**The Secretome of *Caldicellulosiruptor bescii*: Biomass Deconstruction without Conventional Pretreatment**

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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.

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. A comparison of the pangenome of this genus suggests that among the genes important for cellulolytic ability are *celA* and a cluster of genes involved in pectin degradation (1). CelA is a bi-functional glycoside hydrolase that contains a Family 9 endoglucanase and a Family 48 exoglucanase joined by three Family 3 carbohydrate binding modules (CBMs), and while there are two Family 9 and three Family 48 glycoside hydrolases in *C. bescii*, CelA is the only protein that combines both activities. Deletion of CelA resulted in a significant decrease in cellulolytic activity (2). Expression of full length CelA in *C. bescii* revealed that extracellular CelA protein is glycosylated whereas intracellular CelA is not (3). The mechanism and role of protein glycosylation in bacteria is poorly understood and the ability to express CelA *in vivo* in *C. bescii* will allow the study of the mechanism of protein glycosylation in this thermophile. Moreover, heterologous expression of an additional thermophilic endoglucanase (E1) from *Acidothermus cellulolyticus*, enhanced the ability of *C. bescii* to deconstruct biomass demonstrating that while extremely effective, the ability of *C. bescii* to deconstruct plant biomass can be improved (4).

The *C. bescii* genome contains five genes predicted to be involved in pectin deconstruction/ utilization and three exist in a cluster with a predicted transcriptional regulator. Expression of this cluster is significantly up-regulated in cells growing on biomass (5). Most biomass models do not list pectin because of its low abundance in grass walls and in dicot secondary walls (6). Recent work has shown that pectin is synthesized in secondary walls (7), that some pectin biosynthetic enzymes are amplified in grasses (8), and that saccharification of plant biomass can be improved by modifying the structure of pectin (9). Deletion of the pectinase gene cluster in *C. bescii* resulted in a mutant reduced in its ability to grow on dicot and grass biomass (10). The phenotype of the *C. bescii* pectinase mutant provides direct genetic evidence that pectin is a significant barrier to

deconstruction of unpretreated plant biomass by *C. bescii* and that pectin plays an important and underappreciated role in biomass recalcitrance.

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*The BioEnergy Science Center is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.*