Journal of Microbiology (2014) Vol. 52, No. 2, pp. 111–119
DOI 10.1007/s12275-014-3133-1

Microbial Communities in Semi-consolidated Carbonate Sediments of the Southwest Indian Ridge[§]

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(Received Mar 5, 2013 / Revised Jul 29, 2013 / Accepted Sep 10, 2013)

White semi-consolidated carbonate sediments attached to black ferromanganese oxide films were collected approximately 50 km west of a newly discovered hydrothermal field near the Southwest Indian Ridge (SWIR). The biodiversity of the prokaryotic communities within the field was examined using clone library-based culture-independent analysis of the exterior black oxides and the interior white carbonates. Subsequent 16S rRNA gene analysis suggested that Gammaproteobacteria, Acidobacteria, and Thaumarchaeota members dominated the bacterial and archaeal clone libraries. To further characterize the metabolic processes within the microbial community, analyses of the *amoA* (coding the alpha subunit of the ammonia monooxygenase for Archaea) and *aprA* (coding the alpha subunit of the dissimilatory adenosine-5′-phosphosulfate reductase for the sulfate-reducing and sulfur-oxidizing prokaryotes) functional genes were conducted. The functional gene analysis results suggested that Thaumarchaeota and Alphaproteobacteria members were the potential players that participated in N and S cycles in this marine carbonate sedimentary environment. This paper is the first to describe the microbial communities and their potential metabolic pathways within the semi-consolidated carbonate sediments of the SWIR.

Keywords: carbonate sediments, ferromanganese oxides, prokaryotic communities, biodiversity, metabolic process

Introduction

Detailed information about prokaryotic community structures and their spatial variations is essential to the assessment of the biological, geographical, and chemical relationships of marine environments. It also aids in the characterization of the roles that benthic microorganisms play in overall oceanic processes. Previous molecular diversity inventories of prokaryotes in marine pelagic sediments have been conducted for various environments, such as the cold seeps (Li *et al*., 1999; Newberry *et al*., 2004; Arakawa *et al*., 2006; Reed *et al*., 2006), Arctic Ocean (Knoblauch *et al*., 1999; Ravenschlag *et al*., 1999, 2000, 2001; Sahm *et al*., 1999; Bowman and McCuaig, 2003), and hydrothermal fields (Teske *et al*., 2002; López-García *et al*., 2003; Reed *et al*., 2006; Kato *et al*., 2009; Peng *et al*., 2011; Li *et al*., 2013). These studies have collectively provided useful insights into the phylogenetic composition of the prokaryotic community within the ocean. However, investigations to date of microorganisms in marine sediments have revealed only fragmentary evidence of the extant phylogenetic and metabolic diversity (Hagstrom *et al*., 2002) compared with that worldwide (Curtis *et al*., 2002). Moreover, the roles that microorganisms play in the microbial-mediated redox cycling of N and S require further identification. Therefore, more details about the geochemical characteristics and microbial community of marine sedimentary environments are required.

The Southwest Indian Ridge (SWIR), which marks the boundary between the Antarctic and African plates, extends from the Bouvet triple junction in the Atlantic Ocean to the Rodriguez triple junction in the Indian Ocean (Patriat and Segoufin, 1988; Muller *et al*., 1999). Hydrothermal activity along the SWIR has been continually explored over the last several decades (German *et al*., 1998; Scheirer *et al*., 1998; Sohrin *et al*., 1999; Munch *et al*., 2001; Batch *et al*., 2002; Tao *et al*., 2007), as it is both the slowest spreading of the main ridges and the sole modern migration pathway between the diverse vent fauna of the Atlantic and Pacific oceans (German *et al*., 1998).

During the fifth leg of the 2008 Chinese DY115-20 expedition of the R/V Da-Yang-Yi-Hao, a hydrothermal field was discovered at 50.4671°E, 37.6579°S (Tao *et al*., 2008). Many semi-consolidated carbonate sediments were simultaneously discovered at 50.85–51.00°E, 37.60–37.65°S approximately 50 km from the newly found hydrothermal region. These carbonate sediments were characterized by the attachment of flat black oxides to the white carbonates. This paper describes the results of a molecular phylogenetic analysis of these semi-consolidated carbonate sediments using the 16S rRNA gene and functional gene approaches. This information will further extend our knowledge of the structure and role that the microbial community plays in the ocean ridge sedimentary environments.

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[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

Materials and Methods

Sample collection

The semi-consolidated carbonate sediment samples were acquired using a television grab bucket of the R/V Da-Yang-Yi-Hao conducted by the China Ocean Mineral Resource R&D Association in 2008. The sample in this study was discovered at 51.0091°(E), 37.6081°(S) with a water depth of 2,034 m (Fig. 1). A thin black Fe-Mn oxide film was attached to the white semi-consolidated carbonates, and a few living and dead actiniae lived upon its surface. Due to their different compositions and colors, these sediments were divided into two sub-samples: surface black oxides (O) and interior white carbonates (C). The black oxide samples were collected aseptically by scraping from the exterior part of the carbonate sediments with a sterile blade, while the carbonate samples were immediately taken from the interior part of the white semi-consolidated sediments. The samples were stored at -20°C immediately after being taken onboard and then maintained on dry ice during transportation to our laboratory.

Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) analysis

Morphological observations were made using a SEM (Philips XL30) equipped with an EDS (Phoenix EDAX) for geochemical analysis at Tongji University in Shanghai.

DNA extraction

The total microbial community DNA were directly extracted from the oxide and carbonate samples using a Power Soil™ DNA kit (MO BIO Laboratories, USA) following the manufacturer's protocol and stored at -80°C until the analysis. We extracted DNA from two replicate samples and pooled the two replicate DNA extracts together for each sample. Negative controls were also used.

16S rRNA and functional gene clone library construction

Bacterial 16S rRNA genes were amplified using the primers Eubac27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and Eubac1492r (5′-GGTTACCTTGTTACGACTT-3′) (Lane, 1991). The archaeal 16S rRNA gene was amplified by primer pair Arch21f (5′-TTCCGGTTGATCCYGCCGGA-3′) and Arch958r (5′-YCCGGCGTTGAMTCCAATT-3′) (Delong, 1992), with products close to 1,500 and 900 bp, respectively. The functional genes were also amplified by polymerase chain reaction (PCR) using the following primer sets: Arch-amoAF (5′-STAATGGTCTGGCTTAGACG-3′) and Arch-amoAR (5′-GCGGCCATCCATCTGTATGT-3′) for the archaeal *amoA* gene fragment (~635 bp; Francis *et al.*, 2005) and AprA-1-FW (5′-TGGCAGATCATGATYMAYGG-3′) and AprA-5-RV (5′-GCGCCAACYGGRCCRTA-3′) for the *aprA* gene fragment (~396 bp; Meyer and Kuever, 2007).

In a final 50-μl volume, the PCR reaction mixture contained the following: 2.0 μl of template DNA, 5.0 μl of 10× PCR buffer (Sangon, China), 5.0 μl of 2.5 mmol/L deoxy-

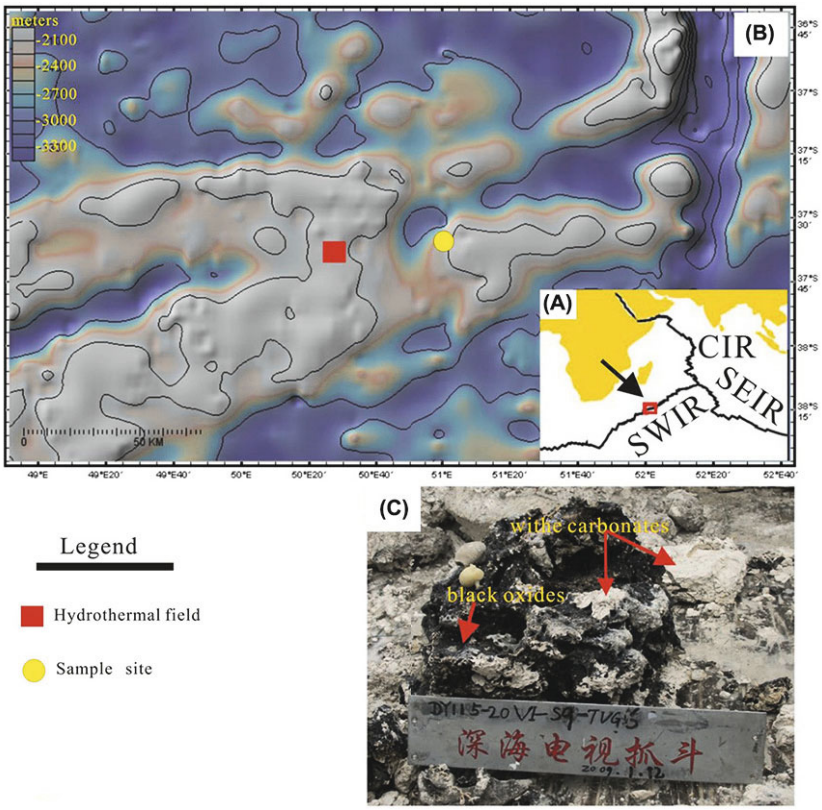

Fig. 1. (A) Location of the Southwest Indian Ridge (SWIR) (B) Sample site and nearby known hydrothermal field at the SWIR. (C) Image of the semi-consolidated carbonate structure. SEIR, Southeast Indian Ridge; CIR, Central Indian Ridge.

