Analysis of the Setomimycin Biosynthetic Gene Cluster from *Streptomyces nojiriensis* JCM3382 and Evaluation of Its α-Glucosidase inhibitory activity using Molecular Docking and Molecular Dynamics Stimulations

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Table S1. Putative *S. nojiriensis* JCM 3382 BGCs[*].

Regions	Type	From	To	Most similar known cluster		Similarity
Region 1	Terpene	40,991	61,998	Avermitilol	Terpene	100%
Region 2	Lanthipeptide-class-iv PKS-like Butyrolactone	119,303	167,470	Streptocollin	RiPP:Lanthipeptide	100%
Region 3	Thiopeptide LAP	168,443	197,954	Lactazole	RiPP:Thiopeptide	55%
Region 4	NRPS-like NRPS	432,526	479,497	Antipain	NRP	100%
Region 5	T2PKS	1,066,919	1,139,413	Spore pigment	Polyketide	58%
Region 6	NRPS/NRPS-like	1,561,900	1,631,094	Tambromycin	NRP	92%
Region 7	NRPS/T1PKS	1,796,196	1,861,151	Coelichelin	NRP	72%
Region 8	Butyrolactone	1,961,621	1,972,592	Neocarzinostatin	Iterative type I + Enediyne type I polyketide	4%
Region 9	NI-Siderophore	3,622,602	3,652,383	Desferrioxamin B/E	Other	100%
Region 10	T2PKS	4,276,901	4,349,362	Setomimycin	Polyketide	100%
Region 11	RiPP-like	4,584,818	4,595,105			
Region 12	NRPS-like/T1PKS/NRPS	5,057,273	5,117,610	Bleomycin A2/B2	NRP + Polyketide + Saccharide	7%
Region 13	CDPS	6,208,634	6,229,392	BD-12	NRP	17%
Region 14	NI-Siderophore	6,607,990	6,640,997	Kinamycin	Polyketide	13%
Region 15	Hydrogen-cyanide	6,812,909	6,825,953	Aborycin	RiPP	21%
Region 16	RiPP-like	6,891,612	6,902,943			
Region 17	Terpene	7,011,883	7,034,099	Toxoflavin Fervenulin	Other	14%
Region 18	Lanthipeptide-class-iv	7,095,057	7,117,990			
Region 19	Terpene	7,404,584	7,431,728	Hopene	Terpene	61%

Region 20	T1PKS/HglE-KS	7,504,501	7,555,916	Hexacosalactone A	Other	9%
Region 21	NRPS/NRPS-like	7,604,900	7,720,913	Leupeptin Pr/Ac	NRP	100%
Region 22	T1PKS	7,832,794	8,024,104	Linearmycin A-C	Type I polyketide	100%
Region 23	Lanthipeptide-class-iv	8,122,015	8,144,813			
Region 24	NRPS-like	8,186,135	8,229,356	Lipstatin	NRP	42%
Region 25	Terpene	8,288,028	8,309,149	Bombyxamycin A/ B	Polyketide	3%
Region 26	Terpene	8,385,926	8,407,281	2-Methylisoborneol	Terpene	100%
Region 27	Terpene	8,477,920	8,498,825	Monensin	Polyketide	5%
Region 28	Melanin	8,514,987	8,542,302	Istamycin	Saccharide	4%
Region 29	NI-Siderophore	8,627,590	8,658,781	Gausemycin A/B	NRP + Saccharide	4%
Region 30	T3PKS	8,688,189	8,729,250	Alkylresorcinol	Polyketide	100%
Region 31	CDPS/NAPAA	8,765,178	8,817,045	ε-Poly-L-lysine	NRP	100%

*Predicted biosynthetic gene clusters of *S. nojiriensis* JCM 3382 (antiSMASH version 7.1.0).

Table S2. Genetic organization of the setomimycin BGCs[*].

***S. nojiriensis* JCM3382**			***S. aurantiacus* JA4570** (GenBank/Identities/Positives/Gaps)	***S. justiciae* RA-WS2** (GenBank/Identities/Positives/Gaps)
QTI46191.1	StmR (199aa)	TetR family transcriptional regulator	EPH44807.1/90%/92%/0%	MCW8383185.1/87%/93%/0%
QTI46192.1	StmP (206aa)	Hypothetical protein		
QTI46193.1	StmJ (504aa)	Drug resistance transporter	EPH44808.1/92%/95%/0%	MCW8383184.1/87%/93%/0%
QTI46194.1	StmK (217aa)	Type II polyketide cyclase (TcmI)	EPH44809.1/75%/86%/0%	MCW8383183.1/69%/83%/0%
				MCW8383182.1/58%/66%/6% (StmP-like)
QTI46195.1	StmL (142aa)	NTP-2 family protein	EPH44810.1/76%/86%/0%	MCW8383181.1/79%/89%/0%
QTI46196.1	StmG (268aa)	Sensory transduction protein(regX3)	EPH44811.1/87%/92%/0%	MCW8383180.1/79%/87%/0%
QTI46197.1	StmI (406aa)	Cytochrome P450	EPH44812.1/97%/99%/0%	MCW8383179.1/92%/95%/0%
QTI46198.1	StmM (64aa)	[3Fe–4S]-like ferredoxin	EPH44813.1/89%/95%/0%	MCW8383178.1/77%/90%/0%
QTI46199.1	StmN (152aa)	NTP-2 family protein	EPH44814.1/86%/91%/0%	MCW8383177.1/76%/83%/0%
QTI46200.1	StmH (216aa)	Type II polyketide cyclase (TcmI)	EPH44815.1/85%/90%/0%	MCW8383176.1/66%/75%/9%
QTI46201.1	StmA (423aa)	β-ketoacyl synthase (KSα)	EPH44816.1/94%/97%/0%	MCW8383175.1/94%/97%/0%
QTI46202.1	StmB (397aa)	Chain length factors (KSβ)	EPH44817.1/93%/96%/0%	MCW8383174.1/90%/94%/0%
QTI46203.1	StmC (88aa)	Acyl carrier protein	EPH44818.1/86%/92%/0%	MCW8383173.1/86%/90%/3%
QTI46204.1	StmD (261aa)	C9 Ketoreductase	EPH44819.1/98%/99%/0%	MCW8383172.1/92%/95%/0%
QTI46205.1	StmE (317aa)	Aromatase/cyclase	EPH44820.1/88%/93%/1%	MCW8383171.1/79%/87%/0%
QTI46206.1	StmF (299aa)	α/β Fold hydrolase	EPH44821.1/85%/90%/1%	MCW8383170.1/79%/85%/0%
QTI46207.1	StmO (63aa)	Hypothetical protein	EPH44822.1/57%/62%/0%	
QTI46208.1	ORF1 (292aa)	4'-phosphopantetheinyl transferase superfamily protein		
QTI46209.1	ORF2 (199aa)	Phosphoribosylanthranilate isomerase		

[*] Genetic organization, putative functions, and amino acid homology of the setomimycin BGCs from *S. nojiriensis* JCM 3382, *S. aurantiacus* JA4570, and *S. justiciae* RA-WS2.

