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

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

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

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

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. 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 H₂, H₂S, Fe²⁺, CO₂, CH₄, 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. 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 microbical 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. 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). 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).

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, Cu²⁺ and Zn²⁺), and volatiles (such as H₂, SO₂, CH₄ and CO₂) 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 H₂, H₂S, CH₄ and Fe⁺²) in the hydrothermal fluids and oxidants (such as O₂ and SO₂⁻²) in seawater generate chemical energy that could support the growth of various chemolithoautotrophic microorganisms in marine hydrothermal fields.

A marine hydrothermal field is thought to be one of the environments where ancient life thrived. 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. An ecosystem in marine hydrothermal fields does not require solar energy. However, it is noted that the ecosystem of present...
marine hydrothermal systems is not completely independent of photosynthetic ecosystems because the main electron acceptor, O₂, is produced by photosynthesis. Geological evidence indicates that microorganisms lived in ancient hydrothermal fields at 3.5-3.2 Gya. Filamentous microfossils were found in a massive ancient volcanogenic sulfide deposit and in rocks. The morphologies of the microfossils resemble some present-day filamentous thermophilic microorganisms detected in both terrestrial and marine hydrothermal fields. Massive sulfide deposits are commonly observed in the present hydrothermal fields. Methane with lighter carbon isotopic composition was detected in 3.5-billion-year-old hydrothermal precipitates. Sulfides with lighter sulfur isotopic composition recognized as products of sulfate reducing microorganisms were also found in 3.5-billion-year-old hydrothermal deposits. Notably, deep-branching Archaea in the phylogenetic tree contain anaerobic hyperthermophilic methanogens and sulfate reducing microorganisms. This fact suggests that these microorganisms retain the characteristics of ancient life on the early Earth. This notion is partially supported by experimental data demonstrating that ancestral enzymes showed high thermal stability. Regarding the physicochemical conditions of the ancient ocean (i.e., oxic or anoxic, hot or cold), which are controversial, 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 H₂ and Fe²⁺ that were generated by rock-water reactions, SO₂ and CO₂ degassed from magma, and carbon compounds (such as CH₄, methanol, formic acid, amino acids and peptides) that would be abiotically produced in the hydrothermal system. 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. 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$_2$S and Fe$^{2+}$ concentrations are controlled by phase equilibrium of diverse minerals in the rocks. 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. For example, higher concentrations of volatiles such as H$_2$, O$_2$ and minerals during the upwelling process. This reaction generates H$_2$S or H$_2$SO$_4$, 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. For example, higher concentrations of volatiles such as H$_2$, H$_2$S 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. 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. 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. The mechanism of magma generation at the hotspot is still controversial. 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 (~350-400°C) and low pH (~5). The basement of mid-ocean ridges consists of basalt (mafic rock); however, in some areas (e.g., Rainbow site in the Mid-Atlantic Ridge), the basement consists of peridotites (ultramafic 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.

Recently, a non-magmatic hydrothermal field, called the Lost City hydrothermal field, was discovered in the western Mid-Atlantic Ridge. 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 100°C 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$_3$O$_4$) and H$_2$O. High concentrations of hydrocarbons (such as methane, ethane, propane and butane) were detected in the fluids of the Lost City as well as the Rainbow site of a magmatic hydrothermal field of the Mid-Atlantic Ridge, 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.
Archaea revealed that diverse not-yet-cultivated techniques based on 16S rRNA gene sequences have limits of life. Culture-independent molecular of these extremophiles has expanded the known various hydrothermal fields. Unsurpassed physiology barophiles), have been cultured and isolated from and piezophiles (pressure-loving; also called thermophiles (heat-loving), acidophiles (acid-loving) 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. 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. 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 CO2 as a sole carbon source, 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 H2, H2S, Fe2+, CH4, NH4+, Mn2+, S0 and/or S2O32− as electron donors and O2, NO3−, SO42−, S2O32−, CO32−, and/or Mn4+ 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. 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., Pyrococcus furiosus 57), hydrogen-oxidizing sulfate-reducers (e.g., Archaeoglobus veneficus 58), and hydrogen-oxidizing iron-reducers (e.g., Geobacter shahamii 59), have been detected in various hydrothermal environments including mid-ocean ridges, back-arc basins and island-arcs. 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., O2) as opposed to respiration. Members of the Thermoprotei, a hyperthermophilic archaeal group, are often detected in hydrothermal environments. This taxonomic group includes fermenting species. 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. Recently, the whole genome sequence of a member of the Korarchaeota, "Korarchaeum cryptofilum", was determined by metagenomic analysis of an enrichment culture. This group had been detected in marine and terrestrial hydrothermal fields by culture-independent methods 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 archaean. 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. It should be noted that amino acids and peptides could be abiotically synthesized in marine hydrothermal systems and CO is provided by magma degassing. 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.

**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. 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. 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.
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, 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 (less than -20 kJ mol⁻¹ is favorable), the microorganism could grow using any of these energy-yielding reactions. As long as H₂, CO₂, CO, Fe³⁺ (potentially generated by photooxidation of Fe²⁺), SO₄²⁻ and S⁰ (potentially generated by photolysis of SO₂), amino acids and peptides 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 first suggested and Chapelle and coworkers 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 or tectonic activity. 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 H₂ concentration in the hydrothermal fluids) and microbiological (the predominance of hyperthermophilic methanogens) evidence. Ueno and his colleagues exhibited geological evidence for the presence of methanogens in an ancient hydrothermal environment. It is highly likely that H₂ and CO₂ 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 122°C. 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 by maximum-likelihood analysis of 1081 homologous positions in the alignment dataset generated using ClustalW, 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 Archaea 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 Archaeoglobi is the sole archaean 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 \(^{2,7}\). 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 hydrothermal 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 \(^{7}\). 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 \(^{15,24}\), 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 abiotically produced 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 not only on microbiological but also on other related scientific fields. Unrecognized, a 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 \(^{85}\). 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 *Archaean* 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.
The Marine Hydrothermal Vent Group-1 (MHVG-1), which is one of the deepest-branching archaeal lineages (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. 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 not be 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. Another useful method is a flow-type cultivation system called continuous culture or chemostat. In fact, several novel thermophiles have been cultured from marine hydrothermal samples using a flow-type cultivation system. 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 TAIQA: Trans-crustal Advection and In-situ biogeochemical processes of Global sub-seafloor Aquifer).

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