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Variability in Microbial Communities in Black Smoker Chimneys at the NW Caldera Vent Field, Brothers Volcano, Kermadec Arc

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Microbial communities in black smoker chimney structures at the NW caldera vent field of the Brothers volcano, Kermadec arc were characterized by using both culture-dependent and -independent techniques. The hydrothermal vent fluid chemistry, as given by end-member salinities and gas contents, differ among the black smoker sites of the NW caldera field, indicating probable phase-separation-controlled variability in the fluid chemistry. Chimney structures collected from typical Cl-depleted and Cl-enriched hydrothermal fluid vents were used for the microbiological investigation. The 16S rRNA gene clone analysis showed that the archaeal rRNA gene communities were similar within interior and exterior substructures of any single chimneys, and even between chimneys having either Cl-depleted or Cl-enriched hydrothermal emissions. By contrast, the bacterial rRNA gene communities varied between chimneys hosting Cl-depleted or Cl-enriched fluids. Cultivation analysis showed significant variation in the viable counts of various microbial components among the chimneys, particularly of H_2- and/or S-oxidizing chemolithotrophs such as the genera *Persephonella* and *Sulfurimonas*. The difference shown by the cultured microbial community structures between the chimneys may be related to the different chemistries of hydrothermal fluids being expelled by the chimney structures, and possibly differences in the subseafloor environments beneath the vent sites, especially when considering different gas inputs and carbon sources. The patterns in cultivated microbial populations in the chimney structures were compared among the chimney structures studied so far from various deep-sea hydrothermal fields including this study. It shows that the patterns from the gas-rich hydrothermal fluid chimneys are quite similar between the geographically and geologically different hydrothermal fields of the Brothers NW caldera vent field, and the Mariner vent field of the Valu Fa Ridge.

Keywords *Aquificales*, Brothers submarine volcano, chemolithoautotroph, community structure, deep sea hydrothermal vents, Kermadec arc

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INTRODUCTION

The steep gradients of physical and chemical conditions in the deep-sea hydrothermal vent environments generate a wide

range of niches and energy sources for microorganisms, leading to a range of phylogenetic diversity and the variation of physiological function of the microbial ecosystems (Karl 1995; Reysenbach et al. 2000a; Takai et al. 2006). Many studies, based on a molecular ecological approach and/or a cultivation-based approach, have demonstrated variability in microbial communities within chimney structures, or mineralized single vent structures (Harmsen et al. 1997; Takai and Horikoshi 1999; Reysenbach et al. 2000b; Takai et al. 2001a; Schrenk et al. 2003; Nakagawa et al. 2004a; Kormas et al. 2006; Pagé et al. 2008), including among different hydrothermal vent fluids and chimneys within a single hydrothermal field (Huber et al. 2002, 2003, 2006; Takai et al. 2004, 2008; Nakagawa et al. 2004b, 2005a, 2006; Perner et al. 2007). The intra-field variability in microbial communities, that is the variability observed within a hydrothermal field, is associated with the physical and geochemical conditions of the various microbial habitats (Huber et al. 2002, 2003, 2006; Butterfield et al. 2004; Takai et al. 2004, 2008; Nakagawa et al. 2005, 2006).

In previous studies, it was shown that the concentration and composition of the gas components in seafloor hydrothermal vent fluids could be one of the potential driving forces behind intra-field variability in active (cultivated) microbial communities (Takai et al. 2004; 2008; Nakagawa et al. 2005; 2006). Dilution of hydrothermal fluids by seawater, the input of hydrothermal fluids from different sources (Butterfield et al. 2004; Huber et al. 2006; Nakagawa et al. 2006), microbial metabolic activity associated with subseafloor hydrothermal fluid flows (Butterfield et al. 2004; Takai et al. 2004; Von Damm and Lilley 2004; Huber et al. 2006) and phase-separation and -segregation of hydrothermal fluids (Massoth et al. 1989; Lilley et al. 1993; Butterfield et al. 1994; Nakagawa et al. 2005; Konno et al. 2006; Takai et al. 2008; de Ronde et al., in prep.) are all mechanisms that could generate variability in the concentration and composition of the gas components in these hydrothermal fluids. With increasing numbers of deep-sea hydrothermal fields discovered in the arc-backarc systems of the western Pacific margin (de Ronde et al. 2001, 2003a, 2005, 2007; Embley et al. 2004; Ishibashi et al. 2006; Konno et al. 2006; Lupton et al. 2006; Nakagawa et al. 2006; Takai et al. 2008), an increasing variability in hydrothermal fluid chemistry has been discovered. Unique hydrothermal fluid compositions have been identified that are commonly coupled with the discovery of new microbial ecosystems, providing new insights into the biogeochemical diversity in the deep-sea hydrothermal environment.

Brothers volcano is a submarine caldera volcano located along the Kermadec intra-oceanic arc, and forms an elongate edifice 11–13 km long by 7–8 km across that strikes NW-SE. The caldera has a basal diameter of ~3 km where the caldera floor is located at a depth of 1850 m, surrounded by 290 to 530 m high walls (de Ronde et al. 2005). Several unique, chemically different sources of hydrothermal plumes had been identified at the NW caldera and cone sites during hydrothermal plume surveys (de Ronde et al. 2001, 2005). In October 2004, seafloor observations and exploration was done at both the NW caldera and cones sites using the manned submersible *Shinkai 6500* (JAMSTEC YK04-09), and confirmation of actively discharging hydrothermal vents fields was made at both sites.

Hydrothermal activity located on the inner NW slope of Brothers caldera is characterized by numerous, vigorous, black smoker discharges in an area covering some 400 × 270 m (de Ronde et al. 2005). During the *Shinkai 6500* dives, black smoker hydrothermal fluids and chimney structures were sampled from four different vent sites. Based on preliminary chemical analysis of the hydrothermal fluids, it is evident that both Cl-depleted and Cl-enriched hydrothermal fluid samples had been successfully recovered. We report the characteristics of microbial communities in the chimney structures hosting these different vent fluid compositions and discuss the potential biogeochemical interactions between the hydrothermal fluids and the microbial community structures.

MATERIALS AND METHODS

Seafloor Survey and Sampling

In October 2004, 2 dives were done by *Shinkai 6500* (dives #851 and #852) at the NW caldera vent field. Hydrothermal fluid samples for geochemical analysis were collected using a "WHATS-II" (Water Hydrothermal-fluid *Atsuryoku* Tight Sampler II) gas-tight fluid sampler (Saegusa et al. 2006), while the *in situ* temperature of the hydrothermal effluent was measured with a self-recording thermometer on the WHATS-II sampler. This study focused on the hydrothermal fluids and the chimney structures from two distinct chimney sites because the hydrothermal fluid chemistry (e.g., chloride concentration and total gas content) of the chimney sites represented the most distinct features of the Cl-enriched and –depleted fluids, respectively, and the relatively intact chimney structures were successfully obtained from the sites.

The first is a chimney with black smoker discharge with a maximum fluid temperature 274°C located at a depth of 1670 m, adjacent to *Shinkai* Marker #5 (chimney 851-3A; 34°51.742'S/179°03.517'E). The second is also a chimney with black smoker discharge, with a maximum fluid temperature of 290°C located at a depth of 1627 m, near Marker #7 (chimney 852-2B; 34°51.720'S/179°03.481'E). In addition to these chimney sites, hydrothermal fluids from several other chimney sites were also sampled and analyzed for hydrothermal fluid chemistry (the data will be published elsewhere). The sulfide-sulfate chimney structures were recovered using the submersible manipulator directly after fluid sampling.

The hydrothermal fluids and the chimney structures were recovered onboard in 3–6 hours after the sampling on the seafloor. In particular, the chimney samples were exposed to the oxygenated deep seawater in the sample boxes during the recovery. However, this sample recovery condition was not a special case for this study and was similar with the previous explorations of other deep-sea hydrothermal fields (Takai et al. 2004, 2008;

Nakagawa et al. 2005, 2006). Immediately upon recovery onboard, a large piece of each chimney sample was sub-sampled for follow-up microbiological investigations, especially where there was clear zonation of different mineral compositions. The microbiological subsampling was performed in one hour after the sample recovery onboard. This was also similar with the cases in the previous studies.

