Pacific Northwest
NATIONAL LABORATORY

PNNL-29249

Identification of plant host and microbe model system for study of rhizophagy cycle

October 2019

Vivian S Lin
Natalie C Sadler
Yuliya Farris
Elizabeth H Denis
Lucas C Webber
Ryan M Francis

U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

DISCLAIMER

PACIFIC NORTHWEST NATIONAL LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC05-76RL01830

Printed in the United States of America

Available to DOE and DOE contractors from the Office of Scientific and Technical Information,
P.O. Box 62, Oak Ridge, TN 37831-0062
www.osti.gov
ph: (865) 576-8401
fax: (865) 576-5728
email: reports@osti.gov

Available to the public from the National Technical Information Service
5301 Shawnee Rd., Alexandria, VA 22312
ph: (800) 553-NTIS (6847)
or (703) 605-6000
email: info@ntis.gov
Online ordering: http://www.ntis.gov

PNNL-29249

Identification of plant host and microbe model system for study of rhizophagy cycle

October 2019

Vivian S Lin
Natalie C Sadler
Yuliya Farris
Elizabeth H Denis
Lucas C Webber
Ryan M Francis

Prepared for
the U.S. Department of Energy
under Contract DE-AC05-76RL01830

Pacific Northwest National Laboratory
Richland, Washington 99354

Identification of plant host and microbe model system for study of rhizophagy cycle

Vivian S. Lin

◆ ***This project aims to establish robust methods using imaging, plant science, and microbiological capabilities for the study of rhizophagy and its potential as a mechanism by which bioenergy plant species can modulate nutrient acquisition from their environment.*** ◆

Introduction and Project Description:

Rhizophagy ("root eating") is a process by which plants actively uptake soil microbes into their roots and use oxidative degradation to extract micronutrients from the cells. This phenomenon has been proposed to be a general mechanism for plants to acquire nutrients from the environment via bacteria and fungi. Determining the conditions under which rhizophagy occurs and its prevalence in different plant species will improve our understanding of nutrient dynamics and plant-microbe metabolic interactions at the molecular level. This project aims to (1) optimize methods for plant and microbial growth, imaging, and analysis for the study of rhizophagy and (2) identify a bioenergy-relevant model system for future rhizophagy studies.

Results and Accomplishments

We optimized plant growth, inoculation, and imaging approaches for tracking microbial colonization of plant roots to identify a potential rhizophagy model system. Tomato (*Solanum lycopersicum*), model C3 grass species *Brachypodium distachyon*, and model C4 grass and bioenergy crop *Setaria viridis* were grown on phytagel or semi-hydroponically in Hoagland's solution with glass bead substrate. Plants were inoculated with green fluorescent protein (GFP) expressing bacteria (*Escherichia coli* Nissle, *Pseudomonas fluorescens* SBW25, and *Paenibacillus polymyxa* SCE2). Whole plant imaging using a Typhoon laser scanner allowed for identification of root areas of interest for plants grown in phytagel and glass chamberslides. Root samples were excised and fixed in paraformaldehyde for fluorescence confocal microscopy. Whole plants were grown in glass chamberslides for live confocal imaging experiments. Non-fluorescent protein expressing bacteria (known endophyte and nitrogen-fixing *Herbaspirillum seropedicae* Z67 and Z78, known plant-growth promoting strains *Bacillus subtilis* 3610 and GB03) were stained with SYBR Gold to visualize bacterial localization in fixed roots. Calcofluor white was used to stain cellulose and identify the outer boundary of the root.

Differences in location and timeline of bacterial colonization of plant roots were observed. We observed strong biofilm formation of *P. polymyxa* on root tips and at lateral root primordia (sites of lateral root emergence) of tomato, *Brachypodium*, and *Setaria*, whereas *E. coli* was rarely observed on root tips; root tips and lateral root sites have previously been identified in the literature as hotspots for microbial activity in the rhizosphere. Both *H. seropedicae* strains showed rapid colonization of *Brachypodium* roots with heavy biofilm formation at the root tip within 3 days post-inoculation, while *B. subtilis* colonized the roots at lower initial densities and displayed stronger biofilm formation after 7 days. *P. fluorescens* also formed biofilms on all parts of the root. Bacteria were frequently observed to cluster at the interfaces of root cells on the outside of the root. We determined that inoculation with high bacterial concentrations (10^7 cells/mL, or ~10^8 total cells) cited in the literature was generally not suitable for our studies. Inoculation of plant roots, particularly for live imaging, was better tolerated at lower cell densities (10^3 for live imaging and 10^6 cells total for isotopic labeling studies).

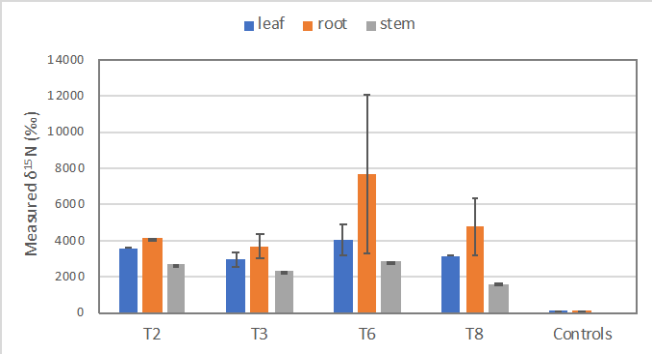
We identified *P. polymyxa* in *Setaria* as a potential model system to target for future studies. *P. polymyxa*, a nitrogen-fixing bacterium and known plant endophyte, was observed to be endophytic in the main root and possibly in root hairs of live *Setaria* roots under N-limited conditions; under N-replete conditions, no bacteria were observed within the root.

