119. Chemical genomic analysis reveals feedstock specific differences in the microbial response to lignocellulosic hydrolysates

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Project Goals
Lignocellulosic derived fermentation inhibitors represent a challenge to biofuel production. We developed a method to biologically assess the quality and inhibitor landscape of hydrolysates using chemical genomics. We are using this approach to understand the different biological responses to hydrolysates produced using different methods and feedstocks. Herein, we specifically investigated the biological response of Saccharomyces cerevisiae to hydrolysates produced from corn stover and switchgrass and discovered a unique chemical genomic profile of each hydrolysates. We have extended this analysis to other systems using E. coli and Z. mobilis chemical genomics to provide a rapid method of assessing the biological response to hydrolysates that yield rich functional insight into fermentation inhibitors, and can inform hydrolysate production strategies.

Abstract text
Lignocellulosic biomass derived fuels and chemicals provide a suite of sustainable bioproducts. Before biomass can be converted to fuel or compounds through biological conversion, it must be converted to fermentable sugars via pre-treatment and hydrolysis, and these sugars converted to fuels by microorganisms. Both pre-treatment and hydrolysis can imbue the resultant hydrolysates with toxicity arising from residual pre-treatment chemicals or biomass derived inhibitors1, which throttle fermentation rates at a substantial economic cost2. Rapid assessment of hydrolysate quality is a key requirement not only to assess the content of fermentable sugars and inhibitors, but also the variation that can arise during production. Analytical chemistry methods can provide detailed compositional analysis of the substrate, but given the complex nature of the plant biomass and chemistry occurring during pre-treatment, it is impossible to quantify all compounds present in the hydrolysate, and ultimately the effects of the composition on the microbial biocatalysts must be inferred. What is needed is an “analytical biology” method to assess the quality of starting substrates, which can provide functional information of the microbial response to a medium and inform process improvements.

Chemical genomics is a reverse-genetics approach that uses genome-wide mutant collections to gain functional insight into the modes-of-actions and cellular targets of bioactive compounds3. Pooled mutant collections are grown in the presence of a bioactive compound, and the individual fitness of these mutants can be assessed by sequencing of their molecular barcodes. Sensitive mutants give clues into the mode-of-action of fermentation inhibitors, and resistant mutants can provide points of rational engineering of tolerance.

We applied chemical genomics to investigate the biological response to hydrolysates produced from either corn stover or switchgrass, which show different fermentation profiles. We challenged the yeast deletion collection with a variety of hydrolysates produced from either ammonia fiber expansion (AFEX) treated switchgrass or corn stover. While variation between batches was low, indicating consistency in production, obvious differences were apparent between hydrolysates made from the different feedstocks.
Deletion mutants in amino acid related processes were more resistant in corn stover produced hydrolysate compared to switchgrass, indicating potential amino acid deficiencies in switchgrass hydrolysates. We also found that deletion mutants in genes involved in ergosterol biosynthesis (ERG6, ERG3) were sensitive to switchgrass hydrolysates. Mutations in these genes affect membrane composition and confer sensitivity to acetic acid. We found that the acetate content of switchgrass hydrolysates was significantly higher (p<0.001) than corn stover. These data suggest that the slower fermentation rates found in switchgrass hydrolysates could arise from amino deficiencies and acetic acid stress.

Chemical genomic profiling of hydrolysates provides detailed information on the biological response of fermentative microbes to lignocellulosic hydrolysates. Using similar mutant collections in E. coli4 and Z. mobilis5, we have further extended this method to diverse industrially relevant microbes. We are presently exploring the effects of more diverse feedstocks and interannual variability on the biological response of fermentative microbes.

References

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