Ecology, Diversity, and Biogeochemical Contribution of Viruses in Methane-Rich Sediments

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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.

In recent years, the fundamental role of viruses in marine ecological networks has become increasingly apparent. However, viral communities in sediment environments are largely unexplored, though initial evidence exists for their large contribution to the dissolved organic carbon pool. In anoxic sediments of cold methane seeps, methane is the primary energy source; anaerobic oxidation of methane coupled to sulfate reduction, performed by a microbial consortium of anaerobic methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB), underpins much of the biodiversity of the seep and beyond. To understand the role of viruses in this environment, we have established a series of microcosm anoxic incubations of multiple sediment depths from four discrete cores collected from sites of active AOM. From these incubations, virus-like particles (VLP) were concentrated and then further purified using an Optiprep density gradient at two distinct densities (30 and 35%). Metagenomic assembly of these samples yielded more than 2200 complete and nearly complete viral genomes along with thousands more contigs longer than 15kb. Initial phylogenetic analyses show that the viral community from this environment is very diverse, mostly uncharacterized, and includes a number of novel distinct clades. We also profiled the diversity of VLP morphologies using transmission electron microscopy (TEM). Seep samples contain a variety of viral capsid morphologies, ranging from the classic head-tail phage to the spindle shapes of viruses known to infect archaea. In addition, incubations amended with anthraquinone-2, 6-disulfonate (AQDS), an external electron acceptor which decouples the process of AOM from sulfate reduction[1], showed an increase in the abundance of spindle-shaped viruses. Parallel incubations lacking

AQDS, in which the SRB partner was not decoupled from the ANME, showed a greater diversity of tailed viruses. These results are the first indication of the potential impact of the ANME-SRB syntrophy on the viral community in the cold seep environment. To further constrain the impact of viruses on AOM and sulfate reduction as well as their impact on the syntrophic relationship between ANME and SRB, we are developing a number of new methods to track viral activity and elemental composition. First, we adapted a protocol[2] for Biorthogonal Non-Canonical Amino Acid Tagging (BONCAT) with click-chemistry for fluorescently labeling newly-synthesized viruses. This will enable the quantification of viral production under different environmental conditions and host physiologies. We are also developing a method that combines viral-BONCAT fluorescence-activated sorting (FACS) with Single Virus Genomics[3] (SVG) in order to identify and sequence the genomes of newly produced viruses in sediments from active host cells. Optimization of protocols combining stable isotope probing with fluorescence identification and nanoscale Secondary Mass Spectroscopy (NanoSIMS) is also ongoing with sediment hosted viruses to assess the proportion of of carbon (methane) and nutrients (nitrogen) through the viral assemblage under different incubation conditions in the laboratory.

References

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