Exploring Mechanisms of Bacterial-Fungal Interactions Using *Ralstonia pickettii* Genomes Obtained from Diverse *Monosporascus* Isolates

Aaron J. Robinson[1,2*] (arobin@lanl.gov), Donald O. Natvig,[2] Demosthenes Morales,[1] Julia M. Kelliher,[1] La Verne A. Gallegos-Graves,[1] Karen W. Davenport,[1] and **Patrick S. G. Chain**[1]

[1]B-10 Biosecurity and Public Health, Los Alamos National Laboratory, Los Alamos, New Mexico; [2]University of New Mexico, Albuquerque

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

Project Goals: The vast taxonomic diversity and the complexity of interactions within the soil microbiome presents many challenges. Many of the interactions between soil-dwelling bacteria and fungi are not yet well-understood, and a more comprehensive understanding of these relationships and their response to environmental pressures would lead to substantial agricultural, environmental, and energy-focused advancements. These potential developments align with the foci of the DOE, and would influence multiple scientific disciplines. 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 decipher the mechanisms behind their communication. Herein we discuss continued efforts towards establishing experimental models to examine and compare bacterial-fungal interactions.

Interactions between fungi and bacteria are both common and diverse. Descriptions of these interkingdom interactions generally fall into two categories: internal and external associations. The total number of descriptions of internal associations, where the bacterial partner is present and often maintained within cells of the fungal host, as well as the taxonomic diversity of these descriptions continue to steadily increase over time. However, the mechanisms that allow or promote these internal associations, as well as their diversity remain largely unknown due to the currently limited number of descriptions and genetic resources.

Genome sequencing results from several *Monosporascus* (Ascomycota; Xylariales) isolates obtained from the roots of plants in the Southwestern U.S. contained a substantial number of bacterial reads, despite the isolates being grown on diverse antibiotics and having been sub-cultured several times before sequencing. The majority of these sequences were classified as *Ralstonia pickettii* (Burkholdariaceae) at both the read and contig levels. Here we show fluorescence *in situ* hybridization (FISH) imaging indicates that *Monosporascus* is capable of harboring and maintaining *R. pickettii* as a bacterial endosymbiont. Attempts at isolating *R. pickettii* from these fungal hosts have been unsuccessful and the persistence of this bacteria in fungal isolates which have been maintained in culture for several years suggest an intimate relationship. The genome sequencing results for three distinct *Monosporascus* isolates each contained enough reads for the *de-novo* assembly of near complete *R. pickettii* genomes. Phylogenetic comparisons indicate that these *R. pickettii* genomes recovered from *Monosporascus* represent three distinct lineages that are closely related to previously identified environmental isolates. Broad-scale evolutionary comparisons conducted with these genome

assemblies also suggest differences both among the endosymbiotic *R. pickettii* lineages and between the fungal-derived and non-fungal-derived *R. pickettii* lineages. The observed diversity of these associations involving closely related bacterial endosymbionts recovered from closely related fungal hosts indicate this association could serve as a valuable model for studying bacterial-fungal interactions.

This work was supported by the U.S. Department of Energy, Office of Science, Biological and Environmental Research Division, under award number LANLF59T.

LA-UR-20-20697