## Crystal Structure of SgcJ, An NTF2-Like Superfamily Protein Involved in Biosynthesis of the 9-Membered Enediyne Antitumor Antibiotic C-1027

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Strain	Description	Source (Reference)				
Streptomyces globisporu	s –					
SB1022	1					
SB1027	S. globisporus $\Delta sgcR1/\Delta sgcJ$ mutant strain	This work				
SB1028	$\Delta sgcJ$ mutant complemented by pBS1146	This work				
SB1029	$\Delta sgcJ$ mutant complemented by pBS1147	This work				
SB1030	$\Delta sgcJ$ mutant complemented by pBS1148	This work				
SB1031	$\Delta sgcJ$ mutant complemented by pBS1149	This work				
SB1032	$\Delta sgcJ$ mutant complemented by pBS1150	This work				
SB1033	$\Delta sgcJ$ mutant complemented by pBS1151	This work				
SB1034	$\Delta sgcJ$ mutant complemented by pBS1152	This work				
SB1035	$\Delta sgcJ$ mutant complemented by pBS1153	This work				
Escherichia coli						
<i>E. coli</i> DH5α	General subcloning host	commercial				
E. coli BR513	Bioassay test strain for BIA	2				
Micrococcus luteus						
M. luteus ATCC 9431	Test strain for testing of the antibacterial activity of C-1027	ATCC				

Table 1. Bacterial strains used in this study

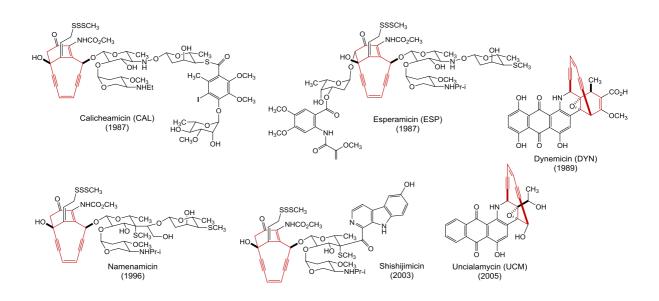
Plasmid	Description	Source (Reference)
pSET152	Integrative Streptomyces vector	commercial
pSET151	Integrative Streptomyces vector	commercial
pJTU4659	Vector containing kanamycin (kan) resistance cassette	This work
pUWL201pw	E. coli-Streptomyces expression shuttle vector	3
pBS1005	Cosmid containing partial C-1027 gene cluster	4
pBS5007	Cosmid containing partial ncs gene cluster	5
pMCSG57	E. coli expression vector	6
pBS1143	pBS1005 in which <i>sgcJ</i> has been inactivated by inserting a kanamycin ( <i>kan</i> )	This work
	cassette	
pBS1144	pSET151 harboring kan cassette (cloned into the XbaI site)	This work
pBS1145	pUWL201pw with oriT fragment inserted into the KpnI site	This work
pBS1146	pBS1145 harboring <i>sgcJ</i> gene (cloned into the <i>Nde</i> I and <i>Eco</i> RI sites)	This work
pBS1147	pBS1145 harboring ncs-orf16 gene (cloned into the NdeI and HindIII sites)	This work
pBS1148	SgcJ mutant (W29A) generated by site-directed mutagenesis of pBS1146	This work
pBS1149	SgcJ mutant (F37A) generated by site-directed mutagenesis of pBS1146	This work
pBS1150	SgcJ mutant (Y72A) generated by site-directed mutagenesis of pBS1146	This work
pBS1151	SgcJ mutant (D111A) generated by site-directed mutagenesis of pBS1146	This work
pBS1152	SgcJ mutant (W118A) generated by site-directed mutagenesis of pBS1146	This work
pBS1153	SgcJ mutant (Y132A) generated by site-directed mutagenesis of pBS1146	This work
pBS1154	pMCSG57 containing <i>sgcJ</i> gene for protein expression	This work
(APC109082)		
pBS1155	pMCSG57 containing ncs-orf16 gene for protein expression	This work
(APC109139)		

