Plant-Microbe Interfaces: A high-throughput bioassay to investigate bacterial-provided benefits to heat stress

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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.

Heat is a major environmental factor that negatively affects plant growth and development. Along with breeding efforts to develop thermotolerant plants, exploiting beneficial plant microbes is a promising alternative approach. In our previous study, we demonstrated that the plant thermotolerant phenotype can be transmitted through the microbiome. To understand the underlying molecular genetics by which plants receive benefits from microbes to heat, we developed a high-throughput bioassay using *Arabidopsis thaliana* to screen individual bacterial strains that confer enhanced thermotolerance to the plant host. Multiple culture systems and heat treatment strategies were tested. The final system used hydroponically cultured seedlings with and without bacteria and was subjected to heat shock followed by recovery. Plants were then harvested to measure total chlorophyll content as an indicator of heat-induced damage. Initial screening results with several genera of rhizobacterial isolates from our inhouse collection found that *Variovorax* strains provided thermal benefit to the host plant. Subsequent screening results with a larger panel of 26 *Variovorax* found 6 strains that confer enhanced host plant thermotolerance. To investigate promising strains further, we examined a set of treatments that includes no heat shock control, non-lethal thermal exposure (i.e., thermoprimed) prior to heat shock, and heat shock with and without bacteria. Thermoprimed plants showed no significant difference (p=0.0587) in chlorophyll content compared to no heat shock control. Chlorophyll degradation by heat shock was observed in both inoculated plants (18%) and uninoculated plants (32.5%), whereas the level of damage was significantly reduced in inoculated plants (p<0.001), indicating bacterial-induced resilience to heat. Not only is this assay amenable to high-throughput screening of individual bacterial isolates, but it can also scale to include multiple *Arabidopsis* genetic variants and microbial consortia.

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