MICROBIAL ECOSYSTEM IN MARINE HYDROTHERMAL SYSTEM IN THE EARLY AND PRESENT EARTH

Shingo Kato[1] and Akihiko Yamagishi[1]
[1] Department of Molecular Biology, Tokyo University of Pharmacy and Life Science, 1432-1
Horinouchi, Hachioji, Tokyo 192-0392, Japan
Fax: +81-426-76-7145
yamagish@toyaku.ac.jp

(Received September 14, 2010; Accepted October 28, 2010)

(Abstract)

Marine hydrothermal fields are thought to be an environment where microorganisms thrived in early Earth. However, the physiological characteristics of these microorganisms are unknown. The study of microorganisms in the present hydrothermal fields will provide clues to elucidate the ancient ecosystem. For a better understanding of the ancient ecosystem, it is important to know the relationship between geological, geochemical and microbiological diversities of the present marine hydrothermal systems. Microbiological breakthroughs for both culture-dependent and -independent methods are needed to reveal the physiology of the microorganisms living in the present marine hydrothermal fields.

(Keywords)
Ancient life, marine hydrothermal system, hyperthermophile, Archaea

1. Introduction

The ancient ecosystem on early Earth is one of the most attractive topics for not only scientists but also the general public. The present ecosystem is mostly fueled by solar energy, and thus is called a photosynthetic ecosystem. In this ecosystem, plants and cyanobacteria play the roles of primary producers that fix inorganic carbon and transform it into organic carbon using solar energy. These organic carbon compounds sustain the growth of various organisms by providing energy and carbon sources. In contrast, in the early Earth before the evolution of photosynthesis, the ancient ecosystem was probably sustained by energy supplied from compounds produced from the Earth. Microorganisms are able to use inorganic- and organic-compounds such as H_2, H_2S, Fe^{2+}, CO_2, CH_4, amino acids and peptides, which were generated or synthesized by abiotic reactions within the Earth's crust, as energy and/or carbon sources. Evidence supporting the presence of ancient life in the early Archaean, at least 3.8-3.2 billion-years-ago (Gya), has been reported [1-7]. However, the characteristics of the ancient ecosystem are still unclear: What microorganisms were there? What did they do? From when, where and how did they thrive in the early Earth? Many researchers have accumulated data in various scientific fields, such as geology, mineralogy, geochemistry, microbiology and molecular biology, and tried to answer these questions. It is necessary to integrate these data to build a comprehensive view of the ancient ecosystem.

A marine hydrothermal field is one of the most probable environments where the ancient ecosystem was present. Along with the accumulation of geological, geochemical and microbiological data for various hydrothermal fields, the diversity of marine hydrothermal systems and diversity of microorganisms living there has been evaluated. Knowledge of the microbial ecosystem in the present diverse hydrothermal systems will provide information about the early microbial ecosystem in ancient hydrothermal systems before the evolution of photosynthesis. This paper reviews the geological, geochemical and microbial diversities of the present hydrothermal systems, and possible metabolic functions of the microorganisms in the ancient hydrothermal system in the early Earth.

2. Marine hydrothermal fields as an analog of habitats in the early Earth

In the late 1970's, a deep-sea hydrothermal field discharging warm fluids was discovered in the Galapagos Rift [8]. Unusual animal communities were found in the hydrothermal field. As soon as this discovery was made, other hydrothermal fields venting high temperature fluids (300ºC or higher) were found in the East Pacific Rise (EPR) [9]. To date, a number of marine hydrothermal fields have been found in various areas of the world (Fig.1). A database of active submarine hydrothermal vent fields (the "InterRidge Vents Database") is available (http://www.Interridge.org/irvents) [10].

In marine hydrothermal systems, cold oxygenated seawater penetrates into the deep sub-seafloor. The penetrating seawater passes through sub-seafloor oceanic crusts, reacts with heated rocks and dissolves ionized metals (such and Fe^{2+}, Cu^{2+} and Zn^{2+}), and volatiles (such as H_2, SO_2, CH_4 and CO_2) degassed from magma (Fig. 2A). The hot fluids, which have dramatically changed physicochemistry compared to the original seawater, rise toward the seafloor and discharge while mixing with seawater under and above the seafloor. Redox reactions between reductants (such as H_2, H_2S, CH_4 and Fe^{2+}) in the hydrothermal fluids and oxidants (such as O_2 and SO_4^{2-}) in seawater generate chemical energy that could support the growth of various chemolithoautotrophic microorganisms in marine hydrothermal fields [11].

A marine hydrothermal field is thought to be one of the environments where ancient life thrived [12]. There are geological records indicating that marine hydrothermal systems were present in the early Earth. All of the compounds required for the growth of microorganisms, i.e., energy and carbon sources and trace elements, are provided in the present hydrothermal system [13]. An ecosystem in marine hydrothermal fields does not require solar energy. However, it is noted that the ecosystem of present

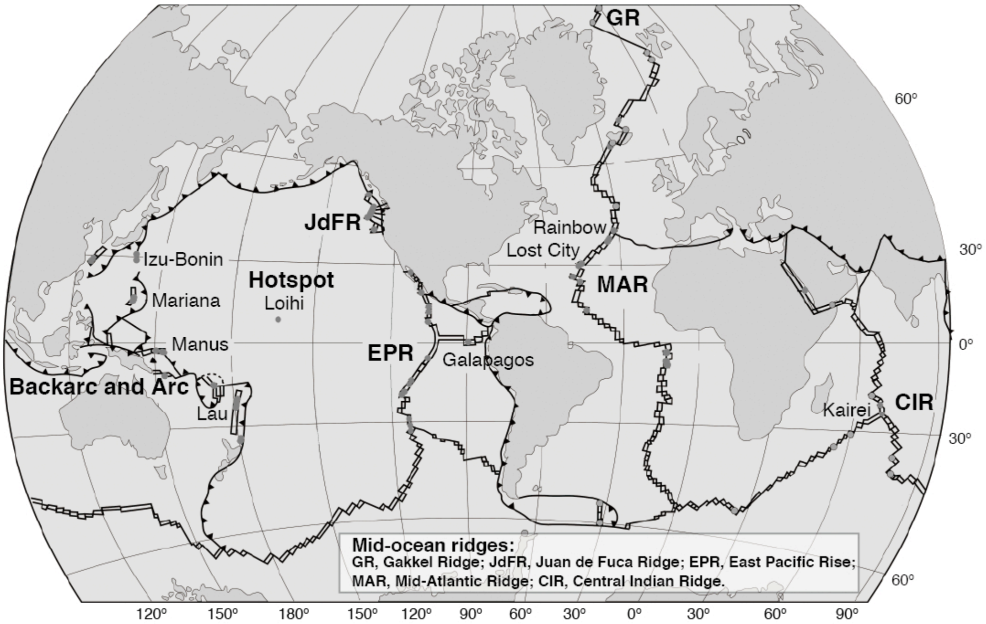

Fig. 1. The location of marine hydrothermal fields. The map was modified from Tivey (2007). The abbreviations are shown in the box at the bottom of the map.

