

## Diversity of microbial metalloid transformation pathways and its geochemical implications

\*Natsuko Hamamura<sup>1</sup>, Sathoshi Mitsunobu<sup>2</sup>

1.Dept. Biology, Faculty of Science, Kyushu University, 2.Faculty of Agriculture, Ehime University

Arsenic (As) and antimony (Sb) are both naturally occurring toxic elements and are considered to be priority pollutants of interest by the USEPA. Although the concentrations of these toxic metalloids in natural systems are generally low ( $\sim 15 \mu\text{g g}^{-1}$  As and  $< 1 \mu\text{g g}^{-1}$  Sb in soils [1]), the elevated levels of As and Sb have been released via natural processes and human activities. Antimony is commonly associated with As in the environment and both elements have similar chemistry and toxicity. Antimony and arsenic can exist in four oxidation states (-III, 0, III and V), while they are mainly found in two oxidation states, trivalent (III) and pentavalent (V) in natural systems. Antimonate [Sb(V)] and arsenate [As(V)] are the thermodynamically stable species in aerobic environments and occur primarily as  $\text{H}_2\text{AsO}_4^-$  and  $\text{HAsO}_4^{2-}$ , or  $\text{Sb}(\text{OH})_6^-$ . In anaerobic environments, the dominant solution species of antimonite [Sb(III)] and arsenite [As(III)] occur as the neutral  $\text{Sb}(\text{OH})_3^0$  and  $\text{As}(\text{OH})_3^0$  at the environmentally relevant pH range. As(III) and Sb(III) are considered more toxic than As(V) and Sb(V) [1].

Despite its toxicity, microorganisms have developed mechanisms to tolerate and utilize these elements for respiratory metabolism. Although many microorganisms have been identified to catalyze As transformations, we have just begun to unveil the full diversity of microbial processes associated with As and Sb geochemical cycling in the environment. In this study, we characterized metalloid transformation pathways associated with As and Sb-impacted environments. The presence of indigenous microbial populations capable of metalloids transformation was examined by using both molecular approach targeting As functional genes and cultivation approach. The genes coding for arsenite oxidase (*aioA*), which catalyzes the oxidation of As(III) coupled to  $\text{O}_2$  reduction, have been recovered from soils from mine tailing. Successful cultivation of various As(III)-oxidizing bacteria confirmed the microbial attribute in As oxidation. In contrast, diverse sequences of anaerobic arsenite oxidase (*arx*) and arsenate respiratory reductase (*arr*) genes were detected from the As impacted lake sediments, while no *aio* genes were recovered. The anaerobic arsenite oxidase, Arx, is known to catalyze arsenite-oxidation coupled to nitrate reduction or photosynthesis. Consistent with the molecular analysis, an anaerobic arsenite-oxidizing nitrate reducer and an arsenate-reducing bacterium were isolated from the lake sediments. The indigenous microbial population associated with Sb transformation was also identified by successful cultivation of aerobic Sb(III)-oxidizing *Pseudomonas*- and *Stenotrophomonas*-related isolates from the mine tailing soils. In addition, anaerobic enrichment cultures capable of reducing Sb(V) were also obtained, in which the precipitation of antimonite as antimony trioxide was observed. This study revealed the diversity and distribution of microbial metalloid redox metabolisms associated with the polluted environments, indicating their contribution to the speciation and mobility of As and Sb *in situ*.

[1] M. Filella, N. Belzile, Y.-W. Chen. Earth Sci. Rev. 57:125-176. (2002)

Keywords: Arsenic, Antimony, arsenite oxidase, arsenate reductase

## Regional variation of CH<sub>4</sub> and N<sub>2</sub> production processes in the deep aquifers of an accretionary prism

\*Makoto Matsushita<sup>1</sup>, Shugo Ishikawa<sup>2</sup>, Yuichiro Hirata<sup>2</sup>, Kenta Magara<sup>2</sup>, Hiroyuki Kimura<sup>3</sup>

1.Department of Environment and Energy Systems, Graduate school of science and technology, Shizuoka university, 2.Department of sciences, Graduate school of integrated science and technology, Shizuoka university, 3.Research Institute of Green Science and Technology, Shizuoka University

Methane (CH<sub>4</sub>), reserved in the subsurface environments such as natural gas and methane hydrates, have recently attracted considerable attention as the important greenhouse gas and energy resource. It is generally considered that CH<sub>4</sub> in subsurface environments is mainly generated by methanogenic archaea (biogenic origin) or through thermal degradation of organic molecules in the sediments (thermogenic origin). The processes of CH<sub>4</sub> production have been investigated by geochemical and microbiological studies at various surface and subsurface environments. Furthermore, it has been reported that a large amount of CH<sub>4</sub> is present in the deep aquifers associated with accretionary prism.

