Methylation by a Unique α-class N4-Cytosine Methyltransfersase is Required for DNA Transformation of *Caldicellulosiruptor bescii* DSM6725: Use for Construction of Mutants That Affect Biomass Utilization

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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 multitalented microbes for 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.

Thermophilic organisms offer special advantages for the conversion of lignocellulosic biomass to biofuels and bioproducts. The use of these complex substrates often requires pretreatment, involving exposure to acid or base at high temperature and the addition of hydrolytic enzymes that partially digest the plant cell walls. Enzymatic pretreatment is expensive and often prohibitive for the production of low value commodity products. Members of the Gram-positive bacterial genus *Caldicellulosiruptor* are anaerobic thermophiles with optimum growth temperatures between 65 °C to 78 °C and are the most thermophilic cellulolytic organisms known. *C. bescii* is capable of using both unpretreated switchgrass and *Populus* for growth. The ability to genetically manipulate these organisms is a prerequisite for engineering them for use in conversion of these complex substrates to fuels and products of interest. Here we report the first example of DNA transformation of a member of this genus, *C. bescii*. We show that restriction of DNA is a major barrier to transformation and that methylation of heterologous DNA with a unique α-class N4-Cytosine methyltransfersase is required for DNA transformation. We have used this genetic system to generate deletions of genes predicted to be involved in biomass utilization and identified a cluster of genes that encode pectinase enzymes that play an important role in biomass utilization.

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