211. Construction and Characterization of Interspecies Yeast Hybrids Using Newly Discovered Species with Native Biofuel Potential

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Project Goals: The DOE Great Lakes Bioenergy Research Center performs basic research that generates the technology to convert lignocellulosic biomass to ethanol and advanced biofuels. Within this broad theme, we are engineering yeast to efficiently convert hexoses and pentoses to ethanol in the presence of the inhibitory toxins found in lignocellulosic hydrolysates. Specifically, we have sought 1) to determine the potential of diverse non- *cerevisiae* strains of *Saccharomyces* for cellulosic ethanol production; and 2) to improve their performance through engineering, hybridization, and experimental evolution. We are now focused on identifying specific genes and alleles from yeasts and other fungi that can be engineered into xylose-fermenting GLBRC "chassis" strains of *S. cerevisiae*, as well as other biofuel strains.

Most research in biofuel production involves improvement of strains of *Saccharomyces cerevisiae*[1]. Recent discoveries and genome projects have made it clear that other species of *Saccharomyces* also have many of the same traits useful for fermentation, but these new species harbor novel genes and alleles[2,3]. The maximum pairwise genetic divergences between *Saccharomyces* species are similar to those between humans and birds[4] and ~60x greater than the most divergent *S. cerevisiae* strains. In addition, natural interspecies hybrids between *S. cerevisiae* and other *Saccharomyces* species can tolerate some stressful fermentative environments better than *S. cerevisiae*, such as during certain winemaking and brewing processes[5,6]. Here we explore the growth properties of 457 non-*cerevisiae* strains in AFEX corn stover hydrolysate (ACSH). We also engineered 6 interspecies hybrids by crossing representatives to strains of *S. cerevisiae* that had been engineered and evolved for xylose metabolism. To explore metabolic diversity and fermentation bottlenecks, we selected the best and the worst growing strains from each species for fermentation in ACSH in microaerobic conditions. They were then compared against the engineered interspecies hybrids and GLBRC benchmark strains.

Our results show that several non-*cerevisiae* species of *Saccharomyces* possess novel and native biofuel potential. *S. paradoxus* and *S. mikatae* had unusually high tolerance to the fermentation inhibitors found in ACSH (over 20% faster growth for some strains). Some strains of *S. eubayanus* and *S. paradoxus* had high natural tolerance to the most prevalent lignotoxin, acetamide, and could slowly metabolize xylose without engineering. Many of these traits proved to be dominant in interspecies hybrids constructed by crossing engineered xylose-fermenting strains of *S. cerevisiae* with these new species. In some cases, the engineered hybrid strains displayed hybrid vigor and novel, industrially desirable properties. For example, *S. mikatae x S. cerevisiae* had unusually high tolerance to acetamide and produced ethanol from ACSH faster and with higher yields than the xylose-fermenting *S. cerevisiae* parent. Finally, by using a gene from a filamentous fungus, we have engineered an acetamide-consuming strain. We conclude that several other yeast and fungal species possess genes and alleles that could be used to improve the performance of *S.*

cerevisiae biofuel strains. Our current research focuses on identifying these genes and developing high-throughput engineering techniques to test their effect on lignocellulosic fermentations.

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This work was funded by the DOE Great Lakes Bioenergy Research Center (DOE Office of Science BER DE-FC02-07ER64494).