192. *In vitro* Synthesis of Xylan Catalyzed by Purified Plant Xylosyl Transferases

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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 multitalented microbes for 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 generation 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.

Secondary cell walls are composed mainly of a cellulose/xylan network impregnated with lignin, resulting in a reinforced structure that is recalcitrant to deconstruction by microbial enzymes. 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. The glucuronoxylan (GX) present in hardwoods including *Populus* and in mature stems of *Arabidopsis* is a homodisperse polymer that has a backbone composed of 1,4-linked β-D-xylosyl (Xyl) residues that are often substituted at *O*-2 with glucuronic acid (GlcA) or 4-*O*-methyl glucuronic acid (MeGlcA) and also contain a distinct acidic reducing-end sequence.

Beginning with the identification of cellulose synthase (CesA) genes from cotton almost two decades ago, the genes that encode enzymes responsible for catalyzing β-1,4 linked backbone formation have been identified for all principal hemicellulosic polysaccharides, with the notable exception of xylan. All of these identified backbone synthases have been cellulose synthase-like (Csl) genes, which are integral membrane proteins from glycosyltransferase family 2, structurally related to CesA. Despite predictions that the β-1,4-xylan synthase will be a member of the Csl family, most of the candidate enzymes are members of other families and none of them have been functionally characterized due to difficulties in heterologous expression of plant GTs. We investigated the function of several GT candidates for the xylan synthase in *Arabidopsis* with our MALDI-TOF MS based assay using heterologously expressed and purified enzymes.
These experiments provide direct biochemical evidence establishing the identity of the *Arabidopsis* gene encoding the glycosyltransferase that catalyzes the transfer of xylosyl units from UDP-xylose to xylan oligosaccharide acceptors in the absence of other proteins *in vitro*, establishing this enzyme as the xylan synthase.

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