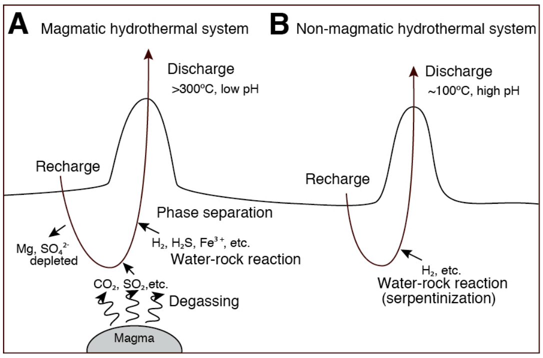

Fig. 2. Illustration of the generation process of hydrothermal fluids. (A) Magmatic and (B) non-magmatic hydrothermal systems are shown, respectively.

marine hydrothermal systems is not completely independent of photosynthetic ecosystems because the main electron acceptor, O_2, is produced by photosynthesis. Geological evidence indicates that microorganisms lived in ancient hydrothermal fields at 3.5-3.2 Gya [1-6]. Filamentous microfossils were found in a massive ancient volcanogenic sulfide deposit [3] and in rocks [6]. The morphologies of the microfossils resemble some present-day filamentous thermophilic microorganisms detected in both terrestrial and marine hydrothermal fields [14,15]. Massive sulfide deposits are commonly observed in the present hydrothermal fields. Methane with lighter carbon isotopic composition recognized as a product of methanogenesis was detected in 3.5-billion-year-old hydrothermal precipitates [1]. Sulfides with lighter sulfur isotopic composition recognized as products of sulfate reducing microorganisms were also found in 3.5-billion-year-old hydrothermal deposits [2,4]. Notably, deep-branching *Archaea* in the phylogenetic tree contain anaerobic hyperthermophilic methanogens and sulfate reducing microorganisms [16]. This fact suggests that these microorganisms retain the characteristics of ancient life on the early Earth [16]. This notion is partially supported by experimental data demonstrating that ancestral enzymes showed high thermal stability [17-19]. Regardless of the physicochemical conditions of the ancient ocean (i.e., oxic or anoxic, hot or cold), which are controversial [20,21], the venting fluid in the ancient hydrothermal fields must have been anoxic and hot. These data suggest that anaerobic (hyper)thermophiles might have thrived in marine hydrothermal fields in the early Earth at 3.2-3.5 Gya. The fluids would have contained H_2 and Fe^{2+} that were generated by rock-water reactions, SO_2 and CO_2 degassed from magma, and carbon compounds (such as CH_4, methanol, formic acid, amino acids and peptides) that would be abiotically produced in the hydrothermal system [22-27]. These compounds can be used as carbon and energy sources by anaerobic (hyper)thermophiles. Thus, anoxic and hot environments in the present hydrothermal fields may be analogous to those in the ancient hydrothermal fields.

3. Diversity of geochemical characteristics and geological settings of hydrothermal systems

To understand the ancient ecosystem in the marine hydrothermal fields of the early Earth, it is important to investigate extant marine hydrothermal systems. After the discovery of marine hydrothermal fields, studies of these hydrothermal systems have revealed an unexpected diversity of geochemical characteristics of venting fluids and geological settings of the fields [28,29]. A variety of physicochemical characteristics of venting fluids are found in the present hydrothermal fields. This physicochemical diversity is mainly generated by the following three processes: (1) water-rock reactions, (2) magmatic input and (3) phase separation.

The type and amount of minerals contained in the host rock influence qualitatively and quantitatively the chemical species contained in hydrothermal fluids that are generated by seawater-rock reactions. In particular, pH and H_2, H_2S and Fe^{2+} concentrations are controlled by phase equilibrium of diverse minerals in the rocks [30,31]. The presence/absence of magmatic input also dramatically controls pH and chemical composition of hydrothermal fluids. The magmatic gases mainly consist of CO_2 and SO_2 with H_2, CH_4 and CO as minor components [32,33]. The SO_2 reacts with H_2, O_2 and minerals during the upwelling process. This reaction generates H_2S or H_2SO_4, the latter lowering the pH of hydrothermal fluids. In sub-seafloor environments, hydrothermal fluids are separated into a vapor phase with a relatively low Cl-concentration and a liquid (or brine) phase with a relatively high Cl-concentration. The process is called phase separation [34]. For example, higher concentrations of volatiles (such as H_2, H_2S and CH_4) are found in the fluids of the vapor phase; in contrast, higher concentrations of Ca^{2+} and Ba^{2+} are found in the fluids of the liquid phase [35,36]. These three processes are strongly influenced by differences in the geological settings of the fields described below.

Marine hydrothermal fields have been found on mid-ocean ridges (e.g., East Pacific Rise, Mid-Atlantic Ridge, Central Indian Ridge), island-arcs (e.g., Izu-bonin Arc, Kermadec Arc, Mariana Arc) and back-arc basin spreading centers (e.g., Lau Basin, Manus Basin, Mariana Trough) (Fig. 1). In contrast to mid-ocean ridges, island-arcs and back-arc basins exist in the subduction zone (Fig. 3). Magmatic input of island-arcs and back-arc basins is different from that of mid-ocean ridges, which constrains the geochemistry of hydrothermal fluid venting, thus affecting the pH and concentration of volatiles in the hydrothermal fluid [37,38]. The fluids of the subduction zone are frequently lower in pH than those of mid-ocean ridges. Another magmatic hydrothermal system called a hotspot has been found, such as the Loihi seamount near Hawaii [39]. The mechanism of magma generation at the hotspot is still controversial [40]. The features of the end-member (i.e., pure hydrothermal fluid, not mixed with seawater) of these venting fluids in these magmatic hydrothermal fields are very high temperature (~350-400ºC) and low pH (~5). The basement of mid-ocean ridges consists of basalts (mafic rock); however, in some areas (e.g., Rainbow site in the Mid-Atlantic Ridge), the basement consists of peridotites (utramafic rock). The basement of island-arcs and back-arc basins consists of andesite or dacite (intermediate- or intermediate-felsic rock). The variety of basement rocks is one of the factors responsible for the geochemical diversity of venting fluids [28,29].

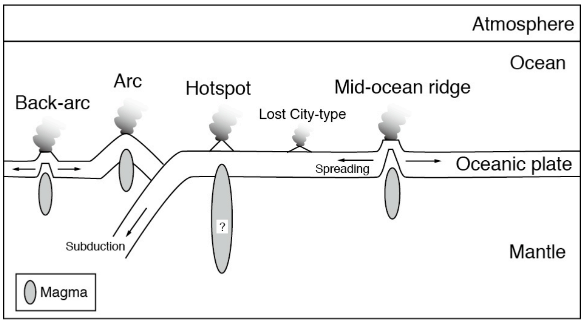

Fig. 3. A variety of geological settings of marine hydrothermal fields.

Recently, a non-magmatic hydrothermal field, called the Lost City hydrothermal field, was discovered in the western Mid-Atlantic Ridge [41]. In contrast to the venting fluids in magmatic hydrothermal fields, the fluids of the Lost City have unique geochemistry. The fluid temperature can reach approximately 100ºC and the pH is very high (~ 11). In the Lost City, the basement rock is mantle peridotite rifted up from deeper regions of oceanic crust. The warm, alkaline fluid is likely to be generated only by seawater-peridotite reactions (Fig. 2B). These are exothermic serpentinization reactions: peridotite containing olivine and pyroxenes (a mineral group high in magnesium and iron) reacts with seawater producing serpentinite, brucite [$Mg(OH)_2$], magnetite (Fe_3O_4) and H_2 [42,43]. High concentrations of hydrocarbons (such as methane, ethane, propane and butane) were detected in the fluids of the Lost City [22] as well as the Rainbow site of a magmatic hydrothermal field of the Mid-Atlantic Ridge [23], which are likely to be produced abiotically by Fischer-Tropsch type reactions: hydrocarbons are produced from H_2 and CO_2 with metal catalysts such as Co, Fe, and Ni, which are rich in peridotites. Although non-magmatic hydrothermal fields have not been found except for the Lost City, other such hydrothermal fields may currently exist, and also may have existed in the early Earth. The pH gradient, i.e., proton gradient, between the seawater and alkaline hydrothermal fluids could have supported ATP synthesis of the first cell [44].

