Examinations of the Fungal Genus *Monosporascus* Reveal its Potential as an Experimental Model for Studying Bacterial-Fungal Interactions

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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.

The genus *Monosporascus* represents an enigmatic group of fungi important in agriculture and widely distributed in natural arid ecosystems. Of the eight described species of *Monosporascus*, two (*M. cannonballus* and *M. eutypoides*) are important pathogens on the roots of members of the Cucurbitaceae in agricultural settings. The remaining six species are capable of colonizing roots from a diverse host range without causing obvious symptoms of disease. To explore evolutionary relationships within the genus and gain insights into potential ecological functions, we sequenced and assembled the genomes of three *M. cannonballus* isolates, one *M. ibericus* isolate and six phylogenetically distinct New Mexico isolates. The assembled genomes were significantly larger than what was expected for the Sordariomycetes, despite having predicted gene numbers similar to other members of the Class. Differences in predicted genome content and organization were observed between endophytic and pathogenic lineages of *Monosporascus*,

The sequencing results from several of these *Monosporascus* isolates contained a significant 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. Phylogenetic comparisons indicate that the *R. pickettii* sequences found within *Monosporascus* represent multiple distinct lineages that are closely related to previously identified environmental isolates. Broad-scale evolutionary comparisons conducted with the limited sequencing data also suggest differences between the endosymbiotic and environmental *R. pickettii* lineages. The diversity