Evaluation of 1,2-Diacyl-3-Acetyl Triacylglycerol Production in Yarrowia lipolytica

CABBI
CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

Background/Objective

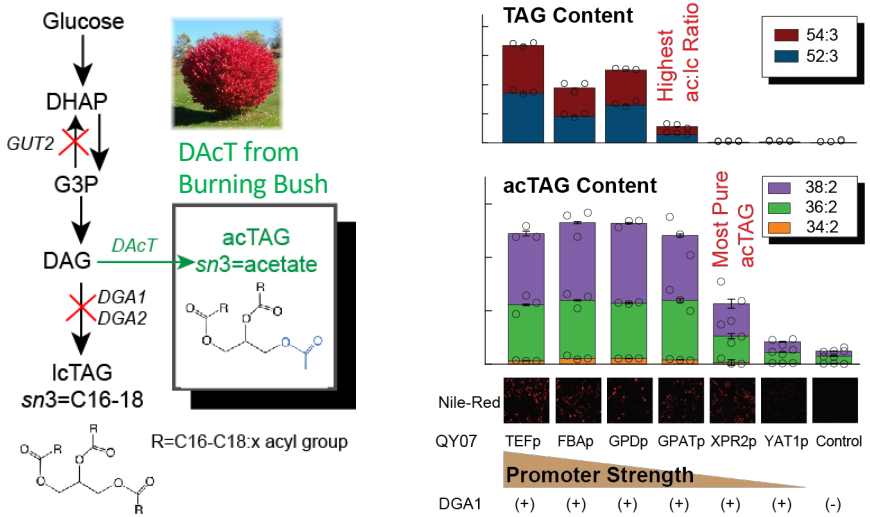
Plants produce many high-value oleochemical molecules, but suitable amounts of land for industrial-scale oil-crop agriculture is not available to meet global demand. Worse, establishing new oil-crop farms often comes with the environmental cost of tropical deforestation. The field of metabolic engineering offers tools to transplant oleochemical metabolism into tractable hosts to produce chemicals using non-agricultural plants. We evaluated strategies to metabolically engineer the oleaginous yeast *Yarrowia lipolytica* to synthesize a foreign lipid, 3-acetyl-1,2-diacyl-sn-glycerol (acTAG), an oil used in low-grade diesels, lubricants, and emulsifiers due to its reduced viscosity and melting point relative to traditional triacylglycerol oils.

Approach

- Engineered *Y. lipolytica* to eliminate lcTAG synthesis, maximized production of acTAG precursor DAG (using the best synthase identified via bioprospecting), then titrated DGA1/2 activity to reduce the lcTAG titer while maintaining lipid body formation.
- Engineered two production strains, one that maximizes acTAG production titer but contains large amounts of lcTAG and another that maximizes the ratio of acTAG:lcTAG, which minimized purification needs.
- Created a techno-economic model for acTAG production and assessed how yield, titer, selectivity, and productivity affected the minimum selling price (MSP).

Results

- The engineered strains produced **4 g/L acTAG** on sugarcane juice, **2.5 g/L acTAG** on oilcane hydrolysate, and **12 g/L acTAG** on laboratory media.
- Predicted feasibility of acTAG production for commercial emulsifiers at 85% theoretical yield, 90 g/L titer, 75% acTAG selectivity, and 1.0 g/L/h productivity.



Titrating DGA1 Transcription minimized lcTAG production while maintaining lipid bodies.

Significance/Impacts

Demonstrates novel strategies for improving non-native, neutral lipid production in *Y. lipolytica* and other metabolic engineering targets for industrialization.

Yan, Q., Jacobson, T.B., Ye, Z., Cortés-Pena, Y.R., Bhagwat, S.S., Hubbard, S., Cordell, W.T., Oleniczak, R.E., Gambacorta, F.V., Vazquez, J.R., Shusta, E.V., Amadon-Noguez, D., Guest, J.S., Pfleger, B.F. Jan. 7, 2023. "Evaluation of 1,2-Diacyl-3-Acetyl Triacylglycerol Production in *Yarrowia lipolytica*." *Metabolic Engineering*. DOI: 10.1016/j.ymben.2023.01.003.