



Distributions and assemblages of microbial communities along a sediment core retrieved from a potential hydrate-bearing region offshore southwestern Taiwan



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ABSTRACT

Assessing the impacts of methane released from hydrate-bearing environments on global carbon cycling would require detailed insights into the distributions and capacities of microbial communities at different horizons of sediment column. In this study, we conducted geochemical, gene abundance and diversity analyses for a sediment core retrieved from a potential hydrate-bearing region off southwestern Taiwan. Geochemical profiles were characterized by a sulfate-to-methane transition with decreasing total organic carbon and nitrogen in sediments, and increasing dissolved inorganic carbon, ammonium and total sulfur in sediments. Bacterial and archaeal 16S rRNA and *amoA* gene abundances decreased with depth. In contrast, ANME-1 and -2 16S rRNA gene abundances increased significantly across the sulfate-to-methane transition and peaked at different horizons below this interface. A total of 124,379 bacterial and 130,351 archaeal reads were recovered through tag-pyrosequencing of 16S rRNA genes and categorized into 9014 bacterial and 6394 archaeal operational taxonomic units on the basis of 97% sequence similarity, respectively. Major bacterial phyla/divisions and archaeal groups (>5% of the total reads) detected included *Chloroflexi*, *Planctomycetes*, OP9, *Deltaproteobacteria*, BHI80-139, MBG-B, *Halobacteria*, MCG, *Thermoplasmata*, ANME-1 and MG-I. The abundance variations of most major OTUs (>0.5% of the total reads) were statistically correlated with those of geochemical parameters. These lines of evidence suggest that the populations represented by the major OTUs or detected by group-specific primers were compartmentalized into different horizons and involved directly or indirectly in the cycling of methane, sulfate, organic carbon and nitrogen. Overall, this study demonstrates that the deep sequencing coverage combined with the quantification of gene abundance and geochemical characterization would enable to uncover the detailed distributions and potential metabolic capabilities of specific groups from complexly structured microbial communities in methane-rich marine sediments.

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1. Introduction

Gas hydrates are naturally occurring solid materials formed from a mixture of gas and water molecules arranged in a cage-like structure under specific pressure–temperature conditions (Sloan, 2003). Methane appears to be the most common gas constituent (Kvenvolden, 1993). To date, methane hydrates are primarily discovered on seafloors, in subseafloors of continental margins, and

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in permafrost regions. Recent estimates indicate that methane hydrates distributed globally might amount up to ~10,000 Gt (Milkov, 2004). Considering that its stability is strongly dependent on local geothermal gradients and fluctuations of seawater temperature, and its gaseous form is effective in heat absorption, such a huge potential capacity of methane hydrates stored in near surface environments would exert profound effects on the climatic fluctuation, carbon sequestration and submarine slope stability over geological time scales as well as provide as an additional energy source accommodating the diminishing petroleum reserves.

Decadal studies have demonstrated that the ultimate methane emission into seawater in hydrate-bearing environments is controlled by a complex reaction network of microbial processes

compartmentalized into different depth intervals (Boetius and Suess, 2004). In general, sulfate reduction is succeeded by sulfate-dependent anaerobic methanotrophy, and methanogenesis with increasing depth (Dale et al., 2008). The metabolic zonation results from the competition for common electron donors (e.g. hydrogen and acetate) during the energy acquisition, thereby coupling these metabolic pathways directly or indirectly with organic mineralization (Parkes et al., 2005). While the major players mediating these electron accepting processes have been documented on the basis of molecular and culturing analyses, community assemblages potentially involved in the hydrolysis and fermentation of organic matters and downstream carbon and nitrogen cycling remain largely unclear. Furthermore, community assemblages have been shown to be complexly structured and vary substantially from site to site or at a centimeter resolution in sediments (Bowman and McCuaig, 2003; Hamdan et al., 2012; Inagaki et al., 2006; Nunoura et al., 2012; Roalkvam et al., 2012; Siebert et al., 2011; Yanagawa et al., 2011). In addition to sequences closely affiliated with culture representatives or mixed populations in enrichments (e.g. *Deltaproteobacterial* sulfate reducers and anaerobic methanotrophic archaea), sequences affiliated with numerous uncultivated lineages (e.g. Marine Benthic Group B (MBG-B), Miscellaneous Crenarchaeotic Group (MCG), Marine Group I (MG-I) and Japan Sea group 1 (JS-1)) have been commonly recovered from such methane-rich sediments. Of these detected groups, ANME (ANAerobic Methanotrophic Euryarchaeota) groups have received far more attentions than others particularly due to their high abundances in methane transition zones and unique identities indicating methane consumption (Boetius et al., 2000; Knittel et al., 2005; Losekann et al., 2007). Despite the general consensus for the inference of potential metabolisms on few numerically dominant groups, the projected community diversity often exceeds those resolvable with the capability combining clone library construction and Sanger sequencing conducted in most case studies in which less than 100 clones are commonly screened. Therefore, determining the abundance changes of less dominant community members in response to the imposed redox and geochemical gradients is often infeasible.

