POSSIBLE OCCURRENCE OF SUBSURFACE LITHOTROPHIC MICROBIAL ECOSYSTEM (SLiME) SUSTAINED BY GEOLOGICALLY DERIVED ENERGY AND CARBON SOURCES: HYPERSLiME IN THE MID OCEAN RIDGES AND ALKALISLIME IN THE MARIANA FOREARC

K. TAKAI & F. INAGAKI

Subground Animalcule Retrieval (SUGAR) Project, Frontier Research System for Extremophiles
Japan Marine Science & Technology Center (JAMSTEC), Yokosuka, Japan

SUMMARY – Subsurface microbial communities supported by geologically derived energy and carbon sources fkom the earth's interior have great interest not only with regard to the nature of primitive life before photosynthesis, but as potential analogs for extraterrestrial life. Here we present the hypothesis that hyperthennophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) dominated by hyperthermophilic methanogens might be present beneath active deep-sea hydrothermal seafloors in the Mid Ocean Ridges (MOR) and that alkaliphilic subsurface lithotrophic microbial ecosystem (AlkaliSLiME) may occur in the deep subsurface biosphere of the serpentine-bearing seamounts in the Mariana Forearc. The existence of HyperSLiME has been recently proved in the geochemical and microbiological investigation of a deep-sea hydrothermal system in the Central Indian Ridge. Exploration of AlkaliSLiME is now underway with the samples obtained from the South Chamorro seamount by means of Ocean Drilling Project Leg#195 and *Shinkai6500* cruise.

1. INTRODUCTION

Recently, there has been an increasing interest in the subsurface biosphere in the Earth as a potential analogous habitat for extraterrestrial life, particularly in our own Solar System, where liquid water and hydrolithologicallyprovided energy and carbon sources may present in the subsurface (Stevens and McKinley, 1995; Kasting and Siefert, 2002; Chapelle *et al., 2002).* Based on terrestrial biology, this is not an unreasonable expectation: on Earth, chemolitho-autotrophic, even methanotrophic and carboxy-dotrophic, microorganisms can gain energy from a variety of reduced inorganic compounds coupling with electron acceptors such as molecular oxygen, nitrate, iron (111), sulphate, sulphur, or carbon dioxide. On today's Earth, these energy sources are readily generated by biotic processes, and many of them can also be supplied from the earth's interior directly associated with magmatism in volcanic and hydrothermal fields in tectonic margins and hot spots. In contrast to electron donors, the production of significant quantities of many electron acceptor species such as nitrate, metal oxides and sulphate ultimately requires the involvement of molecular oxygen, and is thus intimately linked to past or present photosynthesis on Earth. Thus, the finding of microbial ecosystems based on chemolithoauto-trophic primary producers utilizing photosyn-thesis-independent energy generation and carbon sources would have implications not only for understanding early earthly ecosystems prior to emerging photosynthetic life, but as models or potential analogs for geochemically active planets or moons. On the basis of geochemical and thermodynamic considerations, a combination of hydrogen and carbon dioxide might be the most abundant and effective energy and carbon sources in our Solar System, nevertheless the detection of subsurface microbial communities sustained by chemosynthesis (methanogens or homoacetogens) with photosynthesis-independent, lithospheric hydrogen and carbon dioxide has been equivocal and hotly contested.

