Model-driven Metabolic Engineering and 13C-Metabolic Flux Analysis for Non-model Yeast Organisms

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Project Goals: Our project aims to develop new metabolic engineering, omics analysis, and computational modeling tools at 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. Milestones achieved so far include reconstruction of comprehensive genome-scale metabolic models, development of carbon mapping models, and ^{13}C-metabolic flux analysis at genome-scale. The final goal is to develop kinetic models to guide metabolic engineering accounting for reaction kinetics and allosteric regulations.

Unique metabolic capabilities and resilience to inhibitory stressors enable some non-model yeasts to be attractive microbial cell factories. *Rhodosporidium toruloides* is a basidiomycetes yeast that can accumulate large amount of lipids, and *Issatchenkia orientalis* is a promising host for industrial production of organic acids because of its low-pH tolerance. To better assess their metabolic capabilities and to draw comparisons with the model yeast *Saccharomyces cerevisiae*, we reconstructed separate genome-scale metabolic (GSM) models for each non-model yeast. These curated GSM models drew on in-house-measured macromolecular compositions and chemostat growth data which enabled estimating ATP maintenance requirements, and we performed model validation experiments. We applied these GSM models to make suggestions on genetic modifications to bolster targeted product formation for succinic and itaconic acids, as well as to examine the dependence of product formation on oxygen uptake levels. Upon the GSMs, we built carbon mapping models for ^{13}C-metabolic flux analysis (^{13}C-MFA) at genome-scale with labeling data using the tracers U-^{13}C-glucose and 1,2-^{13}C-glucose. Expanding to genome-scale accounts for cofactor balance reveals alternative flux distribution in central metabolism and supplies flux value for biosynthetic reactions. In the future, ^{13}C-MFA fluxes will be used in strain designs, systematic identification of allosteric regulations, and kinetic model development.

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