The advent of tag-pyrosequencing technology provides unprecedented opportunities to tap microbial assemblages at a depth far exceeding (several orders of magnitude) that based on the conventional approaches (Parameswaran et al., 2007; Shendure and Ji, 2008). In most case studies, more than one thousand reads with an average length of more than 300 bps (base pairs) could be easily obtained for individual samples in a single run and processed through downstream analytical pipeline at a relatively faster speed. Although biases against the detection of specific phylogenetic groups could be introduced during PCR and sequencing (Kunin et al., 2010), such a sequencing depth would allow for identifying the variation patterns of both dominant and less abundant community members in environments.

On the basis of the distribution of the bottom simulation reflection, methane hydrates in subseafloor sediments off southwestern Taiwan have been estimated to range up to $2.7 \times 10^{12} \text{ m}^3$ (Lin, 2011), a quantity sufficient for long-term local energy exploitation. The source of these methane hydrates has been suggested to be derived from thermal decomposition and microbial degradation of organic matters buried during the convergence between the Eurasian and Philippine Sea Plates since five million years ago (Oung et al., 2006). Deeply-sourced methane ascends vertically along fracture network to shallow subseafloor environments, providing abundant reducing powers and substrates to drive methane-dependent metabolisms. Additionally, rapid sediment accumulations (Dadson et al., 2003) could supply freshly eroded recalcitrant carbon for hydrolysis and fermentation. Using the pore water and methane profiles, previous studies have revealed that sulfate

reduction and sulfate-dependent methanotrophy are prevalent in the region (Chuang et al., 2006, 2013). These metabolically active zones are confined at different depth intervals at different sites, depending on the interplay between the upward flux of deeply-sourced methane and downward flux of reactive carbon. However, detailed community assemblages potentially involved in sulfate reduction, methanotrophy, methanogenesis, nitrogen metabolisms and organic degradation at any depth resolution have not been reported.

As part of the ongoing gas hydrate exploration program, this study aimed to uncover the compositions of microbial communities along a sediment core extending to a depth of 4.5 m below seafloor in the potential hydrate-bearing region off southwestern Taiwan using the high throughput tag-pyrosequencing technology. A large set of sequence data (~250,000 reads) were integrated together with geochemical profiles and variations of gene abundances to assess community patterns and abundance variations of individual members imposed by the redox and geochemical transitions.

2. Materials and methods

2.1. Site description and sampling

Offshore southwestern Taiwan is located in the transition from the subduction system of the Manila trench to the west to the arc-continent collision system to the east (Liu et al., 2006). A series of fold-and-thrust structures develop with continuous shortening and deformation in the accretionary wedge, providing abundant conduits for gas and fluid migration. Bottom simulation reflection and at least 50 active submarine mud volcanoes and seeps have been identified using seismic profiles, bathymetry and sonar signals (Chiu et al., 2006), suggesting a widespread distribution of gas hydrates in the region. Methane appears to be the major constituent of the gases in pore space or released into seawater (Chuang et al., 2006). Sediment cores were collected from this potential hydrate-bearing region during the Marion Dufresne expedition of leg MD-178-10 in 2010. One CASQ (Wang et al., 2005) box core, MD-178-3280 (22°14.550'N, 119°54.230'E), retrieved from the lower slope unit was selected for our analysis. At this site, the water depth was 1395 m. The overall length of the obtained intact core was 5 m. Samples were collected as soon as the corer was placed on deck and opened. Samples for pore water analyses were subject to centrifugation at $\sim 8200 \times g$ for 15 min. Supernatants were decanted from the tubes, filtered using syringe filters (0.45 μm pore size) and stored in a 4 °C refrigerator until further analyses. For gas samples, sediments were transferred into 20 mL glass vials filled with a saturated NaCl solution. The vials were sealed with butyl rubber stoppers and crimped with aluminum rings. Because the top and bottom samples were subject to potential contamination introduced by manned activities, samples for molecular analyses were obtained from nine depth intervals (50, 100, 150, 200, 250, 300, 350, 400 and 450 cm below seafloor (cmbsf)). At each specific depth interval, sediments were collected using sterilized, disposable knives, kept in 50 mL sterilized centrifuge tubes, and stored in a -40 °C freezer immediately after sampling. All samples were shipped back to the lab on blue ice or dry ice and stored in a 4 °C refrigerator or a -80 °C freezer in the laboratory.

2.2. Geochemical analysis

Anions were determined using an ion chromatograph (4500i, Dionex, USA) equipped with a suppressed conductivity detector and an IonPac AS4A anion exchange column. For methane analyses, 5 mL of NaCl solution was first removed from the vial and replaced

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