Plant-Microbe Interfaces: Beyond symbiosis, fungi can specifically perceive and use lipochitooligosaccharides to organize and modulate the development of microbial communities

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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.

Lipochitooligosaccharides (LCOs) are signaling molecules produced by rhizobial bacteria and fungi. The molecular mechanisms of perception of LCOs by host plants are fairly well characterized in their role as symbiotic molecules. Lysin motif receptor-like kinases (LysM-RLKs) are identified as the LCO-receptors in various plants, and this interaction elicits phenotypes like oscillations in nuclear calcium, and promote root hair branching, or curling in legumes. LCOs are produced by most fungi, including plant and opportunistic human pathogens. However, the role of LCOs outside of symbiosis with plants remains unknown. We test for alternative roles of LCOs to address this question. We examined two species of fungi, namely, *Aspergillus fumigatus*¸ a soilborne fungus that can be an opportunistic human pathogen, and *Laccaria bicolor*, a symbiont of poplar. We determined significant changes in fungal behavior in response to exogenous applications of various types of LCOs, including changes in fungal physiology, metabolomics output, and differential regulation of genes and proteins. Both fungi displayed fewer hyphae branching formation, increased sexual propagation, and delayed growth, indicating that LCOs could be fungistatic compounds. Moreover, upon exposure to LCOs, early transcriptomic and

proteomic changes are observed, as well as the regulation of secreted metabolites that can inhibit or promote the growth of specific bacteria known to inhabit the poplar rhizosphere. Lastly, structural modeling and analysis identified fungal LysM proteins that carry key structural features of known plant LCO-receptors. We describe a molecular dynamics and machine learning-based workflow to predict the relative binding affinity of LysM-LCO complexes. We demonstrate its remarkably high accuracy using validation sets, and therefore, its promising application to identify LysM proteins that can effectively bind to LCOs in fungi and other organisms. Overall, we propose that LCOs are fungistatic compounds produced and used by fungi to organize microbial communities. We hypothesize that these signaling molecules produced by some microbes may be sensed by multiple organisms via membrane-attached LysM proteins. Therefore, the role of LCOs seems to greatly surpass microbe-host plant symbiosis as a trans-kingdom communication signal.

Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR22725. 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 under FWP ERKP730.