213. Directed Engineering and Evolution for Xylose Fermentation and Lipid Biofuels from AFEX-Pretreated Corn Stover Hydrolysate by *Saccharomyces cerevisiae*

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Project Goals

Despite the increased interest and efforts in cellulosic biofuels research, a number of molecular and biochemical barriers remain that prevent the efficient bioconversion of plant feedstocks into ethanol and lipid biofuels. These barriers for the industrial yeast biocatalyst, *Saccharomyces cerevisiae*, include metabolic limitations induced by cellular stress from chemical compounds generated by feedstock pretreatment, which in turn impact fermentation yield and productivity. The second major barrier is the absence of genes required for native *S. cerevisiae* to ferment xylose and produce lipid biofuels. At the DOE Great Lakes Bioenergy Research Center, we have attempted to better understand, address and overcome these barriers in *S. cerevisiae* through evolutionary, synthetic biology and metabolomic approaches.

Abstract

Previously, we screened a large panel of wild and domesticated *S. cerevisiae* strains grown in lignocellulosic hydrolysates generated from Ammonia Fiber Expansion (AFEX™) and alkaline hydrogen peroxide pretreatments. One wild strain, which we named GLBRCY0, was identified for its robust growth properties. Through engineering and experimental evolution, we developed modified derivatives that can ferment xylose into ethanol effectively from AFEX™ corn stover hydrolysate (ACSH). To understand how these strains are able to ferment xylose more rapidly, we performed metabolomic profiling of these strains during ACSH fermentation. This analysis suggested that elevated activities by enzymes in the Pentose Phosphate Pathway are important for this evolved phenotype. We additionally engineered a stress-tolerant, xylose- fermenting yeast strain with the *Euonymus alatus* Diacylglycerol Acetyltransferase (*EaDAcT*) to produce 3-acetyl-1,2-diacyl-*sn*-glycerol (acTAG), which is an oil with diesel like properties. This *S. cerevisiae* strain engineered with *EaDAcT* can produce acTAGs aerobically from both glucose and xylose, as well as from glucose in ACSH. Together, these results indicate that engineered *S. cerevisiae* can effectively produce acTAGs from renewable sugars generated from pretreated plant feedstocks, and our strains have significant promise as industrial biocatalysts for ethanol and lipid biofuels. This work was funded by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494).