

## Leveraging Super High Optical Resolution Microscopy to Probe the Interaction Zone Between *Clostridium thermocellum* and Biomass

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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.**

*Clostridium thermocellum* is one of the most efficient microorganisms for the deconstruction of biomass. To achieve this high level of cellulolytic activity, *C. thermocellum* uses large multienzyme complexes known as cellulosomes to breakdown polysaccharides found in plant cell walls. The attachment of *C. thermocellum* bacterial cells to the nearby substrate via the cellulosome has been hypothesized to be the reason for this high efficiency. The region lying between the cell and the substrate can show great variation and dynamics that is affected by the growth stage of cells and the substrate used for growth. Many aspects of plant cell wall deconstruction by cellulolytic bacteria that directly bind to solid substrates remain unknown and resolving this knowledge gap is crucial for consolidated bioprocessing (CBP) applications. It is imperative to obtain a better fundamental understanding of the interactions that exist between the cellulosomes, bacteria, and the substrate. To address this question, we are utilizing unlimited diffraction microscopy (super resolution microscopy) to probe the distribution of cellulosomes at the microbial substrate interface. Using this technique in conjunction with density-based spatial clustering of applications with noise (DBSCAN), initial results demonstrate an increase in concentration of cellulosomes at the interface between the bacterium and the biomass substrate suggesting this increased concentration is an anchoring point between the bacterium and the substrate allowing other cellulosomes to be shuttled onto the biomass substrate.

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