189. Fermentation Performance of Field-Grown Natural Variant and Transgenic Feedstocks

Kelsey L. Yee,[1,3*] (yeekl@ornl.gov) Miguel Rodriguez Jr.,[1,3] Olivia A Thompson,[1,3] Wellington Muchero,[1,3] Jin-Gui Chen,[1,3] Lee E. Gunter,[1,3] Sara S. Jawdy,[1,3] Charleson R. Poovaiah,[3,5] Hui Shen,[3,6] Mitra Mazarei,[3,5] Holly L. Baxter,[3,5] Nancy L. Engle,[1,3] Angela Ziebell,[2,3] Robert W. Sykes,[2,3] Erica Gjersing,[2,3] Chunxiang Fu,[3,4] Zeng-Yu Wang,[3,4] Mark F. Davis,[2,3] Timothy J. Tschaplinski,[1,3] C. Neal Stewart Jr.,[3,5] Richard A. Dixon,[3,6] Gerald A. Tuskan,[1,3] Jonathan R. Mielenz,[1,3] Brian H. Davison[1,3] and **Paul Gilna[3] (BESC PI)**

[1]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee; [2]National Bioenergy Center, National Renewable Energy Laboratory, Golden, Colorado; [3]BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee; [4]The Samuel Roberts Noble Foundation, Ardmore, Oklahoma; [5]University of Tennessee, Department of Plant Sciences, Knoxville; [6]University of North Texas, Department of Biological Sciences, Denton

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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 researchers provide enabling technologies in characterization, 'omics, modeling and data management in order to 1) understand chemical and structural changes within biomass and 2) to provide insights into biomass formation and conversion.

Populus trichocarpa and switchgrass are dedicated bioenergy feedstocks and their inherent recalcitrance to bioconversion is one of the major economic hurdles for the production of biofuels. Implications of improvements in these feedstocks through use of natural variant and transgenic selection will be discussed with the goal of moving towards an improved phenotype which maintains normal growth characteristics and has increased fermentation yield. Microbial bioconversion assays of the feedstocks (unpretreated and pretreated) were performed with traditional enzymatic hydrolysis and yeast-based fermentation platform: simultaneous saccharification and fermentation (SSF) or separate hydrolysis and fermentation (SHF). In addition, the top ethanol yielding biomass lines from yeast-based fermentation were subjected to a consolidated bioprocessing platform using *Clostridium thermocellum*, a thermophilic bacterial species that produces its own hydrolytic enzymes.

We examined 21 *Populus* sp. variants from two environmentally different common garden field sites. These 21 variants have a mutation that affects the secondary carbon pathway metabolism and leads to reduced lignin content, as low as approximately 16-17% in some variants. In addition, we evaluated field-grown transgenic switchgrass lines with reduced lignin content due to genetic modifications in the lignin biosynthetic pathway. The results demonstrated superior conversion yields for the transgenic switchgrass and the *Populus* natural variants compared to their controls for both yeast-based and *C. thermocellum* fermentations. We concluded that transgenic switchgrass and *Populus* natural variants that have reduced recalcitrance are a valuable resource for producing economical biofuels. We also determined that when characterizing new biomass sources, *in vitro* assays, such as sugar release, should be supplemented with *in vivo* fermentation tests, which we have shown to detect inhibitory compounds.

Finally, we have continued our effort in fermenting the improved feedstock lines with genetically engineered microbes. The engineered and evolved *C. thermocellum* strain (M5170) was found to respond to the apparent reduced recalcitrance of the COMT transgenic switchgrass with no substrate inhibition, producing more ethanol on the transgenic feedstock than the wild-type substrate. Since ethanol was the main fermentation metabolite produced by an engineered and evolved *C. thermocellum* strain, its fermentation ethanol yield on a transgenic switchgrass substrate (g/g) is the highest produced thus far.

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