Identifying microbial traits driving microbial succession and C transformation in the rhizosphere of *Avena sp.*

Heejung Cho[1,2*] (beeologai@berkeley.edu), Kateryna Zhalnina,[2] Ulas Karaoz,[2] Shengjing Shi,[1,3] Benjamin P. Bowen,[2] Richard A. White III,[4] **Mary S. Lipton,[4] Trent R. Northen,[2] Mary K. Firestone,[1,2]** and **Eoin L. Brodie[1,2]**

[1]University of California, Berkeley; [2]Lawrence Berkeley National Laboratory, Berkeley, California; [1,3]Lincoln Science Centre, AgResearch Ltd, Christchurch, New Zealand; [4]Pacific Northwest National Laboratory, Richland, Washington

Our project is designed to advance our understanding of the complex interactions controlling C flow in the rhizosphere by addressing two fundamental topics: 1) How multi-trophic interactions control soil C dynamics, and 2) How changing precipitation regimes alter these interactions, and thus impact flow and fate of soil C.

Plant-soil-microbial interactions strongly impact the dynamics of soil organic carbon (SOC). Plants exude a variety of compounds, supplying rhizosphere microorganisms with readily assimilable substrates and driving microbial succession in response to developing plant roots.

In this study, we examined succession of rhizosphere bacteria throughout *Avena sp*. developmental stages using metagenomic approaches, isolation and metabolomics. To determine the changes in bacterial abundance during root growth, metagenome and 16S rRNA gene reads from rhizosphere and bulk soils during *Avena* developmental stages were mapped to the genomes of thirty-nine bacterial isolates that are phylogenetically representative of Hopland soils and numerically abundant, and to fifty-five moderately complete metagenome-derived genome bins. We classified these bacteria into three different groups based on response in abundance to root growth (positive, negative and no response). We used comparative analyses of these genomes to identify bacterial traits that may contribute to higher abundance in rhizosphere.

Each genome was analyzed for key traits related to C transformation and survival in rhizosphere, such as minimum generation times, polymer degrading enzymes and transporters for different substrates (amino-, fatty-, organic-acids, sugars, nucleotides and plant hormones). We determined that genomes present in bacteria responding positively to root development were slower growers compared to the bacteria with negative responses. Positive responders demonstrated higher numbers of organic and amino acid transporters present in their genomes and fewer genes involved in decomposition of polymeric C. Additionally, multiple functional genes involved in sugar and fatty acid transport, bacterial secretion systems, sugar, amino acid, and organic acid metabolism, motility, pilus formation, and ecdyson metabolism were more abundant in bacteria with positive response to root growth.

We compared closely related *Bradyrhizobial* isolates and genome bins that presented positive, negative and no responses to root development. Genome comparisons among *Bradyrhizobium* isolates and genome bins showed that a *Bradyrhizobium* isolate with higher abundance in rhizosphere had sugar, plant cell-wall driven polysaccharides, amino acids, organic acids and lignin metabolism genes, bacterial secretion system genes, antibiotics exporter genes, and papain-like cysteine protease genes that are absent in *Bradyrhizobial* isolates and genome bins with negative or no response to root development. The presence of these functional genes may play a role in niche optimization and improved fitness, thus shaping rhizosphere bacterial succession.

To investigate the relationship between *Avena* exudates and rhizosphere bacteria and to link identified rhizosphere traits to root chemistry we first analyzed exudate composition of hydroponically-grown plants using LC-MS/MS based metabolomics. We then designed a medium to simulate plant exudates and using this medium we examined the substrate preferences of rhizosphere isolates.

The major fraction of plant exudates was found to be composed of amino- and carboxylic acids, sugars, nucleosides, released at vegetative growth stages and quaternary amines and plant hormones exuded at latter stages of *Avena* growth. Amino acids, sugars and nucleosides were consumed by all analyzed isolates. However, isolates that were preferentially stimulated by plant growth, positive responders, revealed substrate utilization preferences towards aromatic organic acids, such as salicylic, cinnamic, nicotinic, indole-acetic while those not responding to growing roots did not utilize these compounds.

To confirm the genome-predicted trait of rhizosphere-negative isolates more efficiently utilizing polymeric C compared to the positive responders, we evaluated polymeric substrate preferences and enzymatic activities of different isolates using nanostructure initiator mass spectrometry-based enzyme activity assay (Nimzyme) and secretome analysis. Rhizosphere-negative isolates demonstrated higher ability to degrade polymers compared to the rhizosphere-positive bacteria, which revealed monomeric molecules as the preferred source of C.

Together these approaches are being used to predict and test how key traits of soil bacteria interact with root metabolism and soil organic matter to impact C flow in the rhizosphere.

This research is based upon work supported by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under Award Numbers DE-SC0010570 and DE-SC0016247 to UC Berkeley. Part of this work was performed at Lawrence Berkeley National Laboratory under the auspices of the U.S. Department of Energy under Contract DE-AC02-05CH11231 and a portion at the Environmental Molecular Sciences Laboratory (EMSL) sponsored by OBER and located at PNNL under Contract DE-AC05-76RLO 1830.