Optimizing Plant-Microbial Systems for Energy – Mapping Feedstock Quality Genes in *Brachypodium distachyon*

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Project Goals: (1) Quantify relevant genetic diversity for feedstock quality and plant by microbial strain interaction effects. We will phenotype a large germplasm collection of the model grass species *Brachypodium distachyon* to determine the overall genetic diversity for the rate of ethanol production using a range of *Clostridium phytofermentans* strains. (2) Utilize advanced quantitative trait loci (QTL) mapping strategies to identify genetic loci that control biofuel feedstock quality. Genetic linkage mapping will be carried out in immortalized recombinant inbred lines to provide awareness of the number of QTL that influence the traits, and their degree of influence. (3) Identify and evaluate candidate genes for improved energy systems using a multi-tiered approach to tag several QTL. Ultimately, we are interested in identifying the genes that have an effect on plant feedstock quality.

Creation of a sustainable biofuels industry depends on new technologies that are able to release the energy stored in cellulose fibers at a cost that enables widespread use of biofuels as a competitive domestic alternative to fossil fuels. Currently, use of biomass is hampered by the economic costs associated with thermochemical treatment of biomass, enzyme production, and biomass saccharification. This limits not only the use of cellulosic ethanol, but also the advancement of third generation biofuels. The purpose of our work is to develop plant-microbe systems that will reduce the expense of these steps. This will be accomplished in part by elucidating the genetic mechanisms underlying the attributes of plants that lead to facile decomposition with an eye on the interactions between specific feedstock genotypes and microbial systems. *Clostridium phytofermentans* can directly produce ethanol from plant biomass. Using a high throughput format, we quantify ethanol of inoculated biomass as an estimate of plant feedstock quality for our genetic studies [1, 2]. To better understand genetic diversity for feedstock quality, we will concentrate effort on a genetically diverse panel of accessions of the model species *B. distachyon*. To advance from variance components to genetic mechanisms, we will take advantage of high throughput genotyping and the remarkable genome sequence resources available today to map QTLs. We will begin with the most developed populations and expand to explore others whose parents demonstrate not only striking phenotypic divergence but also possess differential interactions with the microbial strains. This will help bring the genetic landscape into focus, further elucidating the number of genes and their relative influence on plant feedstock-microbe interactions. A key advantage of *B. distachyon* as a model system is the relative ease of creating stable transgenic plants. With that, we will make a series of gain- and loss-of-function mutants to test the function of candidate genes derived from mapping experiments. The three project goals are designed to determine the plausibility of specific positive interactions between plant and microbial genotypes. Pairwise comparisons will

be made, varying both plant and microbial genotypes. Similar to adapting crop varieties to different environments, these experiments will link the need for specific feedstock properties to biomass conversion processes. Leveraging the characterization of cellulose-degrading microbes and discovery of plant traits and loci associated with microbial digestibility, we seek to identify the components of a successful plant-microbe system.

References

1. Lee SJ, Warnick TA, Leschine SB, Hazen SP (*In press*) A high-throughput biological conversion assay for determining lignocellulosic quality. In J Normanly, ed, Methods in Molecular Biology: High Throughput Phenotyping in Plants. Springer.
2. Lee SJ, Warnick TA, Pattathil S, Alvelo-Maurosa JG, Serapiglia MJ, McCormick H, Brown V, Young NF, Schnell DJ, Smart LB, Hahn MG, Pedersen JF, Leschine SB, Hazen SP (*In review*) Biological conversion assay using *Clostridium phytofermentans* to estimate plant feedstock quality.

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