRDLHRLET
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus	(226) (195) (195) (191)	226 RVV <mark>LVV</mark> I RVVLVVI RVV <mark>LVV</mark> I	2 SEALSTTY SEALSTTY AEALSTTY	40 Q-PADDTL Q-PADDTL Q-PADDTL	_250 ESLIVRL: ESLIVRL: ESLIVRL:	260, L <mark>FADTAV</mark> LFADTAV LFADTAV	/ <mark>a</mark> avvto / <mark>a</mark> avvto / <mark>a</mark> avvto	270 GRPRP GRPRP GRPRP	28 E <mark>SVLRL</mark> ESVLRL E <mark>SVLRL</mark>	0 _2 .D <mark>A</mark> AWHHTLE .D <mark>A</mark> AWHHTLE .D <mark>A</mark> AWHHTLE	90 300 GTRDLHRLET GTRDLHRLET GTQDLHRLET
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes	(226) (195) (195) (191) (199)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL	260, LFADTAN LFADTAN LFADTAN LFADTAN	/ <mark>A</mark> AVVTC / <mark>A</mark> AVVTC / <mark>A</mark> AVVTC /AAVVTC	270 GRPRP GRPRP GRPRP GRPRP	28 ESVLRL ESVLRL ESVLRL ESVLRL	0 2 D <mark>A</mark> AWHHTLE D <mark>A</mark> AWHHTLE D <mark>A</mark> AWHHTLE D A AWHHTLE	90 300 GTRDLHRLET GTRDLHRLET GTQDLHRLET
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_cobiei	(226) (195) (195) (191) (199) (200)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY AEALSTTY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL	_250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL	260, LFADTAV LFADTAV LFADTAV LFADTAV LFADTAV	/ <mark>a</mark> avvto /aavvto /aavvto /aavvto /avvto	270 RPRP RPRP RPRP RPRP RPRP	28 E <mark>SVLRL</mark> ESVLRL ESVLRL ESVLRL E <mark>S</mark> TLRL	0 2 D <mark>A</mark> AWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE	90 300 GTRDLHRLET GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_scabiei GCS_S_zinciresistens	(226) (195) (195) (191) (199) (200) (225)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY SEALSTTY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-SADDTL	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL ESLIVRL	260 LFADTAN LFADTAN LFADTAN LFADTAN LFADTAN LFADTAN	/ <mark>A</mark> AVVTG / <mark>A</mark> AVVTG / <mark>A</mark> AVVTG / <mark>A</mark> AVVTG / <mark>A</mark> VVTG / <mark>A</mark> AVVTG	_270 GRPRP GRPRP GRPRP GRPRP GRPRP GRPRP	28 E <mark>SVLRL</mark> ESVLRL ESVLRL ESVLRL QA <mark>L</mark> LRL	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DSAWHHTLE	90 300 GTRDLHRLET GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLDT GTRDLHRLET
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2)	(226) (195) (195) (191) (199) (200) (225) (158)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY SEALSTTY CEFCSLCY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-SADDTL Q-SADDTL Q-PTDLG	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSL <mark>I</mark> CNG	260 LFADTAV LFADTAV LFADTAV LFADTAV LFADTAV LFADTAV LFGDGIA	/ <mark>A</mark> AVVTG /AAVVTG /AAVVTG /AAVVTG /AAVVTG AAVVTG	270 RPR P RPR P RPR P RPR P RPR P RPR P RPR P RG	28 ESVLRL ESVLRL ESVLRL ESVLRL ESTLRL QALLRL GTGVRL	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DSAWHHTLE RNGSYLT	90 300 GTRDLHRLET GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTRDLHRLET KTEDWIMYDV
