Intra-Field Variation of Prokaryotic Communities On and Below the Seafloor in the Back-Arc Hydrothermal System of the Southern Mariana Trough

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Abstract
Deep-sea hydrothermal vents harbor diverse prokaryotes. There are a variety of habitat types in a deep-sea hydrothermal field, e.g., active and inactive chimneys, iron-rich mats, venting fluid and hydrothermal plume. Numerous studies have shown the diversity and composition of prokaryotic communities in individual habitats. However, it is still unclear whether and how the characteristics of prokaryotic communities in their respective habitats are different. Previously, we reported 16S rRNA genes in a variety of habitats, i.e., hydrothermally active and inactive chimneys, iron-rich mats, a vent fluid, crustal fluids from boreholes, as well as ambient seawater in a back-arc basin hydrothermal field of the Southern Mariana Trough. Here we summarize the prokaryotic communities in the collected samples at higher taxonomic resolution (up to family level) using the detected 16S rRNA gene sequences and compare them using recently developed bioinformatics tools. The comparative analysis clearly highlights differences in prokaryotic communities among the habitat types on and below the seafloor in the Southern Mariana Trough. Furthermore, descriptions of cultured species and environmental clones close to the detected sequences provide valuable information for understanding of their distribution and potential of ecological roles in deep-sea hydrothermal fields.

Keywords
16S rRNA gene • Archaea • Back-arc basin • Bacteria • Chemosynthetic ecosystem • Deep-sea hydrothermal vents • Prokaryotic community

24.1 Introduction
Deep-sea hydrothermal vents are oases for organisms on the vast seafloor. Hydrothermal fluids are rich in reduced chemical species, such as hydrogen, hydrogen sulfide, methane and ferrous iron, which support the growth of chemolithoautotrophs as energy sources. Chemolithoautotrophs are the primary producers in the chemosynthetic ecosystem in TAIGA, which is designated a great sub-seafloor river (Urabe et al. Chap. 1). Numerous studies have characterized the prokaryotic community structures in various habitat types, such as hydrothermally active chimneys (e.g., Flores et al. 2012a; Kato et al. 2010; Kormas et al. 2006; Nakagawa et al. 2005; Schrenk et al. 2003; Takai and Horikoshi 1999), inactive chimneys (Kato et al. 2010; Suzuki et al. 2004;...
24.2 Re-Analysis of 16S rRNA Gene Sequences

The 16S rRNA gene sequences were recovered from iron-rich mats (YS16 and YS18) (Kato et al. 2009a), active (AFhm, APcsc, APbsc, AAc5) and inactive chimneys (IPtc, IYdc, Inoc) (Kato et al. 2010; Kato et al. 2009a), and crustal fluids (Cowen et al. 2003; Huber et al. 2006; Kato et al. 2009b, 2013; Orcutt et al. 2011), in various deep-sea hydrothermal fields. Through such studies, inter-field variation between prokaryotic community structures in deep-sea hydrothermal system habitats has been revealed (e.g., Takai et al. 2006; Flores et al. 2012a). However, intra-field variation of prokaryotic community structures among these habitat types is still poorly understood.

The Southern Mariana Trough (SMT) is an actively spreading back-arc basin that is located at the southern extension of the Izu-Bonin Arc (Seama et al. Chap. 17). Hydrothermal vents have been found on the back-arc spreading ridge and off-ridge seamounts in the SMT. Furthermore, in this field, several boreholes have been drilled by a seafloor-drilling instrument, the Benthic Multi-coring System (BMS) (Marumo et al. 2008). The tectonics and geochemistry of the SMT and geochemistry of its hydrothermal fluids have been well studied (Seama et al. Chap. 17; Toki et al. Chap. 45). Therefore, the SMT is a model field for studying the relationships among tectonics, geology, geochemistry, and microbial ecology in many habitats including sub-seafloor environments.

We analyzed the prokaryotic community structures in a variety of samples collected in the SMT, such as active and inactive chimneys, iron-rich mats, hydrothermal fluid from an active vent and boreholes, and ambient seawater (Kato et al. 2009a, b, 2010). In particular, the chemosynthetic ecosystem supported by ferrous iron was found in the iron-rich mats and crustal fluids (Kato et al. 2012). However, comprehensive comparative analyses of the prokaryotic community structures among the SMT samples have not been carried out. In this review, we summarize the prokaryotic community structures in the SMT samples at higher taxonomic resolution (up to family level) based on the 16S rRNA gene sequences and compare them using recently developed bioinformatics tools. The results highlight the differences of prokaryotic communities on and below the seafloor in the SMT.

