Cell Wall Characteristics and Ethanol Fermentation Studies of PvMYB4-Over-Expressing Switchgrass Plants

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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). 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.

Overcoming cell-wall recalcitrance for cellulosic ethanol production is the identifying focus of the DOE BioEnergy Science Center. Our previous studies showed that lignin content and wall-bound phenolic ratios had significant impacts on cell wall saccharification efficiency in switchgrass biomass. The aromatic monolignol monomers and wall-bound phenolics share many common biosynthetic reactions, which are genetically controlled by sets of positive and negative transcriptional regulators. Over-expression of a newly characterized transcriptional repressor, *PvMYB4*, dramatically increases, by around 300%, the sugar release efficiency from cell wall residues. To gain more detailed understanding of cell wall recalcitrance and potential impacts on cellulosic ethanol production of utilizing these transgenic lines, we conducted a series of studies utilizing the BESC feedstock characterization pipeline. The results show that, although PvMYB4 over-expressing lines give about three fold enhanced ethanol yields without pretreatment, the total sugar levels of the biomass are the same. Generation of potential inhibitors of fermentation by the thermophile, *C. thermocellum*, during consolidated bioprocessing (CBP) was evaluated under conditions of hot-water pretreatment. Solid-state NMR was used to investigate the changes in the characteristics of the plant cell walls between the transgenic and control plants. Gel permeation chromatography of isolated ball-milled lignin was used to determine the average lignin molecular weight. Together, the data indicate that the aromatic acids embedded in the cell-wall, the lignin content and the lignin polymer size all likely have significant impacts on saccharification efficiency and cellulosic ethanol production. This case study of PvMYB4-overexpressing switchgrass lines demonstrates one successful strategy to overcoming cell wall recalcitrance for cellulosic ethanol production.

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