nucleoside triphosphates, 2.5 mM $MgCl_2$, 0.2 μM of each primer, and 1 U of Taq polymerase (Sangon). Amplification conditions for the archaeal and bacterial 16S rRNA genes were as follows: an initial denaturalization step of 94°C for 4 min, subsequent denaturation at 94°C for 60 sec, annealization at 55°C for 45 sec, extension at 72°C for 60 sec for a total of 30 cycles, and a final extension step of 72°C for 10 min. Amplification conditions for the *amoA* functional gene were as follows: 95°C for 5 min; 30 cycles consisting of 94°C for 45 sec, 53°C for 60 sec, and 72°C for 60 sec; and 72°C for 15 min (Francis *et al*., 2005). Amplification conditions for the *aprA* functional gene were as follows: 95°C for 5 min; 27 cycles consisting of 95°C for 60 sec, 54°C for 60 sec, and 72°C for 3 min; and 72°C for 5 min (Blazejak *et al*., 2006).

For each sample, three PCR products were pooled and purified on a 1% (w/v) agarose gel and extracted using a Gel extraction kit (Omega, USA) following the manufacturer's instructions. Purified PCR products were cloned into the pMD18-T vectors (TaKaRa, China) and transformed into competent *Escherichia coli* DH5α cells (TaKaRa) according to the manufacturer's instructions. The transformed clones were grown in 1 ml of Luria-Bertani culture medium for 1 h at 37°C with shaking (220 rpm) and then coated on agar plates containing ampicillin (100 μg/ml) at 37°C for 12–16 h to form individual bacterial colonies.

Phylogenetic analysis

Clones were randomly selected from the bacteria and archaeal 16S rRNA gene and functional gene clone libraries of the two samples. These clones were then directly sequenced using an ABI 3730 capillary electrophoresis sequencer (Applied Biosystems, USA) by the dideoxynucleotide chain-termination method using the M13f and M13r T-vector universal primers. The inserted sequences were spliced using DNAMAN software (version 6.0). Sequences were trimmed manually for the chimeric sequences using the CHIMERA_CHECK program of the Ribosomal Database Project II (Maidak *et al*., 2001). Nonchimeric sequences were submitted to the Advanced BLAST search program (available through the National Center for Biotechnology Information) to search for closely related sequences in the GenBank database. All of the clone and matched sequences were then aligned by CLUSTAL X 1.83 software and the irregular segments were discarded. Phylogenetic trees were constructed using the neighbor-joining method using by Mega (3.1) software. Bootstrap analysis was used to provide confidence estimates of the tree topologies. The sequences obtained in this study are available under GenBank database accession numbers JN886845–JN886951 for the 16S rRNA gene sequences, JN934396–JN934425 for the *amoA* gene sequences, and JN934426–JN934468 for the *aprA* gene sequences.

Results

Geochemical characteristics

The semi-consolidated carbonate sediments from the SWIR studied here were characterized by the attachment of black Fe-Mn oxide film to the white carbonate surface. SEM observation results showed that the microfossils were embedding in the amorphous oxides on the exterior portion of the carbonate sediments (Fig. 2A and 2B). Fe, Mn, Ca, O, and Cl with minor Na, Mg, Si, and C elements were the major chemical compositions of the black oxides indicated by the EDS analysis (Fig. 2C). However, the interior part was only predominated by microfossils such as coccolith and foraminifera, and the amorphous oxides were absent from this part (Fig. 2D). Moreover, EDS analysis showed that Ca, O, and C were the major elements of the interior white carbonates (Fig. 2E).

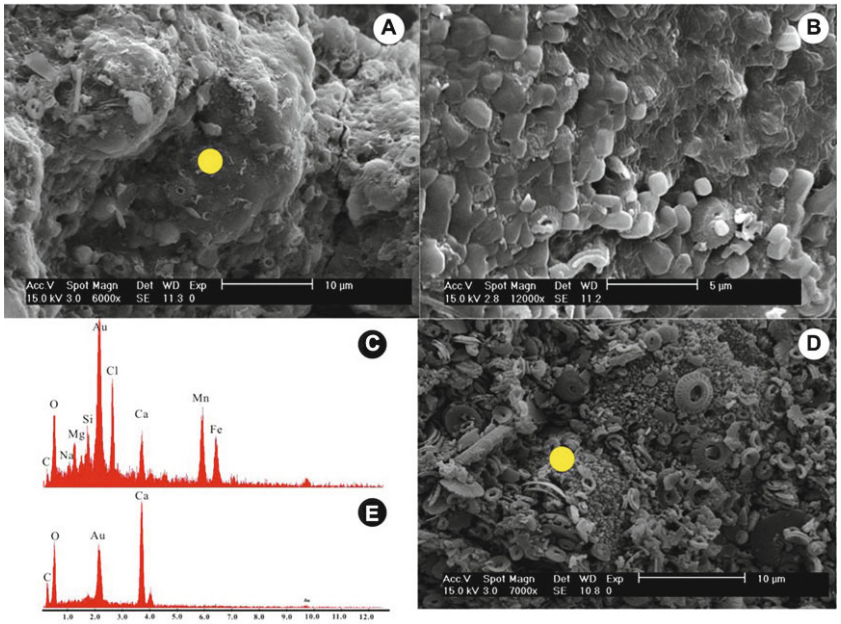

Fig. 2. Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) images of the semi-consolidated carbonate sediments. (A, B) Exterior black oxides. (C) EDS image of the yellow point in A, the Au peak from the Au powder sprayed plating on the sample. (D) Interior white carbonates. (E) EDS image of the yellow point in D, the Au peak from the Au powder sprayed plating on the sample.

