

Supporting Information

Insights into the complex biosynthesis of the leupyrrins in *Sorangium cellulosum* So ce690

Maren Kopp^{a,b}, Herbert Irshik^b, Katja Gemperlein^a, Kathrin Buntin^a, Peter Meiser^{a,c}, Kira J. Weissman^{a,d}, Helge B. Bode^{a,e}, Rolf Müller^{a,b,*}

^aHelmholtz Institute for Pharmaceutical Research, Helmholtz Center for Infection Research and Department of Pharmaceutical Biotechnology, Saarland University, P.O. Box 151150, 66041 Saarbrücken, Germany

^bMicrobial Drugs, Helmholtz Center for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

^cPresent address: URSAPHARM Arzneimittel GmbH & Co. KG, Industriestraße 35, 66129 Saarbrücken, Germany

^dPresent address: AREMS, UMR CNRS-UHP 7214, Nancy Université, Faculté des Sciences et Technologies, Bld des Aiguillettes, BP 70239, 54506 Vandoeuvre-lès-Nancy, France

^ePresent address: Molekulare Biotechnologie, Institut für Molekulare Biowissenschaften, Biozentrum/Campus Riedberg, Goethe Universität Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany

(A)

```
DkxI_DK 1 ----STQLEKEELARAPAA-----EAAAE-----TEELTV-----
DkxI_DW 1 ----HSQLENELARPPS-----AAAE-----TEELTL-----
BlnVIII 1 ---------EETP-----AA-----EEVVL-----
LeuB 1 RLIQGISIRALAAYVEQAALKRL-----VASKGQGGSSADHAADVLEVL-----
MeIF 1 ----S-STGVAA-----PKAA-----WEEGEL-----
TubB 1 ----GCEAQSWSTQAPH-----PPSAS-----TEEGEL-----
MtaF 1 ----GNSGGAA-----VVAKE-----WEEGEL-----
MeIC 1 ---------E-----WEEGSL-----
MtaC 1 ---------A-----WEEGAL-----
AjuK 1 ----VCALETRAADDDR-----GEQS-----WEEGEL-----
AjuC 1 -LELVAAEEVLDVITISGTGGALGSEALGDAESVAPSED-----SETEVLQF-----
Leu9 1 --EGLAARLMEELLQSRANKN-----EF-----SETEVLLV-----
```

(B)

```
MtaG 1 ----MAELSQQGVTLWVEGERLKYRAPKGLSPETLS--LSTHKAALPYLRR--LAAEGESVH-----
MeIG 1 --MSAGELVAELSQQGVTLWVEGDRLYRAPKGLSPETLS--LVAGHKAALQHLRQ--LAAEGESVH-----
TubC 1 --MSAGALLAHAASLGVRLWVEGERLRYAPPGVMTPELQS--RLGGARHELIALLRQ--LQP-----
MtaD 1 --MSTSEFIVYDQKGTIELWIEGDTLRFAPPGHILTEPLRT--ALKDKKEQLLEHLR--SASR-----
MeID 1 --MTTAEFIIEIQKGTDLWVEGDALRFAPPGHILTEPLRA--GLKERKAQLTEHLR--AASQ-----
LeuE 1 --MSLGELFSDLSORGVLWVEGDALRFAPAGALTPNVR--SLATHKAQIVSLLRMEAGTQASDAPIERAPRDLPI-----
DkxJ_DK 1 --MNLGELLAEINRRGLEVRVEGDALRURPKGAATAELRQ--ALAEHKPELLELLRA--HDRGQEEAPIVPVQRTGPA-----
DkxI_DW 1 --MSLGELLAEINRRGLEVRVEGDALRURPKGAATAELRQ--ALTUHKSGLLALLHE--RNR-RETAPIAKALRDGPA-----
AjuL 1 --MTIDELAKLAAAGVSLWVDCGLRFAPPGHILTEPLKA--QLGAHKKATLALRAA--TTEGQEH-----
LeuA 1 --MNTAELLEQLLEIRLSVKGDKLEVSAPKGLTPDLRA--RLAERKAELAMIAQ--SSRAKAEVELPEVQHRPQERHEPF-----
AjuD 1 ----MALITDLAARNVQSATGEQLDVRAPKGLTPELRE--QLAKHKPELLELLRQ--RSSDKAEQKLPTVL-----
BlnV 1 MTTPRITDLELGRQVTLTADGDRLHCRAPKGLTDELLATIRFARDELIALHRA--DRR-----
```

