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DISTRIBUTION OF THE DOMINANT MICROBIAL COMMUNITIES IN MARINE SEDIMENTS CONTAINING HIGH CONCENTRATIONS OF GAS HYDRATES

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ABSTRACT

Methanogens are implicated in the production of methane that accumulates in marine sediments. However, the factors that control the distribution of the microbial communities that influence the presence of methane in these sediments are not well understood. Our objective is to determine the quantity, diversity, and distribution of microbial communities in the context of abiotic (e.g., grain size, presence/absence of hydrates) and geochemical (redox state, organic carbon content) properties in gas-rich marine sediments. To this aim, DNA was extracted from deep marine sediments (25-175 mbsf) cored from continental slope locations including offshore India and the Cascadia Margin. The sediments yielded low levels of DNA (0.3-1.5 ng/g of sediment), and bacterial DNA appeared to be more readily amplified than archaeal DNA. Preliminary analysis of a subset of these samples from India using PhyloChip technology indicated 200-800 distinct archaeal or bacterial taxa in each sample. The PhyloChip detected the presence of methanogens, sulfate reducers, sulfur oxidizers, and other metal reducers, as more prevalent taxa. Infrequently, cores from relatively shallow sediments from central Hydrate Ridge and northern Cascadia (offshore Vancouver Island), and from India's eastern margin contained macroscopically visible, pigmented biofilms. The Hydrate Ridge biofilm consisted of high concentrations (ca. 1-2 μm) cocci in a tetrad when viewed under a microscope. Preliminary terminal-restriction fragment length polymorphism (t-RFLP) characterization of the 16S rDNA amplified from these samples suggested the presence of as many as 7-18 distinct taxa. These discrete biofilm communities are anomalous compared to the normally sparse distribution of cells in the sediments and based on their considerable biomass may factor prominently in the cycling of carbon in the shallow sediments. Understanding the fine-scale distribution and factors that control the presence of sediment communities will provide better parameters for computational models that describe carbon cycling in these systems.

Keywords: gas hydrates, microbial communities, marine sediments, molecular ecology

INTRODUCTION

Methane produced by microorganisms constitutes a significant fraction of the methane that occurs in marine sediments where gas hydrates are present [1]. The diverse communities that populate these formations has been documented by cultures and/or via molecular traces (see, for example, [2,3,4]). Recently, Inagaki et al. explored the biogeography of hydrate-bearing systems by comparing clone libraries developed from sediments where hydrates are abundant with those developed from sediments that lack hydrates [5]. Their findings document the presence of a distinctive microbial community in sediments that have methane hydrates.

We are interested in a finer-scale biogeography, so as to determine how factors such as the presence/absence of hydrates, grain size, and the depositional environment in marine sediments may control the number, type and distribution of microbial communities in sediments. Our aim is to understand the controls on the distribution and activity of all microbes that contribute to the conversion of organic matter to methane. Ultimately, we expect that our data will be used to refine computational models that require biological rate terms that are consistent with sediment conditions in order to accurately describe the dynamics of this large methane reservoir.

MATERIALS AND METHODS

Sample site and sample collection

Deep sediment cores for this investigation were collected as a part of the National Gas Hydrates Program (NGHP) of India [6] using methods consistent with those used previously to obtain cores for microbiological investigations of deep sediments [7]. Forty-six cores were collected for microbiological characterization of which 31 were noted to contain hydrate (Figure 1). Most of the cores were obtained from the Krishna-Godavari (K-G) Basin, a passive margin; however, 12 samples came from a convergent margin offshore the Andaman Islands, the site of the deepest gas hydrates found to date. After collection, samples were kept frozen (-80 C) as whole round cores prior to extraction of DNA.

These samples were collected in concert with samples for geochemical and physical property analyses.