Hydrothermal fluid from each active chimney was sampled in duplicate into two of the WHATS-II gas-tight bottles (150 ml). One of the bottles was used for the analysis of gas components and the other for fluid chemistry and microbiological studies. The bottle devoted to gas chemistry was processed onboard using a high vacuum line within a few hours after the onboard recovery and the preservation in ice. From the other bottle, the fluid sample was divided into sub-samples: 10 ml for pH and H_2S determination (stored into a glass vial with a butyl rubber cap), 20 ml for the enrichment culture (preserved at 4°C under a 100% N_2 gas phase), and 10 ml was fixed with 3.7% (v/v) formalin. The remaining ~100 ml of the fluid sample was then used for chemical analysis.

For the microbiological experiments, the bulk chimney structures were sub-sampled into representative microbial habitats as previously described by Takai et al. (2001a, 2008). For extraction of nucleic acids, the chimney outer surface (1 to 2 mm thick with white and red-brown patches) and chimney inside wall (1 to 2 cm thick) were placed in sterilized 50-ml centrifuge tubes and then frozen at −80°C until processing. For cultivation of microorganisms, each of the chimney sub-samples was suspended in 20 ml of seawater filtered with a 0.22 μm pore size in either the presence or absence of 0.05% (w/v) neutralized sodium sulfide in a 100-ml glass bottle, and then tightly sealed with a butyl rubber cap under a gas phase of 100% N_2 (100 kPa). For microscopic observations, ~1 g of the sub-sample was fixed with 3 ml of filter-sterilized seawater containing 3.7% (v/v) formalin for 24 hours and then stored at -80°C. All samples for cultivation were transferred to the land-based laboratory at JAMSTEC under refrigeration (4°C) within 3 weeks.

Fluid Chemistry Analyses

The procedures and analytical techniques of the hydrothermal fluid chemistry for this study were previously described in Takai et al. (2008). The pH, alkalinity and concentrations of NH_4 and H_2S were determined onboard. Dissolved gas species were extracted and sealed into glass ampoules onboard using a seagoing vacuum line. The sample in WHATS-II bottle was allowed to drop into an evacuated flask and acidified with sulfamic acid in order to aid in the extraction of carbon dioxide. Then, as the gases were released from the fluid, they were pumped through a U-trap chilled to −60°C into a calibrated volume using an all-metal mechanical bellows pump. The U-trap removes all the water vapor, so that only dry gas is pumped into the calibrated volume. Then the pressure was measured at a known temperature with a precision capacitance manometer to the determined total gas volume.

The aliquots of the extracted gases were then sealed into glass ampoules using a torch. At the end of the extraction, the water frozen in the U-trap was melted and then combined with the water in the extraction flask. The mixed water was weighed to get the total sample weight, and then saved in a Nalgene bottle for chemical analysis of magnesium and chloride concentrations. Gas compositions were determined using a gas chromatograph with TCD (for H_2 and N_2) and FID (for CH_4, CO and CO_2) detectors using one of the glass ampoules. Chemical and isotopic composition of helium was determined by high precision mass spectrometry (Lupton et al. 2006).

Total Direct Cell Counts

Microbial community densities in the sub-samples extracted from the hydrothermal fluids and the chimney structures were determined by 4′,6-diamidino-2-phenylindole (DAPI)-staining direct counting. After thawing the hydrothermal fluid samples, they were filtered with 0.22-μm-pore-size 13-mm-diameter polycarbonate filters (Advantec, Tokyo, Japan). The thawed, formalin-fixed chimney sub-sample was vigorously suspended with a vortex mixer. After 5 minutes, 1 ml of formalin-fixed supernatant was added to 1 ml of filter-sterilized phosphate-buffered saline (PBS, pH 7.2) containing DAPI (10 μg/ml), and incubated at room temperature for 30 minutes. After the mixture was filtered, each filter was rinsed twice with 2 ml of filter-sterilized PBS. The filters were examined under epifluorescence using a phase-contrast Olympus BX51 microscope (Tokyo, Japan) with the Olympus DP71 digital camera system. An average total cell count was obtained from more than 100 microscopic fields from three separate filters.

Liquid Serial Dilution Cultures

To estimate the abundance of culturable microorganisms (viable counts) represented by a variety of physiological and metabolic characteristics, a series of serial dilution cultures were done from each of the hydrothermal fluid and chimney sub-samples under the various cultivation conditions (Table 1). All the cultivation experiments to estimate the viable counts were performed within 3 months after the sample collection. The cultivation experiments were conducted in the order of experiments for the strictly anaerobic chemolithoautotrophs (using the slurry samples with Na_2S), for the strictly anaerobic heterotrophs (using the slurry samples with Na_2S), for facultative anaerobic chemolithoautotrophs (using both the slurry samples with and without Na_2S), for facultative anaerobic chemolithoautotrophs (using both the slurry samples with and without Na_2S) and for microaerobic and aerobic chemolithoautotrophs and heterotrophs (using the slurry samples without Na_2S). These conditions are also similar with those in the previous studies (Takai et al. 2004, 2008; Nakagawa et al. 2005, 2006). For aerobic and anaerobic heterotrophs, MJYPV medium (Sako

TABLE 1
Medium and condition of liquid serial dilution cultures

Medium	Gas phase	Cultivation temperature (°C)	pH of medium	Possible electron donor	Possible electron acceptor	Reference
MJYPV	Air	30, 55, 70, 85, 95	7	YE*, TT†	O_2, NO_3	Sako et al. 2003
	100% N_2 (2 atm) (Gas A)	30, 55, 70, 85, 95	7	YE, TT	NO_3, SO_4, fermentation	
	80% H_2+ 20% CO_2 (2 atm) (Gas B)	30, 55, 70, 85, 95	7	YE, TT, H_2	NO_3, SO_4, fermentation	
MJYPS	100% N_2 (2 atm) (Gas A)	55, 70, 85, 95	7	YE, TT	NO_3, SO_4, S^0, fermentation	Takai et al. 2000
	100% N_2 (2 atm) (Gas A)	55, 70, 85, 95	4	YE, TT	NO_3, SO_4, S^0, fermentation	
MJAIS-YTF	80% H_2+ 20% CO_2 (2 atm) (Gas B)	30, 55, 70, 85, 95	6	YE, TT, Formate, H_2	S^0, CO_2	Takai et al. 2005
MJAIS	80% H_2+ 20% CO_2 (2 atm) (Gas B)	30, 55, 70, 85, 95	6.5	H_2	S^0, CO_2	Takai et al. 2003a
MMJ	80% H_2+ 20% CO_2 (2 atm) (Gas B)	30, 55, 70, 85, 95	6.5	H_2	CO_2	Takai et al. 2002
MMJHS	80% H_2+ 20% CO_2 (2 atm) (Gas B)	30, 55, 70, 85, 95	6.5	H_2, S^0, S_2O_3	S^0, S_2O_3, NO_3, CO_2	Takai et al. 2003b
	80% H_2+ 19% CO_2+ 1% O_2 (2 atm) (Gas C)	30, 55, 70, 85, 95	6.5	H_2, S^0, S_2O_3	O_2, S^0, S_2O_3, NO_3, CO_2	
	75% H_2+ 15% CO_2+ 10% O_2 (2 atm) (Gas D)	30, 55, 70, 85, 95	6.5	H_2, S^0, S_2O_3	O_2, S^0, S_2O_3, NO_3, CO_2	
	80% N_2+ 19% CO_2+ 1% O_2 (2 atm) (Gas E)	30, 55, 70, 85, 95	6.5	S^0, S_2O_3	O_2, S^0, S_2O_3, NO_3, CO_2	
	75% N_2+ 15% CO_2+ 10% O_2 (2 atm) (Gas F)	30, 55, 70, 85, 95	6.5	S^0, S_2O_3	O_2, S^0, S_2O_3, NO_3, CO_2	
MMJHFe	80% H_2+ 20% CO_2 (2 atm) (Gas B)	30, 55, 70, 85, 95	6.5	H_2, YE, Formate, Acetate, Pyruvate, Lactate, Citrate	Soluble & insoluble Fe (III), SO_4, CO_2, fermentation	Takai et al. 2008

*YE means yeast extract.
†TT means tryptone.

et al. 2003) supplemented with 10 mM sodium nitrate was used (Table 1).

For anaerobic, heterotrophic sulfur-reducing thermophiles such as members of *Thermococcales, Aciduliprofundum* group and *Thermotogales*, MJYPS medium (Takai et al. 2000) was used with different pHs of 4 and 7 (Table 1). For hydrogen-oxidizing, sulfur-reducing autotrophs and mixotrophs, such as members of the *Desulfurococcales*, *Desulfurobacteriaceae*, *Deferribacteres* and *Epsilonproteobacteria,* MJAIS-YTF medium (Takai et al. 2005) and MJAIS medium (Takai et al. 2003a) were used (Table 1). For mesophilic to hyperthermophilic methanogens, MMJ medium (Takai et al. 2002) was used (Table 1).

