Understanding and Harnessing the Exceptional Robustness of *Yarrowia lipolytica* for the Conversion of Biomass Hydrolysate into Designer Bioesters.

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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 tolerate stressful environments,[1] assimilate a wide range of substrates[2,3] and produce high-value chemicals.[4] In this study, we aim to understand and harness these robust characteristics of *Y. lipolytica* for the conversion of biomass hydrolysate into designer bioesters. Specifically, we aim to understand how these *Y. lipolytica* i) tolerate and effectively assimilate inhibitory biomass hydrolysates for superior lipid accumulation, ii) tolerate organic solvents that are required to produce bioproducts in a two-phase fermentation system and iii) endogenously degrade lipids to produce designer bioesters. From a screen of 57 undomesticated *Y. lipolytica* isolates,[5,6] we selected top-performing strains exhibiting robust growth and lipid accumulation in undetoxified biomass hydrolysate for comprehensive growth and multi-omics characterization. Comparative proteomic analysis of these robust *Yarrowia* strains revealed previously uncharacterized proteins that influence robust utilization of biomass hydrolysate sugars and transcription factors regulating lipid accumulation and degradation. We further engineered these strains to improve their robustness for effective conversion of these sugars for lipid production. Next, transcriptomic analysis of an exceptionally solvent-tolerant *Yarrowia* mutant identified genes conferring high solvent tolerance. These foundational insights provide key mechanisms and genetic targets to engineer robustness in *Yarrowia* strains for production of biofuels and bioproducts from lignocellulosic biomass.

References

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