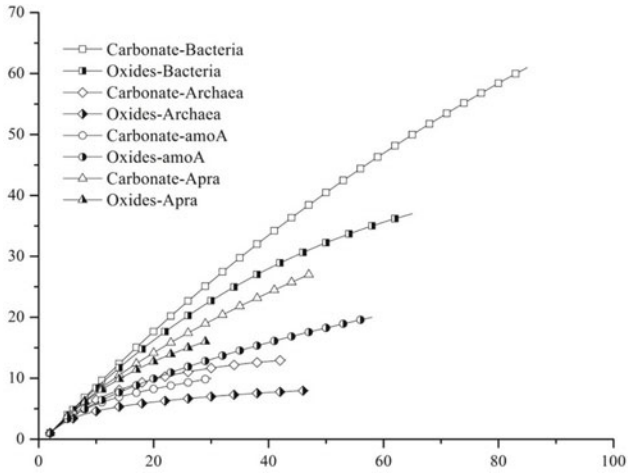

Fig. 3. Rarefaction curves from 16S rRNA and functional genes clone libraries. Phylotypes were defined at 97% sequence similarity.

Diversity of the 16S rRNA and functional genes

To evaluate the biodiversity and phylotype richness of the microbial communities in the carbonates and oxides, statistical analyses for all of the clone libraries were performed using PAST software (Hammer *et al.*, 2001) as follows: rarefaction analysis, Simpson's diversity index, Shannon-Weaver's diversity index, and Margalef's species richness index (Fig. 3 and Table 1). Coverage was calculated with the equation $C=[1-(n1/N)]\times100$, where *n1* is the number of single-occurrence phylotypes within a library and *N* is the total number of clones in the library (Good, 1953). The bacterial clone libraries showed the lowest phylotype recovery ratios by rarefaction analysis, although many more clones were sequenced within them than in the other libraries. Both Simpson's and Shannon-Weaver's diversity calculations indicate the bacterial and *aprA* clone libraries had higher diversity but relatively low coverage values (<50%). In contrast, the values of the Simpson's and Shannon-Weaver's diversity indexes revealed the archaeal and *amoA* clone libraries had lower diversity and relatively high coverage values (>60%). In addition, the two bacterial clone libraries had the two highest Margalef richness index values, while the *amoA* library of the carbonates and archaea library of the oxides had the two lowest Margalef richness index values.

Bacterial 16S rRNA gene sequence profiles

A total of 145 clones (64 and 81 clones from the oxides and carbonates, respectively) were sequenced and divided into 93 phylotypes based on 97% nucleotide identity. Phylogenetically, they are highly diversified and affiliated with the Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Deferribacteres, Nitrospirae, Planctomycetes, Proteobacteria (including alpha, beta, gamma, and delta subdivisions), Verrucomicrobia, Firmicutes, and uncultured taxonomic groups WS3 (Dojka *et al.*, 1998). Members belonging to the Gammaproteobacteria and Acidobacteria generally appeared to be diverse and abundant at both the exterior oxides and the interior carbonates (Fig. 4).

Gammaproteobacteria: Gammaproteobacteria was the second largest segment of bacterial clone libraries of the semi-consolidated carbonate sediments. Seventeen phylotypes including 29 clones compriseing a 20% proportion of the total clones were most closely related to this subdivision (15 and 14 clones from the oxides and carbonates, respectively). Among them, 4 of 17 phylotypes were affiliated with established phylogenetic groups containing cultivable representatives such as *Pseudomonas*, *Alteromonas*, *Serratia*, and *Methylobacter* (Supplementary data Fig. S1). Moreover, one phylotype from the oxides (O-B23) showed high similarity with the Mn-oxidizing bacterium *Pseudomonas* MnB1. Another phylotype branched with a symbiont (sulfur-oxidizer AJ620496) obtained from *Olavius algarvensis* collected from Mediterranean Sea grass sediments (Ruehland *et al.*, 2008). The remaining phylotypes were grouped with clones obtained using culture-independent methods. This class of *Proteobacteria* is common in marine sediments and water column environments.

