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PNNL-30598

Probing Cooperation and Competition between Paenibacillus polymyxa and Setaria viridis under Nutrient Stress

September 2020

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Report

◆ The goal of this project is to use bioorthogonal non-canonical amino acid tagging (BONCAT) to explore utilization of bacterial proteins from the beneficial plant-growth promoting bacterium* Paenibacillus polymyxa *by model C4 bioenergy grass* Setaria viridis *under low nitrogen growth conditions. ◆

Introduction and Project Description:

Chemical biology approaches can be used to elucidate the molecular mechanisms driving complex biological interactions. The goal of this project is to use bioorthogonal non-canonical amino acid tagging (BONCAT) to explore plant host utilization of bacterial proteins from the beneficial plant-growth promoting bacterium *Paenibacillus polymyxa* cultured with the model bioenergy grass *Setaria viridis* under low nitrogen growth conditions. Previous studies have determined that plants are capable of using proteins as a source of N by secreting proteases from their roots as well as taking up intact microbes for proteolytic digestion, possibly in the apoplast. By labeling an organism with a non-canonical amino acid containing a functional click group such as azidohomoalanine (AHA) or homopropargylglycine (HPG), we can specifically tag newly synthesized proteins in a biological system for enrichment and subsequent proteomics analysis; this technique can also be used to characterize the fate of these proteins over time.

Results and Accomplishments:

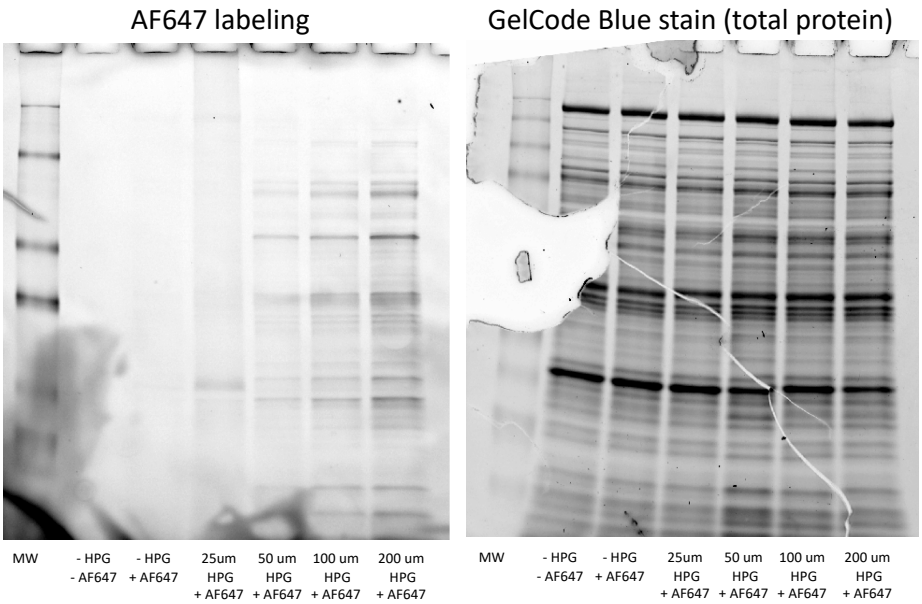
We grew *S. viridis* A10.1 from sterilized seeds in Magenta boxes containing glass beads and Hoagland's media for ~4 weeks. Plants were acclimated for 1 week in normal or N-free media prior to beginning bacterial inoculations.

Our initial attempt to sample plants after 8 days of growth with 3 rounds of inoculations revealed there was insufficient biomass for enrichment and proteomics analysis (25-80 mg root per plant). We therefore doubled the plant growth and inoculation period and decided to pool plant samples in each box to obtain sufficient biomass (~1 g wet weight per pooled sample for roots) at the expense of biological replicates. A second group of plants was grown on a staggered schedule to achieve a total of 4 biological replicates for each treatment. Plants were inoculated a total of 5 times over a 17-day period with live or heat-killed (100 ^{o}C for 5 min) *P. polymyxa* cultured with or without HPG. Heat-killed *P. polymyxa* plated on agar showed no growth after 1 week, confirming the heat treatment was effective at rendering cells non-viable.

Prior to sampling, plants were rinsed in MilliQ water to remove excess media, dead leaf material was discarded. Roots and shoots were collected separately. Due to the need to increase the time period for plant inoculations with BONCAT-labeled bacteria, processing of samples for proteomics analysis could not be completed. Tissues were flash frozen and stored at -70 ^{o}C. for future processing and analysis.

A review manuscript of chemical biology approaches for studying plant-microbe interactions is in preparation for the "Recent Advances in Chemical Biology" special issue of the journal *Molecules*.

Figures/Tables



Fluorescence gel image of lysates of *P. polymyxa* showing dose-dependent labeling of proteins with increasing concentrations of HPG added to culture media. Samples were subjected to click chemistry with AF647-azide. Bands indicate newly synthesized proteins that have incorporated HPG.

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