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

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**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**

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