Mid-ocean ridges have a variety of spreading rates: fast (>80 mm/yr), mid, (60-80 mm/yr), slow (20-60 mm/yr) and ultra-slow (<20 mm/yr) [45]. Studies of mid-ocean ridges showed that the spreading rate quantitatively reflects the type of basement rocks present. Areas with fast- and mid-spreading rates consist of basalts (mafics). In contrast, in regions of mid-ocean ridges with slow- and ultraslow-spreading rates, mantle peridotites (ultramafics) are exposed on the seafloor or close to the seafloor (e.g., the Rainbow site of a segment of the Mid-Atlantic Ridge [23]). The peridotites found in the Lost City, an off-ridge field, may be derived from a part of the Mid-Atlantic Ridge with ultra-slow spreading rate (12 mm/yr) [46]. The difference in the type of basement rocks, mafic or ultramafic, is related to the venting fluid geochemistry at hydrothermal systems as described above. Although there are hydrothermal fields on back-arc basin spreading centers, the relationship between spreading rate and basement rock is not clear.

Thus, the geochemical diversity of fluids is associated with the geological diversity of hydrothermal fields. The diversity of various physicochemical conditions (qualitative and/or quantitative differences in temperature, pH and chemical species) must constrain the composition of microbial communities.

4. Diversity of microorganisms living in marine hydrothermal fields

Diverse microorganisms of both *Bacteria* and *Archaea* are living in marine hydrothermal fields as shown by culture-dependent and -independent methods. Novel extremophiles, including thermophiles (heat-loving), acidophiles (acid-loving) and piezophiles (pressure-loving; also called barophiles), have been cultured and isolated from various hydrothermal fields. Unsurpassed physiology of these extremophiles has expanded the known limits of life. Culture-independent molecular techniques based on 16S rRNA gene sequences have revealed that diverse not-yet-cultivated microorganisms exist in various habitats of hydrothermal fields, such as fluids, sulfide chimneys and microbial mats [47-52]. Furthermore, using the recently developed and more powerful sequencing method "pyrosequencing", the presence of a surprisingly high diversity of microorganisms (especially of *Bacteria*) in hydrothermal fluids was revealed [53]. Although the physiological functions of microorganisms cannot be directly determined from their sequence information such as the 16S rRNA gene and even the whole genome sequence, high phylogenetic diversity of microorganisms in the hydrothermal environments suggests that a variety of physiological functions exist there.

Microorganisms living in marine hydrothermal fields include diverse chemolithoautotrophs that can grow using inorganic materials as energy sources and CO_2 as a sole carbon source [54], which are likely to play important roles as primary producers in the microbial ecosystem in these environments. They are capable of using diverse redox reactions between H_2, H_2S, Fe^{2+}, CH_4, NH_4^+, Mn^{2+}, S^0 and/or $S_2O_3^{2-}$ as electron donors and O_2, NO_3^-, SO_4^{2-}, S^o, $S_2O_3^{2-}$, CO_2, Fe^{3+} and/or Mn^{4+} as electron acceptors. Chemolithoautotrophs synthesize ATP (adenosine triphosphate, the "molecular currency" of intracellular energy transfer) using chemical energy generated by the redox reactions. The redox reactions occur in thermodynamic nonequilibrium conditions created upon mixing reduced hydrothermal fluids with oxygenated seawater. The energy obtained by the redox reactions can be calculated as Gibbs free energy [55]. The amount of energy, as well as reaction rate of the metabolism, constrain which chemolithoautotrophs thrive and what kind of the microbial ecosystem is constructed in the hydrothermal environment. Chemolithoautotrophic hyperthermophilic *Archaea* that use hydrogen as a principal electron donor, such as methanogens (e.g., *Methanopyrus kandleri* [56]), hydrogen-oxidizing sulfur-reducers (e.g., *Pyrodictium occultum* [57]), hydrogen-oxidizing sulfate-reducers (e.g., *Archaeoglobus veneficus* [58]), and hydrogen-oxidizing iron-reducers (e.g., *Geoglobus ahangari* [59]), have been detected in various hydrothermal environments including mid-ocean ridges, back-arc basins and island-arcs [60]. Thus, it seems that the presence or absence of chemolithoautotrophic hyperthermophilic *Archaea* is not associated with differences in global geological settings but in more local environmental factors.

Fermenting microorganisms are also living in marine hydrothermal environments. Fermentation generates ATP from carbon compounds including amino acids and peptides by substrate-level phosphorylation, reactions that do not require exogenous electron acceptors (e.g., O_2) as opposed to respiration. Members of the *Thermococci*, a hyperthermophilic archaeal group, are often detected in hydrothermal environments [48,50,61-63]. This taxonomic group includes fermenting species [64]. In addition, a carbon monoxide (CO) utilizing species belonging to the *Thermococci*, which can grow using CO as sole carbon and energy sources, was isolated from a marine hydrothermal field [65]. Recently, the whole genome sequence of a member of the *Korarchaeota*, "*Korarchaeum cryptofilum*", was determined by metagenomic analysis of an enrichment culture [14]. This group had been detected in marine and terrestrial hydrothermal fields by culture-independent methods [50,52,66-68] but not cultured for over 20 years. The genome sequence includes gene sets that include peptide and amino acid fermentation pathways, suggesting that *K. cryptofilum* is likely to be a peptide- and amino acid-fermenting archaeon [14]. Some members of the *Thermoprotei* can also grow by peptide- and/or amino acid-fermentation, and their 16S rRNA gene sequences have been detected in marine hydrothermal environments [69]. It should be noted that amino acids and peptides could be abiotically synthesized in marine hydrothermal systems [24,25] and CO is provided by magma degassing [32]. This means that peptide- and amino acid-fermenting and CO-utilizing microorganisms could grow as well as chemolithoautotrophs in these environments, and in the absence of any other organisms.

5. Possible metabolic reactions in the ancient ecosystem

Most of the deep-branching microorganisms in the universal phylogenetic tree constructed from 16S rRNA gene sequences are anaerobic hyperthermophiles, implying that anaerobicity and thermophilicity may be the ancestral characteristics of life [16]. Many anaerobic hyperthermophiles have been isolated from hydrothermal fields, especially members of the *Archaea* as shown in Fig. 4. The order of emergence of these archaeal lineages is still controversial.

