

Transferring Ionic Liquid Tolerance from the Rain Forest to *E. coli*

Michael Thelan¹, Michael Thelen^{1*} (MPThelen@lbl.gov) Thomas Rüegg¹, Patrik D'haeseleer¹, Sharon Borglin¹, Kristen DeAngelis¹, Hannah Woo¹, Erika Lindquist², Jane Khudyakov¹, Blake Simmons¹, and Michael Thelen¹

¹ Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA

² DOE Joint Genome Institute, Walnut Creek, CA

www.jbei.org

Project Goals:

Microbes found in natural environments such as forest soils with fast decomposition rates produce highly efficient lignocellulolytic enzymes and are often stress-resistant due to adaptation in fluctuating environmental conditions. Using bacteria isolated from such environments, either directly in biofuel production or to improve existing laboratory strains by genetic engineering, can improve lignocellulose degradation and reduce microbial growth inhibition from toxic byproducts. Pretreatment of plant feedstock with ionic liquids (ILs) has significant advantages over current methods for deconstruction of lignocellulosic feedstocks; however, ILs are toxic to the microorganisms used subsequently for biomass saccharification and fermentation. Based on these considerations, one of our major goals at JBEI is to engineer biofuel microbes to tolerate ILs and chemical inhibitors.

At JBEI we are interested in using microbes that are tolerant to ionic liquids and other chemical inhibitors encountered during biofuel processing. Screening a tropical rain forest soil community for IL-tolerant cellulolytic bacteria identified a novel halotolerant anaerobe that grows in up to 0.5M (~8%) 1-ethyl-3-methylimidazolium chloride, or [C2mim]Cl. By creating a fosmid library containing genomic fragments from this bacterium, we discovered a predicted multidrug-efflux pump that promotes better tolerance to [C2mim]Cl in *E. coli* than in the rain forest isolate. IL-induced changes were found in the native bacterial membrane phospholipids, and in the significant differential expression of 1245 genes revealed by global transcriptomics (RNA-Seq) analysis and metabolic pathway reconstruction. The knowledge of these physiological responses provides us with a first step towards engineering microbial IL tolerance.

This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.