Japan Geoscience Union Meeting 2014
(28 April - 02 May 2014 at Pacifico YOKOHAMA, Kanagawa, Japan)

BGM22-01 Room:415 Time:April 30 09:00-09:15

Plant rhizopsphere is a hotspot for greenhouse gas emissions

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Nitrous oxide (N_2O) is a greenhouse gas that also degrades stratosphere ozone. Marked N_2O emission were detected from soybean root systems with degraded nodules during late growth stage in field-grown soybeans. A model system developed to produce N_2O emissions from soybean fields. Soybean plants inoculated with *nosZ* mutant of *Bradyrhizobium japonicum* USDA110 (lacking N_2O reductase) were grown in aseptic jars. After 30 days, shoot decapitation (D, to promote nodule degradation), soil addition (S, to supply soil microbes), or both (DS) were applied. N_2O was emitted only in the DS treatment. Thus, both soil microbes and nodule degradation are required for the emission of N_2O from the soybean rhizosphere. The N_2O flux peaked at 15 days after DS treatment. A ^{15}N tracer experiment indicated that the N_2O was derived from N fixed in the nodules. As for nitrification, the addition of nitrification inhibitors significantly reduced N_2O flux. Both AOA and AOB were detected by PCR analysis with N_2O emission profile in soybean rhizosphere. The N_2O flux from the *nirKnosZ* mutant rhizosphere was significantly lower than that from *nosZ* mutant, but was still 30% to 60% of that of *nosZ* mutant, suggesting that N_2O emission is due to both *B. japonicum* and other soil microorganisms. Only *B. japonicum nosZ+* strains could take up N_2O. In particular, *Fusarium* spp., a soil fungus may contributed to N_2O emission in soybean rhizoshere. From these results, the organic-N inside of the nodules was mineralized to NH_4^+, and N_2O producing processes (nitrification and denitrification) simultaneously occur in the soybean rhizopsphere. We continue to examine which microbes really mediated N_2O methabolism using isotopic techniques including ^{15}N site preference of N_2O molecules. N_2O emissions from soybeans ecosystems can be mitigated by inoculting *B. japonicum* mutants with increased N_2O reductase activity (Nos++ strains). The mutation of *nasS* gene is responsible for the Nos++ phenotype. We propose that *nasS* mutation might be an effective strategy to induce higher Nos activities in N_2O-reducing rhizobia, such as indigenous isolates from local soybean fields or even from other important leguminous crops such as alfalfa, and thus to mitigate N_2O emission.

Plants have mutualistic symbiotic relationships with rhizobia and fungi by the common symbiosis pathway, in which Ca_2^+/calmodulin-dependent protein kinase (encoded by *CCaMK*) is a central component. Although *OsCCaMK* is required for fungal accommodation in rice roots, little is known about the role of *OsCCaMK* in rice symbiosis with bacteria. Here, we report the effect of a *tos17*-induced *OsCCaMK* mutant (NE1115) on CH_4 flux in low-nitrogen (LN) and standard-nitrogen (SN) paddy fields as compared with wild-type (WT) Nipponbare. Growth of NE1115 was significantly decreased compared with that of WT, especially in the LN field. The CH_4 flux of NE1115 in the LN field was significantly higher (156?407% in 2011 and 170?816% in 2012) than that of WT, although no difference was observed in the SN field. The copy number of pmoA was significantly higher in the roots and rhizosphere soil of WT than those of NE1115. However, mcrA copy number did not differ between WT and NE1115. These results were supported by a ^{13}C-labeled CH_4-feeding experiment. In addition, the natural abundance of ^{15}N in WT shoots (3.05 permile) was significantly lower than in NE1115 shoots (3.45 permile), suggesting higher N_2 fixation in WT due to dilution with atmospheric N_2 (0.00 permile). Thus, CH_4 oxidation and N_2 fixation were simultaneously activated in the root zone of WT rice in the LN field, and both processes are likely controlled by *OsCCaMK*.

Keywords: methane, nitrous oxide, rhizosphere, Bradyrhiozbia, bacteria, stable isotope

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BGM22-02 Room:415 Time:April 30 09:15-09:30

Does microbial ecology expand our understandings of nitrogen cycle in forests?