For hydrogen- and/or sulfur-oxidizing chemolithoautotrophs, such as members of *Aquificales*, *Gammaproteobacteria* and

Epsilonproteobacteria, MMJHS medium (Takai et al. 2003b) was used (Table 1). For anaerobic dissimilatory Fe(III)- and/or sulfate-reducers, such as members of *Archaeoglobales*, *Deferribacteres*, *Thermodesulfobacteria* and *Deltaproteobacteria*, a MMJHFe medium (Takai et al. 2008) was used (Table 1). The microbial growth was identified by turbidity and/or microscopic observation for maximally 2 months. The microorganism present in the most diluted series of the medium at each temperature was isolated by the subsequent extinction-dilution method (Takai and Horikoshi 2000a). The partial sequences of the 16S rRNA genes (approx. 700–1000 bp) of the isolates were determined as described elsewhere (Takai et al. 2004). Although the purity of the isolates was not substantial in this study, it was checked by the cellular morphology on the microscopic observation and the 16S rRNA gene partial sequence.

Nucleic Acid Extraction and 16S rRNA Gene Clone Analysis

Nucleic acids were extracted from the chimney outer surfaces (3 g wet) and the chimney inside walls (10 g wet) with the Ultra Clean Mega Soil DNA kit (MO Bio Laboratory, Solana Beach, CA, USA), following the manufacturer's instructions. A blank tube (with no sample added) was processed as a negative control. Extracted nucleic acids were stored at -80°C.

Bacterial and archaeal 16S rRNA genes were amplified from DNA extracts from subsamples and controls by PCR using LA Taq polymerase with GC buffer (Takara Bio, Otsu, Japan). The Oligonucleotide primers used were Bac27F and 1492R (Lane 1991) for the bacterial rRNA gene and Arch21F and Arch958R (DeLong 1992). Thermal cycling was performed under the following conditions: denaturation at 96°C for 25 sec, annealing at 50°C for 45 sec, and extension at 72°C for 120 sec for a total of 20–25 cycles for bacterial rRNA genes and 30–50 cycles for archaeal rRNA genes, respectively. The PCR cycle numbers represent almost the minimum cycle numbers providing enough amplified products for the cloning based on the preliminary PCR amplification experiments using the same templates.

The amplified rRNA gene products from several separate reactions at the least number of thermal cycles were pooled and purified as previously described (Takai et al. 2001b). Cloning and sequencing were also followed by the procedure described by Takai et al. (2001b). The Bac27F (Lane, 1991) or Arch21F (DeLong 1992) primer was used in partial sequencing analysis.

Single-strand sequences of approximately 500 nucleotides in length were determined. The sequence similarity was analyzed by the FASTA component program of DNASIS (Hitachi software, Tokyo, Japan). The rRNA gene sequences having $\geq$97% similarity by FASTA were assigned to the same phylotype. The partial sequences of the representative phylotypes were determined for both strands (approx. 900 bp), and were applied to sequence similarity analysis against the non-redundant nucleotide sequence databases of GenBank, EMBL and DDBJ using the FASTA.

Phylogenetic Analysis

The sequences of the representative phylotypes from the clone analysis and the isolates were manually realigned according to the secondary structures using ARB (Ludwig et al. 2004). Phylogenetic analyses were restricted to nucleotide positions that could be chosen using the *Bacteria* and *Archaea* filters for the bacterial and archaeal 16S rRNA gene sequences (Hugenholtz 2002). Evolutionary distance matrix analysis (using the Jukes and Cantor correlation method) and neighbor-joining analysis were performed using PHYLIP package (http://evolution.genetics.washington.edu/phylip.html). Bootstrap analysis was performed to provide confidence estimates for phylogenetic tree topologies.

Quantitative PCR Analysis

Determination of the proportion of the archaeal 16S rRNA genes in the whole microbial DNA assemblage was performed by the quantitative fluorescent PCR method using TaqMan probes as described elsewhere (Takai and Horikoshi 2000b). A dilution series of each of the DNA samples was prepared and the samples were assayed using the universal 16S rRNA gene mixture and the archaeal 16S rRNA gene mixture as the respective standards (Takai and Horikoshi 2000b).

Nucleotide Sequence Accession Number

The 16S rRNA gene sequences determined in this study were submitted to DDBJ/EMBL/GenBank and have been assigned the accession numbers shown in Tables 4 and 5, and in Figure 2.

RESULTS AND DISCUSSION

Description of Chimney Samples

Two types of mineralization dominate the numerous clusters of chimneys of the NW caldera field at Brothers volcano. One type is Cu-rich and the other Zn-rich (de Ronde et al. 2003b, 2005). The Cu-rich chimneys typically have a pronounced central conduit that is lined by both fine and coarse-grained chalcopyrite. The outer-most zones to these chimneys, and those intervening between bands of chalcopyrite, are dominated by sphalerite, pyrite and barite, indicating fluctuations in temperature and/or vent fluid composition (de Ronde et al. 2005). The Zn-rich chimneys are the most prevalent within the NW caldera field. The most common examples have very chaotic, porous interiors with 'boxwork'-like textures, having either a barely discernable central conduit, or none at all (de Ronde et al. 2005). They are dominated by sphalerite with pyrite/marcasite and barite, indicative of relatively lower temperatures. A thin band of silica-rich material commonly marks the outermost margin of these chimneys.

Chimneys 851-3A and 852-2B represent actively venting black smoker chimneys. These chimneys host both Cu-rich and Zn-rich mineralization that form distinct zones within the

chimneys. The morphology and mineral composition of the two chimneys are generally similar to each other. More detailed information on the geochemical composition and of these chimneys, including their isotopic signatures, will be reported elsewhere.

Fluid Chemistry

The physical properties and the chemical compositions of the hydrothermal fluids being expelled from the chimneys 851-3A and 852-2B at the time of their sampling are summarized in Table 2. The data in Table 2 are not the endmember chemical compositions extrapolated from various data but are given as the values of the potentially non-diluted hydrothermal fluids that were corrected from the measured values. The correction was based on an assumption that the measured Mg concentration in the fluid was all derived from the ambient seawater during the sampling and represented the dilution ratio of the seawater. Thus, only based on these data, it is difficult to evaluate the hydrothermal fluid chemistry in the NW caldera field at Brothers volcano.

However, the hydrothermal fluid chemistry in the field was characterized by the samples obtained from the same cruise of this study (4 different chimney sites) and also other cruises (many other additional chimney sites). The geochemical characterization of hydrothermal fluids at the NW caldera field demonstrated several different end-member compositions within the vent sites and the potential phase-separation-controlled variability in the hydrothermal fluids (de Ronde et al. unpublished). The hydrothermal fluid chemistry from the chimneys 851-3A and 852-2B shown in Table 2 differed markedly but was close to the endmember fluid compositions found in the NW caldera field at Brothers volcano (de Ronde et al. unpublished). Based on the results in Table 2, the hydrothermal fluid from 851-3A was enriched in Cl, whereas the fluid from 852-2B was Cl-depleted when compared to seawater chlorinity.

The Cl-depleted 852-2B fluid also had higher gas concentrations than the Cl-enriched 851-3A fluid, suggesting that phase-separation into vapor and brine phases has occurred beneath the seafloor. Subsequent re-mixing of these two phases in different proportions from their original ratios may have produced different fluid chlorinities not only between the hydrothermal fluids from these two chimneys (Table 2), but also different to other fluid compositions from other vents within the NW caldera field (de Ronde et al. unpublished).

Gas species dissolved in hydrothermal fluids are considered to be largely of magmatic origin (e.g., Kelley et al. 2002). When comparing the gas components of the hydrothermal fluids between Cl-enriched (851-3A) and Cl-depleted (852-2B) chimneys, the Cl-depleted hydrothermal fluid shows an enrichment in gases (Table 2). However, the extent of enrichment was quite variable among the different gas species even if the data in Table 2 contain some of the sampling and analytical error values (Table 2). The ^3He, CH_4 and H_2S concentrations were enriched in the Cl-depleted hydrothermal fluid by more than 3 times the concentrations in the Cl-enriched one. Even the CO_2 and N_2 concentrations in the Cl-depleted fluid were about twice as high as those in the Cl-enriched fluid, while H_2 was only slightly enriched (Table 2).

