Multi-omic investigation of lipid accumulation mechanisms in *R. toruloides*

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Project Goals: Our project aims to develop new metabolic engineering, omics analysis, and computational modeling tools at the genome scale for strain development, which may be implemented in an automated manner at the Illinois Biological Foundry for Advanced Biomanufacturing. Two non-model yeasts, *Rhodosporidium toruloides* for the production of oleaginous compounds and *Issatchenkia orientalis* for the production of organic acids, are selected as the platform organisms. Milestones achieved so far include reconstruction of comprehensive genome-scale metabolic models, development of carbon mapping models, and ^{13}C-metabolic flux analysis at the genome-scale. The final goal is to develop kinetic models to guide metabolic engineering accounting for reaction kinetics and allosteric regulations.

Oleaginous yeasts are, in recent years, being studied in greater detail for their higher lipid accumulating capacity. In the context of metabolic engineering, they offer the promise of platform hosts that can be used to produce lipid-related biochemicals. Their higher accumulation potential over model yeasts suggests the possibility of achieving production titers that can compete with chemical synthesis routes, thus ensuring economic viability. One such microorganism is the non-model yeast *Rhodosporidium toruloides*, which has been documented to accumulate high titers of intracellular lipids. However, barriers such as lack of precision genetic editing or an incomplete understanding of its metabolic and regulatory topology can hinder the creation of successful overproduction phenotypes. Here we adopt a multi-omic approach to investigate the molecular mechanisms governing lipid accumulation. The *R. toruloides* strain IFO0880 was cultivated in nitrogen-rich and nitrogen-deficient conditions and analyzed via RNA-Seq and lipidomic analysis experiments.

The results from transcriptomic analysis indicate global metabolic shifts were governed by nitrogen limitation. From hierarchical clustering of gene expression data, a pattern emerged where samples grown in high carbon-to-nitrogen ratio clustered together despite different values of the ratio. This trend was observed in both fatty acid biosynthesis and fatty acid utilization pathways implying that culture conditions exerted a greater amount of control on gene expression profiles than the exact function of different pathways. Clustering analysis across different timepoints showed that in the logarithmic stage of growth, all cultures clustered together independent of the carbon-to-nitrogen ratio, while the distinct clusters were only seen in the stationary phase of culture, indicating the role that logarithmic and stationary phase play in switching to a lipid accumulation mode.

In addition, we identified the genes and pathways involved in transcriptional reprogramming during lipid production induced by nitrogen limitation and mapped them to metabolic pathways of *R. toruloides*. We further used bioinformatics analysis to identify networks of co-regulated gene clusters. The multi-omics analysis of regulatory mechanisms of lipid accumulation in such oleaginous yeast thus paves a useful path towards identifying control levers of its metabolism.

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