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Molecular phylogenetic and chemical analyses of the microbial mats in deep-sea cold seep sediments at the northeastern Japan Sea

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Abstract Microbial communities inhabiting deep-sea cold seep sediments at the northeastern Japan Sea were characterized by molecular phylogenetic and chemical analyses. White patchy microbial mats were observed along the fault offshore the Hokkaido Island and sediment samples were collected from two stations at the southern foot of the Shiribeshi seamount (M1 site at a depth of 2,961 m on the active fault) and off the Motta Cape site (M2 site at a depth of 3,064 m off the active fault). The phylogenetic and terminal-restriction fragment polymorphism analyses of PCR-amplified 16S rRNA genes revealed that microbial community structures were different between two sampling stations. The members of ANME-2 archaea and diverse bacterial components including sulfate reducers within Deltaproteobacteria were detected from M1 site, indicating the occurrence of biologically mediated anaerobic oxidation of methane, while microbial community at M2 site was predominantly composed of members of Marine Crenarchaeota group I, sulfate reducers of Deltaproteobacteria, and sulfur oxidizers of Epsilonproteobacteria. Chemical analyses of seawater above microbial mats suggested that concentrations of sulfate and methane at M1 site were largely decreased relative to those at M2 site and carbon isotopic composition of methane at M1 site shifted heavier (^{13}C-enriched), the results of which are consistent with molecular analyses. These results suggest that the mat microbial communities in deep-sea cold seep sediments at the northeastern Japan Sea are significantly responsible for sulfur and carbon circulations and the geological activity associated with plate movements serves unique microbial habitats in deep-sea environments.

Keywords ANME · AOM · Cold seep · Deep-sea · Deltaproteobacteria · Japan Sea · Microbial diversity · Microbial mat

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Introduction

Deep-sea "cold seep" environments have been found in worldwide subduction zones at the continental margins (Kulm et al. 1986; Ohta and Laubier 1987; Sibuet et al. 1988). The nutrient and energy flux from subseafloor environments harbors cell-abundant and diverse microbial community in deep sea. We have studied microbial community structures in cold seep sediments offshore around Japan, such as Japan Trench (Li et al. 1999b; Inagaki et al. 2002, 2004b; Arakawa et al. 2005), Nankai Trough (Li et al. 1999a) and Sagami Bay (Fang et al. 2006), indicating that communities are in most cases composed of ANME archaea and sulfate reducing bacteria within Deltaproteobacteria, which are known to be responsible for anaerobic oxidation of methane (AOM) (e.g., Li and Kato 1999; Boetius et al. 2000; Orphan et al. 2001a, b; Inagaki et al. 2004b; Kato et al. 2005). Yet the distribution of AOM community associated with the

fault activity and its effect on diffused methane remain largely elusive at the West Pacific Margin.

The northeastern margin of the Japan Sea is as the place of the convergent plate boundary between the Amurian and Okhotsk plates (Wei and Seno 1998). Large earthquakes ($M > 7$) frequently occurred during the past few centuries along the continental slope of this margin and a few seismic gaps have been reported (Ohtake 1995). Many active faults, outcrop collapses, and folds have been observed around this area. The location of plate boundary and its tectonic mechanism are, hence, of great interest for geologists. Takeuchi et al. (2000) found widespread microbial mats at the eastern escarpment of the Shiribeshi Trough in the Japan Sea at a depth of 3,145 m during their survey of large earthquakes and bottom disturbances. Additionally, several other microbial mat sites have been found around that area during the multidisciplinary scientific cruise using *DSV Shinkai 6500* (Okamura et al. 2002). The presence of many microbial mats along the faulting event indicates the activity of plate movements; in fact, in 1993, the big earthquake happened nearby this area offshore southwestern Hokkaido (M7.8, 42°47′N–139°12E′).

Preliminary study of the microbial diversity in cold seep sediments from off the Motta Cape site (close to M2 site in this study) showed that the sediments harbored diverse microbial community but methane-consuming archaea were unexpectedly absent (Arakawa et al. 2004). To confirm if the AOM-absent community structure was a common feature, in 2003, we revisited the northeastern Japan Sea and obtained microbial mat sediment samples from two geologically distinct stations: an active fault scarp to the southern base of the Shiribeshi seamount (M1site) and off the Motta Cape field (M2 site) in the Shiribeshi Trough. In this report, we describe comparative results of microbial community structures at two different habitats. Combining the chemical results of sulfate and methane concentrations and the carbon isotopic compositions of diffusing methane, the correlation between microbial community structures and the geochemical and geological characteristics are discussed.

Materials and methods

Sample collection

The microbial mat sediments were collected at two distinct stations (M1 and M2 sites) at the northeastern Japan Sea offshore Hokkaido Island, Japan, during a YK03-05 research cruise using *DSV Shinkai 6500* and *RV Yokosuka* in 2003. M1 site is located at the Shiribeshi Trough at 2,961 m depth where the active fault scarp lies to the south foot of the Shiribeshi seamount (43°20.24′N, 139°39.85′E, dive No. 767). M2 site is located off the Motta Cape field at a depth of 3,064 m (42°42.06′N, 139°40.09′E, dives No. 768 and 769) (Fig. 1). The sediment samples were treated immediately after sampling on board for the isolation of environmental DNA and the pore waters were extracted for the sulfate analysis. Seawater samples for methane analyses were also collected just above the bottom sediments with mats at M1 and M2 sites, and without mat as references by using the Niskin water samplers.