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Forests cover approximately 70% of Japan's total land area, representing a largest reservoir of diversity of organisms including plants, animals, fungi protists and prokaryotes on land. These organisms are closely associated each other in material cycles if not directly. Thus, we need to know how materials are cycling between the organisms in order to address a fundamental question in ecosystem ecology: why do forests have the richest biodiversity on land? However, it is not easy to understand the material cycles in a forest because the forest has the various environmental heterogeneity which greatly affect the cycle. For example, nitrogen dynamics can be different in soils around hills and valleys in forests. Such spatial heterogeneity of the dynamics in the soils has been explained mainly from phenomenological perspectives using abiotic information such as soil moisture, soil temperature or litter quality. However, these perspectives have not fully explained the dynamics. Here, we suggest that such heterogeneity need to be explained in the context of ecology of microbial communities which mediate the nitrogen dynamics. More specifically, we suggest that understanding the nitrogen dynamics based on the physiology, population dynamics and diversity of the microbial communities can provide the mechanistic insights into the nitrogen cycle in forests.

We analyzed the spatial heterogeneity of nitrogen dynamics and associated microbial communities in natural and planted forest soils in Asia. Specifically, we focused on nitrification in which ammonium are oxidized to nitrate and found the close association between gross nitrification rates and population size of nitrifiers in the soils. Additionally, nitrification rates cannot be fully explained by using environmental properties including substrate supply, soil moisture and litter quality, but can be explained by using the population size of nitrifiers. This shows that the better understandings of the microbial ecology allows us to more accurately explain and even predict the spatial heterogeneity of material cycles. In this presentation, we will discuss how information on microbial ecology expands our understandings of nitrogen cycle in forests.

Keywords: microbial ecology, nitrogen cycle, forest

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BGM22-03 Room:415 Time:April 30 09:45-10:00

Diversity of microbial arsenic transformation pathways associated with arsenic cycling in the environment

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Arsenic (As) is a naturally occurring toxic element that is widely distributed in nature. Although the concentrations of As in natural systems are generally low (~15 μg g^{-1} in soil and ~10 μg L^{-1} in surface waters), the elevated levels of As have been released via natural sources (i.e. volcanic activity) and anthropogenic activities due to its increasing industrial use. As can exist in four oxidation states (-III, 0, III and V), while they are mainly found as trivalent [arsenite; As(III)] and pentavalent [arsenate; As(V)] in natural systems. Depending on its oxidation state, As exhibit different mechanisms of toxicity to microorganisms and other biota. As(III) is highly reactive with thiol containing proteins and is considered more toxic than As(V). Despite its toxicity, microorganisms have developed mechanisms to tolerate As and/or utilize the element for respiratory metabolism. Although various microorganisms have been identified to catalyze As transformation including both oxidation and reduction, we have just began to unveil the full diversity of different microbial processes associated with the redox cycling of As in the environment.

To gain insight into microbial roles in the geochemical dynamics of As, the combined geochemical, physiological and molecular biological analyses were applied to examine As-impacted environments and microcosms. Microbial populations were analyzed using 16S rDNA-based molecular approach combined with metagenomic sequencing. The presence of indigenous microbial populations capable of As 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 O_2 reduction, have been recovered from geochemically distinct geothermal habitats (pH 2.6-8) as well as the soils from mine tailing. Successful cultivation of various As(III)-oxidizing bacteria confirmed the microbial attribute in As oxidation *in situ*. In contrast, from the As impacted lake sediments and soils, diverse sequences of anaerobic arsenite oxidase (*arx*) and arsenate respiratory reductase (*arr*) genes were detected, 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 approach, the anaerobic arsenite-oxidizing nitrate reducer and arsenate-reducing bacteria were isolated from the lake sediments.

Our results showed that As redox metabolisms are widespread within phylogenetically and physiologically diverse bacteria, including both chemolithotrophic and organotrophic aerobes and anaerobes. This study revealed the diversity of As transformation pathways associated with geographically and geochemically distinct environments and presented the mechanisms behind microbial processes controlling the redox cycling of As.

