Identifying Potential Detoxification Mechanisms for Furan and Aromatic Aldehydes in Thermophilic, Anaerobic Bacteria Using 2D LC-MS/MS Proteomics

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves 1) designing plant cell walls for rapid deconstruction and 2) developing multitalented microbes for converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC research in biomass deconstruction and conversion targets CBP by studying model organisms and thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction.

Chemical and physical pretreatment of lignocellulosic biomass improves substrate reactivity but also releases microbial inhibitors such as furan aldehydes, low molecular weight fatty acids, and phenolic compounds. Candidate organisms for consolidated bioprocessing (CBP), including *Clostridium thermocellum* and *Caldicellulosiruptor sp.*, will likely require a combination of engineering and evolved adaptation for robust growth and fermentation in the presence of pretreatment inhibitors. Resistance mechanisms to primary inhibitors such as furfural have been described for *Saccharomyces cerevisiae* and ethanologenic *Escherichia coli* but similar responses in cellulolytic thermophiles are poorly characterized. Saccharolytic thermophiles from the genera *Thermoanaerobacter* and *Caloramator*, including a novel isolate from our laboratory, readily grow in the presence of biomass acid-hydrolyzates and demonstrate conversion of furan and aromatic aldehydes to less toxic alcohols. The enzymes responsible for detoxification are unknown but once identified, could potentially be expressed in other organisms relevant to CBP. To identify protein targets for further characterization, the proteomes of two furfural-resistant strains, *Thermoanaerobacter pseudethanolicus* 39E and *Caloramator proteoclasticus* ALDO1, were measured via multidimensional liquid-chromatography mass spectrometry after growing in the presence or absence of 15 mM furfural. While the two anaerobic thermophiles showed similarities in their respective physiological response to the inhibitor, distinct differences were also apparent. Both organisms displayed increases in ABC transporter-related proteins for sugar uptake by two orders of magnitude. *T. pseudethanolicus* 39E upregulated a number of enzymes involved in purine, pyrimidine, and amino acid biosynthesis while *C. proteoclasticus* ALDO1 showed increases in proteins encoded by a large operon responsible for lipid production. Several enzymes involved in redox reactions were also identified in both strains upon exposure to furfural and their role in aldehyde reduction will be further investigated.

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