Chemical analysis

To measure the sulfate concentrations, the pore water samples were diluted 200 times and passed through a 0.1 μm filter, and then sulfate concentrations were measured by ion chromatography (Metrohm Compact IC 761; Metrohm Co. Zhurich, Swiss). The analytical relative standard deviation (RSD%) was 3% and the reproducibility approximately ± 0.01 mM. Concentration and stable carbon isotopic composition ($\delta^{13}C$) of methane in degassed gaseous phase samples were determined by using isotopic-ratio monitoring GC/MS as previously described (Tsunogai et al. 2002a, b).

DNA extraction and clone library analysis of 16S rRNA genes

Total DNA was extracted from 5 g sediment samples using Ultra Clean soil DNA kit (MO Bio Laboratories, Solana Beach, CA, USA). Bacterial and archaeal 16S rRNA genes were amplified by the polymerase chain reaction (PCR) with domain Bacteria- and Archaea-specific primer sets as described previously (Kato et al. 1997). The PCR products were cloned into the TA cloning vector (Invitrogen, Carlsbad, CA, USA). To select representative 16S rRNA gene clones as identical phylotypes, restriction fragment-length polymorphism (RFLP) analysis was performed with restriction enzymes *Rsa* I (GT'AC) and *Msp* I (C'CGG). The selected representative 16S rRNA genes were subsequently sequenced from both strands with a model 3100 DNA Sequencer (Perkin-Elmer/Applied Biosystems Co., Foster City, CA, USA).

Terminal-restriction fragment length polymorphism (t-RFLP) analysis

The fragments of bacterial and archaeal 16S rRNA genes for t-RFLP analysis were amplified by PCR using a primer set of Bac27F and FAM-labeled Bac927R and of Arch21F and FAM-labeled Arch958R, respectively (Inagaki et al. 2001, 2002). Amplified products were purified using a Gel Spin DNA purification kit (MO Bio Laboratories). DNA was precipitated with ethanol, centrifuged, and the pellets were resuspended in double-distilled water. The purified 16S rRNA gene fragments were then digested with *Hha*I at 37°C for 8 h. The electrophoretic patterns of terminal restriction fragments (t-RFs) were analyzed using a model 310 automated sequencer (Perkin-Elmer/Applied Biosystems) equipped with GENESCAN software version 3.1 according to the

