Lignocellulose-Fermenting Microbiomes: A "Compass" for Biofuel Process Development.

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

The question **"What biocatalysts are most effective at cellulosic biomass deconstruction?"** is of considerable fundamental interest, foundational for developing processes for biological conversion of cellulosic biomass, and surprisingly unresolved given the decades of research devoted to the field. Recently, substantial differences in solubilization capability have been demonstrated[1,2] and *Clostridium thermocellum* has been shown to be several-fold more effective than a commercial fungal cellulase preparation at solubilizing several feedstocks under a broad range of conditions.[1,2,3]

Lignocellulose-fermenting enrichments (aka microbiomes) contain a diversity of cellulolytic microbes and cellulase genes.[4] While product formation in such microbiomes is difficult to control, their performance and composition provide a "compass" for developing biofuel production systems featuring defined cultures.

We report here a multi-level investigation of lignocellulose-fermenting microbiomes, featuring:

- A stable, semi-continuous, thermophilic, anaerobic, methanogenic microbiome was established and operated at a 10-day residence time and increasing solids loading. Carbohydrate solubilization was undiminished with increasing solids loading up to 150 g/L, providing a value proof of biological capability.
- A new framework was developed, validated and applied that provides unprecedented insights into the comparative reactivity of lignocellulose substrates and the comparative effectiveness of biocatalysts at mediating lignocellulose solubilization.
- Using this framework, it was demonstrated that both a lab strain of *Clostridium thermocellum* and a prominent cellulolytic isolate obtained from the microbiome have equal ability to access lignocellulose and do so over 2-fold faster than the complex microbiome. This contrasts sharply with conventional wisdom that microbiomes will solubilize complex biomass more rapidly and

completely due to an increased diversity of potential degradative mechanisms; this observation awaits explanation.

- For the first time a comprehensive microbial gene catalog with 1 million genes has been created to give a more complete picture of the functional potential of the microbiome in the digestion of switchgrass
- This study revealed that the functional activity of an anaerobic microbiome, both in terms of protein abundances as well as taxonomic activity, varies significantly during solubilization of the same lignocellulosic biomass across various substrate loadings.

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