Real-time molecular assays for the detection of aerobic methanotrophs in the deep ocean. P. L. Tavormina[1], W. Ussler[2], S.M. Joye[3], C. Scholin[2] and V. J. Orphan[1]. [1]California Institute of Technology, 1200 E. California Boulevard, mail code 100-23, Pasadena, CA 91125; pattytav@gps.caltech.edu, [2]Monterey Bay Aquarium Research Institute, Moss Landing, CA, 95039, [3]Department of Marine Sciences, University of Georgia, Athens, GA 30602-3636.

Introduction: The spatio-temporal behavior of methane in the ocean associated with gas seeps, hydrates, mud volcanoes, and hydrothermal vents, is highly dynamic (see for examples 1, 2, 3). Biogeochemical rate measurements indicate that the majority of methane released from the seabed is biologically oxidized prior to emission to the atmosphere, however the identity, ecophysiology, and distribution of planktonic microorganisms mediating aerobic methane oxidation in the water column is still poorly understood. To effectively characterize these methane-consuming microorganisms and their spatio-temporal distribution relative to localized enrichments of methane in the water column requires high frequency, dynamic sampling and sensitive molecular and chemical detection methods. The newly designed Deep Environmental Sample Processor (D-ESP), is an ideal platform in which to accomplish these objectives. The D-ESP is an autonomous instrument capable of real time sampling and remote surveillance of microorganisms and corresponding physicochemical parameters in the deep-sea (to depths of 4000 m), including methane and hydrothermal vent environments (4, 5).

Here we focus on the first phase of this project, describing the development and testing of high throughput and quantitative molecular detection methods for *in situ* enumeration of marine methanotrophs. In preparation for integration with the D-ESP instrument, we collected and analyzed discrete water column samples from a range of methane seep, hydrothermal vent and non-seep sites using optimized molecular assays targeting *pmo*A, a functional gene central to the aerobic methane oxidation pathway.

Identification of planktonic aerobic methanotrophs: We have sampled the water column above a variety of methane seep and non-seep sites in order to identify methanotrophic bacteria potentially contributing to methane oxidation in ocean waters (6). In the water column above active methane seeps, two clades of gammaproteobacterial methanotrophs are commonly observed. These *pmoA* phylotypes, termed OPU1 and OPU3, vary in relative abundance between seep sites, and are only occasionally recovered in marine sediments (7), suggesting that they may be primarily adapted to a planktonic lifestyle. These clades were initially identified in the oxygen minimum zones of the Eastern Pacific (8), and more recently we have shown that they are generally widespread along the continental margins (9). A third clade, termed Group X and more distantly related to gammaproteobacterial methanotrophs, has also been recovered above some methane seeps. The function of this clade is not yet known, however its recovery demonstrates the potential for additional diversity involved in the cycling of methane in the oceans. The sequences recovered in these surveys provide targets suitable for development of real-time remote surveillance of marine methanotrophs using the Deep Environmental Sample Processor (D-ESP).

Real time quantitation of aerobic methanotroph candidate OPU3: A real-time 5' exonuclease PCR assay (taqman assay) was developed for use with the D-ESP. This assay is sensitive to as few as 10 copies of target DNA, and has a priming efficiency >95% at the bench. Using this assay, methanotrophic lineage OPU3 was quantified through a variety of water column environments including depth profiles above active seep and non-seep sites, and lateral series in the Santa Monica Basin, through the Monterey Canyon system, and in the Fiji hydrothermal vent field (9). OPU3 abundance is approximately 20-fold higher above seeps (>100 copies per milliliter seawater) than in non-seep sites (typically <10 copies per milliliter seawater). This assay is robust and reliable across a range of methane seep sites, ranging from Northern California, to Costa Rica, to the Gulf of Mexico, thus is capable of accurately enumerating OPU3 through a wide range of environments and well-suited to deployment on the D-ESP.

Rapid fingerprinting of methanotroph communities: Population distributions can change in response to environmental factors, and interactions between groups may impact relative metabolic activity and abundance. These dynamics point to the complexities that can exist in methanotroph communities. We have developed a method that can rapidly determine shifts in methanotroph community composition (9). This PCR-based assay amplifies the noncoding spacer region between *pmoC* and *pmoA*, a region that is under relatively low selective pressure and which can therefore change in length over relatively short evolutionary time frames. Similar to ARISA, this assay (Monooxygnease Intergenic Spacer Analysis, or MISA) generates a profile of a methanotrophic popula-

tion in a single PCR reaction. This assay has been used to describe shifts in methanotroph populations laterally in methane seep and non-seep sites, and through depth from surface to seafloor above methane seeps, in non-seep submarine canyons, and through oxygen minimum zones. We have shown that methanotroph lineages OPU1 and OPU3 appear constrained to depths below 200 meters, that both lineages are widespread at seep as well as non-seep sites, and that the lineages are undetected in a subtropical gyre in the Atlantic Ocean (Bermuda Atlantic Time Series, sampled to 300 meters.)

Together, these molecular methods are providing insights into the abundance and distributions of microorganisms participating in the methane cycle through many environments in the world's oceans. The next phase of this work, including real time sampling and processing on the D-ESP, will further enhance our understanding of the dynamics of methane turnover in the ocean.

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