Title: Uncovering Syntrophies within Methane-Oxidizing Microbial Consortia in Sediments: Synthesizing Insights from the Subcellular to the Population Scales

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Project Goals: The overarching goal is to expand the understanding of interactions and fundamental activities involved in cycling of carbon and nutrients by syntrophic methanotrophic archaeal-bacterial consortia and associated viruses in anoxic sedimentary environments. Specific objectives are to (1) quantify energy and nutrient exchange [e.g., nitrogen (N), phosphorus (P), iron (Fe) and vitamins] within AOM consortia and between ANME-bacterial partners; (2) identify virus-host interactions associated with AOM and assess C and N transfer through viruses in methane-impacted sediment ecosystems; (3) model energy and nutrient exchange in AOM consortia and viral-host interactions (i.e., viral activity), and their environmental distribution patterns.

Abstract text:
Microbial-driven anaerobic oxidation of methane (AOM) accounts for up to 80% of methane sequestration in anoxic sediments, preventing this greenhouse gas from reaching the atmosphere. This sequestration is catalyzed primarily by consortia of ANaerobic MEthane-oxidizing 'ANME' archaea and syntrophic bacteria coupled through redox chemistry to sulfur, nitrogen, iron, and manganese. While the importance of AOM consortia for methane-oxidation in sedimentary environments is widely appreciated, the nutritional requirements and interdependencies of these diverse methanotrophic archaeal-bacterial syntrophies and their collective impact on nutrient cycling within the sedimentary ecosystem is not well understood.

Our group has developed several approaches to elucidate the syntrophies between sediment-hosted ANME archaea and their sulfate-reducing bacterial partners (SRB) at the intracellular, intercellular, and population scales, and over time. A key tool in our investigations is the disruption of extracellular electron transfer (EET) between ANME and SRB -- the main syntrophic association between the partners -- through the addition of an external electron acceptor (anthraquinone-2,6-disulfonate, or AQDS) to our incubations [1]. AQDS addition effectively enables ANME to carry out AOM autonomously without the SRB partner; by comparing cells under conditions of intact and disrupted syntrophy, we are able to reveal the nutrient flows dependent upon the syntrophic association.

Our use of 3D electron microscopy and synchrotron-based XRM and XANES spectroscopy enables investigation of ANME-SRB consortia *at the intracellular level,* and reveals storage granules important for nutritional syntrophies. Using serial block electron microscopy (SBEM) to image individual aggregates with nanometer resolution in three dimensions, and applying

supervised machine learning to image segmentation, we are reconstructing the 3D cell volume of several ANME-SRB aggregate morphologies to determine how macromolecular storage compounds (e.g. polyphosphate, carbon or iron storage granules) accumulate or dissimilate within individual archaeal or bacterial cells, and how these nutrients are stored within or shared between cells in the presence or absence of AQDS [2]. Complementing our SBEM imaging, we are using synchrotron-based X-ray fluorescence imaging and XANES spectroscopy at SSRL beamlines 2-3 and 14-3 to investigate elemental speciation in ANME-SRB aggregates. Using the recently upgraded beamline optics, which provide substantially improved signal and resolution, we have identified differences in sulfur-containing macromolecules in AOM consortia incubated under AQDS vs. SO_4^{2-} conditions.

We have developed pulse-chase multi-isotope labeling coupled with FISH-nanoSIMS to investigate consortia *at the aggregate level, and over time,* revealing the growth patterns of consortia as well as the phenotypic heterogeneity of growth patterns between aggregates. By adding $^{15}NH_4^+$, D_2O, $H^{13}CO_3^-$ or $H_2{}^{18}O$ at different incubation periods and tracking isotopic signals enriched in the ANME-SRB biomass using FISH-nanoSIMS, we have obtained some of the first *dynamic* single-cell activity measurements of these slow-growing aggregates. Additional FISH-nanoSIMS analyses reveal cell specific patterns in N_2 fixation among ANME and SRB. ANME archaea have been suggested to be dominant diazotrophs in AOM consortia; however it is not clear whether they are capable of this energetically demanding anabolic process in the absence of their syntrophic partner, nor is it clear how newly fixed nitrogen is partitioned between syntrophs. $^{15}N_2$ FISH-nanoSIMS in the presence and absence of AQDS confirmed active diazotrophic growth by ANME in the absence of an active SRB partner and active transfer of N-containing biomolecules into the extracellular matrix surrounding both partners. Other $^{15}N_2$ labeling experiments revealed a greater degree of variation in diazotrophic activity among diverse AOM consortia, with select SRB syntrophic partners (newly described Seep SRB1g group) shown to also be capable of N_2 fixation in association with ANME-2b [3].

Finally, *at the population level*, our use of proteomic and genomic approaches to compare metabolically-coupled and uncoupled aggregates reveal proteins and pathways important to the syntrophic lifestyle. We find distinct proteomic profiles in both the ANME and SRB in the presence and absence of AQDS. We observed a distinct shift from anabolic activity during metabolic coupling to catabolic activity when the syntrophy is disrupted. Proteome remodeling was observed in many protein families including nitrogen metabolism, amino acid biosynthesis, redox sensing, and cell surface composition. Taken together, our multi-scale approaches and observations will help inform and test model predictions of syntrophic associations and methane metabolism in the environment.

References/Publications

1. Scheller S et al 2016; Science 351: 703-707.
2. McGlynn S et al 2018; Appl Env Microbiol 84 (11): e00399-18
3. Metcalfe et al., 2021; ISME J 15: 377-396

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