Such a system was first reported by Stevens and McKinley in the deep crystalline rock aquifers of the Columbia River Basalt Group (CRB) (Stevens and McKinley, 1995). Based on geochemical and isotopic analyses of the groundwater chemistry, in vitro experiments of basalt-groundwater interaction and culture-based evaluation for microbial population of methanogens and acetogens, the authors proposed that the microbial ecosystem may be supported by primary production of mesophilic methanogens and acetogens utilizing hydrogen produced from in *situ* basalt-groundwater geochemical reaction (Stevens and McKinley, 1995). Anderson *et* al. (1998) argued against the hydrogen-based microbial ecosystems (SLiME hypothesis) in the deep CRB aquifers proposed, citing a lack of evidence supporting the production of hydrogen by the experiments of the basalt-groundwater interactions as proposed by Stevens and McKinley (1995). Subsequently, Chapelle *et al.* (2002) reported the detection of *Archaea*-dominating microbial communities in the groundwater system beneath the Lidy hot springs in Idaho by means of a combination of molecular techniques, and concluded that the microbial communities were derived fiom a hydrogen-based, subsurface lithoautotrophic microbial ecosystem dominated by methanogens. While archaeal rDNA were indeed present, there was no evidence presented either that predominants phylotypes seen were in fact methanogens, or that methanogenesis was in fact occurring, as hypothesized by the authors. In short, there was no cultivation or molecular probing to show abundance of such organisms, there was no isotopic analysis of the methane to suggest a biological origin, and there was no discussion of how a system with nM levels of H_2 could be producing mM amounts of CH_4.

2. HYPERTHERMOPHILIC SUBSUR-FACE LITHOAUTOTROPHIC MICRO-BIAL ECOSYSTEM (HYPERSLIME) BENEATH ACTIVE HYDROTHERMAL SEAFLOOR

Perhaps a more promising site for a hydrogen-driven **SLiME** might be located beneath an active deep-sea hydrothermal seafloor in the Mid Oceanic Ridge (MOR) spreading centers. These environments are located in sediment-poor areas, have very little input of organic carbon, and have the opportunity for production of abundant molecular hydrogen and carbon dioxide provided directly from degassing of magma or reaction between water and superheated rock (Figure 1; Von Damm, 1995). Possible occurrence of microbial communities beneath active deep-sea hydrothermal vent systems (subvent biosphere) has been proposed on the basis of observations of microbial expulsion of hyperthermophiles immediately after submarine volcanic eruptions (Deming and Baross, 1993; Delaney *et al.*, 1998) and of distribution profile of bio-molecules in the deep-sea hydrothermal vent environment (Takai and Horikoshi, 1999; Takai *et al.*, 2001b; Takai and Fujiwara, 2002). Direct measurements to the indigenous microbial communities in the superheated hydrothermal emissions and inside the sulfide chimneys (Takai and Horikoshi, 1999; Huber *et al.*, 2002) and the potential subsurface microbial communities in the core samples penetrating beneath hydrothermal seafloor (Kimura *et al.*, 2003) have also supported the presence of a subvent biosphere. Most of the microbial components determined so far are hyper-thermophilic chemolithoorganotrophs obtaining energy from organic compounds probably provided fiom chemosynthetic microbial and animal communities utilizing photosynthesis-derived electron acceptors (molecular oxygen, nitrate and sulphate) in the near surface zone of habitats. Superheated vent emissions, with minimal dilution by seawater, probably entrain the microbial components indigenous to the subsurface hydrothermal water-rock interface in a temperature range permitting microbial growth (up to around 120 °C; Figure 1). In such systems, the input of photosynthetically-derived energy and carbon sources will be negligible, and molecular hydrogen and carbon dioxide from the earth's interior should be primary energy and carbon sources. However, previous studies of undiluted vent emission have shown that it contains limited information about the subvent biosphere because of the paucity of microbial cells and microbial products (Takai and Horikoshi, 1999; Takai *et al.*, 2003), almost certainly due to the very high temperature (>300 °C), unhibiting the growth and survival of any entrained microbes.

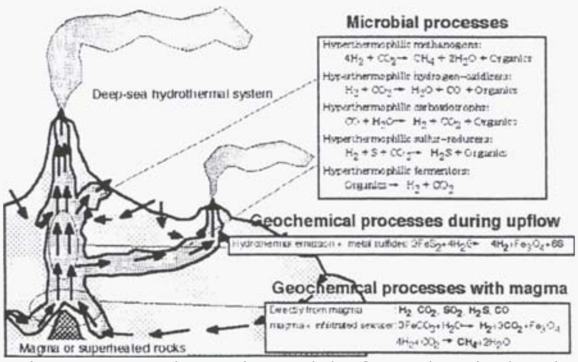

Figure 1. A schematic model of geochemical and microbiological processes for the occurrence of HyperSLiME beneath active hydrothermal seafloor.

