BRC Science Highlight
April 2022

Metabolic Engineering Strategies to Produce Medium-Chain Oleochemicals via Acyl-ACP:CoA Transacylase Activity

Background/objective

Microbial lipid metabolism is an attractive route for producing medium acyl-chain oleochemicals (e.g., fatty alcohols and ketones) that can then be used to make biofuels. The predominant production strategy is to use heterologous thioesterases to create fatty acids, and then convert them back to coenzyme-A (CoA) thioesters to create oleochemicals. This strategy is energetically expensive to the microbe and could be avoided by transferring the acyl-chain directly from the acyl-carrier protein (ACP) to the CoA-thioester (a.k.a. transacylase activity). Here, we demonstrate such an alternative strategy by heterologous expression of enzyme PhaG, which transfers 3-hydroxy acyl-chains between ACP and CoA-thioester forms, to create a pool of acyl-CoA's for producing oleochemicals.

Approach

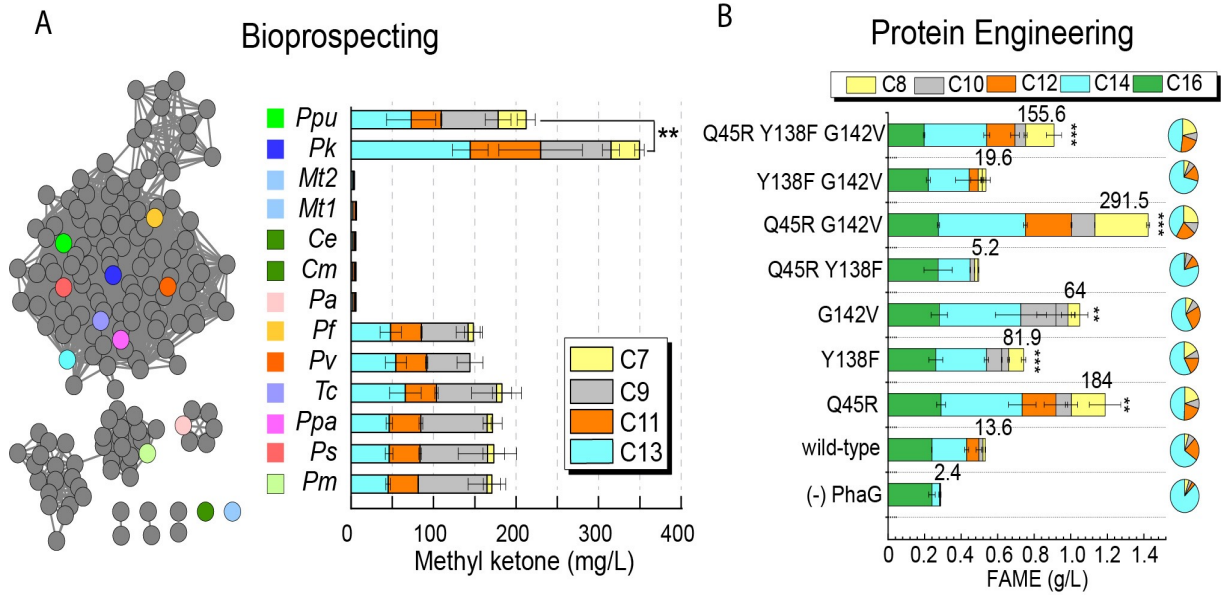
- Used functional genomic approach to examine PhaG transacylase activity.
- Used bioprospecting approach to identify a higher activity PhaG enzyme.
- Adapted a lipoic acid auxotrophic selection strategy to isolate highly active PhaG variant from a random mutagenesis library.
- Tested s*in situ* extraction approach to capture off-gas methyl ketone by employing an absorber system.

Results

- Identified PhaG homolog from *Pseudomonas koreensis* as having the highest activities toward 3-hydroxyacyl-ACP substrates.
- Identified a PhaG Q45R G142V mutant with 4-fold improved activity from wild-type PhaG.
- Measured fatty acid and fatty alcohol production at greater than 1 g/L and methyl ketones at 7.2 g/L.

Significance

This work demonstrates a novel transacylase route for producing acyl-CoA for oleochemical synthesis through bioprospecting and protein engineering that produces fatty acid, methyl ketones and fatty alcohols at greater than 1 g/L.



(A) Comparison of PhaG homolog activities in vivo by evaluating methyl ketone titers. A two-dimensional cluster map displays the sequence similarity of PhaG variants. Colored boxes and dots indicate the sequences tested. (B) FAME analysis of cultures harboring PhaG variants containing combinatorial point mutations.

Yan, Q., Cordell, W.T., Jindra, M.A., Courtney, D.K., Kuckuk, M.K., Chen, X., Pfleger, B.F. Mar. 25, 2022. "Metabolic Engineering Strategies to Produce Medium-Chain Oleochemicals via Acyl-ACP:CoA Transacylase Activity." Nature Communications. 13:1619. DOI: 10.1038/s41467-022-29218-3.

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