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli	(226) (195) (195) (191) (199) (200) (225) (158) (132)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY SEALSTTY CEFCSLCY SDVLARTC	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-SADDTL Q-PTDLG <mark>V</mark> DP	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSL <mark>I</mark> CNG TDRGTI	260 LFADTAV LFADTAV LFADTAV LFADTAV LFADTAV LFADTAV LFGDGIF IFGDGAG	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG	270 RPR P RPR P RPR P RPR P RPR P RPR P RC P RE P RE	28 ESVLRI ESVLRI ESVLRI ESTLRI QALLRI GTGVRI PGIIST	0 2 DAWHHTLE DAWHHTLE DAWHHTLE DAWHHTLE DSAWHHTLE FRNGSYLTE HLHADGSYC	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV ELLTLPNADR
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa	(226) (195) (195) (191) (199) (200) (225) (158) (132) (184)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA ARVLVVC	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY SEALSTTY CEFCSLCY SDVLARTC SEVTAVTE	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-SADDTL Q-SADDTL Q-PTDLG DP RGP S DTHL	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSL <mark>I</mark> CNG TDRGTI <mark>I</mark> DSL <mark>V</mark> GOA	260 LFADTAN LFADTAN LFADTAN LFADTAN LFADTAN LFGDGIA LFGDGAG LFGDGAG	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVTG AAVTG AAVTG	270 RPR P RPR P RPR P RPR P RPR P RPR P RPR P REE	28 ESVLRI ESVLRI ESVLRI ESTLRI QALLRI GTGVRI PGIIST KPIFEM	0 2 DAAWHHTLE DAAWHHTLE JDAAWHHTLE JDAAWHHTLE JDAAWHHTLE FRNGSYLE FHLADGSYL VWTAOTTE	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV ELILT_PNADR DSEGIJGHL
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1 D discoideum	(226) (195) (195) (191) (199) (200) (225) (158) (132) (184) (177)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA ARVLVVC ARVLVVC NRILVVC	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY SEALSTTY CEFCSLCY SDVLARTC SEVTAVTE TEVCSLHE	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-SADDTL Q-SADDTL Q-PTDLG DP RGP S DTHL SNTDGG	_250 ESLIVRL: ESLIVRL: ESLIVRL: ESLIVRL: GSLICNC: GSLICNC: DSLVGQA DOMVASS	260 LFADTAN LFADTAN LFADTAN LFADTAN LFADTAN LFGDGIA IFGDGAG LFGDGAG	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVTG AAVTG AAVTG AYTIGC	270 RPRP RPRP RPRP RPRP RPRP RG SEE SEE NPR-IEE	28 ESVLRI ESVLRI ESVLRI ESTLRI QALLRI GIGVRI FGIIST KPIFEM TPIYEV	0 22 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE RNGSYLLE HILHADGSYC WTAQTIAE MCSINREF	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYD KTEDWIMYD DSEGAIDGHL NTENAMVWDL
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens	(226) (195) (195) (191) (200) (225) (158) (132) (184) (177) (158)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA ANALIVA ARVLVVC NRILVVC NHVLVS	2 SEALSTTY SEALSTTY AEALSTTY AEALSTTY SEALSTTY SEALSTTY SDVLARTC SEVTAVTF TEVCSLHF LEFSSLCY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-FTDLG DP SNTDGG Q-PDDTKL	_250 ESLIVRL: ESLIVRL: ESLIVRL: ESLIVRL: GSLICNC: GSLICNC: DSLVGQA DSLVGQA DQMVASS HAFISAA	260 LFADTAX LFADTAX LFADTAX LFADTAX LFADTAX LFGDGAG LFGDGAG LFGDGAG LFGDGAG LFGDGAG	AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVTA AAVTA AAUTVGS AYIIGC AAVTAGS	270 RPRP RPRP RPRP RPRP RPRP RFRP SEE SEE SEE SEE NPR-IEE DP	28 SVIRI ESVIRI ESVIRI ESVIRI ESTIRI QAIIRI QAIIRI GIGVRI FGIIST KPIFEM TPIYEV APGFKI	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE PRNGSYLE PHLHADGSYC WHTAQTIAE WMTAQTIAE MCSINRSFE AKTGSYFEL	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTRDLHRLET GTRDLHRLET GTRDLHRLET NTENAMVWDL NTENAMVWDL NSEHYIKYDV