Since the different gas species have different vapor-liquid distribution coefficients under various hydrothermal conditions (Chiodini et al. 2001), they did not show the similar distribution behaviors, and therefore ratios of the different gases could be changed during the phase-separation and the re-mixing processes, as previously suggested (Takai et al. 2008). Nevertheless, the extent of enrichment of H_2 in the Cl-depleted fluid is too small if the gas deviation is assumed to follow the vapor-liquid distribution coefficients, since the distribution coefficient of H_2 should be quite similar with that of CH_4 under the physical and chemical conditions of the hydrothermal fluids at the NW caldera field as previously suggested (Takai et al. 2008).

Besides the distribution behavior of H_2 into different phases of the hydrothermal fluid, the chemical equilibrium by iron/sulfide mineral buffer (Seyfried and Ding 1995) and the kinetic reaction of pyrite formation by H_2S oxidation (Rickard 1997) between the hydrothermal fluids and minerals might have an impact on the H_2 concentrations in the fluids. However, based on the thermodynamic and kinetic calculations using the physical and chemical properties of the two Cl-depleted and -enriched hydrothermal fluids in Table 2, it was shown that both the fluid-mineral reactions served to promote more enrichment of H_2 concentration in the 852-2B Cl-depleted fluid than that expected only by the distribution coefficient.

Thus, any other mechanism than the physical and chemical processes is expected to induce the H_2 anomaly between the two fluids. The microbial consumption of H_2 in the subseafloor environments may have a significant impact on the H_2 concentration in the phase-separation affected hydrothermal fluids. If H_2 is assumed to be similarly fractionated into the Cl-depleted and enriched hydrothermal fluids like other less soluble gas species such as ^3He and CH_4 and then to be consumed by the subseafloor microbial communities, the difference in $H_2/^3He$ and H_2/CO_2 values between the Cl-depleted and enriched fluids suggests >10 μM of the H_2 along the subseafloor Cl-depleted fluid flow path.

The phase-separation and partition of hydrothermal fluids seems to be important in the variability of the hydrothermal fluid chemistry at the NW caldera field of Brothers volcano. Moreover, the enriched gas components, particularly H_2 and other gas components such as H_2S, in the Cl-depleted hydrothermal fluids may have an impact on the activity of the microbial communities associated with subseafloor fluid flow and even for seafloor habitats such as the chimneys.

Biomass in the Hydrothermal Fluids and Chimneys

The prokaryotic community density determined by direct counting of each subsample is listed in Table 3. The direct count

TABLE 2
Onboard measurements and estimated chemical compositions (Mg = 0) of hydrothermal fluids from the Brothers volcano NW Caldera field.

	Vent fluid associated with chimney #851-3A	Vent fluid associated with chimney #852-2B	Seawater*
Onboard measurement			
Max. temperature (°C)	274	290	
pH	3	2.8	
Alkalinity (meq/kg)	−0.89	−1.77	
NH_4 (μM)	14.8	8.88	
Estimated chemical composition of non-diluted hydrothermal fluid			
Si (mM)	14.2	12.4	0.12
Na (mM)	526	369	473
K (mM)	73.1	48.8	9.2
Ca (mM)	43.6	33.7	9.5
Fe (mM)	7.3	4.2	0
Mn (mM)	0.75	0.51	0
Mg (mM)	0	0	51.4
Cl (mM)	732	502	545
SO_4 (mM)	0	0	28.2
H_2S (mM)	2.32	7.86	0
CO_2 (mM)	17.8	35.5	2.3
CH_4 (μM)	2.16	6.79	0.01
H_2 (μM)	14.3	16.8	0
N_2 (μM)	510	1100	
^{3}He (pM)	2.72	10.3	
$H_2S/^3He$ ($\times 10^9$)	0.844	0.76	
$CO_2/^3He$ ($\times 10^9$)	6.58	3.45	
$CH_4/^3He$ ($\times 10^6$)	0.787	0.657	
$H_2/^3He$ ($\times 10^6$)	5.22	1.63	
$N_2/^3He$ ($\times 10^9$)	0.187	0.107	
H_2S/CO_2	0.13	0.221	
CH_4/CO_2 ($\times 10^{-3}$)	0.121	0.191	
H_2/CO_2 ($\times 10^{-3}$)	0.803	0.473	
N_2/CO_2 ($\times 10^{-3}$)	28.7	31	

*Data from from Takai et al. (2008).

densities in the hydrothermal fluids obtained from chimneys 851-3A and 852-2B were very low, below 5 x 10^2 cells/ml. In addition, quantitative PCR analysis showed that the amount of whole microbial 16S rRNA gene in both the hydrothermal fluids was also below detection limits (<0.1 fg/ml). The direct count cell densities of all the mineralized chimney samples were much lower than for the chimney outer surface, while only very small amounts of archaeal 16S rRNA genes were detected by quantitative PCR analysis from the internal chimney environment (Table 3).

The direct count prokaryotic densities for the outer surfaces of chimneys 851-3A and 852-2B were comparable with those from chimney exterior surfaces collected from the Myojin Knoll of the Izu-Bonin arc (Takai and Horikoshi 1999), the PACMANUS field of the Manus Basin (Takai et al. 2001a), the Kairei field of the Central Indian Ridge (Takai et al. 2004a), the Iheya North field in the mid-Okinawa Trough (Nakagawa et al. 2005) and the Mariner field in the Lau Basin (Takai et al. 2008). However, a higher cell density was detected for the exterior to chimney 852-2B than was for the 851-3A (Table 3).

Quantitative PCR analysis indicates that the archaeal 16S rRNA gene was a minor fraction in the total prokaryotic 16S rRNA gene community in both chimney surfaces, while the proportion of the archaeal 16S rRNA gene in the total prokaryotic 16S rRNA gene community was increased in the exterior to chimney 851-3A. The number of the archaeal 16S rRNA gene was almost identical between both chimney surface environments, whereas the number of the bacterial 16S rRNA gene was

TABLE 3
Microbiological characteristics of each sub-sample

Sample ID	Prokaryotic community density (cells/g or ml)	Strains obtained from dilution-cultivation test in the medium of								Proportion of rRNA gene for	
		MJYPS Gas A	MMJHS Gas B	MMJHS Gas C	MMJHS Gas D	MMJHS Gas E	MMJHFe Gas B	MJYPV air	MJYPV Gas A	Bacteria (%)	Archaea (%)
4CVW (hydrothermal fluid expelled by chimney 851-3A)	$3.6 \pm 1.3 \times 10^2$	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4C-S (surface part of chimney 851-3A)	$2.1 \pm 0.8 \times 10^7$	Tc95-4C-S (1.0×10^2 cells/g) Tc85-4C-S (1.0×10^5 cells/g) Tc70-4C-S (1.0×10^4 cells/g)	n.d.	n.d.	n.d.	n.d.	n.d.	Ae55-4C-S (5.0×10^3 cells/g) Ae30-4C-S (3.7×10^6 cells/g)	An30N-4C-S (1.0×10^1 cells/g)	94	6
4C-I (interior part of chimney 851-3A)	$6.2 \pm 2.1 \times 10^4$	Tc85-4C-I (1.0×10^3 cells/g) Tc70-4C-I (1.0×10^3 cells/g)	n.d.	n.d.	n.d.	n.d.	n.d.	Ae30-4C-I (1.0×10^1 cells/g)	n.d.	0	100
7CVW (hydrothermal fluid expelled by chimney 852-2B)	$2.6 \pm 1.1 \times 10^2$	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7C-S (surface part of chimney 852-2B)	$9.9 \pm 1.5 \times 10^7$	Tc95-7C-S (1.0×10^2 cells/g) Tc85-7C-S (1.0×10^6 cells/g) Tc70-7C-S (1.0×10^5 cells/g)	0H70-7C-S (1.0×10^5 cells/g) 0H55-7C-S (1.0×10^5 cells/g) 0H30-7C-S (1.0×10^4 cells/g)	1H70-7C-S (1.0×10^6 cells/g) 1H55-7C-S (1.0×10^6 cells/g) 1H30-7C-S (1.0×10^5 cells/g)	10H85-7C-S (1.0×10^2 cells/g) 10H55-7C-S (1.0×10^5 cells/g) 10H30-7C-S (1.0×10^5 cells/g)	1N30-7C-S (1.0×10^5 cells/g)	Fe70-7C-S (1.0×10^3 cells/g) Fe55-7C-S (1.0×10^3 cells/g) Fe30-7C-S (1.0×10^5 cells/g)	Ae70-7C-S (1.0×10^4 cells/g) Ae30-7C-S (1.0×10^2 cells/g)	An30N-7C-S (4.0×10^3 cells/g)	99.3	0.72
#7C-I (interior part of chimney 852-2B)	$4.8 \pm 0.7 \times 10^4$	Tc95-7C-I (1.0×10^2 cells/g) Tc85-7C-I (1.0×10^2 cells/g) Tc70-7C-I (1.0×10^2 cells/g)	0H70-7C-I (1.0×10^1 cells/g) 0H55-7C-I (1.0×10^1 cells/g)	n.d.	n.d.	n.d.	n.d.	Ae30-7C-I (1.0×10^1 cells/g)	n.d.	0	100

*n.d.; not detected.

increased in the exterior to chimney 851-3A. The bacterial 16S rRNA gene population was estimated to be about 5 times higher on the surface of chimney 852-2B chimney than was on the 851-3A, consistent with the difference in direct count prokaryotic density between the exterior surfaces of the two chimneys. These results strongly suggest significant variation in the bacterial components of the microbial communities between the exterior surface environments of these two chimneys.

