Transcriptomic and Metabolomic Analysis of Nitrogen and Carbon Metabolism in *Saccharomyces cerevisiae* and *Rhodotorula toruloides*

William Woodruff[1,2] (ww12@illinois.edu), Sujit S. Jagtap,[1,2] Jing-Jing Liu,[1,2] Anshu Deewan,[1,2] Hanna E. Walukiewicz,[1,2] Eun Ju Yun,[1,3] Yong-Su Jin,[2,4] and **Christopher V. Rao**[1,2]

[1]Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana Champaign, Urbana; [2] DOE Center for Advanced Bioenergy and Bioproducts Innovation (CABBI); [3]Department of Biotechnology, Graduate School, Korea University, Seoul, Republic of Korea; [4]Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana

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Project Goals: The goal of this research is to understand the metabolic response of yeast to changes in nitrogen and carbon availability. Accumulation of valuable lipids and lipid-derived compounds in yeasts occurs during nitrogen starvation, and an improved understanding of nitrogen metabolism may enable development of engineered strains that can accumulate lipids in nitrogen-rich media. At the same time, the effects of varying carbon sources on the metabolism of oleaginous yeasts such as *R. toruloides* remain poorly characterized. In this study, the role of Ure2, a key regulator of nitrogen catabolite repression, was studied in a *ΔURE2* strain of *S. cerevisiae,* and the effects of changing carbon source on the metabolism of *R. toruloides* were investigated using a multi-omics approach.

The production of lipids and valuable oleochemicals in yeast is affected by changes in both nitrogen and carbon availability.[1,2] Lipid accumulation occurs under nitrogen limiting conditions, the response to which is mediated by the nitrogen catabolite repression (NCR) pathway.[2,3] Ure2 is a key transcriptional regulator of this pathway.[4] To investigate the role of this regulator, gene expression and intracellular metabolite concentrations were measured in a *ΔURE2* strain of *S. cerevisiae*. Transcriptomic changes consistent with nitrogen starvation and a selective autophagic response were observed. This mutant strain also accumulated less trehalose and glycogen, and it produced more lipid and ethanol. *URE2* is therefore a potential target for engineering yeast strains capable of lipid accumulation on nitrogen-rich substrates.

While *R. toruloides* can grow on a wide variety of substrates, the choice of medium substantially affects growth rates and product formation, while the metabolic basis for these differences remains poorly understood. To investigate these responses, gene expression and intracellular metabolite concentrations were measured on *R. toruloides* grown on glucose, xylose, acetate, and soybean oil. These substrates were chosen because they can all be obtained from plant biomass. Most observed changes were consistent with upregulation of known substrate utilization pathways; however, poor expression of xylulokinase was observed on xylose. This poor expression results in arabitol accumulation through an arabitol dehydrogenase bypass and opens the possibility of targeted metabolic engineering to improve xylose utilization.[5]

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.*