Plant-Microbe Interfaces: Identification of PtLecRLK1-based signaling cascade in *Laccaria bicolor* root colonization

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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.

Soil-borne microbes can establish mutualistic relationships with host plants, providing a large variety of nutritive and protective compounds in exchange for photosynthesized sugars. However, the molecular events mediating the establishment of these beneficial relationships have yet to be fully characterized. Our previous genetic mapping and whole-genome resequencing studies identified a gene deletion event of a lectin receptor-like kinase gene *PtLecRLK1* that is associated with differential root colonization by the ectomycorrhizal fungus *Laccaria bicolor* among different *Populus* species. We introduced *PtLecRLK1* into the model annual plant *Arabidopsis* and the model perennial plant switchgrass (*Panicum virgatum*), subsequently converting these non-host plants to host plants, allowing colonization by *L. bicolor.* These results have established PtLecRLK1 as a key regulator of *L. bicolor* colonization. Among all proteins currently identified thus far as regulators of *Populus*-*L. bicolor* interactions, PtLecRLK1 is the most promising receptor candidate, responsible for perceiving and transducing signals from *L. bicolor*, which leads to molecular and physiological responses required for root colonization in the host plant. We wanted to define the molecular mechanism of action of PtLecRLK1 in *L. bicolor* root colonization. We hypothesize that PtLecRLK1 perceives signals from *L. bicolor* resulting in phosphorylated downstream components. We applied phospho-proteomics to identify proteins with differential abundance between *L. bicolor*-inoculated and un-inoculated switchgrass *PtLecRLK1* transgenic plants. We have identified several promising phosphorylation targets, including a leucine-rich receptor-like kinase, a cGMP-dependent protein kinase, and a splicing factor. Biochemical assays

are being conducted to validate protein-protein and protein phosphorylation between PtLecRLK1 and these candidate targets. Currently, we are taking a similar phospho-proteomics approach by using recently generated *Populus PtLecRLK1* transgenic plants. We will further examine the biological significance of these protein-protein interactions and protein phosphorylation through genetic validation. Collectively, our studies will help construct the entire PtLecRLK1-based signaling cascade that is responsible for specific molecular and physiological responses leading to *L. bicolor* root colonization, advancing our fundamental understanding of the molecular mechanism underlying the selection and maintenance of a mutualistic relationship between *Populus* and *L. bicolor*.

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