Engineering *Yarrowia lipolytica* to Produce 3-acetyl-1,2-diacyl-sn-glycerol

Qiang Yan[1,2*] (qyan32@wisc.edu), William Cordell,[2] and **Brian Pfleger**[1,2]

[1]DOE Center for Advanced Bioenergy and Bioproducts Innovation (CABBI), Urbana, IL;
[2]Department of Chemical and Biological Engineering, University of Wisconsin at Madison, Madison

https://cabbi.bio/

Project Goals: The goal of this project is to metabolic engineering of *Yarrowia lipolytica* to enhance accumulation of 3-acetyl-1,2-diacyl-sn-glycerol. The engineered Yarrowia strains can be a useful base strain to screen highly active TAG synthase, which can be leveraged in the sustainability teams to pursue high TAG production in plant lines.

The triacylglycerols 3-Acetyl-1,2-diacyl-sn-glycerol (acTAG) have many potential industrial applications such as engine lubricant oil, emulsifiers, food coatings, and plasticizers. As an oleaginous yeast, *Yarrowia lipolytica's* high flux toward native TAG (lcTAG) synthesis serves as an excellent chassis for production of acTAG. The acTAG are unusual triacylglycerols (TAG) with an acetyl group at the sn-3 position instead of the typical long-chain acyl group. Compared to regular TAG, the acetyl group of acTAG confers useful physical and chemical properties such as reduced kinematic viscosity and lower melting points.

The main challenge here is how to modify *Y. lipolytica* to accumulate acTAG while maintaining a high carbon flux toward TAG synthesis. To accomplish this, the presented work provides a two-fold strategy: removing the competing pathways of lcTAG synthesis and identifying highly active acTAG synthases. In the first strategy, we generated a multi-knockout *Y. lipolytica* strain that deleted three acyltransferase, two lipases, and one dehydrogenase. This engineered strain is incapable of producing lcTAG and forming lipid bodies due to removal of three TAG synthase genes. In the second strategy, we evaluated activities of 28 bioprospected acTAG synthase homologs in an engineered *Y. lipolytica* with two deleted acyltransferases and two deleted lipases. We integrated each acTAG synthase homolog randomly in the strain and quantified cellular lipid content using a nile-red staining fluorescence assay. Our preliminary results show that 5 acTAG synthase homologs showed activities greater than the literature enzyme *Ea*DAcT from *Euonymus alatus*. To this end, we engineered a *Y. lipolytica* strain which lacks native TAG synthesis and identified five promising acTAG synthase candidates. We believe these efforts will be beneficial toward enhancing acTAG production in *Y. lipolytica*.

References

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Funding statement: *This work was funded by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and*

Environmental Research under Award Number DE-SC0018420). Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the U.S. Department of Energy.