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SUPPLEMENTARY INFORMATION

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A polyketoacyl-CoA thiolase-dependent pathway for the synthesis of polyketide backbones

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Supplementary Information for

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This file includes:

Supplementary Tables 1-4

Supplementary Table 1. Thermodynamic analysis of the biosynthesis of polyketides through PKT and PKS platforms.

PKT platforms	Standard $\Delta_rG'^{\circ}$ (Min Δ_rG', Max Δ_rG') [kcal/mol][a]	PKS platforms	Standard $\Delta_rG'^{\circ}$ (Min Δ_rG', Max Δ_rG') [kcal/mol][a]
		Acetyl-CoA carboxylase (ACC) acetyl-CoA + HCO_3^- + ATP = malonyl-CoA + ADP + P_i + H^+	-8.1 (-21.6, 5.4)
Thiolase acetyl-CoA + acetyl-CoA = acetoacetyl-CoA + CoA	7.1 (-1.9, 16.1)	PKS III acetyl-CoA + malonyl-CoA = acetoacetyl-CoA + CO_2 + CoA	5.7 (-10.1, 12.4)
Thiolase acetoacetyl-CoA + acetyl-CoA = triacetic acid lactone + 2 CoA	-3.5 (-19.3, 3.2)	PKS III acetoacetyl-CoA + malonyl-CoA = triacetic acid lactone + CO_2 + 2 CoA	-4.8 (-27.4, -0.4)
		PKS III acetyl-CoA + 2 malonyl-CoA = triacetic acid lactone + 2 CO_2 + 3 CoA	1.1 (-33.7, 7.7)
Thiolase 3 acetyl-CoA = triacetic acid lactone + 3 CoA	3.7 (-16.6, 15.3)	ACC + PKS III 3 acetyl-CoA + **2 HCO_3^- + 2 ATP** = triacetic acid lactone + 3 CoA + **2 CO_2** + 2 ADP + 2 P_i + 2 H^+	-15.0 (-66.9, 9.6)
Thiolase + Cyclase (OAC) acetoacetyl-CoA + 2 acetyl-CoA = orsellinic acid + 3 CoA	-15.9 (-36.2, -4.7)	PKS III+ Cyclase (OAC) acetoacetyl-CoA + 2 malonyl-CoA = orsellinic acid + 3 CoA + 2 CO_2	-18.7 (-52.6,-12.1)
Thiolase + Cyclase (OAC) acetoacetyl-CoA + 2 acetyl-CoA = orcinol + CO_2 + 3 CoA	-15.1 (-42.2, -6.2)	PKS III+ Cyclase (OAC) acetyl-CoA + 3 malonyl-CoA = orsellinic acid + 4 CoA + 3 CO_2	-13.0 (-58.3,-4.3)
Thiolase + Cyclase (OAC) 4 acetyl-CoA = orsellinic acid + 4 CoA	-8.9 (-29.2, 6.8)	ACC + PKS III + Cyclase (OAC) 4 acetyl-CoA + **3 HCO_3^- + 3 ATP** = orsellinic acid + 4 CoA + **3 CO_2** + 3 ADP + 3 P_i + 3 H^+	-37.0 (-98.4, 1.3)
Thiolase + Cyclase (OAC) 4 acetyl-CoA = orcinol + 4 CoA + CO_2	-7.9 (-39.5, 5.5)	ACC + PKS III + Cyclase (OAC) 4 acetyl-CoA + **3 HCO_3^- + 3 ATP** = orcinol + 4 CoA + **4 CO_2** + 3 ADP + 3 P_i + 3 H^+	-36.1 (-115.2, -2.7)
Thiolase + DH + KR 4 acetyl-CoA + NADPH + H^+ = 6-MSA + 4 CoA + $NADP^+$ + H_2O	-17.8 (-47.1, 2.4)	PKS I (KS + AT + DH + KR + ACP) 4 acetyl-CoA + **3 HCO_3^- + 3 ATP** + NADPH + H^+ = 6-MSA + 4 CoA + **3 CO_2** + 3 ADP + 3 P_i + $NADP^+$ + H_2O	-45.9 (-122.7, -5.7)
Thiolase + DH + KR 4 acetyl-CoA + NADPH + H^+ = *m*-cresol + 4 CoA + $NADP^+$ + CO_2 + H_2O	-16.8 (-34.7, 19.3)	PKS I (KS + AT + DH + KR + ACP) 4 acetyl-CoA + **3 HCO_3^- + 3 ATP** + NADPH + H^+ = *m*-cresol + 4 CoA + **4 CO_2** + 3 ADP + 3 P_i + $NADP^+$ + H_2O	-45.0 (-128.6, -7.1)

