

The Unique Mechanism of the Dominant Multi-Component Cellulase from *Caldicellulosiruptor bescii*

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus*) to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

The cellulase, CelA, from the thermophile, *Caldicellulosiruptor bescii*, is one of the most active cellulose degrading enzymes known to date. In the saccharification of Avicel, a common cellulose standard, CelA outperforms mixtures of benchmark fungal exo- and endoglucanases. Unlike the secretomes of cellulolytic fungi, which typically comprise multiple, single catalytic domain enzymes for biomass degradation, some bacterial systems employ an alternative strategy that utilizes multi-catalytic domain cellulases. Additionally, CelA is extremely thermostable and highly active at elevated temperatures, unlike commercial fungal cellulases. However, the activity of CelA seems to be diminished when acting on biomass, yet the barriers responsible for this loss of activity are not yet clear. Many of the factors negatively affecting digestion of lignocellulosic materials by *C. bescii* enzyme cocktails containing CelA appear to be significantly different from the performance barriers affecting fungal cellulases. Here, we explored the activity and degradation mechanism of CelA on a variety of pretreated substrates to better understand how the different bulk components of biomass, such as xylan and lignin, impact its performance. Notably, we have determined that lignin content, but not cellulose crystallinity, is an impediment to the cellulolytic activity of CelA.

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