

Bacterial Traits Linking *Avena* Exudate Chemistry and Rhizosphere Bacterial Community Structure During Root Development

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Project Goals: Plant roots are a major source of soil organic carbon, and the rhizosphere is a hotspot of biological activities mediating multiple nutrient cycles. Plant roots exude varying combinations of organic substrates into soil, including amino acids, sugars, organic acids, fatty acids and secondary metabolites. The rhizosphere of growing plant roots is a dynamic interface for complex rhizospheric interactions, involving both one-to-one interactions and multitrophic interactions. Growing plant roots alter soil bacterial community composition by selecting a subset of bacteria from surrounding soil. Our study site, the Hopland UC research field station in Northern California, is a semi-arid annual grassland that has been dominated by *Avena* plants for over one hundred years, and thus has long history of rhizospheric interactions associated with *Avena* roots. Many studies have demonstrated the selection of soil bacterial taxa by growing plant roots and have elucidated the role of individual exudate compounds. However, we still lack clear understanding on how the changing chemistry of plant root exudates interacts with bacterial traits such as substrate utilization preferences and growth strategies to shape bacterial communities in the rhizosphere.

Mapping metagenomes from the *Avena* rhizosphere and bulk soil during plant developmental stages to the genomes of thirty nine bacterial isolates and ninety seven genome bins assembled from the soil metagenomes, we classified bacterial isolates and genome bins into three groups representing positive, negative and neutral responders to root growth. Comparative analyses of these genomes was then used to identify bacterial traits that may be essential for growth and survival in the rhizosphere. This demonstrated that multiple genes involved in amino acid, sugar and sulfonate transport, flagellar protein assembly and function, and chemotaxis were more abundant in bacteria that responded positively to growing *Avena* roots. Fragment recruitment of metagenome reads to isolate genomes was used to identify strain variation and relate recovered isolates to their metagenome counterparts and showed that closely related strains appeared to have divergent responses to root growth. To investigate this we compared the genomes of three closely related *Bradyrhizobium* isolates that exhibited differential responses to root growth (positive or neutral) and highlighted regions unique to the positive responder. These encoded genes for amino acid and carbohydrate transporters, genes coding for xylanase/chitin degradation, and chemotaxis related proteins amongst others, demonstrating that the acquisition of a relatively small number of genes may result in niche optimization and improved fitness.

Exometabolite analyses were used to identify how root exudates changed across *Avena* developmental stages, and demonstrated that exudate chemistry could be classified into three major patterns of exudation corresponding to early developmental stage, vegetative stages and senescence. Further exometabolomic analyses defined the exudate utilization preferences of bacterial isolates and demonstrated that positive, negative and neutral responders to root growth had distinct preferences for specific components of root exudates. Positive responders showed higher uptake of organic acids, and amino acids that corroborated the greater number of transporters for these substrates encoded in their genomes. In particular, aromatic organic acids including indole-3-acetic acids, salicylic acid, cinnamic acid and nicotinic acid were primarily consumed by the positive responders to root growth, suggesting a significant role of these metabolites in shaping the rhizosphere microbial community. Further work is being carried out to confirm the genetic basis and generality of these rhizosphere traits.

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