

Supporting Information

Chalkophomycin Biosynthesis Revealing Unique Enzyme Architecture for a Hybrid Nonribosomal Peptide Synthetase and Polyketide Synthase

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Table S1. Plasmids and strains used in this study.

Strains/plasmids	Descriptions	Reference/ Source
<i>E. coli</i> strains		
DH5 α	General cloning	Commercial source
S17-1	Intergeneric conjugal transfer	Kieser T, 2000
<i>Streptomyces</i> strains		
CB00271	Producing strain of chalkophomycin	This study
CB00271:: Δ <i>chmO</i>	<i>ChmO</i> gene replacement mutant of CB00271	This study
Plasmids		
pOJ260	<i>E. coli</i> vector, nonreplicating in <i>Streptomyces</i> , Am ^R	Kieser T, 2000
pXY5001	pOJ260-based plasmid for <i>chmO</i> inactivation, Am ^R	This study

Table S2. Primers in this study.

Primers	Sequences (5'-3')	Function
NRPS1.1-Up-F*	GCGGCCGCGGATCCTCTAGAGC GGCACAGGGCGAG	Construction of knockout plasmid for <i>chmO</i>
NRPS1.1-Up-R*	GAATGTGAACACACCTCGCGCA CCCC	
NRPS1.1-Tsr-F*	TGCGCGAGGTGTGTTACATTC GAACGGTCTCTGC	
NRPS1.1-Tsr-R*	GGGCCAACTGATTTATCGGTTG GCCGCGAGA	
NRPS1.1-Dn-F*	GCCAACCGATAAATCAGTTGGC CCGCCG	
NRPS1.1-Dn-R*	ACGACGGCCAGTGCCAAGCTT CCTGCCGACGAACTCGA	
pYZNRPS1.1F	CCGCGCTTCGTCTGATCTG	confirmation
pYZNRPS1.1R	TCCAGCAGCACCCCGAC	of mutant $\Delta chmO$

* The red-labeled nucleic acid sequence is the overlapping region with pOJ260 and the homology arm.

Figure S1. Identification of three sets of homologous proteins of GrbED from *S. sp.* CB00271, using query sequences GrbE (WP_006051176.1) (A) and GrbD (WP_006051175.1) (B).

WP_006051176.1 MSQSALSLPIFDSPFVGHSSERYVALIKQFAAQAQFRSAATVDLELLDDDFHRRRLRPFDFEFELKFMKFPV67
 WP_143203106.1 - - - MSPVSR - - - - IKSPADSPNGIVRRYAAANAPFRSATAEELLVNEDNPFRRFVRPHDLGFLDYGTGTFP60
 WP_073800909.1 MDTSP-SPHY - - - - SPLPGDDPNVIVKFAAANTAFRAAEWEHELTTEDNPNYRRFVRPHDDLWGLDYSGSPFM63
 WP_073805873.1 MVATSLIEVP - - - - EISIGDDAHSLVKEFAANTRAFRAAEWEHELTTTEQNPFRRFVRPHDDLGLWLDHSEFM62

WP_006051176.1 REHNVSRLPALASGRTLMSIYELDLARPPLSAEP-ADWQRFDTFYSEDTRLGLAQIAPFLEAYAFLEY133
 WP_143203106.1 TADRAKRLSALLGHRMLRTLVDHADLPLPAGGDGHPSSSDAEAFRSLDNRVRLALQAGQVLENHLHFHW128
 WP_073800909.1 PADAKRLLSGLLGHRLMRLNVYDLDLHLVPARTP-AVAADQKAFYSHDNRLVSALAKPVLHERHLFSF128
 WP_073805873.1 REVNALKLSGLLGHRLMRLNRYADSLLYVSDPTF-AAANDGAAFFYSAPNRAIRALARPILHERHLFT129

