Acta Oceanol. Sin., 2014, Vol. 33, No. 10, P. 85–93
DOI: 10.1007/s13131-014-0543-1
http://www.hyxb.org.cn
E-mail: hyxbe@263.net

Bacterial diversity in sediments of core MD05-2902 from the Xisha Trough, the South China Sea

LI Tao[1,2], WANG Peng[1]*

[1] State Key Laboratory of Marine Geology, Tongji University, Shanghai 200092, China
[2] Guangzhou Marine Geological Survey, Guangzhou 510760, China

Received 6 June 2013; accepted 1 October 2013

Abstract

A sediment core MD05-2902 was collected from the deep-sea basin of the Xisha Trough. The vertical distribution and diversity of bacteria in the core was investigated through ten sub-sampling with an interval of 1 m using bacterial 16S rRNA gene as a phylogenetic bio-marker. Eighteen phylogenetic groups were identified from 16S rRNA gene clone libraries. The dominant bacterial groups were JS1, Planctomycetes and Chloroflexi, which accounted for 30.6%, 16.6%, and 15.6% of bacterial clones in the libraries, respectively. In order to reveal the relationship between biotic and abiotic data, a nonmetric multidimensional scaling analysis was performed. The result revealed that the $\delta^{15}N$, $\delta^{13}C$, total organic carbon and total organic nitrogen possibly influenced the bacterial community structure. This study expanded our knowledge of the biogeochemical cycling in the Xisha Trough sediment.

Key words: Xisha Trough, bacterial diversity, 16S rRNA gene

Citation: Li Tao, Wang Peng. 2014. Bacterial diversity in sediments of core MD05-2902 from the Xisha Trough, the South China Sea. Acta Oceanologica Sinica, 33(10): 85–93, doi: 10.1007/s13131-014-0543-1

1 Introduction

Microbes are considered as an essential component to the sub-seafloor ecosystem for its high biomass, diversity and important ecological roles they play (Zinger et al., 2011). Bacteria, along with archaea, drive many biochemical processes in marine sediments, including utilizing organic matter, producing methane, and reducing sulfate (D'Hondt et al., 2004; Kallmeyer et al., 2012). High bacterial diversity in sub-seafloor habitats have been found (Inagaki et al., 2006; Inagaki et al., 2003; Reed et al., 2002; Teske, 2006; Webster et al., 2006), with a broad distribution from seabed to extending 1 000 m depth sediment (Parkes et al., 1994; Roussel et al., 2008).

Molecular and cultivation surveys are both methods that used to determine the diversity of bacteria in sub-seafloor sediment. As most bacteria are unculturable in laboratory condition (Jorgensen et al., 2012), molecular approaches based on 16S rRNA genes are commonly used (D'Hondt et al., 2007). Bacterial communities have been characterized in a variety of different marine sediments, including coastal and open-ocean sites. For instance, during the Ocean Drilling Program (ODP) cruises, researchers explored the microbial community composition of sediment samples from several marginal seas along the Pacific Ocean, including the Japan Sea (Rochelle et al., 1994), the Nankai Trough (Kormas et al., 2003; Newberry et al., 2004), the Nankai Forearc Basin (Reed et al., 2002), the Sea of Okhotsk (Inagaki et al., 2003), the Peru Margin and the Cascadin Margin (Inagaki et al., 2006; Marchesi et al., 2001; Parkes et al., 2005; Webster et al., 2006). Recently, Fry et al. (2008) and Orcutt et al. (2011) have summarized the average percentages of major subseafloor bacterial groups, and conclude that Gammaproteobacteria, Chloroflexi, and JS1 dominate in sub-seafloor sediment, while Gamma- and Deltaproteobacteria dominate in surface environment, and Alphaproteobacteria or Acidobacteria are sometimes abundant. However, our understanding of the microbial population size, community composition, diversity, distribution, and metabolism is still limited (Batzke et al., 2007; D'Hondt et al., 2007; Fry et al., 2008; Schippers et al., 2005).

The Xisha Trough, at the northwest of the South China Sea, locates between Hainan and Xisha (Paracel) Islands, close to 18°N. The trough is about 430 km long and 14 km wide, and water depth varies from 1 500 m to 3 300 m. The previous geological, geophysical and geochemical evidences have indicated the occurrence of methane hydrate near and in the Xisha Trough (He et al., 2009; Yang et al., 2007).

The objective of this study is to explore the bacterial diversity and the relationships between bacterial community and environmental variables in a core MD05-2902 near the Xisha Trough. Ten sub-samples were selected for 16S rRNA gene clone library construction. In order to uncover the species-environment relationship, a non-metric multidimensional scaling (NMS) analysis of bacterial communities was performed in this study.

2 Materials and methods

2.1 *Site description, sampling and environmental parameters*

The core MD05-2902 (17°57.70′N, 114°57.33′E) was obtained from the deep-sea basin of the Xisha Trough at 3 697 m water depth using the R/V *Marion Dufresne* (chief operator Yvon Balut), during the Chinese–French joint MARCO POLO/IMAGES

Foundation item: The Key Project of China National Programs for Fundamental Research and Development under contract No. 2007CB815904; the National Natural Science Foundation of China under contract No. 41276125; the South China Sea-Deep Program of the National Science Foundation of China under contract No. 91028005; the State Key Laboratory of Marine Geology, Tongji University under contract No. MGK 120202.
*Corresponding author, E-mail: pengwang@tongji.edu.cn

147 cruise (May 15 to June 8, 2005). Deep-sea sediments were collected using a gravity box-coring device-CASQ core sampler. The core is 9.42 m long. Ten sub-samples were cut up by intervals of 1 m under sterile conditions on board, and stored in airtight sterile PVC tubes in a –80°C freezer.

The selected environmental parameters were obtained from the same core previously measured by Wang et al. (2010). The measured environmental variables illustrated in Table 1 generally decreased with depth. TOC varied from 0.41% to 1.42% (mean 0.83%); TON varied between 0.03% and 0.12% (mean 0.07%); C/N ratio ranged between 9.8 and 15 (mean 12.13); $\delta^{13}C$ in most sediments (expect for 3-m sediment) remained relatively constant around –20‰; $\delta^{15}N$ had a narrow range between 4.34‰ and 6.26‰.