Bacterial and Archaeal Phylotypes in the Chimney Surfaces

The bacterial and archaeal 16S rRNA gene community structures of the chimney surface and interior environments are shown in Tables 4 and 5, and summarized in Figure 1. The phylogenetic affiliation of the phylotype is shown in Figure 2. The 16S rRNA gene clone analysis clearly shows that the bacterial rRNA gene community in the surface environment differs markedly between chimneys 851-3A and 852-2B (Table 5 and Figure 1).

The most abundant archaeal phylotype common to both chimneys is pKB3A-1 (Table 4 and Figure 1), which is most closely related to the deep sea hydrothermal vent clone pLM5A-1 from the Mariner vent field of the Lau Basin (Figure 2A; Takai et al. 2008) within the Marine Benthic Group E (MBGE; Takai and Horikoshi 1999; Vetriani et al. 1999), respectively. The predominance of MBGE phylotypes, including phylotypes other than pKB3A-1, in the archaeal rRNA gene communities found in the active vent chimney structures was unique in the Brothers NW caldera vent field (87.1, 100, 96.9 and 100% in clonal frequency for chimney 851-3A interior and exterior surfaces, and chimney 852-2B interior and exterior surfaces, respectively). The predominance of very closely-related archaeal phylotypes had been reported previously from inactive chimney environments of the Iheya North field in the mid Okinawa Trough, the Central Indian Ridge Kairei vent field (Suzuki et al. 2004), and from a large, active chimney in the Mariner field of the Lau Basin (Takai et al. 2008).

Although the MBGE phylotypes are yet-uncultivated and their physiology is still uncertain, it is inferred from the high incidence of the MBGE phylotypes in the inactive chimney structures that this uncultivated group of *Archaea* may be associated with weathering of the metal-rich sulfide deposits under relatively oxidizing conditions (Suzuki et al. 2004; Takai et al. 2008). Thus, the predominance of MBGE phylotypes in both chimney structures may represent the formation of environments favorable for the weathering of metal-rich sulfides under relatively oxidative redox states.

The bacterial 16S rRNA gene community structure in the exterior surface to chimney 851-3A also contained phylotypes potentially associated with weathering of metal sulfide deposits under relatively oxidizing conditions. The phylotypes phylogenetically affiliated within the phyla *Nitrospirae* and *Planctmycetes* found on the surface of chimney 851-3A (Table 5 and Figure 1) represent components rarely detected in active chimney structures. Similar *Nitrospirae* and *Planctmycetes* phylotypes have been obtained from inactive chimneys of the Iheya North field in the mid Okinawa Trough, the Central Indian Ridge Kairei vent field (Suzuki et al. 2004), and from an active chimney in the Mariner field of the Lau Basin (Takai et al. 2008). Nevertheless, within the same environment for chimney 851-3A were bacterial phylotypes within *Aquificaceae*, *Deferribacteres* and *Gammaproteobacteria*, which are all related to chemolithotrophs previously isolated from active chimneys in deep sea hydrothermal environments.

By contrast, the bacterial 16S rRNA gene community structure in the exterior surface of chimney 852-2B was largely composed of the phylotypes within *Epsilonproteobacteria* and *Gammaproteobacteria* (Table 5, Figures 1 and 2B). The epsilonproteobacterial phylotypes of the genera *Sulfurovum* (Inagaki et al. 2004) and *Sulfurimonas* (Inagaki et al. 2003) within the family *Thiovulgaceae* (Campbell et al. 2006) and the gammaproteobacterial phylotypes related with sulfur-oxidizing symbiotic groups predominate in the surface environment to chimney 852-2B.

There was a clear difference in the bacterial 16S rRNA gene community structure between the chimney surface habitats of 851-3B and 852-2B. However, it is difficult to interpret the community differences based solely on the culture-independent, bacterial 16S rRNA gene community analysis. As shown by direct count prokaryotic density characterization and quantitative 16S rRNA gene analysis, the bacterial population was more enriched in the exterior surface of chimney 852-2B than of chimney 851-3A. In all likelihood, the bacterial components of *Epsilonproteobacteria* and *Gammaproteobacteria* represent the enriched populations in the surface habitats of chimney 852-2B. These bacterial components might be chemolithoautotrophic members potentially utilizing H_2 and H_2S as the primary energy sources. Thus, the results from the culture-independent microbial community analysis suggest an increased potential for the microbial community to metabolize the gaseous energy and carbon sources in the habitats associated with the Cl-depleted hydrothermal fluid.

Cultivation Analyses of Microbial Components

We attempted to estimate the viable counts thereby setting a lower limit to the population density of potentially predominant microbial components living in the chimney environments by means of a liquid serial dilution cultivation technique. The viable counts and the phylogenetic affiliation of the cultures and the isolated strains are shown in Table 3 and Figure 3, respectively.

Variability in the cultivated microbial community was evident in the comparison between the surface environments of the chimneys expelling Cl-enriched (851-3A) and Cl-depleted hydrothermal fluids (852-2B) (Figure 3). The viable counts of *Thermococcus* and *Persephonella* species was more than 10^6 cells/g, and the count of *Sulfurimonas* was also more than 10^5