24.3 Bacterial Community Structures

Bacterial community structures in the SMT samples are summarized in Fig. 24.1. The relative abundance of each taxon in the libraries at the phylum level is shown in Fig. 24.1a. For Proteobacteria, the relative abundance in the class level (i.e., Alpha-, Beta-, Gamma-, Delta-, Epsilon- and Zetaproteobacteria) is shown in Fig. 24.1b. For each proteobacterial class except Beta- and Zetaproteobacteria,
Fig. 24.1  Bacterial community structures based on the 16S rRNA gene clone libraries. The detection frequency of each taxon for (a) Bacteria, (b) Proteobacteria, (c) Alphaproteobacteria, (d) Gammaproteobacteria, (e) Deltaproteobacteria, (f) Epsilonproteobacteria, (g) Bacteroidetes, and (h) Uncultured clone groups are shown. (a) “Thermophilic phyla” include Aquificae, Caldithrix, Deinococcus-Thermus, Thermodesulfobacteria and Thermotogae, and “Other phyla” include Chlamydiae, Chlorobi, Acidobacteria, Cyanobacteria, Firmicutes, Fusobacteria, Gemmatimonadota, Spirochaetes and Verrucomicrobia. Colors of the sample ID indicate the sample types: red, active chimney; purple, inactive chimney; yellow, iron-rich mat; orange, natural vent fluid; green, crustal fluid; sky-blue, ambient seawater. See also Suppl. Table 24.1 for the sample description.
Zetaproteobacteria were detected in the two iron-rich mat samples (YS16 and YS18), three crustal fluid samples (Papm3, Fapm1a and Fapm1b) and one inactive chimney sample (Inoc) (Fig. 24.1b). The relative abundance was up to 50 % of the total number of clones. Davis and Moyer (2008) also detected Zetaproteobacteria in an iron-rich mat sample from the Snail site. McAllister et al. (2011) have shown that most of the zetaproteobacterial clones detected in the crustal fluids are not clustered with the other clones detected in the other samples, suggesting the presence of endemic zetaproteobacterial members in the crustal fluids in the SMT (Kato et al. 2009a, b). The sole cultured species in Zetaproteobacteria was *Mariprofundus ferrooxydans*, a neutrophilic iron-oxidizing chemolithoautotroph (Emerson et al. 2007). Given that all or most of the Zetaproteobacteria are iron-oxidizers (Emerson et al. 2010; Fleming et al. 2013), any microbial ecosystem dominated by Zetaproteobacteria is supported by ferrous iron as the energy source (i.e., “iron-based ecosystem”). This is consistent with the thermodynamic calculation of bioavailable energy yields based on geochemical characteristics of the environments: the energy yield obtained from iron oxidation is comparable to or higher than that from oxidation of other reduced chemical species, such as hydrogen, hydrogen sulfide and methane (Kato et al. 2012).

Remarkably, iron-based ecosystems were found at both the Snail and Pika sites, even though their geological settings (on and off-ridge) are different (Seama et al. Chap. 17). Iron-based ecosystems also occur in deep-sea hydrothermal fields with other geological characteristics, such as the Loihi Seamount of Hawaii (Fleming et al. 2013), Tonga–Kermadec Arc (Forget et al. 2010; Hodges and Olson 2009), Lau basin (Li et al. 2012), Juan de Fuca Ridge (Davis et al. 2009) and Mohns Ridge (Övreás et al. 2007). Thus, the development of iron-based ecosystems seems to be independent of these geological differences. Kato et al. (2012) have suggested that higher concentration of ferrous iron than hydrogen sulfide, which can supply more energy for the growth of iron-oxidizers than sulfide-oxidizers, is needed for the development of iron-based ecosystems. In addition, the continuous supply of ferrous iron, the chemical conditions that make ferrous iron dissolve stably (i.e., low pH, low Eh and low concentration of sulfide), and the temperature at which the iron-oxidizers can grow may be also important for the development of iron-based ecosystems. Iron-based ecosystems are likely to be widely distributed in environments that fulfill the above conditions on and below the seafloor.