Table S2. Plasmids and cosmids used in this study

Table S3	Primers	used in	this s	tudy
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Primer	Nucleotide Sequence	Function
sgcJtgtF	5'-ATGACCAGCACCGACTCGACCACCTCCGCTCCCGACGCCG ATTCCGGGGATCCGTCGACC-3'	PCR targeting for replacement of <i>sgcJ</i>
sgcJtgtR	5'-TCAGTCGTTGCCGCGCGGGCTGTTCTGGTAAGCGGCCAGA CTGTAGGCTGGAGCTGCTTC-3'	PCR targeting for replacement of <i>sgcJ</i>
oriT152F	5'-CG <u>GGTACC</u> AGTATGCAGGTCGACGGATCT-3' (KpnI)	oriT amplification
oriT152R	5'-CC <u>GGTACC</u> TTGCTCGTCGGTGATGTAC-3' (KpnI)	oriT amplification
sgcJ201NdeIF	5'-GCAATTC <u>CATATG</u> ACCAGCACCGACTCGA-3' (NdeI)	sgcJ amplification
sgcJ201EcoRIR	5'-G <u>GAATTC</u> TCAGTCGTTGCCGCGCGG-3' ( <i>Eco</i> RI)	sgcJ amplification
ncs16NdeIF	5'-GCAATTC <u>CATATG</u> GGAGTGAAGATGAGTTC-3' (NdeI)	ncs-orf16 amplification
ncs16HindIIIR	5'-CCC <u>AAGCTT</u> TGATGGCAGACGGACGGG-3 ( <i>Hind</i> III)	ncs-orf16 amplification
sgcJW29A-F	5'-ATCGTGGCCGCCGCGGCCGACCACGACGCCGAC-3'	Site-directed mutagenesis for W29A of SgcJ
sgcJW29A-R	5'- GTCGTGGTCGGCCGCGCGGCGGCCACGATGCGCGC-3'	Site-directed mutagenesis for W29A of SgcJ
sgcJF37A-F:	5'- GACGCCGACCGG <del>GCC</del> GCCGACGTCTTCGCCGAG-3'	Site-directed mutagenesis for F37A of SgcJ
sgcJF37A-R	5'- GAAGACGTCGGCGGCCCGGTCGGCGTCGTGGTC -3'	Site-directed mutagenesis for F37A of SgcJ
sgcJY72A-F	5'- TTCGCGGGCCCGGCCAAGGGCACCCGTGTCATC-3'	Site-directed mutagenesi for Y72A of SgcJ
sgcJY72A-R	5'- ACGGGTGCCCTTGGCCGGGGCCCGCGAAGGCGGC-3'	Site-directed mutagenesis for Y72A of SgcJ
sgcJD111A-F	5'- GAGGCGAGCGGCGCCGGCGCCGTCCGGGCCTCC-3'	Site-directed mutagenesis for D111A of SgcJ
sgcJD111A-R	5'- CCGGACGGCGCCGGCGCCGCTCGCCTCGGTCTC-3'	Site-directed mutagenesis for D111A of SgcJ
sgcJW118A-F	5'- GTCCGGGCCTCCGCGCTGGCCGTCGAACAGGAC-3'	Site-directed mutagenesis for W118A of SgcJ
sgcJW118A-R	5'- TTCGACGGCCAGCGCGGAGGCCCGGACGGCGCC-3'	Site-directed mutagenesi for W118A of SgcJ
sgcJY132A-F	5'- CGTCTGGCCGCTGCCCAGAACAGCCCGCGCGGC-3'	Site-directed mutagenesis for Y132A of SgcJ
sgcJY132A-R	5'- CGGGCTGTTCTGGGCAGCGGCCAGACGCCACTG-3'	Site-directed mutagenesi for Y132A of SgcJ
sgcJ-F	5'-TACTTCCAATCCAATGCCATGACCAGCACCGACTCGACC-3'	sgcJ amplification
sgcJ-R	5'-TTATCCACTTCCAATGTTAGTCGTTGCCGCGCGGG-3'	sgcJ amplification
ncs-orf16-F	5'-TACTTCCAATCCAATGCCATGGGAGTGAAGATGAGTTCTG GC-3'	ncs-orf16 amplification
ncs-orf16-R	5'-TTATCCACTTCCAATGTTATGGCAGACGGACGGGGGCT-3'	ncs-orf16 amplification
sgcJidF	5'-ATGACCAGCACCGACTCGA-3'	PCR check of SB1027
sgcJidR	5'-GCTTACCAGAACAGCCCGC-3'	PCR check of SB1027
sgcJ-Sprobe-F	5'-TCCGTTGGTTGGCGGTTTC-3'	Southern hybridization fo SB1027
sgcJ-Sprobe-R	5'-CGATGTGCTCGTAGGTGGG-3'	Southern hybridization fo SB1027

**Figure S1.** Structures of the six known 10-membered enediyne natural products with their enediyne core highlighted in red. Given in parentheses are the years when each of the enediyne structures was established.