Accretionary prisms are thick sediments that are formed at a convergent plate boundary, and it is observed around the world. In Southwest Japan, accretionary prism is distributed in a wide region from the coastal area of the Pacific Ocean side to the mountainous area. Since the sediment contains layers of water-bearing permeable sandstone, groundwater is anaerobically reserved in the deep aquifers. In addition to the groundwater, it has been reported that a large amount of the natural gas, mainly CH<sub>4</sub> and nitrogen gas (N<sub>2</sub>) is present in the deep aquifers. However, the processes of CH<sub>4</sub> and N<sub>2</sub> production in the deep aquifers associated with accretionary prism is poorly understood. The objectives of this study were to identify the origin of the CH<sub>4</sub> that is reserved in the deep aquifers on the basis of the carbon stable isotope analysis. We also determined the processes and potential of microbial CH<sub>4</sub> and N<sub>2</sub> productions using 16S rRNA gene analysis and culture experiments.

The groundwater and natural gas derived from the deep aquifers of accretionary prism were collected from 14 deep wells situated in Shizuoka Prefecture, Japan. CH<sub>4</sub> was the predominant component of the natural gas derived from deep aquifer of the coastal and middle areas of the accretionary prism (>96 vol.%). In contrast, the natural gas collected from deep aquifer of the mountainous area included a considerable amount of N<sub>2</sub> (23-50 vol.%) as well as CH<sub>4</sub>. The stable carbon isotope analysis of CH<sub>4</sub> in the natural gas and the DIC in the groundwater, mainly bicarbonate, showed that CH<sub>4</sub> included in the natural gas of the coastal area is thermogenic origin. On the other hand, it was suggested that the natural gas of the middle and mountainous areas mainly contains CH<sub>4</sub> of biogenic origin. Next generation sequencing analysis of bacterial and archaeal 16S rRNA genes showed the dominance of methanogenic archaea, fermentative bacteria, and denitrifying bacteria in the groundwater. Furthermore, high potential of CH<sub>4</sub> production through anaerobic degradation of organic substrates by syntrophic consortium of H<sub>2</sub>-producing fermentative bacteria and H<sub>2</sub>-using methanogenic archaea was observed from anaerobic cultivation using the groundwater samples collected from the middle and mountainous areas of the accretionary prism. In addition, high potential of N<sub>2</sub> production by denitrifying bacteria was also confirmed from anaerobic cultivation using the groundwater samples collected from the mountainous area.

From these results, it was suggested that CH<sub>4</sub> is produced by a thermogenic process especially in the deep aquifer of the coastal area associated with the accretionary prism, and H<sub>2</sub>-producing fermentative bacteria and H<sub>2</sub>-using methanogenic archaea contribute to significant CH<sub>4</sub> production in the deep aquifer of the middle and mountainous areas. Our results also suggest that N<sub>2</sub> production

by denitrifying bacteria occurs particularly in the deep aquifer of the mountainous area.

Keywords: accretionary prism, deep aquifer, methanogens, fermentative bacteria, denitrification

## Evidence of microbial methane generation in the deep aquifer of accretionary prism in southern Okinawa Island, Japan

\*Kenta Magara<sup>1</sup>, Makoto Matsushita<sup>2</sup>, Shuugo Ishikawa<sup>3</sup>, Yuichiro Hirata<sup>1</sup>, Hiroyuki Kimura<sup>4</sup>

1.Department of Geoscience Shizuoka, University , 2.Department of Environment and Energy Systems, Graduate school of science and technology, Shizuoka university, 3.Department of science, Graduate school of integrated science and technology, 4.Reserch Institute of Green Science and Technology, Shizuoka University

The accretionary prism situated the Pacific Ocean side of Southwest Japan forms thick sediment that accretes onto the nonsubducting tectonic plate at a convergent plate boundary. The sediments contain a deep aquifer that a large amount of anaerobic groundwater and methane are accumulated. The methane is mainly generated by methanogenic archaea (biogenic origin) or thermal degradation of organic matter (thermogenic origin). Anaerobic groundwater and natural gases are observed from in the deep aquifers of the accretionary prism in Shizuoka Prefecture, Miyazaki Prefecture and Okinawa Island, Southwest Japan. The methane, which was collected from deep aquifer in Shizuoka and Miyazaki Prefectures, is composed biogenic origin and thermogenic origin. The methane of biogenic origin is mainly generated by syntrophic consortium of  $H_2/CO_2$ -producing fermentative bacteria and  $H_2/CO_2$ -using methanogenic archaea in deep aquifer of accretionary prism. However, methane generation process in the deep aquifer of accretionary prism in southern Okinawa Island has not been investigated. Therefore, the aim of this study was to identify the methane generation mechanism in the deep aquifer accretionary prism in southern Okinawa Island. In this study, we measured environmental parameters and dissolved ion concentrations of the deep groundwater. To identify the origin of methane, we performed stable carbon isotopic analysis. Furthermore, we also determined the potential of microbial methane production by anaerobic culture experiments and 16S rRNA gene analysis.