Table S3. Comparison of amino acid homology between the N- and C-termini of StmH and StmK.

	StmH-N (Identity/Similarity)	StmH-C (Identity/Similarity)	StmK-N (Identity/Similarity)	StmK-C (Identity/Similarity)
StmH-N	-	-	-	-
StmH-C	39.68%/52.38%	-	-	-
StmK-N	33.86%/48.82%	37.90%/59.68%	-	-
StmK-C	27.73%/46.22%	34.75%/55.93%	33.33%/51.22%	-

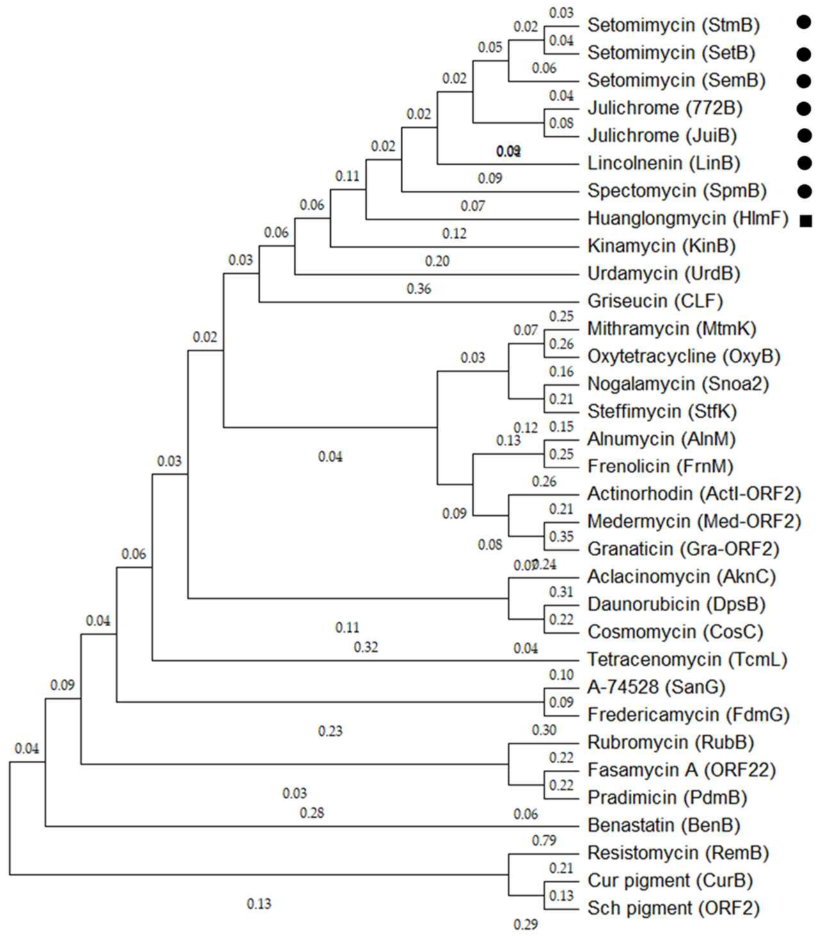

Figure S1. Phylogenetic analysis of a cluster of 42 known KSβ genes in Type II polyketide BGCs. The alignment was created by use of ClustalX and edited manually. The phylogenetic tree was constructed using MEGA11 with the method of maximum likelihood. It indicates that the putative KSβ gene StmB is located in the KSβ group for biaryl nonaketide (●) and huanglongmycin (▪) biosynthesis. Sequences included StmB (QTI46202.1), SetB (EPH44817.1), SemB (MCW8383174.1), LinB (ANS69118.1), SpmB (MCI3903331.1), JuiB (QNL10614.1), 772B (EPJ36720.1), and HlmF (AXL88815.1).

```
ActIII   MATQDSEVALVTGATSGIGLEIARRLGKEGLRVFVCARGEEGLRTTLKELREAGVEADGRTCDVRSVPEIEALVAAVVERYGPVDVLV NNAGRPGGGATAELADELWLDVVETNLTGVFR  120
HedA     --MSRPQTAFVTGVSSGIGLAVARTLAARGIAVYGCARDAKNVSAAVDGLRAAGHDVDGSSCDVTSTDEVHAAVAAAVERFGPIGILV NSAGRNGGGETADLDDALWADVLDTNLTGVFR  118
StmD     MTQQEQRVALVTGATSGIGLAVTRLLAGQGHRVFIGSRTEDKVLTTVKQLQEEQLDVDGTTCDVRSVPEIEALVAAVVERYGPVDVLV NNAGRPGGGATAELADELWLDVVETNLTGVFR  120
BexD     MTDQEGRTAVVTGATSGIGLAVSKLLARRGLRVFLGARTAENVATTVKELRDAGFEADGAACDVRSGEDVTAFVRAAVDRYGPVGVLV NNAGRPGGGVTAEIADELWLDVIETNLTSVFR  120
                    TGxxxGxG                                                                      NNAG PGGxxT

ActIII   VTKQVLKAGGMLERGTGRIVNIASTGGKQGVVHAAPYSASKHGVVGFTKALGLELARTGITVNAVCPGFVETPMAASVREHYSDIWEVSTEEAFDRITARVPIGRYVQPSEVAEMVAYLI  240
HedA     VTREVLRAGGMREAGWGRIVNIASTGGKQGVMYAAPYTASKHGVVGFTKSVGFELAKTGITVNAVCPGYVETPMAERVREGYARHWGVTEQEVHERFNAKIPLGRYSTPEEVAGLVGYLV  238
StmD     LTREVLTTGGLRDKSWGRVINIASTAGKQGVVLGAPYSASKHGVVGFTKALGNELAPTGITVNAVCPGYVETPMAERVRQNYSRLSGAPESAILEKFQAKIPLGRYSTPEEVAGLVGYLA  240
BexD     VTREVLTTGGMVAAGHGRIVNIASTAGKQGVVLGAPYSASKHGVVGFTKALGNELAPAGITVNAVCPGYVETPMAERVRQGYAAAYGTSEDEVLKKFQAKIPLGRYSTPDEVAGLVGYLV  240
                                             YxxxK

ActIII   GPGAAAVTAQALNVCGGLGNY  261
HedA     TDAAASITAQALNVCGGLGNY  259
StmD     SDTAASITSQALNVCGGLGNF  261
BexD     TDTAGSITAQALNVCGGLGNF  261
```

