Development of Consortia of Beneficial Microbes for Switchgrass and Consequences of Their Deployment on Native Soil Microbiome

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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.

The family *Serendipitaceae* represents a diverse group of fungi in the Basidiomycota that encompasses endophytes and lineages that repeatedly evolved ericoid, orchid and ectomycorrhizal abilities. *Serendipitaceae* species provide excellent models for root endophytism, given the availability of sequenced genomes, genetic tractability, and a broad host plant range. Previous research performed in our lab with an Australian strain of *Serendipitaceae* named *Serendipita vermifera* indicates its plant growth promoting abilities in switchgrass in normal or stressed conditions. Considering their proven beneficial impact on plant growth and their ubiquity, we describe *Serendipitaceae* as an effective microbial tool for enhancing switchgrass productivity and stress tolerance. The agronomic utility of these fungi has been hampered by the paucity of strains available, the large majority isolated from Australian orchids. We have addressed this constraint by isolating the first North American strain of *Serendipita*, named *Serendipita vermifera* subsp. *bescii* NFPB0129, from switchgrass root in Ardmore, Oklahoma.

In parallel, more than 500 bacterial strains that were previously isolated from switchgrass roots, from the Tall Grass Prairie Preserve, northern Oklahoma have been screened for plant growth promoting traits such as phosphate solubilizing activity, ACC deaminase activity and nitrogen fixation ability. We found 31 strains with phosphate-solubilizing activity, 39 strains with ACC deaminase activity and 42 strains that harbor the *NifH* gene. These strains were subsequently evaluated for their switchgrass growth promoting ability. The top three performers across these three different categories are selected to make a bacterial consortium. The consortia itself as well as individual members of this consortia were evaluated for switchgrass growth promotion under

greenhouse conditions. Ultimately, we would pair this bacterial consortium with *Serendipita bescii*, the unique endomycorrhiza isolated from switchgrass and evaluate their growth promoting potential in two different field locations in Ardmore, Oklahoma.

Deployment of microbes with known plant growth promoting traits such as this has played a major role in improving the productivity of agronomically important crops for many decades. Around the world, experiments have been designed and conducted in laboratory set up, green house and field conditions to optimize and increase the efficiency of microbes or consortia of microbes to improve productivity of agronomically important crops. However little or no effort has been taken to measure impact of these introduced microbes on native soil or associated rhizosphere microbiome. We are now beginning to acknowledge the critical role played by the soil microbiome in shaping the plant's overall physiology and development. Hence, it is critical to put forth similar emphasis to understand the interactions between introduced microbes with the rhizosphere microbiome. Recent studies have suggested that such microbial cross talk are regulated, available soil nutrients, and defense hormones, among others. Therefore, care must be taken to ensure that synthetic communities are not so aggressive that they invade local ecosystems and negatively affect soil health, and eventually compromise plant productivity.

From this perspective, in our field trial we are going to determine not only the impact of bacterial endophyte and *Serendipita bescii* inoculation on switchgrass growth performance, but also its impact on native soil microbiome by 16rRNA and ITS2 amplicon metagenomics analysis to estimate diversity and relative proportion of bacterial and fungal communities. This will enable us to evaluate how these microbial inoculants interact with the native microbiome, and subsequently establish, compete, and function in agricultural soils for improving plant productivity.

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