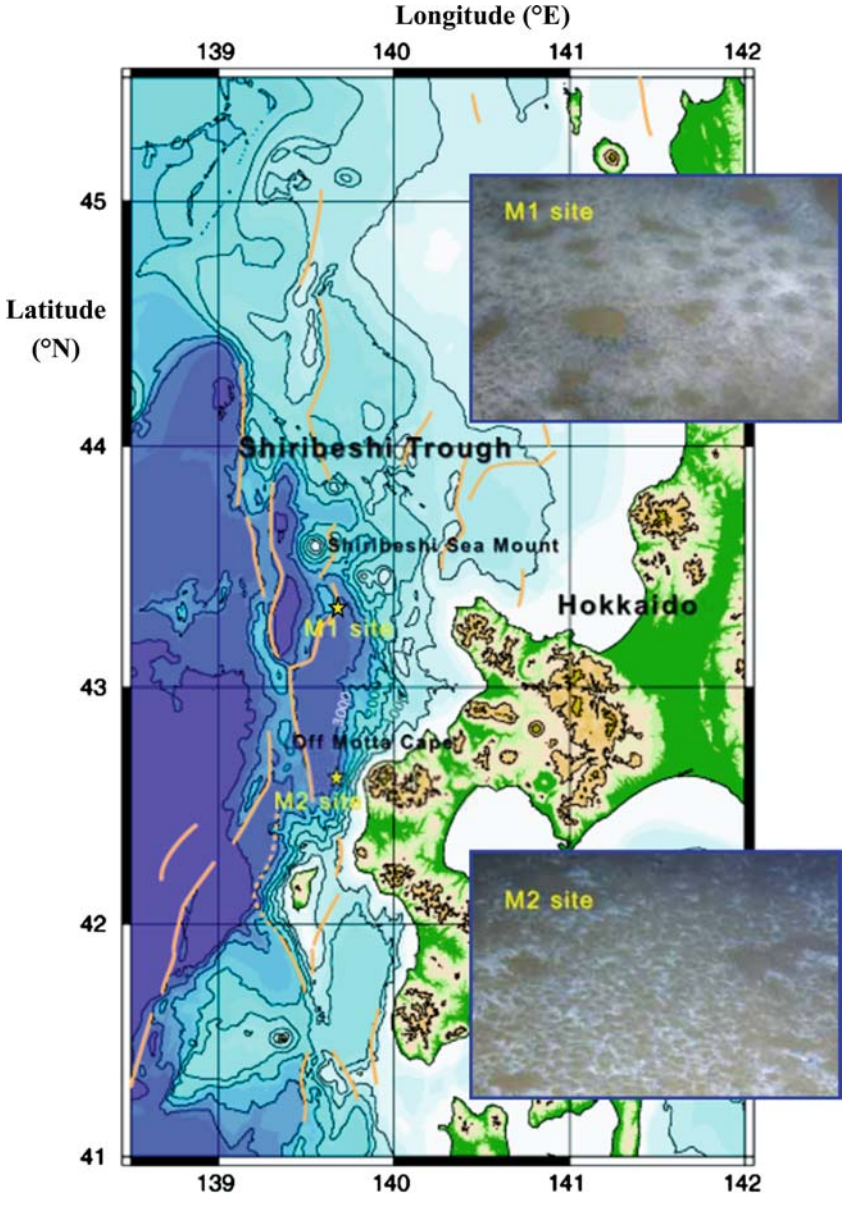

Fig. 1 Location map of sampling sites (*stars*) at the Shiribeshi Trough in the eastern margin of the Japan Sea. *Orange lines* indicated as active faults (data from Okamura et al. 2002). *M1* Fault scarp to the south of the Shiribeshi seamount by dive no. 767, *M2* off Motta Cape by dive no. 768 and 769. M1 was located on the active fault line

manufacturer's protocol (Perkin-Elmer/Applied Biosystems). The precise lengths of the t-RFs were determined by comparison with an internal size standard (GENESCAN-2500 ROX, Perkin-Elmer/Applied Biosystems) added to each digested sample.

Phylogenetic analysis of 16S rRNA genes

The similarity values of 16S rRNA gene sequences were analyzed by DNA Data Base of Japan (DDBJ) and FASTA program of GENETYX-MAC software (version 13.0.3, Software Co., Tokyo, Japan). Sequences were manually aligned and ambiguous positions were removed. Phylogenetic trees were constructed based on the matrix of pairwise evolutional distances calculated by the maximum-parsimony algorithm and the neighbor-joining method using the CLUSTAL W program (Saitou and Nei 1987).

Data deposition

The sequences reported in this paper are available in the DDBJ/EMBL/GenBank nucleotide sequence databases according to the following accession numbers: AB238972 to 239071 for bacterial 16S rRNA gene sequences and AB239072 to 239079 for archaeal 16S rRNA gene sequences.

Results

Chemical analyses of SO_4^{2-} and methane

The concentrations of sulfate in the pore water samples from the microbial mat sediments at both M1 and M2 sites were largely decreased as compared to the reference samples (Table 1), indicating the occurrence of microbial

Table 1 Chemical analyses of ΔSO_4^{2-} values in the microbial mat sediments, and methane values in the bottom water samples just above the mat site

Chemicals	Inside of microbial mat		Control (outside of mat)
	M1 site	M2 site	
ΔSO_4^{2-} (mmol/l)	22.7–26.4	1.9–16.8	0.4[a]
Methane conc. (nmol/kg)	3.4[b]	32.2[b]	1.1–1.3
Methane-$\delta^{13}C$ (‰)	−63.4	−78.0	−48.7 to −51.9

[a]Outside of mat (1 m distance from the microbial mat) for analysis of ΔSO_4^{2-} concentrations
[b]Methane concentration and $\delta^{13}C$ (‰) were measured from the bottom waters just above the microbial mat sites, M1 and M2, using the Niskin Water Sampling system

sulfate reduction. The values of ΔSO_4^{2-} at M1 site (22.7–26.4 mmol/l) were greater than those at M2 site (1.9–16.8 mmol/l), suggesting that the activity of sulfate reduction at M1 site is much higher than at M2 site (Table 1). At both mat sites, the carbon isotopic values ($\delta^{13}C$) of methane (−63% at M1 site and −78% at M2 site) were largely depleted relative to reference sites (−48.7 to −51.9%), indicating the input of biologically produced methane (Kvenvolden 1995). The concentrations of methane at both M1 and M2 sites were higher than at reference sites while the concentration at M1 site was lower than that at M2 site. In addition, the $\delta^{13}C$ value of methane at M1 sites (−63%) was significantly enriched relative to that at M2 site (−78%). The large values of ΔSO_4^{2-}, lower amounts of defused methane, and heavier shift of $\delta^{13}C$ value of methane at M1 site consequently indicate that the activity of AOM coupled with sulfate reduction is higher than at M2 and reference sites.

t-RFLP profiles

To monitor the microbial community structures at M1 and M2 sites, t-RFLP fingerprint analysis was performed as shown in Fig. 2. Bacterial t-RFLP profiles showed that bacterial community structures at M1 and M2 sites were relatively similar (Fig. 2a). Based on the fragment lengths (t-RFs) of cloned sequences, the members of Gamma-, Epsilon-, and Deltaproteobacteria were found to be predominant bacterial component in both samples. The t-RF signal of Betaproteobacteria was only detected from M1 site. Archaeal t-RFLP profiles revealed that the archaeal communities at M1 and M2 sites were different (Fig. 2b). The t-RF signals corresponding to 16S rRNA genes of ANME-2 archaea were predominantly detected from the mat sample at M1 site while M2 sample was dominated by the Marine Crenarchaeota group I (MGI) (Fig. 2b).

