303c Engineering Stress-Tolerant Microbes for Lower Cost Production of Biofuels and Bioproducts

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Efficient fermentation processes to produce ethanol from both the hexose and pentose sugars available in low-cost lignocellulosic biomass are sought to support the expansion of the biofuels industry. Such an expansion is expected to strengthen our nation by lessening dependence on foreign sources of fuel, preserving our environment and national resources, and boosting our rural economy. Stress-tolerant microorganisms are needed that are able to withstand, survive, and function in the presence of stress factors common to fermentations of lignocellulose hydrolysates, including various chemical fermentation inhibitors such as furfural, hydroxymethylfurfural (HMF), and ethanol. Furfural and HMF are key byproducts of the dilute acid pretreatment hydrolysis of lignocellulosic biomass, the most economical method of releasing hemicellulosic sugars for fermentation to ethanol biofuel. The availability of tolerant microbial catalysts would allow efficient fermentation of low-cost acid hydrolysates despite the presence of inhibitory byproducts.

Gathering fundamental knowledge about the metabolic, physiologic, and genetic mechanisms underlying inhibitor tolerance of ethanologenic yeast strains is the first step in our approach to engineering improved strains and/or process conditions that foster tolerance and functionality of microbes during production of ethanol from lignocellulosic materials. Our research has shown that natural strains of the yeasts *Saccharomyces cerevisiae* and *Pichia stipitis* can survive and adapt to the presence of furfural and HMF. We have also shown that exposure to gradually increasing levels of each inhibitor can lead to the development of strains able to tolerate relatively high levels of HMF and furfural (30 mM). Fermentation analysis of adapted strains has revealed that these strains are more efficient than their parent strains in the reduction of the aldehyde functional group of the inhibitors to the corresponding less toxic alcohol, ie. furfural to furfuryl alcohol and HMF to 2,5-bis-hydroxymethylfuran, suggesting the role of in situ detoxification in inhibitor-tolerant strains. Screening a *S. cerevisiae* disruption library identified 65 genes involved in furfural tolerance, and fermentation studies showed that tolerance to furfural and HMF was associated with the successful expression of pentose phosphate pathway genes, a pathway that, like furfural and HMF detoxification, is subject to limitation by the availability of NADPH/NADH cofactors to reduction reactions. Overexpression of a subset of these genes enhances *S. cerevisiae's* tolerance to furfural. Microarray studies comparing HMF-treated wild type and adapted strain cultures have shown that tolerant strains have distinct expression profiles of selected genes compared with that of the parent strain. Genes in all categories of biological process, cellular component, and molecular function were involved; some were HMF-specific while others could be associated with a core set of stress genes, such as those belonging to the pleiotropic drug resistance gene family. This body of work suggests that an understanding of adaptation mechanisms can be utilized to either engineer new strains or to design inoculum production and fermentation process conditions. Process-based strategies to produce a tolerant initial population and then to foster and sustain tolerance during growth and ethanol fermentation will be discussed.