A Comprehensive Genome-Scale Model for *Rhodosporidium toruloides* IFO0880 Accounted for Functional Genomics and Phenotypic Data

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Project Goals: Non-model yeasts are yet under the limelight for metabolic engineering efforts despite possessing advantageous physiological traits as production hosts. To assist the effort in understanding and engineering non-model yeast, a genome-scale metabolic model can be reconstructed capturing the comprehensive metabolism of the organism. As a part of the Genome Scale Engineering project and in conjunction with genome-scale engineering (i.e., CRISPR-based tools) and analysis (i.e., -omics data) techniques, engineered strains can be built, understood, and tested in an automated manner at the Illinois Biological Foundry for Advanced Biomanufacturing. *Rhodosporidium toruloides* can accumulate lipid up to >65% cellular weight and is therefore an attractive host for the bioproduction fatty acids-derived products. This work presents a comprehensive genome-scale model for the oleaginous yeast *Rhodosporidium toruloides* IFO0880 accounted for functional genomics and phenotypic data.

Rhodosporidium toruloides is a basidiomycetes yeast that can accumulate large amount of lipids and natively produce carotenoids. A genome-scale model of *R. toruloides* IFO0880's metabolic network is reconstructed to better assess this non-model yeast's metabolic capabilities by integrating the latest available knowledge with in-house generated biomass composition, growth yield and viability. The model captures the most recent annotations from the latest version of the *R. toruloides* genome with organism-derived macromolecular composition in the biomass description and ATP maintenance requirements. It contains two separate biomass compositions depending on the growth condition (i.e., carbon or nitrogen limitation, respectively). The gene-protein-reaction rules and transporter system assignments are revised leading to gene essentiality prediction accuracy at a level similar to the latest *S. cerevisiae* model (yeast 7.6). The metabolic model is used to predict growth and lipid production phenotypes, contrast predicted metabolic flux redistribution and expression change, and facilitate *in silico* prediction of metabolic engineering strategies for the overproduction of triacylglycerol using the OptForce algorithm. The model predictions were in good agreement with functional genomics and phenotypic data and meaningful regulatory perturbation strategies were obtained from using the strain design algorithm on the model.

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