Phylogenetic analysis of 16S rRNA gene sequences

A total of 55 representative bacterial 16S rRNA gene clones were screened by RFLP analysis among 226 clones, and were sequenced from both strands. Phylogenetic analysis revealed that most of these sequences were affiliated with phyla Proteobacteria, Cytophaga-Flavobacterium-Bacterioides (CFB) group, and Spirochaeta (Fig. 3). There were 40 representative phylotypes belonging to the phylum Proteobacteria, including 193 related clones (based on RFLP results). Among these 193 proteobacterial clones, Gammaproteobacteria (69 clones), Deltaproteobacteria (64 clones) and Epsilonproteobacteria (48 clones) were the most frequently detected subphyla. The members of Alpha- (9 clones) and Betaproteobacteria (3 clones) were detected as minor bacterial components. Within Gamma- and Epsilonproteobacteria, some sequences were closely related to sulfur-oxidizing chemolithoautotrophic bacteria. For example, the Gammaproteobacteria sequences of BJS72-009 (2 relative clones), BJS72-036 (12 relative clones), and BJS72-017 (3 relative clones) from M1 site and BJS82-047 (2 relative clones) from M2 site were closely related to the sequence of symbiotic sulfur-oxidizing bacteria in chemosynthetic-based bivalves (Distel et al. 1994; Fujiwara et al. 2001). Within Epsilonproteobacteria, BJS82-040 (27 relative clones), BJS72-010 (6 relative clones), BJS8R-080 (4 relative clones) and BJS82-018 (1 clone) were closely related to sulfur-oxidizing chemolithoautotroph *Sulfrovum lithotrophicum* (Inagaki et al. 2004a), and BJS8R-056 (9 relative clones) was also related to another known sulfur-oxidizing chemolithoautotroph *Sulfurimonas autotrophica* (Inagaki et al. 2003), respectively. In addition, the potential sulfate reducers belonging to Deltaproteobacteria were also detected as predominant bacterial components (11 different sequences in 64 clones). The sequences within the *Dsulfosarcina*/*Desulfococcus* group and *Desulfobulbus* relatives, which were recognized as AOM-associated sulfate reducers (Orphan et al. 2001a; Knittel et al. 2005), were detected. Co-existence of sulfate-reducers and sulfur-oxidizers implies the significance of microbial sulfur circulation, which has been reported from another deep-sea cold seep environment at the Japan Trench (Li et al. 1999b; Inagaki et al. 2002; Arakawa et al. 2005).

Seven representative clones of archaeal 16S rRNA genes were selected from a total of 113 archaeal clones by RFLP analysis, and then sequenced. Three representative phylotypes including 24 relative clones were affiliated with MGI (DeLong 1992) (Fig. 4), of which members are known to be widely distributed archaea in seawater. All clones from M2 site were affiliated to MGI representing the phylotype of AJS91-004 (10 relative

