Towards Understanding Plant Cell Wall Structure and Properties During Microbial Deconstruction - From Chemical Bonds to Wall Architecture

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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 will address 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 and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. And CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Many aspects of plant cell wall deconstruction by cellulolytic microbes remain unknown. Specifically, cellulolytic bacteria that directly bind to substrates show modalities of deconstruction that are important in the: 1) understanding of cell wall deconstruction and 2) discovery other efficient microbes to enable the complete conversion of biomass to sugars. Closing this knowledge gap will inform consolidated bioprocessing (CBP) applications wherein the microbe both deconstructs and converts biomass to advanced biofuels and biochemicals. For microorganisms which bind to plant biomass, it is known that the proximity of the microbe to the biomass surface and the titer of the enzymes secreted contribute to greater cell wall solubilization than the enzymes alone. Interestingly, the molecular mechanisms governing these complex systems are not yet understood. Our goals are to: 1) obtain in-depth understanding of the barriers that confront biomass deconstruction by biocatalysts, 2) gain the fundamental knowledge needed to inform deconstruction processes by consolidated bioprocessing microorganisms and 3) develop a multi-length scale understanding of plant cell wall deconstruction by combining integrated experimental and computational techniques. This research will increase understanding of the emergent mechanisms by which biocatalysts interact with and utilize biomass; as well as the roles that biomass polymers, cell wall architectural units/surfaces, and tissues play in conversion processes. Ultimately, we aim to gain a holistic, predictive view of biomass deconstruction by studying the physical, chemical, and biochemical phenomena occurring during natural deconstruction.

Understanding biomass synthesis and structure, as well as their relationships to deconstruction, is also vital for reaching these goals. We are working to provide an in-depth understanding of the biochemical and biophysical mechanisms that promote (or inhibit) the deconstruction of plant cell walls during CBP. Here we present our most recent work on understanding cellulolytic synergism in secretomes from thermophilic microbes during deconstruction of biomass and the importance of studying the relationship between microbial binding strength and extent of solubilization focusing on *Caldicellulosiruptor bescii and Clostridium thermocellum*. Understanding the functional diversity of enzymes in the *C. bescii* exoproteome and how inter-molecular synergy between them confers *C. bescii* with its high cellulolytic activity is an important endeavor to potentially confer cellulolytic capability to non-cellulolytic microbes considered for high titer production of biochemicals and biofuels. We found that the combination of three or four of the most highly expressed enzymes in the *C. bescii* exoproteome exhibits such synergistic activity. For example, some discrete combinations of these enzymes mimic and even improve upon the activity of the exoproteome, even though some of the enzymes lack significant activity on their own. Regarding our work with *C. thermocellum*, we have found that modifying the cellulolytic machinery of this microbe reduces its cellulolytic activity but also the strength of binding to biomass significantly, leading to a change in its deconstruction mechanism.

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