Plant-Microbe Interfaces: Pathway prediction and production through cell-free synthetic biology

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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.

Collectively, *Populus* and its microbiome have the potential to produce a large number of highly diverse and unique metabolites. As our definition and understanding of the plant and its microbiome has progressed, so too has the backlog of information and open questions generated from the thousands of uncharacterized proteins and metabolites that comprise these systems. To address this problem, we are developing new tools in order to rapidly test and define the function of uncharacterized proteins and metabolic pathways. Cell-free systems have developed into a powerful tool for synthetic biology and metabolic engineering with applications across multiple disciplines. Developments in cell-free biology have remarkably improved its capacity for expressing proteins as well as created the field of cell-free metabolic engineering. We are advancing cell-free systems as a rapid means for exploring protein function and metabolite production using two complementary approaches. The first, uses crude cell-free extracts to produce proteins related to potential metabolic pathways in newly isolated organisms. Upon modular assembly of the pathway and metabolite analyses, active and inactive pathways are differentiated. The second, focuses on developing cell-free extracts as bioproduction platforms by analytically verifying the metabolic pathways resident in a cell free system and determining which are drawing resources towards and away from the production of specific metabolites. As a result, we have significantly expanded our ability to use cell extracts outside of their native context to solve metabolic engineering problems and provide engineers new tools that can rapidly explore the function of proteins and test novel metabolic pathways.

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