**Using Glycome Profiling on Plant Biomass for Functional Characterization of Cell Wall Hydrolytic Enzymes**

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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). BESC researchers provide enabling technologies in biomass characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion mechanisms.

Microbes, which are responsible for the degradation of plant biomass in the biosphere, produce wide arrays of glycosylhydrolases in order to mobilize the sugars in biomass. However, the hydrolytic activity and specificity of most of these hydrolases remain unknown. Most assays for delimiting the activity of glycosylhydrolases rely upon isolated polysaccharides as substrates for assays, and thus multiple assays are required to fully characterize newly identified putative hydrolases. We describe the use of intact biomass as a substrate for novel glycosylhydrolases, (provided by the Great Lakes Bioenergy Research Center), followed by antibody-based Glycome Profiling to reveal the activities of hydrolases capable of degrading biomass components. The enzymes examined in this study are: CelEcc_CBM3a (an GH5 family enzyme engineered for multi-functionality), XynY (a potentially processive hemicellulase from the GH10 family) and XynA (a xylanase from the GH11 family). Treatment of native or AFEX$^{TM}$ pretreated switchgrass and corn stover biomass with CelEcc_CBM3a, XynY and XynA showed that, in addition to the removal of xylans from the biomass, xyloglucan epitopes were also depleted, suggesting multi-functionality in the activities of these three enzymes. Thus, the biomass/Glycome Profiling-based assay system shows promise as a rapid, moderate throughput method for characterization of putative enzymes for use in biomass deconstruction.

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