### Table 24.1
Summary of dominant bacterial and archaeal taxa for each sample type

| Sample type       | Temperature (°C) | pH<sup>a</sup> | Abundant bacterial taxa<sup>b</sup> | Abundant archaeal taxa<sup>b</sup> | Inorganic energy sources<sup>c</sup> |
|-------------------|-----------------|----------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Active chimney     | 19–270          | 2.9–6.4        | *Aqueiic, Thiovalgaceae* (α), *Campylobacteraceae* (α), *Desulfobulbiae* (δ), *Xanthomonaales* (γ), *Flavobacteriales* (Bact) | *Thermococci, Archaeoglobi*, *Thermoprotei*, *Acdulipodunfudae*, *THSCG*, *Korarchaeota* | H₂, H₂S                           |
| Inactive chimney   | 2–3             | 7.6–8.1        | *Chromatiales* (γ), VC21_Bac22 (Bact) | MGI, DHVEG-6                     | FeS₂, NH₄                         |
| Iron-rich mat      | 33–116          | 5.8–6.7        | *Zetaproteobacteria, SAR406*       | MCG, MBGE                        | Fe²⁺                             |
| Crustal fluid      | 6–40            | 6.4–7.3        | *Zetaproteobacteria, Piscirickettsiaceae* (γ), *Oceanospirillaceae* (γ), *Alteromonadales* (γ), *Pseudomonadales* (γ) | MCG, MGI                         | Fe²⁺, H₂S, NH₄                    |
| Natural vent fluid | 63–69           | 6.2–6.3        | *Alteromonadales* (γ), OP1         | MCG, MGBE, MGI                   | H₂                               |
| Ambient seawater   | 2–3             | 7.6–8.1        | *Pelagibacteraceae* (α), SUP05 (γ) | MGI                              | H₂S, NH₄                         |

<sup>a</sup>Temperature and pH of the venting fluids for active chimneys and iron mat and those of the seawater for inactive chimneys are shown. Data from Toki et al. (Chap. 45).

<sup>b</sup>Abundant taxa in the libraries (>20 % of the total number of clones) are shown. Greek letters in parentheses indicate proteobacterial classes. "Bact" in parentheses indicates the phylum *Bacteroidetes*.

<sup>c</sup>Inferred from the physiological characteristics of the cultured species in the taxa.
24.3.2 Alphaproteobacteria

The relative abundance of each family level taxon of Alphaproteobacteria differed among the sample types (Fig. 24.1c). Hyphomicrobiaceae were relatively abundant in some solid samples, i.e., active and inactive chimneys and iron-rich mats (Fig. 24.1c). Most of the clones in the Hyphomicrobiaceae were not close to the cultured species (<92 % of 16S rRNA gene similarity) and clustered with environmental clones detected in deep-sea basaltic rocks and sulfide chimneys (Kato et al. 2013; Santelli et al. 2008). Rhodospirillaceae were relatively abundant in the iron-rich mat and inactive chimney samples, but not detected in the active chimney samples (Fig. 24.1c). One of the closest cultured species for the clones in the Rhodospirillaceae was a magnetotactic bacterium Magnetovibrio blakemorei with 92 % similarity (Bazylinski et al. 2013). The physiology of the uncultured bacteria in Hyphomicrobiaceae and Rhodospirillaceae was unclear; however, considering the sample types (i.e., sulfide chimneys, iron-rich mats and rocks on the seafloor) where they were detected, their presence was probably related to the presence of iron and/or sulfide minerals (such as goethite, pyrite, chalcopyrite and sphalerite (Ikehata et al. Chap. 22; Kakegawa et al. 2008; Masuda and Fryer, Chap. 21)).

In contrast, Sphingomonadaceae were relatively abundant in two crustal fluid samples (Fig. 24.1c). The detected clones of Sphingomonas were close to Sphingomonas species (>97 % similarity) including oligotrophs, which are widely distributed in nature. In the crustal aquifer, they can survive with low concentrations of nutrients.