Betaproteobacteria: Two phylotypes composed of 4 clones belonged to the Betaproteobacteria and had a proportion of 2.76% within the bacterial library. Our sequence mainly clustered with the pure cultures *Nitrosospira* and *Ralstonia* (Supplementary data Fig. S1).

Deltaproteobacteria: Fourteen phylotypes representing 21 analyzed clones (5 and 16 clones from the oxides and carbonates, respectively) fell into the Deltaproteobacteria class. Among them, two phylotypes were clustered with *Nitrospina* which could reduce SO_4^{2-} and NO_3^- (Supplementary data Fig. S1). The others were related to sequences recovered from a wide range of marine environments such as cold seep (Li *et al.*, 1999), hydrothermal field (unpublished data), ocean crust (Santelli *et al.*, 2008), and marine basalt (Mason *et al.*, 2009).

Table 1. The results of statistical analyses of each library

	Clone number	Phylotypes	Shannon-Weaver	Simpson	Margalef	Coverage (%)
Carbonate-Bacteria	84	61	4.016	0.9799	13.54	27
Oxides-Bacteria	64	37	3.512	0.9673	8.66	42
Carbonate-Archaea	42	13	2.351	0.8878	3.21	69
Oxides-Archaea	46	8	1.714	0.7854	1.83	83
Carbonate-amoA	29	10	2.031	0.8442	2.67	66
Oxides-amoA	57	20	2.389	0.8489	4.70	65
Carbonate-aprA	46	27	3.052	0.9376	6.79	41
Oxides-aprA	28	16	2.633	0.9184	4.50	43

Alphaproteobacteria: Eleven phylotypes, including 18 clones (7 and 11 clones from the oxides and carbonates, respectively) accounting for 12.41% of the total bacterial clones, were most closely related to the Alphaproteobacteria. Members of this class were widely diversified. Among them, Rhodospirillales were the largest fraction and contained 7 phylotypes (12 clones). Members of this order have been detected in *Muricea elongata*, a shallow-water gorgonian (DQ917856; unpublished data), and a pure culture MC2UP-L3 isolated from hydrothermal field sediments of the Southwest Pacific Ocean (Supplementary data Fig. S1; Dong *et al*., 2010). Notably, clones of this order in this study were also related to sulfur-oxidizing bacterial endosymbionts of *Inanidrilus leukodermatus* (AJ890098; Blazejak *et al*., 2006). In addition, one phylotype from the oxides (O-B73) showed high similarity with the iron-reducing bacterium enrichment culture clone HN72, which was isolated from arsenic-contaminated paddy soil of Hunan province, China (AJ269066; unpublished data).
Acidobacteria: A total of 21 phylotypes belonged to Acidobacteria, which comprised the largest proportion (22.07%) of the total bacterial clones (13 and 19 clones from the oxides and carbonates, respectively). However the functions of this group remain unidentified. All of the clones in this phylum were similar to the uncultured counterparts in the public database that were collected from various marine environments (Supplementary data Fig. S2). The most significant part was closest related to the clones derived from hydrothermal region sediments. The remainder were branched with sequences recovered from ocean crust (Santelli *et al*., 2008), polluted harbor (Zhang *et al*., 2008) and southern Cretan margin sediments (Polymenakou *et al*., 2009).
Actinobacteria, Bacteroidetes: In this study, approximately 6.9% of the total clones were affiliated within Actinobacteria. Among them, two phylotypes (O-B25 and O-B49) were related to the uncultured clone I5B from the hydrothermal region (FJ205357; unpublished data) (Supplementary data Fig. S2). Sequences belonging to Bacteroidetes comprised small proportions (6 clones, 4.14% of the total) (Fig. 4), and all of them were from the oxides. Their most close cultured relatives were *Capnocytophaga* and *Cyophaga*.

The Verrucomicrobia, Nitrospirae, Chloroflexi, Firmicutes, Deferribacteres, Planctomycetes, and WS3 contained a small number of clone sequences, most of which were closely related to uncultured clones derived from various marine environments (Supplementary data Fig. S2). Five clones fell within Verrucomicrobia, including one phylotype related to a pure culture of *Coraliomargarita akajimensis* (Yoon *et al*., 2007). The clone sequences within Nitrospirae were related to the culture *Nitrospira*, nitrite-oxidizing bacteria (Burrell *et al*., 1998) that are important in the nitrite oxidization process (Ehrich *et al*., 1995). One clone from the oxides grouped within the Chloroflexi-related clone MSB-3G36 was retrieved from mangrove soil (DQ811860; unpublished data). Sequences of Firmicutes and Deferribacteres were both closely related to the clones recovered from hydrothermal region at the East Lau Spreading Center. A few unidentified bacteria in this study, together with its closest relatives such as clones recovered from the yellow sea surface sediment and ocean crust fluid, provided a novel bacterial subdivision and might indicate special functions.

