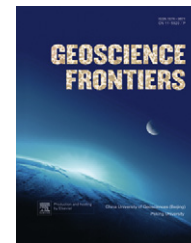
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RESEARCH PAPER

Microbial diversity in cold seep sediments from the northern South China Sea

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Abstract South China Sea (SCS) is the largest Western Pacific marginal sea. However, microbial studies have never been performed in the cold seep sediments in the SCS. In 2004, “SONNE” 177 cruise found two cold seep areas with different water depth in the northern SCS. Haiyang 4 area, where the water depth is around 3000 m, has already been confirmed for active seeping on the seafloor, such as microbial mats, authigenic carbonate crusts and bivalves. We investigated microbial abundance and diversity in a 5.55-m sediment core collected from this cold seep area. An integrated approach was employed including geochemistry and 16S rRNA gene phylogenetic analyses. Here, we show that microbial abundance and diversity along with geochemistry profiles of the sediment core revealed a coupled reaction between sulphate reduction and methane oxidation. Acridine orange direct count results showed that microbial abundance ranges from 10^5 to 10^6 cells/g sediment (wet weight). The depth-related variation of the abundance showed the same trend as the methane concentration profile. Phylogenetic analysis

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indicated the presence of sulphate-reducing bacteria and anaerobic methane-oxidizing archaea. The diversity was much higher at the surface, but decreased sharply with depth in response to changes in the geochemical conditions of the sediments, such as methane, sulphate concentration and total organic carbon. Marine Benthic Group B, *Chloroflexi* and JS1 were predominant phylotypes of the archaeal and bacterial libraries, respectively.

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1. Introduction

Cold seep sediments represent one of the most extreme marine conditions and offer unbounded opportunities to discover the interactions between macroorganisms/microbes and geochemical processes. Defining the diversity and distribution of microbial communities in marine cold seep sediments has been a long-standing challenge in the field of microbial ecology and evolution (Li et al., 1999). Cold seeps are characterized by fluids seepage into surface sediments, and the fluids have elevated methane and/or sulfide concentrations over those of ambient seawater. The first cold seep ecosystem was discovered on the Florida Escarpment in the Gulf of Mexico (Paull et al., 1984). Subsequently, seeping sites were found in different geological settings, including active [e.g. Northern Pacific (Kojima, 2002)] and passive margins [e.g. Florida Escarpment in the Gulf of Mexico (Paull et al., 1984)] at depths ranging from 10 m (Jensen et al., 1992) to at least 7300 m (Jumars and Hessler, 1976).

Microbial chemosynthetic carbon fixation is the basis for the food web at cold seeps. Microbial-driven metabolic processes, such as aerobic oxidation of methane, anaerobic oxidation of methane (AOM), methanogenesis, sulphate reduction, sulfide oxidation, and petroleum hydrocarbon oxidation, have been recognized (Aharon and Fu, 2000; Hinrichs et al., 2000; Formolo et al., 2004; Inagaki et al., 2004; Joye et al., 2004; Niemann et al., 2006). In these processes, AOM presumably coupled to sulphate reduction facilitates formation of carbonates and, in many places, generates extremely high concentrations of hydrogen sulphide in pore waters (Levin, 2005). Methane can also be oxidized aerobically to CO₂ by aerobic methanotrophic bacteria that live at the interface between anoxic, methane-rich sediments and oxic seawater (Reed et al., 2009). In cold seep environments, microorganisms contribute to the growth of invertebrates as symbionts and play an important role in the sulphur cycle (Kennicutt et al., 1985).

Microbial community structures have been investigated in different typological cold seep areas around the world, such as the Gulf of Mexico (Yan et al., 2006), Hydrate Ridge (Knittel et al., 2005), Mediterranean Sea (Heijs et al., 2007; Omoregie et al., 2009), Nankai Trough (Arakawa et al., 2006), Sagami Bay (Fang et al., 2006; Takishita et al., 2007), and Marmara Sea (Ritt et al., 2010). Those previous studies showed that different cold seep areas harboured characteristic microbial community structures. For example, in Sagami Bay, γ -*proteobacteria* and δ -*proteobacteria* were the dominant bacterial phylotypes, and *Euryarchaeotes* including the anaerobic methane oxidation group (ANME)-2a and ANME-2c were detected (Fang et al., 2006). In Nankai Trough cold seep, *Gammaproteobacteria* and *Deltaproteobacteria* were identified amongst the bacteria from three depths of the sediments, but ANMEs and methanogens were only found in the 600-m deep sediments (Arakawa et al., 2006). In the

tropical Timor Sea methane seep, *Alpha*-, *Delta*- and *Gammaproteobacteria* and marine group-I (MG-I) were the dominant bacterial and archaeal phylotypes, respectively (Wasmund et al., 2009). At Hydrate Ridge, there were only four phylogenetic archaeal clusters detected, most of which belonged to ANME (Knittel et al., 2005). In pockmarks and brine seeps of Eastern Mediterranean mud volcanoes, the microbial activity showed strong differences with respect to the rates of AOM and sulphate reduction as well as microbial structures (Omoregie et al., 2009). In the Gulf of Mexico, anaerobic methane-oxidizing communities were influenced by hydrocarbons at the gas hydrate and seep sites, and can be differentiated from those in other normal marine sediments (Yan et al., 2006).

The South China Sea (SCS) is one of the marginal seas around the Pacific Ocean. Tectonically, it is a passive margin setting grading into the SCS Basin and it abuts on the accretionary wedge formed off-shore with the Southwestern Taiwan Island. During the past decades, a number of geophysical and geological cruises were performed in this area, and old and/or active methane seepages were found in the Dongsha area of the SCS (Suess, 2005). These cruises also showed the presence of gas hydrates, and gas hydrate samples were obtained by drilling in the Shenhu area of the northern SCS in 2007 (Zhang et al., 2007).

During the past decades, a number of geophysical, geological, biological and geochemical evidences for the presence of gas hydrates, and cold seeps or fluid and methane vents have been found in the Haiyang 4 Site. Bottom simulating reflectors (BSRs) were found in this region (Song et al., 2001). Methane-derived cold seep carbonates and presence of microbes in these carbonates were reported by Chen (Chen et al., 2005) and Su et al. (2008). Cold seep bivalve communities and bacterial mats were observed by seafloor camera surveys during the Chinese “Haiyang 4” cruises and SO177 cruise (Fig. 1); and an alive bivalve sample was obtained by “Haiyang 4” in 2004 and abundant dead bivalve shells were collected by “SO177” in 2005 (Suess, 2005; Huang et al., 2008). The SO177 cruise also detected high concentrations of methane, sulphate-methane-interface (SMI), and negative chloride ion anomalies in sediment cores (TV-guided multi-core and gravity cores) from the “Haiyang 4” Site area (Suess, 2005; Huang et al., 2008).

A few studies have examined microbial communities in the SCS. The results showed that archaeal and bacterial diversities of the SCS sediments were similar to those in other deep-sea sediments (Xu et al., 2004; Jiang et al., 2007; Li et al., 2008a, b; Zhang et al., 2010). However, little is known about the microbial community composition and its correlation with geochemical conditions in cold seep sediments in the SCS. This knowledge is of great importance for understanding the biogeochemical processes in globe cold seep ecosystems. The objective of this study was therefore to study microbial communities in sediments of a gravity piston core (Core DSH-1) collected from the cold seep in the northern SCS.

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