(C)

```

DkxH_DK 1 -----AEEELGTEVQKKQEQASR-----EAQ6GALS-----AETQGLSEQELTSLTDQELLES---A--TR-----
DkxH_DW 1 -----AGEGMGGFFQKQDKSSE-----QAQDALA-----AETQGLSEQELTSLTDQELLES---A--TR-----
MeID 1 -----LGEPLAPSPSGHVPDK-----AEAGPS-----LENLEQPPQDELGAALDQKLDADLEKL--GDA-----
CtaD 1 -----LGPPLAPSAP- AVPDE-----AVATAQA-----VANLEQPPQDELGAALDQKLDADLEKL--G-----
MtaD 1 -----LKGVPDVPVA-QASKG-----EEAAQQT-----LANLEQPPQDELGAALDQKLDADLEKL--GDG-----
StiD 1 -----GPLEAPPEPPRETAE-----KKDSAST-----AAE-LDALSDDDEVAARLAELK-----AA--NKGRK-----
AjuG 1 -ATEILAPTPETAAPPSPDASSAP-----SPTGSET-----APDPLDALTDEELVALLAELWPARG--SDARSGTLSEGPQRPEPHTA
DkxG_DK 1 -----DAFIPLEFTEEARV-----TSKSDAP-----VESPLEGLAANLAHLSEQL-----SK--N-----
DkxG_DW 1 -----AAFIPLFEASEENT-----QQEQSKP-----VEDHLEGLTANLANLAELQ-----SK-----
StiC 1 -----FATSSAQQRVDPRP-----GETQSDAP-----SVVLDVLSRNEVESRUDERAA-----EASMMD-----
StiH 1 -----FATLFEAPRATTPEP-----VSNDDAR-----WDALDELSDQVASTLAERUNA-----E-----
AjuB 1 LGHVSAEPVPATSSPTLIATVPSP-----VTALPALP-----SLENLSAEASDLAELASASAAL--G-PGMD-----
AjuL 1 LGELYAPLATSIPSPPPASPPNTPVATLFTASSSPDDAQESALHDLSE--SELAQLDDELAS--I--GNPSGS-----
DkxN_DK 1 -----LAAEVLGLKEEAAAPVE-----EAPEDALESLLADVDSDDDTVQAALRRGR-----I-----
DkxN_DW 1 -----AAEVLGLKDDEAAPAAA-----WDAGEDQELDALLADVDDDDQVQAALRRGR-----I-----
LeuC 1 -----EGSALARGQEAPVR-----AAIDDAELGALLEEVDALSGAEINARLQGAR-----IGAAR-----
LeuA 1 -----IERVIDLDFSPGKRPAD-----VAPAALG-----TNLDALSSDEAEELLSQKLQA-----ID-----
AjuA 1 -LRQLDAPTANGATVRGPRTTTS-----TEVRMLGEPLDLPABELSDSEAEALLVEELER-----INY-----
AjuF 1 -----AASRSATPQRAESD-----ADEAMR-----AEVEDLSDDEAALALAEALAE-----VLDDEEMME-----
AjuE 1 ----ADRMQIATVVSAGHDDAREPQ-----ESRDRAALDAALHEVEQLSEDAALALMKGT-----I-----
LeuB 1 ----SELLPGAERPASAAPAGAP-----GDAGRQE-----FTEIEPLSPGELAEALDSEFN-----ITD-----