Archaeal 16S rRNA gene sequence profiles

Of the total 88 clones that were completely sequenced (46 and 42 clones from the oxides and carbonates, respectively), 14 phylotypes were defined. Nearly all of them belonged to Thaumarchaeota. The most interesting member was the pure culture *Nitrosopumilus maritimus* SCM1, a mesophilic archaeon that is able to oxidize ammonia (Könneke *et al*., 2005) (Supplementary data Fig. S3). The other closest relatives were uncultured sequences derived from various deep sea sedimentary environments including hydrothermal fields, Antarctic bathypelagic sediment surface, and marine basalts. Only one phylotype C-A3 from the interior carbonates belonged to Crenarchaeota, and its closest sequence was clone D1Ru (Ehrhardt *et al*., 2007), which was recovered from potentially vast hydrothermal reservoirs of the basaltic flanks of the EPR.

Functional gene phylotypes

Phylotypes of the *amoA* functional gene that were involved in the ammonia-oxidation pathways (ammonia monooxygenase for Archaea) were recovered from the oxides and carbonates. Detection of these functional genes indicated the presence of microorganisms that were potentially involved in the N cycles in the study environment. A total of 86 archaeal *amoA* gene sequences were analyzed and 30 phylotypes were defined based on 97% nucleotide identity. Nearly all of the *amoA* phylotypes were grouped within the Water Column A cluster (Sediments or Shallow Marine Clade *amoA*) defined in an earlier report (Francis *et al*., 2005), the

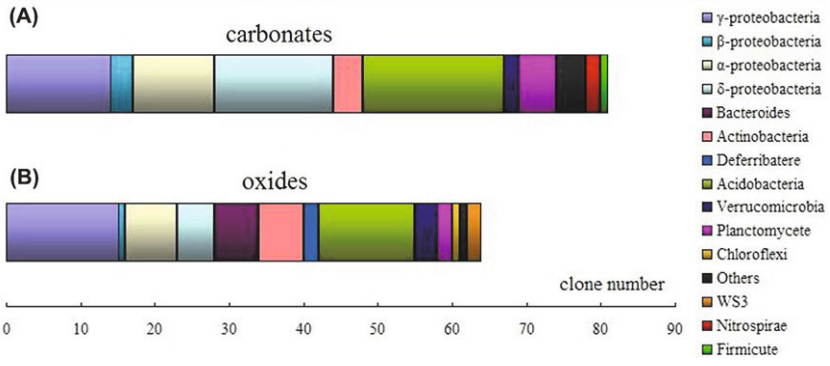

Fig. 4. Bacterial community structures in the semi-consolidated carbonate sediments from the Southwest Indian Ridge based on the 16S rRNA gene clone libraries. (A) Separate oxides and carbonate bacterial community structure. (B) Analysis of the total bacterial clones sequenced in this study.

exception being phylotype *amoA*-O-38, which fell into Water Column B (Deep Marine Clade *amoA*; Francis *et al*., 2005) and exhibited the highest identity to the sequence GOM_WAM1-1 derived from a Gulf of Mexico sample (Supplementary data Fig. S4). Mincer and colleagues (2007) suggested that the phylotypes grouped within Water Column A were related to the Thaumarchaeota, while the Water Column B clusters were related to the pSL12-related group, which included the pSL12 clone obtained from a terrestrial hot spring (Barns *et al*., 1996). This finding was consistent with the analyses of the archaeal 16S rRNA gene, which showed Thaumarchaeota predominance in the microbial community of the present carbonate sediments.

Phylotypes of the *aprA* functional gene that are involved in the sulfate-reduction and sulfur-oxidation metabolic pathways (coding for the alpha subunits of the dissimilatory adenosine-5′-phosphosulfate reductase *aprA*) were also recovered from the oxides and carbonates. The *aprA* gene-based analyses enabled the detection of 43 phylotypes representing 74 clones (based on 97% nucleotide identity), which can participate in the S biogeochemical cycle in the semi-consolidated carbonates sedimentary environments. Mostly of the phylotypes (41 of 43) were indicative for the presence of sulfur-oxidizing alphaproteobacterial species (Supplementary data Fig. S5). Only two phylotypes from the interior carbonates (Apra-C-24 and Apra-C-27) were weakly affiliated with the *aprA* sequence of uncultured prokaryote species that were recovered from agriculturally drained peats (DQ995782; unpublished data). All of the the *aprA* gene sequence identities were rather weak, with percentages of similarity <94%. The dominant potential sulfur-oxidizing Alphaproteobacteria was moderately related to bacteria from the gut microflora of wood-feeding urchin *Asterechins elegans* living in the sulfide-rich wood falls environment (Becker *et al*., 2009). Moreover, no unambiguous SRB was recovered by *aprA* from the present samples despite the fact that Deltaproteobacteria was one of the major components in the interior carbonate bacterial clone library.

