Examining Molecular Mechanisms Selecting for Rhizosphere Bacteria

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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 plant rhizosphere microbiome is both dynamic and complex, with many host and environmental factors governing its assembly and function. Here, we explored the assembly of the model plant *Brachypodium distachyon* root microbiome over time using a synthetic community (SynCom) of 17 microbes. Using 16S ribosomal sequencing, we demonstrate that both time of inoculation (0, 3, 8 days after germination) and duration of the experiment (14, 24 and 29 days) affected microbiome composition in fabricated ecosystems (EcoFABs). Next, we tested the role of root exudates and root glycans in this process. This was initially done by mapping the colonization pattern of a fluorescently tagged member of the SynCom, *Rhizobium* OAE497, in the *B. distachyon* plant mutant PMT that over expresses *p*-Coumaroyl-CoA:monolignol transferase (Petrik et al. 2014) and has an altered root cell wall composition. Further, we discovered a potential role for several plant cell wall-derived glycans in enriching for novel microbes that have proved challenging to culture, including members of the *Acidobacteria,* to develop reduced complexity microbial communities. Finally, we identified specific microbial genes and pathways responsible for colonization under multiple growth

conditions through use of an RB-Tnseq mutant library developed in another SynCom member, *Burkholderia OAS925*. Collectively, these orthogonal approaches deepen our knowledge of the genetic and metabolic basis of rhizosphere assembly.

References

Petrik DL, Karlen SD, Cass CL, et al. p-Coumaroyl-CoA:monolignol transferase (PMT) acts specifically in the lignin biosynthetic pathway in Brachypodium distachyon. *Plant J*. 2014;77(5):713-726. doi:10.1111/tpj.12420

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