24.3.3 Gammaproteobacteria

Piscirickettsiaceae were relatively abundant in crustal fluid samples (Fig. 24.1d). This family contains chemolithoautotrophic sulfur-oxidizing bacteria such as Thiomicrospira spp. and Thioalkalimicrobium spp. The detected clones in the Piscirickettsiaceae were closest to Thiomicrospira spp. with up to 96 % similarity. In contrast, Chromatiales were relatively abundant in active and inactive chimney samples (Fig. 24.1d). The closest cultured species to the clones in Chromatiales were Thiohalomonas spp. or Thioalkalispira spp. (up to 94 % similarity), which are chemolithoautotrophic sulfur-oxidizing bacteria. Members in Chromatiales are facultative anaerobes, which can grow using nitrate as an electron acceptor. In contrast, Thiomicrospira spp. within the Piscirickettsiaceae are strict aerobes. Therefore, the availability of electron acceptors is likely to lead to differences in the relative abundance of the two taxa of sulfur-oxidizing bacteria.

Alteromonadales were relatively abundant in crustal fluid samples and the natural vent fluid sample (Fig. 24.1d). The detected clones in the Alteromonadales were close to Alteromonas spp., Pseudoalteromonas spp., Marinobacter spp., etc. (up to 99 % similarity). These cultured species are mainly chemooorganoheterotrophs. These species may thrive in warm crustal fluids (<40 °C) under the seafloor using organic carbon produced by chemolithoautotrophs. The gammaproteobacterial population of the F2apm1 sample differed from those of the other crustal fluid samples (Fig. 24.1d).

24.3.4 Deltaproteobacteria

Deltaproteobacteria accounted for over 10 % of the total number of clones in 3 out of 4 active chimney samples (Fig. 24.1b, e). Most of the deltaproteobacterial clones from the active chimney samples were affiliated with the Desulfobulbaceae, of which members grow depending on the reduction of oxidized sulfur species (such as sulfate and thiosulfate) or sulfur disproportionation. The closest cultured species to these clones are Desulfobulbus spp., Desulfocapsa spp., etc. (92–97 % similarity). Desulfobulbaceae have been also detected in active chimneys in other hydrothermal fields, such as the hydrothermal fields in the Okinawa Trough, Suiyo Seamount and 9°N EPR (Kato et al. 2013; Kormas et al. 2006; Nakagawa et al. 2004). In contrast, Desulfobulbaceae were rarely detected in the other SMT samples and in inactive chimneys in other hydrothermal fields, such as the hydrothermal fields in the Okinawa Trough, Central Indian Ridge (CIR) and 9°N EPR (Suzuki et al. 2004; Sylvan et al. 2012). These facts suggest that the detected members in Desulfobulbaceae prefer conditions in the active chimneys.

In contrast, Nitrospinaceae were detected in all of the inactive chimneys and iron-rich mat samples (Fig. 24.1e). A nitrite-oxidizing bacterium, Nitrospinae gracilis is the sole cultured species in Nitrospinaceae (Watson and Waterbury 1971). It should be noted that a novel phylum, Nitrospinae has been proposed on the basis of a detailed phylogenetic analysis of the genome sequence of N. gracilis (Lücke et al. 2013). The detected members in the Nitrospinaceae may use nitrite as an electron donor. Nitrate reducers, e.g., members in Chromatiales, can produce nitrite. Remarkably, both Nitrospinaceae and Chromatiales were relatively abundant in the inactive chimney samples, suggesting that redox cycling of nitrogen species is potentially driven by the combination of nitrite oxidizers in the Nitrospinaceae and nitrate reducers in the Chromatiales.
24.3.5 Epsilonproteobacteria

Epsilonproteobacteria accounted for over 15% of the total number of clones in all the active chimney samples (Fig. 24.1b, f). They are likely to play a significant role as primary producers in the ecosystem within the active chimneys in the SMT. This class contains chemolithoautotrophs using hydrogen and/or reduced sulfur species as electron donors (Campbell et al. 2006). The high proportions of Epsilonproteobacteria and Desulfobulbaceae in the Deltaproteobacteria in the clone libraries from the active chimney samples suggest that they contribute to the redox cycling of sulfur.