Some of these deep-branching anaerobic

hyperthermophilic *Archaea* gain energy by methanogenesis, fermentation, and hydrogen-oxidizing and sulfate-, sulfur- or iron-reducing reactions. These members could live in marine hydrothermal environments without any other microorganisms because all essential matter for their growth is abiotically supplied from the marine hydrothermal system. Furthermore, these anaerobic hyperthermophilic *Archaea* have been detected in all types of magmatic hydrothermal fields [60], except the Hotspot (i.e., Loihi seamount) and the non-magmatic hydrothermal field (i.e., the Lost City). It should be noted that quantitative data regarding the abundance of each archaeal group in these environments and the efficiency of each energy-yielding reaction does not directly indicate the order of emergence of these lineages. If the Gibbs free energy of the energy-yielding reactions is less than zero [70] (less than -20 kJ mol^{-1} is favorable [71]), the microorganism could grow using any of these energy-yielding reactions. As long as H_2, CO_2, CO, Fe^{3+} (potentially generated by photooxidation of Fe^{2+})[72], SO_4^{2-} and S^0 (potentially generated by photolysis of SO_2)[73], amino acids [24] and peptides [25] are abiotically supplied from the ancient marine hydrothermal system and ocean surface, any microorganisms using these energy-yielding reactions (both respiration and fermentation) could have existed in the ancient hydrothermal environment as primary producers in the ancient ecosystem before the evolution of photosynthesis.

It is the most likely scenario that hyperthermophilic methanogens dominated in the ancient ecosystems in the hydrothermal fields in the early Earth. In terrestrial environments, Stevens and McKinley [74] first suggested and Chapelle and coworkers [75] reconfirmed that a hydrogen-based subsurface lithoautotrophic microbial ecosystem (SLiME) dominated by methanogens existed in deep continental basaltic crust. Hydrogen, the energy source of methanogens, could be supplied from water-basalt reactions [76] or tectonic activity [77]. The SLiME could sustain itself completely independent of a photosynthetic ecosystem. In marine environments, Takai and coworkers suggested that a hydrogen-based hyperthermophilic SLiME (hyperSLiME) may be present below a deep-sea hydrothermal field, an idea supported by geochemical (the presence of methane with lighter carbon isotopic composition and high H_2 concentration in the hydrothermal fluids) and microbiological (the predominance of hyperthermophilic methanogens) evidence [63]. Ueno and his colleagues exhibited geological evidence for the presence of methanogens in an ancient hydrothermal environment [1]. It is highly likely that H_2 and CO_2 were present in the ancient hydrothermal system. The phylogenetic tree of all life based on small subunit rRNA gene sequences suggests that one of the deepest-branching members of the *Archaea* is a hyperthermophilic methanogen, *Methanopyrus* (Fig. 4). This genus contains the most hyperthermophilic microorganism identified to date, *M. kandleri* strain 116, which can grow at 122ºC [78]. These facts support the presence of hyperthermophilic methanogens in the ancient ecosystem.

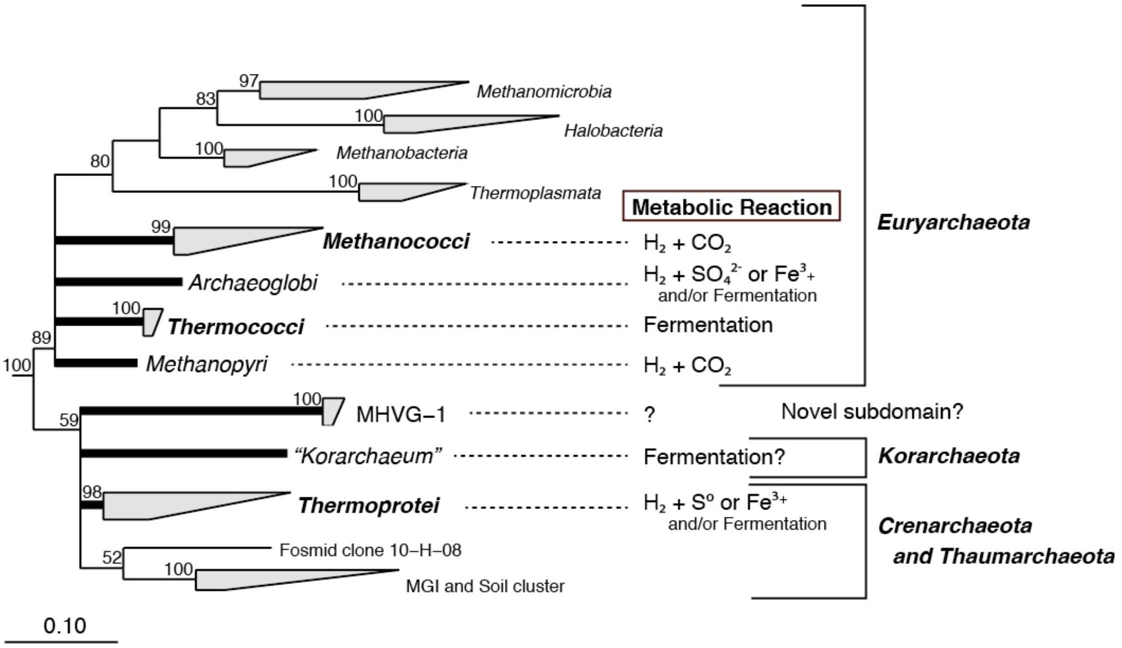

Fig. 4. A phylogenetic tree of the domain *Archaea* constructed based on the 16S rRNA gene sequences. The tree was constructed using Phyml [91] by maximum-likelihood analysis of 1081 homologous positions in the alignment dataset generated using ClustalW [92], using *Thermus thermophilus* (NC_006461), *Aquifex aeolicus* (NC_000918) and *Thermotoga maritima* (NC_000853) as the out-group (not shown). All 16S rRNA gene sequences (total 62 taxa) used for the tree were collected from the whole genome sequences of each archaeon or from fosmid library sequences. Bold branches indicate groups including hyperthermophiles. MHVG-1: Marine Hydrothermal vent group 1. The *Nanoarchaeota* was excluded from the tree to avoid long-branch attraction. Bootstrap values (higher than 50%) based on 100 replicates are shown at branch points. The scale bar represents the expected number of changes per nucleotide position.

Hyperthermophilic hydrogen-oxidizing and sulfate-reducing microorganisms also potentially existed in the ancient hydrothermal fields. It has been found that sulfide minerals in Archaean rocks were isotopically fractionated [2,4,79]. Sulfide precipitates produced by sulfate reducing microorganisms have lower values of $\delta^{34}S$[80]. The Archaean sulfide minerals with relatively lower $\delta^{34}S$ values than seawater sulfate indicate that microbial sulfate reduction occurred before 3.4 Gya [80]. Hyperthermophilic hydrogen-oxidizing and sulfate-reducing *Archaea* and *Bacteria* have been detected in the present marine hydrothermal fields. The *Archaeogolobi* is the sole archaeal group containing hydrogen-oxidizing and sulfate-reducers, e.g., *Archaeoglobus veneficus,* which was isolated from a deep-sea hydrothermal field [58]. Sulfate must have been present in the early ocean, although the concentration and origin are still controversial [79,81]. Thus, hyperthermophilic hydrogen-oxidizing and sulfate-reducing microorganisms could have grown in the ancient hydrothermal fields.

Hyperthermophilic hydrogen-oxidizing and sulfur- or iron-reducing microorganisms, such as members of the *Thermoprotei*, could also have been present in the ancient hydrothermal fields, if Fe^{3+} and S^0 had been present in the Archaean ocean[72,73]. However, the iron or sulfur isotopic fractionations resulting from metabolic reactions catalyzed by hyperthermophilic sulfur- or iron-reducing microorganisms are inconclusive. Further geological evidence and analysis of microbial isotopic fractionation are required to verify the presence of hyperthermophilic sulfur- or iron-reducing microorganisms in the ancient hydrothermal fields.

