Plant-Microbe Interfaces: Experimental characterization of protein movement from plants to ectomycorrhizal fungus

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Project Goals: The goal of the PMI SFA is to characterize and interpret the physical, molecular, and chemical interfaces between plants and microbes and determine their functional roles in biological and environmental systems. Populus and its associated microbial community serve as the experimental system for understanding the dynamic exchange of energy, information, and materials across this interface and its expression as functional properties at diverse spatial and temporal scales. To achieve this goal, we focus on 1) defining the bidirectional progression of molecular and cellular events involved in selecting and maintaining specific, mutualistic Populus-microbe interfaces, 2) defining the chemical environment and molecular signals that influence community structure and function, and 3) understanding the dynamic relationship and extrinsic stressors that shape microbiome composition and affect host performance.

Beneficial interactions between plants and fungi in the below-ground world are important for improving ecosystem stability and crop resilience. The establishment of plant-fungal associations in the rhizosphere requires complex molecular cross-talk between symbiotic partners (Martin et al., 2017). Mycorrhiza-induced small secreted protein MiSSP7 can move from the mutualistic fungus Laccaria bicolor into the roots of Populus to facilitate the establishment of symbiosis with host trees (Plett et al., 2014; Plett et al., 2011). Yet, to our knowledge, there is no prior evidence showing that proteins can move from plant roots into the hyphae of their fungal partners. Recently, we predicted that more than 400 P. trichocarpa small secreted proteins (PtSSPs) could be responsive to symbiosis with L. bicolor (Plett et al., 2017). We hypothesized that some of these PtSSPs can move from plant roots into L. bicolor hyphae. To test this hypothesis, we selected a subset of 14 PtSSPs (i.e., PtSSP1, PtSSP2, …, PtSSP14), based on computational analysis of DNA-binding capability and signal peptides for secretion, for experimental characterization of protein movement from plant roots into fungal hyphae. Transgenic Arabidopsis thaliana and poplar plants were created to overexpress these PtSSPs fused to green fluorescent protein (GFP). The transgenic plants were co-cultured with L. bicolor to assess the movement of the PtSSP-GFP fusion proteins. So far, we have found that PtSSP1, PtSSP5 and PtSSP8 could move from roots of transgenic Arabidopsis and/or poplar into the hyphae of L. bicolor. We are currently establishing a microfluidic platform to monitor the movement of PtSSP-GFP fusion proteins from
transgenic yeast cells into *L. bicolor* hyphae, and using computational approaches to predict the protein domains and 3D structures of these PtSSPs.

In summary, the results from our experiments support our hypothesis that PtSSPs can move from plant roots into the hyphae of *L. bicolor*. In the near future, we will study the impact of these mobile poplar small proteins on fungal gene expression and identify the potential fungal proteins interacting with the poplar small proteins.

**References**


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