**Figure S2.** Sequence analysis of SgcJ and its homologues from the seven known and 34 putative 9-membered enediyne biosynthetic gene clusters (given in parenthesis are accession numbers). (a) Phylogenetic analysis of SgcJ and its homologues. The phylogenetic tree was constructed with neighbor-joining method. Numbers of each node indicated the percentage of bootstrapping of a 1000 replications. The scale bar indicates branch length. (b) Multiple sequence alignment of SgcJ and its homologues. Aligned residues are colored on the basis of the level of conservation (red background with white character shows strict identity, red character similarity and blue frame similarity across groups).



b

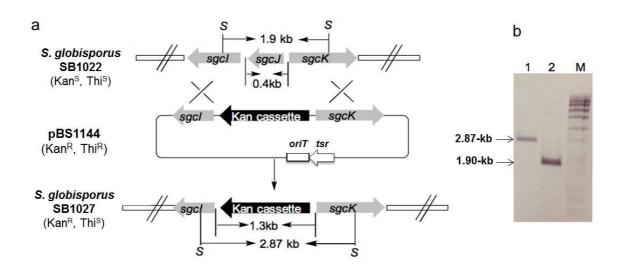
	$\alpha_1 \qquad \alpha_2$
Streptomyces globisporus (SgcJ) (AAL06676)	TT 000000000 00000000 00 1
Streptomyces globisporus (SgcJ) (AAL06676 Actinomadura madurae (MdpJ) (ABY66022	1MTSTDSTTSAPDAALAAVAALDARIVAADHDAD 1MTKSDAAAPSASDPAAVAALTORVVAADAAHODAE
Actinoalloteichus cyanogriseus (WP 026420245)	1
Streptoalloteichus sp. ATCC 53650 (KedJ) (AFV52149	1
Saccharothrix espanaensis (CCH31208	1MITDQRMIVHIGPSKADQAAIAALPQRMIAGWALHDAS
Micromonospora lupini (CCH17058 Saccharomonospora sp. CNQ490 (WP 024875098	
Saccharomonospora sp. CNQ490 (WP 024875098) Salinispora pacifica CNS237 (WP 027650868	
Salinispora pacifica CNT150 (WP 033666428)	
Verrucosispora maris (AEB44798	
Streptomyces sp. LaPpAH-95 (WP 018105847	1MTTDTTAAPTNAVRPHDAAAVRGLIORVVAAMRAODAE
Saccharomonospora halophila (WP 019811731) Saccharomonospora marina (EHR52753	
Streptosporangium roseum (ACZ89676	
Streptomyces sp. CNS615 (WP 017949464	1
Streptomyces clavuligerus (EFG04857	
Streptomyces sp. Tu6071 (EGJ77068	
Streptomyces sp. SA3 actF (WP 010297295 Nocardia sp. CNY236 (WP 028478582	
Salinispora tropica CNB-440 (SpoJ) (ABP55153	
Streptomyces viridosporus (WP 016828682	1
Streptomyces sp. CNT302 (WP 018892902	1
Nocardiopsis sp. CNT312-c (WP 051415461	
Amycolatopsis rifamycinica-a (KDN18888 Amycolatopsis mediterranei RB-a (AGT87402	1MDDYYGPFTSEKEKEALDVPLRLIAANSKNDGY 1MSVSAEDRLSDYYGPFTSEREKEVLGVPLRLVGANAENSAD
Actinoplanes sp. N902-109-a (AGL16702	
Nocardiopsis sp. CNT312-a (WP 028647182	1MARDIRISADDKAVREVADSVMRTWAANDAD
Amycolatopsis mediterranei RB-b (AGT87403	1
Actinoplanes sp. N902-109-b (AGL16703	1MNDAAALVSSAKKWASYYGEHANGRAGAVLTAPLRVRAAMDAGDAC 1MSNDAASLVAQAKEWAGSYGPFPNGEEGAALSAVLRARAAMAEGNAC
Nocardiopsis sp. CNT312-b (WP 028647186 Amycolatopsis rifamycinica-b (KDN18889	
Actinoplanes sp. N902-109-c (AGL16704	1
Nocardiopsis sp. CNT312-d (WP 051415462	1 MPGETTVANGASADHGTSTATRVLAEAGVVEDPEYYKDFPDDLSREVLTVPQLIQAAWLANDAD
Salinispora pacifica (CyaJ) (AGO97200	
Streptomyces sp. CNT-179 (CynJ) (AGO97162 Amycolatopsis mediterranei RB-c (AGT87405	
Amycolatopsis rifamycinica-c (KDN18887	
Catenulispora acidiphila (ACU75234	
Streptomyces carzinostaticus subsp. neocarzinostaticus (Ncs-Orf16) (AAM77985)	1
Actinomadura flavalba (WP 018657673 Actinomadura oligospora (WP 026413625	
Streptomyces globisporus (SgcJ) (AAL06676)	$\alpha \qquad \beta \qquad $