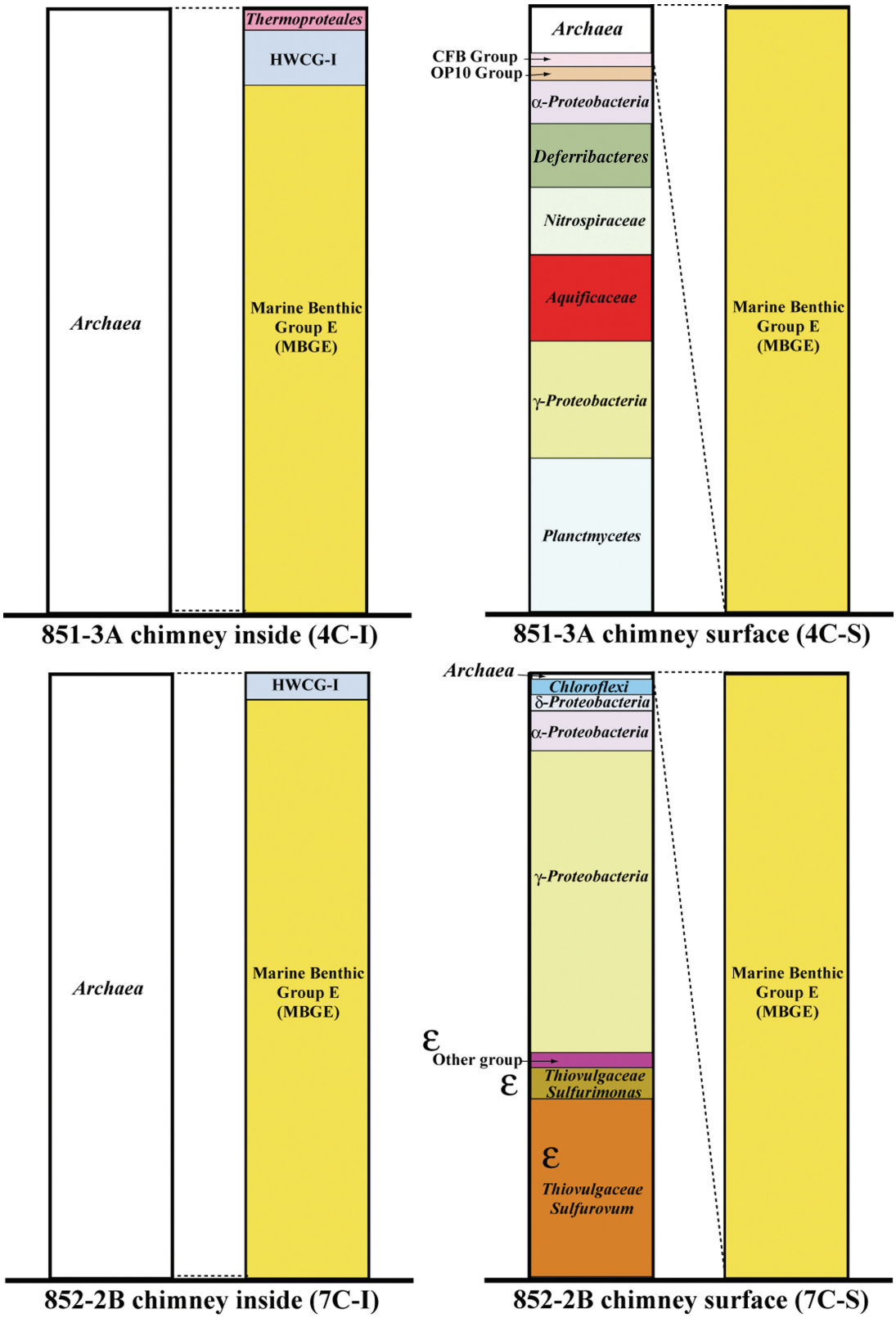

FIG. 1. Composition of the microbial rRNA gene community in the different chimney environments. The phylogenetic grouping was delineated from the phylogenetic trees indicated in Figure 2. The archaeal rRNA gene proportion is based on quantitative PCR analysis.

cells/g in the chimney 852-2B surface environment. Among these major cultivated populations in this environment, only the *Thermococcus* population was detected in the chimney 851-3A surface environment and was 10 times less than the viable count of *Thermococcus* species in the exterior surface to 852-2B (Figure 3).

Instead, mesophilic heterotrophic *Halomonas* species represented the highest viable count on the surface of chimney 851-3A (Figure 3). Other cultivated microbial populations on the surface to 851-3A were the members of *Marinithermus* and *Vibrio* (Figure 3), with all the populations of heterotrophic members. By contrast, other than *Persephonella* and *Sulfurimonas* members, chemolithoautotrophic *Aquifex* and potentially chemolithomixotrophic *Deferribacter* members were cultivated from the surface environments of 852-2B together with other aerobic and anaerobic heterotrophs of *Rhodothermus*, *Desulfuromusa*, *Vibrio* and *Pseudoalteromonas* (Figure 3). In both chimney samples, considerably lower viable counts of microbial components that were similar with those in the surface habitats were noted for the chimney interior environments (Figure 3). These results imply that the microbial community in 852-2B consists of more cultivable and viable components than that in 851-3A. More significantly, an increased population of hydrogen- and/or sulfur-oxidizing chemolithoautotrophs, such as *Persephonella* and *Sulfurimonas* members, was found in the chimney 852-2B which was discharging the Cl-depleted (gas-enriched) hydrothermal fluid.

From the any samples of the Brothers Caldera NW slope field, no methanogen was cultivated. As previously reported by Reysenbach et al. (2006), we sought to cultivate the acidophilic heterotrophs such as the members of thermoacidophilic *Aciduliprofundum* group using MJYPS media at different pH conditions (Table 1) because the microbial habitats in the chimney structures are likely created in the pH range between about 3 (hydrothermal fluids) and about 8 (deep seawater). In addition, the hydrothermal fluids of the Brothers Caldera NW slope field were influenced by potential phase-separation and the chloride concentrations were variable although the variation was relatively small (within 30% against the seawater chloride concentration) (Table 2). Thus, the cultivation test of extremely halophilic heterotrophs such as archaeal halophiles, of which 16S rRNA gene sequences were identified in the PACMANUS field in the Manus Basin (Takai et al. 2001), was also conducted using the HA solid medium (Ihara et al. 1997). These attempts provided no detectable cultivation of any of the acidophiles and extreme halophiles.

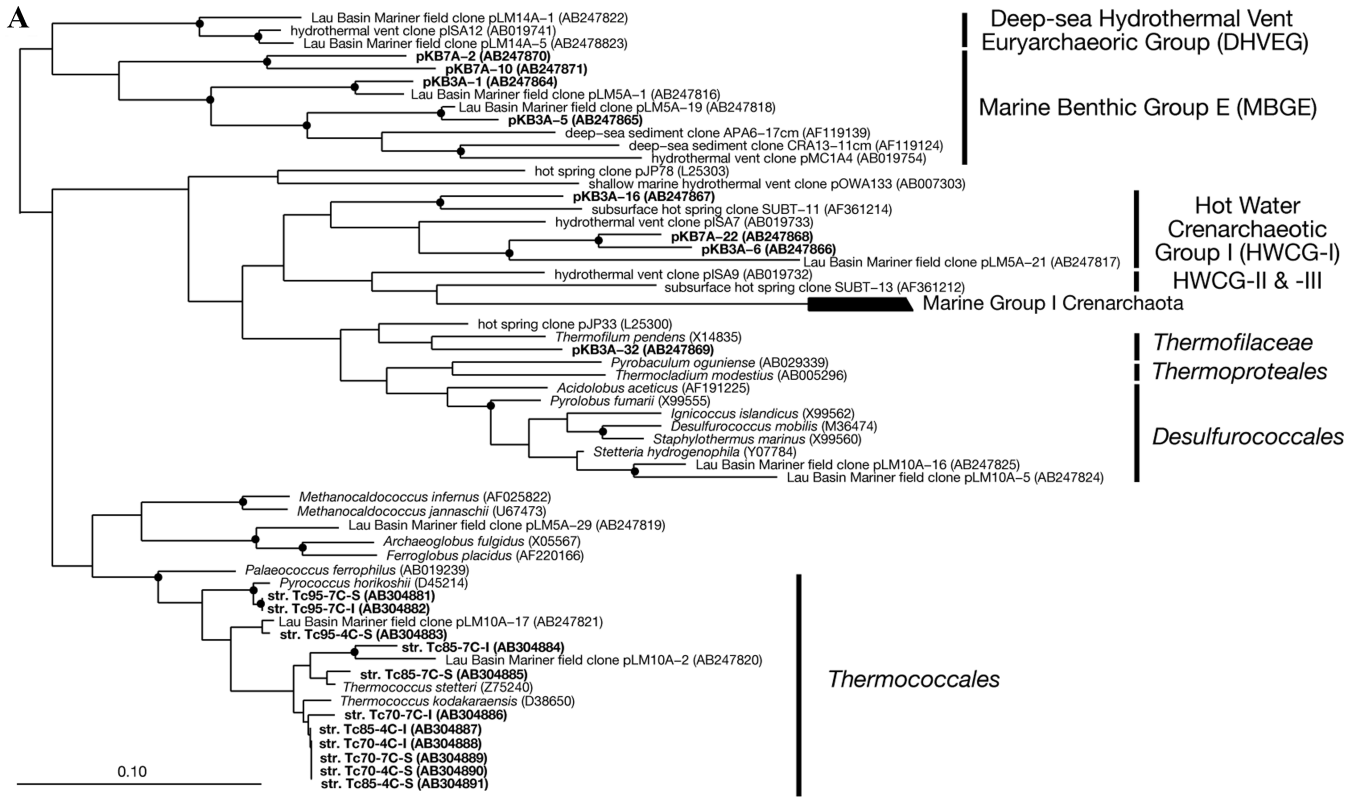

FIG. 2. Phylogenetic trees of representative isolates and clones (phylotypes) within the domain *Archaea* (A) and the domain *Bacteria* (C) inferred from the partial 16S rRNA gene sequences. The trees were constructed from 462 (A) and 762 (B) homologous positions of the rRNA gene sequence by the Neighbor-joining method. The sequences obtained in this study are marked in bold. The numbers in parentheses are DDBJ/EMBL/GenBank accession numbers. The closed circles indicate the branches with more than 50% of bootstrap confidence. The scale bars show 0.1 substitution per nucleotide position.