Amino acid- (or peptide-) fermentation is thought to be an earlier energy-yielding metabolic reaction than methanogenesis or other hydrogen-based respirations because of its metabolic simplicity: amino acid-fermentation requires fewer enzymes than respiration [82]. In addition, the deep-branching archaeal groups, such as *Thermococci*, *Archaeoglobi*, *Thermoprotei* and *Korarchaeota*, include amino acid-fermenting species. The ubiquity of amino acid-fermentation among the deep-branching *Archaea* also supports the idea proposed by Clarke and Elsden [82]. If all matter, including amino acids and trace elements that are needed for the growth of fermenting microorganisms had been continuously and abiotically supplied by the ancient hydrothermal system of the early Earth [13,24], these organisms could have functioned as primary producers in the microbial ecosystem as well as methanogens. However, this hypothesis has a problem: the H_2-producing fermentation could not release energy from the reaction for the growth of microorganisms at high H_2 concentration, whereas the H_2 concentration at the ancient hydrothermal system would be high and abiotic production of amino acids would occur easier at higher H_2 concentration [24]. If there was an anaerobic environment around the ancient hydrothermal field where amino acids accumulated at low H_2 concentration, fermenting microorganisms could have thrived there: it is possible that amino acids adsorbed onto minerals (for example, Si or mineral oxides) in hydrothermal fluids [83], were precipitated, pooled and concentrated on the seafloor where the amino acid fermentation reactions could proceed. It goes without saying that this hypothesis needs additional experimental evidence.

6. Detection, cultivation and characterization of deeper and deeper lineages of life

What can we do to characterize the ancient life in the early Earth? The simplest approach for a microbiologist is to find and describe all prokaryotes existing in the present Earth, construct the correct phylogenetic tree of them and determine the deepest-branching species. The physiological characteristics of the deepest-branching species may be most similar to those of the ancient life in the early Earth. However, this is not enough to unveil the ancient life. It is important to propose hypotheses based on the theoretical, experimental, and observational approaches based not only on microbiological but also on other related scientific fields. Unrecognized, deeper-branching microorganisms may be living in the present hydrothermal fields. Although the characteristics of the deeper-branching species do not directly describe ancient life in the early Earth, it will provide information on early evolution. There may be no end to this approach as a game of hide-and-seek. However, we know that this strategy could help us not only to understand ancient life but also to understand what life is: the limit of life, habitability of life and unexpected novel function of life.

Culture-independent molecular analysis (mainly based on PCR) has suggested that only 1% or less of the microorganisms living on Earth have been cultivated [84]. In fact, while diverse 16S rRNA gene sequences of both *Archaea* and *Bacteria* have been detected in marine hydrothermal fields [47-52], most of this diversity is as yet uncultured. Furthermore, technical limitations of PCR-based culture-independent molecular methods must be considered [83]. The PCR primers used for metagenomic analysis have been targeted to common regions within genes, e.g., 16S rRNA, based on the “known” sequences. If certain uncultured microorganisms have sequence differences in these regions, they may never be detected by traditional PCR analysis even if they are abundant in particular environments. This problem can be solved by using PCR-independent metagenomic analysis, e.g., whole genome shotgun sequencing (WGS). Actually, WGS analysis has revealed the presence of uncultured *Archaea* in an acid mine drainage [85], which had never been recognized because they have unique 16S rRNA gene sequences. To date, there are no reports of microbial communities at deep-sea hydrothermal fields using WGS analysis of environmental DNA. These facts suggest that not-yet-recognized, much more diverse microorganisms are likely to be present in marine hydrothermal fields.

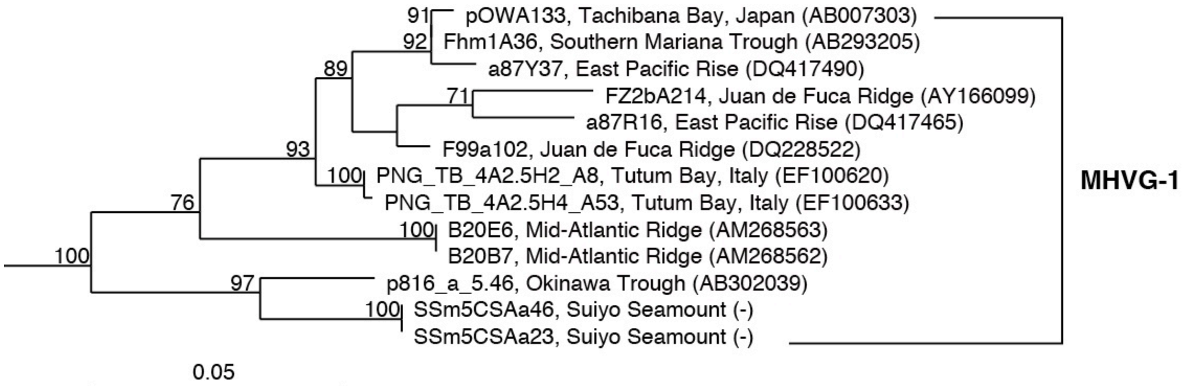

Fig. 5. A phylogenetic tree of the MHVG-1 based on 16S rRNA gene sequences. The tree was constructed using Phyml [91] by maximum-likelihood analysis of 710 homologous positions in the alignment dataset generated using ClustalW [92], using sequences of the *Korarchaeota* as the out-group (not shown). Bootstrap values (higher than 50%) based on 100 replicates are shown at branch points. The scale bar represents the expected number of changes per nucleotide position.

The Marine Hydrothermal Vent Group-1 (MHVG-1), which is one of the deepest-branching archaeal lineages [52] (Fig. 4), was detected in various marine hydrothermal fields including mid-ocean ridges, an island-arc and back-arc basins (Fig. 5). The MHVG-1 *Archaea* may represent hyperthermophiles based on the estimated optimum growth temperature. The optimum growth temperature of 88ºC was calculated based on the GC content of the 16S rRNA gene sequences of MHVG-1 using a correlation between the optimum growth temperature and GC content [86]. The physiological function (e.g., the energy-yielding metabolism) of this deep-branching MHVG-1 *Archaea* would provide useful information about early evolution of life. However, no isolate species of MHVG-1 have been reported. The sequence information alone does not tell us the physiology, e.g., optimal growth temperature and pH, growth rate, morphology, or the complete metabolic function of the microorganism. Classical cultivation methods (e.g., batch culture) might be not suitable for the cultivation, isolation and characterization of MHVG-1. High-pressure cultivation is a proven method for cultivation of novel thermophiles living in marine hydrothermal fields [87]. Another useful method is a flow-type cultivation system called continuous cultivation or chemostat [88]. In fact, several novel thermophiles have been cultured from marine hydrothermal samples using a flow-type cultivation system [89,90]. Such contrivances of the cultivation system may be required to culture as-yet-not-cultivated microorganisms living in marine hydrothermal fields.

7. Conclusion

Since the first discovery of marine hydrothermal systems, subsequent studies have revealed the geological, geochemical and microbiological diversities of hydrothermal systems. These data have provided remarkable insights into the origin and early evolution of life on Earth. However, our knowledge of marine hydrothermal systems is still limited. Marine hydrothermal fields must be more widely distributed in the world's oceans, and novel and unique types of hydrothermal systems are likely to be found. Microbiology of hydrothermal systems is also still developing. The present image of the ancient ecosystem described in this paper and other previous reports is based on limited knowledge. To propose the most reliable hypothesis of the primary producer in the ancient ecosystem, it is important to expand our knowledge of the geology, geochemistry and microbiology of present hydrothermal systems. More exploration of the seafloor, additional understanding of hydrothermal systems and more microbiological breakthroughs for both culture-independent and -dependent methods are needed.