Streptomyces globisporus (SgcJ) (AAL06676)	
Streptomyces globisporus (SgcJ) (AAL06676) Actinomadura madurae (MdpJ) (ABY66022)	
Streptomyces globisporus (SgcJ) (AAL06676)	
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Streptomyces globbsporus (Sgc.) (AAL06676) Actionmadura madurae (Mdp.) (ABY66022) Actinomaturae (Mdp.) (ABY66022) Streptoaltoteichus sp. ATCC 53650 (Ked.) (AFV52149) Saccharothrix espanaensis (CCH31208) Micromonospora lupini (CCH1708) Saccharomonospora sp. CN4304 (WP 02487008) Salinispora pacifica CN1504 (WP 03468408) Salinispora pacifica CN1504 (WP 03686408) Starinspora pacifica CN1504 (WP 03686408) Starinspora pacifica CN1504 (WP 03686408) Starinspora pacifica CN1504 (WP 03686408) Streptomyces sp. LaPAH3 (WP 016308471) Saccharomonospora halophila (WP 01981731) Saccharomonospora halophila (WP 01981731) Streptomyces sp. CN3517 (WP 01749464) Streptomyces sp. CN3517 (WP 01749464) Streptomyces sp. CN3517 (WP 017495464) Streptomyces sp. CN3517 (WP 01749545153) Streptomyces sindaspora tw/1692617852] Salinispora tropica CNB-440 (Spo.) (ABP55153) Streptomyces sp. CN322 (WP 01892902) Nocardiaps sp. CN322 (WP 018921962)	0.0 TT 7 FADVF7EDGTMILPG LFRKGRENIRITHMAAAPAG0YKGTQVTGRDIDARL GGG.IALLTT 7 FADVF7EDGTMILPG VYRKGRDDIAACMAAAPAG0YKGTQVTGRDIDMFLAAD.TALLLT 5 FADVF7EDGTMILPG VYRKGRDDIAACMAAAPAG0YKGTQVTGRDIDMFLAAD.TALLLT 5 FADVF7EDGTMILPG VYRKGRDDIAACMAAAPAG0YKGTQVTGRDIDMFLAAD.TALLLT 6 FAQVF7EDGTMILPG VYRKGRDIRENAAAPAG0YKGTQVTGRDIDVFFEFD.SCVLLT 6 FAQVF7EDGTMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD.SCVLLT 7 FADLF7EDGVMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD.SCVLLT 7 FADLF7EDGVMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD.SCVLLT 7 FADLF7EDGVMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD.SCVLLT 7 FADLF7EDGTMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD.SCVLLT 7 FADLF7EDGTMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD.SCVLLT 7 FADLF7VEDGTMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD.SCVLLT 6 FADVF7EDGTMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD 7 FADLF7VEDGTMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD 7 FADLF7EDGTMILPG VYRKGRDIRENAAAPEG0YKGTQVTGRDIDVFFEGD 7 FADLF7EDGTMILPG VYRKGRDINAAAPTG0YKGTQVTGRDIDVFFEGD 7 FADLF7EDGTMILPG VYRKGRDINAAAPTG0YKGTQVTGRDIDVFFEGD 7 FADVF7EDGTMILPG VYRKGRDINAAAPAG0YKGTQVTGRDIDVFFEGD 7 FAVF7EDGTMILPG VYRKGRDINAAAPAG0YKGTQVTGRDIDVFFEGD 8 FAVF7EDGTMILPG VYRKGRDINAAAPAG0YKGTQVTGRDIDVFFEGD 8 FAVF7EDGTMILPG VYRKGRDINAAAPAG0YKGTQVTGRDIDVFFEGD 8 FAVF7EDGTMILPG VYRKGRDINAAAAPAG0YKGTQVTGRDIDVFFEGD 9 FAVF7EDGTMILPG VYRKGRDINAAAAPAG0YKGTQVTGRDIDVFFEGD 9 FAVF7EDGTMILPG VYRKGRDINAAAAPAG0YKGTQVTGRDIDVFFEGD 9 FAVF7EDGTMILPG VYRKGRDINAAAAPAG0YKGTQVTGRDIDVFFEGD 9 FAVF7EDGTMILPG VYRKGRAVEGMAAAAPAG0YKGTQVTGRDIDVFFEGD 9 FAVF7EDGTMILPG VYRKGRAVEGMAAAPAG0YKGTAVTGED 10 FAAVF7EKGTMILPG VYRKGGRAVEGMAAAPAG0YKGTAVTGED 10 FAAVF7EKGTMILPG VYRKGGRAVEGTAAPK7CAAPAG0YKGTAVTGED 10 VYDTEDGTMVLPG VYRKGGRAVEGTAAPK7CAAAPK7GTAVTGED 10 FAA