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_grise	(226) (195) (195) (191) (200) (225) (158) (132) (184) (177) (158) (158)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA ARVLVVC NRILVVC NHVLIVS SNVLIVS	2 SEALSTY SEALSTY AEALSTY AEALSTY SEALSTY CEFCSLCY SDVLARTC SEVLARTC SEVLAVTF TEVCSLHF LEFSSLCY CEFCSLCY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PTDLG DP RGPSDTHL Q-PDDTKL Q-PTDIG	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTII DSL GQA AFISAA GSI	260 LFADTAN LFADTAN LFADTAN LFADTAN LFADTAN LFADTAN LFADGAG LFGDAG LFGDAG LFGDALS LFGDALS	AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVTA AAVTGS AAVTGS AAVTGS AAVVTC AAVVC	270 RPRP RPRP RPRP RPRP RPRP RGP SEE SEE SEP PVPEIE DD PVPEIE DD CG	28 ESVLRI ESVLRI ESVLRI ESTLRI QAICIRI GIGIIST KPIFE PGIIST APGFKI GTGMRI	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE PRNGSYLE WHTAQTIAF MCSINR SKTGSYFLE FRNGSHVE FRNGSHVE	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTRDLHRLET GTRDLHRLET GTRDLHRLET NGERAIDGHL NTENAMVWDL NSEHYIKYDV DTEDWISYAV
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_sviceus GCS_S_sviceus GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu	(226) (195) (195) (191) (200) (225) (158) (132) (184) (177) (158) (158) (158)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA ARVLVVC NHILVVC NHILVS SNVLVVI	2 SEALSTY SEALSTY AEALSTY AEALSTY CEFCSLCY SDVLARTC SEVTAVTF TEVCSLHF LEFSSLCY SEALSTY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PTDLG DP RGPSDTHL SNTDGG Q-PDDTKL Q-PTDIG O PADTLIG	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTI DSLVGQA DQMVASS HAFISAA GSLISNG ESLIVRL	260 LEADTAV LEADTAV LEADTAV LEADTAV LEADTAV LEADTAV LEGDGIF IFGDGAG LEGDAVS LEGDALS LEADTAV	AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVTC AAVTC AAVTC AAVTC SAAVTR SAAVTR SAAVVTC	270 RPRP RPRP RPRP RPRP RPRP REPRP RPR DP VPEIE DP VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPEIE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD VPE DD DD VPE DD DD VPE DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD DD	28 ESVLRI ESVLRI ESVLRI ESTLRI QAICRI GIGIIST KPIFE PGIIST KPIFE SVLRI SVLRI	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE ERNGSYLE WTAQTIA MCSINRSF AKTGSYLL ERNGSHL DAAWHHTLE	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV ELLTIPNADR DSEGAIDGHL NTENAMVWDL NSEHYIKYDV DTEDWISYAV GT DLHRLDT
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_sviceus GCS_S_sviceus GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu	(226) (195) (195) (191) (200) (225) (158) (132) (184) (177) (158) (158) (158) us(226)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA ARVLVVG ARVLVVG NHILVS SNVLIVS RVVLVVI	2 SEALSTY SEALSTYY AEALSTYY AEALSTYY CEFCSLCY SDVLARTC SEVTAVTF TEVCSLHF LEFSSLCY SEALSTY	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PTDLG DP RGPSDTHL SNTDGG Q-PDDTKL Q-PTDIGV Q PADDTL	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTI DSL GSL CM AFISAA GSL SNG ESLIVRL	260 LEADTAY LEADTAY LEADTAY LEADTAY LEADTAY LEGDGLE LEGDGA LEGDAVS LEGDAVS LEGDALS	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG	270 RPR P RPR P RPR P RPR P RPR P RPR P SEE SEE SEE SEE SEE SEE SEE SEE SEE SE SE SE SE SE SE SE	28 ESVIRI ESVIRI ESVIRI ESVIRI ESTIRI QALIRI QALIRI GTGVRI FIFM TPLYEV APGFKI GTGMRI SVIRI	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DSAWHHTLE PRNGSYLA WHAQTIAE WHAQTIAE MCSINRFE AKTGSYFLE PRNGSHLWF DAAWHHTLE	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV ELLTLPNADR DSEGAIDGHL NTENAMVWDL NSEHYIKYDV DTEDWISYAV GT DLHRLDT
