10. Improving Cellulolytic Activity of the C. thermocellum Secretome by Engineering the CipA Scaffoldin

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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.

The extremely high cellulolytic activity of Clostridium thermocellum is based on a cellulase system that is composed of two sub-systems, one being a consortium of free, individual enzymes and the other being composed of large, multi-enzyme complexes (cellulosomes) in which individual enzymes are organized into supramolecular machines for the depolymerization of crystalline cellulose. The most basic step in the organization of cellulosomes is the binding of multiple individual enzymes, through specific cohesin-dockerin interactions, to the primary scaffoldin protein CipA. The enzyme-laden CipA molecules are in turn bound, also through specific modular interactions, to secondary scaffoldins to generate even larger protein complexes, or cellulosomes. Our studies and those of others have demonstrated that CipA plays an essential role in efficient cellulose degradation by C. thermocellum, whereas the secondary scaffoldins are considerably less important. In this study, we present a novel approach to improve C. thermocellum cellulose-degradation performance via modifications of CipA. By characterizing these mutants, we found that the secretome activities of some mutants, both on Avicel and on process-relevant biomass (pretreated corn stover) were greatly enhanced (30 to 80% higher than that of the parent on Avicel and 3 to 25% higher on pretreated corn stover), paving the road for further improvements in the cellulolytic performance of C. thermocellum.

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