WP_006051176.1 LTKDVDTSLEDVGAASAR--ITRIVDDELAFWSDTEFERLMRNDYIEGLRLFIMVORWALAVSKRRRLA196
 WP_143203106.1 LDGERTPLRPG-GAEAVTRHVQVDLEERRAPGRGRALDVARSSGRHRDAATFTLTLTAAARPAVQSSAVG193
 WP_073800909.1 LAEGRTPLRPG-GAEAVATSHVIGAFEQRGAAGNKAIDAVERTVGRKEAGTFLMLQLAARFLPAMNAAV195
 WP_073805873.1 LDEVVRVPLETAELAAALKRHLIECYRRRSAPGDAFEAAARSTRARKESSTFLLQLFSAFLPASNAAV196

WP_006051176.1 RAGASGFFDMLTPDERPSLHRET- - - - PDDALFERYVASAGVTRRKHAYWQFYLPTSTAKCNLLLYAL261
 WP_143203106.1 - RGLLGDDYCTAHAPRLRGLLDDLDRDRADREAHRYRALLTGAGLSAEQPYAYWQFLLGSSSLGGANLHLSV259
 WP_073800909.1 - RAALGEYDLACHTLPRPFLLDDEYRSWVDSAAATYKMLLEGGLKTTPAAAYWQLYLLTSSLARGNHLHL261
 WP_073805873.1 - RNALGEYDRACHPTLRLRLLDGEYQAQWVEASQSYEKMLAGAELKTTAEAYWQLYLLGTSSLARGNHLHFT262

WP_006051176.1 ARRPDRAGFGLGAFAAAEAELVAFGLALERACF--L-OPAGRNHTDAEALKSALEQRCARLRVA325
 WP_143203106.1 SRDHARIGFELGAWVHHRLDEAVATAAYAEIQEAF- - - LGVDTGYFDTTPALAEGLRELVLGRLLTPLV326
 WP_073800909.1 SVNRENLFALFGLGAWVHHKMDLSLTRERFAADAFATC-LGVDTYFDTTPALAEGLRELVLGRLLTPLV327
 WP_073805873.1 SANHERLFAFLGALHKKVDEQAVRAFLSAVLADG-LGVGTGYFDTQDQKALTEADLGLQLVDELVLVAPL328

WP_006051176.1 DNFGAAARAQYVQGIVAVGERLAAARGRWDLGELQLRWLASIREYVAFAHRIISARIDAECPGIDRETTFVE392
 WP_143203106.1 ERFYGDEAVSFHHGFGFEDARRLASLGDGLDEVLQVLDWADRDLREYKIKAEKLDALISESGIEVDLETTFVE393
 WP_073800909.1 ARYGDDEVVEGFGFGFEDAAARFALAWETDMVEQVTWADSIQEQDKAIRIDKYLDSDNNIVVDLDTTFVE394
 WP_073805873.1 ERFGRRVIEGYVAGFADATWLADLDWRDVAEQLTWADRIEYQDKAAKLDHHTISEGIEVDLDTTFVE395

WP_006051176.1 PHEMCSTTHVHNDHRLVTIEEGOMLFWNVGMQMAHMKGDMVLIPDGRHLHGSTVSNECTYHQPIIF459
 WP_143203106.1 SHEETSTTHVHNDHRLVVVEEGQMHFWNVVGMQIMHDEGDKLLIPALRHLHGSTMVSGTCTYHQPIIF460
 WP_073800909.1 SNEETSTTHVHNEHRLVMVIEEGQMHFWNVVTHKIEFNOGDKVLIPVSLRHLHGSTVLSGECTYHQPIIF461
 WP_073805873.1 SDEETSTTHVHNEHRLVMVIEEGQMHFWNVVTHKIALNEGDKLLIPVSLRHLHGSTVLSGECTYHQPIIF462

WP_006051176.1 DAWIEALVAELDEPATATT
 WP_143203106.1 DELLSQV- - - - -
 WP_073800909.1 DDMLNQIF- - - - -
 WP_073805873.1 EAMLLQLS- - - - -

[illegible]

Figure S2. Phylogenetic analysis of ChmQ with other known methyltransferases.