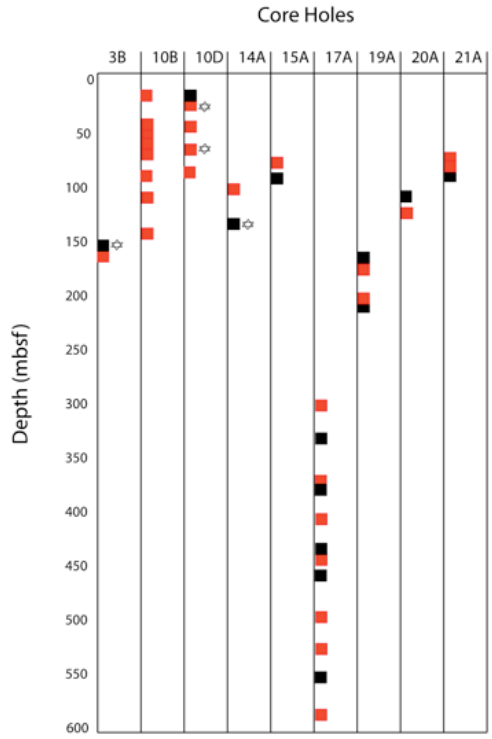

Figure 1. NGHP samples collected for microbiological studies shown as hydrate-bearing (red) or non-hydrate-bearing (black). * = samples from which DNA has been extracted. Mbsf = meters below seafloor.

Microbiological analysis

Deep sediment cores for this investigation were subcored to obtain pristine material for DNA extraction. DNA was extracted and quantified (as described in [7]) prior to analysis using molecular biology techniques. As a preliminary estimate of microbial diversity in the samples we used terminal restriction fragment length polymorphism (t-RFLP) to separate unique sections of the extracted, amplified 16S rDNA that was most prominent in the samples [8]. A more thorough microbial diversity assessment was performed on samples by characterizing part of the original extracted, amplified 16S rDNA using high-density oligonucleotide microarrays [9]. This microarray technology (called PhyloChip) works by labeling the DNA amplified from the sample and then permitting the possible hybridization of the labeled DNA to 500,000 probes on the chip. The probes represent 9000 taxa from two microbial domains (Bacteria and Archaea).

RESULTS AND DISCUSSION

DNA extractions

DNA representing both Bacteria and Archaea was successfully extracted from the NGHP samples and amplified using 16S rDNA-based primers. On electrophoretic gels the appearance of bacterial DNA was brighter than archaeal DNA (Figure 2). The brighter bands may be due to the ease with which bacterial DNA was extracted or amplified relative to archaeal DNA, or due to higher numbers of bacteria. We also noted that dilutions of the original DNA extracts yielded better amplification as noted by brighter bands in the gels. This may be a result of achieving the appropriate concentration of DNA in the polymerase chain reaction mixture or because the diluted DNA extracts also contained lower levels

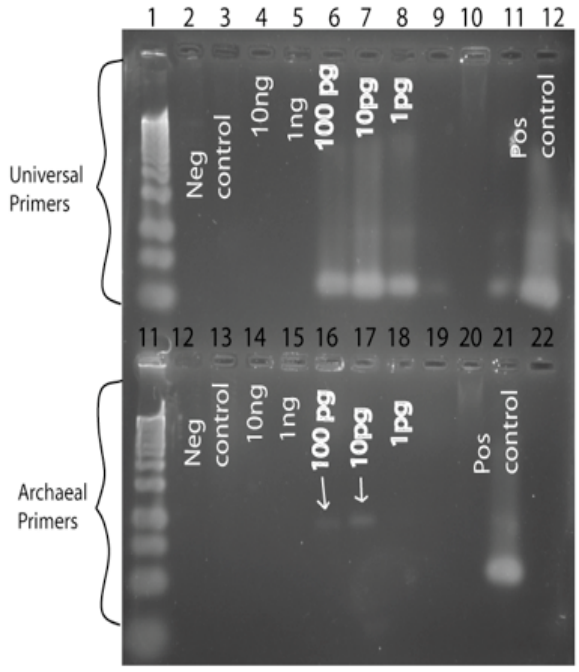

Figure 2. Electrophoretic gels showing DNA (arrows point to the bright DNA bands) from NGHP samples amplified using either universal or archaeal primers. Note that bands are brighter when lower levels of DNA (e.g., 10-100 picogram) were amplified relative to higher levels of DNA (e.g., 1-10 nanogram).

of compounds such as humic acids that can interfere with the amplification reactions.