Keywords: arsenite oxidase, arsenate reductase, microbial arsenic transformation, soil microbiology

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BGM22-04 Room:415 Time:April 30 10:00-10:15

Biosignature found in iron oxide mineralogy of iron-oxidizing microbe origin?

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Recently, many iron mats have been discovered at deep-sea hydrothermal fields in all over the world. It has been thought that microbes, especially iron-oxidizing microbes, are the key players for forming the iron mats. However, there was no direct evidence to this, due to cultivation difficulty of iron oxidizers. Recently, '*Mariprofundus ferrooxidans*' that belong to the Zetaproteobacteria was successfully isolated. From this isolation, it has been proved that this microbe can oxidize ferrous iron as the electron donor, and can widely be observed in various deep-sea low-temperature hydrothermal fields. Therefore we have investigated how these microbes contributed to the formation of the iron mat using mineralogical and culture independent approaches.

We tried to clarify mineralogical properties of natural or lab-prepared iron oxides of iron-oxidizing microbes by using XAFS, SEM and EDX. Natural samples were collected at 3 sampling sites: iron mats from deep-sea hydrothermal fields in the Mariana Volcanic Arc, Mariana Trough and the Okinawa Trough. Lab-prepared iron-oxide synthesis was carried out using chemoautotrophic bacterium Mariprofundus ferrooxydans PV-1 (ATCC BA-1020) and was cultured by diffusion cell's method (Kikuchi et al., 2011, 2014). SEM observation showed similar morphology to all samples, which have distinctive plait-like structure, and at where iron oxides precipitate around distinctive materials. Although each natural iron-oxide sample was precipitated at different environments and with different dominant microbial species within the natural samples, XAFS showed identical spectrum. Regardless of medium employed in the cultivation, lab-prepared iron oxides also showed similar spectrum to natural samples. XANES fitting suggested that iron mats consist of ferrihydrite and iron-organic complex being the same as the lab-prepared iron oxides. These results strongly supported the iron-oxidizing chemolithoautotrophs had significant ecological roles in producing the iron mat. These mineralogical analyses may help to find biosignature in the deep-sea environments.

Keywords: iron-oxidizing bacteria, Biosignature, Mineralogical property, deep-sea, hydrothermal fields

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BGM22-05 Room:415 Time:April 30 10:15-10:30

The trench biosphere observed from the transect water sampling for the Japan Trench

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We have discovered the presence of the trench biosphere that harbored distinct microbial populations comparing to those in the upper water masses in the Challenger Deep, Mariana Trench (Nunoura et al. in preparetion). The deep locates under the oligotrophic ocean and is isolated from the other trenches while the Japan Trench locates under eutrophic ocean and in a series of long trenches in north Pacific. Therefore, the Japan Trench has one of the best environments to test the universality of the occurrence of trench biosphere. In this study, we conducted CTD casts in 8 stations across the Japan Trench in 2011 after the big earthquake and analyzed microbial structures for each sample in order to examine the occurrence of the trench biosphere.

Keywords: Japan Trench, nitirification

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SUP05 contribution for Carbon and Nitrogen cycles in semi-closed water mass

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In the deep sea hydrothermal plume, significantly elevated microbial biomass has been reported depending on chemolithoautotrophic activities by hydrothermal reduced chemicals. The potential energetic is sulfur, methane and hydrogen oxidation, and microbial production is up to date. The most important microbes in the plume is SUP05 phylotype (genus Thioglobes), which is known to have sulfur and H2 oxidation pathway, RubisCO carbon assimilation pathway, and denitrification pathways. In this study, we compared the bicarbonate and inorganic nitrogen species with SUP05 cell densities in the hydrothermal plume of the TOTO caldera hydrothermal field with half-closed water mass system in the Southern Mariana Trough. The cell densities of SUP05 is strong negative correlation with bicarbonate and nitrate, however, the correlation slope indicated the nitrogen assimilation but not the nitrogen respiration (denitrification). Only the nitrogen assimilation occurred in the plume is also supported by the lack of denitrification genes in the plume sample with the metagenomic analysis.

