

## **Field-relevant Isolates from the Oak Ridge FRC and their Deep Characterization and Functional Analysis.**

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<http://enigma.lbl.gov>

**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. To isolate key microbes or stable consortia and develop approaches to characterize and dissect function of these key microbes from Oak Ridge Field Research Center, TN. Determine if environmental correlations similar to those found in the field are observed in the laboratory with wide diversity of single microbes or defined consortia.**

In the past few years, we have developed both low and high throughput strategies to recover isolates representative of the genetic and functional potential observed in the field. Currently, we have in our collection close to 1000 diverse isolates obtained from background and contaminated ORR sites. These include isolates from field groundwater and sediment as well as laboratory enrichment and bioreactor studies conducted with field-sourced inocula. The ever increasing isolate collection includes representatives from diverse phylogenetic orders that nearly cover the diversity of our field site. Closely matching the site geochemistry to media composition has resulted in an increase of both the number and diversity of strains isolated. For example, our ability to measure the small molecule composition of the field site through development of sediment metabolomics and our ability to extract and characterize bioavailable natural organic carbon have enabled us to design media with carbon sources that closely resemble the quality and quantities of in situ nutrients. We are currently developing methods to isolate the rare biosphere, the phylogenetically diverse, uncharacterized clade members, and difficult to grow organisms. Representative isolates from processes of interest such as sulfate reduction have been obtained. *Desulfoporosinus*, a strictly anaerobic sulfate reducer, was isolated. Methodologies utilizing biorthogonal non-canonical amino acid tagging (BONCAT) in combination with fluorescent in situ hybridization (FISH) are being developed to cultivate translationally active phylogenies of interest. Certain strains cannot be isolated as monocultures and we are developing methods for highly rarified co-cultures for downstream analyses.

Several high-throughput tools and techniques are being developed to characterize different phenotypes and metabolisms of these diverse environmental isolates. For example, a high-throughput cultivation pipeline was used to measure the inhibitory toxicity of 80 inorganic ions against representative ENIGMA isolates and the resulting data was used to identify compounds that potentially limit the range of these organisms in the field. In addition, an automated high throughput DAP-seq analysis protocol is being developed to swiftly assess regulatory proteins and their targets in a large number of isolates that show importance in metal resistance, denitrification and sulfate reduction functions.

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