Additional Information for:

Heterologous expression of the atypical tetracycline chelocardin reveals the full set of genes required for its biosynthesis

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Table S1

Bacterial strains and plasmids used in this study[a]

Strain or plasmid	Relevant characteristics	Reference or source
Escherichia coli		
DH10β	F$^-$ *endA*1 *recA*1 *galE*15 *galK*16 *upG* *rpsL* Δ*lacX*74 Φ80*lacZ*ΔM15 *araD*139 Δ(*ara-leu*)7697 *mcrA* Δ(*mrr-hsdRMS-mcrBC*) λ$^-$	Invitrogen
ET12567	F$^-$ *dam*13::Tn*9,* *dcm*6, *hsdM*, *hsdR*, *recF*143::Tn*1I,* *galK*2, *galT*22, *ara*14, *lacY*1, *xyl*5, *leuB*6, *thi*1, *tonA*31, *rpsL*136, *hisG*4, *tsx*78, *mtl*1 *glnV*44	(1)
GB2006	δM109 *rpsL*- Δ*rfuA*	Gene Bridges
Amycolatopsis sulphurea		
NRRL 2822	WT producer of CHD	ARS Culture Collection
Streptomyces rimosus M4018	Producer of OTC	(2)
Streptomyces albus del14	host strain for heterologous expression	(3)
Plasmids		
pAB03	pSET152-derived, containing ΦBT, Apr^r	(4)
pAB03oxyDP	*oxyD* and *oxyP* cloned into pAB03	(5)
pAB03otcR	*otcR* cloned into pAB03	This study
pAB03oxyDP-otcR	*oxyD*, *oxyP* and *otcR* cloned into pAB03	This study
pAB03SARP	*SARP* cloned into pAB03	This study
pAB03oxyDP-SARP	*oxyD*, *oxyP* and *SARP* cloned into pAB03	This study

pOJ436	pSET152-derived cosmid, containing ΦC31, Apr^r	(6)
pKC1139	bifunctional *oriT* RK2 vector, pSG5 ori, Apr^r	(6)
pOJ456	pOJ436-derived cosmid, ΦC31 integrase cassette replaced with pSG5 replication cassette, Apr^r	This study
pOJ456CHD12	pOJ456 cosmid carrying CHD BGC	This study
pAB03e*	pAB03 vector with P_{ermE*} promoter instead of actII-ORF4/PactI activator/promoter system	Acies Bio
pAB03e*chdR	pAB03e* carrying *chdR* gene	This study
pAB03e*oxyD	pAB03e* carrying *oxyD* gene	This study
pAB03e*oxyDP	pAB03e* carrying *oxyD* and *oxyP* genes	This study
pAB03e*oxyDPchdR	pAB03e* carrying *oxyD*, *oxyP* and *chdR* genes	This study
pOJ436e*chdR	pOJ436 carrying a 1.8 kb fragment from pAB03e*chdR containing *chdR* gene under the control of P_{ermE*} promoter	This study
pOJ436e*oxyDP	pOJ436 carrying a 3.2 kb fragment from pAB03e*oxyDP containing *oxyD* and *oxyP* genes under the control of P_{ermE*} promoter	This study
pOJ436e*oxyDPchdR	pOJ436 carrying a 4.7 kb fragment from pAB03e*oxyDPchdR containing *oxyD*, *oxyP* and *chdR* genes under the control of P_{ermE*} promoter	This study
pOJ436CHD12	pOJ436 carrying CHD BGC	This study
pOJ436e*chdRCHD12	pOJ436e*chdR carrying also CHD BGC	This study
pOJ436e*oxyDPCHD12	pOJ436e*oxyDP carrying also CHD BGC	This study

pOJ436e*oxyDPchdRCHD12	pOJ436e*oxyDPchdR carrying also CHD BGC	This study

[a] Apr[r], apramycin resistant; Kan[r], kanamycin resistant.