2.2 *DNA extraction and bacterial 16S rRNA gene clone library construction*

Bulk DNA were extracted from 5 g sediment samples (wet weight), following the method described by Zhou et al. (1997). The gene fragment of bacterial 16S rRNA were amplified using universal primers: Eubac 27F (AGAGTTTGATCMTGGCTCAG) and Eubac 1492R (GGTTACCTTGTTACGACT) (DeLong, 1992). PCR was performed under the following conditions: 95°C initial denaturation for 3 min; 30 cycles of 94°C denaturation for 1 min, 55°C annealing for 1 min and 72°C elongation for 1.5 min; and finally 72°C elongation for 10 min. The cleaned PCR amplicons were cloned into pMD-18T Vector (TaKaRa Bio Inc., Japan). Vector primers T7 and SP6 were then used to re-amplify the 16S rRNA gene inserts from individual clones to generate template DNA for restriction fragment length polymorphism (RFLP) analysis using *Msp*I (Fermentas, Canada). Electrophoresis was performed in a 3% gel. Afterwards, the gel was stained with Ethidium bromide and developed the RFLP profiles. The occurrence frequency of every band pattern was calculated in RFLP profiles, and at least one representative of each RFLP pattern was sent out for sequencing.

Sequencing reactions were performed with primers T7 and SP6 on an ABI3770 automated sequencer (Applied Biosystems, US). We assembled about 1 470 bp sequence fragment using the SeqMan NGen tool from DNASTAR software. All 16S rRNA gene sequences were checked for chimeras using the CHIMERA_CHECK program of the Ribosomal Database project (Maidak et al., 2001). All possible chimeric sequences were removed from further analysis. The chimera-clean sequences were grouped into operational taxonomic units (OTUs) based on a cut-off of 3% with the DOTUR program (Schloss and Handelsman, 2005). The OTUs were blasted against GenBank, and the top hit was selected as the closest relative for each OTU. Sequences from each OTU and its relative were aligned using the ClustalX program (Version 1.8). Phylogenetic analysis was conducted by PAUP software (Version 4.0 b10) (Swofford, 1999). The phylogenetic relationship was calculated using the neighbor-joining algorithm with Juke-Cantor correction.

2.3 *Accession number of nucleotide sequences*

Bacterial sequences had been deposited in the GenBank database with the accession numbers: EU048608–EU048636 and EU385861–EU385962.

2.4 *Statistical analysis*

Percentage of Good's coverage (*C*) was calculated according to the formula following:

$$C = 1 - \frac{n_1}{N},$$

where n_1 is the number of OTU that occur merely once in clone library and N is the total number of OTUs in the library. Shannon-Wiener diversity index (H') and species richness estimator (S_{ACE}) were calculated using the SPADE software (Chao and Shen, 2003). NMS analysis with Sørensen distance measures was performed using PCORD version 5.0 (mJm Software) on relative abundance of bacterial taxa to determine the environmental stress effected on bacterial communities, including the variables of total organic carbon (TOC), total organic nitrogen (TON), C/N ratio, $\delta^{13}C$ and $\delta^{15}N$. In addition, SPSS (Statistical Product and Service Solutions) software package was used to calculate Pearson correlations.

3 Results

3.1 *Library analysis*

Ten bacterial 16S rRNA gene clone libraries were constructed (Table 2). Approximately 100–150 clones of each clone library were used for the RFLP analysis and totally 150 clones encompassing all RFLP patterns were processed by sequencing. The Shannon-Wiener diversity index of clone libraries ranged from 2.4 to 3.2, averaging 2.9±0.1 (SE). The ACE richness estimates ranged from 25 to 84, averaging 46±8 (SE). The Shannon-Wiener diversity index and species richness indicated that the bacterial community was with high diversity. The values of Good's coverage ranged from 81.4% to 98.2%, suggesting that the bacterial clone libraries were robust to represent the community diversities.

Table 1. Measured environmental variables

Depth/m	$\delta^{15}N$/‰	$\delta^{13}C$/‰	C/N ratio	TOC(wt%)	TON(wt%)
0	6.26	–20.56	13.3	1.36	0.10
1	5.25	–20.30	10.7	0.98	0.09
2	5.40	–19.77	11.6	1.42	0.12
3	5.54	–30.11	12.5	1.27	0.10
4	4.43	–20.04	10.0	0.52	0.05
5	4.34	–20.42	12.7	0.66	0.05
6	4.50	–20.27	11.0	0.68	0.06
7	4.88	–21.02	14.7	0.47	0.03
8	4.43	–21.39	15.0	0.48	0.03
9	5.10	–20.97	9.8	0.41	0.04

Note: Data in this table were cited from Wang et al. (2010).

Table 2. Major characteristics of 16S rRNA gene clone libraries

Layers	No. of clones	Good's coverage/%	Shannon-Wiener diversity index	ACE species richness (95% C.I.)
0 m	118	95.7	3.1	30
1 m	137	88.1	3.2	54
2 m	112	81.4	3.1	84
3 m	142	84.6	2.7	71
4 m	128	98.2	3.0	28
5 m	88	92.4	2.9	29
6 m	144	83.3	3.4	64
7 m	130	94.6	2.7	32
8 m	132	89.4	2.9	48
9 m	133	97.0	2.4	25

3.2 *Phylogenetic diversity analysis*

From the phylogenetic tree shown in Fig. 1, most 16S rRNA gene sequences were clustered into 18 phyla (Fig. 1). JS1, Planctomycetes and Chloroflexi were dominant in all libraries, accounting for 30.6%, 16.6%, and 15.6% of total bacterial clones, respectively. Deltaproteobacteria, Actinobacteria, WS3, and OP8 were also common in the core, representing 5.1%, 4.9%, 4.6%, and 4.4% of total bacterial clones. Other groups accounted for a small fraction of clone libraries. Unclassified branches were also observed (Fig. 1).

Members of JS1 dominated in most bacterial libraries, except for 0-m and 6-m libraries (Fig. 2). Most sequences in JS1 were closely related (98%–99% similarity) to clones retrieved from Forearc Basin and Japan Trench cold seep (Li et al., 1999; Reed et al., 2002).

Members of Planctomycetes dominated in 0-m, 3-m and 6-m bacterial libraries (Fig. 2). Most of them were closely related (92%–99% similarity) to clones that retrieved from marine and territorial habitats, including the Peru Margin and Forearc Basin (Inagaki et al., 2006; Reed et al., 2002), the Nankai Trough and Japan Trough (Newberry et al., 2004), the Victoria Harbor, and ultra-oligotrophic lake (Ena et al., 2001). A minor portion of this phylum was close (85%–96% similarity) to anaerobic ammonia oxidation bacteria, including *Candidatus Anammoxoglobus propionicus* and *Candidatus Scalindua brodae*.

