Comparative proteomics unveils functional signatures of cellulose formation (*Populus*) and deconstruction (cellulolytic microorganisms) at a cellular level

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Project Goals: Addressing the roadblock of biomass recalcitrance will require a greater understanding of plant cell walls from synthesis to deconstruction. This understanding would generate models, theories and finally processes that will be used to understand and overcome biomass recalcitrance. This grand challenge calls for an integrated research approach, which is illustrated by the project described below. BESC research is multidisciplinary by design and multi-institutional in composition.

The availability of complete genome sequences for both plant model systems (such as *Populus*) as well as cellulolytic bacteria has provided a means to further unveil the functional intricacies of *in situ* biomass degradation at an unprecedented level. Our current work not only develops and demonstrates advanced "shotgun" mass spectrometry (MS) techniques that dive even deeper into the proteomes of relevant bioenergy-related organisms, but also furthers our understanding of the molecular machinery employed by both plants and microorganisms as they either establish (plant) or degrade (microbe) a variety of simple and complex biomass feedstocks. To accomplish these goals, we have established a robust, high-throughput approach for the comprehensive quantitative analysis of deep LC-MS measurements utilizing both label-free and stable isotope-labeling strategies. In particular, we have examined the enzymatic features of the cellulolytic apparatus both shared between and unique to several different cellulose-degrading bacteria (i.e., *C. thermocellum*, *C. bescii* and *C. obsidiansis)* over the course of fermentative growth on simple (e.g., Avicel, cellobiose) and complex (e.g., *Populus*, switchgrass) feedstocks alike. For these microbial systems, we have characterized cellulolytic machinery for both cell-attached (i.e., cellulosome) and cell-free enzymatic systems, with a particular focus on multi-domain glycosidases, extracellular solute binding proteins, and uncharacterized proteins, all of which remain vitally important to sugar release and utilization from biomass.

More recently, we have demonstrated a robust quantitative methodology utilizing selected reaction monitoring (SRM) on a triple quadrupole MS to pursue absolute quantification of targeted proteins. Employing SRM as a quantification tool enabled the accurate stoichiometric determination of key structural and enzymatic constituents of *C. thermocellum*'s cellulosome. These measurements enhance our understanding of the link between absolute cellulosome abundance and overall enzymatic activity – a result which could help direct future molecular engineering endeavors. This MS technique has several advantages over ELISA-based quantification methods including high-precision, ease of use, high-throughput, as well as the ability to quantify proteins in samples ranging in proteomic complexity (purified cellulosomes to whole-cell lysates).

Though much work thus far has investigated biomass deconstruction, recent efforts in our lab have focused on uncovering the mechanisms of biomass formation. In this regard, we have developed an experimental approach that combines an enhanced cell lysis and proteome solubilization protocol with state-of-the-art LTQ-Velos MS technology to achieve the deepest proteome coverage of *Populus* to date. In response to the enhanced genomic complexity inherent

to eukaryotic species, especially plants, we have optimized our post-data acquisition informatics to resolve ambiguous protein identifications resulting from the substantive genetic redundancy exhibited by *Populus* spp. Clustering redundant proteins prior to mapping peptides to the proteome allowed us to more thoroughly characterize *Populus* organ-specific protein information, focusing on comparing and contrasting the proteomes of leaf (young and old), stem, and root samples. Taken together, these MS-based proteome approaches provide remarkable insight into the fundamental mechanisms of biomass formation by plants as well degradation by cellulolytic microbes as a step to enhance biofuel production.

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