Understanding Robustness of *Yarrowia lipolytica* for Undetoxified Biomass Hydrolysate Utilization

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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. Robustness is an important phenotype for bioenergy microbes to acquire but is difficult to engineer. The oleaginous yeast, *Yarrowia lipolytica*, is an exceptionally robust microbe that can naturally tolerate stressful environments, assimilate a wide range of substrates, and produce high-value chemicals. In this study, we aim to understand and harness the robust characteristics of *Y. lipolytica* for utilizing mixed C5 and C6 sugars in undetoxified switchgrass biomass hydrolysates. From a screen of 57 undomesticated *Y. lipolytica* isolates, we selected top-performing strains exhibiting robust growth and lipid accumulation in biomass hydrolysates and subjected them to comprehensive growth, lipid production, and multi-omics characterization. We then engineered these top performing strains through a combination of targeted pathway engineering followed by adaptive laboratory evolution to further enhance their inherent robustness. Multi-omics profiling of these robust *Yarrowia* strains revealed novel genotype, regulation and cellular processes that positively impact their utilization of biomass hydrolysate sugars. These foundational studies provide key insight into the genetic mechanisms responsible for robustness in *Yarrowia* strains, enabling targeted engineering strategies to be deployed for enhanced production of biofuels and bioproducts from lignocellulosic biomass.

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