Co-Fermentation of Glucose, Xylose and Cellobiose by the Beetle-Associated Yeast, *Spathaspora passalidarum*

Tanya M. Long,[1] Yi-Kai Su,[2] Alan Higbee,[1] Jennifer Headman,[1] Laura B. Willis,[1,3] and **T.W. Jeffries**[1,2,3*] (twjeffri@wisc.edu)

[1]Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, [2]Biological Systems Engineering, University of Wisconsin-Madison, [3]Institute for Microbial and Biochemical Technology, Forest Products Laboratory, Madison, WI

http://genome.jgi.doe.gov/Spapa3/Spapa3.home.html

The goal of this project is to uncover basic regulatory and genetic mechanisms that will enable the efficient conversion of lignocellulosic feedstocks to higher value fuels and chemicals.

Fermentation of cellulosic and hemicellulosic sugars from herbaceous or woody biomass could resolve food vs. fuel conflicts inherent in the bioconversion of grains. However, the inability to co-ferment these sugars is a major challenge to the economical use of lignocellulose as a feedstock for renewable fuels. Simultaneous co-fermentation of mixed sugars is problematic for almost all microbes – including yeasts that ferment xylose and cellobiose natively – because glucose represses utilization of other sugars. Recent studies have shown that *Saccharomyces cerevisiae* can be engineered to ferment xylose in the presence of cellobiose (1), but competition by glucose for xylose transport still presents a problem.

Surprisingly, the ascomyceteous, beetle associated yeast, *Spathaspora passalidarum* (2, 3), which ferments xylose and cellobiose natively, can also co-ferment these two sugars in the presence of glucose when appropriate conditions are employed. This could be highly advantageous in simultaneous saccharification and fermentation (SSF) processes. Under appropriate conditions, *S. passalidarum,* will simultaneously assimilate glucose and xylose aerobically; it will simultaneously co-ferment glucose, cellobiose and xylose under oxygen limitation and it has a specific ethanol production rate on xylose more than 3 times faster than the corresponding rate on glucose. Moreover, adapted strains of *S. passalidarum* can co-ferment glucose and xylose from acid and enzymatic hydrolysates containing significant amounts of acetic acid. Metabolome analysis of *S. passalidarum* before onset and during the fermentations of glucose and xylose showed that the concentration of glycolytic intermediates is significantly higher on xylose than on glucose.

We examined the co-fermentation of xylose/cellobiose mixtures, or glucose/xylose/cellobiose mixtures, in duplicate bioreactors under oxygen limitation. In the absence of glucose, xylose and cellobiose were metabolized at essentially similar rates until xylose was depleted. In the presence of glucose, co-utilization of cellobiose and xylose were delayed, but all three sugars were co-utilized at similar rates and sugars were consumed by 68 h. The maximum ethanol production rate from xylose and cellobiose was 1.07 g/l·h and from all three sugars was 0.73 g/l·h. Ethanol yields during the phase of maximum production rate were 0.43 g/g and 0.42 g/g for xylose/cellobiose and glucose/xylose/cellobiose mixtures respectively.

Two strains of *S. passalidarum*, AF8 and E7, showed co-utilization of glucose and xylose in AFEX corn stover and maple hydrolysates. The AF8 strain was able to ferment 93% of raw AFEX hydrolysate containing 10% of monosaccharide and could tolerate 1.6-fold more acetic acid than the parent. The maximum ethanol productivity of AF8 was about 2.5 times higher than the parental adapted strain AF2. The E7 strain was able to produce about 38 g/l ethanol from maple hydrolysate medium containing 65 g/l

of xylose and 35 g/l of glucose in 59 h in 2-l bioreactors. The ethanol yields from AFEX corn stover and maple hydrolysate were 0.33 and 0.38, respectively. Our results showed that adaption can improve strains performance and adapted *S. passalidarum* can still co-ferment glucose and xylose in hydrolysates.

Understanding metabolic flux during fermentation is an important challenge in identifying factors that limit efficient ethanol production. Metabolite profiling has long been a useful tool for identifying bottlenecks, however obtaining meaningful data in sufficient replicates can be arduous and the results are often limited in scope. Metabolomics is a rapidly developing field, and HPLC-MS/MS provides a powerful tool to efficiently identify and quantitate a large number of metabolites simultaneously. We have used this approach to simultaneously determine the concentrations of more than 40 intracellular metabolites during the cultivation of *S. passalidarum* on glucose, or xylose under aerobic and oxygen limiting conditions. Metabolites were analyzed during early growth phase and oxygen limited fermentation in order to better define the differences between the growth and ethanol production. The metabolite levels exhibited during fermentation on glucose and xylose reflect the higher fermentation rates observed with xylose and suggest a regulatory mechanism.

These features combined with metabolomic analytical tools make *S. passalidarum* very attractive for SSF applications and for studying regulatory mechanisms enabling bioconversion of lignocellulosic materials by yeasts.

References

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