Figure S2. Sequence alignment among various type II PKS KRs. Sequences included hedamycin (HedA), actinorhodin (ActIII), BE-7585A (BexD), and setomimycin (StmD) KRs. Multiple-sequence alignment was performed using Clustal Omega. The SDR proteins motif (TGxxxGxG, PGxxxT, NNAG and YxxxK) were shown in red.

```
StfQ  MPTLGKDTLQYTATVAAPAPLVFDLVAGLENWPQF HGPSVHAEPLGRRDGAEEFQHWWVIDDRTVRTWRAR WRFDSQALRIG YTYDPAEPAAPAQHGEWTFRRLSDASTEVRVE YEL—L   118
StmE  MPSE-LRTSEHEIKIAGPAENVYRLLAQAEKWPQL FPPNIYVDHVERGEREERIRI-WATAGDTVKHWTSHRTLDPDKLRIGFRQEVSTHPVAAMGGSWQVEALPSGETLVRLS HDFRAA   118
BexL  -----MREAEHDILVDAPADEVYRLVAEVANWPRI FPPTVFVDHVERGEGTERIRI-WATANGEPKNWTSRRELDPAARRISFHQEVSTPPVAEMSGTWIVEPVSAATAKVRLL HAYRAV   114
                 ::   : .**  *: *:*   :**::. *.:..: : * :  *.::  *.   .    : * ::  :*    **.:  : :   .     * * .. :  . : **: :

StfQ  GDEGDA-AL----ADRELRELVDCVT-DAAERHEERRDLVVDFEDPLFVAGAVDDAYTYLYEADKWPERIPHVARLVMEERVP NIQFFDMDTTTPDGSAHTTRSVRVCLPGDKIVYKQIQ   232
StmE  DD—DSLTWLEEVVDRNSRSELDSLKALAESRDAAADELTCSFEDTVQIRGAAQDVYDFIYEANLWADRLPHVITVRLDEPTPGVQT LEMDTLSKDGSSHTTKSHRVGLAPHRIAYKQTT   236
BexL  GDDPGGLAWIDRAVDTNSRSELAALKHNVEL —VTNPELTFSFTDTVRIDAPAKDVYDFVDQAALWAERLPHVSSVDLREPSPGLQV LRMDTRAKDGSVHTTESVRVCFPHHRIVYKQTT   232
      .*  .. :     .* : *. : .:.  .          :*. .* * : : . ..*.* :: :*  * :*:***  : : *  *.:* : *** : *** ***.* ** :   .:*.***

StfQ  LPKLLTGHTGHWKFTPTREGFV-LGARHTATIKP-SALPILGEGTTVLDARKYLRRVLSANSMG NLRLAKAFAEERAGV---   309
StmE  LPALITLHTGHWTFTPNDD-GVAASSQHTFTVNTDNIAKVLGADATVQDAIAYVRTALSTNSKA TLGIAKEYAEGLAGAGRS   317
BexL  LPALMTLHTGRWDFAEEPGGGTTASSEHTVVLNTANIAKVLGAGAGVAEAREFVRTALSTNSRA TLGFAKDHAEARP-----   309
      ** *:* ***:* *:        .  .:.** .::  .    :** .: * :*  ::* .**:** ..* :** .**
```

Figure S3. Sequence alignment of StmE and polyketide ARO/CYCs. StmE is extremely conserved with R70 in the monodomain ARO/CYCs TcmN, WhiE, and ZhuI, whereas StfQ is substituted with a bulky W72. StmE is also highly conserved with F35, F81, and H103, which are seen in BexL rather than StfQ

(a)

```
TcmI      MAYRALMVLRMDPAD-AEHVAAAFAEHDT-TELPLEIGVRRRVLFRFHDLYMHLIEADD-DIMERLYQARSHPLFQEVNERVGQYLTPYA-QDWEELKDSKAEVFYSWTAPDS----- 109
AbxD      -MATTLIVARLKPGDHRDQISRLFAESDT-TELPDLVGVQERRLLTFKDLYFHLVRTDE-ALSKTLTPQHDHPLFRSISEAMDEYVTPYE-GAWGSVEQASARQFYHWKRGLGRVQP- 113
StmK-C    -TGTVVTITRMDQAV-IPAVTSIFREFES-AESSRPTGIRRRQLFAFKDLCIHIQEYTGSNGADIAQQVASDIWSGKVVRDLPEL-APEQ-----PAANGPASRFYGWEAS------- 102
StmH-N    -MHSSVIVGRIKPGS-TEAVAGVFAGRDG--EPSGTRNLLRRQLFEYKGIYIHLQDHTQENLTEVVEHGS--------DEAFTPFLDAYDPVAGTAPSGQVATRFYEWHGQPVSDEHR 106
StmK-N    -MRTMVTVAKMDQNS-IPEVSKLFAEFDS-TEIPNMIGLRRRQLFIHDGIQIHLYDFAEKSDQAALEKVKTDPRMGRLAIDMKAFVTPYEPETWNSPADSTAFCFHDWEGEPIENPGP 115
StmH-C    -MYSTVIVARLNPAD-IGTVAKLFGDFDTGTELPHTMGTRRRQLFAYNGLYFHIQDFESDNGGELIERAKSDPRFVQIQQDLLPYISVLDPETWRSPSDAMASRFYTWQVTA------ 110
               : : ::.       ::  *   :   *     .  .* *: ...: :*:                           .                   *  *: *
```