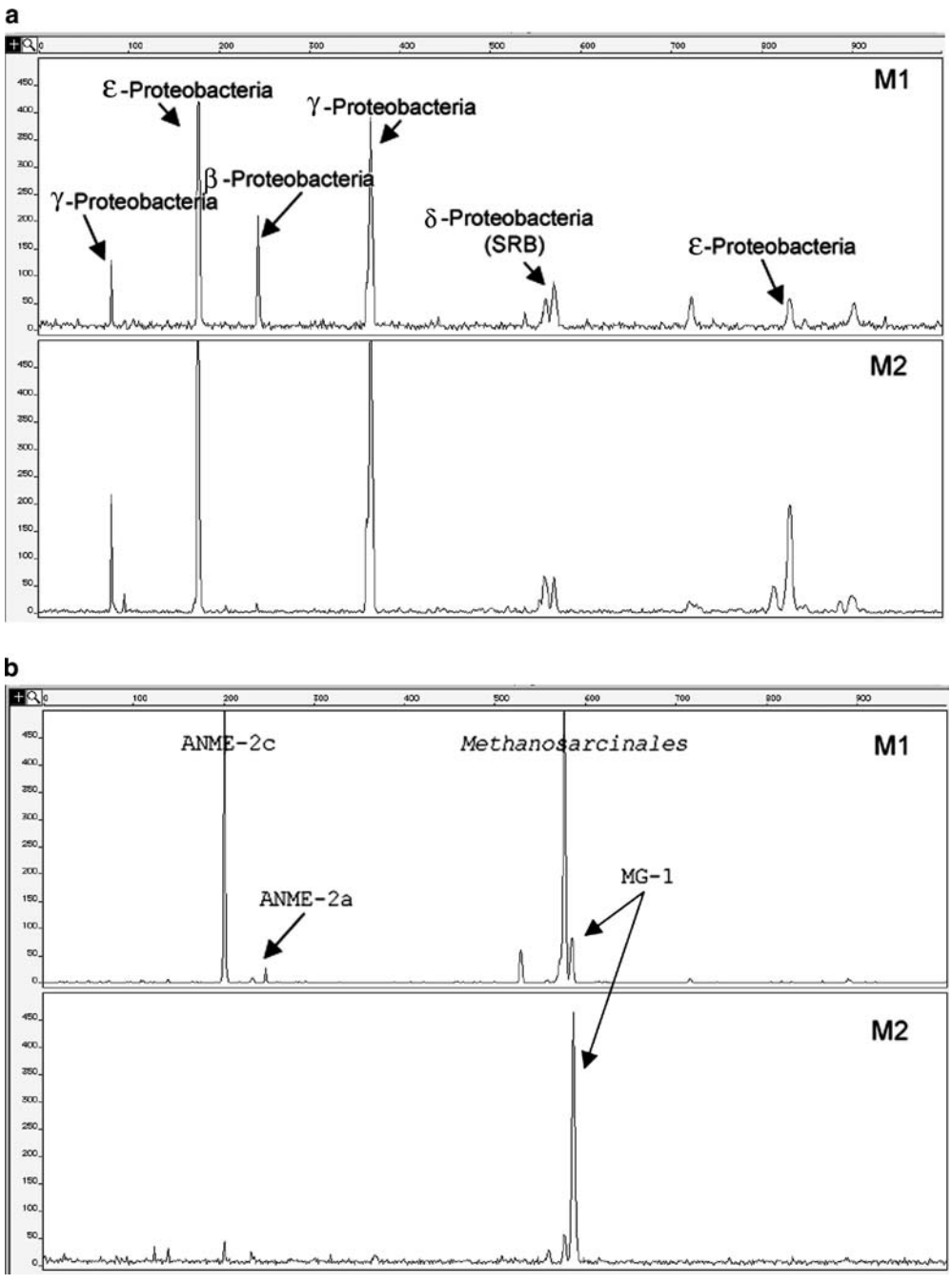

Fig. 2 Comparison of t-RFLP profiles between the microbial mats at M1 and M2 sites. **a** Bacterial communities. **b** Archaeal communities. The length of fragments (*x*-axis) and relative fluorescence intensity of peaks (*y*-axis) are indicated

clones), whose result was consistent with t-RFLP analysis. Phylotypes of AJS72-003 (14 relative clones), AJS72-022 (1 clone), AJS72-117 (71 relative clones) were all detected from M1 site, and were affiliated to ANME-2 cluster within *Methanosarcinales* (Fig. 4), indicating the occurrence of AOM at M1 site.

Discussion

Recent molecular ecological surveys in deep-sea cold seep environments demonstrate that energy and carbon substrates transported from subseafloor environment by geological activities harbor specific microbial communities responsible for carbon and sulfur circulations (e.g., Li and Kato 1999; Boetius et al. 2000; Inagaki et al. 2002, 2004b; Arakawa et al. 2005). In this study, we evaluated microbial community structures at two geologically distinct cold seep sediments (M1 and M2 sites) at the northeastern Japan Sea. Although white colored microbial mats were observed at both cold seep sites, the community structure and chemical characteristics appeared to be clearly different. At M1 site, on one hand, the members of ANME-2 archaea and Deltaproteobacteria dominated, which clearly indicated the occurrence of AOM accompanied with sulfate reduction. On the