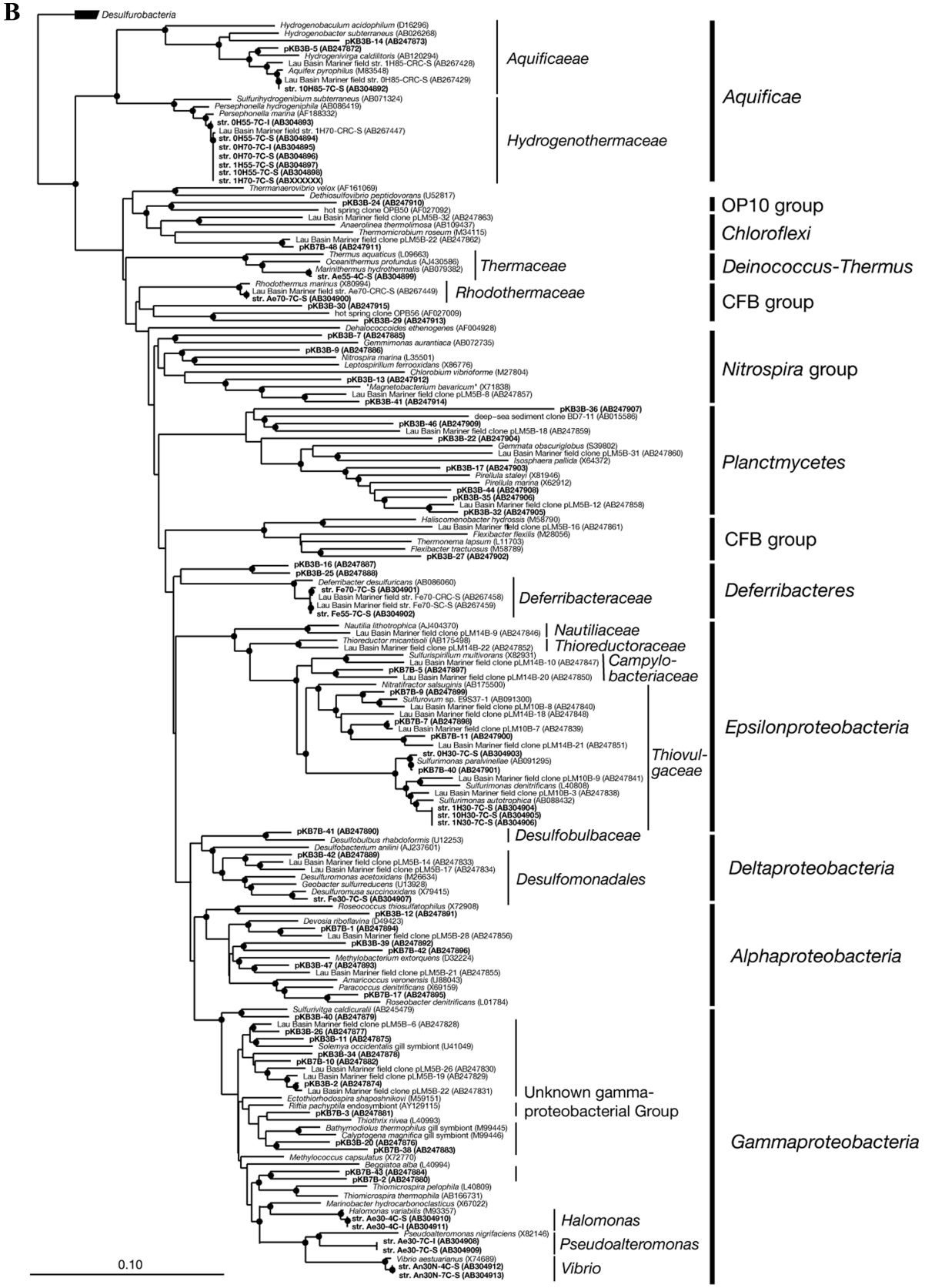

FIG. 2. (*Continued.*)

TABLE 4
Distribution of representative archaeal 16S rRNA gene clones (phylotypes) in the various chimney environments

Phylogenetic affiliation	Representative clone	Accession #	Number of clones from 851-3A inside	851-3A exterior	852-2B inside	852-2B exterior
Crenarchaeota						
Hot Water Crenarchaeotic Group I (HWCG-I)						
	pKB3A-16	AB247867	2	—	—	—
	pKB7A-22	AB247868	—	—	1	—
	pKB3A-6	AB247866	1	—	—	—
Thermoproteales, Thermofilaceae						
	pKB3A-32	AB247869	1	—	—	—
Euryarchaeota						
Marine Benthic Group E (MBGE)						
	pKB7A-2	AB247870	1	—	1	—
	pKB7A-10	AB247871	—	—	3	—
	pKB3A-1	AB247864	23	17	27	24
	pKB3A-5	AB247865	3	7	—	6
TOTAL			31	24	32	30

Fluid chemistry suggests that microbial H_2 consumption possibly occurs more significantly in subseafloor environments associated with Cl-depleted hydrothermal fluids than with Cl-enriched ones. In addition, both culture-independent and dependent microbiological characterization demonstrates the increased abundance of hydrogen- and/or sulfur-oxidizing chemolithoautotrophs such as the *Aquificales*, *Epsilonproteobacteria* and *Gammaproteobacteria* members in the chimney environments associated with Cl-depleted hydrothermal fluid. Our combined application of geochemical and microbiological studies of the hydrothermal fluids and chimneys does not necessarily point to a correlation between the variability in the phase-separation-controlled hydrothermal fluid chemistry and the microbial community structure and activity in both subseafloor and seafloor environments. However, since it is still quite difficult to directly access high temperature subseafloor hydrothermal environments, the linkage between the chemistry of high temperature hydrothermal vent fluids and the proximal

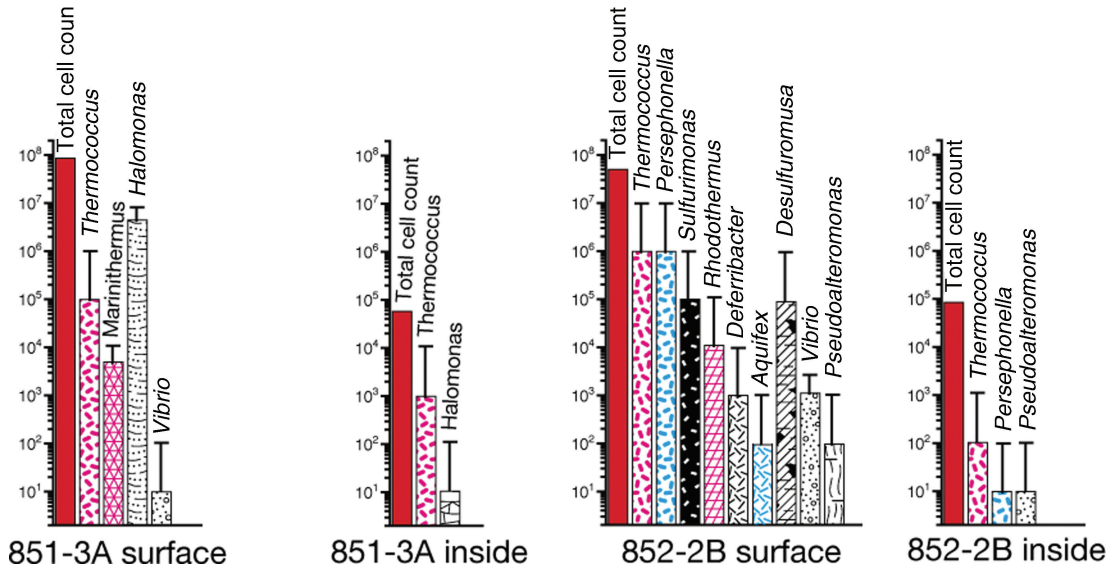

FIG. 3. Total cell count and viable count determined using DAPI staining and serial dilution cultivation in the chimney environments. The phylogenetic grouping of the isolates was delineated from the phylogenetic tree shown in Figure 2.

TABLE 5
Distribution of representative bacterial 16S rRNA gene phylotypes in the various chimney exterior environments.

