Title: Spatially-resolved Multi-omics Analyses Reveal Key Taxa Responding to Root Depth in the Sorghum Rhizosphere

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Project Goals: The Pacific Northwest National Laboratory Persistence Control Scientific Focus Area aims to gain a fundamental understanding of factors governing the persistence of engineered microbial functions in rhizosphere environments. From this understanding, we will establish design principles to control the environmental niche of native rhizosphere microbes. In our first funding period, we are examining the efficacy of genome reduction and metabolic addiction to plant root exudates in environmental isolates as persistence control strategies using the bioenergy crop sorghum and defined microbial communities as a model ecosystem. Effective persistence control will lead to secure plant–microbe biosystems that promote stress-tolerant and highly productive biomass crops.

Abstract: The rhizosphere, the zone of soil influenced by plant roots, is closely associated with a complex microbial community of bacteria, archaea, viruses, and fungi[1]. Using a correlative surface imaging approach with *Brachypodium distachyon*, a genomics model for grasses[2], we have demonstrated that the root surface is metabolically heterogeneous with hot spots for bacterial attachment[3]. Recently, we developed a 3D root cartography platform to map root exudate metabolites and microbes onto a 3D image of the root, generated by X-ray computed tomography (XCT)[4]. The aims of this ongoing experiment are to 1) map the distribution of exuded metabolites and the soil microbiome in the 3D space of the sorghum root and rhizosphere, 2) examine if there are hotspots for microbial colonization, and 3) determine if these hotspots correlate with exudation of specific metabolites.

We have employed this multi-omics and imaging platform to collect spatially resolved root morphology, metabolite, and microbial species data for the bioenergy crop *Sorghum biocolor* (L.) Moensch. We collected XCT root profiles, metabolomics data, and 16S microbial profiles for three plants grown in soil collected from the Prosser, WA field site administered by the Soil Microbiome Science Focus Area at PNNL. While an integrated analysis linking spatial root morphology to metabolomic and taxonomic signatures remains in progress, we applied a network approach to the 16S taxonomic data to gain a more complete view of the role of individual taxa within the root microbial. Using amplicon data from three plants and across a profile of eight root depths we inferred a species co-abundance network and interrogated this network to identify which taxa occupied important and central positions and which taxa may shift their importance specifically as a function of depth. In a complete network of all data

Proteobacteria, Crenarchaeota, Bacteroidota and Firmicutes shows the greatest number of links to other taxa while Bdellovibrionota and Myxococcota occupied positions as bridge taxa, linking other larger groups of species. When removing data from the lowest depths and remaking the network we found that certain taxa exhibited drops in their centrality suggesting that they may be crucially important specifically at lower depths. Taxa that may be critical at lower depths included Chloroflexi and Gemmatimonadota. In contrast, Myxococcota showed an increase in its centrality when depth data was removed, suggesting it may be more important at shallow depths. This network analysis will be critical in future studies that seek to better understand both beneficial and antagonistic interactions between root microbiomes and how these interaction networks shift as a function of depth.

We anticipate this research will enable testing our hypothesis of community complementation for genome-reduced and, thus, niche-restricted biocontainment strains. Specifically, we hypothesize that species with the capacity for genetic or metabolic complementation are more likely to complement lost functions of niche-restricted strains when physically proximal in the rhizosphere. To test this hypothesis, we will assess the abundance of engineered strains directly in the sorghum rhizosphere that contains simplified microbiome communities.

References/Publications

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