Keywords: Chemolithoautotroph, SUP05, TOTO, metagenomics

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BGM22-07 Room:415 Time:April 30 11:00-11:15

From who, where, how many and what to ’Earth science’

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Glancing at 50 years’ history of aquatic microbial ecology since Wright and Hobbie proposed an uptake kinetics using radio-labeled glucose, I may pose issue(s) of consideration for microbial ecology as an earth science.

Keywords: 14C-glucose uptake vs. 3H-Thymidine uptake, Production vs. Respiration, sec vs. year

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BGM22-08 Room:415 Time:April 30 11:15-11:30

Microbial potential and carbon cycle in deep aquifer of the accretionary prism of Southwest Japan

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The accretionary prism situated along the Pacific side of Southwest Japan forms thick sediments. The sediment contains deep aquifers that anaerobic groundwater is accumulated. In addition to the anaerobic groundwater, it has been reported that dissolved natural gases composed mainly of methane are present in the deep aquifers. The groundwater and natural gases are collected from deep wells (150-1500 m depth) which are drilled at the accretionary prism. In the past study conducted in a deep well situated Shimada, Shizuoka Prefecture, Japan, it has been shown that methane has been produced by subterranean microbial community in deep aquifer associated with accretionary prism. However, microbial and geochemical studies have not yet been performed at other areas of accretionary prism. In this study, we collected groundwater and natural gases from 14 deep wells of Shizuoka Prefecture, and we performed measurements of physical and chemical parameters, anaerobic cultivations of microbial communities and 16S rRNA gene analysis to understand microbial potential and carbon cycle in subterranean environments of the accretionary prism.

The temperature of groundwater samples ranged from 24.2 to 49.3 ℃, and pH was weakly alkaline. Oxidation-reduction potential suggested -325 to -114 mV at all deep wells. Electric conductivity ranged widely from 92 to 2,110 mS m^{-1} at each groundwater sample. NO_3^-, SO_4^{2-} and S^{2-} in groundwater was below the detection limit. Dissolved organic carbon (DOC) ranged from <0.3 to 50 mg l^{-1}. From componential analysis of the natural gases, methane was predominant gas component at many sites (>90%). On the other hand, we detected several natural gas samples contained a large amount of N_2 (20-50%). Stable carbon isotopic analysis of methane in the natural gases and dissolved inorganic carbon (DIC) in groundwater suggested that methane of biogenic origin are contained in the natural gases at a lot of sites.

Anaerobic incubations using groundwater amended with organic substrates revealed the high potential of H_2 and CO_2 generation by H_2-producing fermentative bacteria. Furthermore, methane generation by syntrophic consortium of H_2-producing fermentative bacteria and H_2-using methanogen was also observed in 3-5 days after the start of incubation.

Bacterial 16S rRNA gene analysis indicated the dominance of H_2-producing fermentative bacteria. The presence of denitrifying bacteria was also observed at the sites where N_2 is contained in the natural gas samples. In archaeal 16S rRNA gene analysis, H_2-using methanogens dominant in the groundwater.

From these date, it was shown that carbon cycle that methane has been produced from organic matters which are contained in the sediments by syntrophic consortium of H_2-producing fermentative bacteria and H_2-using methanogens exist in wide area of the subterranean environments of the accretionary prism. In addition to methane production, the presence of denitrification using NO_3^- or NO_2^- and organic matter or methane was also suggested at a few site.

Keywords: accretionary prism, deep aquifer, methanogenesis, fermentation, syntrophic biodegradation, subsurface environment

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BGM22-09 Room:415 Time:April 30 11:30-11:45

The global methane cycle revealed through geomicrobiological analysis

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Methane is one of the major end products of anaerobic microbial metabolism. Based on stable carbon and hydrogen isotopic compositions of methane, geochemical studies have systematically classified the origin of methane; 1) biological pathways consisting of carbon dioxide reduction coupled to molecular hydrogen oxidation and methyl-type fermentation, and 2) abiological pathways such as thermal degradation of organic matter and Fischer-Tropsch type reaction. In contrast, regarding methane consumption, recent advances in subseafloor biosphere research have unveiled the complexity of processes involved in the transformation, migration and fate of methane. Particularly, it has been recognized that marine sediments with high methane flux harbor novel lineages of microorganisms, the physiological traits of which are largely unknown due to their resistance to cultivation. Recent advances in subseafloor biosphere research indicate that microbes play much more important roles in methane production and consumption than previously assumed. Though these biogeochemical processes are not fully understood, future combined approach of geochemistry and geomicrobiology will shed light on the global methane cycle on Earth.

