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

Xiaohan Yang[1]* (yangx@ornl.gov), Hua Cheng,[1] Linling Li,[1] Haiwei Lu,[1] Xiaoli Hu,[1] Md Mahmudul Hassan,[1] Zhenzhen Qiao,[1] Guoliang Yuan,[1] Jin Zhang,[1] Degao Liu,[1] Jennifer Morrell-Falvey,[1] Jessy Labbé,[1] Wei Shen,[2] Shi-You Ding,[2] Muneeba Khalid,[1] Scott T. Retterer,[1,3] Julie Mitchell,[1] Jin-Gui Chen,[1] Wellington Muchero,[1] Gerald A. Tuskan,[1] and **Mitchel J. Doktycz**[1]

[1]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN; [2]Department of Plant Biology, Michigan State University, East Lansing, MI; [3]Center for Nanophase Materials Sciences, Oak Ridge National Lab, Oak Ridge, TN

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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

Martin, F.M., Uroz, S. and Barker, D.G. (2017) Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. *Science* **356**, eaad4501.

Plett, J.M., Daguerre, Y., Wittulsky, S., Vayssières, A., Deveau, A., Melton, S.J., Kohler, A., Morrell-Falvey, J.L., Brun, A. and Veneault-Fourrey, C. (2014) Effector MiSSP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZ6 protein and represses jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences* **111**, 8299-8304.

Plett, J.M., Kemppainen, M., Kale, S.D., Kohler, A., Legué, V., Brun, A., Tyler, B.M., Pardo, A.G. and Martin, F. (2011) A secreted effector protein of Laccaria bicolor is required for symbiosis development. *Current Biology* **21**, 1197-1203.

Plett, J.M., Yin, H., Mewalal, R., Hu, R., Li, T., Ranjan, P., Jawdy, S., De Paoli, H.C., Butler, G., Burch-Smith, T.M., Guo, H.-B., Ju Chen, C., Kohler, A., Anderson, I.C., Labbé, J.L., Martin, F., Tuskan, G.A. and Yang, X. (2017) *Populus trichocarpa* encodes small, effector-like secreted proteins that are highly induced during mutualistic symbiosis. *Scientific Reports* **7**, 382.

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