```

(D)

```

DkxH_DK 1 -----MKRALKKQETQSKLDAHEQEH--
DkxH_DW 1 -----MSTQG-----SDTGYSDDLKRALKKQDAQSKLDAHEREH--
LeuB 1 -----MPTR-----DYAELMKQALKKEETEAKLRALKQAATE--
AjuB 1 MNSGEKREEA---TTASAPTVKRALAAQDTRARLAEEQEHAP
MeIE 1 -----MST-----EQNEHSARLARALVALEKQARLEASEREKRE--
CtaE 1 -----MST-----EQNEHSARLARALVALEKQARLEASEREKRE--
MtaE 1 -----MSSSV-----EQNEHSARLARALVALEKQAKLEASEREKRE--
StiD 1 -----MSTTPNMPEQGEDLGRKRALIALEKQAKLDAAERKTE--
AjuA 1 -----MEH-----KARITSAALAAKKIQDLDAAVERSR--
AjuC 1 -----MNST-----ETPPQARLEALAAHKRTIRLDAVERKAT--
StiE 1 -----MST-----GSQDHRSLKQSFLETERLOAKVDSLERAKSE--
StiJ 1 -----MSV-----KTKDNQDLKRALYTDKLOAKVDALERAKSE--
DkxN_DK 1 -----MTPLCRAALAAKTIRARVDGLERAKS--
DkxN_DW 1 -----MSND-QTPASLEAMTPLCRAALAAKTIRTRIDGLERART--
AjuG 1 -----MSR-----QGSPDADLSAQKRALLAKKLQTKLDTERAAT--
LeuC 1 -----MIT-----TTPARKELSOLERAAYAKHLRAQLDKYR-----
AjuH 1 -----MSSP-----PLPLDRHAVKHALREQQARVDTSERRER--
DkxI_DK 1 -----MAKLS-----TRISELPVKKLAYLA-SQLRSKELLAA-----
DkxI_DW 1 -----MAKLS-----TRISEMPVKKLAYLA-SQLRRAKELLAA-----
AjuF 1 -----MSRST-----AREGRISAKLALLA-QQSRKTEGAEELLGS--
LeuB 1 -----MSTLL-----ERAAGMSVAKLAYLA-QQRAKRVVT-----

```

Fig. S1. (A) Multiple sequence alignment of C-terminal docking domains present at PKS/NRPS-NRPS junctions in mixed systems ^{1,2}. (B) Multiple sequence alignment of N-terminal docking domains (' β -sheet type' ¹) present at PKS/NRPS-NRPS junctions in mixed systems (potential partners of those shown in (A) (color-coded)). (C) Multiple sequence alignment of C-terminal docking domains present at PKS-PKS junctions in mixed systems ³. (D) Multiple sequence alignment of N-terminal docking domains present at PKS-PKS junctions in mixed systems (potential partners of those shown in (C) (color-coded)). Key to pathways: Dkx, DKxanthene (DK = *Myxococcus xanthus* DK 1622; DW = *Stigmatella aurantiaca* DW 4/3-1); Blm, bleomycin; Leu, leupyrrin; Mel, melithiazol; Mta, myxothiazol; Tub, tubulysin; Aju, ajudazol; Cta, cystothiazole; Sti, stigmatellin. In each case, the subunit name is given.

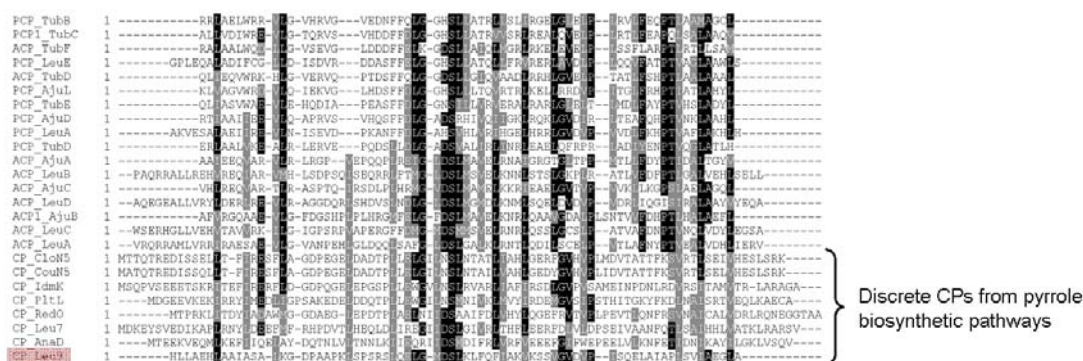


Fig. S2. Multiple sequence alignment of ACPs and PCPs derived from myxobacterial pathways alongside discrete CPs involved in pyrrole biosynthesis from diverse bacteria. Key to pathways: Tub, tubulysin; Leu, leupyrrin; Aju, ajudazol; Clo, clorobiocin; Cou, coumermycin; Idm, idanomylin; Plt, pyloluteorin; Red, undecylprodigiosin; Ana, anatoxin. In each case, the subunit or protein name is given.