The groundwater and natural gas samples were collected from 4 deep wells constructed in southern Okinawa Island. The chemical analysis of the groundwater suggested that the deep aquifers are affected by seawater or seawater and rainwater. Analysis of the natural gas showed that methane is more than 93 vol% in all gas samples. Stable carbon isotopic analysis of methane in the natural gas and dissolved inorganic carbon (mainly bicarbonate) in the groundwater suggested that the methane was derieved from both thermogenic and biogenic origin. Analysis of 16S rRNA gene showed that the dominance of  $H_2/CO_2$ -producing fermentative bacteria and  $H_2/CO_2$ -using methanogenic archaea in the groundwater. Anaerobic cultivation targeting syntrophic consortium of the fermentative bacteria and the methanogenic archaea revealed high potential of microbial methane generation was obserbed in all groundwater samples.

From these results, it was shown that the methane generation is conducted by syntrophic consortium of  $H_2/CO_2$ -producing fermentative bacteria and  $H_2/CO_2$ -using methanogenic archaea in the deep aquifer of accretionary prism in southern Okinawa Island. Furthermore, our results suggested that methane generation has been conducted by subterranean microbial community in deep aquifer of accretionary prism in wide range of the Pacific Ocean side of Southwest Japan.

Keywords: accretionary prism, deep aquifer, methane production, fermentative bacteria, methanogenic archaea, syntrophic consortium

## Dry heat tolerance of a terrestrial cyanobacterium, *Nostoc* sp. HK-01

\*Shunta Kimura<sup>1</sup>, Kotomi Inoue<sup>1</sup>, Hiroshi Katoh<sup>2</sup>, Seigo Sato<sup>1</sup>, Kaori Tomita-Yokotani<sup>1</sup>

1. Graduate School of Life and Environmental Sciences, University of Tsukuba, 2. Life Science Research Center, Mie University

Terrestrial cyanobacteria have played an important role in carbon and nitrogen circulation on the earth since ancient times. Terrestrial cyanobacteria have a dry heat tolerance from 60 to over 100°C, but the detailed function of the tolerance has not yet been determined. Dry heat tolerance is important for terrestrial cyanobacteria to survive on land. A terrestrial cyanobacterium, *Nostoc* sp. HK-01 has a dry heat tolerance up to 100°C for 10 h. We elucidated that akinete (dormant cell) is the cell type which has a tolerance to dry heat in *Nostoc* sp. HK-01. Some active substances may exist in the akinete. Here, we will show a candidate active substance related to dry heat tolerance in *Nostoc* sp. HK-01.

Keywords: Akinete, Dry heat tolerance, *Nostoc* sp. HK-01, Terrestrial cyanobacteria

Microbial community succession and hydrogen utilization in hydrogen-gas rich hydrothermal plume of Kairei field, CIR

\*Michinari Sunamura<sup>1</sup>, Akira Ijiri<sup>2</sup>

1.University of Tokyo Dept. of Earth & Planetary Science, 2.JAMSTEC

Deep sea hydrothermal plume is important interface between solid earth and ocean. In the hydrothermal plume, various and large energy is yielded for chemolithoautotrophic microorganisms through the oxidation-reduction reaction between the reduced chemicals in the hydrothermal fluids and oxygen in the seawater. The microbial community structures varied among different hydrothermal systems (Sunamura and Yanagawa 2015) and epsilon proteobacteria has been specifically detected in the hydrogen-rich hydrothermal plume of Kairei hydrothermal field (KHF). Here, I report the microbial community structure in the hydrothermal plume and statistical analysis based on physical, chemical, and biological parameters in the hydrothermal plume of KHF.

As results, we found 16 microbial families, e.g. *Cenarchaeaceae* (Archaea), *Helicobacterceae* (epsilon proteobacteria), SUP05 (gamma proteobacteria), SAR324 (delta proteobacteria), and 3 methylotrophs, in 251 microbial families are determined to be the active and important microbial groups in the hydrothermal plume. Based on the ratio (20/1) of hydrogen and methane gas in the end member fluids of KHF, we estimate the consumed hydrogen gas from the hydrogen and methane gas concentration in each hydrothermal plume samples. The estimated consumed hydrogen gas is strongly correlated with *Helicobacterceae* population ( $R=0.65$ ,  $n=28$ ). Relatives of *Helicobacterceae* are known to utilize hydrogen gas/reduced sulfur compounds as electron donor. Our result suggest the *Helicobacterceae* in the hydrothermal plume of KHF obtain energy from hydrogen gas and the variation of H<sub>2</sub>/CH<sub>4</sub> ratio in the plume would due to their activity.

