

Proteomic Analysis of Robust *Yarrowia lipolytica* Isolates Reveals Key Processes Impacting Sugar Utilization and Lipid Degradation during Growth on Biomass Hydrolysate

Authors: Caleb Walker^{1*} (fyz692@vols.utk.edu), Stephanie R. Thompson², Richard Giannone³, Patricia J. Slininger², Bruce S. Dien², and Cong T. Trinh¹ (ctrinh@utk.edu)

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

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:

Lipid accumulation from biomass hydrolysate sugars (i.e., mainly glucose and xylose) and then subsequent degradation are complex phenotypes making them difficult to control and engineer in *Yarrowia lipolytica*. Yet, the phenotypic diversity of the undomesticated *Y. lipolytica* clade illuminates desirable traits not found in well-characterized laboratory strains¹. Recently, draft genomes were assembled for five non-conventional *Y. lipolytica* strains with superior xylose utilization, lipid accumulation, and growth on undetoxified biomass hydrolysates in comparison to the conventional laboratory strain CBS7504². Here, we explored the genetic diversity of these strains and identified singleton and unique genes shared by strains exhibiting desirable phenotypes. Next, bioreactor growth characterization using a switchgrass biomass hydrolysate revealed that the unconventional strain YB420 used xylose to support cell growth and maintained lipid levels while the laboratory strain CBS7504 degraded biomass and lipids when xylose was the remaining carbon source in the biomass hydrolysate. Proteomic analysis identified carbohydrate transporters, xylose metabolic enzymes and pentose phosphate pathway proteins stimulated during the xylose uptake stage for both strains after glucose was depleted. Further, we distinguished proteins in lipid metabolism (e.g., lipase, NADPH generation, lipid regulators, beta-oxidation) activated by YB420 (lipid maintenance phenotype) or CBS7504 (lipid degradation phenotype) when xylose was the remaining carbon source in the biomass hydrolysate. This work highlights the importance of studying nonconventional robust isolates to better understand and engineer complex phenotypes such as lipid accumulation from the non-preferred biomass hydrolysate sugars and subsequent degradation or conversion to other products.

References/Publications

1. Quarterman, J., Slininger, P. J., Kurtzman, C. P., Thompson, S. R. & Dien, B. S. A survey of yeast from the *Yarrowia* clade for lipid production in dilute acid pretreated lignocellulosic biomass hydrolysate. *Applied microbiology and biotechnology* 101, 3319-3334, doi:10.1007/s00253-016-8062-y (2017).

2. Walker, C. et al. Draft Genome Assemblies of Five Robust *Yarrowia lipolytica* Strains Exhibiting High Lipid Production, Pentose Sugar Utilization, and Sugar Alcohol Secretion from Undetoxified Lignocellulosic Biomass Hydrolysates. *Microbiol Res Announc* 7, e01040-01018 (2018).

Funding statement: *This work is supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DESC0019412.*