

## **ENIGMA: Capturing the Diversity of Subsurface Microbiota – Choice of Carbon Source for Microcosm Enrichment and Isolation of Groundwater Bacteria**

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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.

**Abstract:** Improved and innovative enrichment/isolation techniques that yield relevant isolates representing the diversity of environmental microbial communities would significantly advance exploring the physiology of ecologically important taxa in ecosystems. Traditionally, simple organic carbon (C) or yeast extract is used as C source in culture medium for microbial enrichment/isolations. In natural environment however, composition and properties of available natural organic C influences growth and assembly of microbial communities. In this study, we fed 8 different organic C to subsurface groundwater microbes from Oak Ridge Reservation Field Research Center (ORR-FRC) background site for a 30-day incubation period to investigate the response of indigenous microbial communities to different C. Filtered groundwater was the cultivation ‘medium’ and was amended with simple organic C (glucose, acetate, benzoate, oleic acid, and cellulose) that are either traditionally used as C source in bacterial culture medium or proxies of C in natural environments; naturally occurring undefined complex C (bacterial cell lysate and sediment-derived natural organic matter (NOM)); as well as a vitamin mixture, commonly used ingredient in culture medium. Our results clearly indicated that natural complex C substrates fared better in enriching diverse bacterial strains compared to other C sources. Microcosms amended with small organic C (glucose, acetate, benzoate, or oleic acid) showed significantly lower biodiversity, dominated by only a few phyla such as *Proteobacteria* and *Bacteroidetes* which are commonly isolated and already have diverse representative isolates. Microcosms amended with natural complex C (cell lysate or NOM) displayed significantly higher biodiversity, three phyla (*Verrucomicrobia*, *Planctomycetes*, and *Armatimonadetes*) that are poorly represented in culture were abundantly enriched. Further, NOM and cell lysate amended enrichments led to cultivation of many unique bacterial strains

across 4 phyla, 13 orders, and 39 genera. Several of these isolates have only 85-90% similarity (by 16S rDNA sequencing) to published strains.