BRC Science Highlight
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Metabolic Engineering of *Rhodotorula toruloides* IFO0880 Improves C16 and C18 Fatty Alcohol Production from Synthetic Media

Background/objective

The oleaginous yeast *Rhodotorula toruloides* has been increasingly explored as a platform organism to produce fatty acid derivatives. Fatty alcohols, a fatty acid derivative widely used in the production of detergents and surfactants, can be produced in yeasts by expressing a heterologous fatty acyl-CoA reductase. In this study, several metabolic engineering approaches were investigated to improve the titer of fatty alcohol production in *R. toruloides*.

Approach

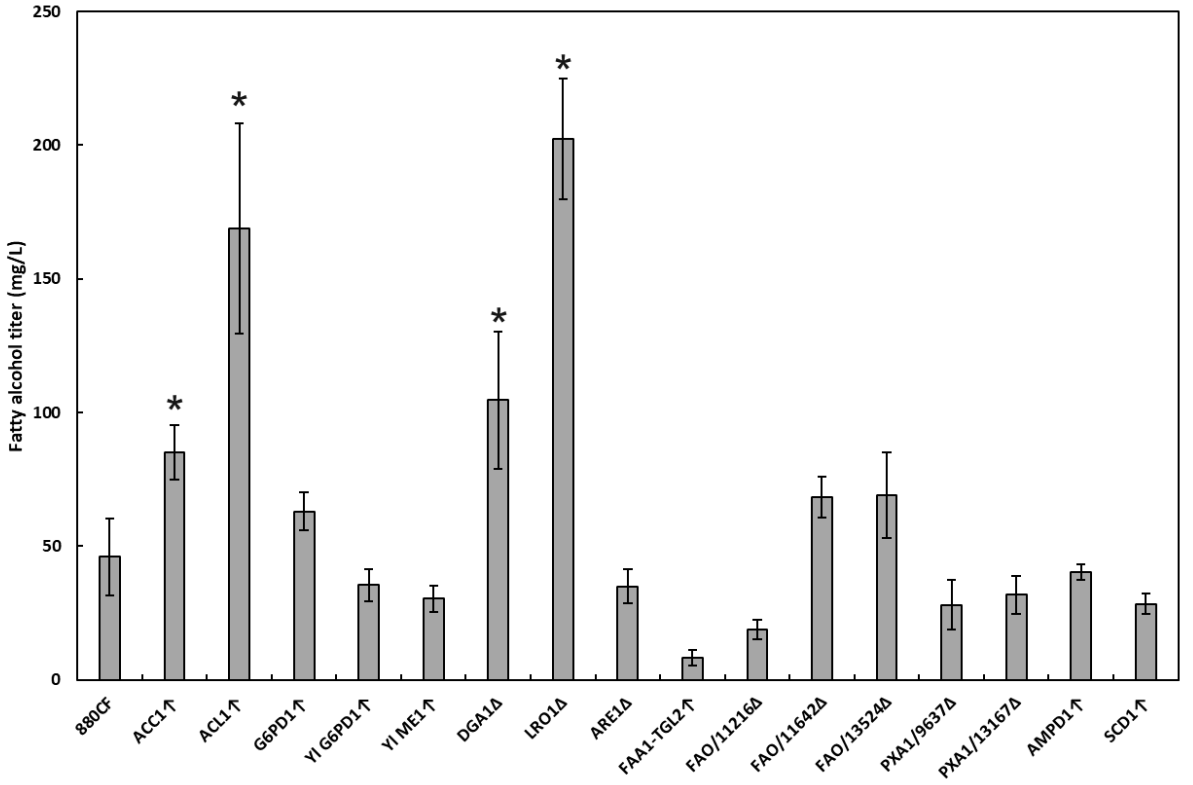
- Transformed *R. toruloides* IFO0880 with *Marinobacter aqueolei* fatty acyl-CoA reductase (MaFAR) for fatty alcohol production and *Streptococcus pyogenes* Cas9 (SpCas9) for creating knockout (Δ) mutants.
- Evaluated the fatty alcohol production effects of the overexpression and deletion of genes related to fatty alcohol precursor production, fatty alcohol degradation, and lipid formation. Top producing strains were then tested in fed-batch bioreactor fermentations.

Results

- The top engineered strain, 880CF-LRO1Δ, produced 3.7 g/L fatty alcohols in fed-batch fermentation with a yield of 0.024 g/g glucose.
- Deleting gene DGA1 and overexpressing genes ACC1 and ACL1 also increased fatty alcohol production in *R. toruloides.*
- Lipidomics analysis revealed while DGA1Δ decreased TAG formation, LRO1Δ increased TAG formation, contrary to expectation for knockout of this acyltransferase.

Significance

- This work demonstrates the highest fatty alcohol titer achieved from synthetic media in *R. toruloides,* the largest metabolic engineering effort so far undertaken in this yeast, and the first lipidomic characterization.



Increase in fatty alcohol titer achieved through metabolic engineering of *R. toruloides* IFO0880 expressing MaFAR.

Schultz, J.C., Mishra, S., Gaither, E., Mejia, A., Dinh, H., Maranas, C., Zhao, H. Feb. 19, 2022. "Metabolic Engineering of Rhodotorula toruloides IFO0880 Improves C16 and C18 Fatty Alcohol Production from Synthetic Media." Microbial Cell Factories 21:26. DOI: 10.1186/s12934-022-01750-3.

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