4-5-2022

Fungal highways enable migration and communication of engineered bacteria in soil

Eric Young

Nilesh Sharma

Felipe Carrillo

Jaclyn Thompson

Allison Taggart

See next page for additional authors

Authors

Eric Young, Nilesh Sharma, Felipe Carrillo, Jaclyn Thompson, Allison Taggart, Jacob Beal, Miles Rogers, and Natalie Farny

FUNGAL HIGHWAYS ENABLE MIGRATION AND COMMUNICATION OF ENGINEERED BACTERIA IN SOIL

Eric M. Young, Chemical Engineering, WPI, USA
emyoung@wpi.edu
Nilesh Sharma, Chemical Engineering, WPI, USA
Felipe Carrillo, Biology and Biotechnology, WPI, USA
Jaclyn Thompson, Chemical Engineering, WPI, USA
Allison Taggart, Synthetic Biology, Raytheon BBN Technologies, USA
Jacob Beal, Synthetic Biology, Raytheon BBN Technologies, USA
Miles Rogers, Synthetic Biology, Raytheon BBN Technologies, USA
Natalie Farny, Biology and Biotechnology, WPI, USA

Key Words: soil microbiome, synthetic biology, genetic circuits, fungal highways.

The soil microbiome is essential for natural chemical cycles, contains immense biosynthetic capacity, and interfaces with civilization through agriculture, the built environment, and national defense. Understanding and harnessing the soil microbiome is therefore critical on multiple fronts. One potential use of the soil microbiome is quantification of soil chemical state at depth across broad areas. However, inducible circuits that function in soil and delivery of circuits underground is currently impossible – for example, if the typical genetic circuit host *E. coli* even survives in soil it cannot penetrate into the soil or maintain burdensome plasmids for long. However, the study of natural soil microbiomes has revealed that bacterial migration and long-distance chemical signaling occurs naturally, facilitated by filamentous fungal highways. If these properties could be harnessed, then delivery and sensing underground would be possible.

Here, we report an interkingdom microbial consortium that enables genetic circuit delivery and function in soil. We harnessed the bacterial and chemical transmission properties of fungal highways to deliver a motile engineered bacteria down into soil where it senses a chemical signal and sends that signal to the surface. Several innovations were required. First, we selected an alternative bacterial host – *Pseudomonas putida*. *P. putida* is a motile soil bacterium with developed genetic techniques, but few inducible systems. We tested several genetic circuits for function in *P. putida*, eventually identifying quorum sensing circuits Lux and Las as the most robust, achieving 15-fold induction in soil. Next, we prototyped several fungal species for growth rate, soil penetration, and compatibility with *P. putida*, finding that *Lyophyllum atratum* extends *P. putida* soil survival and accelerates migration.

Finally, we tested the utility of fungal highways for bacterial migration and signal propagation. Bacterial migration in response to a salicylic acid chemoattractant was 15 mm/day with fungi whereas no migration was observed in response to water as control. Quorum sensing signal propagated 35 mm and fluorescent protein expression lasted for 48 hours in response to quorum sensing molecules in soil without fungi. With the fungal partner, signal propagated 80 mm and fluorescent protein expression lasted for 120 hours (Figure 1).

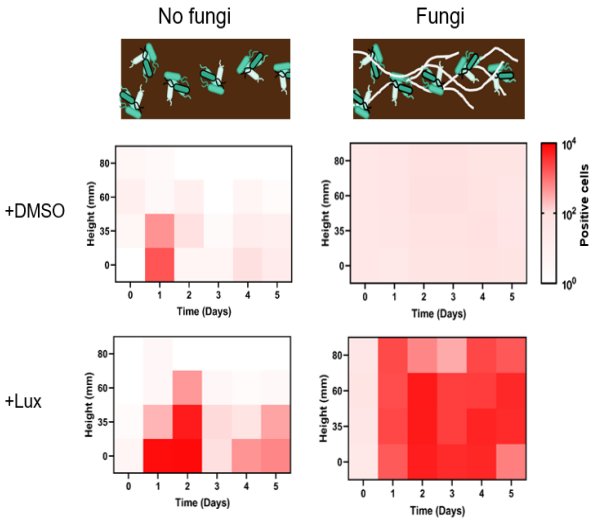

Figure 1 – Function of an underground chemical sensor based on an engineered soil microbiome. Presence of the fungal partner and the inducer are both required for the cells to produce RFP and propagate signal 8 cm to the surface.

These results show that interkingdom networks are key to engineering robust genetic circuit function in soil. Thus, this study builds the foundation for synthetic biology solutions in agriculture, environmental remediation, and chemical detection at depth.

Supported by the Defense Advanced Research Projects Agency (DARPA) under Contract No. HR0011-19-C-010. The views, opinions, and/or findings expressed are those of the author(s) and should not be interpreted as representing the official views or policies of the Department of Defense or the U.S. Government. This document does not contain technology or technical data controlled under either U.S. International Traffic in Arms Regulation or U.S. Export Administration Regulations. Approved for public release, distribution unlimited (DARPA DISTAR case 35633, DATE 1/4/2022)