Characterization of viral assemblages in methane-saturated sediments and their Spatio-temporal Dynamics

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Project Goals:

The overarching goal is to characterize the role of viruses in the ecology and biogeochemical cycling in methane saturated sediments , especially their impact on the syntrophic archaeal-bacterial consortia that perform the anaerobic oxidation of methane (AOM). Specific objectives are to (1) Characterize the viral community in methane-saturated sediments and their environmental distribution patterns using viral metagenomics and transmission electron microscopy; (2) Identify virus-host interactions associated with AOM and potential viral auxiliary metabolic genes (AMG) involved in key sedimentary biogeochemical cycles. (3) Develop activity-based methods for fluorescently-labeling viruses for microscopy and flow sorting to achieve single virus level resolution of newly produced viruses and their genomic diversity; (4) Quantify viral activity and constrain how the production of new viruses relates to host physiology and AOM.

Viral communities in sediment and soil environments are largely unexplored, though initial evidence exists for their large contribution to the dissolved organic carbon pool. In methane-saturated anoxic sediments, the process of anaerobic oxidation of methane by syntrophic consortia of methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB), is the dominant pathway by which methane is oxidized prior to release to the atmosphere.

To understand the role of viruses in this process, 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, we have assembled viral metagenomes that yielded more than 3,400 complete and nearly complete viral metagenome-assembled genomes (vMAGs), along with thousands more contigs longer than 15kb. The viral community from this environment is very diverse and the majority of the vMAGs are unclassified followed by Siphoviruses and Halovirus-like viruses. Our data are dominated by site-specific abundance patterns rather than clustering by sediment depths. Auxiliary metabolic genes (AMG) involved in heme metabolism and assimilatory sulfate reduction are highly abundant throughout our dataset, pointing at the potential role of viruses in sulfur metabolism and possibly extracellular electron transfer.

To further characterize the impact of viruses on AOM and sulfate reduction a set of incubations were amended with $^{15}NH_4^+$, $^{13}CO_3$ and L-Homopropargylglycine (HPG). An additional set of incubations were also amended with anthraquinone-2,6-disolfonate (AQDS), an external electron acceptor that decouples the syntrophic relationship between the ANME and SRB. TEM analysis of a previous incubation series showed preliminary differences in viral capsid morphologies between decoupled and non-decoupled samples. The current incubation series seeks to track such changes in the viral community over time, and correlate it with metabolic activity of the hosts, viral production rates and the rate of elemental transfer between hosts and virus-like particles (VLP). Biorthogonal Non-Canonical Amino Acid Tagging (BONCAT)[2] with click-chemistry was used to fluorescently label newly-synthesized viruses and quantify viral production. BONCAT methodology was optimized for maximizing the ratio of viral signal to noise, which is crucial for flow cytometry detection and sorting of positive BONCAT tagged viral particles. Finally, we present preliminary results of a method that combines optimized viral-BONCAT with fluorescence-activated sorting (FACS) on an Influx cell sorter with Single Virus Genomics[3] (SVG) that can be used to identify and sequence the genomes of newly produced viruses in sediments from active host cells, correlated to geochemical data from the incubation.

References

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