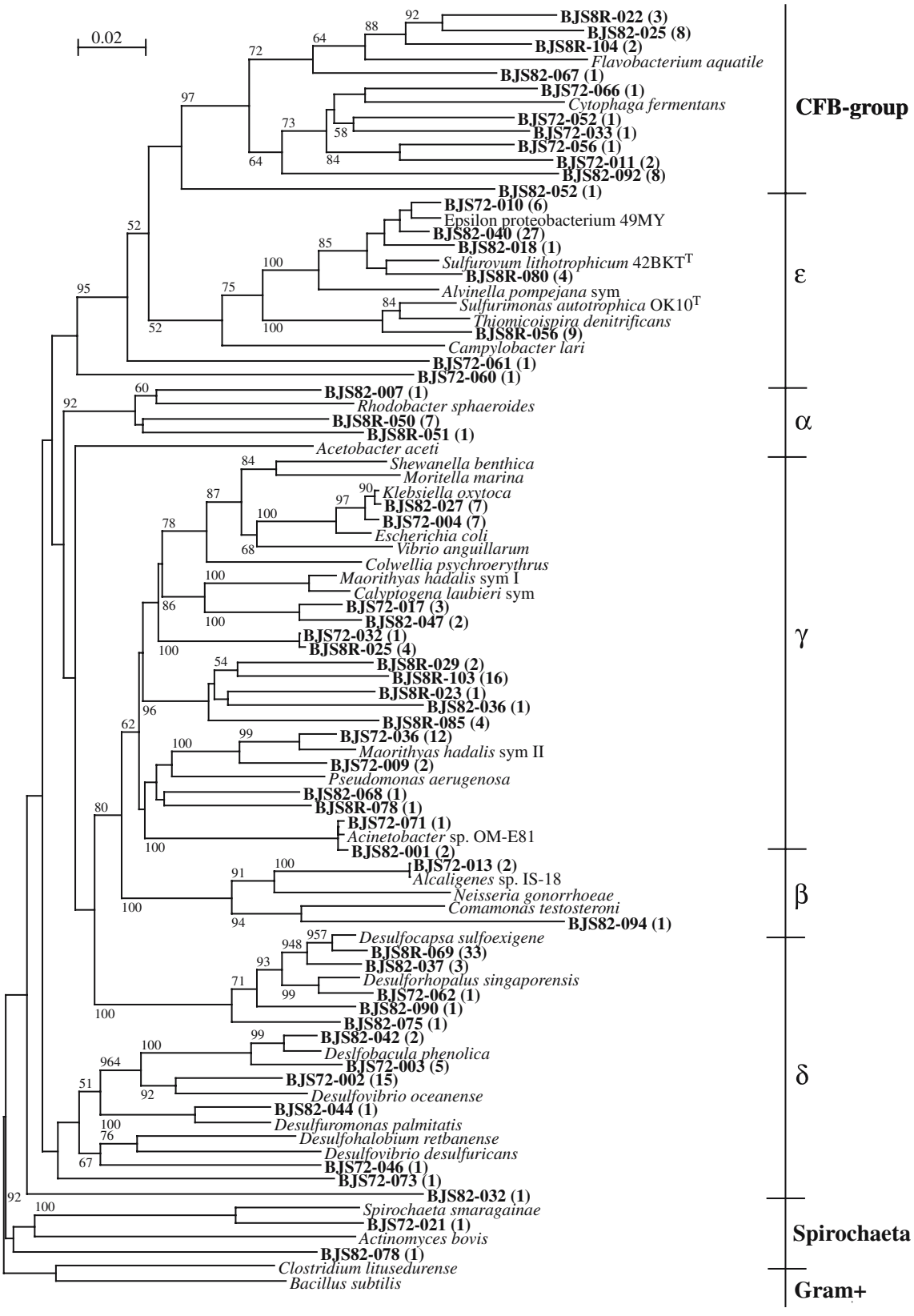

Fig. 3 Phylogenetic analysis of bacterial 16S rRNA gene sequences from microbial mats. The sequences from M1 and M2 sites are indicated as BSJ72- and BST82-, BST8R-numbers, respectively, as shown in *bold* characters (*numbers in parenthesis* show the identical clone numbers). The values for 1,000 bootstrap trial replications are indicated as nodes in the trees (%). The *scale bar* represents 0.02 nucleotide substitutions per sequence position. *Symbol characters* are indicated for the subphyla of Proteobacteria