One approach we have taken to address this problem has been the fabrication of a microbial habitat called an *in situ* colonization system (ISCS), designed to trap and contain cellular material in the vent waters (Takai *et al.*, 2003). In recent studies of the vent fields of the Central Indian Ridge (CIR) we analysed the geochemical and isotopic properties of the vent waters, and deployed the ISCS systems at several sites. The combined geochemical, isotopic, and microbiological evidence to support the idea of an active HyperSLiME community, one that is existing primarily on hydrogen as an energy source, CO_2 as the carbon source, and producing methane as the major product.

The chemical compositions of the vent emissions from the two chimneys were in keeping with the hypothesis that the two different chimney sites have the same hydrothermal fluid origin based on characterization of major chemical composition, but the isotopic composition of the methane in the two sites showed that the methane of the divergent flow was significantly lighter, and suggested to us a biological contribution to the methane at least beneath the divergent vent site.

Microscopic cell counts revealed that the ISCS successfully captured cells: the cell counts of the vent fluid fiom the main vent was ~ 10^3 ml^{-1}, while the ISCS material contained ~ 10^7 g^{-1}. ***Thermococcales*** members were the most predominant components of the viable microbial population in all the ISCSs. The second most abundant viable components were thermophilic to hyperthermophilic ***Methanococcales.*** As the distance fiom the vent increased, the proportion of the total hyperthermophilic ***Methanococcales*** decreased.

Cultivation independent methods were also used to assess the proportion of various groups present, and yielded results consistent with the cultivation experiments. The proportion of rDNA that could be attributed to ***Archaea,*** on the basis of clone sequencing and quantitative PCR (Takai and Horikoshi, 2000), was highest in the hottest vent (>99%), dominant in the divergent vent ISCS (>55%), and minor in the samples away fiom the vent. Of the archaeal clones that were obtained and identified, the major types that were found were ***Methanococcales,*** with ***Thermococcales*** always present as a minor component. This was consistent with the percentage of the cell counts determined by fluorescence in situ hybridization (FISH) analysis, which showed the ***Methanococcales*** being the dominant community member in the vent fluid samples.

The combination of geochemical, isotopic, and microbiological (both cultivation dependent, and cultivation independent) results made a very strong case for the existence of a viable HyperSLiME community in the subvent region of the CIR. The nature and extent of such communities on a global basis remains to be determined, but judging from reports of methane isotopic results from other MOR deep-sea hydrothermal systems (Shanks, 2001), there may well be other similar systems waiting to be found; they may be the rule rather than the exception in the CIR hydrothermal fields.

3. ALKALIPHILIC SUBSURFACE LITHOTROPHIC MICROBIAL ECO-SYSTEM (ALKALISLIME) BENEATH SERPENTINE SEAMOUNTS

Deep-sea hydrothermal seafloor and its subseafloor terrain in the tectonic margins are considered to be the primary regions on Earth where the potential energy and carbon sources for the active subsurface biosphere are constantly provided fkom the Earth's interior. However, some places other than hydrothermal systems associated with magmatism, like the subducting oceanic lithosphere occurring in the Mariana Forearc, could serve as a host for SLiME on Earth where the geochemical and mineralogical components are being generated and recycled. Called a 'Subduction Factory', the Mariana subduction system composed of the subducting oceanic slab and the upper mantle generates liquid water, carbon dioxide, hydrogen, methane, and other reduced chemical components under much lower temperature conditions than hydrothermal systems, corresponding to the following reactions (Dobson ***et al.***, 2002; O'Hanley, 1996):