Microbial diversity

We performed t-RFLP on DNA from four sediment samples extracted from the K-G Basin amplified using archaeal primers and then digested

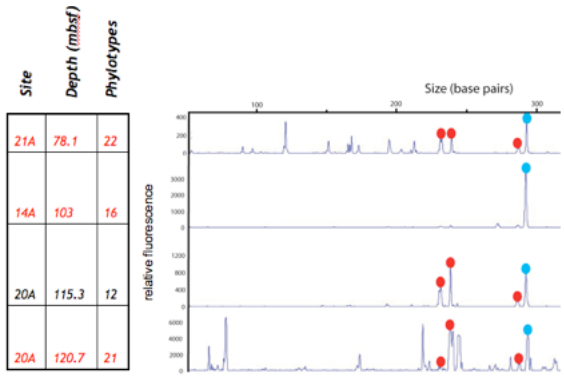

Figure 3. Electropherograms of t-RFLP analysis of four samples from the K-G Basin. Peaks identify distinctive archaeal 16S rDNA fragments cut by the HaeIII restriction enzyme. Peaks with a blue dot were common to each of the samples whereas peaks with a red dot were only present in three of the four samples. The table shows sample origin, depth, and number of phylotypes detected. Red = samples contained hydrate; black = samples without hydrates.

with HaeIII (Figure 3). In this limited set of samples, we detected as many as 22 phylotypes from hydrate sediments and 12 phylotypes from the single non-hydrate sediment.

In our preliminary evaluation of the data none of the HaeIII-cut peaks that we detected correspond to known archaeal peaks as cut by the same enzyme and registered in the Michigan State University Ribosomal Database Project II (http://rdp.cme.msu.edu/). The small sample size analyzed to date precludes inferences on whether the presence of hydrate or depth of the sample played any role in determining the types of microbes present.

Results of the hybridization of archaeal DNA from four samples to the PhyloChips indicated the presence of additional microbial taxa (Figure 4). Over 200 archaeal taxa were detected using the PhyloChip and methanogenic taxa were among the most dominant with anaerobic methane oxidizing archaea also present. For the samples tested, the archaeal communities from the non-hydrate samples were more similar to each other than they were to the hydrate samples; however, these similarities were not strong. A greater number of positive hybridizations were noted for the bacterial DNA (up to 800 taxa detected), with sulfate reducers, sulfur oxidizers, and metal reducers detected with the greatest frequency and anaerobic ammonia oxidizers, acetogens, and aerobic methanotrophs also represented (data not shown). For the four samples of bacterial DNA that were hybridized to the PhyloChip, there was no clear distinction between the two samples from sediments that contained hydrate and the two samples from sediments that lacked hydrate. As for the t-RFLP data these analyses will be easier to interpret when additional samples have been considered.

