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Supplemental Information

Polyketide Proofreading

by an Acyltransferase-like Enzyme

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Inventory of Supplemental Information

Supplemental information for
Polyketide Proofreading by an Acyltransferase-Like Enzyme

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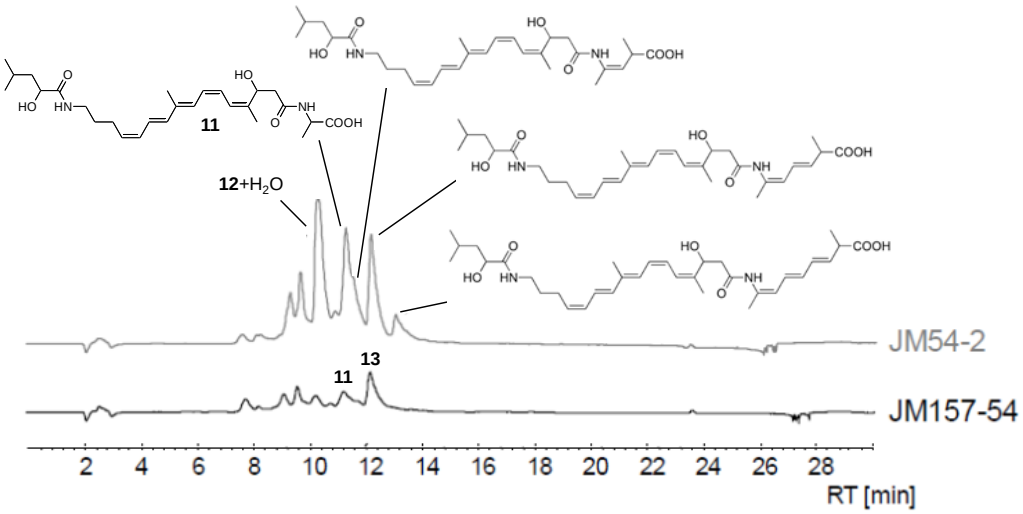
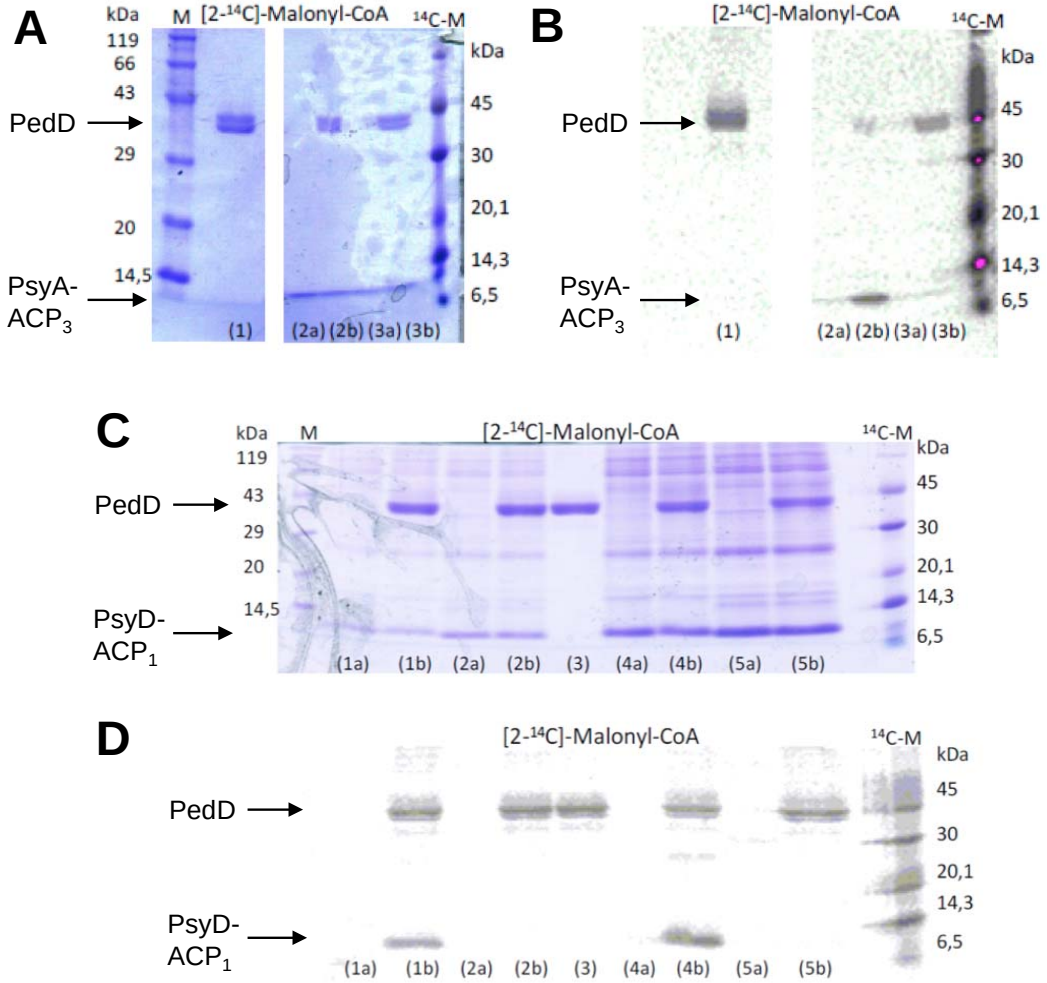
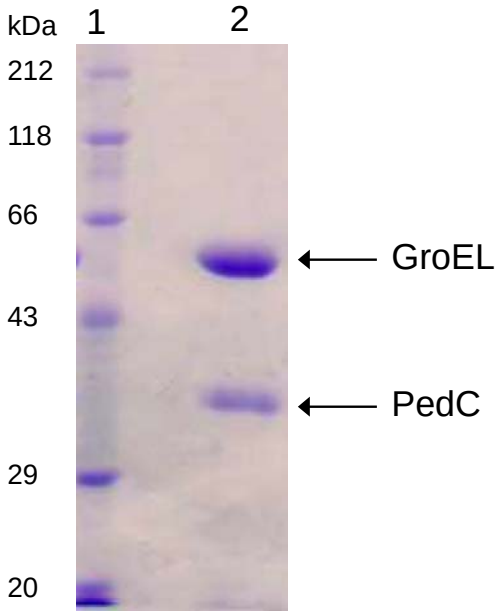
Supplemental Figure 1, related to Figure 2: HPLC traces of crude extracts of *B. amyloliquefaciens* JM54-2 (bacillaene TE deletion mutant) in comparison to JM157-54 (TE/*baeB* double mutant). Compounds were identified by LC-HRMS and LC-NMR analysis (Moldenhauer, et al., 2007; Moldenhauer, et al., 2010). In JM157-54 extracts only small amounts of compounds **11** and **13** could be detected.



