**Using Gel Microdroplets to Investigate Bacterial Influence on Fungal Spore Germination**

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

**Project Goals: The vast taxonomic diversity and the complexity of interactions within the soil microbiome present a unique challenge. Many of the interactions between soil-dwelling bacteria and fungi are not yet well understood, and a more comprehensive understanding would lead to substantial agricultural, environmental, and energy-focused advancements. These potential developments align with the focus areas of the DOE, and would influence multiple scientific fields. The aim of this Science Focus Area (SFA) is to better understand the diverse and abundant interactions within the soil rhizosphere, specifically between fungi and bacteria, and to decipher the mechanisms behind their communication. Herein, we discuss a pipeline for the effective interrogation and monitoring of bacterial:fungal partners that will provide insight into the environmental conditions and biotic associations that affect fungal spore germination.**

An understanding of the interactions between microbes inhabiting the soil rhizosphere is becoming increasingly pertinent. Bacterial:fungal interactions are at the forefront of this undertaking, specifically regarding how bacteria can promote the growth of fungal species that are used for biocontrol, and how bacteria can inhibit the growth of pathogenic fungi. Elucidating which bacteria promote or inhibit fungal spore germination would have substantial implications in understanding impacts on plant fitness, agricultural practices, and more broadly, ecosystem-level nutrient flux. In order to address this challenge, our lab has developed a pipeline to rapidly assess bacterial:fungal partners of interest during the early stages of fungal growth.

Gel microdroplet (GMD) technology was previously optimized by Los Alamos National Laboratory for use in the screening of algae, bacteria, and various microbiomes. These microsphere agarose droplets range from 20-70μm in diameter and are used to encapsulate viable cells for cultivation and downstream molecular assays. For this project, we have employed GMDs to interrogate bacterial:fungal interactions by co-capturing bacterial cells with fungal spores. These GMDs allow us to test how fungal spore germination is affected by various ratios of bacteria to fungal spores while maintaining close proximity between the captured cells and allowing for nutrient exchange. In addition, the GMDs are able to be efficiently interrogated and sorted via flow cytometry and visualized using microscopy; and finally, the GMDs have demonstrated improved genomic recovery for downstream analyses compared to traditional culturing techniques.

We have successfully co-captured several genera of fungal spores and bacteria within GMDs, and monitored their growth by flow cytometry and microscopy. We optimized the

targeted flow cytometry-based enrichment of the GMDs of interest in order to achieve an 80% success rate in isolating only the GMDs containing the desired bacterial:fungal ratios. The sorted GMDs can now be monitored either in bulk or individually on novel static culture microscopy slides. Initial experiments established that the GMDs are best suited for monitoring the early stages of spore germination, as the fungal hyphae can easily extend out of the droplets when they become too long. The ease, utility, and speed of the GMD screening process makes this pipeline an ideal candidate for observing altered fungal germination rates in the presence of various bacterial partners. This technology will be applied to fungal and bacterial partners of interest, and will be coupled with time-lapse transcriptomics to better understand the mechanisms through which bacteria can affect fungal spore germination.

*This SFA is supported under the Computational Biosciences Program of the Office of Biological and Environmental Research in the DOE Office of Science through grant DE-AC52-06NA25396*

LA-UR 19-20592