

Plant-Microbe Interfaces: Fungal and plant small secreted effector proteins play a key role in the development of the *Populus-Laccaria* ectomycorrhizal symbiosis

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Project Goals: The goal of the PMI SFA is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. *Populus* and its associated microbial community serves as the experimental system for understanding how these molecular events manifest themselves within the spatially, structurally, and temporally complex scales of natural systems. To achieve this goal, we focus on 1) characterizing host and environmental drivers for diversity and function in the *Populus* microbiome, 2) utilizing microbial model system studies to elucidate *Populus*-microbial interactions at the molecular level and dissecting the signals and pathways responsible for initiating and maintaining microbial relationships and 3) develop metabolic and genomic modeling of these interactions to aid in interpreting the molecular mechanisms shaping the *Populus*-microbial interface.

Mycorrhizal symbiosis between perennial plants and fungal associates has critical implications for diverse phenomena including global carbon, water and nutrient cycling. As such, characterizing the molecular genetics underlying such interactions holds tremendous potential in engineering biological systems for enhanced carbon sequestration and sustainable biomass production.

The crosstalk between the two partners is fundamental for the timing, establishment and maintenance of beneficial relationships. Many fungal lineages within the pathogenic/mutualistic continuum have evolved elaborate protein-based signals to manipulate their hosts to foster their needs during symbiosis. These signals, called effectors, which are typically fungal strain- or species-specific, contain a secretion signal motif and are less than 250 amino acids (aa) in size. We have previously described protein-based effectors in the ectomycorrhizal fungus *Laccaria bicolor*. Indeed, we showed that *L. bicolor* relies on Mycorrhizal-induced Small Secreted Proteins (MiSSP) to establish the mutualistic interaction with *Populus*. In particular, MiSSP7 interacts with the jasmonic acid (JA) co-receptor PtJAZ6 of *P. trichocarpa*, blocking JA signaling and promoting mutualism. We showed that PtJAZ6 is able to interact with *Populus* NINJA and TOPLESS proteins as well as the bHLH transcriptional factor, as was found in *Arabidopsis* leaves. Our results emphasize that the JA-mediated signaling pathway is a hub that plant-interacting microbes have to control in order to colonize plant tissues.

In addition, plant-encoded small proteins have also been implicated in affecting symbiotic associations. Recently, deep RNA-seq data and re-annotation of the *Arabidopsis* and *Populus*

genomes showed evidence for new small proteins in *Populus*. We identified 417 plant-based putative small secreted proteins (SSPs) that were significantly regulated during *Populus-Laccaria* interactions. This SSP set was over-represented by proteins predicted to be localized in the nucleus. Of the 417 SSPs regulated during *P. trichocarpa-L. bicolor* mutualism, only 3% were also significantly up- or down-regulated in the pathogenic interaction between *Populus* and *Melampsora laricini-populina*. Furthermore, several *Populus* SSPs were shown to enter the *L. bicolor* nucleus and affect the growth and/or branching of the fungal hyphae.

Our results suggest that both the fungal and plant partners could use small proteins as signals to mediate this mutualistic association. Our ultimate goal aims at identifying the protein-coded signaling circuitry that regulates the early steps of this symbiotic association, in both *L. bicolor* and *Populus*,

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