Keywords: deep sea hydrothermal plume, microbes, hydrogen gas

## Hyperpycnal sediment in Lake Inawashiro, northeast Japan

\*Yoshio Inouchi<sup>1</sup>, Takashi Suzuki<sup>1</sup>

1.Faculty of Human Sciences, Waseda University

In Lake Inawashiro, northeastern Japan, there are characteristic layers which show relatively thick dark brown color underlain by white layer. These layers have, in general, few centimeters in thickness and differ in thickness from main sediment which have a few millimeter in thickness. In this presentation, we discuss the origin of these characteristic sediments, namely event sediments. Lake Inawashiro, in central Fukushima prefecture, is an acidtrophic lake and has an area of 103.24km<sup>2</sup>, maximum depth 94.6m and mean depth 51.5m. Main part of the cored sediment is composed of bar code like sediments with thin dark and light colored layers. In addition to several tephra layers, about 30 characteristic layers were observed. Grain size measurement and sediment grain observation using microscope were carried out in each 5mm thick sample. The result shows that reverse grading at lower part and normal grading at upper part, and that the characteristic layers sometimes contain plant fragments and diatom fossils in addition to many mineral grains. Preliminary observation of diatom assemblages in characteristic layers show presence of attached diatom and acidophilic diatom, however, further observation is needed. In one of the characteristic layers, inner sedimentary structure of erosion between light colored layer and dark layer. Furthermore, diatom assemblages of general thin alternations of light and dark layers show only presence of planktonic diatoms. In conclusion, these facts show the characteristics of hyperpycnal flow deposits. Consequently, those event sediments in Lake Inawashiro are derived by hyperpycnal flow caused by abrupt drastic flooding

Keywords: Lake Inawashiro, hyperpycnal, event sediment, lake bottom sediment

## "Hot spot of methanogenesis" on the deep-seafloor after the mega-earthquake

\*Eiji Tasumi<sup>1</sup>, Hidetaka Nomaki<sup>1</sup>, Katsunori Yanagawa<sup>2</sup>, Yuuta Konno, Sanae Sakai<sup>1</sup>, Miho Hirai<sup>1</sup>, Katsunori FUJIKURA<sup>1</sup>, Takuro Nunoura<sup>1</sup>, Ken Takai<sup>1</sup>

1.Japan Agency for Marine-Earth Science and Technology, 2.Kyushu University

We investigated the deep-sea microbial ecosystem after the 2011 Off Tohoku Earthquake and tsunami. In the series of study, we found several "hot spot of methanogenesis" on the deep-seafloor after the mega-earthquake and demonstrated the methylotrophic methanogenesis in the deep-sea surface sediment.

On the 2011 cruise, we found a lot of large microbial mats on the 5,000 m-depth deep-seafloor. The surface sediment cores (< 25 cm) were collected from microbial mats and analyzed their chemical and microbial profiles. On the top of the cores (a few centimeters), decomposing dead body of marine lives such as echinoderms that contains trimethylamine N-oxide, a precursor of trimethylamine (TMA), in the body were accumulated. In the surface layer (< 15 cm), high concentration of ammonium, TMA, and isotopically light methane was detected. In this layer, heterotrophic microbes such as *Bacteroides*, *Firmicutes*, and *Spirochaeta* were dominated. These results suggest that huge amount of organic matter had been recently supplied on the deep-seafloor. In addition, a large number of *mcrA* gene were also detected. Most of which were identified as those of *Methanococoides* sp. that can grow on methyl compounds as the sole energy source. From the results of investigation in 2011, we hypothesized that high concentration of methane in the microbial mat sediments were generated by methylotrophic methanogen.

On the 2012 cruise, we conducted in-situ incubation to prove our hypothesis. In-situ incubation cores with <sup>13</sup>C substrate, <sup>13</sup>C-bicarbonate, <sup>13</sup>C-acetate, <sup>13</sup>C-monomethylamine (MMA) were set on the deep-seafloor where a large microbial mat had been found in 2011 and measured methanogenesis activity. During three days of incubation, significant activity was detected only in the incubation core supplied MMA.

We hope that our results provide important hints to understand the ecology and evolution of methanogenic/methanotrophic archaea in deep-sea environments.

Keywords: deep sea, methanogenesis, mega-earthquake