



COLLEGE OF
ARTS AND SCIENCES
Biology

COKER HALL
CAMPUS BOX 3280
CHAPEL HILL, NC 27599-3280

T 919.962.2077
F 919.962.3690
biology.unc.edu

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

December 7, 2018

Dear Jaydev Upponi,

Enclosed please find our manuscript, "**Monitoring bacterial colonization and maintenance on the roots of *Arabidopsis thaliana* in a floating, hydroponic system**", which we are submitting to the Journal of Visual Education.

Rhizosphere colonization by plant growth-promoting bacteria (PGPB) can increase the health or development of host plants in the presence of diverse stressors compared to uncolonized plants, at least in laboratory conditions. However, when tested in field settings, treatments of plants with PGPBs often don't provide substantial benefits to crop growth. In an effort to understand this discrepancy between lab and field studies, we developed a hydroponic plant-growth assay to quantify species presence and visualize the spatial distribution of bacteria during initial colonization and following transfer into differing growth environments.

We validated our system's reproducibility and utility with the well-studied PGPB *Pseudomonas simiae*. We wanted a system that would be applicable to a range of bacteria species, and thus that had sufficient adaptability to account for the differing growth needs to a range of bacteria while also permitting exploration of field-like environmental stressors. Further, we wanted to support the health and development of the plant host. The hydroponic system we describe here allows bacteria to colonize in nutrients that are most conducive to their growth or in conditions of interest. We show that we can measure the presence of multiple bacterial species using this assay, which provides an alternative to sequencing-based studies of bacterial colonization of plant roots.

Future studies using this system may improve our understanding of how bacteria behave in multispecies plant microbiomes over time and in changing environmental conditions, with the potential to improve crop productivity.

The main manuscript has four figures and one supplemental figure.

We look forward to hearing from you.

Sincerely,

Elizabeth Shank, PhD
Assistant Professor of Biology, UNC-CH