(b)

```
StmK-C  TGTVVTITRMDQAVIPAVTSIFREFES-AESSRPTGIRRRQLFAFKDLCIHIQEYTGSNG  59
StmH-N  MHSSVIVGRIKPGSTEAVAGVFAGRDG--EPSGTRNLLRRQLFEYKGIYIHLQDHTQENL  58
StmH-C  MYSTVIVARLNPADIGTVAKLFGDFDTGTELPHTMGTRRRQLFAYNGLYFHIQDFESDNG  60
StmK-N  MRTMVTVAKMDQNSIPEVSKLFAEFDS-TEIPNMIGLRRRQLFIHDGIQIHLYDFAEKSD  59
          : * : ::.      *: :*   :   *     .  ***** ...: :*: :.  ..
```

Figure S4. Comparison of the amino acid sequences of TcmI, AbxD, StmH, and StmK proteins. (a) Alignment of the complete amino acid sequence of TcmI, AbxD, and the N-terminus and C-terminus of StmH and StmK. (b) Alignment of the N-terminus and C-terminus of StmH and StmK with each other. Among the three amino acids that play a role in controlling and catalyzing cyclizaion and aromatization in TcmI and AbxD, R40 and H51 are conserved in both the N-terminus and C-terminus of StmH/StmK, whereas Y49 is only conserved in the N-terminus and C-terminus of StmH, and, the C-terminus of StmK of D27 is substituted with Asp.

(a)

```
StmF    MTTAPVAPPPAR----SIDQRHITLDADGIMLSARLAQPVHVPPRATIVALHGAGMSAGYFDGPAHPETSLLAVAAELGFTAVAIDRPGYGRSAGRLPYGQGVVAQSHTLAAALRRLVAQ
SetF    MTTAPVAPFTGRSADPRIDRRHLTLDADGVTLSARLARPAHVPPRATVVALHGAGMSAGYFDGPAHPETSLLTLAAELGFTAVAVDRPGYGDSAGRLPHGQGVAAQADTLAAALRGLLAR
SemF    MTTAPPAPPPPAP---VVDRRLITLDADGIPLSARLALPTHVPPRATVVALHGAGMSAAYFDGPAHPETSLLTLAAELGFAAVAVDRPGYGASAGQLPYGQGVGDQAATLSAALRSLSAF
AlpS    -------------------------------MASRTKARAARPARVTLNCLAHAGAGVASYR--------DWASAVGDGVDVHALPLPGR-EARRREPRLTTRADLLADLLPGLLTTARR
                                       :::*    .  *.*.*: .*  ** ... :           : *.  *. . *:  **   :  : *           * ..*

StmF    YETGAGIFLMGHSFGSKPALQIAADGTVPEIIGLDLSGCGAEYLVPPTAPGTRAGSWKLN--WGPLRLYPPGTFQSSIGVVAPAPERELADAAQWPTVFAALAERIRVPVRFTFAEHEAW
SetF    HETGAGIFLMGHSFGCKPALRIAADGTVPGLLGLDLSGCGAEYLAAPGAPATRAGSWKLN--WGPLRLYPPGTFQSSIDVVAPAPVRELADAAHWPTVFPALAERVRVPVRFTFAEHEAW
SemF    CAPDAGIFLVGHSFGSKPALRMAADGRVPGLLGLDVSGCGDEYLVPPTTPGTRAGSWKLN--WGPLRLYPPGTFQAGLAVVAPPPERELADAARWPEEFAALAGRVRVPVRFTFAEHEAW
AlpS    GPYALYGHSMGALVGYTLTRALADAGAPP--LFLAVGACPPPHTTTALVDGADLPDEDLLPLLDTIGSLPPGASASPGGLWRRTFLPVLRDDLRLAKSLRNAALDPVTGGPVDVPVLVFA
               . :*  .* . :  :*  *  *  : * :..*   : ... . .:   . .*    ..:   ***:  :   :   .    * *  : .  :   *    .   . ..

StmF    WRRD-HQALTRLRSRLVAAPRVLIDHQPGAGHNISLGWAARAYHLRALGFAEECLHRRSPGTEQRA-
SetF    WRRD-HEALTRLRNRLAAAPRVLIDHQPDAGHNISLGWAARAYHLRALGFAEECLRRYAADGKVVHP
SemF    WRRD-APTLARLRNRLDAAPRVRIDHQPDAGHNISLGWAARSYHLRALSFAEECLRRRTGGEGRQP-
AlpS    GSDDPLAAPAALRHWQQWTTDLIELHTVSGGH----FFASSAGLAQHVGRACRDHVNAQPAGGRR--
           *   : : **     :. :   *  ..**     :*: :   : :. * .   .
```

