

## **Intracellular Metabolite Pool Size Quantification in Oleaginous Yeast *Yarrowia lipolytica* using Acetate as Carbon Source**

Nian Liu<sup>1,\*</sup> (nianl@mit.edu) and Gregory Stephanopoulos<sup>1</sup>

<sup>1</sup>Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

**Project Goals:** This project aims to develop a cost-effective process that uses the oleaginous yeast *Yarrowia lipolytica* as a biocatalyst to convert carbon sources into fatty acids for biodiesel production. In particular, we are interested in using acetate as an inexpensive, renewable carbon source and understanding fundamentally how the metabolic pathways are coordinated to produce lipids. From this, we will be able to identify bottlenecks within the metabolic network and provide suggestions on engineering strategies that help improve the lipid yield, titer, and productivity.

The use of the oleaginous yeast *Yarrowia lipolytica* as a biocatalyst to convert acetate into fatty acids for biodiesel production is a renewable process and can potentially be cost-effective. However, due to the limited understanding of its metabolism on acetate, engineering the strain to achieve high lipid yield, titer, and productivity can be difficult. This work serves to provide a fundamental understanding of the metabolism through the quantification of intracellular metabolite pool sizes. Differences across two strains were compared in this study: the wild type strain (WT) and a previously engineered lipid overproducing strain (ACCDGA). A total of 17 intracellular metabolites in the glycolytic, tricarboxylic, and pentose phosphate pathways were quantified. Reducing cofactors (i.e. NADH, NADPH) and energy metabolites (i.e. ATP, ADP, AMP) were also analyzed. Our results indicate that the ACCDGA strain has higher reducing capacity and increased acetyl-CoA usage, both contributing to higher lipid production. Furthermore, upon transitioning from the growth phase to the lipid production phase (nitrogen depletion in media), both strains shift into a metabolic state that increases generation of the reducing cofactors favorable for lipid production. Finally, both strains are in an energy deficient state when cultured on acetate as the sole carbon source. This indicates that ATP requirements must be considered when engineering the strain.