9. Acknowledgments

We thank the editors of this journal for giving us the opportunity to publish this review. This research was funded by the Ministry of Education, Culture, Science and Technology (MEXT), Japan, through a special coordination fund (Project TAIGA: Trans-crustal Advection and In-situ biogeochemical processes of Global sub-seafloor Aquifer).

10. References

1. Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S. and Isozaki, Y. Evidence from fluid inclusions for microbial methanogenesis in the early archaean era, Nature 440, 516-519 (2006).
2. Ueno, Y., Ono, S., Rumble, D. and Maruyama, S. Quadruple sulfur isotope analysis of ca. 3.5 Ga Dresser Formation: New evidence for microbial sulfate reduction in the early Archean, Geochimica et Cosmochimica Acta 72, 5675-5691 (2008).
3. Rasmussen, B. Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit, Nature 405, 676-679 (2000).
4. Shen, Y., Buick, R. and Canfield, D. E. Isotopic evidence for microbial sulphate reduction in the early Archaean era, Nature 410, 77-81 (2001).
5. Schopf, J. W. Fossil evidence of Archaean life, Philosophical Transactions of the Royal Society B: Biological Sciences 361, 869-885 (2006).
6. Ueno, Y., Isozaki, Y., Yurimoto, H. and Maruyama, H. Carbon isotopic signatures of individual Archean microfossils(?) from Western Australia, International Geology Review 43, 196-212 (2001).
7. Schidlowski, M. A 3,800-million-year isotopic record of life from carbon in sedimentary rocks, Nature 333, 313-318 (1988).
8. Corliss, J. B., Dymond, J., Gordon, L. I., Edmond, J. M., Von Herzen, R. P., Ballard, R. D., Green, K., Williams, D., Bainbridge, A., Crane, K. and Van Andel, T. H. Submarine

thermal springs on the Galapagos Rift, Science 203, 1073-1083 (1979).

9. Spiess, F. N., Macdonald, K. C., Atwater, T., Ballard, R., Carranza, A., Cordoba, D., Cox, C., Garcia, V. M. D., Francheteau, J., Guerrero, J., Hawkins, J., Haymon, R., Hessler, R., Juteau, T., Kastner, M., Larson, R., Luyendyk, B., Macdougall, J. D., Miller, S., Normark, W., Orcutt, J. and Rangin, C. East Pacific Rise: Hot springs and geophysical experiments, Science 207, 1421-1433 (1980).
10. Beaulieu, S. E. Interridge global database of active submarine hydrothermal vent fields: Prepared for Interridge, version 2.0. Http://www.Interridge.Org/irvents, (2010).
11. Jannasch, H. W. and Mottl, M. J. Geomicrobiology of deep-sea hydrothermal vents, Science 229, 717-725 (1985).
12. Nisbet, E. G. and Sleep, N. H. The habitat and nature of early life, Nature 409, 1083-1091 (2001).
13. Kelley, D. S., Baross, J. A. and Delaney, J. R. Volcanoes, fluids, and life at mid-ocean ridge spreading centers, Annual Review of Earth and Planetary Sciences 30, 385-491 (2002).
14. Elkins, J. G., Podar, M., Graham, D. E., Makarova, K. S., Wolf, Y., Randau, L., Hedlund, B. P., Brochier-Armanet, C. L., Kunin, V., Anderson, I., Lapidus, A., Goltsman, E., Barry, K., Koonin, E. V., Hugenholtz, P., Kyrpides, N., Wanner, G., Richardson, P., Keller, M. and Stetter, K. O. A korarchaeal genome reveals insights into the evolution of the archaea, Proceedings of the National Academy of Sciences 105, 8102-8107 (2008).
15. Huber, R., Eder, W., Heldwein, S., Wanner, G., Huber, H., Rachel, R. and Stetter, K. O. *Thermocrinis ruber* gen. nov., sp. nov., a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park, Appl. Environ. Microbiol. 64, 3576-3583 (1998).
16. Woese, C. Bacterial evolution, Microbiol. Rev. 51, 221-271 (1987).
17. Watanabe, K., Ohkuri, T., Yokobori, S. and Yamagishi, A. Designing thermostable proteins: Ancestral mutants of 3-isopropylmalate dehydrogenase designed by using a phylogenetic tree, Journal of Molecular Biology 355, 664-674 (2006).
18. Iwabata, H., Watanabe, K., Ohkuri, T., Yokobori, S.-I. and Yamagishi, A. Thermostability of ancestral mutants of *Caldococcus noboribetus* isocitrate dehydrogenase, FEMS Microbiology Letters 243, 393-398 (2005).
19. Shimizu, H., Yokobori, S., Ohkuri, T., Yokogawa, T., Nishikawa, K. and Yamagishi, A. Extremely thermophilic translation system in the common ancestor commonote: Ancestral mutants of glycyl-trna synthetase from the extreme thermophile *Thermus thermophilus*, Journal of Molecular Biology 369, 1060-1069 (2007).
20. Holland, H. D. The oxygenation of the atmosphere and oceans, Philosophical Transactions of the Royal Society B: Biological Sciences 361, 903-915 (2006).
21. Robert, F. and Chaussidon, M. A palaeotemperature curve for the Precambrian oceans based on silicon isotopes in cherts, Nature 443, 969-972 (2006).
22. Proskurowski, G., Lilley, M. D., Seewald, J. S., Fruh-Green, G. L., Olson, E. J., Lupton, J. E., Sylva, S. P. and Kelley, D. S. Abiogenic hydrocarbon production at Lost City hydrothermal field, Science 319, 604-607 (2008).
23. Holm, N. G. and Charlou, J. L. Initial indications of abiotic formation of hydrocarbons in the Rainbow ultramafic hydrothermal system, Mid-Atlantic Ridge, Earth and Planetary Science Letters 191, 1-8 (2001).
24. Amend, J. P. and Shock, E. L. Energetics of amino acid synthesis in hydrothermal ecosystems, Science 281, 1659-1662 (1998).
25. Imai, E., Honda, H., Hatori, K., Brack, A. E. and Matsuno, K. Elongation of oligopeptides in a simulated submarine hydrothermal system, Science 283, 831-833 (1999).
26. McCollom, T. M., Ritter, G. and Simoneit, B. R. T. Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions, Origins of Life and Evolution of Biospheres 29, 153-166 (1999).
27. McCollom, T. M. and Seewald, J. S. Abiotic synthesis of organic compounds in deep-sea hydrothermal environments, Chem. Rev. 107, 382-401 (2007).
28. Tivey, M. K. Generation of seafloor hydrothermal vent fluids and associated mineral deposits, Oceanography 20, 50 (2007).
29. Nakamura, K. and Takai, K. Physical and chemical diversity of seafloor hydrothermal systems and presentation of associated chemolithoautotrophic ecosystem, Journal of Geography (in Japanese) 118, 1083-1130 (2009).
30. Seyfried Jr, W. E., Ding, K. and Berndt, M. E. Phase equilibria constraints on the chemistry of hot spring fluids at mid-ocean ridges, Geochimica et Cosmochimica Acta 55, 3559-3580 (1991).
31. Seyfiied Jr, W. and Ding, K. Phase equilibria in subseafloor hydrothermal systems: a review of the role of redox, temperature, pH and dissolved Cl on the chemistry of hot spring fluids at mid-ocean ridges, pp. 248-273, in Humphris, S., Mullineaux, L., Zierenberg, R. and Thomson, R. Eds., Seafloor hydrothermal systems: Physical, chemical, biological, and geological interactions, Amer Geophysical Union, Washington DC, 1995.
32. Symonds, R. B., Rose, W. I., Bluth, G. J. S. and Gerlach, T. M. Volcanic-gas studies; methods, results, and applications, Reviews in Mineralogy and Geochemistry 30, 1-66 (1994).
33. Alt, J. Subseafloor processes in mid-ocean ridge hydrothermal systems, pp. 85-114, in Humphris, S., Mullineaux, L., Zierenberg, R. and Thomson, R. Eds., Seafloor hydrothermal systems: Physical, chemical, biological, and geological interactions, Amer Geophysical Union, Washington DC, 1995.
34. Massoth, G. J., Butterfield, D. A., Lupton, J. E., Mcduff, R. E., Lilley, M. D. and Jonasson, I. R. Submarine venting of phase-separated hydrothermal fluids at axial volcano, Juan de Fuca Ridge, Nature 340, 702-705 (1989).
35. Foustoukos, D. I. and Seyfried, W. E., Jr. Fluid phase separation processes in submarine hydrothermal systems, Reviews in Mineralogy and Geochemistry 65, 213-239 (2007).
36. Foustoukos, D. I. and Seyfried, J. W. E. Quartz solubility in the two-phase and critical region of the NaCl-KCl-H_2O system: Implications for submarine hydrothermal vent systems at 9º50'N East Pacific Rise, Geochimica et Cosmochimica Acta 71, 186-201 (2007).
37. Ishibashi, J. and Urabe, T. Hydrothermal activity related to arc-backarc magmatism in the Western Pacific, Backarc Basins: Tectonics and Magmatism, 451-495 (1995).
38. Gamo, T., Ishibashi, J., Tsunogai, U., Okamura, K. and Chiba, H. Unique geochemistry of submarine hydrothermal fluids from arc-back-arc settings of the Western Pacific, Geophysical monograph 166, 147-161 (2006).
39. Karl, D. M., Mcmurtry, G. M., Malahoff, A. and Garcia, M. O. Loihi seamount, Hawaii: A mid-plate volcano with a distinctive hydrothermal system, Nature 335, 532-535 (1988).
40. Dyment, J., Lin, J. and Baker, E. T. Ridge-hotspot interactions: What mid-ocean ridges tell us about deep earth processes, Oceanography 20, 102-115 (2007).
41. Kelley, D. S., Karson, J. A., Blackman, D. K., Fruh-Green, G. L., Butterfield, D. A., Lilley, M. D., Olson, E. J., Schrenk, M. O., Roe, K. K., Lebon, G. T., Rivizzigno, P. and Party, T. A.-S. An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30ºN, Nature 412, 145-149 (2001).
42. Kelley, D. S., Karson, J. A., Fruh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Summons, R. E. and Sylva, S. P. A serpentinite-hosted ecosystem: The Lost City hydrothermal field, Science 307, 1428-1434 (2005).
43. Boetius, A. Lost City life, Science 307, 1420-1422 (2005).
44. Martin, W., Baross, J., Kelley, D. and Russell, M. J. Hydrothermal vents and the origin of life, Nat Rev Micro 6, 805-814 (2008).
45. Dick, H. J. B., Lin, J. and Schouten, H. An ultraslow-spreading class of ocean ridge, Nature 426, 405-412 (2003).
46. Blackman, D. K., Cann, J. R., Janssen, B. and Smith, D. K. Origin of extensional core complexes: Evidence from the Mid-Atlantic Ridge at atlantis fracture zone, J. Geophys. Res. 103, 21315-21333 (1998).
47. Takai, K. and Horikoshi, K. Genetic diversity of archaea in deep-sea hydrothermal vent environments, Genetics 152, 1285-1297 (1999).

