

*Trichoderma sp.**Alternaria sp.**Alternaria sp.**Fusarium sp.*

SCIENCE FOCUS AREA

Bacterial:Fungal Interactions and Their Role in Soil Functioning

Illuminating underlying mechanisms to harness their biotechnological potential

In soil microbiomes, bacteria and fungi play key roles in biogeochemical and nutrient cycling. Little is known, however, about the relationships between these microorganisms, how they operate, and how they vary under changing environmental conditions. Better understanding is key to improving the ability to predict and potentially steer the function of soil ecosystems toward enhanced productivity for sustainable energy sources and soil carbon storage.

In addressing this knowledge gap, a Science Focus Area (SFA) project, called Bacterial:Fungal Interactions (BFI) and Their Role in Soil Functioning, aims to significantly advance understanding of the diversity and breadth of BFI, as well as the range of molecules, genes, and pathways used in establishing the interactions. Led by Los Alamos National Laboratory (LANL), the SFA is supported by the U.S. Department of Energy's (DOE) Genomic Science program within DOE's Office of Biological and Environmental Research (BER).

Connecting Microbial Diversity to Phenotypic Responses

The BFI SFA's goals are to investigate microbial interactions at a fundamental level, to establish the types of interactions both phylogenetically and functionally, and to characterize these interactions at the molecular level.

Ultimately, the insights gleaned into the mechanisms underlying BFI will inform predictive models of their function in soil and their responses to environmental perturbations, such as changing climate and agricultural practices. Such model development could then lead toward exploiting resident bacterial-fungal networks for ecosystem steering.

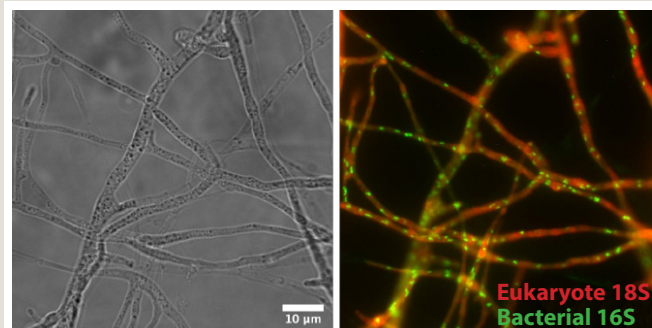
Leveraging Multiple DOE Resources

In this study, SFA researchers are combining advanced microbiology with state-of-the-art omics and bioimaging technologies, the



Symbiotic Interactions.

Fungi and their associated bacteria establish a symbiosis with plant roots (e.g., arbuscular mycorrhiza and ectomycorrhiza, as shown) that is essential for plant survival and regulation of plant productivity.



Endobacterial Subways. (Left) Confocal microscopy imaging of endobacteria in fungal mycelia. (Right) Stained with fluorescence in situ hybridization (FISH), using a universal probe targeting the 16S rRNA (green), co-stained with a probe targeting fungal 18S rRNA (red).

Key SFA Research Questions

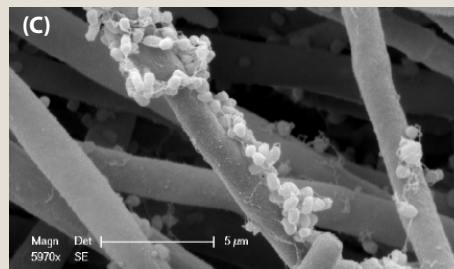
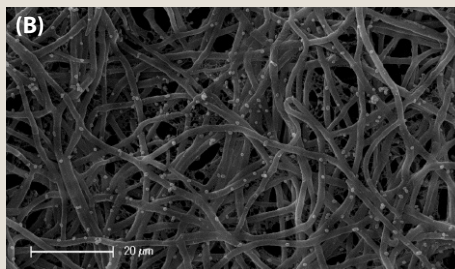
The SFA's central hypothesis is that BFI affect the flux of nutrients within and between cells, organisms, and ultimately the soil ecosystem. Guiding research questions are organized under four assumptions:

- **There is a diverse fungal microbiome (bacteria associated with fungi).** What is the diversity of bacterial:fungal relationships and of the fungal microbiome? Are there specific bacterial and fungal genetic determinants that lead to positive or negative interactions?
- **Molecular signaling and cascades drive intracellular BFI. What are the consequences of bacterial endosymbiosis on fungal fitness?** Can fungi survive and thrive in a changing environment without endobacteria? How do bacteria enter fungal cells? What are the specific metabolic signals and the underlying genetic controls regulating these interactions under changing environmental conditions? What cues are involved for recognition of partners?
- **Molecular signaling and cascades drive specific extracellular BFI.** Which physiological and structural features are characteristic of extracellular BFI? Can any bacterium form such associations? How do molecular effectors differ between intra- and extracellular BFI?
- **Complex behavior is an outcome of these interactions.** How do BFI regulate the exploitation and transfer of nutrients? What is the fungal soil interactome (molecular components dictating the interaction)? What is the role of fungus-driven bacterial dispersal in the structure and differentiation of the soil microbiome?