(b)

```
SpmF    MTGPQPGGVALAPQSPSTAGAPTPTRPPSAARRPAGPAPRPAPPVPLPPWTPSPGVRQVTLAAGDGITLSGLLALPRGERPRATVLALHGAGTSAAYFDGQAHPGTSLLTLAADLGYAVL
JuiF    --------------------------------------------MTVAQPAVAGGVRGISL-DADGITLSALLALPPQAPCRAVIVALHGAGMSSAYFHGSAHPDTSFLGLATSLGFGVV
HlmB    ----------------------MTKAPP------KAPPTAPPTAPPTAPAAVSAKVGRITL-QAGGITVSALLARPLYAPPRATVVALHGAGMSAAYFHGQTRPETSLLTLAADLGFAAV
LinF    ----------------------MTAA------------------GSAVAARPPATVRRITL-DADGTPLSALLALPEHTPPRATVVALHGAGMSAAYFHGTARPDTSFLALAADLGFAAV
SemF    ----------------------MTTAPP--------------APPP---PAPVVDRRLITL-DADGIPLSARLALPTHVPPRATVVALHGAGMSAAYFDGPAHPETSLLTLAAELGFAAV
StmF    ----------------------MTTAPV--------------APPPAR----SIDQRHITL-DADGIMLSARLAQPVHVPPRATIVALHGAGMSAGYFDGPAHPETSLLAVAAELGFTAV
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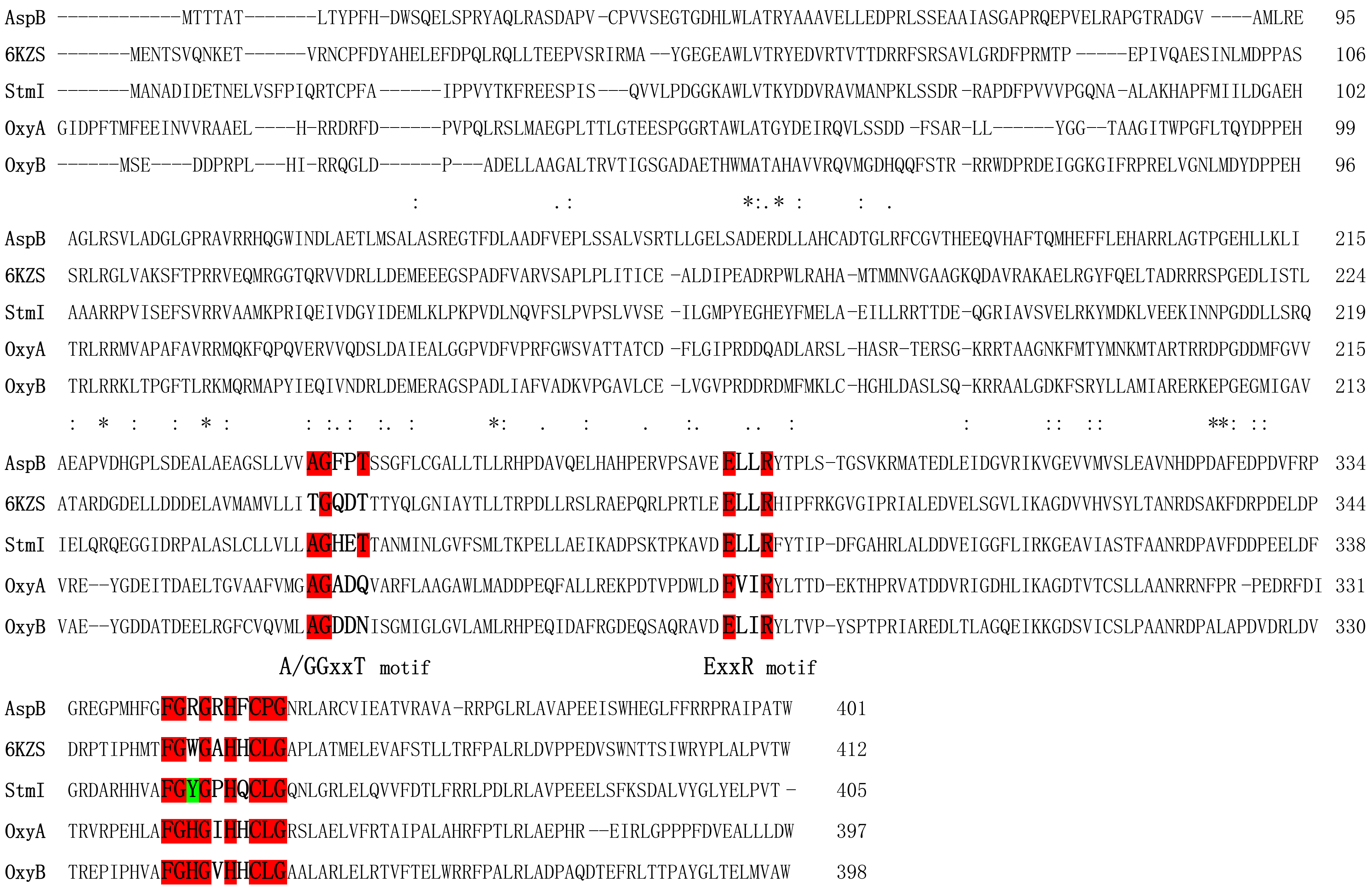
```
SetF  ----------------------MTTAPV--------------APFTGRSADPRIDRRHLTL-DADGVTLSARLARPAHVPPRATVVALHGA GMSAGYFDGPAHPETSLLTLAAELGFTAV
                                                                     ::*  ..*  :*.  ** *     **.::*****  * *:. **.* ::* **:* :*:.**: .:
SpmF  ALDRPGYGLSAARLPQGLPVAEQAATVRAALRGHAALHDTGAGVLVL AHSFGGKVALRMAADDRGDPSDPVLLGLDVSGCGERYARPLAELTGPAGGVRWSGHWGPLRLYPPHTFRASAR
JuiF  AVDRPGYGRSARQLPEGQGVADQATTLGAALRFLAARYDTGSGLFLL AHSFGGKAALRIAADR----AVPELLGLDVSGCGAEYAVPLGDRE-PHGRQSRRLNWGPLRLYPPGTFRASGG
HlmB  AVDRPGYGLSSAELPRGQTAAEQAATLTAALGDLADRYDTGAGLFLL AHSFGGKPALRMAAEG----TLPALLGVDVSGCGDAYTAPPTEPG-T-RDGGWRRSWGPLRLYPPGTFRSGAA
LinF  ALDRPGYGLSARRLPEGQGVMEQATTIAAAVRDLTARHLPGAGVFLL AHSFGGKPALGIAADG----LVPGLLGLAVSGCGDEYLVPPTATT-G-RAAQWRLNWGPLRLYPPGTFRASGA
SemF  AVDRPGYGASAGQLPYGQGVGDQAATLSAALRSLSAFCAPDAGIFLV GHSFGSKPALRMAADG----RVPGLLGLDVSGCGDEYLVPPTTPG-T-RAGSWKLNWGPLRLYPPGTFQAGLA
StmF  AIDRPGYGRSAGRLPYGQGVVAQSHTLAAALRRLVAQYETGAGIFLM GHSFGSKPALQIAADG----TVPEIIGLDLSGCGAEYLVPPTAPG-T-RAGSWKLNWGPLRLYPPGTFQSSIG
SetF  AVDRPGYGDSAGRLPHGQGVAAQADTLAAALRGLLARHETGAGIFLM GHSFGCKPALRIAADG----TVPGLLGLDLSGCGAEYLAAPGAPA-T-RAGSWKLNWGPLRLYPPGTFQSSID
      *:****** *: .** *  .   *: *: **:          .:*::::.**** * ** :* *:        * ::*: :****  *                ********* **::.
SpmF  MVSPVPPRELRDAARWPGAFAALAGRVRVPVRFTFAEYEALWHC DAPALARLRAGLRAAPRVSVDRQPGAG HNISLGWAARAYHLRALAFADDCLRR---------------
JuiF  VVAPVPSLELADAARWPGDFAGFAGRVRVPVRFTFAEHEFWWQH DARAVARLRSRLSGAPRVVTDHQPDAG HNISLGWSARAYHLRVLGFVEECLRWRESRQRATPEEQEGT
HlmB  VVARVPPGELADAARWPAEFGRLAGRIRVPVRFTFAEYETWWRH DPPALDRLRSRLSAAPRVVIEHQPDAG HNISLGWAARAYHLKALGFLEESMRHHGVGRESRT ------
LinF  VVAPAPPRELADAGRWPETFRRIGGRIRVPVRFTFAEHEHWWRH DRRAVAQLRSRLHAAPRVLVDHQPDAG HNISLGWTARAYHLGVLGFVADCLRPDGDGKEHPA ------
SemF  VVAPPPERELADAARWPEEFAALAGRVRVPVRFTFAEHEAWWRR DAPTLARLRNRLDAAPRVRIDHQPDAG HNISLGWAARSYHLRALSFAEECLRRRTGGEGRQ -P-----
StmF  VVAPAPERELADAAQWPTVFAALAERIRVPVRFTFAEHEAWWRR DHQALTRLRSRLVAAPRVLIDHQPGAG HNISLGWAARAYHLRALGFAEECLHRRSPGTEQRA ------
SetF  VVAPAPVRELADAAHWPTVFPALAERVRVPVRFTFAEHEAWWRR DHEALTRLRNRLAAAPRVLIDHQPDAG HNISLGWAARAYHLRALGFAEECLRRYAADGKVVHP -----