Discussion

This study reported the distribution of the prokaryotic communities inhabiting both the exterior black Fe-Mn oxides and interior white carbonates of the semi-consolidated sediments, and we focused on microorganisms involved in the nitrogen N and S cycles using the 16S rRNA and functional gene approaches. Previous research in modern submarine hydrothermal fields focused on restricted areas with high metal (e.g. Fe and Mn) contents along the mid-ocean ridge crests (e.g., Boström and Peterson, 1966; López-García *et al*., 2003). However, scientific surveys of microbial communities in areas with a moderate distance to the known vents are still lacking.

The geochemical characteristics of the highly biogenic carbonate sediments (containing 80–87% $CaCO_3$; Cave *et al*., 2002), which were putatively influenced by the hydrothermal activity, were previously studied at the rainbow hydrothermal field at the mid-Atlantic ridge. These samples were characterized by their enrichment of P, V, Mn, Fe, and Cu caused by fallout from the hydrothermal plume (Cave *et al*., 2002). In the present study, these sediments were collected from an area approximately 50 km from the newly discovered hydrothermal field at the SWIR. SEM and EDS analyses proved that the black exterior portion of the semi-consolidated carbonate sediments consisted of Fe and Mn oxides with some biodetritus. However, there was not enough geochemical evidence to prove that these Fe and Mn oxides were collected from the hydrothermal fields at the SWIR.

Clone analyses of the 16S rRNA gene indicated that the bacterial microflora associated with different regions (the exterior oxides and interior carbonates) of the semi-consolidated sediments were highly diversified. The results showed that 93 different phylotypes representing 145 clones analyzed fell into the alpha, beta, gamma, and delta subdivisions of Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Deferribacteres, Nitrospirales, Planctomycete, Verrucomicrobia, and uncultured taxonomic groups WS3. All of the bacterial groups detected in this study may be common in deep-sea sedimentary environments and participate in a variety of *in situ* biogeochemical processes such as C, N, S, Fe, and Mn cycling.

Bacteria belonging to the Gammaproteobacteria and Acidobacteria appeared to be diverse and abundant in both the exterior oxides and the interior carbonates. The fact that the bacterial clone library was dominated by Gamma-proteobacteria and Acidobacteria was already reported in hydrothermal sediment samples from the rainbow field at MAR (López-García *et al*., 2003). Li *et al*. (2009) also suggested that Gammaproteobacteria and Acidobacteria were the two major groups in the bacterial clone libraries of the surface sediments of the Pacific Arctic Ocean. Gammaproteobacteria members are metabolically versatile and abundant in various environments including the cold deep ocean, hydrothermal region, nodule province, polluted continental area, and water column.

Acidobacteria has been considered as diverse as other bacterial phyla such as Proteobacteria (Hugenholtz *et al*., 1998; Quaiser *et al*., 2004), a finding that was supported by molecular studies revealing the complex diversity and coherence of the phylum and its wide distribution throughout aquatic and terrestrial habitats (Quaiser *et al*., 2007). However, cultivable strains of Acidobacteria remain limited (Sánchez-Peinado *et al*., 2010), so it is difficult to infer their function in this carbonate sedimentary environment. In the present study, most of their closest relatives were the uncultured clones that were recovered from various metal-rich environments such as the hydrothermal region, ocean crust, and polluted shelf and harbor sediments. Therefore, Acidobacteria members could have metal-resistance capabilities.

Some differences were observed in the relative abundance of sequences from the exterior oxides versus the interior carbonates. Bacteroidetes were found in the exterior oxides only, while the Deltaproteobacteria and Acidobacteria were more frequently found in the interior carbonates. The heterogeneous bacteria distribution might be due to the different physicochemical environments at the different regions of the semi-consolidated sediments. Related to Acidobacteria, Fierer *et al*. (2007) demonstrated in field and lab studies that relative numbers of this phylum were negatively corre-

lated with organic carbon availability but that relative numbers of Bacteroidetes were positively correlated with organic carbon availability. The distribution of Acidobacteria and Bacteroidetes noted in this study might indicate the greater organic carbon availability in the interior carbonates than in the exterior black oxides.

Microorganisms are believed to play a vital role in the cycling of Fe and Mn in marine environments (Stein *et al*., 2001; Xu *et al*., 2005). One laboratory study proved that bacteria colonization of the metal oxide surface can enhance catabolic rates (e.g., the transfer of electrons between the cells and metal substrate) (Little *et al*., 1997). Wide phylogenetic diversity of bacteria capable of metal (Fe and Mn) reduction has been recognized using culture-dependent and -independent methods to examine *Bradyrhizobium*, *Caulobacter*, *Deferribacter*, *Marinobacter*, and *Ralstonia.* These bacteria were discovered in various natural environments such as marine sediments (Edwards *et al*., 2003), nodule province sediments (Xu *et al*., 2005) and freshwater ferromanganese micronodules (Stein *et al*., 2001).