48. Schrenk, M. O., Kelley, D. S., Delaney, J. R. and Baross, A. J. A. Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney, Appl. Environ. Microbiol. 69, 3580-3592 (2003).
49. Kato, S., Kobayashi, C., Kakegawa, T. and Yamagishi, A. Microbial communities in iron-silica-rich microbial mats at deep-sea hydrothermal fields of the Southern Mariana Trough, Environmental Microbiology 11, 2094-2111 (2009).
50. Kato, S., Takano, Y., Kakegawa, T., Oba, H., Inoue, K., Kobayashi, C., Utsumi, M., Marumo, K., Kobayashi, K., Ito, Y., Ishibashi, J. and Yamagishi, A. Biogeography and biodiversity in sulfide structures of active and inactive vents at deep-sea hydrothermal fields of the Southern Mariana Trough, Appl. Environ. Microbiol. 76, 2968-2979 (2010).
51. Kato, S., Yanagawa, K., Sunamura, M., Takano, Y., Ishibashi, J., Kakegawa, T., Utsumi, M., Yamanaka, T., Toki, T., Noguchi, T., Kobayashi, K., Moroi, A., Kimura, H., Kawarabayasi, Y., Marumo, K., Urabe, T. and Yamagishi, A. Abundance of *Zetaproteobacteria* within crustal fluids in back-arc hydrothermal fields of the Southern Mariana Trough, Environmental Microbiology 11, 3210-3222 (2009).
52. Takai, K. and Sako, Y. A molecular view of archaeal diversity in marine and terrestrial hot water environments, FEMS Microbiology Ecology 28, 177-188 (1999).
53. Huber, J. A., Welch, D. B. M., Morrison, H. G., Huse, S. M., Neal, P. R., Butterfield, D. A. and Sogin, M. L. Microbial population structures in the deep marine biosphere, Science 318, 97-100 (2007).
54. Nakagawa, S. and Takai, K. Deep-sea vent chemoautotrophs: Diversity, biochemistry and ecological significance, FEMS Microbiology Ecology 65, 1-14 (2008).
55. McCollom, T. M. and Shock, E. L. Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems, Geochimica et Cosmochimica Acta 61, 4375-4391 (1997).
56. Kurr, M., Huber, R., König, H., Jannasch, H. W., Fricke, H., Trincone, A., Kristjansson, J. K. and Stetter, K. O. *Methanopyrus kandleri*, gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110ºC, Archives of Microbiology 156, 239-247 (1991).
57. Stetter, K., K Nig, H. and Stackebrandt, E. *Pyrodictium* gen. nov., a new genus of submarine disc-shaped sulphur reducing archaebacteria growing optimally at 105 degree C, Systematic and Applied Microbiology 4, 535-551 (1983).
58. Huber, R., Jannasch, H., Rachel, R., Fuchs, T. and Stetter, K. O. *Archaeoglobus veneficus* sp. nov., a novel facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers, Systematic and Applied Microbiology 20, 374-380 (1997).
59. Kashefi, K., Tor, J. M., Holmes, D. E., Gaw Van Praagh, C. V., Reysenbach, A. L. and Lovley, D. R. *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor, Int J Syst Evol Microbiol 52, 719-728 (2002).
60. Takai, K., Nakagawa, S., Reysenbach, A. L. and Hoek, J. Microbial ecology of mid-ocean ridges and back-arc basins, Geophysical monograph 166, 185-213 (2006).
61. Kormas, K. A., Tivey, M. K., Von Damm, K. and Teske, A. Bacterial and archaeal phylotypes associated with distinct mineralogical layers of a white smoker spire from a deep-sea hydrothermal vent site (9ºN, East Pacific Rise), Environmental Microbiology 8, 909-920 (2006).
62. Reysenbach, A.-L., Longnecker, K. and Kirshtein, J. Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic Ridge hydrothermal vent, Appl. Environ. Microbiol. 66, 3798-3806 (2000).
63. Takai, K., Gamo, T., Tsunogai, U., Nakayama, N., Hirayama, H., Nealson, K. H. and Horikoshi, K. Geochemical and microbiological evidence for a hydrogen-based, hyperthermophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) beneath an active deep-sea hydrothermal field, Extremophiles 8, 269-282 (2004).
64. Zillig, W. and Reysenbach, A.-L. *Thermococci*, pp. 341-348, in Boone, D. R., Castenholz, R. and Garrity, G. Eds., Bergey's manual of systematic bacteriology, 2nd edition, Springer, New York, 2001.
65. Sokolova, T. G., Jeanthon, C., Kostrikina, N. A., Chernyh, N. A., Lebedinsky, A. V., Stackebrandt, E. and Bonch-Osmolovskaya, E. A. The first evidence of anaerobic CO oxidation coupled with H_2 production by a hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent, Extremophiles 8, 317-323 (2004).
66. Barns, S. M., Fundyga, R. E., Jeffries, M. W. and Pace, N. R. Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment, Proceedings of the National Academy of Sciences 91, 1609-1613 (1994).
67. Barns, S. M., Delwiche, C. F., Palmer, J. D. and Pace, N. R. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences, Proceedings of the National Academy of Sciences 93, 9188-9193 (1996).
68. Auchtung, T. A., Takacs-Vesbach, C. D. and Cavanaugh, C. M. 16S rRNA phylogenetic investigation of the candidate division "*Korarchaeota*", Appl. Environ. Microbiol. 72, 5077-5082 (2006).
69. Reysenbach, A.-L. *Thermoprotei*, pp. 169-210, in Boone, D. R., Castenholz, R. and Garrity, G. Eds., Bergey's manual of systematic bacteriology, 2nd edition, Springer, New York, 2001.
70. Jackson, B. E. and Mcinerney, M. J. Anaerobic microbial metabolism can proceed close to thermodynamic limits, Nature 415, 454-456 (2002).
71. Schink, B. Energetics of syntrophic cooperation in methanogenic degradation, Microbiol. Mol. Biol. Rev. 61, 262-280 (1997).
72. Braterman, P. S., Cairns-Smith, A. G. and Sloper, R. W. Photo-oxidation of hydrated Fe^{2+} significance for banded iron formations, Nature 303, 163-164 (1983).
73. Farquhar, J., Savarino, J., Airieau, S. and Thiemens, M. H. Observation of wavelength-sensitive mass-independent sulfur isotope effects during SO_2 photolysis: Implications for the early atmosphere, J. Geophys. Res. 106, 32829-32839 (2001).
74. Stevens, T. O. and Mckinley, J. P. Lithoautotrophic microbial ecosystems in deep basalt aquifers, Science 270, 450-455 (1995).
75. Chapelle, F. H., O'neill, K., Bradley, P. M., Methe, B. A., Ciufo, S. A., Knobel, L. L. and Lovley, D. R. A hydrogen-based subsurface microbial community dominated by methanogens, Nature 415, 312-315 (2002).
76. Stevens, T. O. and Mckinley, J. P. Abiotic controls on H_2 production from basalt-water reactions and implications for aquifer biogeochemistry, Environmental Science & Technology 34, 826-831 (2000).
77. Wakita, H., Nakamura, Y., Kita, I., Fujii, N. and Notsu, K. Hydrogen release: New indicator of fault activity, Science 210, 188-190 (1980).
78. Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H., Nakagawa, S., Nunoura, T. and Horikoshi, K. Cell proliferation at 122ºC and isotopically heavy CH_4 production by a hyperthermophilic methanogen under high-pressure cultivation, Proceedings of the National Academy of Sciences 105, 10949-10954 (2008).
79. Ohmoto, H., Kakegawa, T. and Lowe, D. R. 3.4-billion-year-old biogenic pyrites from Barberton, South Africa: Sulfur isotope evidence, Science 262, 555-557 (1993).
80. Detmers, J., Bruchert, V., Habicht, K. S. and Kuever, J. Diversity of sulfur isotope fractionations by sulfate-reducing prokaryotes, Appl. Environ. Microbiol. 67, 888-894 (2001).
81. Habicht, K. S., Gade, M., Thamdrup, B., Berg, P. and Canfield, D. E. Calibration of sulfate levels in the Archean ocean, Science 298, 2372-2374 (2002).
82. Clarke, P. H. and Elsden, S. R. The earliest catabolic pathways, Journal of Molecular Evolution 15, 333-338 (1980).
83. Lambert, J.-F. Adsorption and polymerization of amino acids on mineral surfaces: A review, Origins of Life and Evolution of Biospheres 38, 211-242 (2008).
84. Amann, R., Ludwig, W. and Schleifer, K. Phylogenetic identification and in situ detection of individual microbial cells without cultivation, Microbiol. Rev. 59, 143-169 (1995).
85. Baker, B. J., Tyson, G. W., Webb, R. I., Flanagan, J., Hugenholtz, P., Allen, E. E. and Banfield, J. F. Lineages of acidophilic archaea revealed by community genomic analysis, Science 314, 1933-1935 (2006).
86. Kimura, H., Ishibashi, J.-I., Masuda, H., Kato, K. and Hanada,

