

3. Biomass Deconstruction by Members of the 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 (CBP)]. 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.

A major obstacle, and perhaps the most important economic barrier to the effective use of plant biomass for the production of fuels, chemicals and bioproducts, is our current lack of knowledge of how to efficiently and effectively deconstruct plant cell wall polymers for their subsequent use as feedstocks. Plants represent a desired source of renewable energy and hydrocarbons because they fix CO₂, making their use in such processes carbon-neutral. Plant structure, however, is a barrier to deconstruction and this characteristic is often referred to as recalcitrance. Members of the bacterial genus *Caldicellulosiruptor* are the most thermophilic cellulolytic organisms so far described and have the ability to grow on lignocellulosic biomass without conventional pretreatment. Here, we describe two studies using deletions in *Caldicellulosiruptor bescii* to examine biomass hydrolysis.

Different species vary in their ability to degrade cellulose, and the presence of CelA, a bi-functional glycoside hydrolase correlates well with cellulolytic ability in members of this genus. *C. bescii*, which produces CelA and expresses it constitutively, is among the most cellulolytic. In fact, CelA is the most abundant extracellular protein produced in *C. bescii*. In contrast, *C. hydrothermalis*, which does not contain a CelA homolog, or members of the common bacterial cellulases (GH48 or GH9 glycoside hydrolases), is the least cellulolytic of the *Caldicellulosiruptor* species so far described.

The enzyme contains two catalytic units, a Family 9A-CBM3c processive endoglucanase and a Family 48 exoglucanase joined by two Family 3b carbohydrate binding domains. While there are two GH9 and three GH48 glycoside hydrolases in *C. bescii*, CelA is the only protein that combines both enzymatic activities. A deletion of the *celA* gene in *C. bescii* resulted in a dramatic reduction in the microorganism's ability to grow on crystalline cellulose (Avicel) and diminished growth on lignocellulosic biomass. A comparison of the overall endoglucanase and exoglucanase activities of the mutant compared to the wild type suggests that the loss of the endoglucanase activity provided by the GH9 domain is perhaps compensated by other enzymes produced by the cell. In contrast, enzymatic activity on Avicel by the mutant strain resulted in a 15-fold decrease in sugar release compared to the parent and wild type strains. Taken together, these data suggest that the exoglucanase activity of the GH48 domain of CelA plays a major role in biomass degradation within the suite of *C. bescii* biomass degrading enzymes.

The combination of microbial digestion and plant biomass analysis provides an important platform to

identify plant wall structures whose presence reduces the ability of microbes to deconstruct plant walls and to identify enzymes that specifically deconstruct those structures. A deletion of a gene cluster encoding enzymes involved in pectin degradation was constructed in *C. bescii* and the resulting mutant was reduced in its ability to grow on both dicot and grass biomass, but not on soluble sugars. We believe that saccharification of plant biomass can be improved by modifying the structure of pectin. Most efforts targeting improved deconstruction of plant biomass for biofuel production have focused on crystalline cellulose, lignin, and xylan—the major hemicellulose in grass (e.g., switchgrass) walls and in dicot (e.g., Populus wood) secondary walls. Indeed, most models of biomass used in the biofuels field do not list pectin because of its low abundance in grass walls and in dicot secondary walls. Recent work, however, has shown that pectin is synthesized in secondary walls and that some pectin biosynthetic enzymes are amplified in grasses. Plant biomass from three phylogenetically diverse plants, Arabidopsis (an herbaceous dicot), switchgrass (a monocot grass), and Populus (a woody dicot) were used in the analysis. These biomass types have cell walls that are significantly different from each other in both structure and composition. Glycome profiling, using a large and diverse set of plant glycan-directed monoclonal antibodies, of the biomass remaining after growth of the bacterial mutant compared to the wild type revealed differences in the way the mutant utilizes these plants for growth. While pectin is a relatively minor component of the grass and woody dicot substrates, these analyses provide direct evidence that pectin plays an important role in the recalcitrance of all three types of biomass.

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