Understanding the Hyperactive Multi-Component Cellulase: CelA

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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 multi-talented microbes or converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. BESC research in biomass deconstruction and conversion targets CBP by studying thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction and manipulating these microorganisms for improved conversion, yields, and biofuel titer.

Unlike fungal systems, which typically comprise a number of single catalytic domain enzymes for biomass degradation such as family 7 and family 5 glycoside hydrolases (GH), some bacterial systems utilize an alternative strategy with tethered multi-catalytic domain cellulases. The cellulase CelA from the thermophile *Caldicellulosiruptor bescii,* which is one of the most active cellulose degrading enzymes known to date, is one such example. In the saccharification of a common cellulose standard, Avicel, CelA outperforms mixtures of commercially relevant exo- and endoglucanases. The modular architecture of CelA is defined as: GH9-CBM3-CBM3-CBM3-GH48 and the enzyme is extremely thermostable and highly active at elevated temperatures.

From transmission electron microscopy studies of biomass following incubation with CelA, we have discovered morphological features that suggest CelA utilizes a biomass digestion mechanism different from the common surface ablation strategy driven by processivity and we propose that CelA and possibly other multi-functional glycoside hydrolases, act in a novel manner when compared to traditional fungal enzyme systems. We have explored the activity of CelA on a variety of pretreated substrates in order to better understand how the different bulk components of biomass, such as xylan and lignin impact CelA activity and how the effect of those bulk components may differ between traditional fungal enzymes and CelA. We have also examined the impact of cellulose crystallinity on the respective cellulolytic activity of these two cellulase systems.

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