Members in Chloroflexi dominated in 2-m, 4-m, 5-m and 9-m bacterial libraries (Fig. 2). Phylogenetic analysis suggested that this phylum could be divided into two sub-phyla of Chloroflexi. Sequences from sub-phylum IV were closely related to a clone isolated from Peru Margin deep-sea sediment (Inagaki et al., 2006). Sequences from sub-phylum II shared 89%–98% similarity to clones that detected in deep-sea sediments from the Peru Margin and Forearc Basin, and Baby Bare Seamount hydrothermal sediment (Inagaki et al., 2006; Reed et al., 2002).

Members of Deltaproteobacteria, OP8, WS3 and Actinobacteria represented a small portion of bacterial clone libraries (Fig. 2). Sequences belonging to Deltaproteobacteria were related to clones isolated from the Wadden Sea intertidal mud flat, and deep-sea sediments from the West Pacific Ocean, the Barents Sea, and the Japan Trough (Lösekann et al., 2007; Li et al., 1999; Zeng et al., 2005). OP8-associated sequences were closely related (95% similarity) to a clone retrieved from the Peru Margin (Inagaki et al., 2006). Members of WS3 were related to (83%–94% similarity) clones detected at Peru Margin methane hydrate–bearing sites (Inagaki et al., 2006) and the Scotland west coast shallow marine sediment (Freitag and Prosser, 2003). Actinobacteria phylotypes were related (85%–98% similarity) to clones from various environments, including the Forearc Basin deep-sea sediment (Inagaki et al., 2006), the Gulf of Mexico hypersaline sediment (Lloyd et al., 2006), the Iceland hot spring (Marchesi et al., 2001), the Mediterranean cold seep sediment (Heijs et al., 2007), and the Mid-Atlantic Ridge hydrothermal sediment (López-García et al., 2003).

3.3 *Statistical analysis of bacterial communities*

NMS ordination (P<0.05) suggested that environmental variables correlated with Axis 1 and Axis 2 (Fig. 3), which explained 75.6% of the variance. A bi-plot overlaid on the ordination identified those environmental parameters that correlate with the community structure. δ^{15}N (r^2=0.494), TOC (r^2=0.213) and TON (r^2=0.176) had an $r^2 > 0.1$ to Axis 1. The correlation between environmental variables and Axis 2 were as follows: δ^{15}N (r^2=0.147), δ^{13}C (r^2=0.212) and TON (r^2=0.169). The C/N ratio lacks significant correlation with both axes.

The similarity between biotic and abiotic parameters was investigated by correlation analysis (Table 3). The correlation matrix revealed that the measured chemical parameters were closely correlated to certain bacterial taxa. δ^{15}N was positively correlated with Delta-, Alphaproteobacteria and OP11, and negatively correlated with WS3. δ^{13}C and Chloroflexi were positively correlated, while δ^{13}C and Planctomycetes were negatively correlated. The correlation matrix shows a positive influence of TOC on OP11, Delta-, Alpha-, and Gammaproteobacteria, and a negative influence on JS1, WS3 and BRC-1. TON represented a positive correlation with Delta-, Gammaproteobacteria, OP3 and OP11, and a negative correlation with OP10, JS1 and BRC-1.

4 Discussion and conclusions

4.1 *Bacterial diversity*

Based on the profile of bacterial community structure (Fig. 2), it is clear that JS1, Planctomycetes and Chloroflexi dominated in the core MD05-2902 from the Xiasha Trough, which is similar with previously results that found at the methane hydrate-containing sites along the Pacific Ocean (Inagaki et al., 2006). But it is hard to conclude that the dominance of these three groups represents a diagnostic feature of methane hydrate-containing environments. First, aside from indirect evidences, methane hydrate has not been found at this site; second, it remains unclear whether certain bacterial groups involve in methane hydrate metabolism. The first report of hydrate-associated bacteria in Forearc Basin are dominated by an unclassified phyla (recognized as candidate division JS1 later) (Reed et al., 2002). The result is similar to the result from the Cascadia Margin hy-

drate-bearing sediments (Nunoura et al., 2008). Later, Inagaki et al. (2006) also found that JS1, together with Planctomycetes and Chloroflexi, dominated at methane hydrate bearing sites in the Peru Margin and the Cascadia Margin. However, along the gas hydrate occurrence zone (GHOZ) in the Andaman Sea, more than 50% of bacterial clones are identified as members of Firmicutes (Briggs et al., 2012).

The phylum Chloroflexi distributes diversely and widely. Members in this phylum have been detected in various habitats, including hot and cold environments (Forschner et al., 2009; Hugenholtz et al., 1998; Takai et al., 2008; Zhang et al., 2012), not restricted to sub-seafloor sediments (Blazejak and Schippers, 2010; Inagaki et al., 2006; Kormas et al., 2003; Webster et al., 2006). Currently, Chloroflexi has been divided into at least five sub-phyla: *Anaerolinaea* (sub-phylum I), *Dehalococcoidetes* (sub-phylum II), *Chloroflexi* (sub-phylum III), *Thermomicrobia*, and an unclassified sub-phylum IV (Hugenholt and Stackebrand, 2004; Hugenholtz et al., 1998). Generally, at deep seafloor environments, Chloroflexi-affiliated sequences belong to sub-phyla II and IV (Fry et al., 2008). In this study, the majority phylotypes in Chloroflexi were also clustered into sub-phylum II and IV. Chloroflexi-related bacteria have been found to be a major component of the microbial community in organic-rich sediments (Inagaki et al., 2006), and they are also abundant above and in the sulfate-methane transition zone (Nunoura et al., 2009). Less is known about the biogeochemical roles of Chloroflexi in sub-surface environments owing to the lack of sufficient isolates (Fry et al., 2008). *Dehalococcoides*