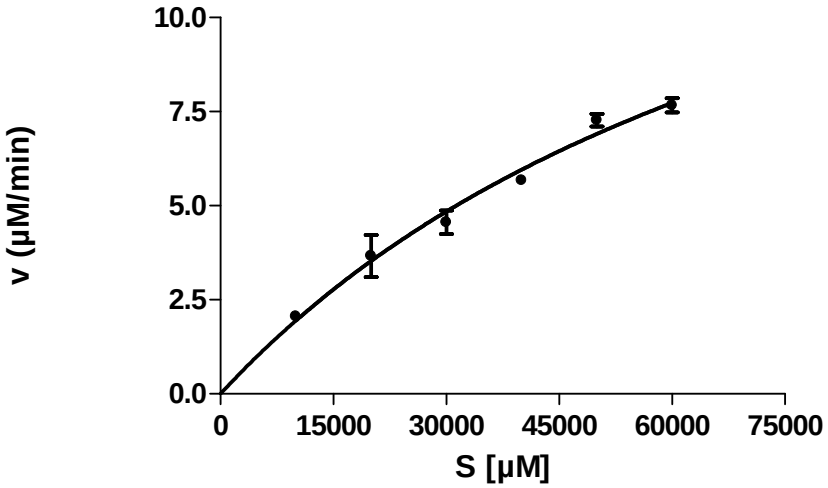
Supplemental Figure 2, related to Figure 4. Acyltransfer assay using PedD, radiolabeled malonyl-CoA, and two integrated ACPs of the multimodular psymberin PKS. **A:** SDS-PAGE analysis of assays containing the ACP of PsyA, module 3. Lane 1, PedD only; lane 2a, *holo*-ACP; lane 2b, PedD + *holo*-ACP; lane 3a, *apo*-ACP; lane 3b, PedD + *apo*-ACP. All assays contained [2-^{14}C]-malonyl-CoA. **B:** Autoradiogram of the same gel. **C:** SDS-PAGE analysis of assays containing the ACP of PsyD, module 1. Lane 1a, *holo*-ACP; lane 1b, PedD + *holo*-ACP; lane 2a, *apo*-ACP; lane 2b, PedD + *apo*-ACP; lane 3, PedD; lane 4a, *holo*-ACP; lane 4b, PedD + *holo*-ACP, lane 5a, *apo*-ACP; lane 5b, PedD + *apo*-ACP. For assays of lanes 4a to 5b, the ACP was concentrated 10× using Vivaspin ultrafiltration columns (molecular weight cut-off 5000). **D:** Autoradiogram of the same gel. All assays contained [2-^{14}C]-malonyl-CoA.