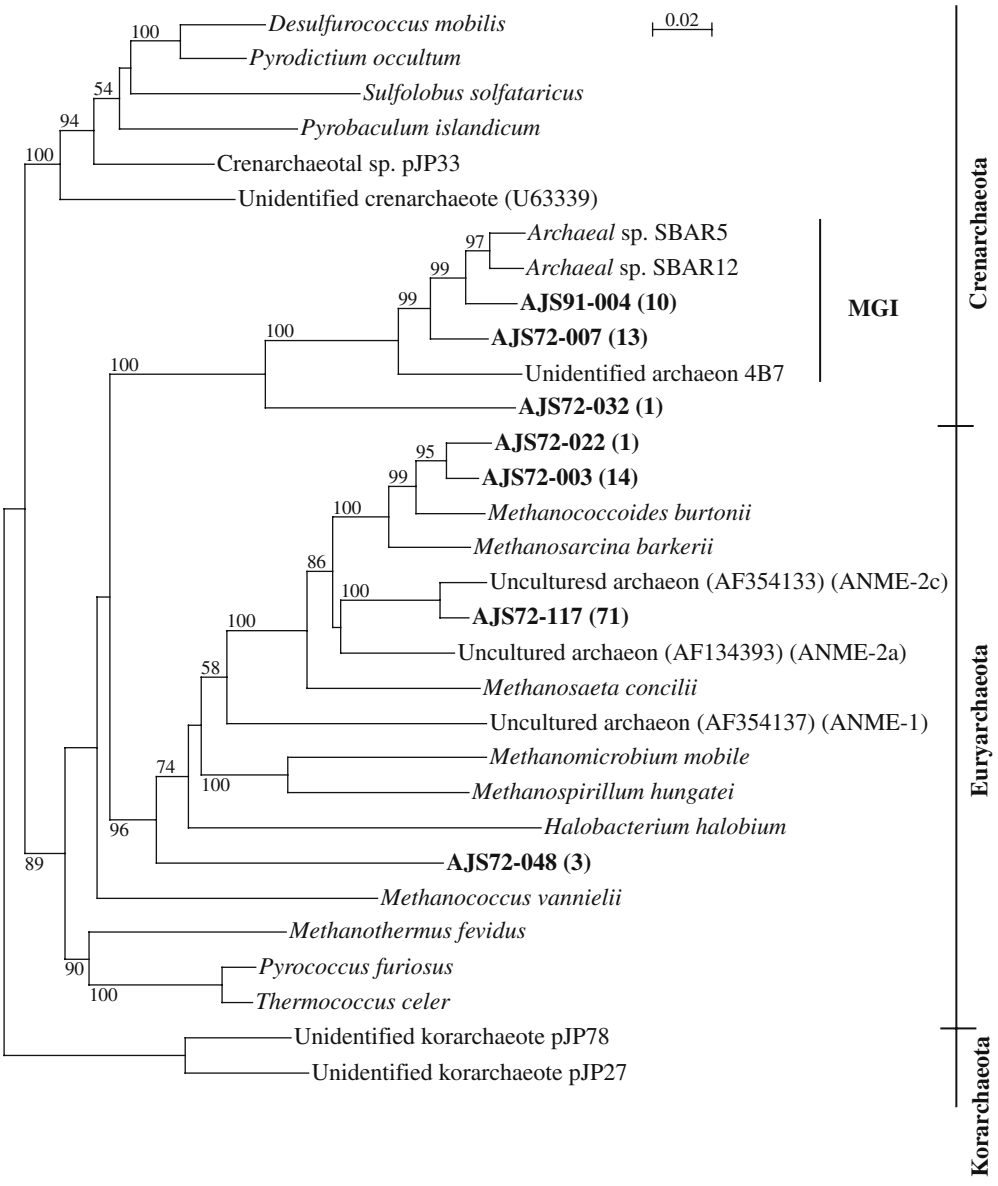

Fig. 4 Phylogenetic analysis of archaeal 16S rRNA gene sequences from microbial mats. The sequences from M1 and M2 sites are indicated as ASJ72- and AST91- numbers, as shown in *bold* characters (*numbers in parenthesis* show the identical clone numbers). The values for 1,000 bootstrap trial replications are indicated as nodes in the trees (%). The *scale bar* represents 0.02 nucleotide substitutions per sequence position

other hand, at M2 site, although the bacterial community structure was quite similar to that at M1 site, no ANME signals were obtained. Geochemical analyses strongly support the notion that the effect of microbial AOM and sulfate reduction is stronger at M1 site than at M2 site. Although high concentration of methane (32.2 nmol/kg, Table 1) was discharged from the mat at M2 site, why was the AOM community not detected at the M2 site?

As shown in Fig. 1, M1 site is located on the active fault line while M2 site located off the Motta Cape field is relatively far from the fault line. Therefore, if the formation of microbial habitat associated with the cold seep at M2 site is younger than at M1 site, it can be reasonably explained that AOM members have not yet migrated as new seep sites appeared off the Motta Cape field. Indeed, the geologic activity in the research field is very high as proved by the occurrence of frequent earthquakes, and AOM members are not movable as free-living microbes. In addition, since AOM reaction requires a strictly anaerobic condition, the transportation may be limited to beneath the seafloor and its rate is expected to be very slow. In other words, the presence of a mature AOM community may be a microbiological indicator of the stable cold seep environment and the AOM habitats in deep-sea environments are strongly associated with plate movements (e.g., subduction system).

One of the important features common at M1 and M2 mat sites is the presence of Delta- and Epsilonproteobactertia. As indicated by t-RFLP analysis, the bacterial community structures at M1 and M2 sites were similar and the sulfur-metabolizing bacteria were predominantly detected. The members of Deltaproteobacteria are most likely sulfate reducers while the phylotypes within Epsilonproteobacteria were closely related to recently isolated chemolithoautotrophic sulfur-oxidizers. These observations suggest that the steep redox front between the anoxic sediment and overlying oxic seawater is present at the cold seep site, which should be the preferential habitat for both sulfur-oxidizing and -reducing microorganisms.

It is also worthwhile to note that the carbon isotopic values of methane at M1 and M2 sites were largely depleted relative to those at reference sites (Table 1). It may indicate that methane produced by methanogens significantly affect the quality of diffusing methane at microbial mat sites. The $\delta^{13}C$ values of methane at reference sites (approx.1 m distance) were strongly ^{13}C-enriched, implying that if the ^{13}C-depleted methane at the mat areas is produced by methanogens their habitat may be in subseafloor sediments just beneath the microbial mat.

In conclusion, this study demonstrates that microbial communities associated with cold seep sediments play an important role for carbon (AOM) and sulfur (sulfate reduction and sulfur oxidation) cycles and the geological setting may control the community structure and distribution. Since we examined only surface mat samples, vertical distribution of microbial community in sediments remains elusive. To verify the 2-demintional distribution of AOM community (and potential habitat of methanogens) beneath the microbial mat, sediment core samples from a series of sampling stations along the transect of the active fault would be required. These are our ongoing efforts.

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References

Arakawa S, Kato C, Sato T, Nogi Y, Yoshida Y, Usami R, Horikoshi K (2004) Microbial diversity of bacterial mat samples collected at a depth of 3100 m in the Japan Sea. Mar Biotechnol 6:S185–S189

Arakawa S, Mori M, Li L, Nogi Y, Sato T, Yoshida Y, Usami R, Kato C (2005) Cold-seep microbial communities are more abundant at deeper depths in the Japan Trench land slope. J Jap Soc Extremophiles 4:50–55

Boetius A, Ravenschlag K, Schubert CJ, Ricket D, Widdel F, Gieseke A, Amann R, Jorgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626

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

Distel DL, Felbeck H, Cavanaugh CM (1994) Evidence for phylogenetic congruence among sulfur-oxidizing chemoautotrophic bacterial endosymbionts and their bivalve hosts. J Mol Evol 38:533–542

Fang J, Arakawa S, Kato C, Schouten S (2006) Microbial diversity of cold-seep sediments in Sagami Bay, Japan as determined by 16S rDNA and lipid analyses. FEMS Microbiol Ecol (in press)

Fujiwara Y, Kato C, Masui N, Fujikura K, Kojima S (2001) Dual symbiosis in the cold-seep thyasirid clam Maorithyas hadalis from the hadal zone in the Japan Trench, western Pacific. Mar Ecol Prog Ser 214:151–159