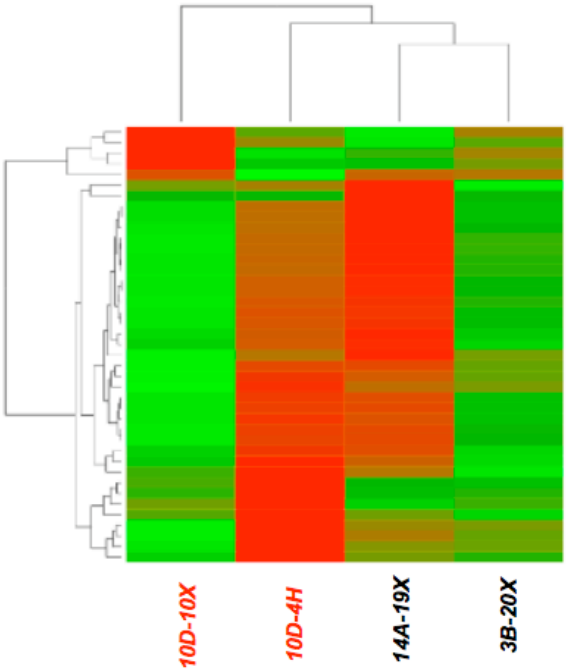

Figure 4. Heat map and dendrogram showing the response of archaeal groupings (y-axis) exhibiting the highest standard deviation between four samples (lower x-axis) from the K-G Basin from which archaeal DNA was extracted and amplified. The color gradient (green to red) displays increasing hybridization of the labeled sample DNA to the probes on the PhyloChip. 10-D samples (red) are from hydrate sediments whereas 14A and 3B samples (black) are from non-hydrate sediments.

Biofilm analyses

We are also investigating a unique biofilm that occurs in marine sediments rich in methane (Figure 5). This biofilm often appears as a pink-orange slimy material along fracture faces in shallow (less than 20 mbsf) marine sediments has been observed at Hydrate Ridge (offshore Oregon), on Leg 311 (offshore Vancouver Island), and in some of the cores from NGHP (offshore India). Preliminary assessment of the microbial communities in the biofilm by DNA extraction, amplification with archaeal and bacterial primers, cloning and sequencing indicate the presence of one archaea related to ANME-1, and three bacterial species related to *Cladothrix*, *Ornithinimicrobium*, and an unidentified delta-proteobacterium.

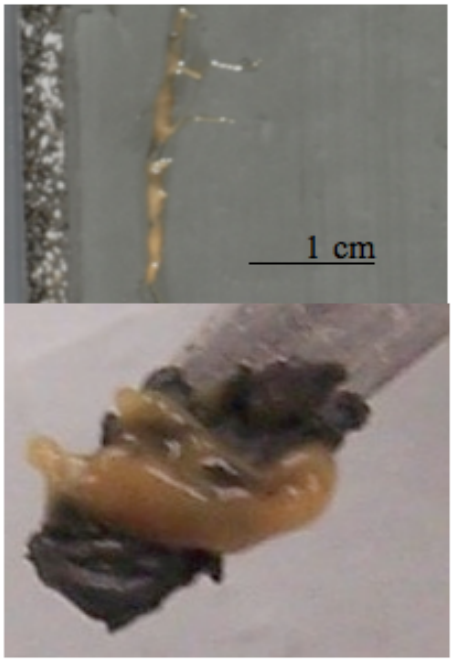

Figure 5. Photos of unusual biofilm from methane-rich marine sediments showing presence within a fracture (upper panel) and as collected on a spatula (lower panel).

SUMMARY

Our findings complement those reported previously, most notably by Inagaki et al. [5] that indicate the presence of diverse microbial communities in sediments containing methane hydrates. Bacterial DNA is more readily extracted and amplified from the sediments than archaeal DNA. Molecular signatures from t-RFLP profiles indicate that certain taxa reoccur in different sediments. PhyloChip analysis suggests that numerous specific taxa are present in the sediments including archaea (methanogens, anaerobic methanotrophs, thermophiles) and bacteria (sulfate reducers, sulfur oxidizers, metal reducers, anaerobic ammonia oxidizers, acetogens, aerobic methanotrophs). Data from the PhyloChip hybridizations indicate that microbes capable of biogeochemical cycling of carbon, sulfur, nitrogen and metals are present in these sediments. Our future research will include the multivariate analysis of communities with respect to the geochemistry and geology of the sediments in order to determine the environmental factors that control microbial community diversity. We also plan to determine the identity and biogeochemical processes associated with the shallow-sediment biofilms.

ACKNOWLEDGEMENTS

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