Supplemental Figure 3, related to Figure 5. SDS-PAGE analysis of purified PedC used for the hydrolysis assays. Samples were stained with Coomassie brilliant blue for visualization. Lane 1, molecular weight marker; lane 2: purified PedC with GroEL after affinity chromatography.



Supplemental Figure 4, related to Figure 5. Rate versus substrate concentration for the hydrolysis of 3-hydroxybutyryl-SNAC (**5**) by PedC. Calculated kinetic parameters are K_m = 88.48±27.97 mM, k_{cat} = 76.48 min^{-1}, v_{max} = 0.01912±0.0041 mM min^{-1}, and k_{cat}/K_m = 14.41 $M^{-1}s^{-1}$.



Supplemental Table 1. Constructs and their respective antibiotic resistance cassettes, additives, inducer and expression strains.

construct	plasmid	additive	inducer	expression host
PedC (C-terminal strep tag)	pHN38 + pGro7	arabinose (0.5 mg mL^{-1})	anhydrotetracycline (200 ng mL^{-1})	*E. coli* BL21DE3
PedC (N-terminal MBP tag)	pH66	glucose monohydrate (2 g L^{-1})	IPTG (1 mM)	*E. coli* BL21DE3
PedC (C-terminal His_6 tag)	pET-pedC + pG-Tf2	tetracycline (50 ng mL^{-1})	IPTG (1 mM)	*E. coli* BL21-Gold(DE3)
PedD (N-terminal His_8 tag)	pKZ178	-	IPTG (0.5 mM)	*E. coli* BL21DE3
GroES/L	pGro7	arabinose (0.5 mg mL^{-1})	-	*E. coli* BL21DE3
RhiG and mutants (N-terminal His_6 tag)	pNB121, pNB146, pNB147	-	IPTG (0.5 mM)	*E. coli* BL21DE3
PsyD-ACP1 (N-terminal His_8 tag)	pHN66, pHN78	-	IPTG (0.75 mM)	*E. coli* BL21DE3
PsyA-ACP3 (N-terminal His_8 tag)	pHN60, pHN77	-	IPTG (0.75 mM)	*E. coli* BL21DE3
PedN (N-terminal His_8 tag)	pKZ124, pKZ161	-	IPTG (0.4 mM)	*E. coli* BL21DE3
PedI3 (N-terminal His_8 tag)	pKZ123, pKZ176	-	IPTG (1 mM)	*E. coli* Rosetta gami 2 (DE3) pLysS
BaeB (N-terminal His_8 tag)	pKJ7	-	IPTG (0.5 mM)	*E. coli* BL21DE3

Supplemental Table 2: Sequence of PCR primers used for cloning of obtained constructs. Introduced restriction sites are marked with bold letters.

