

Plant-Microbe Interfaces: Linking diversity and function in fungal communities associated with *Populus trichocarpa*

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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.

<http://PMI.ornl.gov>

This project aims to utilize novel sampling efforts and analyses to characterize the associated fungal community of *Populus trichocarpa* and link these fungi to their diverse functions. To this end, we focus on 1) sampling ectomycorrhizal fungi (EMF) from natural *P. trichocarpa* populations along a latitudinal gradient to create a culture collection and gather relevant material for identification and targeted –omics sequencing; 2) metatranscriptomic sequencing of bulk soil and root tips to analyze functional diversity within functional guilds of *Populus* with a focus on symbiotic and endophytic fungi; and 3) genomic sequencing of novel *Populus* associates to characterize the ectomycorrhizome of *Populus* and extend *Populus-Laccaria* findings to new model systems for future experimentation.

Previous sampling efforts focused on assembling a unique culture collection, representative of root endophytic and rhizospheric fungi. To expand this collection to mycorrhizal fungi, we focused on the collection, isolation, and characterization of major EMF fungal associates from a variety of *P. trichocarpa* genotypes from across major watersheds in the Pacific Northwest. EMF fungi are essential mutualistic partners with *Populus* that have shown to significantly expand nutrient uptake and acquisition of the plant host while also providing protection against antagonistic parasites. We recently conducted sampling of several watersheds to increase the representation of EMF in the collection and to enable detailed studies of functional diversity within this ecological guild. This effort consisted of five core watersheds on the Squamish (BC), Snohomish (WA), Puyallup (WA), Columbia (OR and WA), and Willamette (OR) rivers. This resulted in over 100 EMF sporocarp collections and sampling of bulk soil from 8 different *Populus* sites. All sporocarp collections were plated on modified Melin-Norkrans medium,

photographed, spore printed, and dried for identification and accession into a fungal herbarium. A consortium of taxonomic experts assessing morphological features and using ITS sequencing is currently identifying species. Taxonomic groups that were found associated with *Populus* include Russulaceae (18 coll.), *Inocybe* (12 coll.), *Boletus* (4 coll.), *Laccaria* (4 coll.), *Hebeloma* (3 coll.), *Amanita* (3 coll.), *Tricholoma* (2 coll.), *Scleroderma* (1 coll.), and *Clavulina* (1 coll.). Russulaceae comprised the highest taxonomic diversity of any EMF lineage sampled with at least 10 distinct species confirmed as *Populus* associates. Three of these members are host-restricted to *Populus*. Some species of EMF, such as *Inocybe geophylla*, were found at all sample sites, indicating that they may be ubiquitous associates of *P. trichocarpa*. We were also able to confirm *Laccaria bicolor* as a natural associate of *Populus* in a monodominant stand. Sterile tissue from the center of sporocarps was removed and frozen for transcriptome and genome sequencing. Representative isolates are being prepared for full genomic and transcriptomic sequencing at the DOE-JGI.

Many plant-associated fungi host symbiotic endobacteria with reduced genomes. While endobacteria play important roles in plant/fungal/endobacterial systems, the active physiology of fungal endobacteria has not been elucidated. We used integrated proteomics and metabolomics to characterize the endobacterium *Candidatus Glomeribacter* sp. and its host, the root endophytic fungus *Mortierella elongata*. In a nitrogen-poor condition, *M. elongata* had constrained growth, but hosted a large and growing endobacterial population. The active endobacterium likely extracted malate from the fungal host as the primary carbon substrate for energy production and biosynthesis of phospho sugars, nucleobases, peptidoglycan, and several amino acids. The endobacterium obtained nitrogen by importing a variety of nitrogen-containing compounds. Furthermore, nitrogen limitation significantly perturbed the carbon and nitrogen flows in the fungal metabolic network. *M. elongata* regulated many pathways by concordant changes on enzyme abundances, post-translational modifications, reactant concentrations, and allosteric effectors. Such multimodal regulations may be a general mechanism for metabolic modulation.

With this additional sampling and the development of integrated –omics analyses, we are positioned to address the fundamental hypothesis that molecular signaling pathways are conserved among different microbial and plant cohorts. The mycorrhizal lifestyle is thought to have arisen multiple times on the evolutionary tree, and we will determine whether similar mechanisms have as well. We aim to investigate the specific genomic determinants in fungi and plants that contribute to the symbiosis through comparative genomics, transcriptomics, proteomics, and metabolomics analyses, and, ultimately, characterize key components (e.g., nutrient distribution and C/N dynamics) of the mutualistic lifestyle.

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