Consolidated Bioprocessing Conversion of Genetically Modified Switchgrass

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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. BESC research in biomass deconstruction and conversion targets CBP by studying model organisms and thermophilic anaerobes to understand novel strategies. This grand challenge calls for an integrated research approach, which is illustrated by the project described below.

Consolidated bioprocessing (CBP) conversion comprises biomass fermentative microorganisms that produce some or all of the needed biomass hydrolytic enzymes, mitigating the cost of added enzymes. It has been shown previously that down regulation of the caffeic acid O-methyltransferase (COMT) gene in switchgrass (*Panicum virgatum*) reduced lignin content, reduced S/G ratio, increased sugar release, and improved the yeast-based SSF conversion of the modified switchgrass line compared to the wild-type switchgrass (Fu, et al. 2011). As expected, after either dilute acid or hot water pretreatment of switchgrass provided by the Noble Foundation, yeast-based SSF conversion showed the COMT transgenic switchgrass yielded at least 25% more ethanol than the wild-type regardless of the pretreatment process. To follow-up this work, we examined the fermentation of both wild-type and COMT transgenic switchgrass lines using anaerobic bacteria including *Thermoanaerobacterium saccharolyticum* and very active consolidated bioprocessing (CBP) microorganisms *Clostridium thermocellum*, *Caldicellulosiruptor obsidiansis*, and *Caldicellulosiruptor bescii*. Comparison of the wild-type switchgrass to a modified COMT plant line after either dilute acid or hot water pretreatment showed that the transgenic switchgrass yielded over 20% greater total fermentation products on a (g/g) substrate basis when fermented by *Thermoanaerobacterium saccharolyticum* after addition of enzymes ala the SSF mode. Biomass fermentation by *C. thermocellum*, *C. obsidiansis*, or *C. bescii* requires no added industrial enzymes but switchgrass fermentation showed differential inhibition with wild-type and COMT transgenic switchgrass. Specifically, wild-type and COMT transgenic switchgrass were pretreated with dilute acid (0.5% H_2SO_4, 180°C, 7.5 min.) followed by water washing of the solids. Bioconversion of the solids using *C. thermocellum* showed partial inhibition of fermentation with efficient hydrolysis liberating free sugars, but only partial conversion of these sugars to fermentation products. The COMT transgenic feedstock showing greater inhibition compared to the wild-type switchgrass. However, full fermentation capabilities for *C. thermocellum* were restored after an extra hot water extraction of soluble components from the wild-type and transgenic switchgrass and the COMT line produced about 25% more total products than from the wild-type switchgrass, as seen with yeast and *Thermoanaerobacterium saccharolyticum* SSF conversion. However, using the same biomass sources processed identically with extensive water extraction, both *Caldicellulosiruptor* species failed to ferment the transgenic switchgrass (ca. 7-10% of expected) while successfully fermenting the wild-type switchgrass. This suggests *Caldicellulosiruptor* can be used as an indicator for fermentation inhibition with different biomass sources.

References: Fu et al., PNAS 108:3803-3808 (2011)

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