Straptomyces globisporus (Sgc.J) (AAL06676) Actinomadura madurae (Md.D.J) (ABY66022) Actinomatorae (Md.D.J) (ABY66022) Streptoalloteichus sp. ATCC 53650 (Ked.J) (AFV52149) Saccharothrix espanaensis (CCH31200 Micromonospora lupini (CCH17058) Saccharothrix espanaensis (CCH31200 Salinispora pacifica CN1500 (WP 024875096) Salinispora pacifica CN1500 (WP 024875096) Stantispora pacifica CN1500 (WP 03468424) Verrucositgora maria (AEB44768) Streptomyces sp. L04PA41950 (WP 03686424) Streptomyces sp. L04PA1950 (WP 031696424) Streptomyces sp. L04PA1950 (WP 031696424) Streptomyces sp. L04PA1950 (WP 03169424) Streptomyces sp. L04PA1950 (WP 03297425) Streptomyces sp. L04PA1950 (JABP5155) Nocardia sp. CNY236 (WP 038974252) Salinispora tropica CNH 340 (Sp.01) (ABP5155) Streptomyces sp. CN132c (WP 03897452) Streptomyces sp. KN 3450 (WP 03897452)	0.0 TT 7 FADVFTADGTMILPG LFRKGRENIRITHMAAAPAGPYKGTQVTGRPUDARLDGFFT 7 FADVFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUDARLDGFFTALLGG 7 FADVFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUDARFTLAAD.TALLIT 7 FAQVFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUDARFTLAAD.TALLIT 7 FADVFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUDARFTLAAD.TALLIT 9 FADVFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUDARFTLAAD.TALLIT 10 IADLFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUTARFTDJRFFGDE.SGVLTG 10 IADLFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUTARFTDJRFFGDE.AALLIT 10 IADLFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUTARFTDJRFFGDE.AALLIT 10 FADVFTDGTMILPG VYRKGRDEIRAYMADAPAGOYKGTQVTGRPUTARFTDJRFFGDE.AALLIT 10 FADVFTDGTMILPG VYRKGRDEIRAYMAOAFTCGRVTGRPUTARFTDJRFFGDE.AALLIT 10 FADVFTDGTMILPG VYRKGRDEIRAYMAOAFTCGRVTGRPUTARFTDJRFFGDE.AALLIT 10 FADVFTDGTMILPG VYRKGRDEIRAYMAOAFTCGRVTGRPUTARFTDJRFFGDE.AALLIT 10 FADVFTDGTMILPG VYRKGRDEIRAYMAOAFTCGRVTGRPUTARFTDJRFFGDE.AALLIT 10 FADVFTDGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFGDE.AALLIT 10 FADVFTDGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFANZ 10 FADVFTDGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFANZ 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFANZ 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFANZ 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFANZ 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFANZ 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTQVTGRPUTARFTDJRFFANZ 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTNVTGRPUTARFTDJRFFANZ 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTNVTGRPUTARFTDJRFFIND 10 FADVFTDGGTMILPG VYRKGRDEIRAYMAOAFTAGOYKGTNVTGRPUTARFTDJRFFIND 10 FADVFTDGGTMILPG VYRKGRANAGOTAGFYKGTNVTGRPUTARFTJSFLST.SALLIT 10 FADVFTDGGTMILPG VYRKGRANAAFTAGFYKGTNVTGRPUTAFTDJSFLSTLSSTLIVTT 10 FADVFTDGGTMILPG VYRKGRANAAFTAGFYKGTNVTGTPTJLSSTLIVTFLSST.SALLIT 10 FADVFTDGGTMILPG VYRKGRANAAFTAGFYKGTNVTGTPTJLSSTLIVTFLSST.SALLIT 10 FADVFTDGGTMULPG VYKGRANAAFTAGFYKGTNVTGTPTJLSSTLIVTFLSST.SAVVIT 10 FADVFTRGGTNULPG VYRKGRANAAFTA
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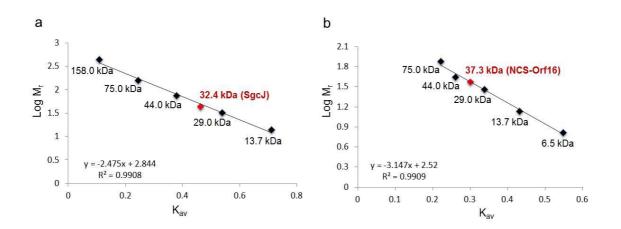
## b (continued)