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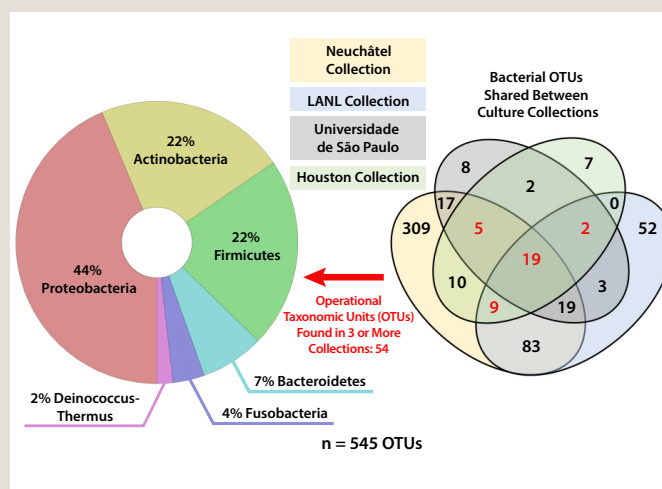
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Fungal Highways. The unsaturated and complex three-dimensional nature of soils restricts bacterial activity and dispersal. In these conditions, some bacteria engage with filamentous fungi in cooperative interactions, using the fungal hyphae to disperse. Similar to a highway interchange (A), these interactions are referred to as “fungal highways” as seen in (B) and further magnified view in (C). [Image A courtesy Kevin Payravi, Wikimedia Commons. Image B courtesy Anael Simon, University of Neuchâtel. Image C reprinted by permission from Simon, A., et al. 2015. “Exploiting the Fungal Highway: Development of a Novel Tool for the In Situ Isolation of Bacteria Migrating Along Fungal Mycelium,” FEMS Microbiology Ecology 91(11). Copyright 2015, Oxford University Press.]

development of novel bioinformatics algorithms, and application of novel spatial modeling methods. A LANL exascale computing partnership with DOE’s National Energy Research Scientific Computing Center (NERSC) and Joint Genome Institute (JGI) is conducting a full reanalysis of available fungal and metagenomic data to identify sequence signatures and possibly gene functions relevant to bacterial and fungal interactions. Tools from DOE’s Systems Biology Knowledgebase (KBase) are being utilized, and, in return, novel software developed by the BFI SFA is being integrated into KBase.



Fungal Microbiome. A phylogenetically broad range of fungal samples from various culture collections was screened for bacterial signals. The results showed Proteobacteria (44%), Actinobacteria (22%), and Firmicutes (22%) as the most frequently detected across all examined isolates.

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Coming soon: lanl.gov/science-innovation/science-programs/office-of-science-programs/biological-environmental-research/sfa-bacteria-fungal.php

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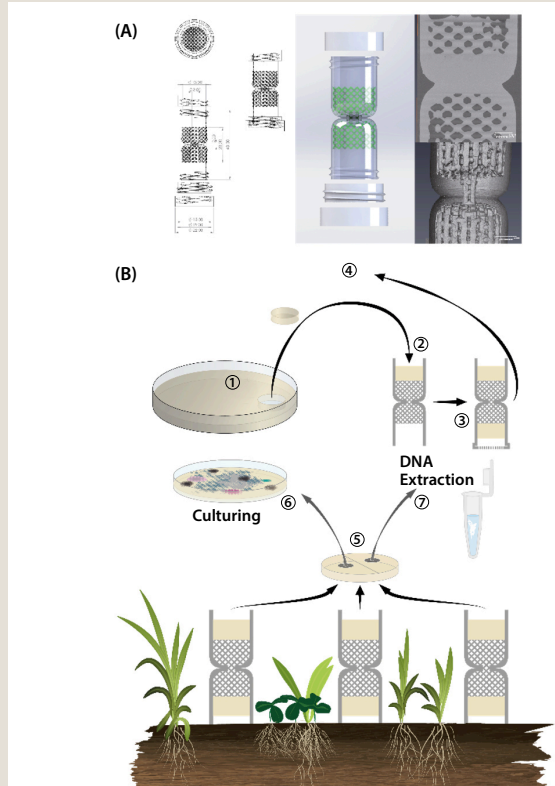
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Three-Dimensional (3D) Fungal Highway Columns. (A) Schematic representation of a novel, 3D-printed sampling device. (B) Once printed, the device can be autoclaved and any kind of agar-based medium (1) can be placed on the top (2) and on the bottom (3) as target and attracting media, respectively. Once a filtering mesh is placed in direct contact with the attracting medium, the device can be placed on the environmental sample (4). After incubation, the target medium (5) can be collected and used to isolate bacteria and fungi by re-culturing (6) or for direct DNA extraction (7).