

Harnessing robustness of *Yarrowia lipolytica* for effective utilization of undetoxified biomass hydrolysates

Seunghyun Ryu^{1*} (sryu2@utk.edu), Josh C. Quaterman,² Caleb Walker¹, Richard Giannone,³ Patricia J. Slininger,² Bruce S. Dien,² and **Cong T. Trinh**¹

¹University of Tennessee, Knoxville, TN 37996; ²National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL 61604; ³Oak Ridge National Laboratory, Oak Ridge, TN 37831

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. Even though the oleaginous yeast *Yarrowia lipolytica* has been extensively studied for high lipid accumulation, its robustness to enable effective utilization of undetoxified biomass hydrolysates is not fully understood. In our study, we first discovered that *Y. lipolytica* has native metabolism, capable of degrading complex sugars such as xylose, arabinose, and cellobiose that are dominant in plant biomass. Through bioinformatic, metabolic, and transcriptomic analyses of *Y. lipolytica*, we identified and activated the endogenous genes encoding for metabolic enzymes and sugar-specific transporters that assimilate these complex sugars. Interestingly, we found that xylose assimilation is synergistically activated by glucose with xylitol accumulation whereas arabinose assimilation is synergistically activated by xylose with arabitol accumulation. By tuning the expression of native xylitol dehydrogenase and arabitol dehydrogenase of *Aspergillus oryzae* in *Y. lipolytica*, the engineered strains could enhance xylose and arabinose utilization and eliminate xylitol and arabitol accumulation. Remarkably, by tuning expression of sugar-specific transporters, the assimilation efficiency of not only the pentose sugars but also cellobiose was improved. To further enhance robustness of *Y. lipolytica* for effectively utilizing undetoxified biomass hydrolysates, we next screened a comprehensive set of 57 undomesticated *Y. lipolytica* isolates belonging to the 13 unique species in *Yarrowia* clade. Unlike the model strain W29, we found some *Yarrowia* isolates, *Y. lipolytica* YB-392, YB-419, and YB-420, capable of exhibiting robust growth and lipid accumulation in up to 90% undetoxified dilute acid-pretreated switchgrass hydrolysates. Some isolates, e.g., *Candida phangngensis* PT1-17, were also identified as the top lipid producers that can accumulate neutral lipids up to 3-fold higher than W29. We sequenced the whole genome of these novel *Y. lipolytica* strains and in the process of activating and rewiring their robust metabolism for improved complex sugar assimilation, lipid accumulation, and designer bioester synthesis in undetoxified biomass hydrolysates.

References¹⁻⁵

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