S. Selective phylogenetic analysis targeting 16S rRNA genes of hyperthermophilic archaea in the deep-subsurface hot biosphere, Appl. Environ. Microbiol. 73, 2110-2117 (2007).
87. Takai, K., Miyazaki, M., Hirayama, H., Nakagawa, S., Querellou, J. and Godfroy, A. Isolation and physiological characterization of two novel, piezophilic, thermophilic chemolithoautotrophs from a deep-sea hydrothermal vent chimney, Environmental Microbiology 11, 1983-1997 (2009).
88. Suzuki, K., Nakamura, K., Kato, S. and Yamagishi, A. Experimental approach to obtain a comprehensive understanding of the biogeochemistry of a seafloor hydrothermal system, Journal of Geography (in Japanese) 118, 1131-1159 (2009).
89. Houghton, J., Seyfried, W., Banta, A. and Reysenbach, A. Continuous enrichment culturing of thermophiles under sulfate and nitrate-reducing conditions and at deep-sea hydrostatic pressures, Extremophiles 11, 371-382 (2007).
90. Postec, A., Lesongeur, F., Pignet, P., Ollivier, B., Querellou, J. and Godfroy, A. Continuous enrichment cultures: Insights into prokaryotic diversity and metabolic interactions in deep-sea vent chimneys, Extremophiles 11, 747-757 (2007).
91. Guindon, S. and Gascuel, O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood, Systematic biology 52, 696-704 (2003).
92. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., Mcgettigan, P. A., Mcwilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. Clustal W and Clustal X version 2.0, Bioinformatics 23, 2947-2948 (2007).