Isolates and their Deep Characterization

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Project Goals: ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies) uses a systems biology approach to understand the interaction between microbial communities and the ecosystems that they inhabit. To link genetic, ecological, and environmental factors to the structure and function of microbial communities, ENIGMA integrates and develops laboratory, field, and computational methods. Deep characterization of isolates from the Oak Ridge Field Research Site is essential to understanding their role in microbial community assembly and key biological processes in this environment. The project goal is to develop and optimize high throughput and genome wide tools, to interrogate phenotypes and genotypes of the field isolates.

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Ground water and sediment samples from the Oak Ridge Field Research Site have yielded a large number of isolates that demonstrate desirable phenotypes for carbon utilization, metal reduction and resistance, denitrification and sulfate reduction. We aimed to characterize these isolates and obtain a genotype to phenotype understanding of molecular mechanisms for stress tolerance, cellular interactions and other biological phenomena. To assess the role of individual genes in fitness towards environmental parameters, we have developed a high-throughput genetic pipeline based on randomly barcoded transposon site sequencing (RB-TnSeq) and have applied this strategy to over 30 bacteria, including 10 ENIGMA FRC isolates. The resulting data have been used to identify phenotypes for previously hypothetical genes, improve hundreds of annotations of transport proteins and catabolic enzymes, fill in gaps in amino acid biosynthetic pathways, and identify novel catabolic pathways. To understand signaling and regulation, we have developed the DAP-seq methodology and examined bacterial response regulators in a high throughput and genome-wide manner. Our current studies, focused on denitrifying *Pseudomonas putida* strains have allowed us to elucidate multiple two-component signaling and the regulation of genes in response to metals and other environmental parameters. Coupled with other functional genomics studies (e.g. RNAseq) we map complex regulatory networks that underlie the response to key environmental factors such as metals. These data sets are also valuable in predicting and validating regulatory motifs. We have also examined the physiology of isolates in a co-culture format. Three of the isolates (*P. fluorescens* strains N1B4, N2E2, N2E3) were selected for in-depth analysis based on growth in pairwise co-cultures relative to monocultures with relevant genetic tools such as transposon mutant libraries. Our results show strain N1B4 (with truncated denitrifying pathway) grows more quickly and to greater density in co-culture than in monoculture. It was also found that some genes involved in nitrate reduction, sulfate permeability, molybdenum utilization, and anaerobic reduction are important for growth under

these conditions. In addition, a few uncharacterized genes were also shown to be positively correlated to growth. We are also using RB-TnSeq to study interactions between isolates where we determine which genes are important for susceptibility to the inhibitory compounds produced by other isolates. Barcoded transposon mutant libraries were grown in the presence of spent media from other strains. Improved fitness of a mutant indicates that the disrupted gene is related to the susceptibility of a compound. Preliminary metabolomics analysis (NIMS and RP-LC-MS) of a spent medium with known inhibitory activity has provided candidate inhibitory compounds. Analysis with MAGI provides a link between the metabolomics data and gene annotations to help predict which genes produce the compounds of interest. Together these methodologies and tools allow a deep understanding of the mechanisms and the interdependencies of key biological activities in these environments, and also a better understanding of community architecture.

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