Investigating the Role of Polysaccharide Methylation in the Plant Cell Wall

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus)* to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in **biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.**

One of the core goals of the Plant Biomass Formation and Modification Focus Area in BESC is to understand the molecular mechanisms that control biomass recalcitrance. The thick recalcitrant secondary cell walls of *Populus* are principally composed of cellulose, glucuronoxylan (GX), and lignin with lesser amounts of pectic polysaccharides and glycoproteins. Interactions among these components are believed to be responsible for the resistance of plant biomass to enzymatic deconstruction to fermentable sugars. The GX present in hardwoods including *Populus* and in mature stems of the model plant *Arabidopsis thaliana* 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). Recently, we identified a new gene (*GXMT1*) which belongs to a family in *Arabidopsis* and encodes a xylan methyltransferase. Plants carrying a mutation in this gene synthesize xylan in which the degree of GlcA methylation is reduced to ~25% of wild-type levels. Our previous results (Urbanowicz et al., 2012), suggests that *O*-methylation plays a role in the polysaccharide's ability to associate with lignin and or other glycopolymers and thereby impacts biomass recalcitrance. Reducing *O*-methylation by directed breeding or genetic manipulation has considerable potential to affect the recalcitrance of lignocellulosic biomass by modulating the interactions of glucuronoxylan with the other components of the secondary cell wall without any negative effects on plant development or fitness. However, our knowledge of the enzymes involved in polysaccharide methylation and the role of these non-glycosyl substituents in plant cell wall untrastructure remains enigmatic.

As part of an expanded investigation into the identification and characterization of polysaccharide methyltransferase gene families, we isolated homozygous T-DNA insertion *Arabidopsis* mutant lines for several candidates that contain methyltransferase motifs that are highly expressed during both primary and secondary cell wall formation. Cell wall material from mutant plants was fractionated to enrich for different polysaccharides including pectins, arabinogalactans (AGPs) and glucuronoxylan. Fractions enriched in glucuronoxylan were analyzed from mutants in genes expressed during secondary cell wall formation to quantify structural differences with a focus on xylan *O*-methylation. To identify structural changes in the non-hemicellulosic cell wall glycopolymers we developed a robust the fractionation procedure that yielded high amounts of pure, intact soluble polymers. The neutral and acidic sugar content of the isolated glycopolymers was analyzed using an optimized HPAEC-PAD method, which discriminates methylated verses unmethylated saccharides. To confirm that the changes in methylation were specific, NMR structural characterization was performed. Our discovery and characterization of new methyltransferases extends the portfolio of structural targets that can be modified to increase the economic value of lignocellulosic biomass by modulating biopolymer interactions in the cell walls of biomass crops such as poplar and switchgrass.

References

Urbanowicz, Breeanna R., et al., "4-O-methylation of glucuronic acid in Arabidopsis glucuronoxylan is catalyzed by a domain of unknown function family 579 protein." *Proceedings of the National Academy of Sciences* 109.35 (2012): 14253-14258.

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