Table S2

Sequences of the oligonucleotide primers for PCR experiments used in this study[a]

Primers	Sequence
CobU1	5'-TCCTCACTGCAGGTCGAGTACC-3'
CobU2	5'-CGGGAAGTCGCGGTATGC-3'
glu1	5'-CGCGCTGGTCAAAGTCTACG -3'
glu2	5'-CTGGACGCCTCGCCGTAC-3'
chdRF	5'-TATATACATATGAAGGACAATCTCGCGAGA-3'
chdRR	5'-TATATATCTAGAGGACCTCCGCATCAGGC-3'
otcR-Fw	ATATATTCTAGATCCGATTAATTAAGGAGGACACATATGGACTTCAAGGCACTCG GC
otcR-Rv	TATATATCTAGATTCAAGACGCCGACCTCAACAC
SARP-Fw	ATATATTCTAGATCCGATTAATTAAGGAGGACACATATGAAATTCAACCTGCTTG GTCCG
SARP-Rv	TATATATCTAGATTCACACATGCGCGGGTG

[a] Restriction sites are underlined.

Table S3

Overexpression of SARPs *otcR* and *chdB* in *A. sulphurea* WT strain

Strain	**(CD)CHD concentration [mg/L]**	**Comment**
WT/pAB03	546 ± 76	
WT/pAB03otcR	390 ± 89	0.7× CHD production
WT/pAB03+SARP	812 ± 117	1.5× CHD production
WT/ pAB03oxyDP	68 ± 23	
WT/ pAB03oxyDP+otcR	130 ± 29	1.9× CDCHD production
WT/ pAB03oxyDP+SARP	103 ± 7	1.5× CDCHD production

