**Bioinformatic Guided Enrichments to Develop Representative Rhizosphere Communities**

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http://mCAFEs.lbl.gov

**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

We aim to understand rhizosphere microbial communities at sufficient molecular resolution to predict responses to environmental and genetic perturbations. To this end, representative microbial enrichments and isolates are critical to facilitate downstream investigations with gene editing and computational models. In m-CAFEs, we are leveraging our expertise in metagenomics and fabricated ecosystems across labs to guide our enrichment strategies to obtain ecologically relevant yet challenging-to-cultivate taxa that are important to plant-microbe interactions in the rhizosphere.

We used soil from the rooting zone (0-10cm) at Angelo Coast Range Reserve, a well-studied field-site showing distinct microbial processes across depth[1], to grow the model grass *Brachypodium distachyon* in EcoFABs. EcoFABs are fabricated ecosystems designed to investigate the molecular basis of microbial interactions with plants (see Zhalnina *et al.* poster). In parallel, we also grew the same plant in conventional growth systems (i.e. pots, tubes) to assess the impacts of growth chambers on plant growth. After 2 weeks of incubation, 16S rRNA amplicon analysis of the microbial community revealed that the rhizosphere community was enriched in members of *Actinobacteriota, Bacteriodota,* and *Firmicutes* across all growth systems. This represents the community possibly enriched from root exudates and further guides our enrichment strategies.

We are using an enrichment strategy that focuses on reproducing geochemical properties of the Angelo soil and employing carbon sources representative of rhizosphere environments, to mimic environmentally relevant conditions. These enrichments are used to generate reduced complexity consortia that are stable and reproducible and strain isolation (particularly when novel microorganisms are enriched), and targets for genetic manipulation using m-CAFEs genetic tools. To increase the probability of novelty and diversity for editable targets, native soil was used as inoculum directly from the field-site to initiate enrichments. We leveraged metagenomic insights from Angelo soil to select a wide variety of enrichment substrates.. Our preliminary results revealed stark differences corresponding to different carbon sources and we aim to expand our enrichments to also include different incubation conditions beyond carbon sources such as pH and salinity.

Finally, in our third approach, we collaborated with scientists from the DOE Joint Genome Institute to use the GALT prospector platform[2] with the same Angelo soil. Using commercially available media, we were able to isolate field-abundant yet rarely cultivated taxa such as *Acidobacteria* and *Gammatimonadetes* using this massively parallel approach.

These enrichment approaches we have employed are distinct yet complementary to obtain multiple relevant rhizosphere microbial communities and isolates which are now in the pipeline to be tested for phenotypic impacts on plants (see Lin *et al.* poster) and are prime candidates for metabolic modeling (see Dukovski *et al.* poster) as well as for gene editing (see Diamond *et al.* poster).

**References**

1. Diamond, Spencer, et al. "Mediterranean grassland soil C–N compound turnover is dependent on rainfall and depth, and is mediated by genomically divergent microorganisms." *Nature microbiology* 4.8 (2019): 1356-1367.
2. https://www.galt-inc.com/

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