Title: Crabtree/Warburg-like aerobic xylose fermentation by engineered *Saccharomyces cerevisiae*

Authors: Sae-Byuk Lee[1,2,3,4], Mary Tremaine[1], Michael Place[1,3,4], Lisa Liu[1], Austin Pier[1], David J. Krause[1,2,3,4], Dan Xie[1], Yaoping Zhang[1], Robert Landick[1,5,6], Audrey P. Gasch[1,3,4], **Chris Todd Hittinger**[1,2,3,4], and **Trey K. Sato**[1,*] (tksato@glbrc.wisc.edu)

Institutions: [1]DOE Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, WI; [2]Wisconsin Energy Institute, J. F. Crow Institute for the Study of Evolution, University of Wisconsin-Madison, Madison, WI; [3]Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; [4]Center for Genomic Science Innovation, University of Wisconsin-Madison, Madison, WI; [5]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI; [6]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI

Project Goals: We aimed to identify genetic modifications that enhance metabolic flux and the conversion of xylose into fermentative end-products by yeast through directed evolution.

Abstract:

Bottlenecks in the efficient conversion of xylose into cost-effective biofuels have limited the widespread use of plant lignocellulose as a renewable feedstock. The yeast *Saccharomyces cerevisiae* ferments glucose into ethanol with such high metabolic flux that it ferments high concentrations of glucose aerobically, a trait called the Crabtree/Warburg Effect. In contrast to glucose, most engineered *S. cerevisiae* strains do not ferment xylose at economically viable rates and yields, and they require respiration to achieve sufficient xylose metabolic flux and energy return for growth aerobically. Here, we evolved respiration-deficient *S. cerevisiae* strains that can grow on and ferment xylose to ethanol aerobically, a trait analogous to the Crabtree/Warburg Effect for glucose. Through genome sequence comparisons and directed engineering, we determined that duplications of genes encoding engineered xylose metabolism enzymes, as well as *TKL1*, a gene encoding a transketolase in the pentose phosphate pathway, were the causative genetic changes for the evolved phenotype. Reengineered duplications of these enzymes, in combination with deletion mutations in *HOG1*, *ISU1*, *GRE3*, and *IRA2*, increased the rates of aerobic and anaerobic xylose fermentation. Importantly, we found that these genetic modifications function in another genetic background and increase the rate and yield of xylose-to-ethanol conversion in industrially relevant switchgrass hydrolysate, indicating that these specific genetic modifications may enable the sustainable production of industrial biofuels from yeast. We propose a model for how key regulatory mutations prime yeast for aerobic xylose fermentation by lowering the threshold for overflow metabolism, allowing mutations to increase xylose flux and to redirect it into fermentation products

Funding Statement: This material is based upon work supported by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Numbers DE-SC0018409 and DE-FC02-07ER64494.