

Harvesting the Root Microbiome of Grasses toward Sustainable Increases in Crop Production

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Project Goals:

The global demand for increased food commodities and energy supply highlight the necessity to further improve crop productivity. A variety of plant functions and traits are co-dependent on the microbial communities that exist within and around them. Characterizing, manipulating and /or mimicking the plant microbiota provide a promising path toward increasing crop production. The extent to which the host selects its root microbial community remains poorly understood, especially across vastly divergent species. Grasses constitute our main source of food, feed, and bioenergy, but how their microbiomes relate to, or may differ from other plant microbiomes remain unclear.

In order to determine the influence of plant host species on the assembling of both the rhizospheric (RZ) and endophytic (EC) microbiome, we monitored the bacterial root microbiome of *Arabidopsis thaliana* Col-0, *Brachypodium distachyon* Bd21 and *Setaria viridis* A10-1 through 16S amplicon sequencing. For a rigorous comparison, all three species were grown under identical conditions in the same wild soil. We observed, as expected, that bacterial communities vary mainly according to plant compartment (rhizosphere or endophytic compartment [EC]) and soil digs, and that all three plant species assemble an extremely similar bacterial endophytic communities. However, unweighted UniFrac distance analysis indicates that *Brachypodium distachyon* (Bd) and *Setaria viridis* (Sv) share a slightly more similar microbial EC communities compared to *Arabidopsis thaliana* (At). The *Brachypodium* EC compartment was especially marked by the presence of *Tenericutes*. The relative abundance of *Tenericutes* was much lower in *Setaria* and null in *Arabidopsis*. In an attempt to refine the variation of the bacterial root microbiome across species from the phylum level to the Operational TaxonomicUnit level, we established a stringent regularized generalized linear model to identify differentially abundant (DA) OTUs across species, and across compartments. One fourth of the DA OTUs identified were assigned to the *Anaeroplasmatales* order, which belongs to the phylum *Tenericute*, and these were found primarily more abundant in Bd_EC samples when compared to At_EC samples, thus confirming the robustness of our model. Most of the non-redundant DA OTUs identified in our data set are of very low abundance. Our result indicate that the bacterial EC_root microbiome across plant species, even widely divergent species is highly similar at the 16S level and that the minor differences observed are mostly attributed to low

abundance OTUs. In addition, we are conducting in parallel fungal censuses as fungal communities associated with plant roots are also central to the host health, survival and growth. Fungal and bacterial communities most likely act in concert to influence plant health and development. Interestingly, the *Tenericute* OTUs detected in our 16S census cluster with sequences from Mollicutes-related bacteria (Mre) known to associate with arbuscular mycorrhizal fungus (AMF), and are mainly absent in *Arabidopsis* samples, in accordance with its lack of association with AMF.

We determined that the root microbiome is relatively conserved across plant species however it remains to be assessed if the microbiome may also present functional conservation across species. The Dangl lab has previously isolated ~600 bacterial strains from the rhizoplane and endophytic compartments of *Arabidopsis thaliana* and evaluated the effect of these isolates on plant health under various starvation stresses. Isolates shown to promote growth of *Arabidopsis* or alleviate nutrient stress response are being tested on *Setaria viridis*. However, our root-associated bacterial isolate collection was derived from plants grown in soils with relatively high nutrient values. To better the relationship between the microbiome and the plant nutritional stress response, and increase our odds to identify bacterial strains promoting plant health under nutrient stress we established a field experiment in which *Setaria* was grown under low and high Nitrogen side by side with various *Sorghum* accessions. The root (rhizoplane + EC) and, rhizosphere of those plants, as well as bulk soil, have been collected for bacterial isolation and 16S census.

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