

Plant-Microbe Interfaces: Determining the rate and consequences of horizontal gene transfer in the rhizosphere by simulating lateral spread of salicylate catabolism genes.

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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.

Horizontal Gene Transfer (HGT) is one of the main drivers of prokaryotic evolution. HGT is particularly common in the rhizosphere, due to the abundance and diversity of microbes and niches. Plants and microbes are continuously engaged in an evolutionary arms race. While the slower-growing plants benefit from nurturing useful microbes and deterring antagonistic ones, shorter microbial generation times provide an opportunity for microbes to evade host controls. Horizontal transfer of pathways that regulate plant-microbe interactions are one of the fastest evolutionary paths. However, many details about the ecological and evolutionary impact of this transfer remain unclear. In this study, we assess the breadth, rates, and consequences of HGT in a synthetic soil bacterial community of low complexity, both in vitro and in situ using *Populus trichocarpa* as a model host.

In a first set of experiments, we tracked the abundance and association of a conjugative plasmid in a constructed bacterial community in vitro, using the Hi-C technique to crosslink plasmid DNA to the genome of its bacterial host. When employing capture probes that enrich for genome/plasmid interactions, preliminary data suggest that the target plasmids can be tracked even when harbored only transiently and by a very low percentage of the total microbial population (<0.01%). Analysis of plasmid association time courses will elucidate the dynamics and network effects of its transfer. By varying the constituents of the microbial community, the environmental conditions, and the identity of the plasmids being tracked, we hope to increase our understanding of the mechanics and impact of this process in nature.

In a second approach, we seek to investigate the consequences of successful HGT events in the rhizosphere. Plants such as *Populus* are hypothesized to exude salicin and related compounds to

modulate its microbiome. Several *Pseudomonas* spp. isolated from *Populus* roots were engineered to obtain energy and carbon from salicyl alcohol, a degradation product of salicin. To determine how the acquisition of such a pathway could alter a strain's niche and/or behavior, both engineered and wild-type *Pseudomonas* strains were labeled with DNA barcodes and inoculated onto otherwise sterile plant roots. DNA barcode sequencing was very sensitive, even for rhizosphere samples, and so localization and abundance of the barcoded strains could be tracked with high spatial resolution. These and future experiments with different substrate pathways will provide insights into the evolutionary and ecological consequences of gene acquisitions by HGT on plant roots.

Combining our two approaches, we aim to determine the holistic effects of MGEs in complex, diverse environments, and investigate microbial evolutionary responses to mechanisms by which plants shape the rhizosphere microbiome.

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