

234. Contribution of multifunctional enzymes to biomass hydrolysis

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Project Goals: We are using genomic, biochemical, computational and structural approaches to understand the rate-limiting constraints on enzymatic hydrolysis of biomass prepared by pretreatment methods developed in the GLBRC and the other BRCs. Independent of the pretreatment used, it would be desirable to decrease the complexity of enzyme cocktails required. To address this goal, we are investigating how multi-functional enzymes might be used to simplify the composition of enzyme cocktails. Recent work in collaboration with the Joint Genome Institute (JGI) and the Joint BioEnergy Institute (JBEI) has greatly expanded the number of multi-functional enzymes known and has also given a new method for time-resolved studies of biomass.

Abstract: Higher plants are composed of a complex mixture of cellulose, xylan and mannan in addition to other recalcitrant polymers such as lignin. This complexity provides an effective barrier to deconstruction of plant cell walls. In order to overcome this complex structure, microbes secrete enzyme cocktails containing a wide range of different substrate activities. One potential way to reduce the number of enzymes needed, while retaining deconstruction ability, is by the incorporation of high activity enzymes with broad substrate specificity. The glycoside hydrolase (GH) 5 family, one of the largest GH families, offers a promising starting point for further investigation of the relationships between substrate specificity and hydrolytic activity. Our initial work showed that CelE (Cthe_0797), a GH5 enzyme from *Clostridium thermocellum*, can hydrolyze all three major polysaccharides found in higher plant cell walls. Using this enzyme as an anchor point, we explored phylogenetically related GH5 enzymes using a combination of gene synthesis, automated protein translation, biochemical assays, and structure determination. This work has revealed additional naturally evolved multifunctional GH5 enzymes. The enzymes exhibit a breadth of catalytic properties, thermal stabilities, and pH optima. Structural analysis has suggested several residues that may play a role in multifunctionality. Cocktails including these enzymes are also effective in the deconstruction of pretreated biomass. The unique structural and catalytic properties of CelE and other newly discovered multifunctional enzymes suggest a new approach for targeted improvement of enzyme cocktails for biomass deconstruction.

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Appendix A: Additional Abstracts

- ▶ Breakout Abstracts
- ▶ Plenary Abstracts

BREAKOUT ABSTRACTS