		η1	β5	β6	
Streptomyces globisporus (SgcJ) (AAL06676)	TT	eee		▶ T T	• .
Streptomyces globisporus (SgcJ) (AAL06676) 99	GILAPGETE.	ASGDGAVR		QDGQWRLAAYQNSF	RGND
Actinomadura madurae (MdpJ) (ABY66022) 98	GVLAAGESE	VSDEOAIR	ASWLAVK	RDGEWRLTAYONSE	SVOTLPTPGASAK
Actinoalloteichus cyanogriseus (WP 026420245) 97	GVLAEGESE	VSDDOAIR	ASWLVVR	ODGEWRLAAYONTE	AVHRLPTPGATGR
Streptoalloteichus sp. ATCC 53650 (KedJ) (AFV52149) 99	GVLKPGTTE	VTEDSAIR	ASWVVVR	ODGEWRLAAYONSF	RDDR
Saccharothrix espanaensis (CCH31208) 102	GVLAPGDTE	VTAERAIR	SSWVVVK	QDGEWRLATYQNSE	ANT
Micromonospora lupini (CCH17058) 99	GVIAAGSTE	LAAADAIRA	ASWILVK	RNDAWVLAVYQNCE	RDPA
Saccharomonospora sp. CNQ490 (WP 024875098) 95	GVMEEGQTE	VSPERAIR	ASWVAVR	DDGQWRLAAYQNSE	к
Salinispora pacifica CNS237 (WP 027650868) 99				RDGEWRLAAYQNTF	
Salinispora pacifica CNT150 (WP 033666428) 99	GVLESGESE'	VSSKGAVRI	ASWLVVK	RDGEWRLAAYQNTF	ANAA
Verrucosispora maris (AEB44798) 99	GVLAPGETE	VAAERAIRA	ASWLLVR	TPNGWQLTAYQNSF	RDAR
Streptomyces sp. LaPpAH-95 (WP 018105847) 104				RDGEWLVDCYANTF	
Saccharomonospora halophila (WP 019811731) 104				QDGEWLIAAYQNTF	
Saccharomonospora marina (EHR52753) 102				RGGEWYLAAYHNSF	
Streptosporangium roseum (ACZ89676) 102				RDGEWRLAAYQNSF	
Streptomyces sp. CNS615 (WP 017949464) 101				RDGQWFLAAYQNSF	
Streptomyces clavuligerus (EFG04857) 97				DQGRWRLAAYQNTF	
Streptomyces sp. Tu6071 (EGJ77068) 96				EGADWRLAAYQNTF	
Streptomyces sp. SA3 actF (WP 010297295) 96				EGADWRLAAYQNTF	
Nocardia sp. CNY236 (WP 028478582) 88	GIRPTTADS			DGDSWSLASYTNAF	
Salinispora tropica CNB-440 (SpoJ) (ABP55153) 98				RDGQWLLTAYQNSF	
Streptomyces viridosporus (WP 016828682) 100				QDGEWLIAAYQNTE	
Streptomyces sp. CNT302 (WP 018892902) 104				.QDGEWLIAAYQNTF	
Nocardiopsis sp. CNT312-c (WP 051415461) 113				RDGEWKLAAYQNSE	
Amycolatopsis rifamycinica-a (KDN18888) 98				RDNEWQLAVYQNSF	
Amycolatopsis mediterranei RB-a (AGT87402) 106 Actinoplanes sp. N902-109-a (AGL16702) 113				. KDGEWFLAGYQNSF	
Nocardiopsis sp. CNT312-a (WP 028647182) 94				RDGQWQLAGYQNSF	
