Rhizosphere Biogeography of *Brachypodium distachyon* and Microbial Plant-Growth Promoting Traits

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Project Goals: Understanding the interactions, localization, and dynamics of grass rhizosphere communities at the molecular level (genes, proteins, metabolites) to predict responses to perturbations and understand the persistence and fate of engineered genes and microbes for secure biosystems design. To do this, advanced fabricated ecosystems are used in combination with gene editing technologies such as CRISPR-Cas and bacterial virus (phage)-based approaches for interrogating gene and microbial functions *in situ*—addressing key challenges highlighted in recent DOE reports. This work is integrated with the development of predictive computational models that are iteratively refined through simulations and experimentation to gain critical insights into the functions of engineered genes and interactions of microbes within soil microbiomes as well as the biology and ecology of uncultivated microbes. Together, these efforts lay a critical foundation for developing secure biosystems design strategies, harnessing beneficial microbiomes to support sustainable bioenergy, and improving our understanding of nutrient cycling in the rhizosphere.

Abstract:

The rhizosphere is a critical interface between plants and microbial communities that mediates ecosystem processes such as nutrient cycling and carbon stabilization. Plants exude a variety of nutrient-rich compounds (exudates) that maintain a selection of microbes from the surrounding soil environment. The rhizosphere microbial community, in turn, can directly influence plant growth and development through plant-growth promoting and nutrient scavenging functions. We assessed the distribution of microbial communities in the rhizosphere (biogeography) of microbes colonizing the roots of *Brachypodium distachyon* in fabricated ecosystem devices (EcoFABs) as a result of spatially-distinct root exudation. In addition, we also examined key rhizobial isolates for their plant-growth promoting traits.

Distinct parts of the root can vary in exudation patterns. Specifically, fine roots, occurring at the tips of the root system, have higher exudation rates than coarser roots that occur near the base. However, the influence of these spatial patterns on the biogeography of rhizosphere microbial communities is not well understood. We analyzed the microbial community of *Brachypodium distachyon* at two distinct areas: roots collected at the tips of the primary root and roots collected at the base. Furthermore, we grew plants in unamended natural soil and compared results among plants grown in standardized fabricated ecosystems known as EcoFABs, as well as

in traditional pots and test tubes. Our results showed similar microbial community distributions across the different growth chambers and a higher degree of reproducibility within EcoFAB grown plants. We were also able to enrich for less characterized lineages such as *Verrucomicrobia* and *Acidobacteria* in addition to *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Genome-resolved metagenomics indicate higher abundances of biofilm formation, flagellar assembly, and simple sugar transporter genes in microbial communities from the base and root tips in comparison to bulk soil. Furthermore, bulk soil and plant-base microbiomes were highly abundant in genes related to prokaryotic defense and transcription.

Next, we used rhizosphere samples from each growth chamber to enrich for rhizobacteria on single and mixed carbon media to examine the influence of carbon source on microbial community composition. We used media that reflected known *B. distachyon* exudates (i.e., glutamine, succinate, and asparagine) and sequentially transferred cultures every three days into fresh media to form reduced complexity communities amenable to targeted rhizosphere assembly studies. Our results indicate that different carbon sources resulted in the enrichment of differing abundances of genera. For example, we found that cultures from glutamine and succinate contained higher abundances of *Burkholderia* and *Enterobacter* but that mixed carbon sources such as R2A yielded smaller abundances of each but with a higher diversity of genera present. Furthermore, we measured key plant-growth promoting traits in axenic isolates obtained from these enrichments. Initial assessment of indole-3-acetic acid (IAA) phytohormone production indicated that all of our recovered isolates are capable of producing auxin to some degree (0.3 µg IAA ml^{-1} to 10.54 µg IAA ml^{-1}). Further assays will include measuring other plant-growth promoting traits related to nutrient acquisition such as phosphorus solubilization and siderophore production.

Our research reveals patterns in the distribution of bacteria and gene abundances in different areas of the root. Furthermore, we showed the comparable performance of EcoFAB devices with regards to traditional plant growth chambers and also their higher reproducibility in plant-microbial studies. Lastly, reduced complexity communities formed using sequential transfers in media containing single and mixed known *B. distachyon* exudates indicate a common ability to produce indole-3-acetic acid, a phytohormone known to induce growth in plant roots.

Funding statement.
This material by m-CAFEs Microbial Community Analysis & Functional Evaluation in Soils, (m-CAFEs@lbl.gov) an SFA led by Lawrence Berkeley National Laboratory is based upon work supported by the U.S. Department of Energy, Office of Science, Office of Biological & Environmental Research under contract number DE-AC02-05CH11231