In the present study, a total of 10 clones accounting for 6.7% of the total bacterial 16S rRNA gene clones were potentially Fe(III) and Mn(IV) reducers grouped with *Caulobacter*, *Deferribacter*, *Marinobacter*, *Ralstonia*, and Nitrospirae. Their presence in the exterior oxides was consistent with the occurrence of the black Fe-Mn oxides because they could use these oxides as terminal electron acceptors coupled with organic matter oxidation (Mitra *et al*., 1998). However, it is interesting to note that these bacteria were also recovered from the interior white carbonates. This result suggests that the Fe/Mn-reducing biochemical reactions could also occur in the interior portion of the semi-consolidated carbonate sediments. Hence, both potentially metal oxidizing and reducing bacteria were present in this study environment, and the formation of these black Fe/Mn oxides might be related to these microorganisms.

In contrast to the broad taxonomic coverage of this bacterial community, nearly all of the archaeal 16S rRNA sequences fell into Thaumarchaeota. The finding that Thaumarchaeota was dominating in the archaeal clone library was similar to finding of reports from the deepest 11,000-m Mariana trench sediments (Kato *et al*., 1997), Pacific nodule province sediments (Xu *et al*., 2005; Liao *et al*., 2011), and Antarctic bathypelagic sediments (Gillan and Danis, 2007). This result further supported the conclusion that Thaumarchaeota members were widely distributed. Archaea in the global ocean biosphere (Bowman and McCuaig, 2003; Herndl *et al*., 2005; Gillan and Danis, 2007). Furthermore, microbial nitrification is a key component of the global N biogeochemical cycle (Dang *et al*., 2008).

Increasing numbers of studies have reported that Thaumarchaeota members were among the most important ammonia-oxidizing organisms and played an important role in the N cycle of marine environments (Francis *et al*., 2005; Mincer *et al*., 2007). Our 16S rRNA phylogenetic inference indicated that these communities might be potential players that are able to oxidize ammonia. Our analysis of the *amoA* functional gene, which was involved in the metabolic pathways of archaeal ammonia oxidation, further supported this conclusion. The *amoA* phylotypes in the present study were mainly grouped in Water Column A, which is related to Thaumarchaeota.

The *aprA* functional gene analysis results showed that no unambiguous sulfate-reducing microorganisms were recovered from the present samples, although Deltaproteobacteria was a major component of the interior carbonate bacterial clone library. However, it can still be concluded that Alphaproteobacteria rather than other bacterial phyla was the important sulfur-oxidizer within these semi-consolidated sediments. This result was also consistent with that of the 16S rRNA gene analysis, which showed that a significant portion of Alphaproteobacteria branched with a sulfur-oxidizing bacterial endosymbionts of *Inanidrilus leukodermatus*.

The presence of sulfur oxidizers in the microbial community of semi-consolidated carbonate sediments indicated that these microorganisms must have had a source of hydrogen sulfide. We suggest that three possible hydrogen sulfide sources may exist in this study area: direct discharge of the hydrothermal fluid nearby this site; volatile discharge from magma beneath the ocean crust, which frequently occurs in the ocean ridge area; and, since the sediment structure was semi-consolidated, the microbial sulfate reduction zone might be much more shallow in this area than in other normal deep sea sediments.

Conclusion

The current study reported on the distribution and diversity of the prokaryotic communities inhabiting semi-consolidated carbonate sediments collected from the SWIR. The 16S rRNA gene analysis suggested that the bacteria microflora associated with the different regions (exterior oxides and interior carbonates) of the semi-consolidated sediments were highly diversified. Phylotypes belonging to Gammaproteobacteria and Acidobacteria appeared to be diverse and abundant in these samples. In contrast to the broad taxonomic coverage of bacterial community, archaeal 16S rRNA sequences were dominated by Thaumarchaeota. Functional gene analyses of *amoA* and *aprA* showed that members of Water Column A (related to Thaumarchaeota) and Alphaproteobacteria were the potential players that participate in N and S cycles in this carbonate sedimentary environment. This paper is the first to describe the results of a molecular phylogenetic analysis of semi-consolidated carbonate sediments collected from the SWIR, and provides more information to characterize the roles that benthic microorganisms play in the deep-sea processes at the SWIR.

Acknowledgements

Special thanks go to all the participants of the cruise of R/V DA YANG YI HAO conducted by China Ocean Mineral Resource R&D Association (COMRA) in 2008. Financial support for this research came from the National Natural Science Foundation of China (grant 41172309 and 41272370 to Peng), the Frontier Project of Chinese Academy of Science (grant SIDSSE1301 to Peng), and the Open Fund of Key Laboratory of Marine Spill Oil Identification and Damage

Assessment Technology (grant 201307 to Li). We are greatly indebted to two anonymous journal reviewers for their constructive remarks.

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