Targeted Isolation Using Field-Informed Approaches

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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.

Isolation of microorganisms representative of key environmental metabolisms is essential to developing a fundamental understanding of ecological processes., yet, the scientific community estimates that we are able to culture fewer than 2% of microorganisms on Earth in the laboratory.[1] To recover field-relevant isolates from Oak Ridge Reservation Field Research Center (ORR FRC), we combined several statistical analyses using our environmental and sequencing metadata to A) identify high-priority targets for isolation based on abundance, community correlation, and other metrics, B) select samples and enrichments with increased probability to yield those targets, and C) inform growth medium composition as well as enrichment and isolation approaches. We have employed these targeted isolation techniques (in addition to high-throughput, untargeted approaches- ENIGMA SFA poster by Kuehl et al.) to not only increase isolate diversity in the ENIGMA culture catalogue, but also to recover key isolates from the fieldsite that are suspected to play integral roles in carbon and nitrogen cycling in the terrestrial subsurface.

Here, we present several isolation and enrichment successes resulting from this targeted approach, including organisms exhibiting nitrate- and sulfate-reducing metabolisms, complex carbon-transforming metabolisms, high metal tolerance, and acidophiles. Several of our isolates are novel and rarely cultivated/ previously uncultivated clades. We also present ongoing efforts in enrichment and isolation of ammonia oxidizing- and nitrous oxide reducing- microorganisms (see ENIGMA SFA poster by Hunt et al.). In addition, we have several ongoing efforts enriching and isolating for metabolisms and characteristics representative of field observations at the ORR

FRC. The results of this work will facilitate development of novel, tractable genetic systems, community interaction studies, and environmental simulations to connect phenotype and genotype to field observations.

References

1. Steen, A. D. et al. High proportions of bacteria and archaea across most biomes remain uncultured. *ISME J.* 13, 3126–3130 (2019).

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