Comparative multiomic analysis of an *Escherichia coli* ethanologen during fermentation of lignocellulosic hydrolysates and synthetic hydrolysate reveals effects of multiple inhibitors and stress responses on ethanol production

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Project Goals: The Great Lakes Bioenergy Research Center (GLBRC) aims to identify and overcome key barriers to the sustainable conversion of lignocellulosic biomass to biofuels. Toward that goal, we seek to understand the effects of lignocellulosic hydrolysates on microbial physiology and gene regulation. We compare *Escherichia coli* K-12 ethanologenic strains grown in hydrolysates prepared from corn stover pretreated by ammonia fiber expansion (AFEX-CS hydrolysate or ACSH) to strains grown in a synthetic hydrolysate (SynH) that mimics ACSH and contains similar concentrations of sugars, amino acids, and other small molecules, but lacks the lignotoxins found in authentic ACSH. Comparative analysis of multiomic data allows us to understand the regulatory and metabolic changes associated with growth in ASCH, and to identify the effects of single or multiple inhibitory compound(s) on *E. coli* growth, xylose utilization, and ethanol yield.

A comparison of model ethanologenic *E. coli* strains in ACSH and SynH was done by multiomic analysis to understand the effects of multiple inhibitors and stress responses in hydrolysate on strain performance in the conversion of lignocellulose to biofuel. Growth and gene-expression profiling revealed complicated patterns of metabolic physiology and cellular stress responses throughout an exponential growth phase, a transition phase, and the metabolically active stationary phase that was remarkably similar in ACSH and SynH. Transcriptomic data indicated that genes associated with numerous stress responses were highly expressed in ACSH and SynH, including pathways involved with mitigation of osmotic stress. A number of stress-related genes were uniquely activated in ACSH relative to SynH, including genes encoding efflux pumps associated with export of aromatic hydroxylates, heavy metals and small toxic molecules. Although the glucose in the ASCH and SynH was consumed completely during the fermentation, xylose was utilized more efficiently in SynH than ASCH. This result suggests that xylose utilization is inhibited in ACSH by compounds not present in SynH.

To understand the stresses associated with growth in ACSH, and their effects on xylose utilization and gene expression, we are currently testing a second generation SynH containing potential inhibitors, such as phenolic compounds (lignotoxins), acetate, and

higher osmolarity, as well as osmoprotectants such as betaine found in ACSH. These compounds will be investigated individually and collectively to assess their effects on *E. coli* growth, xylose utilization, and ethanol yield. The comparative analysis of these multiomic data will allow for the identification and removal of the bottlenecks associated with the conversion of lignocellulosic biomass to biofuel.

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