Genome-scale Model Reconstruction and 13C-Metabolic Flux Analysis for Non-model Yeast Organisms *Rhodosporidium toruloides* IFO0880 and *Issatchenkia orientalis* SD108

Hoang V. Dinh[1*] (hvd5034@psu.edu), Patrick F. Suthers[1], John I. Hendry[1], Yihui Shen[2], Tianxia Xiao[2], Anshu Deewan[3], Sujit Jagtap[3], Zia Fatma[3], Joshua D. Rabinowitz[2], Christopher Rao[3], Costas D. Maranas[1], and **Huimin Zhao**[3]
[1]The Pennsylvania State University, University Park, Pennsylvania; [2]Princeton University, Princeton, New Jersey; [3]University of Illinois at Urbana-Champaign, Urbana-Champaign, Illinois

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Project Goals: Our project aims to develop new metabolic engineering, omics analysis, and computational modeling tools on a 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 production of oleaginous compounds and *Issatchenkia orientalis* for production of organic acids, are selected as the platform organisms. To guide metabolic engineering, we aim to develop kinetic models accounting for reaction kinetics and allosteric regulations. Milestones achieved so far include reconstruction of comprehensive genome-scale metabolic models and development of large-scale carbon mapping models for ^{13}C-metabolic flux analysis used in kinetic parameterization.

Non-model yeasts are promising microbial cell factories due to their unique metabolic capabilities. *R. toruloides* is a basidiomycetes yeast that can accumulate large amount of lipids while *Issatchenkia orientalis* is a promising host for industrial production of organic acids thanks to its low-pH tolerance. To better assess these yeasts' metabolic capabilities, we reconstructed separate genome-scale metabolic models (GEMs) for each organism. Model reactions and genes were drawn from genome annotations. Biomass descriptions were derived from in-house-measured macromolecular composition and ATP maintenance requirements (calculated from chemostats data). We curated the model based on the available experimental data and ensure its quality with standardized tests (i.e., memote). Following the genome-scale models, we built carbon mapping models that are capable of explaining ^{13}C-labeling data and network cofactor balances. Flux distributions were predicted using the mapping model and labeling data (U-^{13}C-glucose and 1,2-^{13}C-glucose) capturing central carbon flux differences between the two yeasts. Energy metabolism involving reduced cofactors can also be elucidated.

This work is supported by U. S. Department of Energy Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018260.