Phylogenetic affiliation	Representative clone	Number of clones from		
		Accession #	851-3A exterior	852-2B exterior
Aquificae				
Aquificaceae				
	pKB3B-5	AB247872	4	—
	pKB3B-14	AB247873	2	—
OP10 Group				
	pKB3B-24	AB247910	1	—
Nitrospirae				
Nitrospiraceae				
	pKB3B-7	AB247885	1	—
	pKB3B-9	AB247886	1	—
	pKB3B-13	AB247912	1	—
	pKB3B-29	AB247913	1	—
CFB Group				
	pKB3B-27	AB247902	1	—
	pKB3B-29	AB247913	1	—
Chloroflexi				
Anaerolineae				
	pKB7B-48	AB247911	—	1
Planctmycetes				
	pKB3B-36	AB247907	1	—
	pKB3B-46	AB247909	1	—
	pKB3B-22	AB247904	1	—
	pKB3B-17	AB247903	2	—
	pKB3B-44	AB247908	1	—
	pKB3B-35	AB247906	1	—
	pKB3B-32	AB247905	2	—
Deferribacteres				
	pKB3B-16	AB247887	1	—
	pKB3B-25	AB247888	3	—
Epsilonproteobacteria				
Campylobacteraceae, Sulfurospirillum group				
	pKB7B-5	AB247897	—	1
Thiovulgaceae, Sulfurovum group				
	pKB7B-9	AB247899	—	3
	pKB7B-7	AB247898	—	7
	pKB7B-11	AB247900	—	1
Thiovulgaceae, Sulfurimonas group				
	pKB7B-40	AB247901	—	2
Deltaproteobacteria				
Desulfobulbaceae				
	pKB7B-41	AB247890	—	1
Desulfomonadales				
	pKB3B-42	AB247889	1	—
Alphaproteobacteria				
	pKB3B-12	AB247891	1	—
	pKB7B-1	AB247894	—	1
	pKB3B-39	AB247892	1	—

(*Continued on next page*)

TABLE 5
Distribution of representative bacterial 16S rRNA gene phylotypes in the various chimney exterior environments. (*Continued*)

Phylogenetic affiliation	Representative clone	Number of clones from		
		Accession #	851-3A exterior	852-2B exterior
	pKB7B-42	AB247896	—	1
	pKB3B-47	AB247893	1	—
	pKB7B-17	AB247895	—	1
Gammaproteobacteria unknown group				
	pKB3B-40	AB247879	1	—
	pKB3B-26	AB247877	1	—
	pKB3B-11	AB247875	2	1
	pKB3B-34	AB247878	1	—
	pKB7B-10	AB247882	—	8
	pKB3B-2	AB247874	2	2
	pKB7B-3	AB247881	—	4
	pKB3B-20	AB247876	1	—
	pKB7B-38	AB247883	—	2
	pKB7B-43	AB247884	—	1
	pKB7B-2	AB247880	—	1
TOTAL			36	38

microbial communities in the chimneys acts as a good first-order proxy for sub-seafloor biogeochemical interaction.

Interfields Variability

This study has shown variability in the fluid chemistry of the hydrothermal discharge and the microbial community structures and activities in the chimneys of the Brothers NW caldera site. Hydrogen gas concentration in the Cl-depleted hydrothermal fluid is considered to be a geochemical indicator of potential subseafloor microbiological activity associated with phase-separation of the parental hydrothermal fluid. Although the relevance between variability in the microbial communities of the chimneys and of the subsurface environments was not immediately evident in this study, the increased abundance of hydrogen- and/or sulfur-oxidizing chemolithoautotrophs in a chimney environment associated with discharging Cl-depleted fluid may be analogus to possible H_2 (and H_2S) consumption by subseafloor microbial communities.

Intrafield variability in the active microbial community potentially associated with phase-separation-induced chemical variation has been demonstrated for the Iheya North field in the Okinawa Trough (Nakagawa et al. 2005) and for the Mariner field in the Lau Basin (Takai et al. 2008). In an inter-fields comparison of various physical, chemical and microbiological characteristics (Table 6), the composition and abundance of the cultivated microbial populations in the chimney structures from the Brothers volcano NW caldera field are more closely related to those in chimney structures from the Mariner field than those from the Iheya North field. It is also notable that many physical and chemical features of the high temperature hydrothermal fluids of the Brothers NW caldera and the Mariner fields are relatively similar (Table 6).

The abundance of *Aquificales* (*Aquifex* and/or *Persephonella*) populations in chimneys hosting Cl-depleted hydrothermal fluids is a common feature in both hydrothermal systems of the Southwestern Pacific and the Iheya North field of the Okinawa Trough (Table 6). However, detailed comparison of viable counts of *Aquificales* species under different cultivation conditions clearly shows the uniqueness of the microbial communities of the Brothers NW caldera and the Mariner fields (Table 6). A considerable proportion of the *Aquifex* and *Persephonella* populations in the both fields is resistant to a higher partial pressure of O_2 in the gas phase (10%; Table 6). Members of *Aquifex* and *Persephonella* have been known as microaerophiles sensitive to $>10\%$ of partial pressure of O_2 (Huber et al. 1992; Götz et al. 2002; Nakagawa et al. 2003) and have rarely been cultivated under a condition of 10% partial pressure of O_2. A population of these *Aquificales* members resistant to high O_2 partial pressures may be linked with some physical and chemical features in the chimney structures, or to the hydrothermal fluids of each field. Further investigation of geographically and geologically distinct hydrothermal systems and their inter-field comparison will shed light on the previously unresolved linkage between geological, physical, chemical and microbiological settings of deepsea hydrothermal systems.

TABLE 6
Inter-fields comparison of cultivated microbial populations in chimney structures highlighting various physical and chemical characteristics of the hydrothermal fluids

	Kermadec arc Brothers volcano NW caldera field Chimney 852-2B	Kermadec arc Brothers volcano NW NW caldera field Chimney 851-3A	Lau Basin Mariner field Crab restaurant chimney	Lau Basin Mariner field Monk chimney	Central Indian Ridge Kairei field Fugen chimney	Mid Okinawa Trough Iheya North field North big chimney	Mariana arc TOTO caldera Sulfur chimney
Geological setting	Intraoceanic arc	Intraoceanic arc	Barckarc	Barckarc	Mid Ocean Ridge	Backarc	Intraoceanic arc
Water depth (m)	~ 1650	~ 1670	~ 1900	~ 1900	~ 2400	~ 1000	~ 2900
Max. measured fluid temperature (°C)	290	274	279	288	360	311	170
Measured fluid pH	2.8	3	2.66	2.39	3.4	4.83	5.3
Evidence seen for boiling at the seafloor	No	No	Yes	Yes	No	Yes	No
Input of magmatic volatiles	Yes	Yes	Probably	Probably	Possibly	Possibly	Yes
Concentration in end-member fluid:							
H_2S (mM)	7.9	2.3	9.7	6.9	4	4	14.6†
H_2 (μM)	16.8	14.3	43.7	48.2	2500	~ 200*	~ 10*,†
CH_4 (μM)	6.8	2.2	7.8	7	199	~ 70000*	2†
CO_2 (mM)	35.5	17.8	69.6	36.4	8	~ 70*	15.3†
Cl (mM)	502	732	531	597	642	511	502-505†
Presence of pyrrotite in chimney	No	No	No	No	Yes*	Yes*	No
Viable counts in surface:							
Thermococcus (cells/g)	10^6 - 10^7	10^5 - 10^6	10^6 - 10^7	10^5 - 10^6	2×10^5 –6×10^6	3.5×10^7	10^6–10^7 (ISCS)
Methanococcales (cells/g)	< 10	< 10	< 10	<10	1.5×10^2–2.5×10^3	6.5×10^2	<10
Aquifex under 1% O_2 (cells/g)	< 10	< 10	10^6–10^7	10^2–10^3	<10	3.0×10^4	<10</TB>
Aquifex under 10% O_2 (cells/g)	10^2–10^3	< 10	10^2–10^3	10^2–10^3	<10	<10	<10
Persephonella under 1% O_2 (cells/g)	10^6–10^7	< 10	10^6–10^7	10^2–10^3	7–2×10^2	1.2×10^7	<10
Persephonella under 10% O_2 (cells/g)	10^5–10^6	< 10	<10	<10	<10	<10	<10
Thermophilic *Epsilonproteobacteria* (cells/g)	<10	<10	<10	<10	4–1.5×10^2	1.5×10^6	3.0×10^2–3.0×10^3 (ISCS)
Mesophilic *Epsilonproteobacteria* (cells/g)	10^5–10^6	<10	10^5–10^6	10^5–10^6	4–1.5×10^2	3.9×10^6	3.0×10^3–3.0×10^4 (ISCS)
References	This study	This study	Takai et al., 2008	Takai et al., 2008	Takai et al., 2004; Gamo et al., 2002	Nakagawa et al., 2005	Nakagawa et al., 2006; Gamo et al., 2004

*unpublished data.
†these are the measured values.

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