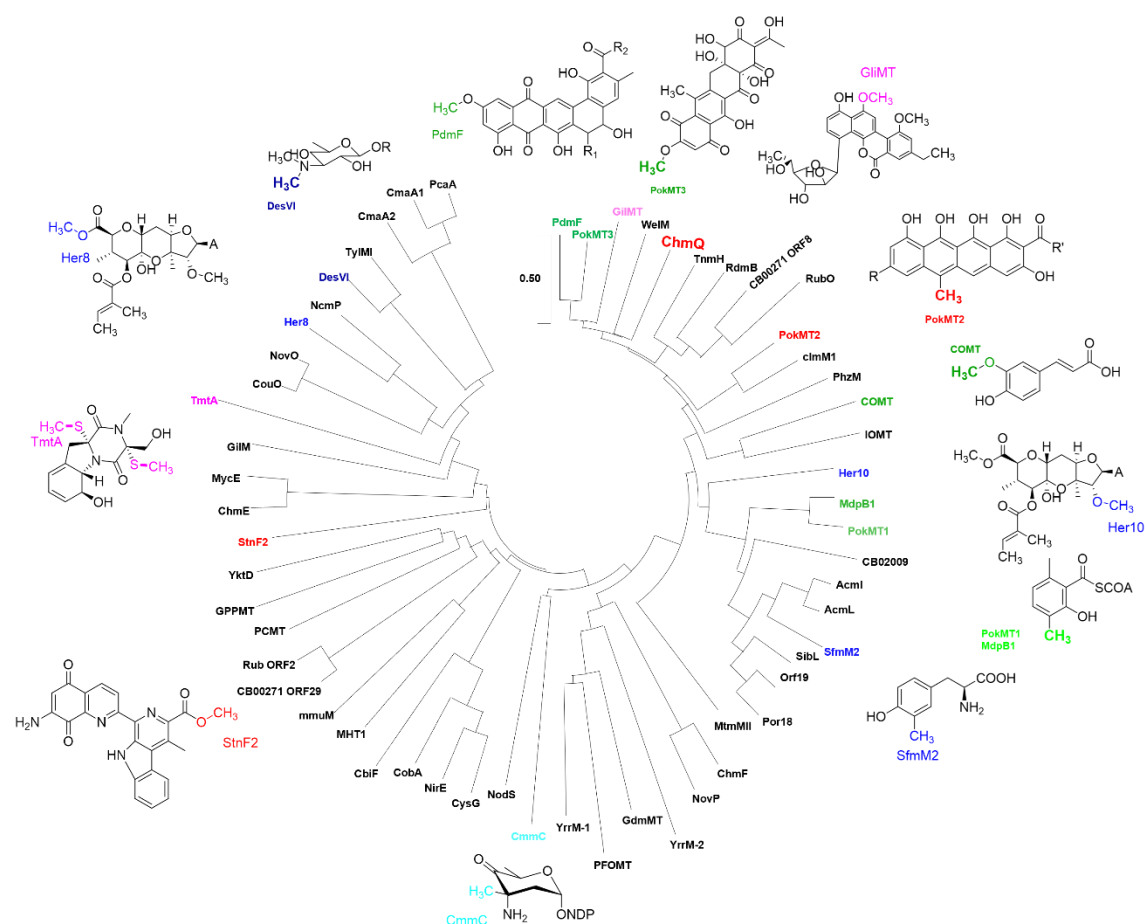


Figure S4. Phylogenetic analysis of the KS domain of ChmP with other known KSs using NaPDoS2 webtool.