To track nutrient transfer from microbes to host plants, we inoculated tomato and *Brachypodium* with bacteria grown in ^{15}N-labeled media. We also generated 50-70% killed labeled bacteria by freeze-thawing the inoculum twice to evaluate the effect of microbial cell death and necromass formation on ^{15}N uptake. Analysis of plant tissues (root, stem, and leaf) using isotope ratio mass spectrometry (IRMS) showed uptake of ^{15}N into all plant tissues compared to control tissues collected prior to bacterial inoculation. Incorporation of ^{15}N appeared to be highest in root

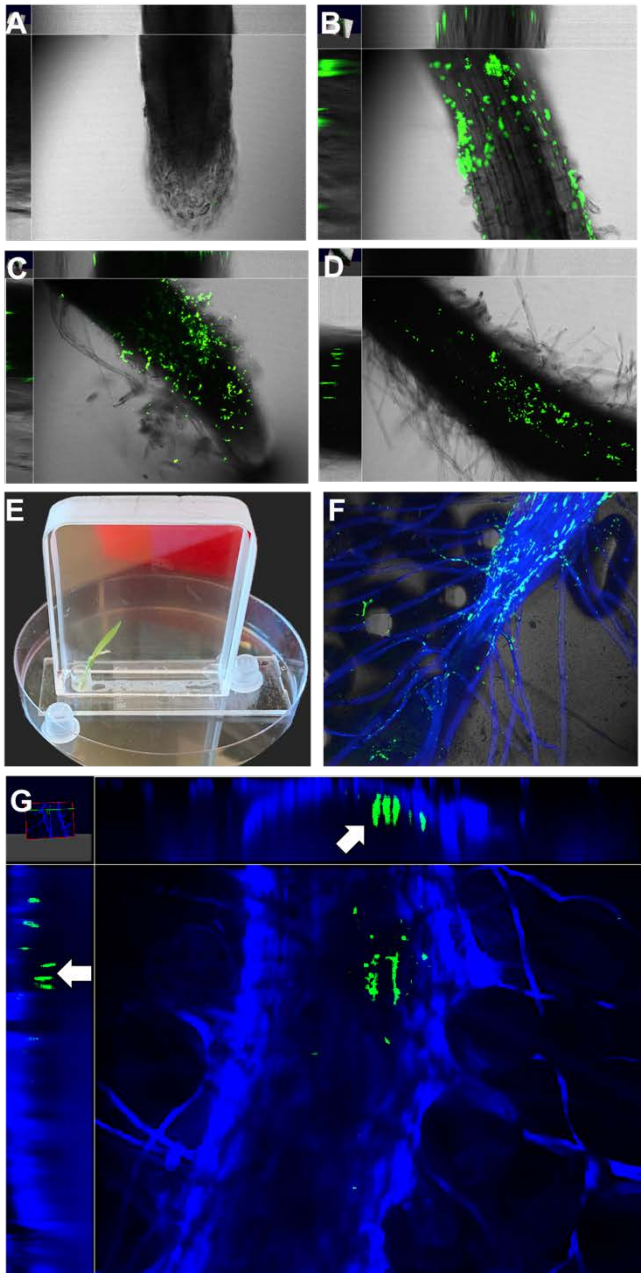
tissues compared to stem or leaf. To account for potential biofilm formation on roots, we sterilized the outside of roots using a brief bleach treatment; microscopy confirmed the absence of any fluorescent bacteria as well as no obvious damage to root tissue. No difference in ^{15}N content was observed for roots treated with bleach compared to roots rinsed in water prior to sampling.

We anticipate that future studies on plant-microbe interactions will benefit from the imaging and isotope analysis approaches explored in this preliminary effort. Confocal microscopy live imaging results from this project will be included in a future publication on approaches to studying microbial interactions (Sadler, N. C. *et al*. "A micro to meso scale view of soil microbial assembly, phenotypes, and interactions," in preparation).

Figures/Tables



Isotope ratio mass spectrometry (IRMS) results for tissues of tomato plants inoculated with "necromass" (T2, T3) or live (T6, T8) ^{15}N-labeled *E. coli*.



Confocal fluorescence microscopy images of roots and bacteria. Tomato with (A-B) GFP *E. coli* and (C-D) GFP *P. polymyxa* at root tip and along the length of the root, 7 days post inoculation. (E) *S. viridis* grown in a soil chip with 0.1 mm silica bead porous structured media & polyacrylamide particles for moisture retention. (F) Live imaging of *Pseudomonas fluorescens* SBW25 mNeon-GFP (green) biofilm on *S. viridis* root. (G) *S. viridis* (21 days old) incubated in N-free minimal media with GFP tagged *P. polymyxa* for 48 hr. Blue fluorescence from Calcofluor white stain was used to determine the outer root boundary. White arrows on Z-stacking images indicate bacteria that have infiltrated the root.

Pacific Northwest National Laboratory

902 Battelle Boulevard
P.O. Box 999
Richland, WA 99354
1-888-375-PNNL (7665)

www.pnnl.gov