      :*:  *  ** **.:**  *  :. *:**********:*  *:  *  :: :**  * .****  ::**.*********:**:*** .*.*  :.::
```

Figure S5. Sequence alignment of AlpS and biaryl polyketide type II TEs. AlpS and setomimycin type II TEs (a) and their respective biaryl polyketide TEs (b). The conserved GHSMG motifs are marked by azure colors, and the Ser-His-Asp triad is highlighted in red. Sequences included AlpS (AAR30163.1), SemF (MCW8383170.1), StmF (QTI46206.1), SetF (EPH44821.1), LinF (ANS69114.1), SpmF (MCI3903327.1), JuiF (QNL10618.1), and HlmB (AXL88811.1).

(a)



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AspB -------------MTTTAT--------LTYPFH-DWSQELSPRYAQLRASDAPV-CPVVSEGTGDHLWLATRYAAAVELLEDPRLSSEAAIASGAPRQEPVELRAPGTRADGV----AMLRE   95
6KZS -------MENTSVQNKET------VRNCPFDYAHELEFDPQLRQLLTEEPVSRIRMA--YGEGEAWLVTRYEDVRTVTTDRRFSRSAVLGRDFPRMTP-----EPIVQAESINLMDPPAS   106
StmI -------MANADIDETNELVSFPIQRTCPFA------IPPVYTKFREESPIS---QVVLPDGGKAWLVTKYDDVRAVMANPKLSSDR-RAPDFPVVVPGQNA-ALAKHAPFMIILDGAEH   102
OxyA GIDPFTMFEEINVVRAAEL----H-RRDRFD------PVPQLRSLMAEGPLTTLGTEESPGGRTAWLATGYDEIRQVLSSDD-FSAR-LL------YGG--TAAGITWPGFLTQYDPPEH   99
OxyB ------MSE----DDPRPL---HI-RRQGLD------P---ADELLAAGALTRVTIGSGADAETHWMATAHAVVRQVMGDHQQFSTR-RRWDPRDEIGGKGIFRPRELVGNLMDYDPPEH   96
                                               :                .:                        *:.* :       :  .

AspB  AGLRSVLADGLGPRAVRRHQGWINDLAETLMSALASREGTFDLAADFVEPLSSALVSRTLLGELSADERDLLAHCADTGLRFCGVTHEEQVHAFTQMHEFFLEHARRLAGTPGEHLLKLI   215
6KZS  SRLRGLVAKSFTPRRVEQMRGGTQRVVDRLLDEMEEEGSPADFVARVSAPLPLITICE-ALDIPEADRPWLRAHA-MTMMNVGAAGKQDAVRAKAELRGYFQELTADRRRSPGEDLISTL   224
StmI  AAARRPVISEFSVRRVAAMKPRIQEIVDGYIDEMLKLPKPVDLNQVFSLPVPSLVVSE-ILGMPYEGHEYFMELA-EILLRRTTDE-QGRIAVSVELRKYMDKLVEEKINNPGDDLLSRQ   219
OxyA  TRLRRMVAPAFAVRRMQKFQPQVERVVQDSLDAIEALGGPVDFVPRFGWSVATTATCD-FLGIPRDDQADLARSL-HASR-TERSG-KRRTAAGNKFMTYMNKMTARTRRDPGDDMFGVV   215
OxyB  TRLRRKLTPGFTLRKMQRMAPYIEQIVNDRLDEMERAGSPADLIAFVADKVPGAVLCE-LVGVPRDDRDMFMKLC-HGHLDASLSQ-KRRAALGDKFSRYLLAMIARERKEPGEGMIGAV   213
      :  *  :   :  * :          : :.:  :. :       *:   .   :     .    :.    ..  :                :       ::  ::          **: ::

AspB AEAPVDHGPLSDEALAEAGSLLVVAGFPTSSGFLCGALLTLLRHPDAVQELHAHPERVPSAVEELLRYTPLS-TGSVKRMATEDLEIDGVRIKVGEVVMVSLEAVNHDPDAFEDPDVFRP   334
6KZS ATARDGDELLDDDELAVMAMVLLITGQDTTTYQLGNIAYTLLTRPDLLRSLRAEPQRLPRTLEELLRHIPFRKGVGIPRIALEDVELSGVLIKAGDVVHVSYLTANRDSAKFDRPDELDP   344
StmI IELQRQEGGIDRPALASLCLLVLLAGHETTANMINLGVFSMLTKPELLAEIKADPSKTPKAVDELLRFYTIP-DFGAHRLALDDVEIGGFLIRKGEAVIASTFAANRDPAVFDDPEELDF   338
OxyA VRE--YGDEITDAELTGVAAFVMGAGADQVARFLAAGAWLMADDPEQFALLREKPDTVPDWLDEVIRYLTTD-EKTHPRVATDDVRIGDHLIKAGDTVTCSLLAANRRNFPR-PEDRFDI   331
OxyB VAE--YGDDATDEELRGFCVQVMLAGDDNISGMIGLGVLAMLRHPEQIDAFRGDEQSAQRAVDELIRYLTVP-YSPTPRIAREDLTLAGQEIKKGDSVICSLPAANRDPALAPDVDRLDV   330
                                 A/GGxxT motif                         ExxR motif