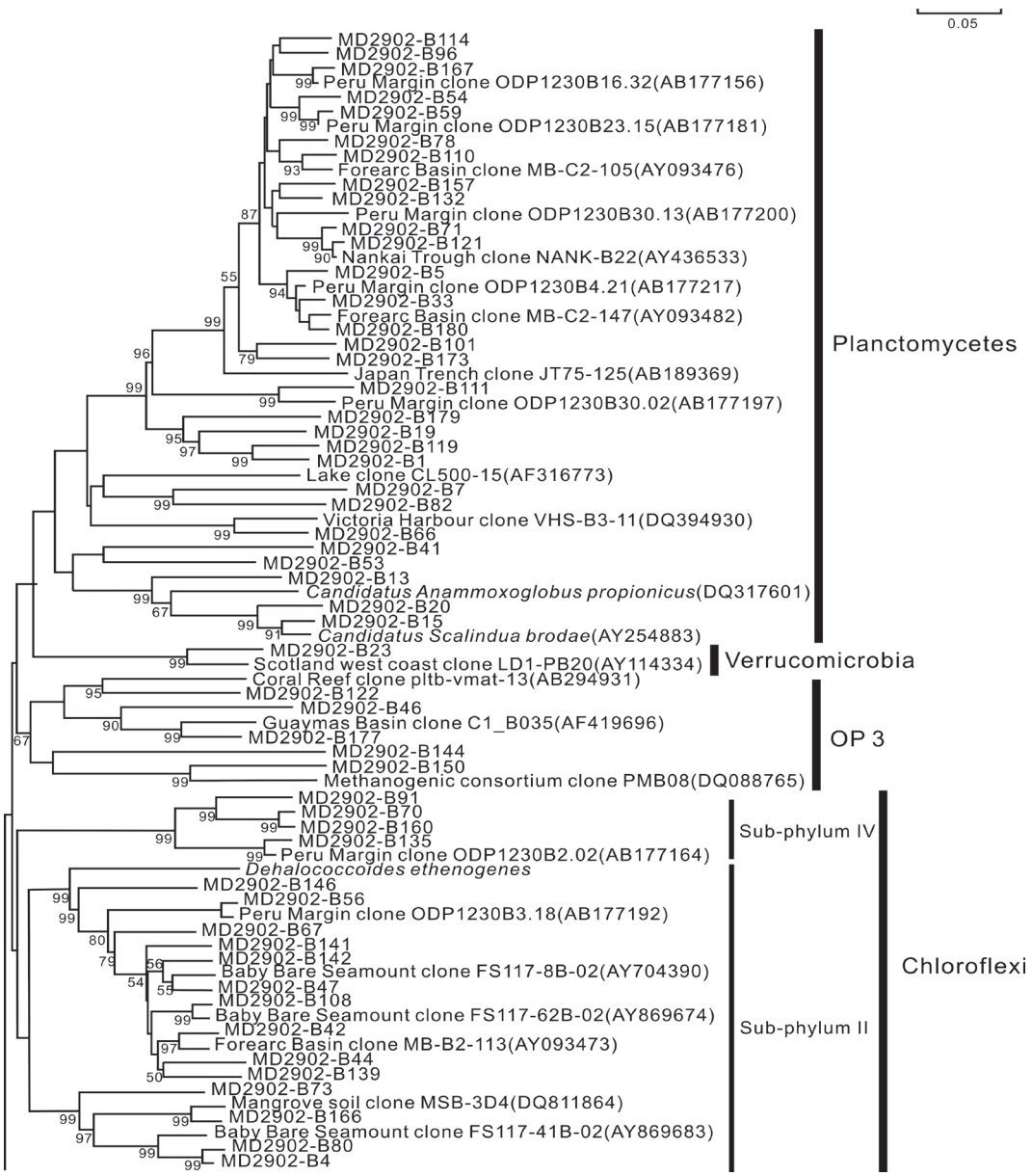

Fig.1.

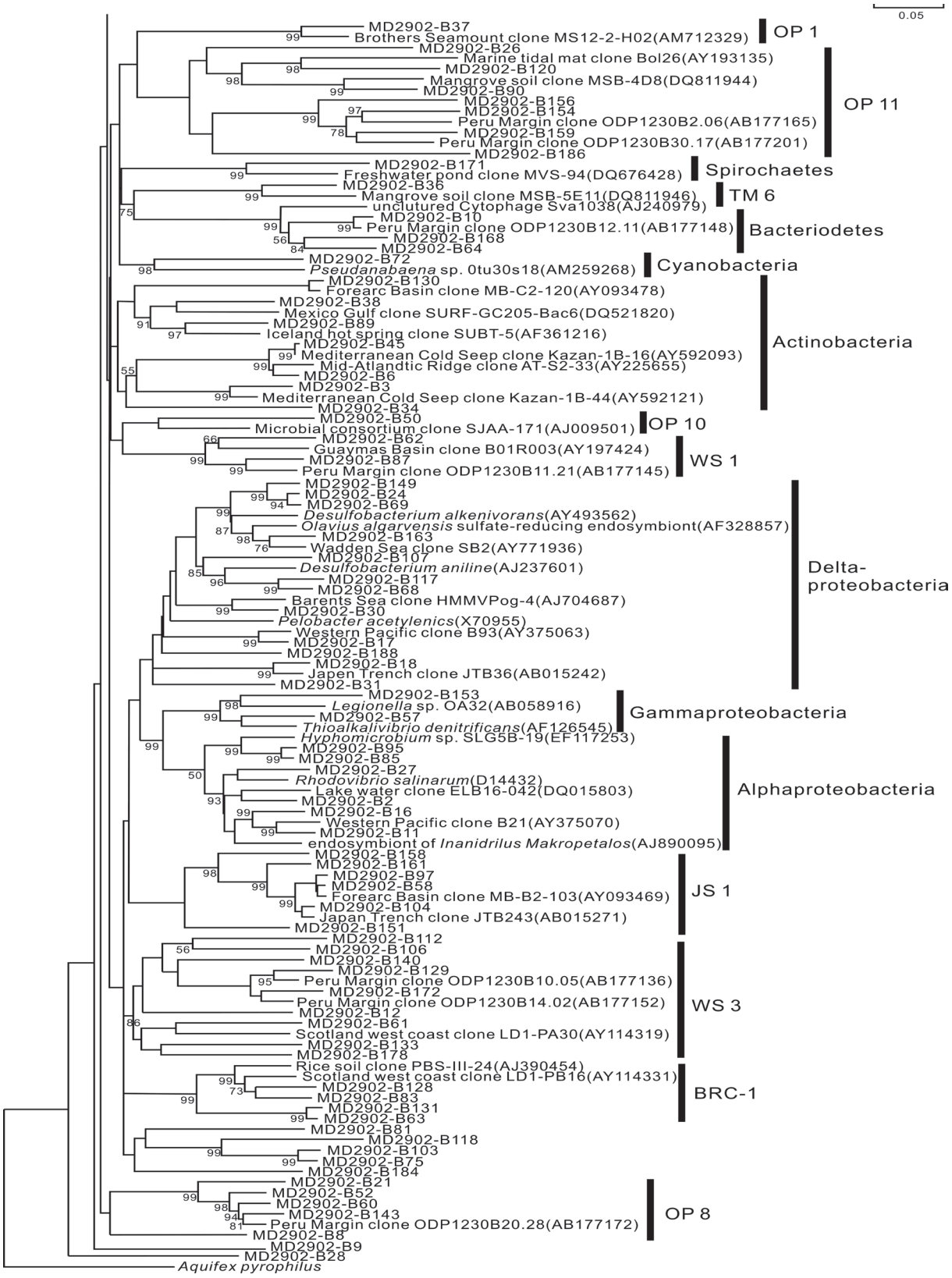

Fig.1. Phylogenetic relationships of 16S rRNA gene sequences obtained from bacterial clones as determined by neighbor-joining analysis. The clones in this study is indicated by MD2902-Bxx (2902 represents core number, B bacterial clone and xx clone number). The scale bar represents 0.05 sub-stitution per nucleotide position.