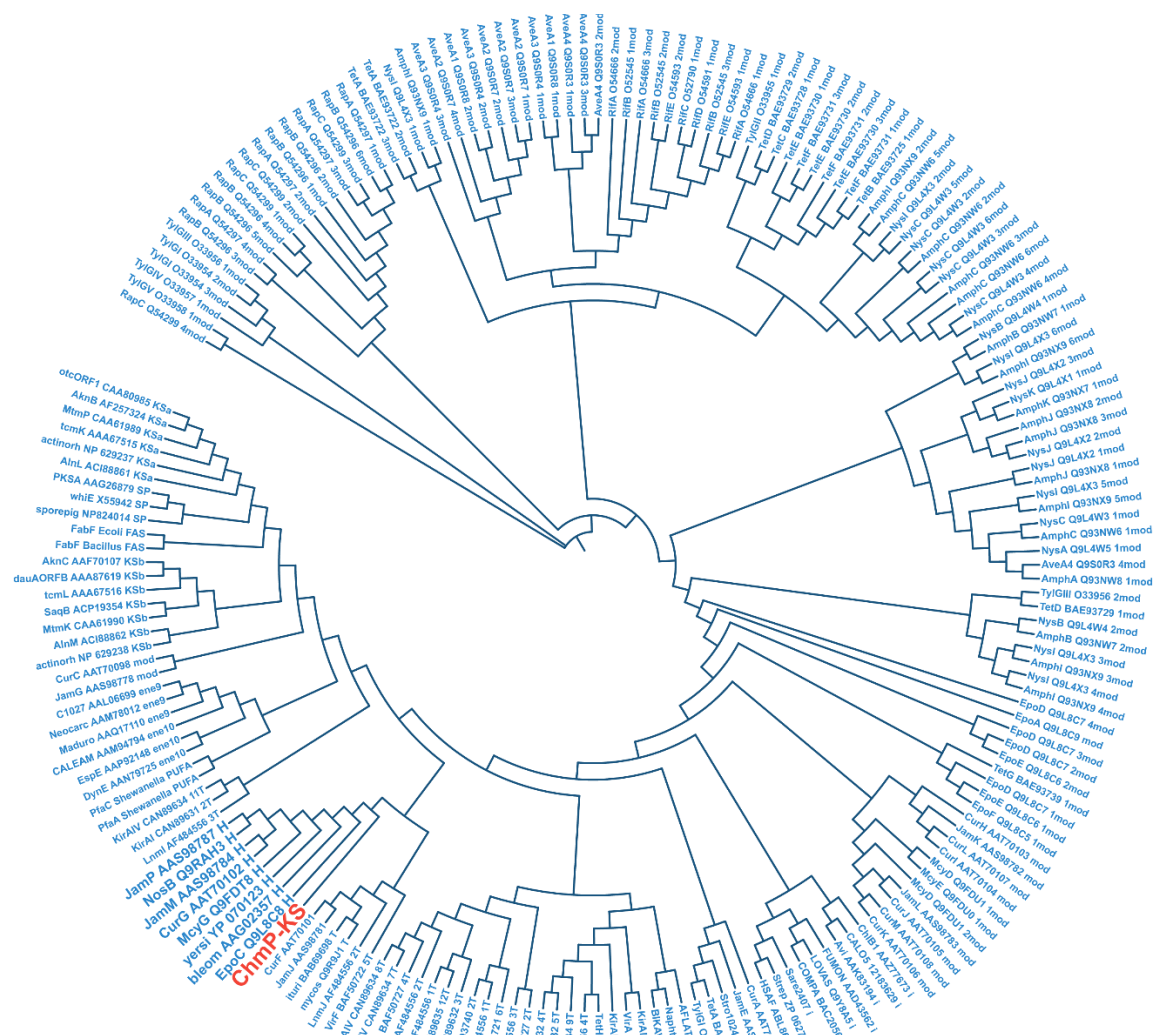


Figure S5. Sequence alignment of the R⁰ domain of ChmL from *S. sp.* CB00271 and *Streptomyces sp.* MNU77.