Primer name	Sequence (5'-3')
FP-cat-*Bam*HI	AAA**GGATCC**GACAGCTTATCATCGGCAATA
RP-cat-*Xba*I	AAA**TCTAGA**GGCGTAGAGGATCTGGAGC
RP-H-vor-Promotor-*Xba*I	AAA**TCTAGA**TTTCTCCATCTCATACGTACTGTGGTG
FP-H-vor-Promotor-*Not*I	AAA**GCGGCC**GCCATCCGATTACGTTTATCGAAATTACG
RP-nach-BaeB-2-*Kpn*I	AAA**GGTACC**AGAAGGCGGTCAAATGGATCA
FP-nach-BaeB-2-*Apa*I	AAA**GGGCCC**GTTCTATCTAACTAGCTTTTCTTTTGAGG
RP-Promotor-*Apa*I	AAA**GGGCCC**TGTCACCATTCCCATTTAAAAGAT
FP-Promotor-*Bam*HI	AAA**GGATCC**TAACAACGTTTATGTGAGACTAAACC
FP-erm-*Xba*I	AAA**TCTAGA**CGAGGAATTTGTATCGATAAGAAATAG
RP-erm-*Apa*I	AAA**GGGCCC**ATAATAGGAATTGAAGTTAAATTAGATGCT
BaeB_low-GC_pHis8-3_for	**GGATCC**ATGGATCATACATATGAAGTGCATCAA
BaeB_low-GC_pHis8-3_rev	**AAGCTT**TTACTTAAAATGAAACAGCCCTTTTTG
pedC_FP	ATGGTA**GGTCTC**AAATGAAAGACTTGCAAAATATACAGAACAC
pedC_RP	ATGGTA**GGTCTC**AGCGCTACGTTGGTCGAGTTCGAGCAGA
pedC-5	GATA**CATATG**AAAGACTTGCAAAATATACAGAAC
pedC-3	GATT**CTCGAG**ACGTTGGTCGAGTTCGAGC
pedD_FP	AAA**GAATTC**AAATCGTACCTTTTTCCCGGG
pedD_RP	AAA**AAGCTT**TCACCACACCTTTTCAACTAAA
ped I3_pRSETf	AAA**AGATCT**GAGCAGAAGGTA TATGCGGTCATT
pedI3_pRSETr	AAA**AGATCT**TCATATCAGGCTCCGTACGTACTG
pedN_pRSETf	AAA**AGATCT**ATCCGCGAACGCATTTTCAATGTGATTGCGAGA AATACGCTTGAAGTCCTT
pedN_pRSETr	AAA**AGATCT**TCACTGTACATACTGGCTGAG
PsyA-ACP3 for	**GGATCC**ACGTCGAGCGGGGAACTTGCGACAGTGG
PsyA-ACP3 rev	**CGGCCG**TTAAACGCATACCGCTTCGAGCTGCTGCGC
PsyD-ACP1 for	**GGATCC**ACTTCGTCGCCAAAGGGCAATCTGACCG

PsyD-ACP1 rev	**CGGCCG**TCAAGGTATACACGCTTCGATGTGGGCAGCCAA
Expr_tAT_F	**GAATTC**AGGACGAAATCTATGCCGTA
Expr_tAT_R	**AAGCTT**TACGATCAGCGGCTTTGTT

Supplemental References

Moldenhauer, J., Chen, X.H., Borriss, R., and Piel, J. (2007). Biosynthesis of the antibiotic bacillaene, the product of a giant polyketide synthase complex of the trans-AT family. Angewandte Chemie, International Edition in English 46, 8195-8197.

Moldenhauer, J., Götz, D.C.G., Albert, C.R., Bischof, S.K., Schneider, K., Süssmuth, R.D., Engeser, M., Gross, H., Bringmann, G., and Piel, J. (2010). The final steps of bacillaene biosynthesis in *Bacillus amyloliquefaciens* FZB42: Direct evidence for beta,gamma-dehydration by a *trans*-acyltransferase polyketide synthase. Angew. Chem. Int. Ed. 49, 1465-1467.