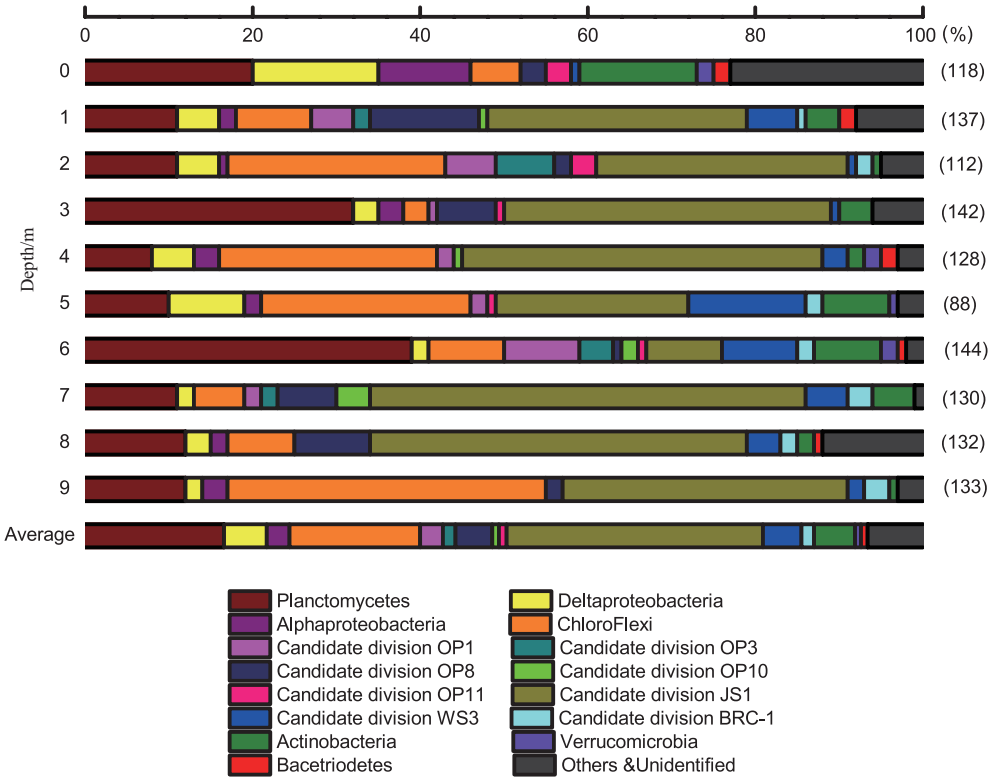

Fig.2. Bacterial community composition based on bacterial 16S rRNA gene clone libraries. Number of clones in a clone library is shown in parentheses.

Table 3. Correlation matrix (Pearson coefficient) between bacterial taxa and chemical parameters

	$\delta^{15}N$	$\delta^{13}C$	C/N	TOC	TON
Planctomycetes	0.167	**–0.492**	–0.026	0.276	0.257
Deltaproteobacteria	**0.506**	0.212	0.202	**0.494**	**0.401**
Alphaproteobacteria	**0.696**	–0.040	0.118	**0.442**	0.349
Gammaproteobacteria	0.265	0.249	–0.247	**0.497**	**0.613**
Chloroflexi	–0.261	**0.402**	**–0.619**	–0.294	–0.168
OP1	–0.199	0.293	–0.346	0.170	0.294
OP3	0.065	0.308	–0.123	0.376	**0.452**
OP8	0.195	–0.268	0.329	0.087	0.064
OP10	–0.259	0.190	0.173	–0.395	**–0.403**
OP11	**0.635**	0.048	0.157	**0.823**	**0.758**
JS1	–0.397	–0.234	0.137	**–0.454**	**–0.441**
WS1	0.240	–0.332	**–0.518**	–0.092	0.010
WS3	**–0.642**	0.302	0.032	**–0.401**	–0.391
TM6	0.134	0.136	–0.269	0.137	0.247
BRC-1	–0.356	0.124	0.138	**–0.476**	**–0.481**
Actinobacteria	0.372	0.080	0.317	0.308	0.189
Spirochaetes	–0.291	0.139	–0.221	–0.128	–0.075
Verrucomicrobia	–0.070	0.314	–0.211	0.001	0.004
Bacetriodetes	0.138	0.332	–0.184	0.085	0.126

Note: Values (p<0.05, two-tailed test) are in bold.

ethenogenes is the closest member to Chloroflexi, which could reduce dechlorinates tetrachloroethene to ethane (Maymó-Gatell et al., 1997). None of the Chloroflexi-related phylotypes were closely related to cultivable species. Hence, no inference could be drawn about the potential biochemical processes of this group in this study.

The phylum JS1 was identified for the first time in the Japan Sea (Rochelle et al., 1994), with a broad distribution in anoxic sedimentary biospheres, including sub-seafloor sediments, mud volcanoes, hydrothermal sediments, and tidal sediment

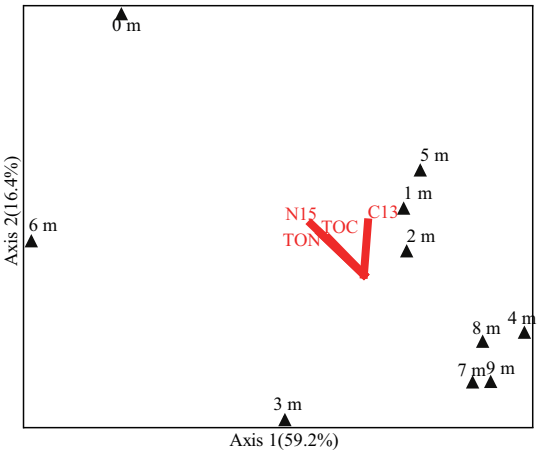

Fig.3. NMS bi-plots for the measured chemical parameters and sampling sites. TOC represents total organic carbon, TON total organic nitrogen, N15 $\delta^{15}N$, and C13 $\delta^{13}C$. Numbers represent the sampling depth.

