

Multi-OMICs Profiling Reveals Key Genes and Cellular Processes Underlying Ionic Liquid Robustness in *Yarrowia lipolytica*

Authors: Caleb Walker^{1*} (fyz692@vols.utk.edu), Richard Giannone², Alice Dohnalkova³, Mary Lipton³, Young-Mo Kim³, Samuel Purvine³, Jennifer Kyle³, Chaevien Clendinen³, and **Cong T. Trinh**¹ (ctrinh@utk.edu)

Institutions: ¹University of Tennessee, Knoxville; ²Oak Ridge National Laboratory, Oak Ridge, TN; ³ Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington.

Project Goals: To elucidate and harness the exceptional robustness of novel, undomesticated *Y. lipolytica* isolates from genetic diversity screening as a bioenergy-relevant microbial platform for efficient conversion of undetoxified biomass hydrolysates into designer bioesters with continuous recovery using solvent extraction.

Abstract text: Microbial solvent tolerance is a desirable phenotype for novel strategies in bioconversion of renewable substrates to replace petroleum-derived chemicals and fuels. In comparison to conventional solvents, ionic liquids (ILs) such as 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) have emerged as a novel reaction medium with superior results in bioprocessing due to their ability to dissolve a wider range of compounds and their adjustable properties for enzyme stabilization and activation. ILs, however, are toxic to microbial growth (e.g., 1%-5% (v/v) IL) which must be overcome for whole-cell biocatalysis in ILs. Previously, we generated the most IL-tolerant mutant reported to date with robust growth in up to 18% (v/v) [EMIM][OAc]^{1,2}. Here, we used multi-omics and morphological characterization to understand the superior IL-tolerance of these *Y. lipolytica* strains. Specifically, we demonstrated a new criterion to predict key genetic targets from dynamic RNA-sequencing that confer IL-tolerance using both single-gene and dual-gene overexpression library enrichment techniques. Additionally, we discovered *Y. lipolytica* reconfigures membrane composition and cellular compartments to tolerate high concentrations of ILs and shed light on proteins and regulatory machinery responsible for these processes. Taken together, our work demonstrates a new approach to identify genetic targets for reverse engineering robustness and highlights overlooked cellular processes underlying robust phenotypes.

References/Publications

1. Walker, C., Ryu, S. & Trinh, C. T. Exceptional solvent tolerance in *Yarrowia lipolytica* is enhanced by sterols. *Metabolic Engineering* 54, 83-95, doi:<https://doi.org/10.1016/j.ymben.2019.03.003> (2019).
2. Walker, C., Ryu, S., Haridas, S., Na, H., Zane, M., Labutti, K., Lipzen, A., Barry, K., Grigoriev, I., Trinh, C.T.*, 2020. Draft Genome Assemblies of Ionic Liquid Resistant *Yarrowia lipolytica* PO1f and its superior evolved strain YICW001. *Microbiol Resour Announce*, 9(999): e01356-19.

Funding statement: *This work is supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DESC0019412.*