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Taming the uncultured: Modeling microbial life under very low redox potential in the subsurface and deep-sea biosphere

SABRINA BECKMANN[1], IBRAHIM FARAG[2], SAMIKSHYA GIRI[3], MALIQUE BOWEN[2], CHRISTINA BAUGHAN[4], KARA HEATHMAN[3], ZOE SIFTAR[3], NANCY PROUTY[5] AND JEN BIDDLE[2]

[1]Oklahoma State University (OSU)
[2]University of Delaware
[3]Oklahoma State University (OSU), College of Art and Sciences
[4]University of Arizona
[5]USGS, Pacific and Coastal Marine Science Center
Presenting Author: sabrina.beckmann@okstate.edu

The subsurface biosphere is a hot spot for microorganisms that carry out anaerobic methane oxidation which plays an important role in the long-term storage and removal of methane from the marine and estuarine carbon cycle. Recent research identified the significance of new metabolic pathways that are used by microbial communities in deep-sea carbonates to carry out methane oxidation (Beckmann *et al.*, 2020, ISMEJ). Questions remain on the search of potential electron acceptors that microbes use to oxidize methane. We used river sediment (Broadkill River, DE) and deep-sea carbonates (Pacific Ocean, Queen Charlotte Fault) and a culturing-dependent approach maintaining a very low redox potentials down to -570 mV, a variety of electron acceptors and C1-C5 organic compounds with the goal to enrich a higher diversity of methane oxidisers. We successfully enriched distinct methanotrophic consortia where the main methane oxidizers vary in response to the prevalent redox potentials from ANME to Bathyarchaeota for -310 mV and -570 mV, respectively, from the subsurface of the river sediment. The final acceptor of electrons is likely sulfate, mediated by *Dehalococcoides.* The microbial community structures showed high degree of similarity across the enrichments and the major players primarily perform substrate-level phosphorylation, including the Coatesbacteria, Patescibacteria, Actinobacteria and Bacteriodeta along with others. The metagenomes of these enrichments have shown that the Coatesbacteria and CPR are performing supporting functions for the microbial community, including the production of cofactors such as vitamin B12 for the *Dehalococcoidia.* In enrichments using deep-sea carbonates, isoprene initiated the methane-oxidation in enrichment cultures at low redox potential (from -250 to -500mV) suggesting an important reductive role of isoprene or isoprenoid compounds in the subsurface. We are currently characterizing the microorganism's metabolizing methane and isoprene in the enrichment cultures. This analysis showed that we have a relatively stable anoxic community, whose members appear to shift in abundance based on the prevalent reduction potential alone. These unique enrichments demonstrate the influence of life at low reduction potential, which is common throughout the subsurface, and show how microbes are partnered to gain the most energy out to fuel their metabolic processes in energy limited systems.