Examining organic acid production and model-driven strategies in *Issatchenkia orientalis*

Patrick F. Suthers[1*] (pfs13@psu.edu), Teresa A. Martin[2], Zia Fatma[2], Vinh G. Tran[2], Hoang V. Dinh[1], Costas D. Maranas[1], and **Huimin Zhao**[2]
[1]The Pennsylvania State University, University Park, Pennsylvania; [2]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 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.

Many platform chemicals can be produced from renewable biomass by microorganisms, with organic acids making up a large number of these chemicals. Intolerance to the resulting low pH growth conditions and inhibitors in biomass hydrolysates, however, remains a challenge for the industrial production of organic acids by microorganisms. The unique metabolic capabilities and resilience to inhibitory stressors enable some non-model yeasts to be attractive microbial cell factories. The non-model yeast *Issatchenkia orientalis* is a promising host for industrial production because it is tolerant of low pH conditions.

Here, we explore engineering synthetic pathways in *I. orientalis* to produce 21 different organic acids from glucose under aerobic or microaerobic conditions. These organic acids include seven of the eight organic acids identified by the US Department of Energy as part of the top twelve bio-based building-blocks. We use a genome-scale metabolic (GSM) model for *I. orientalis* SD108 to blueprint pathways and to examine yields and dependences of product formation on oxygen uptake levels. Based on the model, we determined that the production of 3-hydroxypropionic acid in *I. orientalis* can be enhanced by microaerobic fermentation conditions. Our experimental results have recently supported this finding. We also use constraint-based methods to assess the potential of computationally designing growth-coupled strains and to propose genetic modifications that bolster product formation. We identified growth-coupled strategies for 15 of the substrate-product pairs. We detail our work to introduce pathways *in vivo* and the use of a recently developed CRISPR/cas system for *I. orientalis*. We also highlight recent updates to the GSM model.

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