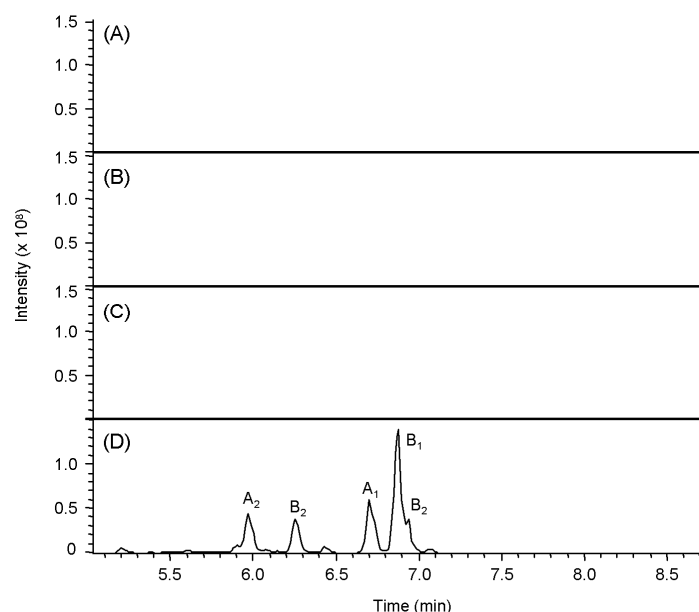


Fig. S3. Base peak chromatogram ($m/z = 730-740$) of the (A) *leu5*⁻, (B) *leu18*⁻, and (C) *leu13*⁻ mutants. (D) Base peak chromatogram ($m/z = 730-740$) of *S. celluloseum* So ce690 wild type, showing peaks corresponding to the leupyrrins A1, A2, B1 and B2.