Amycolatopsis sp. CN1312-a (WP 02804/162) 94 Amycolatopsis mediterranei RB-b (AGT87403) 111				.GSGGWLIAAYQNSK	
Actinoplanes sp. N902-109-b (AGL16703) 110				O.GGDWKLLSHQTSE H.DGDWRVASWQSSE	
Nocardiopsis sp. CNT312-b (WP 028647186) 111				R.DGEWMLLSYOSSE	
Amycolatopsis sp. CN1312-b (WP 020047180) 111 Amycolatopsis rifamycinica-b (KDN18889) 111				7.DGEWRVVSQQTSE	
Actinoplanes sp. N902-109-c (AGL16704) 116				RDDGTPALVSHQSSP	
Nocardiopsis sp. CNT312-d (WP 051415462) 128				R.SGRWHLMSHOSSE	
Salinispora pacifica (CyaJ) (AG097200) 119				GEDWRLVSHQSSF	
Streptomyces sp. CNT-179 (CynJ) (AG097162) 115				.DERWRLLCHHSTE	
Amycolatopsis mediterranei RB-c (AGT87405) 116				.GDGLRLLSHQTSP	
Amycolatopsis rifamycinica-c (KDN18887) 117				NADGDLNLFSHQSSE	
Catenulispora acidiphila (ACU75234) 97				IDGOWLLAAYONSE	
Streptomyces carzinostaticus subsp. neocarzinostaticus (Ncs-Orf16) (AAM77985) 103				RDGAWLVEAYHNSE	
Actinomadura flavalba (WP 018657673) 97				DGDDWLITAYHNSE	
Actinomadura oligospora (WP 026413625) 90				. KDGQWLITAYQNTF	

**Figure S3.** Construction and confirmation of the  $\Delta sgcJ$  mutant strain *S. globisporus* SB1027. (a) Construction of the  $\Delta sgcJ$  mutant strain *S. globisporus* SB1027. S, *StuI*; Kan<sup>R</sup>, kanamycin resistant; Kan<sup>S</sup>, kanamycin sensitive, Thi<sup>R</sup>, thiostrepton resistant; Thi<sup>S</sup>, thiostrepton sensitive. (b) Southern analysis confirming the genotype of SB1027. M, DNA ladder; lane 1, SB1027; lane 2, SB1022.



**Figure S4.** Molecular weight estimation of SgcJ and NCS-Orf16 in solution by size exclusion chromatography. (a) SgcJ and (b) NCS-Orf16 estimated on a Superdex 200 16/600 column calibrated against molecular weight standards. The apparent molecule weights of SgcJ and NCS-Orf16 were estimated to be 32.4 kDa and 37.3 kDa, suggesting that both SgcJ (calculated molecular weight of 14.6 KDa) and NCS-Orf16 (calculated molecular weight of 15.3 KDa) are dimers.



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