1. Experimental Systems to Model Nutrient and Carbon Exchange in Plant-Microbe Symbiosis

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http://www.bio.anl.gov/molecular_and_systems_biology/protein_expression/

Project Goals: The Argonne Environment Sensing and Response (ESR-SFA) program addresses the hypothesis that cellular behavior can be modeled through an understanding of the biological interface with the environment and the cellular responses that originate from the cell/environment interaction. The overall objective of the ESR-SFA program is to identify the molecular basis of cellular transport and sensory pathways that mediate the response to environmental nutrients. This study describes experimental model systems comprised of Aspen, Laccaria bicolor, and Pseudomonas species will be used to minimize system complexity that permit manipulation of nutrient parameters under controlled conditions. All three components of this model system have completed genome sequences and are able to be cultured as monocultures, co-cultures, and tertiary or community-based cultures, thus enabling characterization of molecular interactions within and between the individual systems.

The FY13-15 ESR-SFA science plan uses a experimental model system comprised of Aspen, *Laccaria bicolor*, and *Pseudomonas* sp. The initial experiments proposed examined growth parameters using four selected Pseudomonas fluorescens strains (Pf-5, Pf0-1, SBW25, and WH6). These bacterial strains were isolated from the environment and previous studies indicate these are Plant Growth Promoting Bacteria (PGPB) that occupy the rhizosphere of many plant species and may improve ecosystem productivity. A three-component system is a complex experimental model but enhances the relevance for extrapolation of characterized molecular responses to organism interactions in natural ecosystems. The first objective was implementation of systematic experiments to characterize various experimental systems at a pilot scale to provide a foundation for the design and analysis of large-scale community experiments. In addition to characterizing the experimental models and community properties, the experiments were designed to validate methods for efficient extraction of community RNA and to measure organism/community parameters.

In one approach, we compared Phytagel and expanded perlite growth matrices for 3-component co-cultures. The Phytagel box system is well characterized for plant growth in Magenta boxes and provides reproducible, controlled results. Introduction of each bacterial strain is efficient in this system; however, access to the roots is limited by the density of the Phytagel. A vertical plate 3-component configuration allows clear visualization of the root systems as they interact with both fungi and PGPB. This system greatly improves recovery of plant root samples as they can be peeled from the surface of the gel; however, the plants within this vertical system do not develop as much biomass as either the Phytagel or perlite systems, indicating reduced overall plant health. The perlite system provides the best overall results. While plants need to be removed from the perlite to be visualize root development plant, fungal and bacterial growth and health are optimal, most likely due to the increased root aeration. Roots in particular exhibit a dramatic increase in biomass when grown in perlite; biomass is further increased when colonized by either *Laccaria* alone or *Laccaria* in combination with PGPB strains Pfl01 or PF-5. Such enhancements are not as apparent in the other two systems.

A parallel series of experiments determined the impact of *Pseudomonas fluorescens* PGPB strains on Aspen growth in phosphorus- or nitrogen-limited media. Control and nutrient-limited seedlings were grown with and without bacterial inoculation on vertical petri-plates in controlled environmental conditions. We examined the impact of several *P. fluorescens* strains (WH6, SBW25, Pf0-1, and PF-5) on the *in vitro* growth of Aspen seedlings at control and limited concentrations of nitrogen (4mM versus1mM) and phosphorous (1.5mM versus 20μM). Growth rate and morphology were assessed as primary indicators of plant health and additional parameters such as number of leaves, plant shoot height, root length and root structure such as branching pattern and rootlets, were also recorded to allow for more detailed evaluation of PGPB impact.

Nutrient limitation of both nitrogen and phosphorus was observed to decrease the number of leaves, but increase root length and branching structure. The number of seedling rootlets decreased significantly during phosphorus stress, but increased during nitrogen stress. All plants in nutrient-limited media showed and improvement in growth metrics when inoculated with PGPB relative to uninoculated controls under nutrient stress. The specific effects of PGPB colonization on plant growth and root morphology were found to be strain dependent and included alteration of plant root morphology, total root biomass, and aboveground biomass. Root length, for example, increased in control plants colonized with PF-5 and Pf0-1, while plants colonized with SBW25 exhibited enhanced root branching relative to the controls. An increase in the number of leaves was observed in control seedlings colonized with all bacterial strains. Similar strain-dependent effects were observed under both nutrient limiting and nutrient replete conditions. These preliminary results demonstrate some of the profound effects of PGPB on plant growth and morphology. Further understanding of nutrient limitation effects is expected to increase our insight into community structure and carbon cycling in terrestrial ecosystems.

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