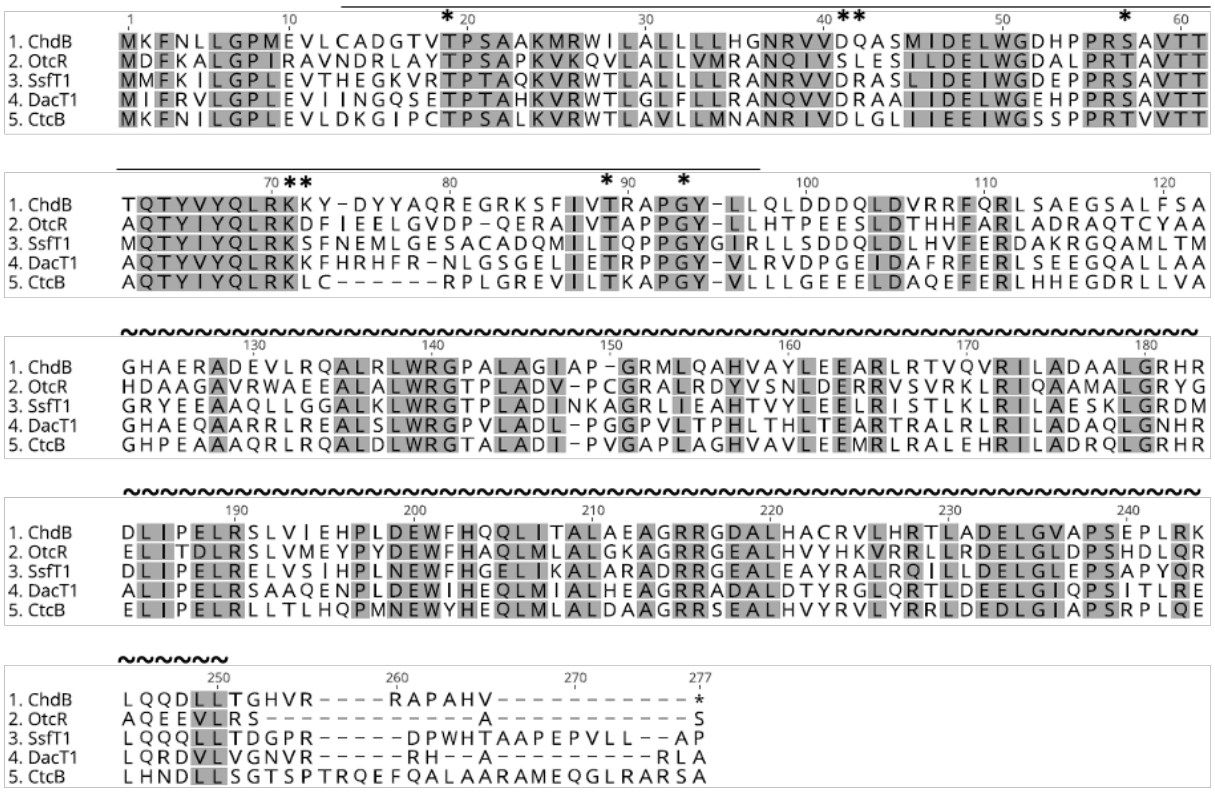

Figure S1. Protein alignment of ChdB with closest homologs present in BGC encoding type II PKS. Close homologs of *Streptomyces* antibiotic regulatory protein (SARP) from oxytetracycline, SF2575, dactylocycline and chlorotetracycline BGC, OtcR, SsfT1, DacT1, CtcB, respectively are presented. Gray colour denotes the similarity of the conserved amino acid residues. The OmpR/PhoB-type DNA-binding domain with a typical fold of the helix-turn-helix is marked with —, whereas the conserved DNA-binding sites are marked with *. Additional DNA-binding domain marked with ~ contains three tetratricopeptide repeats (TPRs) and two C-terminal helices. The TPR motif generates a right-handed helical structure with an amphipathic channel that is thought to accommodate an alpha-helix of a target protein.

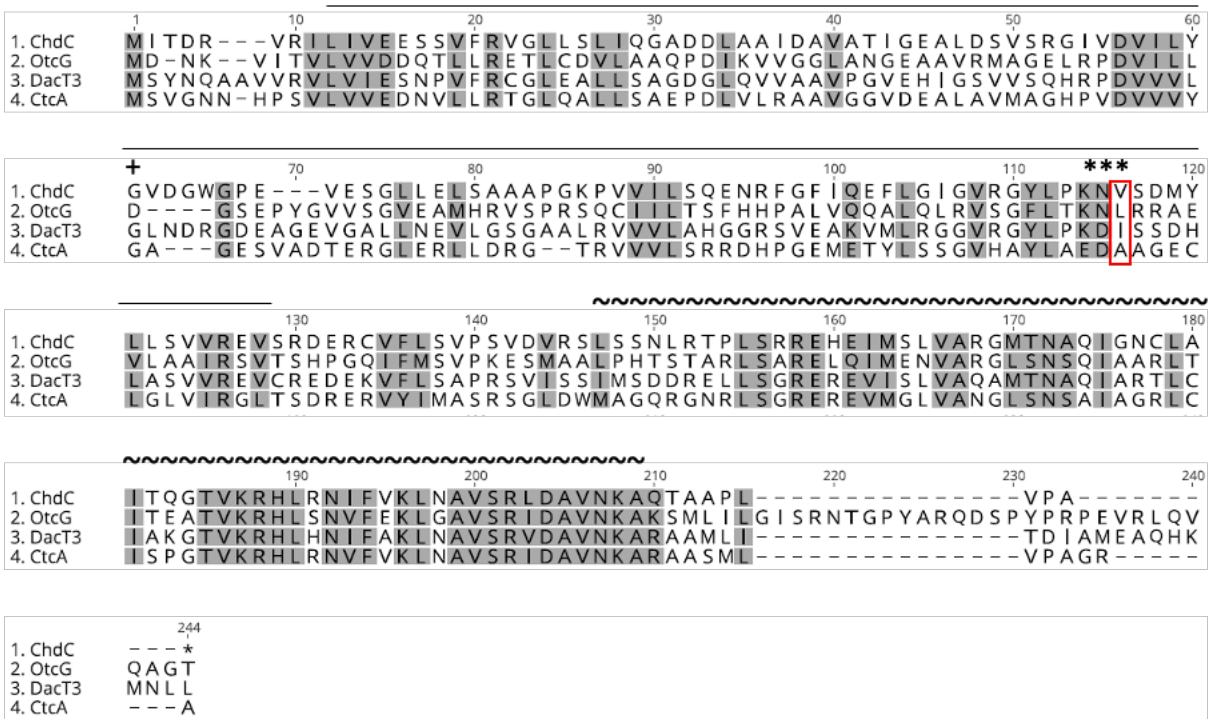

Figure S2. Protein alignment of ChdC with closest homologs present in BGC encoding type II PKS. Close homologs from oxytetracycline, dactylocycline and chlorotetracycline BGC, OtcG, DacT3 and CtcA, respectively are presented. Gray colour denotes the similarity of the conserved amino acid residues. The Sigma-70 domain involved in binding to the -35 promoter element via a helix-turn-helix motif is marked with ~, the signal receiver domain is marked ——, the TTA codon is marked with red rectangle, the phosphorylation site is marked with +, and the dimerization interface is marked with ***. (7)

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