[a] The standard Gibbs free energy change of reaction ($\Delta_r G'^{\circ}$) was estimated under standard conditions (298.15 K, pH 7.3, and ionic strength 0.25). Specifically, $\Delta_r G'^{\circ}$ was computed from values of standard Gibbs free energy of formation ($\Delta_f G'^{\circ}$) for compounds involved in the reaction. Values for $\Delta_f G'^{\circ}$ were obtained from the MetaCyc database (https://metacyc.org/), which estimates $\Delta_f G'^{\circ}$ using the group contribution method. Minimum and maximum $\Delta_r G'$ values were calculated assuming standard conditions with minimum and maximum metabolite concentrations set to 0.00001 M and 0.02 M, respectively.

Supplementary Table 2. Plasmids and strains used in this study

Plasmids/strains	Genetic characteristics	Source
Plasmids		
pCDFDuet-1	CDF ori with P_{T7}; Sm^R	Novagen
pCDF-P1-2-PS	pCDFDuet-1 carrying *2-ps* from *Gerbera hybrid*	This study
pCDF-P1-HMGS	pCDFDuet-1 carrying *hmgs* from *Staphylococcus aureus*	This study
pCDF-P1-EcFadA	pCDFDuet-1 carrying *fadA* from *E. coli*	This study
pCDF-P1-PaaJ	pCDFDuet-1 carrying *paaJ* from *E. coli*	This study
pCDF-P1-PcaF	pCDFDuet-1 carrying *pcaF* from *Pseudomonas putida*	This study
pCDF-P1-FadAx	pCDFDuet-1 carrying *fadAx* from *Pseudomonas putida*	This study
pCDF-P1-DcaF	pCDFDuet-1 carrying *dcaF* from *Acinetobacter* sp. ADP1	This study
pCDF-P1-AtoB	pCDFDuet-1 carrying *atoB* from *E. coli*	This study
pCDF-P1-BktB	pCDFDuet-1 carrying *bktB* from *Ralstonia eutropha*	This study
pCDF-P1-DH-KR	pCDFDuet-1 carrying *dh-kr* from *Aspergillus terreus*	This study
pCDF-P1-2-PS-P2-MCS	pCDF-P1-2-PS carrying *mcs* from *Bradyrhizobium japonicum*	This study
pCDF-P1-2-PS-P2-Acc	pCDF-P1-2-PS carrying *accABCD* from *E. coli*	This study
pET-P1-RppA	pETDuet-1 carrying *rppA* from *Streptomyces griseus*	This study
pCDF-P2-MCS	pCDFDuet-1carrying *mcs* from *Bradyrhizobium japonicum*	This study
pCDF-P2-ACC	pCDFDuet-1 carrying *accABCD* from *E. coli*	This study
pCDF-P1-OAC	pCDFDuet-1 carrying *oac* from *Cannabis sativa*	This study
pCDF-P1-BktB-P2-OAC	pCDF-P1-BktB carrying *oac* from *Cannabis sativa*	This study
pCDF-P1-FadB	pCDFDuet-1 carrying *fadB* from *E. coli*	This study
pCDF-P1-PaaH	pCDFDuet-1 carrying *paaH* from *E. coli*	This study
pCDF-P1-BktB C90S	pCDFDuet-1 carrying *bktB C90S* mutant	This study
