

125. Comparative Fermentation of *Saccharomyces cerevisiae* and *Zymomonas mobilis* in Lignocellulosic Hydrolysates Produced from Corn Stover and Switchgrass to Investigate the Effect of Interannual Climate Variability on Hydrolysate and Microbial Performance

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Project Goals: Different biomass feedstocks will be necessary for sustainable biofuel production from lignocellulose. Therefore, it is crucial to understand microbial responses to hydrolysates produced from different feedstocks. We produced lignocellulosic hydrolysates using AFEX™-pretreated corn stover and switchgrass harvested from three different years with significantly different levels of precipitation and then performed comparative fermentations to study microbial responses to these different feedstock hydrolysates. The chemical compositions of these lignocellulosic hydrolysates were analyzed and the comparative fermentation experiments were carried out using *Saccharomyces cerevisiae* and *Zymomonas mobilis* strains. The growth, glucose and xylose utilization, and ethanol production were monitored during fermentation, and RNA samples were collected for RNAseq analysis to generate high sensitivity profiles and further define microbial responses in these different hydrolysates.

In order to identify and overcome key barriers in the sustainable conversion of lignocellulosic biomass to biofuels, we have produced more than 21 batches of hydrolysates using AFEX™- pretreated corn stover and switchgrass harvested from three different years with significantly different levels of precipitation (Y2010 a wet year, Y2012 a dry year, and Y2013 an average year). A three-tiered strategy has been implemented to understand microbial responses to these diverse feedstock hydrolysates: Tier I - Hydrolysate Compositional Analysis. After hydrolysates were produced using our standard protocol, the concentrations of sugars, phenolics, organic acids, aldehydes, and other well-known inhibitors in these hydrolysates are analyzed by HPLC, GC/MS and LC/MS. This provides us basic knowledge about the variation of compounds in different hydrolysates. Tier II - Chemical Genomics for Fingerprinting of Hydrolysates. *S. cerevisiae* and *Z. mobilis* deletion collections have been used for chemical genomics to generate biological fingerprints of the hydrolysates. Tier III - First-pass Multiomics Fermentation. We conduct fermentation experiments using engineered yeast and *Z. mobilis* strains on hydrolysates that show significant differences in Tier I and II studies. Cell growth is monitored, and samples for endproduct (HPLC-RID) and RNAseq are collected. The endproduct analysis will determine the efficiency of converting sugars (both glucose and xylose) into ethanol whereas the RNAseq will generate high sensitivity gene expression profiles for responses of *S. cerevisiae* and *Z. mobilis* in these different hydrolysates. In collaboration with JGI, RNAseq data will be analyzed to determine how microbial responses (e.g., stress responses, expression of efflux pumps and regulators, etc.) vary among the different feedstocks.

Hydrolysate compositional analysis showed that overall corn stover hydrolysates (ACSH) contain higher levels of lignocellulose-derived inhibitors than switchgrass hydrolysates (ASGH). Chemical genomics with a *S. cerevisiae* deletion mutant library indicated distinct chemical genomics profile when different feedstock hydrolysates were used. Fermentation experiments also showed that *S. cerevisiae* and *Z. mobilis* have different responses to these different hydrolysates. Specifically *Z. mobilis* showed very similar growth and glucose/xylose utilization in ACSH and ASGH, while *S. cerevisiae* grew significantly

better in ACSH than ASGH. There are also effects of interannual climate variability on microbial fermentation performance, especially when *S. cerevisiae* was used for fermentation. In collaboration with JGI, RNAseq data analysis is underway to determine how microbial responses (e.g., stress responses, expression of efflux pumps and regulators, etc.) vary among the different feedstocks. This information will help us to identify the extent to which different feedstocks produce different microbial responses and how bottlenecks for the conversion of biomass to biofuel vary among different feedstocks.

We are currently expanding our studies to other feedstocks, including miscanthus, sorghum, and mixed-prairie. The chemical compositional analysis and comparative fermentation with these diverse feedstocks to determine microbial responses will generate knowledge to enable development of successful and sustainable lignocellulosic biofuel technologies.

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