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_sviceus GCS_S_sviceus GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu	(226) (195) (195) (191) (199) (220) (225) (158) (132) (132) (158) (158) (158) (158) (158)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA AVLVVC NRILVVC NRILVVC NRILVVC SNVLVVI	2 SEALSTY SEALSTYY AEALSTYY AEALSTYY CEFCSLCY SDVLARTC SEVTAVTF TEVCSLHF LEFSSLCY CEFCSLCY SEALSTY 310	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PTDLGV DP RGPSDTHL SNTDGG Q-PTDIGV Q-PTDIGV Q PADTL	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTII DSLVGQA DQMVASS HAFISAA GSLISNG ESLIVRL	260 LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEGDGIF IFGDGAG LEGDAVS LFGDALS LFGDALS LFGDALS	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG	,270 RPRP RPRP RPRP RPRP RPRP RERP REPRP REPR - IEE DD VPEIE DD VPEIE QG SRPR P 3	28 ESVIRI ESVIRI ESVIRI ESVIRI ESTIRI QALIRI GTGVRI PGIIST KPIFEM TPLYEV APGFKI GTGMRI SVIRI	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE ERNGSYLLE RUSYLLE RUSYLLE RNGSHLE DAAWHHTLE 360	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV ELLTIPNADR DSEGAIDGHL NSEHYIKYDV DTEDWISYAV GT DLHRLDT
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_lividans GCS_S_sviceus GCS_S_sviceus GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu	(226) (195) (195) (191) (199) (220) (225) (158) (132) (158) (158) (158) (158) (158) (158) (266)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA KYALVVG ARVLVVC NRILVVC NRILVVC NRULIVS RVVLVVI 301 RADOTHU	2 SEALSTY AEALSTYY AEALSTYY AEALSTYY CEFCSLCY SDVLARTC SEVTAVTF TEVCSLHF LEFSSLCY CEFCSLCY SEALSTY 2310	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PTDIGW Q-PTDIGW Q-PTDIGW Q PADTL 	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTII DSLVGQA DQMVASS HAFISAA GSLISNG ESLIVRL	260 LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEGDGIF IFGDGAG LFGDAVS LFGDAVS LFGDALS LFGDALS LFADTAN 30	AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVVTC AAVTA ALTVGS AAVTA AAVTG AAVTC AAVVTC AAVVTC AAVVTC AAVVTC	,270 RPR P RPR P RPR P RPR P RPR P RPR P RPR P RPR SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE SEE	28 ESVIRI ESVIRI ESVIRI ESVIRI QALIRI QALIRI QALIRI QALIRI GIGURI STRI SVRI 50	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE ERNGSYLE ERNGSYLE ERNGSHLY DAAWHHTLE JAAO	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV ELLTIPNADR SEGAIDGHL NTENAMVWDL NSEHYIKYDV GT DLHRLDT 375 DEEMBSCIIC
