Understanding Exceptional Ionic Liquid Tolerance 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 biocatalysis in organic solvents such as ionic liquids (ILs) is attractive for making high-value chemicals. However, IL toxicity at a level of 0.5% ~1% (v/v) can drastically reduce microbial activity. In this study, we engineered a mutant *Yarrowia lipolytica* YlCW001 that can thrive in up to 18% (v/v) 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]), lethal to almost all microorganisms. Remarkably, YlCW001 also exhibits universal tolerance in most commonly used ILs beyond [EMIM][OAc]. Scanning electron microscopy revealed that ILs significantly damage cell wall and/or membrane of wildtype *Y. lipolytica* while YlCW001 maintains healthy cellular morphology even in high concentration of ILs up to 18% (v/v). By performing comprehensive metabolomics, lipidomics, and transcriptomics to elucidate this unique phenotype, we discovered that both wildtype *Y. lipolytica* and YlCW001 prominently exhibit upregulation of most glycerophospholipids (GPs), sphingolipids, and sterols under IL-stressful environment. However, the mutant reconfigured membrane composition and structure by increasing the content of GPs and sterols more than the wildtype. By modulating the sterols pathway, we validated that sterols is a key component of the cell membrane that enables *Y. lipolytica* to tolerate high IL concentrations. This study provides a fundamental understanding of exceptional robustness of *Y. lipolytica* and helps guide metabolic engineering of *Y. lipolytica* as a microbial manufacturing platform for production of high-value chemicals in organic solvents.

References[1,2]

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