Engineering Improved Cellulases for CBP Microbes

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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.

Several recent studies have shown that certain cellulases in *Clostridium thermocellum* and *C. bescii* are some of the most efficient cellulases for biomass degradation. Being able to understand and improve these cellulases, and ultimately create a more efficient microbe to overcome biomass recalcitrance would be a huge step in improving the CBP process. Here we show how we use basic understanding, rational protein design and engineering to improve cellulases in *C. thermocellum* and *C. bescii*. We demonstrate how computer simulations and design can bring new understanding and help in the enzyme engineering process.

More specifically, new evidence in the function of X1 domains in CbhA from *C. thermocellum* has motivated the creation of a more efficient chimera using domain swapping. This improved chimera exhibits twice the activity of the wild type CbhA. Additionally, new understanding in the mechanisms and strengths of multi-catalytic domain cellulases, such as CelA (*C. bescii*) has led to more efficient minisomes able to convert 55% of cellulose at 60°C. Finally, we report that the energy required for product expulsion in the GH48 from CelA, one of the most active cellulases ever reported, is lower than in other GH48 cellulases. This result could partially explain the superior nature of this cellulase over other GH48-borne cellulases and inspire mutational strategies in other GH48 cellulases.

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