**Phenotypic and Genomic Characterization of Natural Isolates of *Saccharomyces cerevisiae* for Growth Tolerance in Lignocellulosic Hydrolysates**

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**Project Goals: While cellulosic biofuels have tremendous potential for relieving the global energy demand, a number of hurdles prevent the efficient bioconversion of lignocellulose into ethanol and other biofuels. One well-known example is the cellular stress that is imposed from side products generated from biomass pretreatment, which impact fermentation yield and productivity. While a combination of directed engineering and evolution can improve stress tolerance of microbial strains, this approach can be time consuming. An alternative approach is to utilize a microbial strain with endogenous stress tolerant properties, which could then be engineered and evolved for improved biofuel-producing properties. At the Great Lakes Bioenergy Research Center, we have adopted this approach by identifying environmental isolates of the ethanologenic yeast, *Saccharomyces cerevisiae*, with growth tolerance to variety of lignocellulosic hydrolysates. Upon publishing these results, the complete set of phenotypic data will be made publically available to facilitate strain selection for specific applications in lignocellulosic biofuel production.**

Here, we report the results of our phenotypic analysis of approximately 200 natural and industrial isolates of *S. cerevisiae* across lignocellulosic hydrolysates prepared from a variety of biomass pretreated by Ammonia Fiber Expansion (AFEX™), alkaline hydrogen peroxide, ionic liquid, or dilute acid, or lab media containing relevant stress-inducing compounds, such as ethanol, acetic, p-Coumaric and ferulic acids. In addition, we developed an automated software tool that can rapidly analyze growth curves and report growth properties for strain comparison. Using this approach, we identified 3 natural *S. cerevisiae* isolates that maintain rapid growth in multiple stress conditions. In collaboration with the Joint Genome Institute, we have utilized NextGen sequencing and developed mapping tools to resequence the genomes of these diploid strains. Comparative analyses of these genome sequences revealed interesting structural features,

including regions of homozygosity and novel gene sequences. Further phenotypic and sequence analyses may uncover genetic properties that confer strain-specific stress tolerance. *This work was funded in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494).*