Inagaki F, Takai K, Komatsu T, Kanematsu T, Fujioka K, Horikoshi K (2001) Archaeology of archaea: geomicrobiological record of Pleistocene thermal events concealed in the deep-sea subseafloor environment. Extremophiles 5:385–392

Inagaki F, Sakihama Y, Inoue A, Kato C, Horikoshi K (2002) Molecular phylogenetic analyses of reverse-transcribed bacterial rRNA obtained from deep-sea cold seep sediments. Environ Microbiol 4:277–294

Inagaki F, Takai K, Kobayashi H, Nealson KH, Horikoshi K (2003) *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing ε-proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa Trough. Int J Syst Evol Microbiol 53:1801–1805

Inagaki F, Takai K, Nealson KH, Horikoshi K (2004a) *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing epsilon-proteobacterium isolated from hydrothermal sediments from the mid-Okinawa Trough. Int J Syst Evol Microbiol 54:1477–1457

Inagaki F, Tsunogai U, Suzuki M, Kosaka A, Machiyama H, Takai K, Nunoura T, Nealson KH, Horikoshi K (2004b) Characterization of C1-metabolizing prokaryotic communities in methane seep habitats at the Kuroshima Knoll, the Southern Ryukyu arc, by analyzing *pmoA, mmoX, mxaF, mcrA*, and 16S rRNA genes. Appl Environ Microbiol 70:7445–7455

Kato C, Li L, Tamaoka J, Horikoshi K (1997) Molecular analyses of the sediment of the 11,000-m deep Mariana Trench. Extremophiles 1:117–123

Kato C, Arakawa S, Usami R, Sato T (2005) Microbial diversity in the cold seep sediments and sulfur circulation (in Japanese). Gekkan Chikyu 27:939–948

Knittel K, Losekann T, Boetius A, Kort R, Amann R (2005) Diversity and distribution of metanotrophic archaea at cold seeps. Appl Environ Microbiol 71:467–479

Kulm LD, Suess E, Moore JC, Carson B, Lewis BT, Ritger SD, Kadko DC, Thornburg TM, Embly RW, Rugh WD, Massoth GJ, Langseth MG, Cochrane GR, Scamman RL (1986) Oregon subduction zone: venting, fauna, and carbonates. Science 231:561–566

Kvenvolden KA (1995) A review of the geochemistry of methane in natural gas hydrate. Org Chem 23:997–1008

Li L, Kato C (1999) Microbial diversity in the sediment collected from cold-seep areas and from different depth of the deep-sea. In: Horikoshi K, Tujii K (eds) Extremophiles in deep-sea environments. Springer, Tokyo, pp 55–88

Li L, Guenzennec J, Nichols P, Henry P, Yanagibayashi M, Kato C (1999a) Microbial diversity in Nankai Trough sediments at a depth of 3843 m. J Oceanogr 55:635–642

Li L, Kato C, Horikioshi K (1999b) Microbial diversity in sediments collected from the deepest cold-seep area, the Japan Trench at a depth of 6,400 m. Mar Biotechnol 1:391–400

Ohta S, Laubier L (1987) Deep biological communities in the subduction zone of Japan from bottom photographs taken during "Nautile" dives in the Kaiko project. Earth Planet Sci Lett 83:329–342

Ohtake M (1995) A seismic gap in the eastern margin of the Sea of Japan as inferred from the time-space distribution of past seismicity. Island Arc 4:156–165

Okamura Y, Satake K, Takeuchi A, Gamo T, Kato C, Sasayama Y, Nakayama F, Ikehara K, Kodera T (2002) Tectonic, geochemical and biological studies in the eastern margin of the Japan Sea—preliminary results of Yokosuka/Shinkai 6500 YK01–06 Cruise. JAMSTEC J Deep Sea Res 20:77–114

Orphan VJ, Hinrichs K-U, Ussler W III, Paull CK, Taylor LT, Sylva SP, Hayes JM, DeLong EF (2001a) Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Appl Environ Microbiol 67:1922–1934

Orphan VJ, House CH, Hinrichs KU, McKeegan KD, DeLong EF (2001b) Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science 293:484–487

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetics trees. Mol Biol Evol 4:406–425

Sibuet M, Juniper SK, Pautot G (1988) Cold-seep benthic communities in the Japan subduction zones: geological control of community development. J Mar Res 46:333–348

Takeuchi A, Okamura Y, Kato Y, Ikehara K, Zhang J, Satake K, Nagao T, Hirano M, Watanabe M (2000) Large earthquakes and bottom disturbances in the Okushiri ridge along the eastern margin of Japan Sea. JAMSTEC J Deep Sea Res, 16:29–46

Tsunogai U, Kouzuma F, Nakagawa N, Gamo T, Kaneko T (2002a) Development of multi-bottle gas-tight water sampler WHATS for sampling sea-floor venting fluids. JAMSTEC J Deep Sea Res 21:91–95

Tsunogai U, Nakagawa F, Komatsu D, Gamo T (2002b) Stable carbon and oxygen isotopic analysis of atmospheric carbon monoxide using CF-IRMS by isotope-ratio monitoring of CO. Anal Chem 74:5695–5700

Wei D, Seno T (1998) Determination of the Amurian plate motion, mantle dynamics and plate interactions in East Asia. In: Geodynamics Series, American Geophysical Union, Washington, 27:337–346