AspB  GREGPMHFGFGRGRHFCPGNRLARCVIEATVRAVA-RRPGLRLAVAPEEISWHEGLFFRRPRAIPATW   401
6KZS  DRPTIPHMTFGWGAHHCLGAPLATMELEVAFSTLLTRFPALRLDVPPEDVSWNTTSIWRYPLALPVTW   412
StmI  GRDARHHVAFGYGPHQCLGQNLGRLELQVVFDTLFRRLPDLRLAVPEEELSFKSDALVYGLYELPVT-   405
OxyA  TRVRPEHLAFGHGIHHCLGRSLAELVFRTAIPALAHRFPTLRLAEPHR--EIRLGPPPFDVEALLLDW   397
OxyB  TREPIPHVAFGHGVHHCLGAALARLELRTVFTELWRRFPALRLADPAQDTEFRLTTPAYGLTELMVAW   398
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FGHGXHXCLG/GXXXC motif

(b)

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JuiI  MSE---ITAESVPEFPILRTCPFSVPETYRELEGTGGRIHRVRMSDGRPAWLVTKHDDARAVLSDARFSSEKLRPGFPELSPGGLKALTYFSPFLVNMDGPEHAQARRAVLGEFSVRRIN  117
SemI  MTNTDIDETSELVSFPIQRTCPFAIPPVYTKFRE-EAPVSQVVLPDGGKAWLVTKYDDVRAVMSNPKLSSDRRKKDFPVVVPGQNAALAKHAPFMIILDDPEHAEARRPVISEFSVRRVA  119
SetI  -----------------------MPPVYTKFRE-ESPITQVVLPDGGKAWLVTKYDDVRAVMANPKLSSDRRAPDFPVVVPGQNAALAKHAPFMIILDGAEHAAARRPVISEFSVRRVA  95
StmI  MANADIDETNELVSFPIQRTCPFAIPPVYTKFRE-ESPISQVVLPDGGKAWLVTKYDDVRAVMANPKLSSDRRAPDFPVVVPGQNAALAKHAPFMIILDGAEHAAARRPVISEFSVRRVA  119
SpmI  MSDAPVQETAGPIRFPITRTCPFAIPETYERLRE-EEPVSRVEMSDGRHAWMLTRHADVRAALVDPRLGSDRSDPGYPSISSGGKSAFAHFAPFMISLDGPAHSAARSPVISEFSMRRVN  119
LinI  MLNLDPEEVAKLPHYPVTRRCPYEIPEVYTRLRT-EEPVSKVVMSDGQPVWFLSRYEDVRAVLSDPRFGADRLAEGFPNLALGQREGLSKQPKFMISMDGAEHSAVRRRVISDFSVRRVA  119
                                   :* .* .:.      : :* : **  .*::::: *.* *.: : ::.:::   .:* :  *   .::    *:: :*.  *: .*  *:.:**:**:

JuiI  AMRPRIRRIVDAAIDRMLEQPGRPVDLAAHLSQPVPTLVLSAFLGVPAEDLDAIERNTGKMLHEARTEDEQRAAAEALHAHLDLVIAAKEENPGDDFLSRQIDRSRREHGDAADRFELAS  237
SemI  AMKPRIQEIVDHFIDEMLKLP-RPVDLNQVFSLPVPSLVVSEILGMPYEGHEYFMELAEVLLRRTTDEQGRINISVQLRAYMDKLVEEKIANPGDDLLSRQIELQRR -NGG-IDRPQLAS  236
SetI  AMKPRIQEIVDGFIDDMLKMP-KPVDLNQVFSLPVPSLVVSEILGMPYEGHEYFMELAEILLRRTTDEQGRIAVSVELRKYMDKLVEEKIENPGDDLLSRQIELQRQ -QGG-IDRPQLAS  212
StmI  AMKPRIQEIVDGYIDEMLKLP-KPVDLNQVFSLPVPSLVVSEILGMPYEGHEYFMELAEILLRRTTDEQGRIAVSVELRKYMDKLVEEKINNPGDDLLSRQIELQRQ -EGG-IDRPALAS  236
SpmI  TLRPRIQEIVDEAIDKILELP-RPVDLVQHLALIVPRLVITERIGASPDYLERFYALAAGMLQRSTTGEERDAIAREMRANMDRLVAEKEADPGDDLLSREIARQRAETGD -VDRPGLAS  237
LinI  ELRPTIQKLVDDCVDRILDLP-QPVDLVAELALPVPTLLLAELVGANHADHAYFIDLVHRMLWRKTSGEERVQISIGLRKYFDDLIAEKEAHPGDDLISRQIALQREETGE -IDREGLNS  237
         ::* *:.:**  :* :*. * :****   ::  ** *:::  :*         :   .  :* .     : :   :  ::  :* ::  *  .****::**:*  .*   *   **  * *

JuiI  LVQLLQIAGHASSAAMISLSVLTLLSEPEQLKQLTADPSRTPAAAE ELLRFLSITDTGPLRLALEDVEIGGVRIRAGDGVLIPTLPANRDAGAFPDPDRFDIGRLPGTRHVA FGYGAHQC  357
SemI  LCLLVLLAGHETTANMINLGVFSMLTRPELLDVIKADPSKTPKAVD ELLRFYTIPDFGAHRLAMDDVEIGGVLIRKGEAVIASTFAANRDPAVFDDPEELDFSRD -SRHHVAFGYGPHQC  355
SetI  LCLLVLLAGHETTANMINLGVFSMLTKPELLAEIKADPSKTPKAVD ELLRFYTIPDFGAHRLALDDVEIGGVLIRKGEAVIASTFAANRDPAVFDDPEELDFGRD -ARHHVAFGYGPHQC  331
StmI  LCLLVLLAGHETTANMINLGVFSMLTKPELLAEIKADPSKTPKAVD ELLRFYTIPDFGAHRLALDDVEIGGFLIRKGEAVIASTFAANRDPAVFDDPEELDFGRD -ARHHVAFGYGPHQC  355
SpmI  LAQLLLLAGHESTSEMISLGIATLLTHPEQLARMVADPSRTPAVIE ELLRYFSVVEIGMGRVATEDVELGGVRIKAGEGVIASNVAANHDPRAFPDPDVFDPDRD -ARQHVAQGYGPHQC  356
