Natural Genetic Variations Influence Ionic Liquid Tolerance by *Saccharomyces cerevisiae*

Trey K. Sato[1*] (tksato@glbrc.wisc.edu), Douglas Higgins[2,3], Mary Tremaine[1], Scott Bottoms[1], Quinn Dickinson[1], Jenna Fletcher[1], Margaret Agnew[1], Maria Sardi[1,4], Audrey P. Gasch[1,4], Steve Singer[2,3], Blake A. Simmons[2,5], Robert Landick[1,6] and Michael P. Thelen[2,3],

[1]DOE Great Lakes Bioenergy Research Center, University of Wisconsin, Madison, WI; [2]Deconstruction Division, Joint BioEnergy Institute, Emeryville, CA; [3]Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, CA; [4]Department of Genetics, University of Wisconsin, Madison, WI; [5]Biomass Science and Conversion Technology Department, Sandia National Laboratories, Livermore, CA; [6]Department of Biochemistry, University of Wisconsin, Madison, WI.

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 other biofuels. One barrier for the industrial yeast biocatalyst, *Saccharomyces cerevisiae*, is the inhibition of fuel production incurred from chemical compounds in pretreated lignocellulosic feedstocks. These compounds often cause cellular stress, which in turn impacts biofuel yield and productivity. These stressors are not only degradation products generated during the pretreatment process, including acetic acid and lignin-derived phenolics, but are also compounds used in feedstock pretreatment, such as the ionic liquids (ILs) 1-ethyl-3-methylimidazolium -chloride and -acetate ($[C_2C_1im]Cl$ and $[C_2C_1im][OAc]$. At the DOE Great Lakes Bioenergy Research Center and Joint BioEnergy Institute, we have collaborated to employ genetic, phenotypic and screening approaches to determine the genetic variation that drives a range of IL tolerance in natural isolates of *S. cerevisiae*.

Abstract

Imidazolium ILs effectively solubilize lignin and cellulosic components of biomass, enabling the subsequent enzymatic hydrolysis of cellulose into fermentable sugars. Although the majority of $[C_2C_1im]$ ILs can be recovered after the pretreatment process, residual IL can remain in hydrolysates at concentrations that significantly impair yeast growth, viability, and fermentation. We phenotyped a large panel of wild and domesticated *S. cerevisiae* strains for growth tolerance in lab media containing $[C_2C_1im]Cl$ and $[C_2C_1im]OAc$. While the canonical lab strain, BY4741, grew poorly in lab media containing 125 mM $[C_2C_1im]Cl$, we identified two yeast strains with significantly greater tolerance relative to other strains. Genomic DNA fragments from one of these tolerant strains, 378604X, was then cloned into fosmids, which were subsequently transformed into the intolerant BY4741 strain and selected for improved growth in the presence of 125 mM $[C_2C_1im]Cl$. From this selection, we identified two genes that conferred greater IL tolerance when overexpressed in BY4741. Furthermore, one of these genes, *SGE1*, was required for $[C_2C_1im]Cl$ tolerance in both BY4741 and 378604X strains. Interestingly, *SGE1* is has non-synonymous polymorphisms between the BY4741 and 378604X strains, as well as between other wild and domesticated strains with a range of IL tolerance. Expressing the *SGE1* allele from 378604X into the BY4741 *sge1Δ* strain conferred IL tolerance, while expressing the $SGE1^{BY4741}$ allele in the 378604X *sge1Δ* strain imparted IL sensitivity. The Sge1

protein is predicted as a multi-spanning transmembrane protein previously characterized as a drug and cationic dye pump, suggesting that the 378604X strain retains a natural genetic variant with greater tolerance to $[C_2C_1im]Cl$. These results exemplify the importance of strain background when selecting a biocatalyst for metabolic engineering, as well as highlight the possibilities that subtle differences protein sequences can confer phenotypic improvements relevant to biofuel production. **This work was funded by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494) and Joint BioEnergy Institute (DOE BER Office of Science DE-AC02-05CH11231).**