Title: Understanding microbial establishment in the rhizosphere using quantitative trait-locus mapping and CRISPR Cas editing

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Project Goals: The Secure Ecosystem Engineering and Design (SEED) Science Focus Area (SFA), led by Oak Ridge National Laboratory, combines unique resources and expertise in the biochemistry, genetics, and ecology of plant-microbe interactions with new approaches for analysis and manipulation of complex biological systems. The long-term objective is to develop a foundational understanding of how non-native microorganisms establish, spread, and impact ecosystems critical to U.S. Department of Energy missions. This knowledge will guide biosystems design for ecosystem engineering while providing the baseline understanding needed for risk assessment and decision-making.

Abstract Text:

Plant-microbe interactions in the rhizosphere are essential to ensure plant health and productivity. Recently, commercial biofertilizers and biopesticides have become available to enhance plant growth and pest resistance. However, these products have inconsistent performance as the microbes present in these products fail to survive and proliferate when competing against native microbes present in the rhizosphere. Bacteria have multiple traits encoded in their genome that provide them with advantages or disadvantages under different field conditions. For this reason, we need to investigate the genetic determinants for microbial establishment and persistence in the rhizosphere at a genome-wide scale.

In this project, we are applying two high-throughput methods, bacterial genome shuffling and CRISPR-mediated genome editing, to interrogate bacterial genotype-phenotype relationships. We use as a model system the bacterium *Bacillus velezensis,* a sporulating Gram-positive bacterium that has been shown to promote plant growth by secreting beneficial secondary metabolites and acting as antagonist of pathogenic fungi.

First, we are using genome shuffling to recombine isolates of *B. velezensis* and construct a strain panel for bacterial quantitative trait-locus (QTL) mapping. We have demonstrated genome shuffling in *Bacillus velezensis* FZB42, an important and robust root colonizer, and are extending this approach to investigate recombination between strains of *B. velezensi*s. Based on greenhouse studies conducted in the SEED SFA, we are prioritizing parental *B. velezensis* strains with variable plant phenotypes and developing genetic tools for these strains to enable shuffling.

Our second approach uses CRISPR-Cas mediated genome editing to obtain genome-wide knockout/knockdown libraries. By knocking out/down different genes, we can study how the loss of a function impacts microbial interactions with plants and the environment. We are validating Cas9 and dead Cas9 (dCas9) library generation in the Gram-positive model organism *B.*

subtilis using a library targeting 10-15 gRNA per annotated coding region. These experiments will be used to train a computational model of CRISPR Cas efficiency to predict successful gRNA integration and cutting in other bacterial genomes. In parallel, we are expanding the genetic tools available for *B. velezensis* by improving transformation efficiency via natural competency and electroporation. We are also characterizing genetic parts (e.g., promoters, origins of replication, etc.) to engineer a robust Cas9, dCas9, and gRNA expression system in this non-model organism.

Genome-scale genetics in *B. velezensis* will improve our understanding of the genetic factors affecting microbial establishment, persistence, and functionality. This information will be helpful to predict the effect of biostimulants on plant health and productivity.

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