LinI  LAQLLLIAGYESSASMIALGVHTFLTRPDWLAAIRTDPARTSVAVE ELLRFYSILDVAAGRVALEDVEIGGETIRAGDGVMASVFAANRDPSAFPDPDRLDLERG -ARHHVAFGYGPHQC  356

JuiI  LGQNLVRAELQVVLDRLFHRIPDLRLATAPEALPYKYFGQFFGPVELPVTW  408
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SemI   LGQNLGRVELQVVFDTLFRRLPDLRLAVPAEELNFKSDALVYGLYELPVTW  406
SetI   LGQNLGRLELQVVFDTLFRRLPELRLAVPEEELSFKSDALVYGLYELPVTW  382
StmI   LGQNLGRLELQVVFDTLFRRLPDLRLAVPEEELSFKSDALVYGLYELPVTW  406
SpmI   LGRNLARVELQTVFDTLFRRIPGLRLAVGVEELPFKYDALVHGLRELPVTW  407
LinI   LGQNLSRLELQIVFDTLFARVPTLRLAVDEADLPFKYDALAFGLYELPVTW  407
       **:** * *** *:* ** *:* ****.    * :*  .  .*  ******
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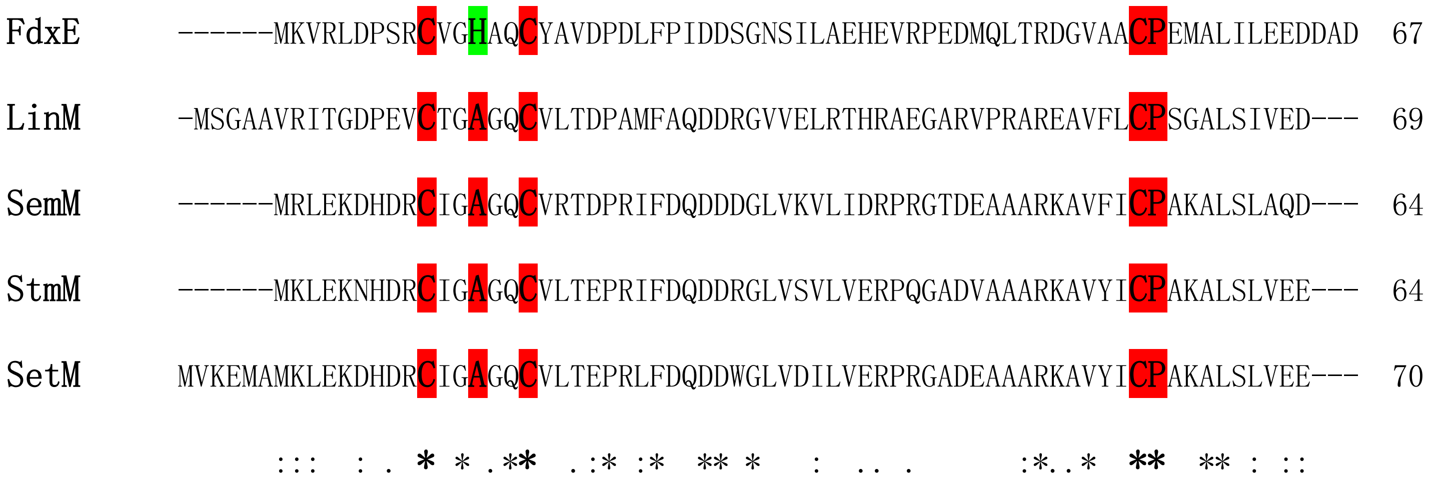
Figure S6. Sequence alignment of P450 eznymes. Sequence alignment of actinomycetes P450s and StmI (a) and their respective biaryl polyketide P450s (b). Typical P450-fold, with helix L containing the signature sequence ^{348}FGHGXHXCLG^{357} containing the proximal axial thiolate ligand of the heme iron, ^{355}Cys, is conserved, and the highly conserved ^{244}A/GGxxT^{248} motif and the ^{283}ExxR^{286} motif in the K-helix, and the ^{351}GxxxC^{355} motif in the heme binding ring is also conserved in StmI and its corresponging enzymes. All of them are marked in red. Amino acids conserved in biaryl polyketide P450 that are specific to the motif region are coloured green. Sequences included OxyA (GIF17054.1),OxyB (Q8RN04.1),AspB (WP_030881046.1), 6KZS (WP_003970936.1), JuiI (QNL10608.1), SemI (MCW8383179.1), SetI (EPH44812.1), StmI (QTI46197.1), SpmI (MCI3903336.1), and LinI (ANS69122.1).



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FdxE        ------MKVRLDPSRCVGHAQCYAVDPDLFPIDDSGNSILAEHEVRPEDMQLTRDGVAACPEMALILEEDDAD   67
LinM        -MSGAAVRITGDPEVCTGAGQCVLTDPAMFAQDDRGVVELRTHRAEGARVPRAREAVFLCPSGALSIVED---   69
SemM        ------MRLEKDHDRCIGAGQCVRTDPRIFDQDDDGLVKVLIDRPRGTDEAAARKAVFICPAKALSLAQD---   64
StmM        ------MKLEKNHDRCIGAGQCVLTEPRIFDQDDRGLVSVLVERPQGADVAAARKAVYICPAKALSLVEE---   64
SetM        MVKEMAMKLEKDHDRCIGAGQCVLTEPRLFDQDDWGLVDILVERPRGADEAAARKAVYICPAKALSLVEE---   70

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Figure S7. Sequence alignment of FdxE and biaryl polyketide ferredoxins. The genes which encode [3Fe–4S] or [4Fe–4S] single cluster containing ferredoxins have unusual iron–sulfur cluster binding motif sequences, CXX?XXC(X)nCP, where '?' indicates a variable amino acid residue. Rather than a cysteine residue, which is highly conserved in [4Fe–4S] clusters, alanine or glycine residues at this position in [3Fe–4S] ferredoxin. The conserved CXXA/GXXC(X)nCP motif of [3Fe-4S] ferredoxin is highlighted in red. In FdxE, the Ala residue of the CXXA/GXXC(X)nCP motif is substituted by a His residue and is coloured green. Sequences included StmM (QTI46198.1), SetM (EPH44813.1), SemM (MCW8383178.1), LinM (ANS69121.1), and FdxE (O53937.1).