

A Bacterial Pioneer Leaves a Complex Legacy

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Project Goals: The goal of this project, performed in the Microbial Communities group in the Deconstruction Division at JBEI, is to identify enzymes critical for polysaccharide hydrolysis in microbial consortia adapted to grow on biomass substrates and use these new enzymes to improve the conversion of biomass to biofuels

Cellulases have traditionally been identified and characterized from fungal and bacterial isolates. In natural environments, microbial consortia are responsible for lignocellulose deconstruction and may produce enzymes previously unobserved in isolates. To obtain new enzymes, a cellulolytic consortium was established by adapting aerobic compost microbial communities to grow with crystalline cellulose as the sole carbon source at elevated temperature. Soluble cellulases from this consortium provided the base for an enzymatic mixture, called Jtherm, that deconstructed biomass at temperatures up to 80°C. The consortium was scaled to 300 L to enable large-scale production of Jtherm. Time series metagenomic analysis of the consortium grown at 300 L indicated that an uncultivated Paenibacillaceae population was abundant early in the cultivation but became <1% abundant when the culture was harvested. Recovery of the genome of this Paenibacillaceae population, named *Candidatus* “Reconcillibacillus cellulovorans”, from the metagenomic data demonstrated that it contained a putative operon with genes for a unique suite of multi-domain glycoside hydrolases (GH9, GH48, GH6/5 and GH10) and a polysaccharide monooxygenase (AA10). All of these proteins were multi-domain cellulases containing from one to three CBM3 binding modules in addition to the catalytic subunits, a structure similar to glycoside hydrolases from *Caldicellulosiruptor*. Biochemical purification of the Jtherm components demonstrated that the active components were the *C. ‘R. cellulovorans’* GH9, GH6/5 and GH48, which were arranged in protein complexes that fundamentally differed from cellulosomes isolated from anaerobic Firmicutes. Recombinant expression of the three components of the complexes demonstrated that the GH9 and GH6/5 proteins had substantial activity on crystalline cellulose. This work demonstrates that the detailed study of microbial consortia can provide a deep understanding of community dynamics and provide access to novel protein structures for biomass deconstruction.

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