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_viridochromogenes GCS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu	(226) (195) (195) (191) (199) (200) (225) (158) (132) (158) (158) (158) (158) (158) (158) (158) (301) (266)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA KYALVVG ARVLVVC NRILVVC NRULVS SNVLIVS RVVLVVI 301 RADGTHE	2 SEALSTY SEALSTYY AEALSTYY AEALSTYY CEFCSLCY SDVLARTC SEVTAVTF TEVCSLHF LEFSSLCY CEFCSLCY SEALSTTY 310	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADTL Q-PTDLGW DP RGPSDTHL SNTDGG Q-PDDTKL Q-PTDIGW Q PADDTL 	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTII DSL GQA DQMVASS HAFISAA GSLISNG ESLIVRL 33 MWEWLR	260 LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEGDGAC LEGDGAC LEGDAVS LEGDAVS LEGDAVS LEGDAVS LEADTAN 30	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVTA AALTVGS AAVTA AALTVGS AAVTA AALTVGS AAVTA AALTVGS AAVTA AAVTG 3A0 	_270 RPR P RPR P RPR RPR RPR RPR RPR RPR RPR RPR RPR	28 ESVLRI ESVLRI ESVLRI ESVLRI ESTLRI QALLRI QALLRI QALLRI GIGVRI STRIMA SVLRI 50 PGGTEV	0 22 DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE ERNGSYLLE KUTSYLLE AKTGSY	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV SELLTLPNADR DSEGAIDGHL NTENAMVWDL NSEHYIKYDV DTEDWISYAV GT DLHRLDT 375 DEWPSGLISY
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_viridochromogenes GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu	(226) (195) (195) (191) (199) (200) (225) (158) (132) (158) (132) (158) (158) (158) (158) (158) (158) (266) (266) (262)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA MYALVVC ARVLVVC NRILVVC NRILVVC NRVLVVI 301 RADGTHF	2 SEALSTY SEALSTYY AEALSTYY AEALSTYY SEALSTYY SEALSTYY SEALSTYY SEALSTYY CEFCSLCY SEVTAVTF TEVCSLHF LEFSSLCY CEFCSLCY SEALSTYY 310 VMDRCPR	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADTL Q-PADTL Q-PADTL Q-PADTL Q-PADTL Q-PADDTL 	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTII DSLVGQA DQMVASS HAFISAA GSLISNG ESLIVRL 	260 LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEGDGAR LEGDGAR LEGDARS LEGDARS LEGDARS LEGDARS LEGDARS LEADTAN 30 REED	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVTA AALTVGS AAVTA AALTVGS AAVTA AALTVGS AAVTA AALTVGS AAVTA AAVTG 3A0VTG 340 	_270 RPR P RPR	28 ESVLRI ESVLRI ESVLRI ESVLRI GIGVRI GIGVRI GIGVRI SVLRI 50 PGGTEV PGGTEV	0 22 DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE DAAWHITLE ERNGSYLE KUTGSYFLE AKTGSYFLE AKTGSYFLE AKTGSYFLE AKTGSYFLE AKTGSYFLE DAAWHITLE 	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KIEDWIMYDV EELLTLPNADR DSEGAIDGHL NTENAMVWDL NSEHYIKYDV GT DLHRLDT 375 CEWPSGLLSY CEWPSGLLSY CEWPSGLLSY
GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_sviceus GCS_S_viridochromogenes GCS_S_cabiei GCS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_vicieus	(226) (195) (195) (191) (200) (225) (158) (132) (158) (132) (158) (158) (158) (158) (158) (266) (266) (266) (266) (262)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA XYALVVC ARVLVVC NRILVVC NRILVC NRVLVVI 301 RADGTHF RADGTHF REDGTHF	2 SEALSTY SEALSTYY AEALSTYY AEALSTYY SEALSTYY SEALSTYY SEALSTYY SEVTAVTF TEVCSLHF LEFSSLCY CEFCSLCY SEALSTYY 310 VMCREPR VMDRRGPR	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PTDLG Q-PTDLG Q-PDDTKL Q-PTDIG Q-PDDTKL Q-PTDIG Q PADDTL 320 AVQETVTA AVQETVTA	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTII DSLVGQA DQMVASS HAFISAA GSLISNG ESLIVRL 	260 LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEGDGAC LEGDGAC LEGDAVS LE	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVIIGC SAAVIA ALIVGS AAVIIGC SACVMR 3AAVVTG 340 	_270 RPR P RPR P RPR P RPR P RPR P RPR P RPR P RPR P RPR P RPR RPR RPR	28 SVLRI ESVLRI ESVLRI ESVLRI QALLRI QALLRI QALLRI QALLRI TPLYEV APGFKI GTCMRI SVLRI 50 PGGTEV PGGTRV PGGTRV	0 2 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE PRIGSYLLE PRIGSYLLE RNGSYFLE RNGSYFLE RNGSHUY DAAWHHTLE 360 LEYMEQTME LEYMEQTME LEYMEQTME	90 300 GTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KIEDWIMYDV EELLTLPNADR DSEGAIDGHL NTENAMVWDL NSEHYIKYDV GT DLHRLDT 375 DEWPSGLLSY CGWPSGLLSY CGWPSGLLRY
