

ENIGMA: Characterization of Microbial Strains from Contaminated Groundwater and from Contaminated Sediments Using Environmental Concentrations of Metals at the Oak Ridge Reservation

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Project Goals: ENIGMA -Ecosystems and Networks Integrated with Genes and Molecular Assemblies use 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.

The goal is to identify mechanisms that enable the growth of microbial communities in nitrate-, metal-contaminated and molybdenum-limited environments. A strategy was employed to isolate microorganisms from the ORR contaminated environment using environmentally relevant enrichment conditions. Strains of interest were then selected from the isolates using high throughput growth and biochemical assays. Mechanisms of resistance are being characterized in the strains of interest using genome sequencing, RB-TnSeq, metallomics and metabolomics approaches, in order to understand the role that they play in communities within this special ORR contaminated environment.

Abstract: The contamination plumes surrounding the S-3 ponds at Oak Ridge Reservation (ORR) are an extreme environment that has elevated concentrations of nitrate (up to 233 mM) and multiple metals including Al (up to 20.7 mM), Mn (up to 3.1 mM), U (up to 576 μ M), and Ni (up to 157 μ M). We hypothesize that microorganisms that live in this hostile environment have nitrate reduction and metal resistance mechanisms with unique properties that enable them to survive here. The goal of this project is to isolate microorganisms under environmentally relevant conditions from ORR contaminated groundwater and sediment, and to characterize the nitrate reduction and metal resistance properties of these isolates. To this end, comprehensive elemental analysis of over 50 elements was performed on two sediment cores, one outside and one within the contamination plumes, revealing differences in concentrations and populations of metals between the two locations. A mix of multiple metals approximating the contaminated environment was then used in a series of high-throughput enrichments to isolate microorganisms from both ORR groundwater and sediment. In depth characterization of selected isolates have uncovered a diversity in pH preference, carbon source utilization, metal tolerance, and nitrate utilization properties between the isolates. This includes metal resistant isolates capable of concentrating multiple metals on the cell surface, and an isolate, XG196, that is capable of

efficiently reducing nitrate when growing in low pM concentrations of molybdenum, a metal required in the catalytic center of nitrate reductase. Further in-depth characterization of the isolates is underway using multiple avenues including the development of a genetic system for MT124, construction of RB-TnSeq libraries for MT58 and other isolates, and genome sequencing of the strains. Also, compilation of the sequencing and characterization data in KBase will aid in further analysis of the isolates for pathways of interest. Finally, to fully understand the role and impact of these isolates at ORR, a cloud-based charting and mapping system called Google Fusion Tables was created by integration of ENIGMA's previous 100-survey topography, geochemical, biological and isolate-specific data streams. This could be used to rapidly analyze isolates, sequencing, and/or geochemical data using custom geospatial maps in a very user-friendly way.

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