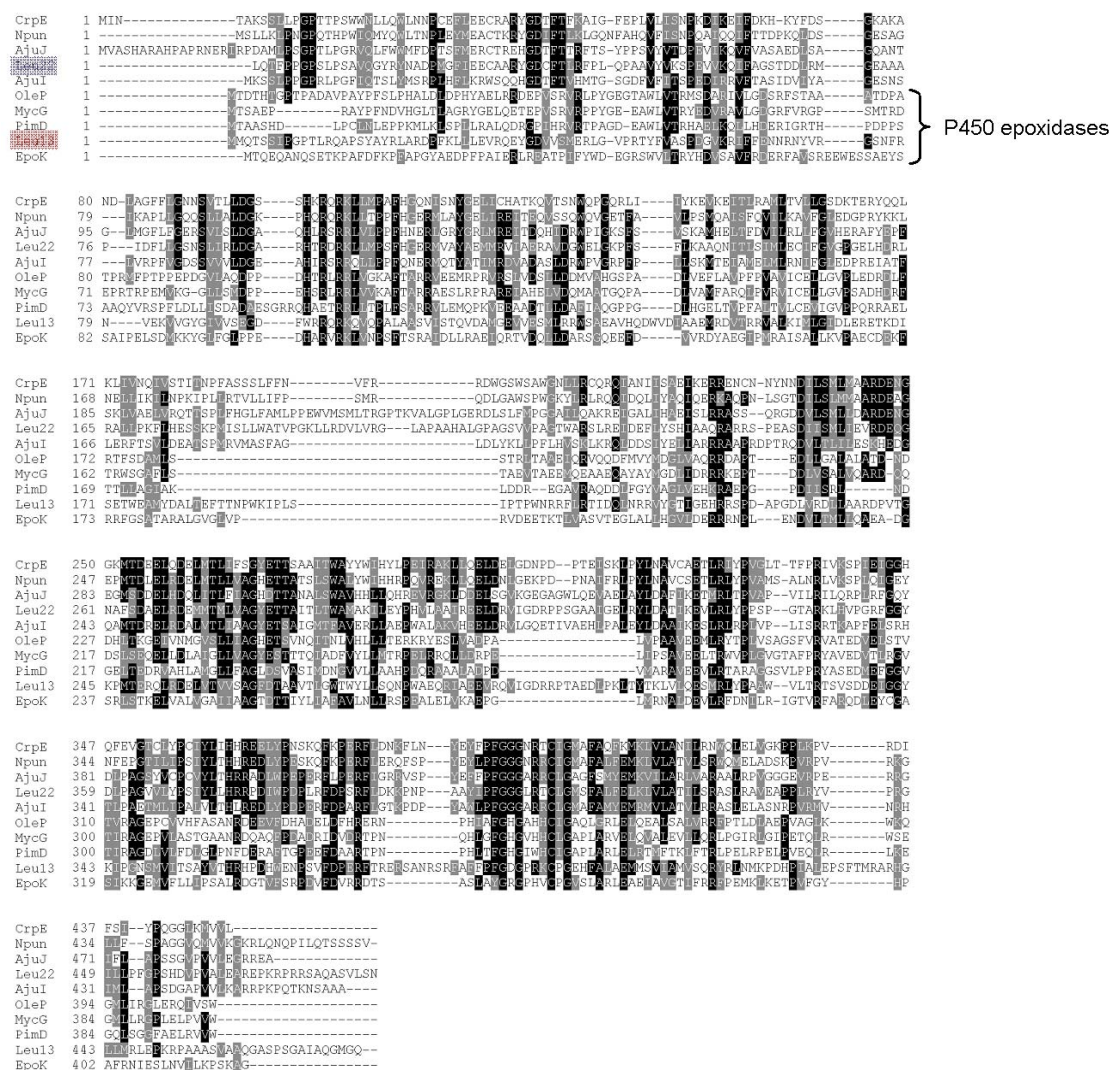


Fig. S4. Multiple sequence alignment of cytochrome P450 enzymes from various secondary metabolic pathways. This analysis shows that Leu13 aligns with other bonafide epoxidases ⁴⁻⁷, while Leu22 is a member of a distinct group which includes the hydroxylase AjuJ ⁸. Key to clusters: Crp, cryptophycin; Npun, unidentified cluster *Nostoc punctiforme* ATCC 29133; Aju, ajudazol; Leu, leupyrrin; Ole, oleandomycin; Myc, mycinamicin; Pim, pimaricin; Epo, epothilone.

Reference List

- 1 C. D. Richter, D. Nietispach, R. W. Broadhurst and K. J. Weissman, *Nat. Chem. Biol.*, 2007, **4**, 75-81.
- 2 Y. Li, K. J. Weissman and R. Müller, *ChemBioChem*, 2010, **11**, 1069-1075.
- 3 P. Meiser, K. J. Weissman, H. B. Bode, D. Krug, J. S. Dickschat, A. Sandmann and R. Müller, *Chem. Biol.*, 2008, **15**, 771-781.
- 4 A. M. Rodriguez, C. Olano, C. Mendez, C. R. Hutchinson and J. A. Salas, *FEMS Microbiol. Lett.*, 1995, **127**, 117-120.
- 5 M. Inouye, Y. Takada, N. Muto, T. Beppu and S. Horinouchi, *Mol. Gen. Genet.*, 1994, **245**, 456-464.
- 6 M. V. Mendes, N. Anton, J. F. Martin and J. F. Aparicio, *Biochem. J.*, 2005, **386**, 57-62.

- 7 H. Ogura, C. Nishida, U. Hoch, R. Perera, J. H. Dawson and P. R. Ortiz de Montellano , *Biochemistry-US* 2004, **43**,14712-14721.
- 8 K. Buntin, S. Rachid, M. Scharfe, H. Blöcker, K. J. Weissman and R. Müller, *Angew. Chem. Int. Ed.*, 2008, **47**, 4595-4599.