Comparative Genomics, Transcriptomic Analysis, and Ecophysiology of Individual Environmental Methane-oxidizing Syntrophic Consortia

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Project Goals: Short statement of goals. (Limit to 1000 characters)

Our goal is to utilize multiple analytical strategies to substantially expand our understanding of the key microorganisms, metabolic strategies, and interspecies relationships involved in the formation and oxidation of methane in the environment. Our research incorporates novel meta'omics strategies, state-of-the-art microscopy, and stable isotope geochemistry spanning spatial scales of nanometers to millimeters. Applied in tandem, these complementary methodologies have the potential to provide a new and more holistic 'eco-systems level' understanding of the factors which regulate methane cycling in anoxic sedimentary ecosystems. These analyses will be combined with characterizations of field samples and geochemically-characterized laboratory microcosms in order to further our understanding of methane cycling in anoxic habitats.

The anaerobic oxidation of methane (AOM) is a major contributor to the global methane cycle. At sites of methane seepage, AOM is catalyzed by diverse syntrophic partnerships between anaerobic methanotrophic (ANME) archaea and sulfate-reducing deltaproteobacteria (SRB), frequently found in well-structured multi-celled consortia. These organisms are not yet available in pure culture, however environmental studies have identified multiple genera of ANME and deltaproteobacterial partners often co-existing in the same sediment environment. The physiological differences and potential for niche differentiation between distinct ANME groups is poorly understood. Our earlier work using conventional environmental metagenomics resulted in a subset of reassembled genomes from these groups, enhancing our understanding of ANME and SRB metabolism. Much of the information on fine scale population structure and information regarding specific syntrophic pairings is lost however when reconstructing genomes from bulk sediment metagenomes. To address these more targeted ecologically relevant questions, we developed assays that combine fluorescent activated cell (consortia) sorting

(FACS) with bioorthogonal noncanonical amino acid tagging (BONCAT) of anabolically active AOM consortia in sediment and sequenced the genomes of multiple individual consortia of ANME-SRB recovered from the same sample. Using this unique sequence dataset from the BONCAT sorted aggregates, we have examined fundamental questions regarding the clonal nature of within consortia archaea and bacteria and the broader microheterogeneity that exists between the genomes of closely related ANMEs recovered from different aggregates. All ANME lineages across the dataset were observed to harbor large multiheme cytochromes that appear to be a hallmark of these organisms, and distinct from closely related methanogen relatives. Examination of potential interspecies interactions including metabolic complementation between the syntrophic partners is ongoing. These single aggregate genomes have also been used in concert with environmental metatranscriptomics of sediment microcosms maintained under conditions supporting syntrophic sulfate-coupled AOM as well as active archaeal methane oxidation with AQDS in the absence of an active bacterial partner. This approach revealed distinct differences between the transcriptional responses of individual aggregates containing distinct ANME types in the same sediment under different conditions. These observations are now being combined with microscopy and nanoSIMS-based analyses of different spatial arrangements between ANME and SRB subgroups within aggregates in order to better understand variations in ecophysiology between co-occurring ANME groups and the interplay between syntrophic metabolism and microbial community structure.

References

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