

Using Microfluidics to Quantify the Spatiotemporal Dynamics of Plant Colonization by Beneficial Bacteria.



Contact: Scott T. Retterer (rettererst@ornl.gov); (865) 405-4066

Funding Source: DOE Office of Biological and Environmental Research, Genomic Science Program, National Science Foundation

Background

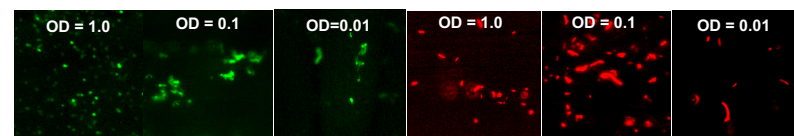
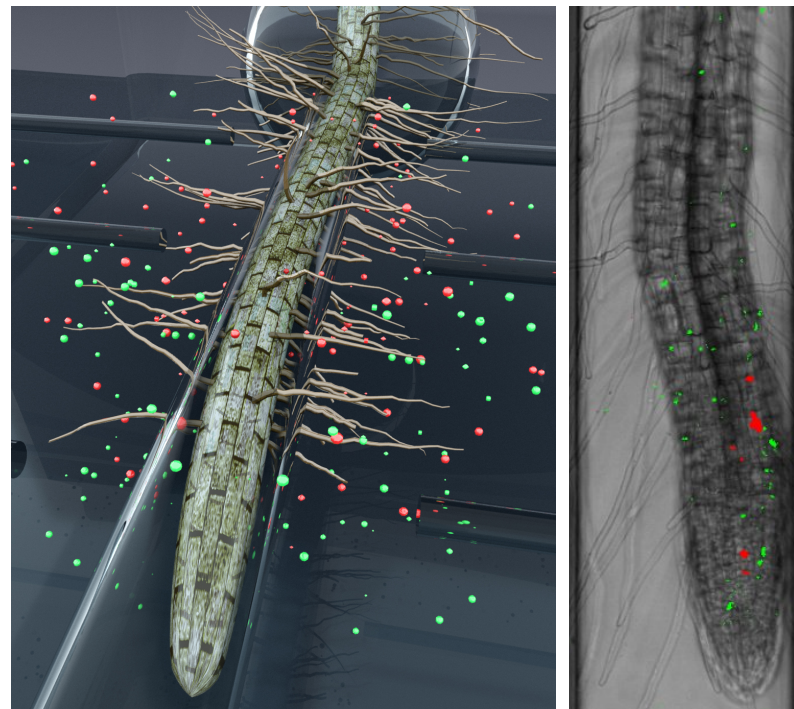
- The attachment and spatial organization of plant-growth promoting bacteria on plant roots is inherently difficult to study *in situ* given the opacity of soil and disparity between root and microbial length scales.
- Microfluidics enable visualization of plant-microbe interactions which can provide mechanistic insight into the physical and biochemical cues that shape their development.

Science

- In a custom microfluidic platform, we treated *A. thaliana* seedlings with two bacterial species isolated from the microbiome of *P. deltooides*. The endophyte, *Variovorax sp. CF313*, exhibited a uniform distribution along the root while the rhizosphere isolate, *Pantoea sp. YR343*, preferred to colonize newly developed tissue.
- The population of bacterial cells associated with the plant root after 4 days of co-culture was independent of the initial inoculum concentration, yet the inoculum concentration dictated the size of bacterial micro-colonies formed on the root and the root's morphological response to the bacterial treatment.

Significance

- The ability to watch and quantify the spatiotemporal organization of biological systems opens the door to formulating and answering questions about community development that cannot be addressed in natural systems.



Microfluidic platforms can be used to visualize plant-microbe interactions (top left) An artistic interpretation of a plant root and microbial community illustrates the habitat created by the microfluidic system. (top right) *Pantoea sp. YR343* (green) and *Variovorax sp. CF313* (red) form micro-colonies on an *A. thaliana* root tip (scale bar = 100 μ m). (bottom row) After four days of co-culture, representative fluorescent images of YR343 (green) and CF313 (red) bacterial micro-colonies show that the optical density of the bacterial inoculum affects the size of bacterial colonies associated with the root (images are 200 μ m squares).

Quantifying the Spatiotemporal Dynamics of Plant Colonization by Beneficial Bacteria in a Microfluidic Habitat



Jayde Aufrecht,^{1,2} Collin M. Timm,¹ Amber Bible,³ Jennifer L. Morrell-Falvey^{1,2,3}, Dale A. Pelletier,¹ Mitchel J. Doktycz,^{1,2,4} and Scott T. Retterer^{1,2,4}

¹ Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN; ² Bredesen Center, ³Biochemistry and Cellular and Molecular Biology Departments, University of Tennessee, Knoxville, TN; ⁴ Center for Nanophase Materials Research, Oak Ridge National Laboratory, Oak Ridge, TN.

Abstract

Plant–microbe interactions underpin processes related to soil ecology, plant function, and global carbon cycling. However, quantifying the spatial dynamics of these interactions has proven challenging in natural systems. Currently, microfluidic platforms are at the forefront of innovation for culturing, imaging, and manipulating plants in controlled environments. Using a microfluidic platform to culture plants with beneficial bacteria, visualization and quantification of the spatial dynamics of these interactions during the early stages of plant development is possible. For two plant growth–promoting bacterial isolates, the population of bacterial cells reaches a coverage density of 1–2% of the root’s surface at the end of a 4 d observation period regardless of bacterial species or inoculum concentration. The two bacterial species form distinct associations with root tissue through a mechanism that appears to be independent of the presence of the other bacterial species, despite evidence for their competition. Root development changes associated with these bacterial treatments depend on the initial concentrations and species of the bacterial population present. This microfluidic approach provides context for understanding plant–microbe interactions during the early stages of plant development and can be used to generate new hypotheses about physical and biochemical exchanges between plants and their associated microbial communities.