$Mg_{48}Si_{34}O_{85}(OH)_{62}$ (Antigorite: scaly serpentine) = $14MgSiO_4$ (Olivine) + 20 $MgSiO_3$ (Enstatite) +31 H_2O

Peridotite (Olivine, orthopyroxene, clinopyroxene, spinel, garnet) + H_2O = Serpentine + H_2 + Magnetite

During this process, called serpentinization, a part hydrogen gas may reduce coexisting carbon dioxide to produce methane or hydrocarbon by the Fischer-Tropsch reaction in presence of metal oxides as catalysts (Berndt ***et al.,*** 1996).

$CO_2 + 4H_2 = CH_4 + 2H_2O$
$2CO_2 + 3H_2 = 2CH_2COOH$

Although these geochemical reactions occur in deeper zone of the crust near the upper mantle, the chemical products can be transported with the serpentine diapir due to their buoyancy. In fact, the geochemical analysis of the sediments and core-liner gases in the south Chamorro seamount, ODP leg. 195 suggested that high concentrations of the C_1 through C_6 hydrocarbons (presumably produced by the Fisher-Tropsh reaction) were rising upward with deep upwelling fluids (Leg. 195 Shipboard scientific party, 2002). Such geochemically produced substrates including water, hydrogen, carbonate, methane and hydrocarbons could supply the needed energy and carbon sources for chemolithoautotrophic or hydrocarnonotrophic microorganisms and support the microbial activities in deep subseafloor environments.

Another outstanding physical aspect defining the subseafloor serpentine biosphere is the unusual high alkaline pH of the interstitial water (Leg. 195 Shipboard scientific party, 2002), a maxim pH of 12.5 was determined in the subseafloor serpentine mud of South Chamorro seamount. This is the world's highest pH known value in naturally occurring environments and exceeds the highest pH limit for growth of life (Takai ***et al.***, 2001a). As compared to the generation mechanisms of various hydrolithospheric energy and carbon sources, the formation process of the deep subseafloor alkaline water is still unclear. It is now hypothsized that extra amounts of hydroxyl ion dissociation occur during the dehydration and serpentinization processes and that supersaturated carbonate ions with extraordinary high pressures may cause the highly elevated pH in the serpentine diapir. In addition, the acidifying effect of CO_2 and bicarbonate ion may be strongly excluded by the rising fluid flow. These geochemical settings might provide an extremely alkaline microbial habitat for the deep serpentine biosphere.

On the basis of the geological and geochemical characteristics described above, we hypothesize that an alkaliphilic subseafloor lithoautotrophic microbial ecosystem that we call 'Alkali-SLiME' exists beneath the serpentine seamount in the Mariana Forearc. The South Chamorro seamount is located in the southwestern edge of the Mariana foerarc graben and is the only previously known site of active serpentine/blueschist mud volcanism in the world (Fryer, 1996; Fryer and Mottl, 1997) (Figure 2). Bathymetric and petrologic features of the serpentine seamounts have been well characterized investigated by dredged holes, submersible dives, and ODP leg. 195 (Figure 2).

During the ODP leg. 195, a total of six holes were drilled at the south Chamorro seamount (Site 1200). Geochemical analyses and microbiological sampling were performed using three holes, 1200D (22.0 m), 1200E (54.4m), and 1200F (16.3 m). The in situ temperatures measured during Leg. 195 seem to be low for prosperous occurrence of a chemolithotroph-dependent, hyper-alikaliphilic microbial ecosystem (eg. 2.0 °C at 41 mbsf at 1200A). To sustain an active microbial loop of the deep subseafloor ecosystem starting from primary production by methanogens based on hydrogen geologically provided from serpentinization an6 re-energized by the subsequent fermentative decomposition, at least moderate temperatures of habitats (>30 "C) are likely necessary since most of the presently known hydrogen-dependent methanogenic and fermentative microorganisms grow well above **30** °C. Based on the temperature gradient in the south Chamorro seamount calculated (0.01-0.07 °C/m), over 3,000 m depth will be required to prove the hypothesis for the presence of a previously unknown, discrete 'Alkali-SLiME' in deep serpentine biosphere. Geochemical products generated by serpentinization processes and the Fisher-Tropsh reactions are almost certainly continuously supplied to the environment, where they can be available for the chemolithoautotrophic and hydrocarbonotrophic microorganisms under hyper-alkaline conditions, and we fully expect that such microbial communities will be present in the south Chamorro deep subseafloor environments (Figure **3**).

