

Aboveground Effects of Beneficial Microbe Co-Culture with *Populus* Cell Wall Chemistry Variants

Udaya C. Kalluri* (kalluriudayc@ornl.gov)¹, Anna Matthiadis¹, Mindy Clark¹, Sara Jawdy¹, Lee Gunter¹, Wellington Muchero¹, Dale Pelletier², Jessy Labbe¹, Timothy Tschaplinski¹, and Gerald A. Tuskan¹

¹Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, TN; ²Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

<https://cbi.ornl.gov>

Project Goals: The Center for Bioenergy Innovation (CBI) vision is to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain. CBI will address strategic barriers to the current bioeconomy in the areas of: 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Beneficial microbes associated with bioenergy feedstock crops are the subject of immense recent interest from research as well as industrial sectors for their potential in improving bioenergy crop productivity and robustness. The goal of present research is to understand the effect of *Populus* cell wall chemistry variation on interactions with beneficial microbes. Previous studies have shown that modifications of lignin and cellulose content can have quantifiable effects on associated microbiome in *Populus* (Beckers et al. 2016; Kalluri et al., 2016; Veach et al. 2018). In the present study, we tested two hypotheses on beneficial plant-microbe interactions: first, plant cell wall phenotype (e.g. high/low lignin) alone is a predictor of functional interactions with microbes, and second, microbes with a known root proliferative effect have a positive impact on aboveground biomass production.

Towards this end, effects of single and mixed microbe cultures on plant growth, stem and root biomass and chemistry were studied in a 15-week long greenhouse experiment. The study included sixteen distinct genotypes of *Populus trichocarpa* (representing extreme % lignin and % C6 sugar phenotypic variants) and bacterial and fungal isolates including *Pantoea*, *Variovorax*, *Paraburkholderia*, *Pseudomonas*, *Mortierella*, and *Laccaria*. These microbes have previously been shown to have root proliferative effects on *Populus* in short-term co-culture experiments (Labbe et al. 2014; Timm et al. 2016; Bible et al. 2016). Leaf, stem and root samples were harvested from six biological replicates for dry biomass, cell wall composition, metabolome, microbiome and transcriptome analyses. Our analyses of aboveground properties, including changes in plant height, stem diameter and leaf chlorophyll changes in plants, with or

without co-culture, show that there was no uniform pattern of response across a given phenotype class; rather a significant genotype-specific effect was observed. While a significant gain in stem biomass was observed as a result of co-culture with *Variovorax*, *Paraburkholderia*, *Mortierella*, and *Laccaria*, a significant overall negative impact on above-ground growth with *Pantoea* was observed across most *Populus* genotypes. Contrary to a theoretical expectation that co-culture with root-growth promoting microbes can have a beneficial aboveground growth effect, several genotypes displayed contrasting stem growth responses to the same microbial treatments. Ongoing analyses are expected to shed light onto the basis of the strong genotype-specific response, and clarify the interconnections among plant genotype, chemistry, above- and belowground- biomass responses to microbes. These insights and approaches will be useful for identifying genetic underpinnings of favorable plant responses, identifying microbes with broadly favorable aboveground effects and for informed design of non-transgenic approaches to maximizing bioenergy crop productivity.

References

1. Beckers et al. Proc Natl Acad Sci U S A. 2016. doi: 10.1073/pnas.1523264113.
2. Kalluri et al. Frontiers in Plant Sciences. 2016. doi: 10.3389/fpls.2016.01455.
3. Veach AM et al. Plant and Soil. 2018. 429: 349–361.
4. Timm et al. 2016. Frontiers in Plant Sciences. doi.org/10.3389/fpls.2016.00497
5. Bible et al. 2016. Frontiers in Microbiology 2016: doi:10.3389/fmicb.2016.00491.
6. Labbe et al. 2014. Frontiers in Plant Sciences, Plant-Microbes interactions 5(579):1-10.

The Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.