Plant-Microbe Interfaces: The contribution of host chemotype as a driver of rhizosphere microbial community structure

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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 serve 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.

A diverse community of bacteria and fungi are closely associated with the roots of plants in the rhizosphere. Beyond carbon exchange between plant roots and their microbiota, the degree to which rhizosphere microbiomes are shaped by other plant host properties is relatively unexplored. *Populus* spp. trees produce characteristically high levels of salicylic acid that is complexed into higher-order salicylate conjugates that are actively involved in host defense, but also may be utilized as a nutritive source by exogenous soil microbial taxa or be inhibitory to others. We conducted a greenhouse-based experiment using 12 *Populus trichocarpa* genotypes that vary in higher-order salicylate concentration and composition (i.e., total phenolics, catechin, salicylic acid, salicortin, salicin, α-salicyloylsalicin, tremuloidin, and populin) to better understand how the genotype and chemotype of *P. trichocarpa* influence rhizosphere microbiome assembly and composition. We planted genotypes in soils originating from 2 locations: Corvallis and Clatskanie, Oregon that differed in their soil physical properties and nutrient concentrations. To assess the relative importance of host genotype vs. soil origin, we planted 5 replicate cuttings per genotype (N = 120) in these 2 soil types (2:1 sterile sand:soil inoculum) and allowed plants to grow for 4 months under greenhouse conditions. At the end of the experiment, leaf chlorophyll content, leaf growth (number of new leaves since transplant), and net photosynthetic rate differed across genotypes and soil type ($P < 0.01$). *P. trichocarpa* chemotypes were confirmed for roots from experimental samples via GC-MS analysis, and

showed host total salicylate concentrations varying from 1221 – 10610 μg/g FW, tremuloidin varying from 17 – 225 μg/g FW, and populin ranging from 0 – 8.9 ug/g FW plant tissue, depending on plant genotype. Total ectomycorrhizal colonization of root tips differed between genotypes ($P = 0.01$), but not soil type. Specifically, the genotype with the lowest root salicortin and salicin concentrations had significantly greater ectomycorrhizal colonization (10 ± 1.5% colonized) compared to three other genotypes with greater higher-order salicylate concentrations. Bacterial diversity within rhizospheres differed among genotypes and soil origin ($p < 0.01$), whereas fungal diversity did not differ among genotypes, but did differ by soil origin ($p < 0.01$). Bacterial diversity decreased with root tremuloidin concentrations ($p = 0.04$) and increased with populin concentration ($p = 0.02$), whereas fungal diversity was not correlated with any higher-order salicylate ($p > 0.09$). Regarding microbial community composition within rhizospheres, soil origin accounts for the majority of variation for bacteria (perMANOVA: $R^2 = 0.52$, $p = 0.001$) and fungi (perMANOVA: $R^2 = 0.40$, $p = 0.001$) with genotype secondarily influential (perMANOVA: bacteria $R^2 = 0.09$, $p = 0.003$; fungi $R^2 = 0.09$, $p = 0.02$). Furthermore, based on distance-based redundancy analyses, bacterial community composition was influenced by both tremuloidin and populin, whereas fungal community composition was influenced by salicortin and salicylic acid ($p < 0.05$). These results suggest that not only do salicylic acid metabolites produced by *Populus trichocarpa* roots directly impact belowground microbial community structure, but also that that bacteria and fungi respond differentially to derivatives of salicylic acid, specifically populin and tremuloidin.

The Plant-Microbe Interfaces Scientific Focus Area is sponsored by the Genomic Science Program, U.S Department of Energy, Office of Science, Biological and Environmental Research.