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GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_viridochromogenes GCS_S_sviceus GCS_S_scabiei GCS_S_zinciresistens THNS_S_coelicolor_A3(2) FabH_E_coli CHS_M_sativa Steely1_D_discoideum PhID_P_fluorescens RppA_S_griseus Consensu GCS_S_coelicolor_A3(2) GCS_S_lividans GCS_S_viridochromogenes GCS_S_coelicolor_A3(2) THNS_S_coelicolor_A3(2)	(226) (195) (195) (191) (200) (225) (158) (132) (158) (132) (158) (158) (158) (158) (158) (158) (266) (266) (262) (262) (270) (271) (296) (227)	226 RVVLVVI RVVLVVI RVVLVVI RVVLVVI ANALIVA AVLVVC ARVLVVC NRILVVC NRILVVC NRILVVC NRILVVC NRILVS RVVLVVI 301 RADGTHF RADGTHF RADGTHF RADGTHF RADGTHF RADGTHF RADGTHF	2 SEALSTY SEALSTY AEALSTY AEALSTY AEALSTY CEFCSLCY SEVTAVTF TEVCSLHF LEFSSLCY SEALSTY (2FCSLCY SEALSTY (310 VMLFESPE VMDRRGPR VMDRRGPR VMDRRGPR VMDRRGPR	40 Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PADDTL Q-PTDLGV DP RGPSDTHL SNTDGG Q-PTDIGV Q-PTDIGV Q-PTDIGV AVQETVTA AVQETVTA AVQETVTA AVQETVTA AVQETVTA AVQETVTA AVQETVTA	250 ESLIVRL ESLIVRL ESLIVRL ESLIVRL GSLICNG TDRGTII DSLVGQA DQMVASS HAFISAA GSLISNG GSLISNG GSLINRL ,3 MWEWLRL MWEWLRL MWEWLRL MWEWLRL MWEWLRL	260 LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEADTAN LEGDGLE LEGDAVS LEGDAVS LEGDAVS LEGDAVS LEGDAVS LEGDAVS RYEDD RYEDD RYEDD RYEDD RYEDD RYEDD RYEDD RYEDD RYEDD	AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG AAVVTG 3A0 	270 RPR P RPR -	28 SVLRI ESVLRI ESVLRI ESVLRI ESVLRI QALLRI GTGVRI PGIIST KPIFEM TPLYEV APGFKI GTGMRI 50 PGGTEV PGGTRV PGGTRV PGGTRV PGGTRV PGGTRV PGGTRV PGGTRV	0 22 DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE DAAWHHTLE CRNGSYLI ERNGSYLI ERNGSYLI CSNUTA CSNUT	90 300 GGTRDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET GTQDLHRLET KTEDWIMYDV EELLTLPNADR DSEGAIDGHL NSEHYIKYDV DTEDWISYAV OTEDWISYAV GT DLHRLDT 3755 DEWPSGLLSY DGWPSGLLSY DGWPSGLLSY DGWPSGLLRY DGWPSGLLRY DGWPSGLLRY DTWPSGLLDH WDP-HAFRF
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Figure S1. Sequence comparisons among selected type III PKSs. a) Sequence alignments for Gcs and selected proteins. Catalytic triad residues are boxed in black. Gcs arginine residues selected for mutation are boxed in red. b) Percent sequence identities of aligned type III PKSs and FabH proteins.



Figure S2. Size exclusion chromatography of Gcs. Mobile phase consisted of 20 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 0.5 mM TCEP. The peak around 45 mLs represents material eluting near the void volume. The major protein peak eluted at 74 mLs. To calculate the apparent molecular weight, this elution volume was fit to the equation, mLs = $-12.447*\ln(MW) + 129.52$ (MW in kDaltons). An apparent molecular weight of 90,000 Daltons is consistent with a dimer for the His-tagged protein whose calculated molecular weight is 44,000 Daltons.



