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 (Harman 2007). In this work we carry out the mutagenesis of all the genes of the *Trichoderma atroviride* genome to characterize their function under different growth conditions and in interactions with bacteria and the rhizosphere. To do this, we used t-DNA and *Agrobacterium tumefaciens* to insert barcodes throughout the fungal genome (Coradetti et al., 2018).

The preliminary t-DNA-seq in *T. atroviride* showed that more than 75% of the reads obtained from the genome have inserted barcodes, and we did not observe bias due to %GC content or an enrichment of insertions in any contigs. The barcode analyses showed that 7,115 of the 11,816 predicted genes in the *T. atroviride* genome have at least one insertion. We then performed a Bar-seq experiment under nutrient deficiency conditions to assess fitness of our insertional library of mutants. We observed that under glucose deficiency, strains with mutations in 67 genes showed lower fitness, while mutants in 36 genes have a positive fitness under these conditions.

Under nitrogen starvation, mutants in 53 genes had a negative effect while 36 mutations had a positive effect on fitness. Under phosphate deficiency, only mutants in 13 genes had a significant change in fitness. These results pave the way to deciphering genes and pathways important for nutritional signaling and utilization in *T. atroviride*, as well as tools to define processes important for interactions with other organisms in rhizosphere communities. .

References

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