How Fatty Acid Biosynthesis is Initiated in Pseudomonas putida KT2440

Background

- *Pseudomonas putida* KT2440 is an industrially-relevant bacterial host for the conversion of biomass-derived sugars and aromatic compounds into various value-added products which includes the fatty acid-derived bioplastic polyhydroxyalkanoates (PHAs).
- Despite numerous reports of *P. putida* KT2440 engineered to produce PHAs, a comprehensive understanding of fatty acid (FA) biosynthesis in this strain is lacking which undermines rationale engineering of the system.

Approach

- We used a combination of genetic, biochemical, structural, and computational approaches to determine all three means by which FA biosynthesis is initiated in *P. putida* KT2440.

Results

- *P. putida* KT2440 harbors three pathways to initiate fatty acid biosynthesis.
- FabH1 and FabH2, are canonical KASIII enzymes with differing specificity to acyl-CoAs.
- A third novel pathway proceeds through MadB-catalyzed decarboxylation of malonyl-ACP.
- Functional homologs of MadB are prevalent in the domain Bacteria.

Significance

- Elucidation of fatty acid initiation pathways in *P. putida* KT2440 will aid in future engineering efforts especially those aimed towards improving the production of FA-derived products such as PHAs.

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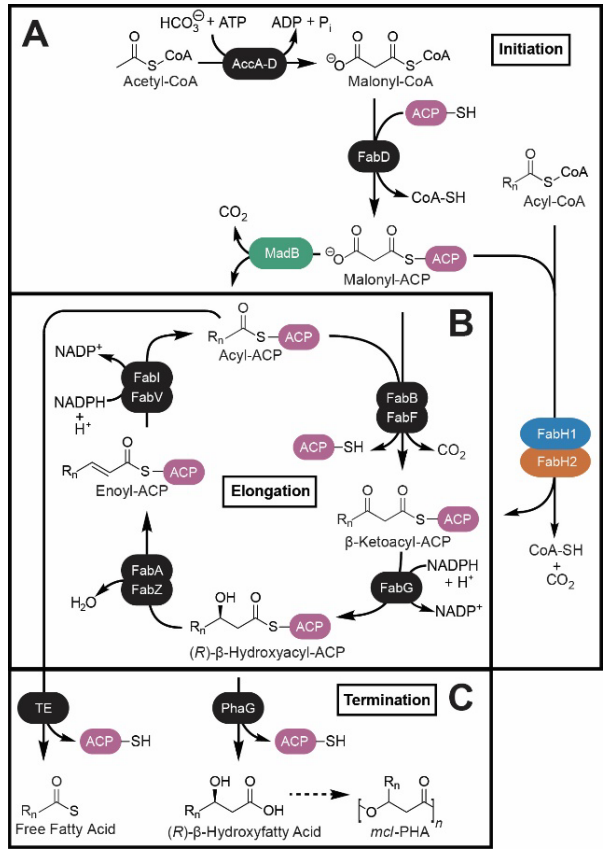
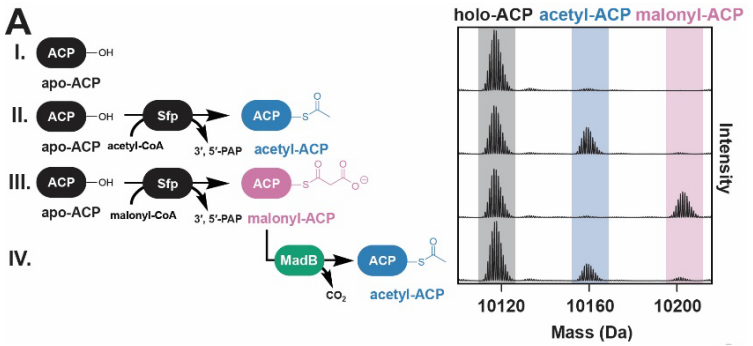


Proposed FA biosynthesis pathway in *P. putida* KT2440. (**A**) Initiation culminates either with the synthesis of a β-ketoacyl-ACP species (β-acetoacetyl-ACP via FabH1 or β-keto-decanoyl-ACP via FabH2), or generation of acetyl-ACP from decarboxylation of malonyl-ACP by MadB. (**B**) Each catalytic cycle extends the growing acyl-ACP chain by two carbons donated from malonyl-ACP. (**C**) FA biosynthesis is terminated when one of the elongation intermediates is consumed to generate a fatty acid-derived product. The production of medium-chain-length polyhydroxyalkanoate from (*R*)-β-hydroxyacyl-ACP and the liberation of free FA by a thioesterase (TE) are shown as examples.



Biochemical characterizations of MadB (malonyl-ACP decarboxylase). Enzymatic reactions (I, II, III, IV) demonstrating the conversion of malonyl-ACP to acetyl-ACP by addition of MadB as detected by LC-MS.