pCDF-P1-BktB H350A	pCDFDuet-1 carrying *bktB H350A* mutant	This study
pCDF-P1-BktB C380S	pCDFDuet-1 carrying *bktB C380S* mutant	This study
pCDF-P1-BktB$_{_EcFadA}$	pCDFDuet-1 carrying *bktB* mutant with fragment switch from *EcfadA*	This study
pCDF-P1-EcFadA$_{_BktB}$	pCDFDuet-1 carrying *EcFadA* mutant with fragment switch from *BktB*	This study
***E. coli* Strains**		
E. coli BL21 (DE3)	Host strain for enzymes expression	Lab collection
E. coli JC01 (DE3)	MG1655, Δ*ldhA*::FRT Δ*poxB*::FRT Δ*pta*::FRT Δ*adhE*::FRT Δ*frdA*::FRT	Lab collection
E. coli JC01 (DE3) Δ*atoB*	*E. coli* JC01 (DE3) Δ*atoB*::FRT	This study
JC01 (DE3) Δ*atoB* BktB	*E. coli* JC01 (DE3) Δ*atoB* with pCDF-P1-BktB	This study
JC01 (DE3) Δ*atoB* 2-PS	*E. coli* JC01 (DE3) Δ*atoB* with pCDF-P1-2PS	This study
JC01 (DE3) Δ*atoB* 2-PS-MCS	*E. coli* JC01 (DE3) Δ*atoB* with pCDF-P1-2PS-P2-MCS	This study
JC01 (DE3) Δ*atoB* 2-PS-Acc	*E. coli* JC01 (DE3) Δ*atoB* with pCDF-P1-2PS-P2-Acc	This study
JC01 (DE3) Δ*atoB* RppA	*E. coli* JC01 (DE3) Δ*atoB* with pET-P1-RppA	This study
JC01 (DE3) Δ*atoB* RppA-MCS	*E. coli* JC01 (DE3) Δ*atoB* with pET-P1-RppA and pCDF-P2-MCS	This study
JC01 (DE3) Δ*atoB* RppA-Acc	*E. coli* JC01 (DE3) Δ*atoB* with pET-P1-RppA and pCDF-P2-Acc	This study
JC01 (DE3) Δ*atoB* BktB-OAC	*E. coli* JC01 (DE3) Δ*atoB* with pCDF-P1-BktB-P2-OAC	This study

Supplementary Table 3. Accession numbers of enzymes used in this study

Enzyme	Organism source	Genbank Accession No.
2-PS	*Gerbera hybrid*	CAA86219.2
HMGS	*Staphylococcus aureus*	WP_000151982
EcFadA	*E. coli*	WP_016232377
PaaJ	*E. coli*	NP_415915.1
PcaF	*Pseudomonas putida*	WP_010952485.1
FadAx	*Pseudomonas putida*	WP_010953196.1
DcaF	*Acinetobacter sp. ADP1*	WP_004926569.1
AtoB	*E. coli*	WP_000786547.1
BktB	*Ralstonia eutropha*	WP_011615089.1
MCS	*Bradyrhizobium japonicum*	WP_018645152.1
AccA	*E. coli*	NP_414727.1
AccB	*E. coli*	NP_417721.1
AccC	*E. coli*	NP_417722.1
AccD	*E. coli*	NP_416819.1
OAC	*Cannabis sativa*	AFN42527.1
FadB	*E. coli*	AYG21354.1
PaaH	*E. coli*	NP_415913.1

Supplementary Table 4. PDB Accession numbers of enzymes used in this study

Enzyme	Organism source	PDB Accession No.
BktB	*Ralstonia eutropha*	4NZS
EcFadA	*E. coli*	No structure is aviable, use *Salmonella typhimurium* FadA (PDB:3GOA) as template (primary sequecne identity = 95.09%) for homology-modelling.