(Blazejak and Schippers, 2010; Fry et al., 2008; Inagaki et al., 2006; Orcutt et al., 2011; Webster et al., 2004; Webster et al., 2007). JS1 often co-occurs with Chloroflexi in anoxic sediment zones (Briggs et al., 2012; Orcutt et al., 2011), which was also observed in this study. But the ecological niche for JS1-affiliated bacteria is still controversial. One opinion is that the phylum JS1 prefers hydrate-containing sediment with a high content of organic matter (Inagaki et al., 2006). An alternative holds the idea that this phylum is not restricted to hydrate-containing sediments, but adapts to surviving in sediments with reasonable concentration of TOC (Webster et al., 2004; Webster et al., 2007). Some JS1-associated bacteria, may involve in the biochemical processes of benzene mineralization, anaerobic methane oxidation, and removal of hydrocarbon (Alain et al., 2006; Dhillon et al., 2003; Phelps et al., 1998; Teske et al., 2002; Webster et al., 2007).

Members of Planctomycetes have been identified in diverse freshwater, hot spring, marine and soil habitats (Inagaki et al., 2006; Kormas et al., 2003; Neef et al., 1998; Penton et al., 2006; Reed et al., 2002; Wang et al., 2002). Planctomycetes-affiliated phylotypes are not common in deep-sea sediments (Orcutt et al., 2011), but abundant in hydrate rich sediments from the Peru and Cascadian Margins (Inagaki et al., 2006). Members of this phylum were also predominant at surface and sub-surface sediments in the South China Sea (Li et al., 2008a, b; Zhang et al., 2012). The ecological roles of Planctomycetes in the Xisha Trough could be partly inferred from the closest relative *Candidatus anammoxoglobus propionicus* and *Candidatus Scalindua brodae* (Kartal et al., 2007; Schmid et al., 2003). Both of them are autotrophic anaerobic ammonium oxidizers, which participate in the autotrophic conversion of ammonium and nitrite to N_2 under anoxic conditions (Penton et al., 2006).

4.2 *Correlation between bacterial community composition and environmental variables*

Multivariate analyze is propitious to interpret the trends in microbial community composition and explore the environmental factors that determine the community structure. NMS is usually used for this purpose (Clarke and Ainsworth, 1993), which ranks distances between objects, and nonlinearly plots the objects onto a two-dimensional ordination according to their ranked difference (Ramette, 2007). In this study, the NMS result indicated that Axis 1 is associated with $\delta^{15}N$, TOC and TON, and Axis 2 related to $\delta^{15}N$, $\delta^{13}C$ and TON.

Organic matter, mixture of complex compounds, could be utilized by microorganism as energy source (Boudreau, 1992). The finding that organic matter influences the bacterial community structure has been observed in the Andaman Sea, the North Sea and the Arctic Mid-Ocean Ridge (Briggs et al., 2012; Jorgensen et al., 2012; Sapp et al., 2010). In this study, both TOC and TON were influencing factors for the bacterial community structure. The total supply of organic carbon affected the relative abundance of several bacterial groups, including OP11, Delta-, Alpha-, Gammaproteobacteria, JS1, WS3 and BRC-1. The total amount of organic nitrogen had a significant impact on groups containing Delta-, Gamma-proteobacteria, OP3, OP11, OP10, JS1 and BRC-1.

Generally, marine sedimentary organic matter is mixed of marine and terrestrial components. The $\delta^{13}C$ values can be used to assess the origin of the two sources. Leventhal (2004) suggested that the $\delta^{13}C$ values of –20‰ represents for "pure" marine origin and –27‰ indicates for "pure" terrestrial origin. $\delta^{13}C$ in most sediments were close to –20‰, which suggested that organic matter in these sediments primarily originated from ocean; however, $\delta^{13}C$ in the 3-m sediment reached –30‰, presenting a terrestrial source. Marine organic components could be degraded faster than terrestrial ones in sediments (Orcutt et al., 2011), and thus the marine organic compounds are easier to be used and subsequently affects the relative abundance of bacterial taxa, such as Chloroflexi and Planctomycetes.

Bacteria could grow with ammonium as sole nitrogen source and produce biomass significantly depleted in ^{15}N. Lehmann et al. (2002) suggested that aerobic degradation of organic matter leads to the residual biomass enriched in ^{15}N isotopes, whereas decomposition of organic matter under anoxic conditions results in depletion of ^{15}N. The $\delta^{15}N$ profile revealed that values of $\delta^{15}N$ decrease along with the increasing of depth (Wang et al., 2010), and depletion of ^{15}N may be responsible for the decrease of $\delta^{15}N$. It is known that the oxygen content is relative higher in surface sediment while decrease dramatically with increasing depth for oxygen depletion by microbes. Thus, it is speculated that the oxygen level is a critical factor controlled some bacterial metabolic behaviors. In this study, compared to other bacterial groups, Delta-, Alphaproteobacteria, OP11 and WS3 were highly correlated with $\delta^{15}N$. The Delta- and Alphaproteobacteria were abundant in the surface sediment, but their relative abundances decreased rapidly as the depth increases (Fig. 2). Members of these two subgroups might prefer to aerobic environment because they were usually dominated in surface sediment (Fry et al., 2008; Orcutt et al., 2011).

In summary, JS1, Planctomycetes and Chloroflexi were predominant at the bacterial communities in the core MD05-2902. Those three groups comprised 30.6%, 16.6%, and 15.6% of bacterial clones, respectively. Most of them were closely related to uncultured clones retrieved from similar environments, therefore, the possible geochemical cycles participated by bacteria in this study are largely unknown. The NMS analysis of bacterial communities and measured chemical parameters indicated that the $\delta^{15}N$, $\delta^{13}C$, TOC and TON probably affected the bacte-

rial community in Xisha Trough, and these variables positively or negatively correlated to certain bacteria phyla.

Acknowledgements

We thank the crew on the MD-147-MARCO POLO cruise of the R/V *Marion Dufresne* of the French Polar Institute (IPEV). We also extend our thanks to three anonymous reviewers for their constructive comments and suggestions, which have helped improve this paper.

