Plant-Microbe Interfaces: Identification and characterization of Proteolytic Cleavage Product (PCP) peptides that function as key signaling molecules for Plant-Microbe Interactions

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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.

The ectomycorrhizal fungus, *Laccaria bicolor,* forms mutualistic association with roots of *Populus* species, in which *L. bicolor* provides *Populus* access to mineral nutrients from the soil, such as complex organic nitrogen, in exchange for fixed carbon derived from photosynthesis. This mutualistic interaction is enabled by the extensive crosstalk between fungi and the plant host. Proteolytic cleavage products (PCPs) are emerging as key signaling molecules that mediate cell-to-cell crosstalk. PCPs are post-translationally processed products of proteins that are involved in various biological processes that occur between and within plants, fungi, and bacteria. The discovery and characterization of PCPs have been challenging because these signaling molecules usually function at extremely low concentrations and undergo extensive post-translational processing that are not well understood. Moreover, PCPs originating from small open reading frames are often overlooked in gene predication/annotation tools due to their small size, and thus are missing in the reference database. In this study, we utilized a molecular weight based selective enrichment strategy, combined with high-performance tandem mass spectrometry and *de novo*-assisted database searching to improve the identification of PCPs. We benchmarked the qualitative and quantitative performance of the purposed approach using reference synthetic peptides.

Initial work focused on evaluating this approach to identify PCPs from different tissues of *Populus* interacting with *L. bicolor*. In total, we identified 1660 *Populus* and 2870 *L. bicolor* PCPs. Besides

qualitative identification of well-known PCPs, the LC-MS/MS method was able to capture a total of 157 PCPs that were significantly more abundant in root tips with established ectomycorrhiza as compared to root tips without established ectomycorrhiza and extramatrical mycelium of *L. bicolor*. These PCPs mapped to 64 *Populus* proteins and 69 *L. bicolor* proteins, with several of them previously implicated in biologically relevant associations between plant and fungus, including a variant of the Mycorrhiza-Induced Small Secreted protein MiSSP7.6. We then extended this approach to identify the PCPs involved in regulating *Populus* and *L. bicolor* interaction at high nitrate concentrations. Even though ectomycorrhizal interactions are often prevalent in the presence of nitrate, little is known about this symbiotic interaction when soils are exposed to extremely high level of nitrate from anthropogenic sources like fertilizers. Experimental observation shows that the rate of *L. bicolor* colonization in *Populus* root is regulated at high nitrate concentration. To better understand this regulatory mechanism, PCPs were extracted and identified from the root tissue of *Populus* with/without *L. bicolor* interaction that were further treated with different concentrations of nitrate. In total, we identified 1443 PCPs in root tissue of *Populus* with *L. bicolor* interaction, out of which 126 PCPs were differentially abundant in different concentrations of nitrate. While the data-analyses are still underway, some of these differentially abundant PCPs mapped to 19 *Populus* proteins and 79 *L. bicolor* proteins, with several of them being oxidative stress-related proteins. Overall, PCPs identified in these studies help further our understanding of molecular progression involved in selecting and maintaining a symbiotic relationship between *Populus* and *L. bicolor*. Moreover, the method implemented in this study provides an avenue for identifying novel PCPs in other biological system.

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