204. 'Caldi World': Unraveling the Mystery and Mechanisms of Plant Biomass Deconstruction by the Bacterial *Genus Caldicellulosiruptor*

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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). BESC research in biomass deconstruction and conversion targets CBP by studying model organisms and thermophilic anaerobes to understand novel strategies and enzyme complexes for biomass deconstruction.

Exploration of plant biomass deconstruction mechanisms beyond the fungal and cellulosomal enzyme paradigms has accelerated in recent years due in part to renewed interest in second- generation biofuels. An alternative strategy, consisting of modular, multi-functional carbohydrate active enzymes, is used by the extremely thermophilic bacterial genus *Caldicellulosiruptor* (T_{opt} 70~80°C) to harvest energy from a wide variety of C_5 and C_6 sugars found in plant biomass.

Recently, interest in this genus led to an exploration of biodiversity amongst globally isolated species and determined that the genus comprises a spectrum of weakly to strongly cellulolytic microorganisms. Common to all species is the ability to degrade amorphous cellulose, xylan and pectin as inferred from enzyme inventory and confirmed by growth physiology. Accessory enzymes, not common to all species, enable unique mechanisms by which individual species approach plant biomass deconstruction. Previous comparative proteomic screening implicated many cell associated proteins and enzymes as being integral for plant biomass deconstruction.

Many enzymes are found attached to the cell's S-layer, in addition to flagella and type IV pili being implicated in attachment to biomass. Two unique proteins directly downstream of the type IV pilus operon have been cloned and characterized as novel cellulose binding proteins. Taken together, cell-surface associated and modular, multifunctional enzymes participate in a process by which the cell strips away layers of lignin and polysaccharides, leaving behind biomass that resembles the starting material ('onion peeling effect'). This mechanism was discovered in *C. bescii* and confirmed in other strongly cellulolytic *Caldicellulosiruptor* species. Plant biomass originating from monocots will support growth of *C. bescii*, *C. kronotskyensis* and *C. saccharolyticus* as the only carbon source, and will also support growth on high loading levels (up to 20% w/v). A comparison of fermentation products from *Caldicellulosiruptor* species when grown on crystalline cellulose (Avicel) or untreated switchgrass demonstrates flexibility in their metabolism to handle only C_6 or a combination of C_5 and C_6 sugars. In addition to a better understanding of the enzymatic capacity and cellular metabolism of the genus *Caldicellulosiruptor*, a genetics system was developed for *C. bescii*. A unique restriction- modification

system was identified (CbeI – M. CbeI) and also determined as the barrier for DNA transformation. Transformation of *C. bescii* was enabled by a Caldi-*E. Coli* shuttle vector treated with M. CbeI and has facilitated the deletion of a lactate hydrogenase and also initial steps for metabolic engineering of Caldi strains. Overall, the genus *Caldicellulosiruptor*, by virtue of their diverse physiology and enzymatic capacity make an attractive model system for high temperature plant biomass deconstruction, and with the advances in genetic systems for the genus, can now be considered as promising platforms for biofuels production.

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