We are now proposing a drilling expedition of AlkaliSLiME that we call: "Deep-STAR (Deep-

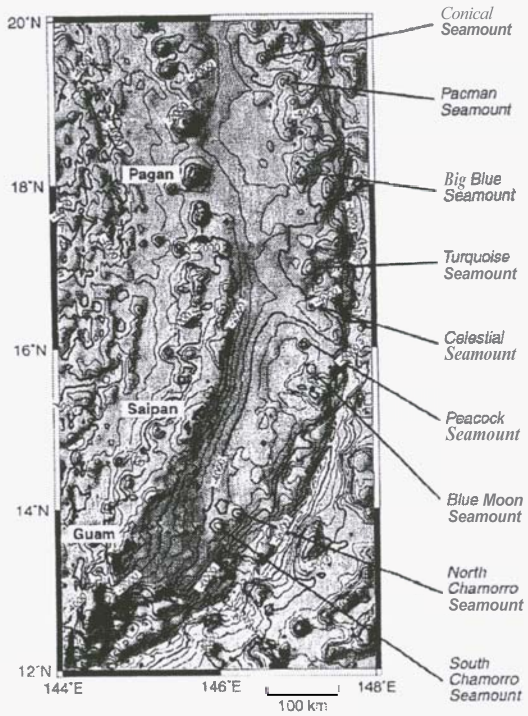

Figure 2. Bathmetry map of the southern Mariana forearc.

Subseafloor Traversal for Animalcule Retrieval) Cruise", at the south Chamorro serpentine seamount in the Mariana Forearc. Preliminary site survey for microbial components, potentially indigenous and entrained from deep subseafloor with upwelling serpentine diapir, will be conducted by means of the manned submersible *Shinkai6500* in 2003. The detection and abundance of alkaliphilic methanogens, methanotrophs and hydrocarbonotrophs will be the primary scientific objective. The microbiological site survey will proceed the successful future operation of our proposing "Deep-STAR Cruise".

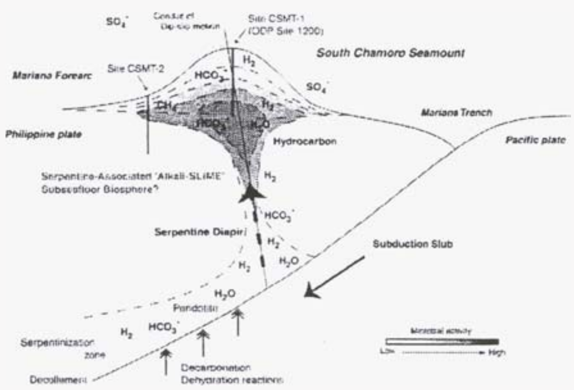

Figure **3**. Schematic figure of possible presence of serpentine-associated Alkali-SLiME subseafloor biosphere beneath the south Chamorro seamount.