References

Alain K, Holler T, Musat F, et al. 2006. Microbiological investigation of methane- and hydrocarbon-discharging mud volcanoes in the Carpathian Mountains, Romania. Environ Microbiol, 8(4): 574–590

Batzke A, Engelen B, Sass H, et al. 2007. Phylogenetic and physiological diversity of cultured deep-biosphere Bacteria from Equatorial Pacific Ocean and Peru Margin sediments. Geomicrobiol J, 24(3–4): 261–273

Blazejak A, Schippers A. 2010. High abundance of JS-1- and Chloroflexi-related bacteria in deeply buried marine sediments revealed by quantitative, real-time PCR. FEMS Microbiol Ecol, 72(2): 198–207

Boudreau B P. 1992. A kinetic model for microbic organic-matter decomposition in marine sediments. FEMS Microbiol Ecol, 102(1): 1–14

Briggs B R, Inagaki F, Morono Y, et al. 2012. Bacterial dominance in subseafloor sediments characterized by methane hydrates. FEMS Microbiol Ecol, 81(1): 88–98

Chao Anne, Shen Tsung-Jen. 2003. Program SPADE (Species Prediction and Diversity Estimation). http://chao.stat.nthu.edu.tw

Clarke K R, Ainsworth M. 1993. A method of linking multivariate community structure to environmental variables. Mar Ecol Prog Ser, 92: 205–219

D'Hondt S, Jørgensen B B, Miller D J, et al. 2004. Distributions of microbial activities in deep subseafloor sediments. Science, 306(5705): 2216–2221

D'Hondt S, Inagaki F, Ferdelman T, et al. 2007. Exploring subseafloor life with the Integrated Ocean Drilling Program. Scientific Drilling, 5: 26–37

DeLong E F. 1992. Archaea in coastal marine environments. Proc Natl Acad Sci USA, 89(12): 5685–5689

Dhillon A, Teske A, Dillon J, et al. 2003. Molecular characterization of Sulfate-Reducing Bacteria in the Guaymas Basin. Appl Environ Microbiol, 69(5): 2765–2772

Ena U, Vergin K L, Young L, et al. 2001. Unusual bacterioplankton community structure in ultra-oligotrophic Crater Lake. Limnol Oceanogr, 46(3): 557–572

Forschner S R, Sheffer R, Rowley D C, et al. 2009. Microbial diversity in cenozoic sediments recovered from the Lomonosov Ridge in the Central Arctic Basin. Environ Microbiol, 11(3): 630–639

Freitag T E, Prosser J I. 2003. Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. Appl Environ Microbiol, 69(3): 1359–1371

Fry J C, Parkes R J, Cragg B A, et al. 2008. Prokaryotic biodiversityand activity in the deep subseafoor biosphere. FEMS Microbiol Ecol, 66(2): 181–196

He Lijuan, Wang Jiyang, Xu Xing, et al. 2009. Disparity between measured and BSR heat flow in the Xisha Trough of the South China Sea and its implications for the methane hydrate. J Asian Earth Sci, 34(6): 771–780

Heijs S K, Haese R R, Wielen P W J J, et al. 2007. Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean Cold Seep. Microb Ecol, 53(6): 384–398

Hugenholt P, Stackebrand E. 2004. Reclassification of *Sphaerobacter thermophilus* from the subclass Sphaerobacteridae in the phylum Actinobacteria to the class *Thermomicrobia* (emended description) in the phylum Chloroflexi (emended description). Int J Syst Evol Micr, 54(6): 2049–2051

Hugenholtz P, Pitulle C, Hershberger K L, et al. 1998. Novel division level bacterial diversity in a Yellowstone Hot Spring. J Bacteriol, 180(2): 366–376

Inagaki F, Nunoura T, Nakagawa S, et al. 2006. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. Proc Natl Acad Sci USA, 103(8): 2815–2820

Inagaki F, Suzuki M, Takai K, et al. 2003. Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. Appl Environ Microbiol, 69(12): 7224–7235

Jorgensen S L, Hannisdal B, Lanzén A, et al. 2012. Correlating microbial community profiles with geochemical data in highly stratified sediments from the Arctic Mid-Ocean Ridge. Proc Natl Acad Sci USA, 109(42): E2846–E2855

Kallmeyer J, Pockalny R, Adhikari R R, et al. 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. Proc Natl Acad Sci USA, 109(40): 16213–16216

Kartal B, Rattray J, van Niftrik L A, et al. 2007. Candidatus "*Anammoxoglobus propionicus*" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. Syst Appl Microbiol, 30: 39–49

Kormas K A, Smith D C, Edgcomb V, et al. 2003. Molecular analysis of deep subsurface microbial communities in Nankai Trough sediments (ODP Leg 190, Site 1176). FEMS Microbiol Ecol, 45(2): 115–125

Lehmann M F, Bernasconi S M, Barbieri A, et al. 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. Geochim Cosmochim Acta, 66(20): 3573–3584

Leventhal J S. 2004. Isotopic chemistry of organic carbon in sediments from Leg 184. In: Prell W L, Wang P X, Blum P, et al., eds. Proceedings of the Ocean Drilling Program, Scientific Results, College Station, TX (Ocean Drilling Program), 184, 1–13, doi:10.2973/odp.proc.sr.184.215.2004

Li Lina, Kato C, Horikoshi K. 1999. Microbial diversity in sediments collected from the deepest cold-seep Area, the Japan Trench. Mar Biotechnol, 1(4): 391–400

Li Tao, Wang Peng, Wang Pinxian. 2008a. Bacterial and archaeal diversity in surface sediment from the south slope of the South China Sea. Acta Microbiologica Sinica (in Chinese), 48(3): 323–329

Li Tao, Wang Peng, Wang Pinxian. 2008b. Microbial diversity in surface sediments of the Xisha Trough, the South China Sea. Acta Ecologica Sinica (in Chinese), 28(3): 1166–1173

Lloyd K G, Lapham L, Teske A. 2006. An anaerobic methane-oxidizing community of ANME-1b Archaea in hypersaline gulf of Mexico sediments. Appl Environ Microbiol, 72(11): 7218–7230

López-García P, Duperron S, Philippot P, et al. 2003. Bacterial diversity in hydrothermal sediment and epsilonproteobacterial dominance in experimental microcolonizers at the Mid-Atlantic Ridge. Environ Microbiol, 5(10): 961–971

Lösekann T, Knittel K, Nadalig T, et al. 2007. Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano, Barents Sea. Appl Environ Microbiol, 73(10): 3348–3362