Keywords: subseafloor biosphere, methane, methanogen, methanotroph

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BGM22-10 Room:415 Time:April 30 11:45-12:00

Isotope systematics among H_2, CH_4 and H_2O in fluid associated with serpentinization

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Serpentinite-hosted hydrothermal systems have attracted considerable attention as sites of abiotic organic synthesis and as habitats for the earliest microbial communities, because hydrothermal fluids derived from ultramafic rocks are characterized by high concentrations of H_2 and CH_4. During water-rock reactions, Fe (II) in olivine of ultramafic rock is oxidized to Fe (III), which accompanies the reduction of water to yield H_2. Methane and hydrocarbons are often observed in serpentinite-hosted hydrothermal systems and are thought to be produced from H_2 and CO_2 via Fischer-Tropsch-type (FTT) reactions. On the other hand, H_2 and CH_4 can be consumed and produced by microorganisms such as methanogens and methanotrophs around the hydrothermal systems. When we collect and analyze samples, those chemical compositions could have been altered due to microbial activities. Therefore, it is very difficult to clarify processes related to H_2 and CH_4 around the serpentinite-hosted hydrothermal systems.

Isotopic compositions are useful tool to discriminate origins and reaction pathways of chemical components. As representative controlling factors of isotopic compositions are temperature equilibrium, isotopic compositions of substrate, and isotopic fractionation, the dynamics of isotopic compositions are complicated in natural environments. Therefore, polyphasic aspects, such as hydrological, geological and microbiological interpretations, are needed. However, even complete hydrogen isotopic analysis of H_2, CH_4 and H_2O from serpentinite-hosted systems and basic laboratory experiments has been reported in only a few studies. As the isotope systematics among H_2, CH_4 and H_2O in fluid associated with serpentinization remain unexplored, I will present the review of some previous studies and results of explorations of hydrothermal systems at Mid Cayman Ridge during YK13-05 cruise.

Keywords: serpentinization, stable isotope, hydrogen, methane

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BGM22-11 Room:415 Time:April 30 12:00-12:15

Acetate-oxidation activities in the deep subseafloor biosphere associated with coalbeds off the Shimokita Peninsula

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The IODP Expedition 337 was the first riser-drilling expedition dedicated to subseafloor microbiology using the drilling vessel Chikyu. During Expedition 337, we penetrated a 2466 m deep sedimentary sequence at Site C0020A with a series of coal layers at 2000 m bellow the seafloor (mbsf) off the Shimokita Peninsula, Japan. One of the primary scientific objectives of Expedition 337 was to understand ecological roles of subseafloor microbial activity in biogeochemical carbon cycles associated with the deeply buried coalbeds in the ocean. It has been hypothesized that immature coalbeds (i.e., lignite) release substantial dissolved organic compounds such as volatile fatty acids or hydrocarbons during the burial alternation process, which compounds may play important roles for supporting microbial population and activity in the deep sedimentary habitat. Alternatively, it is also conceivable that deep subseafloor microbial activities may contribute to the hydrocarbon reservoir system.

To examine those hypotheses, we measured methanogenic and acetate-oxidation activities by radiotracer incubation experiments using 2 cm^3 of the innermost sediment core samples that were supplemented with ^{14}C-labelled substrate ([2-^{14}C]-acetate) immediately after core recovery. Activities of aceticlastic methanogenesis were observed in the sediment above the coalbed layers (>1990 mbsf), ranging from 0.2 to 4 pmol cm^{-3} d^{-1}. The highest activity was observed in a coalbed horizon at 1990 mbsf; however, no aceticlastic methanogenesis activities were observed below the 2 km-deep coalbeds. Activities of acetate oxidation to CO_2 were measured by $^{14}CO_2$ production rate from [2-^{14}C]-acetate. Interestingly, the acetate-oxidation activities were observed in sediments above the coalbeds, which values were generally higher than those of methanogenesis with the maximum value of 150 pmol cm^{-3} d^{-1} at 1800 mbsf. The rates gradually decreased with increasing depth from 1800 mbsf and reached bellow the detection limit in 2 km-deep coalbeds. The occurrence of relatively high acetate oxidation at around 1800 mbsf above the coalbeds indicates the presence of available electron acceptors (e.g., glauconitic iron oxides) in the deep sedimentary habitat.