Epsilonproteobacteria were also detected in some crustal fluid samples (Fig. 24.1b, f). Hydrogen sulfide was detected in the crustal fluids (~5 μM; Toki et al. Chap. 45). These results suggest that, in addition to iron, the reduced sulfur species is used as an energy source for prokaryotic communities in the crustal fluids below the seafloor. In contrast, Epsilonproteobacteria were rarely detected in the iron-rich mat samples, suggesting that hydrogen and reduced sulfur species are not available in these habitats. Instead, iron may be the major energy source for prokaryotic communities in the iron-rich mats as mentioned above.

Epsilonproteobacteria were detected but only in low numbers in two inactive chimney samples, even though reduced sulfur species were rich in the chimneys. This suggests that the detected Epsilonproteobacteria cannot use the reduced sulfur species in the inactive chimneys surveyed. Alternatively, conditions, such as temperature, pH and Eh, in the inactive chimneys might not be suitable for Epsilonproteobacteria. In fact, autotrophs within the Epsilonproteobacteria use the reductive tricarboxylic acid cycle for carbon fixation, and this cycle appears to be the dominant pathway in habitats characterized by warm (over 20 °C) and anaerobic (or microaerobic) conditions (Nakagawa and Takai 2008; Hügler and Sievert 2010).

24.3.6 Bacteroidetes

Bacteroidetes were relatively abundant in the clone libraries from the active and inactive chimney samples (Fig. 24.1a, g). In particular, most of the clones detected in one inactive chimney (IYdc) were classified as belonging to the VC21_Bac22 group. This group was also detected in some of the other active and inactive chimney samples (Fig. 24.1g). The clones in this group are distant from the closest cultured species (<90% similarity). The group was found to be relatively abundant in some inactive chimneys at other hydrothermal fields (Suzuki et al. 2004; Sylvan et al. 2012). Furthermore, this group was also relatively abundant in the clone libraries from massive sub-seafloor sulfide deposits collected in the SMT using BMS (S. Kato, in preparation). These results imply that the appearance of this group is related to the presence of sulfide minerals.

24.3.7 Uncultured Bacterial Clone Groups

The uncultured clone groups detected in the SMT samples were affiliated with GN02, GN04, KSB1, NPL-UPA2, OD1, OP1, OP3, PAUC34f, SAR406, SBR1093, TM6, TM7 and WS3. Metagenomic analyses have shown the metabolic potential of some groups, i.e., OD1 (Wrighton et al. 2012), OP1 (Takami et al. 2012), TM6 (McLean et al. 2013) and TM7 (Marcy et al. 2007). Furthermore, a recent study has reported the near-complete genomes of GN02, OP3, SAR406 and WS3, in addition to OD1 and OP1, using single-cell genomic analysis; however, details of metabolic potential have not been described yet (Rinke et al. 2013). Further cultivation or metagenomic analysis is needed to elucidate the metabolic function of the uncultured groups detected in the SMT.

24.4 Archaeal Community Structures

Diverse archaeal 16S rRNA gene sequences were detected in the SMT samples (Fig. 24.2 and Suppl. 24.2). In addition to cultured thermophilic Euryarchaeota and Crenarchaeota that are typical in deep-sea hydrothermal vents, the following groups were detected: Marine benthic groups A and E (MBGA and MBGE), Marine groups I, II and III (MGI, MGII and MGIII), Marine hydrothermal vent groups (MHVG and MHVG-1), Deep-sea euryarchaeotic group (DSEG), Deep-sea hydrothermal vent euryarchaeotic group (DHVEG) and Terrestrial hot spring crenarchaeotic group (THSCG). The dominant taxonomic groups for each sample type are shown in Table 24.1.

24.4.1 Thermophilic Archaea and Related Groups

Clones closely related to cultured thermophiles in the Euryarchaeota and Crenarchaeota, such as members in Thermococci, Archaeoglobi and Thermoprotei, were detected only in the active chimney samples (Fig. 24.2a). These cultured archaea include hydrogen-oxidizers, S0-reducers, sulfate-reducers and fermenters. In addition, Korarchaeota were detected only in one active chimney sample (AFhm). Whole genome analyses have revealed that the Korarchaeota contains peptide fermenters (Elkins et al. 2008). THSCG was also detected in all of the active chimney samples (Fig. 24.2a). A metagenomic analysis has
suggested that THSCG contains a hydrogen- or CO-utilizing acetogen (Nunoura et al. 2011). These thermophilic archaea have been widely detected in active chimneys within various hydrothermal fields (Takai et al. 2006).

