Correlative Compositional Imaging and Protein Profiling in Marine Anaerobic Methane Oxidizing Microbial Communities

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Project Goals: Multiple experimental lines of inquiry are being applied in an effort to shed light on the metabolic activity and genomic capacity of organisms responsible for the anaerobic oxidation of methane. Characterization by secondary ion mass spectrometry, transmission electron microscopy, and fluorescence *in situ* hybridization reveals organism specific composition and structure, while metaproteomics and histological approaches reveal biochemical functionality and distribution within these uncultured multicellular consortia.

Large amounts of methane are oxidized in anoxic marine sediments by symbiotic microorganisms whose physiology is largely unknown. These uncultured organisms, comprised of consortia of methane-oxidizing archaea (ANME) and sulfate reducing bacteria (SRB), form highly structured physical associations and participate in a syntrophic relationship. To characterize these metabolic partnerships, complimentary microscopic and spectrometric techniques are being developed, providing high resolution compositional and phylogenetic information. These analyses indicate significant differences between cell types and create the opportunity for conducting comparative and quantitative image analysis between samples experiencing shifts in environmental conditions. Integration of these approaches with metagenomics and metaproteomics has the potential to result in direct correlation of form and function in these organisms.

Methodologies appropriate for isolating high concentrations of biological material from environmental samples have been recently developed by our team, making possible the application of high resolution imaging techniques including electron microscopy and spatial mass spectrometry. These techniques have proven suitable for the analysis of microbial consortia with a diversity of imaging methodologies. Characterization of ANME/SRB consortia by transmission electron microscopy (TEM) reveals a diversity of cellular morphologies and intercellular components. Many of these intercellular features can be understood partially from comparison with those present in organisms in pure culture, however other features appear to be novel and undescribed. In addition, TEM analysis reveals the presence of an inorganic iron-bearing phylosilicate crust which surrounds some, but not all of the aggregate types. Material composition of these features, as well as compositional differences between organism types are being probed by secondary ion mass spectrometry (nanoSIMS) at spatial resolutions previously not

possible. These investigations indicate the presence of internal polyphosphate storage granules in ANME cells, and numerous storage granules with unknown composition in the associated SRB partner. In addition, high resolution imaging reveals close physical contact between cells of different types, underscoring the possibility of metabolic coupling between them. Concomitant to these imaging techniques, taxonomic identification of individual microorganisms in prepared thin sections of the consortia is made possible by employing florescence *in situ* hybridization (FISH). The pairing of these techniques gives the ability to study these highly structured consortia at unprecedented spatial resolution and deliver information as to individual organism roles within this complex ecosystem.

As an extension to these studies, histological approaches are being developed to query the presence of functional gene products. The enzymes methyl-coenzyme M reductase (Mcr) as well as nitrogenase (Nif) are being targeted to probe enzyme distribution within microbial consortia. These enzymes represent carbon and nitrogen entry points into the metabolism of ANME/SRB consortia and their identification holds the potential to directly reveal specialization and resource partitioning at the cellular level.

In tandem to conducting cell specific image based analyses, environmental metaproteomic data from methane-seep sediments and microcosm experiments containing ANME/SRB consortia are being used to survey the distribution of expressed proteins. Preliminary proteomic profiling of methane seep sediments reveal many enzymes known to be involved in methane oxidation and sulfur reduction pathways. These analyses indicate that it is possible to extract a significant number of proteins from environmental samples, and furthermore that these proteins can be identified using a locally created metagenomic database. We have found that the efficiency of protein extraction from soil microbes is influenced strongly by the type of soil matrix in which they reside and early results show that protein extraction is inhibited by the presence of clays. These findings will help tailor microcosm setups for in-depth exploration and validation of partnership between ANME and SRB consortia in deep sea methane seep sediments. The ability to profile proteomes from samples of variable environments allows for the identification of gene products of known and unknown function, and will thus be of value in identifying the metabolic modes utilized in the anaerobic oxidation of methane.

Collectively, these investigations indicate that many of the processes occurring in anaerobic methane oxidizing microbial communities are discernable utilizing culture independent techniques and aid in understanding the role of these organisms in global elemental cycling.

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