Leveraging natural variation in *Saccharomyces cerevisiae* to elucidate the toxicity mechanisms of lignocellulosic hydrolysate and advanced biofuels.

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PROJECT GOALS: Explore natural genetic and phenotypic variation in *Saccharomyces cerevisiae* to elucidate the stress response to lignocellulosic hydrolysate and advanced biofuels such as butanol and isobutanol.

ABSTRACT:

The increased demand for alternative fuels is driving the development of more efficient and economical production of biofuels. This requires the use of non-food based plant biomass to produce advanced biofuels such as butanol and isobutanol. A major challenge of implementing this new energy source is that the chemically treated plant material, known as lignocellulosic hydrolysate, contains a variety of toxic compounds that affect fermenting microbes by inhibiting growth, metabolism, and alcohol production, all of which decrease the economic efficiency of lignocellulosic biofuel production. In addition, butanol and isobutanol are toxic even at small concentrations, making end product toxicity a significant limiting factor. We are using multiple genomic strategies to identify mechanisms of toxicity and tolerance that can be then use to engineer tolerance into industrially relevant microbes. Using a collection of 165 genetically and phenotypically diverse strains of *Saccharomyces cerevisiae*, we are exploring natural genetic and phenotypic variation to understand lignocellulosic hydrolysate tolerance. First, by comparing and contrasting the transcriptional responses of tolerant and sensitive strains exposed to these stresses, we are identifying the primary toxin targets and their effects on cellular physiology. Second, we are exploring how genetic background affects engineering strategies. We are measuring phenotypic variation in the response to gene over-expression by introducing a library of 5,000 barcoded, high-copy plasmids that each expresses a different yeast gene, into four different yeast strains. By exploring background-specific effects on the fitness contribution of each gene to toxin and end-product tolerance, we hope to uncover important strategies for engineering. Finally, we are using Genome-wide Association (GWA) and bulk segregant analysis to identify connections between genotype and phenotype. By applying multiple genomic strategies and integrating the results, we expect to identify strategies for improving tolerance to the stresses found in the production of advanced biofuels.