### 24.4.2 Thaumarchaeota and Related Groups

*Thaumarchaeota* were detected in most of the samples (Fig. 24.2a). All of the clones of *Thaumarchaeota* detected in the ambient seawater, the iron-rich mat and the natural vent fluid samples were closely related to *Nitrosopumilus maritimus*, an ammonia-oxidizer belonging to MGI (Könneke et al. 2005). In contrast, the clones that were not affiliated with MGI but placed in deeper positions within the *Thaumarchaeota* (AK31 and D-F10 clusters; Suppl. 24.2) were detected in one active chimney (AFhm) and one crustal fluid samples (Papm3) (Fig. 24.2b). These clones are relatively close to the ammonia-oxidizers, e.g., *Nitrososphaera* spp. (Tourna et al. 2011) or *Candidatus* “Nitrosocaldus yellowstonii” (De La Torre et al. 2008) (Suppl. 24.2). Furthermore, MBGA were detected in the two iron-rich mat samples and one crustal fluid sample (Papm3) (Fig. 24.2a). This group is related to the pSL12 clade that may contain ammonia oxidizers (Mincer et al. 2007). These putative ammonia-oxidizing archaea potentially play a role as primary producers in prokaryotic ecosystems in the SMT, which is consistent with the high bioavailable energy yields obtained from ammonia oxidation (Kato et al. 2012).

### 24.4.3 Thermoplasmata

*Thermoplasmata* accounted for over 15% of the total number of clones in one active sulfide (APcsc) and two crustal fluid samples (Papm3 and F2apm) (Fig. 24.2a, c). Although *Thermoplasmata* contains methanogens (Dridi et al. 2012; Iino et al. 2013), the detected clones were distant from the methanogens (Suppl. 24.2).

DHVEG-2 was detected in two of the active chimney samples (AFhm and APcsc; Fig. 24.2c). DHVEG-2 is an ubiquitous group in deep-sea hydrothermal vents and contains a thermoacidophilic sulfur- and iron-reducing chemooorganoheterotroph, *Aciduliprofundum boonei* (Flores et al. 2012b; Reysenbach et al. 2006). The detected members in DHVEG-2 are probably thermophiles as well as *Archaeoglobi*, *Thermoprotei* and THSCG.

All of the *Thermoplasmata* clones detected in two crustal fluid samples (Papm3 and F2apm1) were affiliated with MGIII or the WCHD3-02 cluster, respectively (Fig. 24.2c). Considering the temperature of the habitats where these clones in MGIII and the WCHD3-02 cluster were detected, they may not be thermophiles.

### 24.4.4 Other Archaeal Clone Groups

MBGE accounted for 20% or higher of the total number of clones in the two iron-rich mats, the natural vent and one of the crustal fluid samples (Papm3), and it was also detected in two active chimney samples (AFhm and APbsc) and another crustal fluid sample (Fapm1a) (Fig. 24.2a). MBGE has been detected in deep-sea sediments including marine hydrothermal fields (e.g., (Nercessian et al. 2005; Vetriani et al. 1999)). MBGE was not detected in the inactive chimney samples of...
the SMT, although they dominated in the libraries from inactive chimneys collected from some hydrothermal fields in the Okinawa Trough and CIR (Suzuki et al. 2004).

MCG was detected in the iron-rich mat, the natural vent fluid and the crustal fluid samples except Fapm1b (Fig. 24.2a). The relative abundance was high (11.2–80.8 %) in each clone library. MCG is abundant and widely distributed in marine sediments (Kubo et al. 2012). Single-cell genome-sequencing analyses suggest that MCG includes an anaerobic protein-degrader (Lloyd et al. 2013). However, the detected MCG clones show up to 83 % similarity with the 16S rRNA gene in the MCG genome. Further cultivation or metagenomic analysis is needed to know the ecophysiology of the MCG in the SMT.

