The Filamentous Fungus *Trichoderma atroviride* as a Model System for Understanding Fungal Genetics, the Plant-Fungal Symbiosis, and Interactions With Diverse Bacteria

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Project Goals: Understanding the interactions, localization, and dynamics of grass rhizosphere communities at the molecular level (genes, proteins, metabolites) to predict responses to perturbations and understand the persistence and fate of engineered genes and microbes for secure biosystems design. To do this, advanced fabricated ecosystems are used in combination with gene editing technologies such as CRISPR-Cas and bacterial virus (phage)-based approaches for interrogating gene and microbial functions *in situ*—addressing key challenges highlighted in recent DOE reports. This work is integrated with the development of predictive computational models that are iteratively refined through simulations and experimentation to gain critical insights into the functions of engineered genes and interactions of microbes within soil microbiomes as well as the biology and ecology of uncultivated microbes. Together, these efforts lay a critical foundation for developing secure biosystems design strategies, harnessing beneficial microbiomes to support sustainable bioenergy, and improving our understanding of nutrient cycling in the rhizosphere.

The plant rhizosphere is ecologically important and houses diverse microbes including Archaea, bacteria, and fungi. Filamentous fungi in the genus *Trichoderma* are ubiquitous in soil, and have well characterized mycoparasitic, biocontrol, and plant growth promoting effects. However, much remains unexplored regarding the gene function of different *Trichoderma* species, their association with plants, and their interactions with other rhizosphere bacteria and fungi. Here we take three separate but complementary approaches to understand *Trichoderma* and its ecological role in the rhizosphere.

Traditional methods of assigning gene function are tedious and slow. To expedite this process, we are leveraging Random Barcode Transposon-site Sequencing (RB-TnSeq) technology to identify gene function in *T. atroviride* IMI in a massively parallel fashion. We currently have a ~140 million uniquely bar-coded plasmid library in *Agrobacterium tumefaci*ens with selection for transformation into *T. atroviride.* We anticipate that we will have an insertional *T. atroviride* library in 2021 for utilization in fitness experiments with different stressors, carbon and nitrogen sources, as well as interactions with other microbes and plants. Our ultimate goal is to develop RB-TnSeq libraries for different species of *Trichoderma* and other rhizosphere-associated fungi to investigate the function of genes within these organisms in shaping rhizosphere communities and plant interactions.

We are also investigating how *Trichoderma* associates with the model temperate grass *Brachypodium distachyon*, in the EcoFAB. The EcoFAB, or fabricated ecosystem, is an easily

constructed chamber that allows for overt control of the microbial community. In addition, we will use a variant of the EcoFAB specifically designed to separate plant roots from a given nutrient source, the so called MycoFAB, which enables the exploration of how fungi access nutrients to trade with their plant hosts and microbial neighbors. By preventing roots from accessing essential nutrient sources, such as insoluble phosphates, plants can be reliably inoculated with fungi such as *Trichoderma*, or even arbuscular mycorrhizal fungi, which are known to access these nutrients. For EcoFAB experiments, seeds of *B. distachyon* are germinated on filter paper for approximately 4 days, and the roots are subsequently dipped in a suspension of fungal conidia, and transferred into the EcoFAB, which is housed in a Magenta box to retain axenic conditions. Our preliminary results indicate that substrate strongly influences the directionality of the association: *T. atroviride* had the most detrimental effect on *B. distachyon* when grown in a hydroponic system, but when grown in sand, the fungus trended toward having a positive impact on the plant. Next steps include exploring how seedling age, watering regime, and nutrient availability shift the plant-fungal symbiosis. In addition, we will evaluate interactions with *B. distachyon* and our synthetic bacterial community (syncom) with wild type and available *T. atroviride* mutants predicted to disrupt plant-fungal interactions.

Lastly, we are exploring how TnSeq can be used to study microbial species interactions. Previous studies have demonstrated that *Trichoderma* species can exert strong antagonistic effects on a diverse range of microbes. Preliminary data using spent media experiments from *Trichoderma* growth and subsequent exposure to bacterial TnSeq libraries showed both a decrease and enrichment for specific bacterial metabolic pathways. We will compare the effect of *T. atroviride* IMI and selected *T. atroviride* mutants on rhizosphere plant growth promoting bacteria (PGPB), plant pathogens, and non-plant associated bacteria, to determine whether interactions are broadly inhibitory or more specifically inhibitory against a particular species.