Title: Bacterial quantitative trait-loci (QTL) mapping – a novel method for identification of genetic determinants affecting establishment of allopatric bacteria

Authors: Delyana P. Vasileva[1*] (vasilevadp@ornl.gov), Leah H. Burdick,[1] Jared C. Streich,[1] Dawn M. Klingeman,[1] Hari B. Chhetri,[1] Daniel A. Jacobson,[1] Joshua K. Michener,[1] and **Paul E. Abraham**[1]

Institutions: [1]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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 tissues are intimately associated with hundreds of thousands of microbes. Members of these microbial communities can profoundly affect plant health and microbiome manipulation has the potential to improve the productivity of biomass plantations. However, microbial inoculants often perform inconsistently in field settings because the newly introduced bacteria fail to survive and proliferate in the presence of the plant and associated microbiome. These failures are attributed to poor understanding of the complex genetic networks and environmental factors involved in microbial establishment. Strains of *Bacillus velezensis* provide a good model system to study this process because they stimulate growth of a broad array of plant species and are currently available as commercial root and foliar inoculants.

To study the genetic architecture of *B. velezensis* establishment in plant ecosystems, we are using a novel genetic method, bacterial QTL mapping. QTL mapping techniques, which rely on crosses between distinct strains or lines, have a long history as powerful tools to untangle the genetic basis of complex phenotypes in eukaryotes. However, the lack of native systems for sexual recombination has prevented the use of this method for similar analyses in bacteria. Our approach uses genome shuffling by protoplast fusion to remove the barriers to recombination on a genome scale between genetically diverse bacterial cells.

As a proof-of-concept, we demonstrated the capacity of this method to produce QTL populations in *B. subtilis* where protoplast generation has been well characterized. Using *B. subtilis* strains with different genetic distances as parents, we showed that a single round of shuffling produces multiple random recombination events across the genome. Recursive protoplast fusion generated further genetic diversity. The resulting libraries of shuffled strains can be sequenced and phenotyped in a high-throughput manner to map causal genetic variants. We are currently expanding this approach to *B. velezensis* strains to identify genetic variants that correlate with successful establishment in plant-associated communities. This work will allow predictive control of microbial establishment, engineering of plant-microbe systems with optimized performance, and risk mitigation in the use of microbial inoculants.

Funding statement: The Secure Ecosystem & Engineering Design Scientific Focus Area is sponsored by the Genomic Science Program, U.S Department of Energy, Office of Science, Biological and Environmental Research, under FWP ERKPA17. Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract no. DE-AC05-00OR45678. This program is supported by the U. S. Department of Energy, Office of Science, through the Genomic Science Program, Office of Biological and Environmental Research, under FWP ERKP123.