ENGINEERING ZYMOMONAS MOBILIS FOR BIOFUELS PRODUCTION

Min Zhang, National Bioenergy Center, National Renewable Energy Laboratory, Golden, Colorado, 80401, USA
min_zhang@nrel.gov

Key words: xylose utilization, hydrolysate toxicity, biofuels

Introduction. The use of renewable lignocellulosic biomass as a feedstock offers the opportunity to reduce our current dependence on fossil fuels and chemicals. The lignocellulosic biomass, such as agricultural and forestry residues, are composed of cellulose, hemicellulose, and lignin. Hemicellulose contains D-xylose and L-arabinose to a lesser extent. D-xylose is the second most abundant sugar in nature after D-glucose. Cost effective conversion of lignocellulosic biomass to fuels requires effective fermentation of both glucose and xylose (and arabinose). Processing lignocellulose for biofuel production results in the release of the major fermentable sugars glucose and xylose. However, the primary processing steps required for this conversion also produce a range of compounds that can inhibit the subsequent microbial fermentation such as acetic acid and furfural. *Zymomonas mobilis* is known for its high specific glucose uptake rate, rapid catabolism and high ethanol yield as compared to yeast (1). However, its modest substrate utilization range (glucose, fructose and sucrose) has limited its use in utilizing sugars derived from lignocellulosic biomass for biofuels production.
The objective of this research is to engineer *Z. mobilis* for efficient conversion of the biomass sugars to ethanol and other fuel molecules.

Methods. We established essential genetic engineering methods to engineer *Z. mobilis* for pentose sugar utilization. To enhance its tolerance to inhibitors in hydrolysate from pretreated biomass, we conducted systematically studies to identify the key inhibitors. We further used the both forward genetics and omics/reverse genetics to identify genes contribute the hydrolysate tolerance. With synthetic biology tools we engineered *Z. mobilis* for hydrocarbon production.

Results. *Z. mobilis* has been engineered to efficiently convert the second and third most abundant plant derived sugars, xylose and arabinose, to ethanol at high yield (2). However, utilization of the pentose sugars is strongly inhibited by the toxic compounds present in the hydrolysate. A systematic study was conducted to identify the key inhibitors through chemical analysis and fractionation of the hydrolysate in combination of growth assay and fermentation (3). More recently, we have been applying systems biology and genomic tools to investigate and improve its tolerance to the specific inhibitors present in biomass hydrolysate obtained from diluted acid pretreatment. We identified a number of genes are upregulated and down-regulated in the presence of inhibitors. Acetate inhibited the growth and biomass of the genomically integrated xylose-utilization recombinant strain 8b of *Z. mobilis* on glucose, but not the glucose utilization and ethanol yield. However, acetate inhibited the growth, biomass, xylose utilization and ethanol production. It looks that *Z.mobilis 8b* is under stressful condition when using xylose as carbon source. By using mini-transposon mutagenesis we created a random mutation (knockout and overexpression) library and screening for improved phenotypes in the presence of inhibitors as well as hydrolysate. We identified several genes related to these phenotypes. We are further investigating the impact of the genes through RNA-seq. With its capabilities for utilizing the vast majority of the biomass sugars, we asked a question - can we redirect carbon to make other fuel molecules or intermediates in addition to ethanol? What other fuel molecules or intermediates can be produced using *Z. mobilis* by applying metabolic engineering and synthetic biology tools? We applied synthetic biology tools and introduced hydrocarbon synthesis genes and demonstrated hydrocarbon production in addition to ethanol.

Conclusions. Using various genetic engineering tools we are able to engineer the excellent glucose fermentor, *Z. mobilis* to effectively utilize pentose sugars and convert them to ethanol and hydrocarbons. It is one of the top fermentation organisms currently developed for the cellulosic biomass to ethanol conversion process. In addition, we are able to identify a number of genes related to enhanced hydrolysate toxicity through both forward genetics and omics/reverse genetics.

Acknowledgements. Funding for this work was provided by the Bioenergy Technologies Office (formerly known as Office of the Biomass Program) in the Department of Energy's Office of Energy Efficiency and Renewable Energy.

References.

1. Rogers PL, Lee KJ, Tribe DE (1979). *Biotechnology lett.* vol.1:165-170.
2. Zhang M, Eddy C, Deantda K, Finkelstein M, Picataggio S (1995) *Science*, vol 267:240-243.
3. Franden, MA, Pienkos, PT, Zhang, M (2009) J. Biotechnol. Vol 144:259-267.