4. CONCLUSION

We present here the hypothesis that hyperthermophilic subsurface lithoautotrophic microbial ecosystem (HyperSLiME) dominated by hyperthermophilic methanogens might be present beneath active deep-sea hydrothermal seafloor in the Mid Ocean Ridges (MOR) and that alkaliphilic subsurface lithotrophic microbial ecosystem (AlkaliSLiME) may occur in the deep subsurface biosphere of the serpentine seamounts in the Mariana forearc. The existence of HyperSLiME has been recently proved in the geochemical and microbiological investigation of a deep-sea hydrothermal system in the CIR. As described above, the existence of HyperSLiME is likely assumed as widely distributed in the global MOR spreading center rather than as the exceptional incidence in the CIR. In addition to the drilling expedition of "Deep-STAR Cruise" at the south Chamorro serpentine seamount, we have been proposing another drilling expedition for exploring the subvent biosphere beneath the sediments-hosted, backarc-lifting deep-sea hydrothermal systems in the Okinawa Trough. It is already apparent that the very diverse and complex subvent biosphere, a different mode of subvent microbial world, might be embedded beneath the matured hydrothermal seafloor (Takai *et al.,* 2002; Takai *et al.,* 2003; Takai and Inagaki, 2003). The future operation of the proposing drilling expedition will provide further important insight into elucidation of the microbial ecosystem and the geochemical impact associated with deep-sea hydrothermal systems.

On a final note, we cite another subsurface environment promising the occurrence of SLiME. "The Lost City" hydrothermal field, discovered in 2001 at an off-axis of the Mid Atlantic Ridge **(MAR)** along the Atlantic Transform Fault (ATF) (Kelley *et al.,* **2001),** probably hosts a chimeric SLiME of HyperSLiME and AlkaliSLiME. The hydrothermal activity in the Lost City was completely different from other on-axis hydrothermal activities in the MAR and CIR because the hydrothermal liquid circulation of the Lost City is not at all involved in magmatism (Kelley *et al.,* 2001). This implies that the hydrothermal activity in the Lost City is substantially derived fiom the hydrolithic interaction directly with the upper mantle, peridotite but not with the basalt. This hydrolithic process is a serpentinization, completely identical to the geological and geochemical process assumed in the deep subsurface of the serpentine seamounts in the Mariana Forearc. One can briefly assume that a hyperthermophilic, alkaliphilic SLiME must be present just below the Lost City hydrothermal system. The off-axis area in the MOR located on similar geological geochemical settings as in the Lost City were the potential Garden of Eden for the primordial life in the Precambrian Era together with the MOR spreading centers and the subduction factories. These places still harbour the descendants fiom the Garden of Eden. Similar places are known to exist uniformly in other planets and moons of our Solar System (Mckay, 2001). Already, extraterrestrial SLiME is a topic in science and no more in science fiction.

5. REFERENCES

Anderson, R.T., Chapelle, F.H. and Lovley, D.R. (1998). Evidence against hydrogen-based microbial ecosystems in basalt aquifers. *Science,* Vol. 281,976-977.

Berndt, M.E., Allen, D.E. and Seyfried Jr., W.E. (1996). Reduction of CO_2 during serpentinization of olivine at 300 °C and 500 bar. *Geology,* Vol. 24,351-354.

Chapelle, F.H. *et al.* (2002). **A** hydrogen-based subsurface microbial community dominated by methanogens. *Nature,* Vol. 415, 312-315.

Delaney, J.R. *et al.* (1998). The quantum event of oceanic crustal accretion: impacts of diking at Mid-Ocean Ridges. *Science,* Vol. 281,222-230.

Deming, J.W. and Baross, J.A. (1993). Deep-sea smokers: Windows to a subsurface biosphere. *Geochim. Cosmochim. Acta,* Vol. 57,3219-3230.

Dobson, D. *et al.* (2002). Simulation of subduction zone seismicity by dehydration of serpentine. *Science,* Vol. 298, 1407-1410.

Fryer, **P.** (1996). Evolution of the Mariana convergent plate margin system. ***Rev.*** *Geophys.,* Vol. **34,** 89-125.

Fryer, **P.** and Mottl, M.J. "Shinkai 6500" investigation of a resurgent mud volcano on the Southeastern Mariana forearc. *JAMSTEC Deep Sea Res.,* Vol. 13, 103-114.

