Title: Identifying persistence control strategies in plant-growth promoting soil bacteria with RB-TnSeq, CRISPRi, and proteomics

Authors: Aaron J. Ogden[1*] (Aaron.Ogden@pnnl.gov), Ritu Shrestha[1*] (ritu.shrestha@pnnl.gov) Joshua R. Elmore[1], Valentine V. Trotter[2], Adam M. Deutschbauer[2], **Robert G. Egbert**[1]

Institutions: [1]Pacific Northwest National Laboratory, Richland, WA. [2]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA

Website: https://genomicscience.energy.gov/research/sfas/pnnlbiosystemsdesign.shtml

Project Goals: The Persistence Control Science Focus Area at PNNL is focused on developing fundamental understanding of factors governing the persistence of engineered microbial functions in rhizosphere environments. From this understanding, we will establish design principles to control the environmental niche of native rhizosphere microbes for the model bioenergy crop sorghum through data-driven genome reduction and engineered metabolic addiction to plant root exudates. These principles will lead to secure plant–microbe biosystems that promote secure, stress-tolerant, and highly productive biomass crops.

Abstract text: Microbial amendments to agricultural soil is becoming an increasingly common strategy to improve crop yield, stress resilience, and carbon sequestration. To prevent escape of these microbes or their genetic contents into unintended agricultural, ecological, or industrial systems, the scientific community requires a better understanding of which genes are dispensable via genomic reduction to limit their ability to grow outside of their intended niche. We hypothesize that identification and genomic reduction of genes essential for growth on common rhizosphere carbon- and nitrogen-sources will result in bacterial strains with novel increased niche-specificity, and are therefore more likely to grow only in their intended environment (e.g. in association with a desired plant). To identify these niche-specific genes and validate their necessity in multiple environments we have implemented LC-MS/MS protein profiling, Random Barcoded Transposon-Sequencing (RB-TnSeq)[1] and CRISPR interference (CRISPRi)[2] using two model plant-growth-promoting soil bacteria, *Pseudomonas fluorescens* SBW25 and *Pseudomonas putida* KT2440. We first identified proteins that are differentially regulated in both strains using protein profiling upon growth in a defined lab media (M9), soil extract (SE), and soil. Our results indicate that each media type results in a unique protein-level metabolic response. However, we also demonstrated a strong correlation between proteins that change significantly during growth in SE and soil, suggesting SE is an appropriate surrogate for soil phenotypes. Further, we showed that KT2440 and SBW25 protein-level responses to both SE- and soil-growth are similar, suggesting both organisms adapt similarly to each environment. We also identified multiple proteins associated with nitrogen and central carbon metabolism were significantly altered in both SBW25 and KT2440 in SE and soil.

We employed RB-TnSeq, a technique that allows rapid and high-throughput screening of mutant populations, to identify genes required for utilization of rhizosphere-relevant carbon and

nitrogen sources, as well as for adaptation to common abiotic soil conditions (e.g. temperature stress). Media types chosen for RB-TnSeq include a defined lab media (RCH2), SE, and soil supplemented with different carbon, and nitrogen sources found in the rhizosphere. Of particular importance is N-acetylglucosamine (GlcNAc), an abundant breakdown product of chitin found ubiquitously in soil. Identification and elimination of GlcNAc catabolic genes is therefore likely to reduce microbial fitness in soil, limiting their niche to other carbon and nitrogen sources (e.g. root exudates of a specific plant). RB-TnSeq successfully identified two GlcNAc importers (Pflu5028 and 2096), as well as catabolic genes (Pflu5025 and 5026) as essential for GlcNAc utilization. We also identified genes necessary for utilization of carbon sources (e.g. mannitol, acetate, lactate, xylose) and nitrogen sources (e.g. urea, nitrate, nitrite), and demonstrate that orthologous genes in SBW25 and KT2440 exhibit similar mutant phenotypes.

To identify gene suppression combinations that control environmental persistence, we developed a multi-gene CRISPRi system in SBW25. Employing single-transcript, multi-guide RNAs for nuclease-dead ddCpf1, we optimized multiplex CRISPRi in SBW25, , including the use of truncated guides [3], by targeting fluorescent protein expression. In addition to green, red, and orange fluorescent proteins, we targeted proteins involved in nitrogen reduction (NarB), urea metabolism (UreC), nitrogen utilization (NtrC) and mucoid production (MucA). Our results indicate that two guides per gene provides higher gene repression compared to a single guide per gene. Also, truncated guides exhibited either improved or equivalent knockdown efficiency for multiple genes when compared to full length guides. We plan to use multiplex CRISPRi in SBW25 to repress up to six different soil-relevant phenotypes simultaneously and we expect these results will guide the creation of genome-reduced strains with engineered environmental niches. Looking forward, we expect our multi-omics and genetics platform to enable persistence control engineering in phylogenetically diverse bacterial isolates from the sorghum rhizosphere.

References

1. Wetmore, Kelly M., et al. "Rapid quantification of mutant fitness in diverse bacteria by sequencing randomly bar-coded transposons." MBio 6.3 (2015).
2. Wang, Mugui., et al. “Multiplex Gene Editing in Rice Using the CRISPR-Cpf1 System.” Mol Plant, Vol 10, Issue 7, (2017): 1011-1013.
3. Fu, Yanfang, et al. "Improving CRISPR-Cas nuclease specificity using truncated guide RNAs." Nature biotechnology 32.3 (2014): 279-284.

Funding statement: *This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (BER), as part of BER’s Genomic Science Program (GSP), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Secure Biosystems Design Science Focus Area "Persistence Control of Engineered Functions in Complex Soil Microbiomes". A portion of this work was performed in the William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by BER and located at PNNL. PNNL is a multi-program national laboratory operated by Battelle for the DOE under Contract DE-AC05-76RL01830.*