

Signaling Between Switchgrass and Fungal Endosymbionts in the Genus *Serendipita*

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus*) to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

Serendipitales (previously *Sebacinales*) are root-associated symbiotic fungi with growth promoting abilities. This association is termed mycorrhiza (Gk. mykós, “fungus,” and riza, “roots”). *Serendipita vermifera* has been shown to be tremendously beneficial in enhancing biomass yield and drought tolerance in switchgrass, an important bioenergy crop. Considering their proven beneficial impact on plant growth and their apparent ubiquity, *Serendipitales* fungi should be considered as a previously hidden, but amenable and effective microbial tool for enhancing plant productivity and stress tolerance. Unfortunately, the agronomic utility of these fungi are currently hampered by the paucity of strains available, the large majority being isolated from Australian orchids. Of particular relevance to this study, our group has addressed this constraint by isolating the first North American strain of *Serendipita* named as *Serendipita vermifera* ssp. *bescii* from Oklahoma that is presumably better adapted to the specific agro-climatic conditions of the Southern Great Plains region of the central United States (Ray and Craven, U.S. Patent pending).

Development of mycorrhizal symbiosis involves the differentiation of both symbionts to create novel symbiotic interfaces within the root cells. Further, recent evidence indicates that the plant and mycorrhizal fungi perceive each other prior to their physical interaction. While this perception is real, underlying factors facilitating this signal perception are currently unknown. The aim of this project was to explore the signals used by the symbiotic partners for the development of the symbiosis. The overarching goal is to design crop-fungus combinations for optimal and sustainable performance by identifying these key signaling components. To accomplish this, we investigated

the transcriptome profile of the symbiotic interface – i.e., switchgrass roots at different stages of fungal colonization by the use of RNA-Seq technology.

Panicum virgatum Cultivar Alamo (AP13) seedlings were colonized *in vitro* with two different strains of *Serendipitaceae* namely *Serendipita vermifera* strain MAFF305830 (Orchid, Australia, 1988) and *Serendipita vermifera* ssp. *bescii* (Switchgrass, USA, 2014). Plants were harvested at three different stages of colonization namely (a) pre-colonization, (b) early stage colonization (c) late stage colonization; RNA was extracted from roots by standard protocol. Stranded RNA-Seq library(s) were created and quantified by qPCR. Sequencing was performed using Illumina HiSeq 2500. Raw fastq file reads were filtered and trimmed using the JGI QC pipeline. Following trimming, reads under the length threshold were removed. Filtered reads from each library were aligned to the reference genome using HISAT version 0.1.4-beta. DESeq2 (version 1.8.1) was subsequently used to determine which genes were differentially expressed between pairs of conditions at adjusted $p < 0.05$.

We report that switchgrass seems to perceive fungal symbiont prior to physical contact. Following that, the plant defense system reorganizes to accommodate the fungal symbiont. Consequently, the nutrient transport machinery is adjusted for the fungal symbiont. Two fungal strains differentially induced plant gene expression, demonstrating unique responsiveness of plant genotype to these fungal symbionts. Understanding such responsiveness will facilitate to design crop-fungus combinations for optimal sustainable agricultural practices.

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