DHVEG-6 dominated in the clone library from one inactive chimney sample (IYdc) and was also detected in two active chimney samples (AFhm and APcsc) (Fig. 24.2a). DHVEG-6 has been detected in various environments including acid mines and marine hydrothermal fields (e.g., Baker et al. 2010; Takai and Horikoshi 1999). The metagenome analyses of Candidatus Parvarchaeum spp. in DHVEG-6 suggest that they are aerobic host-associated/symbiotic archaea interacting with members of cell wall-less Thermoplasmatales (Baker et al. 2010). However, clones related to the cell wall-less Thermoplasmatales spp. were not detected in the SMT samples.

### 24.5 Comparison of Community Structures

The PCoA shows that bacterial community structures are similar within the same sample type, except F2apm1 (Fig. 24.3a). This result is generally consistent with the detection pattern of each taxon (Fig. 24.1), especially of each dominant taxon (Table 24.1). In other words, the bacterial community structures are different among the sample types, i.e., habitat types. Epsilonproteobacteria (putative hydrogen-oxidizers and sulfide-oxidizers) were dominant in the libraries from active chimneys, Zetaproteobacteria (putative iron-oxidizers) were dominant in those from iron-rich mats, and both Zetaproteobacteria and Epsilonproteobacteria were mainly detected in crustal fluids (Fig. 24.1, Table 24.1). Members of the Zetaproteobacteria and Epsilonproteobacteria may use H$_2$S or Fe$^{2+}$ dissolved in the venting hydrothermal fluids. Our previous study has shown that the diversity in bacterial community structures is consistent with the thermodynamic calculation of bioavailable energy yields from oxidation of reduced chemical species, such as hydrogen, hydrogen sulfide and iron, for each habitat (Kato et al. 2012).

In addition, the bacterial community structures clearly differed between active and inactive chimney samples (Fig. 24.3a). Epsilonproteobacteria and thermophilic bacteria (e.g., Aquificae) were rarely detected in the inactive chimneys; Chromatiales in Gammaproteobacteria (putative sulfide-oxidizers) was relatively abundant (Fig. 24.1, Table 24.1). The members in Chromatiales potentially use sulfide minerals such as pyrites, which are the main component of the chimneys (Kakegawa et al. 2008; Ikehata et al., Chap. 22), as their energy sources, even if the supply of hydrothermal fluids ceases. The difference between active and inactive chimney samples may reflect the temporal change before and after the cease of hydrothermal activity, i.e., temperature, pH and Eh, in addition to availability of...
reduced chemical species (Kato et al. 2010), which is consistent with the previous study of 9°N EPR (Sylvan et al. 2012).

In contrast to the bacterial community structures, the archaeal community structures seem not to be clearly distinguished by the sample types, especially for inactive chimneys, some borehole fluids and ambient seawater samples (Fig. 24.3b). Thermophilic archaea (e.g., Thermococcus and Archaeoglobi) were dominant in the libraries from the active chimney samples (Fig. 24.2, Table 24.1). The detection of thermophilic archaea is consistent with the high temperature of venting fluids from the active chimneys. In contrast, they were rarely detected in the iron-rich mat and crustal fluid samples; MBGE and MCG are mainly detected there (Fig. 24.2, Table 24.1). In addition to temperature, the difference in pH, Eh and availability of reduced chemical species may cause the observed difference in the archaeal communities between the active chimneys and the others, as per the case of the bacterial communities.

24.6 Concluding Remarks

These comprehensive comparative analyses show the differences in the prokaryotic community structures among the habitat types on and below the seafloor in the SMT. These differences are likely to be associated with the relative abundance of putative chemolithoautotrophs. The geological difference between the on- and off-ridge hydrothermal sites might not constrain the community structures. Detailed phylogenetic analysis suggests the presence of endemic taxa for each habitat type. The detected prokaryotes are likely to play a significant role in the cycling of carbon, nitrogen, sulfur and/or iron in these habitats. Our results indicate that the prokaryotic community structure in sulfide chimneys dramatically changes before and after cessation of hydrothermal venting. In addition, diverse yet-uncultured bacteria and archaea were detected in the SMT hydrothermal fields. However, DNA-based molecular analyses targeting 16S rRNA genes cannot provide direct evidence for physiology, activity or productivity of prokaryotes. Further studies using RNA-based analyses, in situ incubation experiments, isolation and cultivation are needed to understand prokaryotic ecology within the hydrothermal fields.

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