GLBRC Targeted Metabolomics: Enabling the Science of Lignocellulose Bioconversion

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Project Goals: "GLBRC targeted metabolomics" refers to a number of assays that have been developed to enable and guide research focused on identifying and overcoming limitations that reduce the efficiency of lignocelluose conversion to biofuels. Measurement of inhibitory as well as growth promoting compounds, and of compounds arising from microbial metabolism found in lignocellulosic hydrolysates aims to elucidate the causes of the inhibition of microbial utilization of xylose in these feedstock materials and to identify other growth limiting factors. These methods have also been applied to understanding metabolism of inhibitors by various microbes. Measurement of intracellular metabolites and cofactors associated with conversion of sugars to biofuels are used to inform researchers of the metabolic consequences of these inhibitors, the efficacy of strain improvement efforts and to guide future genetic augmentations by identifying potential points of restricted metabolic throughput.

Abstract: The Great Lakes Bioenergy Research Center (GLBRC) in collaboration with the research group of Dr. Joshua J. Coon (Coon Laboratories) has developed a number of assays for specific compounds of interest aimed at enabling efforts to improve the efficiency of lignocellulose deconstruction and conversion to biofuels. During anaerobic fermentation in hydrolysates prepared from Ammonia Fiber Expansion (AFEX®)-pretreated corn stover (ACSH) specialized yeast and bacterial strains are capable of complete conversion of glucose to ethanol, but convert xylose much less efficiently. Xylose can be completely converted during growth in laboratory medium, indicating that components of ACSH inhibit xylose utilization. To identify the mechanism underlying the reduced efficiency of xylose conversion we have developed methods for characterizing the chemical composition of ACSH and other hydrolysates. To understand the effects of ACSH inhibitors on cell physiology, we use a combination of assays to

determine most of the metabolic intermediates associated with bioconversion of sugars to fuels. These methods are also used to investigate the consequences of genetic changes and strain improvement efforts primarily aimed at developing strains that convert xylose to biofuels effectively in ACSH.

A combination of ion exchange chromatography and ion pairing chromatography coupled to tandem mass spectrometric detection gives measurements of most of the critical components of central energy metabolism in cellular extracts. HILIC chromatography with tandem mass spectrometry detection is used to measure amino acid abundances in cellular extracts and also in highly complex and concentrated extracellular media such as lignocellulosic hydrolysates. Intracellular products associated with xylose assimilation are determined using methoximation/trimethylsilylation and GC-MS, enabling assessment of the functionality of this pathway as engineered into *S. cerevisiae* with the goal of achieving viable rates of xylose bioconversion in a known ethanologen with no endogenous capability to assimilate and utilize xylose. Products of lignin and carbohydrate decomposition referred to as "lignotoxins" in hydrolysates (and their metabolites after microbial action) are measured using reversed phase chromatography routinely coupled to tandem mass spectrometry for targeted analysis of 36 known components. If the ability to detect and potentially identify unknown components is also desired, full scan high resolution / accurate mass spectrometry with fast polarity switching and data dependent MS/MS can be used allowing simultaneous determination of known compounds and nontargeted analysis. Lignotoxin analysis has enabled several lines of research. Headspace solid phase microextraction (HS-SPME) with GC-MS is used for determination of volatile and semivolatile fermentation products and components of hydrolysates and that are not easily measured by other means such as acetaldehyde, furfural and acetamide. Levels of isobutanol production in microbial cultures are routinely measured using headspace sampling GC-MS. Recently we have also developed a HILIC-UHPLC-MS/MS method for determination of most of the compounds in the methylerythritol 4-phosphate (MEP) pathway leading to production of isoprenoids. Many of these methods employ stable isotope labeled internal standards (SILIS) to give the most accurate and robust absolute quantitation possible.

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