Huber, **J.A.,** Butterfield, D.A. and Baross, J.A. (2002). Temporal changes in archaeal diversity and chemistry in a Mid-Ocean Ridge subseafloor habitats. *Appl. Environ. Microbiol.,* Vol. 68, 1585-1594.

Kasting, J.F. and Siefert, J.L. (2002). Life and the Evolution of Earth's. *Science,* Vol. 296, 1066-1068.

Kelly, **D.S.** *et al.* (2001). ***An*** off-axis hydrothermal vent field near the Mid Atlantic Ridge at 30°N. *Nature,* Vol. 412, 145-149.

Kimura, H. *et al.* (2003). Distribution of microorganisms in the Subsurface of the Manus Basin Hydrothermal Vent Field in Papua New Guinea. *Appl. Environ. Microbiol.,* Vol. 69, 644-648.

Leg. 195 Shipboard Scientific Party. (2002). ***Mariana convergent margin/West Philippine seaseismic observatory.*** Proc. ODP Int. Rep. 195.
Mckay, C.P. (2001). The deep biosphere: lessons for planetary exploration. In: Subsurface microbiology and biogeochemistry, J.K. Fredrickson and M. Fletcher (Eds)., Wiley, New York, pp. 315-327.

O'Hanley, D.S. (1996). ***Serpentinites: Records of petrologic and tectonic history.*** Oxford Univ. Press, New York.

Shanks, 111, K.L. (2001). Stable isotopes in seafloor hydrothermal systems. In: ***Stable isotope geochemistry,*** J.W. Valley and D.R. Cole (Eds)., Mineralogical Society of America, Washington DC, pp. 469-526.

Stevens, T.O. and McKinley, J.P. (1995). Lithoautotrophic microbial ecosystems in deep basalt aquifers. ***Science,*** Vol. 270,450-454.

Takai, K. and Horikoshi, K. (1999). Genetic diversity of archaea in deep-sea hydrothermal vent environments. ***Genetics,*** Vol. 152, 1285–1297.

Takai, K. and Horikoshi, K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. ***Appl. Environ. Microbiol.,*** Vol. 66, 5066-5072.

Takai, K. and Fujiwara, Y. (2002). Hydrothermal vents: biodiversity in deep-sea hydrothermal vents. In: ***Encyclopedia of Environmental Microbiology, G.*** Bitton (Ed)., Vol. 3, Wiley, New York, pp. 1604-1617.

Takai, K. and Inagaki, F. (2003). Deep biosphere and hydrothermal activity: potential roles in the co-evolution of earth and life. ***J. Geography,*** Vol. 112,234-249 (Japanese with English abstract).

Takai, K. ***et al.*** (2001a). ***Alakaliphilus transvaalensis*** gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from a deep South African gold mine. ***Int. J. System. Evol. Microbiol.,*** Vol. 51, 1245–1256.

Takai, K. ***et al.*** (2001b). Distribution of archaea in a black smoker chimney structure. ***Appl. Environ. Microbiol.,*** Vol. 67, 3618–3629.

Takai, K., Inoue, A. and Horikoshi, K. (2002). ***Methanothermococcus okinawensis*** sp. nov., a thermophilic, methane-producing archaeon isolated from a Western Pacific deep-sea hydrothermal vent system. ***Int. J. System. Evol. Microbiol.,*** Vol. 52, 1089-1095.

Takai, K. ***et al.*** (2003). Isolation and phylogenetic diversity of members of previously uncultivated ε-***Proteobacteria*** in deep-sea hydrothermal fields. ***FEMS Microbiol. Lett.,*** Vol. 218, 167-174.

Von Damm, K.L. (1995). Controls on the chemistry and temporal variability of seafloor hydrothermal fluids. In: ***Geophysical Monograph 91 Seafloor Hydrothermal System,*** S.E. Humphris ***et al.*** (Eds)., American Geographical Union, Washington DC, pp. 222-247.