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BGM22-12 Room:415 Time:April 30 12:15-12:30

Temperature effect of sulfur isotope fractionation by sulfate reducers when used glucose as electron donor

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Sulfate reducing microbe (SRM) is responsible for over 50 % of organic carbon remineralization in marine sediments and thus plays a prominent role in sulfur cycle. Based on a large number of culture experiments of SRM, sulfur isotope fractionation by SRM changes depending on environmental factors including temperature, sulfate concentration and availability of electron donor. The isotope fractionation is recorded in sedimentary sulfates and sulfides. Hence, the sulfur isotopic fractionation is useful to reconstruct ancient environmental condition. However, the mechanism controlling the degree of the sulfur isotopic fractionation is still unclear. Particularly, we have to consider the physiology. Previous culture experiments of SRM indicated that the temperature effect varies with species of SRM. However, there is little temperature control experiments using various electron donor with same strain. We carried out temperature control experiments at 25 ℃, 30 ℃ and 37 ℃, by sulfate reducing bacteria DSM 642 using glucose as electron donor. Our results revealed growth rate of DSM 642 is fastest at 30 ℃, when using glucose as electron donor. Growth rate is the fastest at 37 ℃ when using lactate as an electron donor. Sulfate reduction rate is thought to primary factor controlling isotope fractionation. In addition, growth rate and sulfate reduction rate have basically positive correlation. Accordingly, the shift of sulfur isotope fractionation by temperature must be changed when used glucose as electron donor. This result indicates that we should pay attention not only sulfate reduction pathway but also oxidation pathway of electron donor. We report temperature dependency of sulfur isotope fractionation by DSM 642 using glucose as electron donor at the first time, to elucidate the mechanism controlling the degree of the sulfur isotopic fractionation during microbial sulfate reduction.

Keywords: sulfur isotope, sulfate reducing bacteria

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A hot-alkaline DNA extraction method for deep subseafloor communities

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Many of the DNA-based researches have greatly enhanced our understanding on stratified nature in subseafloor microbial communities. An important prerequisite for DNA-based microbial community analysis is even and effective cell disruption for DNA extraction. With a commonly used DNA extraction kit, in average, roughly two-third of subseafloor sediment microbial cells remain intact (i.e., the cells are not disrupted), indicating that microbial community analyses may be biased at the DNA extraction step, prior to subsequent molecular analyses. To address this issue, standardized a new DNA extraction method using alkaline treatment and heating by precisely monitoring microbial cell numbers in the treated samples. Upon treatment with 1 M NaOH at 98oC for 20 min, over 98% of microbial cells in subseafloor sediment samples collected at different depths were disrupted. However, DNA integrity tests showed that such strong alkaline and heat treatment also cleaved DNA molecules into short fragments that could not be amplified by PCR. Subsequently, we optimized the alkaline and temperature conditions to minimize DNA fragmentation and retain high cell-disruption efficiency. The best conditions produced a cell disruption rate of 50-80% in subseafloor sediment samples from various depths, and retained sufficient DNA integrity for amplification of the complete 16S rRNA gene (i.e., ˜1,500 bp). The optimized method also yielded higher DNA concentrations in all tested samples compared with extractions using a conventional kit-based approach. Comparative molecular analysis using real-time PCR and pyrosequencing of bacterial and archaeal 16S rRNA genes showed that the new method produced an increase in archaeal DNA and its diversity, suggesting it provides better analytical coverage of subseafloor microbial communities than conventional methods.

Keywords: Subseafloor microbial community, DNA extraction, bias, archaea