

#### 4. Building the Cell Wall: Insights Into Xylan Synthesis Using Recombinant 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 biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and Populus) to develop varieties that are easier to break down into fermentable sugars. We are using both testing and generating of large numbers of natural and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways.

Xylans are the dominant hemicellulosic polysaccharide found in the plant kingdom, second only to cellulose in abundance, and are present in load-bearing secondary cell walls of dicots and in both primary and secondary cell walls of grasses and cereals. These highly acetylated cell wall polysaccharides are a vital component of the plant cell wall that functions as a molecular scaffold, providing plants with mechanical strength and flexibility. Due to its abundance and complex interactions with cellulose and lignin, xylan has profound effects on the recalcitrance of biomass to saccharification, making it a prime target for genetic manipulation. However, the mechanisms by which plants synthesize xylan are poorly understood. A major impediment to the structural and functional characterization of plant carbohydrate active enzymes (CAZymes) has been the lack of highly purified and active enzymes. We have recently overcome these limitations by carrying out heterologous expression of plant CAZymes in Human Embryonic Kidney cells. This breakthrough technology has allowed us to successfully express and purify several different enzymes involved in hemicellulose biosynthetic pathways. Our ability to consistently obtain high-level expression of functional plant glycosyltransferases is a major milestone for BESC. We combined purified, recombinant CAZymes with high-throughput glycosyl-, methyl- and acetyltransferase assays to investigate their biochemical activities. Using this approach, we have demonstrated that *Arabidopsis thaliana* IRREGULAR XYLEM 10-L has UDP-Xyl:  $\beta$ -(1,4)-xylosyl transferase activity and is a xylan synthase that elongates the xylan backbone. Further, we show that ESKIMO1/TRICOME BIREFRINGENCE 29 (TBL29/ESK1) catalyzes the subsequent addition of O-acetyl groups from acetyl-CoA to the 2-position of xylosyl backbone residues, making it the only plant polysaccharide O-acetyl transferase that has been biochemically characterized to date. These two enzymes can be used in combination to synthesize and O-acetylate xylan in vitro. These advances represent a significant achievement for the field of cell wall polysaccharide biosynthesis, but also provide the plant science community with a new and powerful tool-box to produce recombinant proteins for biochemical analysis

*The BioEnergy Science Center is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.*