Maidak B L, Cole J R, Lilburn T G, et al. 2001. The RDP-II (Ribosomal Database Project). Nucleic Acids Res, 29(3): 173–174

Marchesi J R, Weightman A J, Cragg B A, et al. 2001. Methanogen and bacterial diversity and distribution in deep gas hydrate sediments from the Cascadia Margin as revealed by 16S rRNA molecular analysis. FEMS Microbiol Ecol, 34(3): 221–228

Maymó-Gatell X, Chien Y-t, Gossett J M, et al. 1997. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science, 276(5318): 1568–1571

Neef A, Amann R, Schlesner H, et al. 1998. Monitoring a widespread bacterial group: in situ detection of planctomycetes with 16S

rRNA-targeted probes. Microbiology, 144(12): 3257–3266
Newberry C J, Webster G, Cragg B A, et al. 2004. Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. Environ Microbiol, 6(3): 274–287
Nunoura T, Inagaki F, EDelwiche M, et al. 2008. Subseafloor microbial communities in methane hydrate-bearing sediment at two distinct locations (ODP Leg 204) in the cascadia margin. Microbes Environ, 23(4): 317–325
Nunoura T, Soffientino B, Blazejak A, et al. 2009. Subseafloor microbial communities associated with rapid turbidite deposition in the Gulf of Mexico continental slope (IODP Expedition 308). FEMS Microbiol Ecol, 69(3): 410–424
Orcutt B N, Sylvan J B, Knab N J, et al. 2011. Microbial ecology of the Dark Ocean above, at, and below the seafloor. Microbiol Mol Biol R, 75(3): 361–422
Parkes R J, Cragg B A, Bale S J, et al. 1994. Deep bacterial biosphere in Pacific Ocean sediments. Nature, 371(6496): 410–413
Parkes R J, Webster G, Cragg B A, et al. 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. Nature, 436(7049): 390–394
Penton C R, Devol A H, Tiedje J M. 2006. Molecular evidence for the broad distribution of anaerobic Ammonium-Oxidizing Bacteria in freshwater and marine sediments. Appl Environ Microbiol, 72(10): 6829–6832
Phelps C D, Kerkhof L J, Young L Y. 1998. Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. FEMS Microbiol Ecol, 27(3): 269–279
Ramette A. 2007. Multivariate analysis in microbial ecology. FEMS Microbiol Ecol, 62(3): 142–160
Reed D W, Fujita Y, Delwiche M E, et al. 2002. Microbial communities from methane hydrate-bearing deep marine sediments in a Forearc Basin. Appl Environ Microbiol, 68(8): 3759–3770
Rochelle P A, Cragg B A, Fry J C, et al. 1994. Effect of sample handling on estimation of bacterial diversity in marine sediments by 16S ribosomal-RNA gene sequence analysis. FEMS Microbiol Ecol, 15(1–2): 215–225
Roussel E G, Bonavita M A C, Querellou J, et al. 2008. Extending the sub-sea-floor biosphere. Science, 320(5879): 1046
Sapp M, Parker E R, Teal L R, et al. 2010. Advancing the understanding of biogeography-diversity relationships of benthic microorganisms in the North Sea. FEMS Microbiol Ecol, 74(2): 410–429
Schippers A, Neretin L N, Kallmeyer J, et al. 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. Nature, 433(7028): 861–864
Schloss P D, Handelsman J. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol, 71(3): 1501–1506
Schmid M, Walsh K, Webb R, et al. 2003. Candidatus "*Scalindua brodae*", sp. nov., Candidatus "*Scalindua wagneri*", sp. nov., two new species of anaerobic ammonium oxidizing bacteria. Syst Appl Microbiol, 26(4): 529–538
Swofford D L. 1999. PAUP: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0. Sunderland, Massachusetts: Sinauer Associates
Takai K, Nunoura T, Ishibashi J I, et al. 2008. Variability in the microbial communities and hydrothermal fluid chemistry at the newly discovered Mariner hydrothermal field, southern Lau Basin. J Geophys Res, 113: G0203
Teske A, Hinrichs K U, Edgcomb V, et al. 2002. Microbial diversity of hydrothermal sediments in the Guaymas Basin: Evidence for anaerobic methanotrophic communities. Appl Environ Microbiol, 68(4): 1994–2007
Teske A P. 2006. Microbial communities of deep marine subsurface sediments: Molecular and cultivation surveys. Geomicrobiol J, 23(6): 357–368
Wang Jenny, Jenkins C, Webb R I, et al. 2002. Isolation of Gemmata-like and Isosphaera-like planctomycete bacteria from soil and freshwater. Appl Environ Microbiol, 68(1): 417–422
Wang Peng, Li Tao, Hu Anyi, et al. 2010. Community structure of Archaea from deep-sea sediments of the South China Sea. Microb Ecol, 60(4): 796–806
Webster G, Parkes R J, Cragg B A, et al. 2006. Prokaryotic community composition and biogeochemical processes in deep subseafloor sediments from the Peru Margin. FEMS Microbiol Ecol, 58(1): 65–85
Webster G, Parkes R J, Fry J C, et al. 2004. Widespread occurrence of a novel division of Bacteria identified by 16S rRNA gene sequences originally found in deep marine sediments. Appl Environ Microbiol, 70(9): 5708–5713
Webster G, Yarram L, Freese E, et al. 2007. Distribution of candidate division JS1 and other Bacteria in tidal sediments of the German Wadden Sea using targeted 16S rRNA gene PCR-DGGE. FEMS Microbiol Ecol, 62(1): 78–89
Yang Tao, Jiang Shaoyong, Ge Lu, et al. 2007. Geochemical characteristics of sediment pore water from site XS-01 in the Xisha trough of South China Sea and their significance for gas hydrate occurence. Front Earth Sci China, 1(2): 212–217
Zeng Runying, Zhao Jing, Zhang Rui, et al. 2005. Bacterial community in sediment from the western Pacific Warm Pool and its relationship to environment. Sci China (Ser D), 48(2): 282–290
Zhang Yong, Su Xin, Chen Fang, et al. 2012. Microbial diversity in cold seep sediments from the northern South China Sea. Geoscience Frontiers, 3(3): 301–316
Zhou Jizhong, Davey M E, Figueras J B, et al. 1997. Phylogenetic diversity of a bacterial community determined from Siberian tundra soil DNA. Microbiology, 143(12): 3913–3919
Zinger L, Amaral-Zettler L A, Fuhrman J A, et al. 2011. Global patterns of bacterial beta-diversity in seafloor and seawater ecosystems. PLoS ONE, 6(9): E24570