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Figure S3. LC-MS of germicidin A produced by type II fatty acid synthase and Gcs. All LC traces were analyzed at 260 nm. All reaction consists of all or minus one of the following components: apo/holo-AcpP, AcpS, FabD, FabH and Gcs along with free CoA, malonyl-CoA, isobutyryl-CoA and ethylmalonyl-CoA. a) Reaction consisting of all components. The insert is the positive ESI mass spectrum of the major peak (germicidin A) labeled with a red asterisk. b) Reaction without malonyl-CoA. c) Reaction without ethylmalonyl-CoA. d) Reaction without isobutyryl-CoA. e) Reaction without AcpP. f) Reaction without AcpS. g) Reaction without FabD. h) Reaction without FabH. i) Reaction without Gcs.



Figure S4. LC-MS analysis of formation of 3-oxo-4-methyl-pethyl-CoA by FabH. All traces were analyzed at 260 nm. a) FabH reaction using malonyl-CoA (1) and isobutyryl-CoA (4) to form free CoA (2) and 3-oxo-4-methyl-pentyl-CoA (3). The right panel shows the positive ESI mass spectrum of peaks. b) Control reaction without FabH. c) CoA standards of malonyl-CoA, free CoA, and isobutyryl-CoA.



Figure S5. Kinetics curves of Gcs. Rate of formation of pyrone product from reactions containing a) acetoacetyl-CoA b) 3-oxo-4-methyl-pentyl-CoA and c) 3-oxo-4-methyl-pentyl-AcpP. Reactions with acetoacetyl-CoA proceeded with 3 μ M Gcs for five minutes while all other reactions proceeded with 0.3 μ M Gcs for one minute. Red circles indicate reactions contained 1 mM methylmalonyl-CoA and blue squares indicate reactions contained 1 mM ethylmalonyl-CoA.



Figure S6: Gcs binding specificity towards AcpP Is not significantly affected by mutations to arginine surface residues. Biolayer interferometry measurements of Gcs and mutants binding directly to biotinylated AcpP immobilized onto streptavidin sensors. a) Gcs wild type, b) R276A, c) R277A, d) R280A, e) R317A, and f) R276/317A. Shift data, aligned to the start of the association step, are shown in blue and fitted binding curves are in red. g) Example of the raw data including immobilization of biotinylated-AcpP onto the streptavidin biosensors followed by equilibration to a baseline in 1X PBS buffer before association and dissociation of with Gcs wild-type.

Primer name	Primer sequence	Constructed plasmid
R276A_F	ttcgtgatggacgctcgcgggccgcg	pDHS9753
R276A_R	cgcggcccgcgagcgtccatcacgaa	
R277A_F	tgatggaccgggccgggggg	pDHS9807
R277A_R	cccgcggcccggtccatca	
R280A_F	gcgcgggccggcggtgcag	pDHS9790
R280A_R	ctgcaccgccggcccgcgc	
R317A_F	cccggcgggaccgcggtgctggagta	pDHS9791
R317A_R	tactccagcaccgcggtcccgccggg	
pET_F	ctcgatcccgcgaaatta	
pET_R	ccggatatagttcctcctttc	
∆63-96_R	ggacggtctgcgacgcgggcgcgacggcggcctccagggggggg	pDHS9794
∆63-96_F	cctggaggccgccgtcgcgcccgcgtcgcagaccgtccaggagcgc	
∆63-97_R	cctggacggtctgcgacgccgcgacggcggcctccagggggagca	pDHS9795
∆63-97_F	cctggaggccgccgtcgcggcgtcgcagaccgtccaggagcgcacc	
∆63-98_R	tcctggacggtctgcgacgcgacggcggcctccagggggagca	pDHS9793
Δ63-98_F	tggaggccgccgtcgcgtcgcagaccgtccaggagcgcaccgcc	

Table S1. Primers used for mutagenesis of Gcs.

References

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