24. Discovery of a Novel Colony Invading Phenotype of *Pseudomonas stutzeri* RCH2

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Project Goals: Project Goals: The Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) program broadly seeks to understand the interactions between environmentally relevant microorganisms and their environment. Presumably one of the most significant influences on microbial fitness is the microbial environment. Our goal in this project is to develop a generalizable approach combining large-scale microbial interaction screening, chemogenomics and metabolomics to better understand fitness in the context of other organisms.

Chemogenomics, using DNA-barcoded transposon mutant libraries, has proven to be a powerful approach to link genes to function under defined environmental conditions.We hypothesized that this approach could be used to find genetic determinants of microbial interactions. To expedite the discovery of relevant microbial interactions we performed a large-scale interaction mapping experiment using acoustic printing of soilisolates. Acoustic printing uses acoustical energy generated from a piezoelectric source to eject nanoliter droplets from multi-well plates into precisely guided locations on agarose gels.Using arrays of precisely grown bacterial colonies, we rapidly identified microbial interactions that affected colony size and morphology. We observed that *Pseudomonas stutzeri* RCH2 displays an invasive, rugose-forming phenotype when cultured   in rich media on colonies of *Pseudomonads* and *Bacilli* bacteria isolated from the Oak Ridge Field Research Center. A DNA-barcoded transposon mutant library of RCH2 was used for co- culture mutant fitness assays to identify regulators of RCH2 colony invasion. Mutants that were highly sensitive and associated with this phenotype were genes encoding glutamate-5-kinase, gamma-glutamyl phosphate reductase, OHCU decarboxylase and formyltetrahydrofolate deformylase. The latter two genes are involved in purine metabolism, suggesting a role for purine metabolites in the colony invasion phenotype in RCH2. To further investigate the role of nucleobases in colony invasion, we performed metabolic profiling of scraped colonies using LC- qTOF-MS. As predicted from the mutant fitness profiling experiments, purine metabolites such as adenine were found at high levels in invaded colonies and were also consumed by the addition   of RCH2. We are now constructing targeted mutants to further investigate the role of nucleobases in regulating the community behavior of P. *stutzeri* RCH2.