

Democratization of fungal highway columns as a tool to investigate bacteria associated with soil fungi

Pilar Junier^{1*} (pilar.junier@unine.ch), Olivia C. Trautschold², Nicholas G. Parra-Vasquez², Saskia Bindschedler¹, Ilona Palmieri¹, Fabio Palmieri¹, Aislinn Estoppey¹, Danae Bregnard¹, Wafa M. Kooli¹, Andrea Lohberger¹, Geoffrey L. House³, La Verne Gallegos-Graves³, Julia M. Kelliher³, Armand E. K. Dichosa³, Hang N. Nguyen⁴, Simone Lupini⁴, Jean F. Challacombe⁵, Jamey D. Young⁶, Debora F. Rodrigues⁴, and **Patrick S. G. Chain**³

¹Institute of Biology, University of Neuchâtel, Neuchâtel Switzerland; ²Materials Science and Technology, Los Alamos National Laboratory, Los Alamos New Mexico; Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico; ⁴Civil and Environmental Engineering, University of Houston, Houston, Texas; ⁵College of Agricultural Sciences, Colorado State University, Fort Collins, Colorado; ⁶School of Engineering Vanderbilt University, Nashville, Tennessee.

<https://genomicscience.energy.gov/research/sfas/lanlbfi.shtml>

Project Goals: Bacteria-fungi interactions (BFI) are recognized as essential for numerous environmental processes. These interactions are modulated not only by the nutritional conditions of the surrounding environment, but also by its physicochemical constrains. In soils, one of these physical constrains relates to the unsaturated nature of the substrate and the consequence of this for the dispersal and activity of bacteria. Developing new tools to investigate how bacteria overcome dispersal limitation at a cellular scale is important to unravel the mechanisms by which BFI affect processes at an ecosystem scale. This is not only relevant for soils, but also for other unsaturated matrices in which microbial activity is an important driver of function. In many unsaturated systems, bacteria and fungi engage in a cooperative interaction in which bacteria use fungal hyphae to disperse. This interaction is referred to as fungal highways. Here we present the design and validation of a sampling system obtained by additive printing to enrich interacting bacterial-fungal couples that form these fungal highways.

Abstract

Fungi and bacteria form different types of associations that are central to numerous environmental processes. Although many bacteria are able to move by different mechanisms, occasionally requiring the production of specific appendages, most bacterial motility types are only efficient in the presence of a liquid film (1). Thus, in soil and other water-unsaturated matrices, bacterial dispersal is highly limited (2). In contrast, the filamentous growth of fungi is an ideal morphology for colonization of unsaturated matrices. Some bacteria can use the liquid film surrounding hyphae for their dispersal in a mechanism known as fungal highways (3). In order to investigate this dispersal mechanism, a sampling device was designed to select and isolate both bacteria and their fungal highway partner directly from an environmental sample by including an attracting and a target medium in a unsaturated column. These devices, called fungal highways columns, have been used to investigate the diversity and function of fungal highways in soils (4). However, one major limitation for the routine and standardized use of such devices as a scientific instrument for investigating fungal highways in soils has been the variability and time-consuming manual production of these devices by experienced scientists. In this study we present the results of the design and testing of a fungal highway column generated by additive 3D printing. This new model

considers the main features of the manually built device, namely the possibility to include any type of targeting and attracting media, an hourglass shape to avoid the formation of a continuous water film, and a built-in modular lattice that simulates the presence of soil particles and air-filled gaps.

References

- (1) Harshey RM. Bacterial motility on a surface: many ways to a common goal. *Annu Rev Microbiol* 2003 57 249 73.
- (2) Or D, Smets BF, Wraith JM, et al. Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. *Adv Water Res* 2007 30 1505 27.
- (3) Kohlmeier S Smits TH Ford R et al. Taking the fungal highway: mobilization of pollutant-degrading bacteria by fungi. *Environ Sci Technol* 2005 39 4640 6
- (4) Simon A, Binschedler S, Job D et al. Exploiting the fungal highway: development of a novel tool for the in situ isolation of bacteria migrating along fungal mycelium. *FEMS Microbio Ecol* 2015 fiv116.

This study was supported by a U.S. Department of Energy Biological and Environmental Research Science Focus Area grant (grant no. DE-AC52-06NA25396).