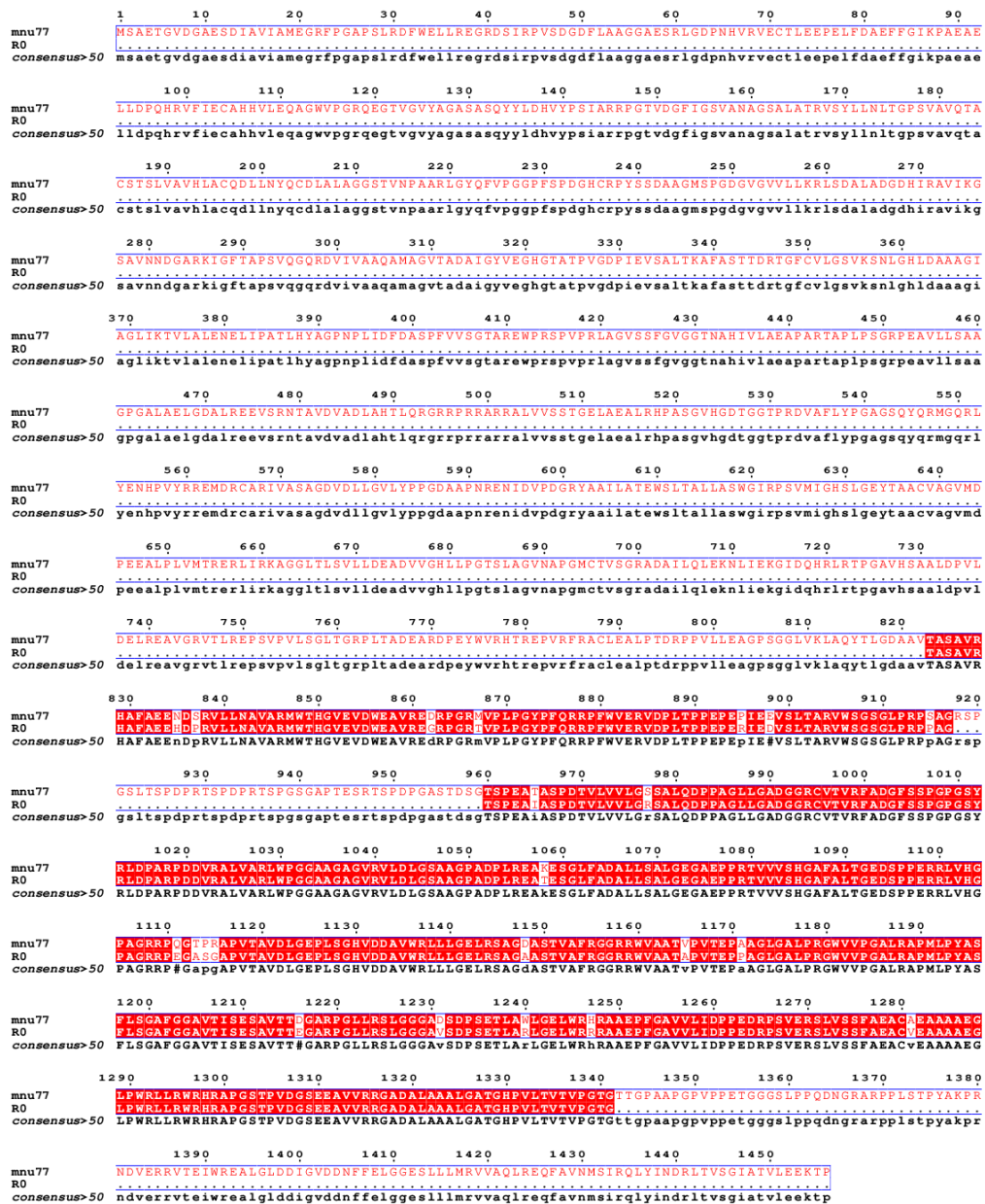
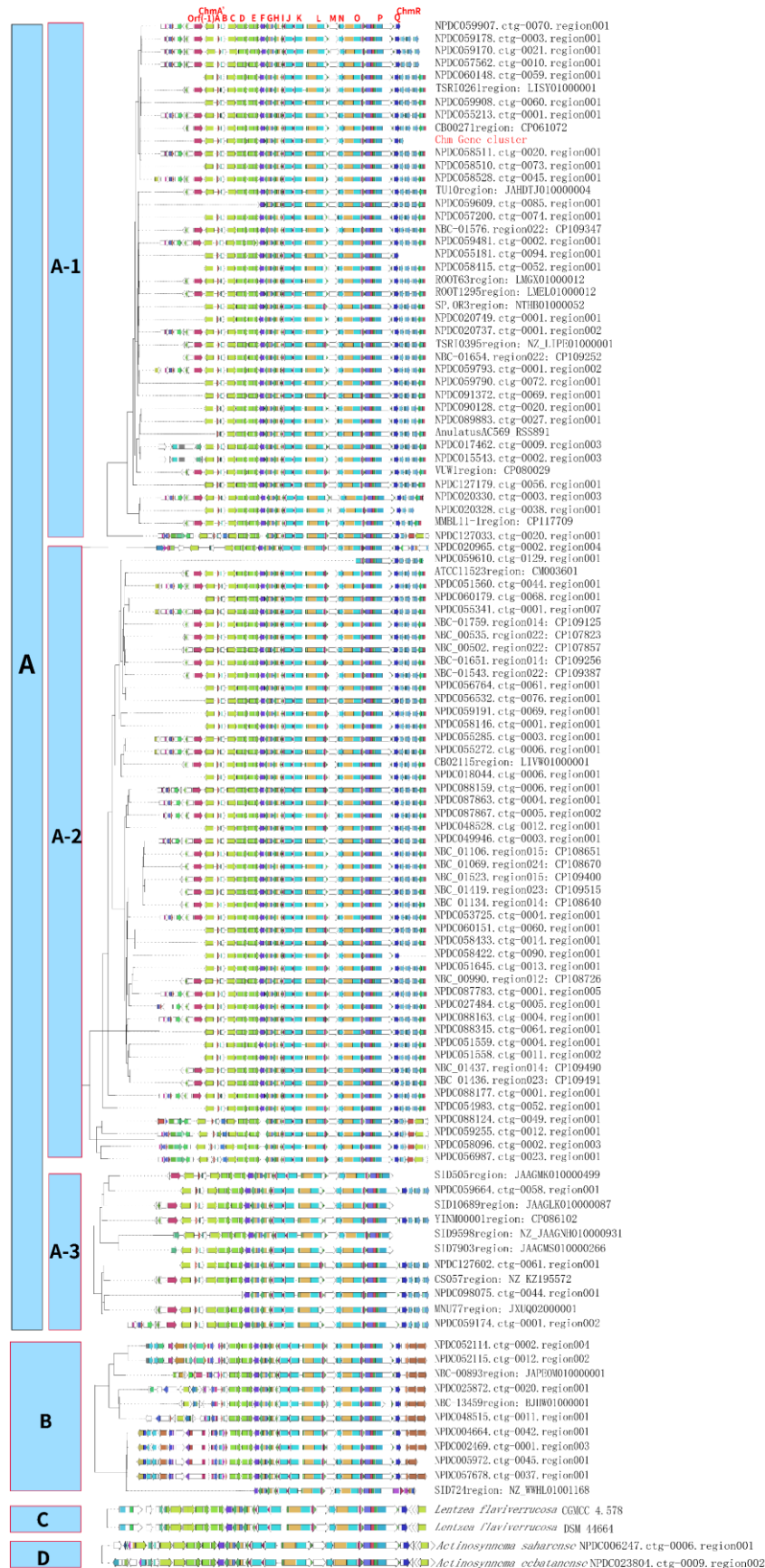


Figure S6. Phylogenetic analysis of *chm* gene cluster from *S. sp.* CB00271 and 116 identified *chm*-type gene clusters from the public databases.



References.

1. Kieser, T.; Bibb. M.J.; Buttner, M.J.; Chater, K.F.; Hopwood D.A. (2000). Practical *Streptomyces* genetics. *John Innes Foundation, Norwich, United Kingdom*.