Abstracts

ASME Sessions

A1-1

Distinctive microbial assemblages and their ecological function in permanently ice-covered lakes of the Dry Valleys, Antartica

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Permanently ice-covered lakes in the McMurdo Dry Valleys of Antarctica have been paid attention for their physical, chemical and biological significances. We carried out the comparative study on bacteria community structures and biogeochemistry with certain depths in five lakes. All lakes showed more homogenous bacterial assemblages in epilimnion, whereas a certain lake had distinctive ones in hypolimnion. In Lake Bonney, 30 m depth of the East and West Lobe was dominated by two distinct *Firmicutes* classes. While lineages of *Cholobi* are detected only at the depth of 18 m in Lake Miers, candidate division WM88 occurred at 15 m depth of Lake Fryxell. Furthermore, Lake Fryxell was dominated by various uncultured bacterial lineages belonging to not only well known *Bacteroidetes, Actinobacteria* and *Planctomycetes* but also candidate divisions, so-called 'microbial dark matter' including JS1, WM88 and SAR 406. To understand the implication of their ecological roles in this lake, shotgun metagenomics were conducted. This talk will present to uncover unexpected metabolic features linked to the microbial ecological function in this hypersaline ecosystem, Antarctica.

A1-2

Evolutionary genetic traits for thermal adaptation in *Bacillales*

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The order Bacillales, one of the most thriving microorganisms on Earth, has actively evolved under a variety of environmental conditions such as temperature, pH, salinity, and pO2. In this context, many taxanomic and physiological studies on individual strains belonging to this order have been performed, but their evolutionary genetic traits still remain unclear. Herein, 85 thermophiles and mesophiles in this order Bacillales, comprised of ten genera, were selected for understanding molecular evolution for thermal adaptation. Comprehensive phylogenomic analysis allowed us to reconstruct the phylogenetic tree of the order Bacillales. Based on this, a multiple Pan-genome analysis revealed 134 core genes that 85 strains commonly have, which have been analyzed genetically and functionally. Unlike mesophilic counterparts, thermophiles have much shorter genome size with higher G + C composition of the core genes, which is highly correlated with thermophily in this order. Secondly, amino acid contents and codon usage frequencies in the core genes are biased depending on temperature and pH optima for their growth. Moreover, such genetic features could be rationalized by theoretical and empirical data with several mutant *E. coli* strains, implying that thermophiles preferentially share genes with other prokaryotes having properties in common, including genome size, genome G+C composition, asymmetric amino acid preference, and gene context. Therefore, such a conditioned phylogenomic approach enabled us to test current theories for thermal adaptation in prokaryotes, but also to provide a new insight into discovering the general outlines of the prokaryotic tree of life in this order Bacillales.

A1-3

Steep redox gradient and biogeochemical processes driven by deeply-rooted fluids in a terrestrial mud volcano

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Mud volcanoes provide an accessible channel to witness source characteristics of fluids and microbial communities thriving in deep subsurface environments. How the deeply sourced reducing materials shape physico-chemical context and biogeochemical processes in such geological features remains largely unknown. This study conducted geochemical and molecular analyses to characterize the redox transition, biogeochemical fluxes and microbial communities of samples collected from a methane-rich mud volcano in Taiwan. Our results revealed that oxygen penetration was confined within the top 4 mm of fluids/muds regardless of the stationinvestigated. Oxidation of sulfide, methane and hydrocarbon appear to be the main mechanisms accounting for oxygen consumption. Beneath the oxic zone, sulfur oxidation, sulfate reduction, anaerobic oxidation of methane and methanogenesis were compartmentalized into different depth intervals in the pool periphery, forming a metabolic network that efficiently cycles methane and sulfur. Community members affiliated with various Proteobacteria capable of aerobic oxidation of sulfur, methane and methyl compounds were more abundant in the anoxic zone with diminished sulfate and high methane, suggesting a requirement of alternative electron acceptors. Overall, this study demonstrates the distribution pattern for a suite of oxidative and reductive metabolisms along a steep redox gradient imposed by deep fluids in mud volcano ecosystems.

A1-4

H_2 accumulation by Ni limitation in *Cyanothece*

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Ni is an essential co-factor in uptake hydrogenase, an enzyme existing in all the diazotrophic cyanobacteria examined. We thus investigated the effects of Ni availability on H_2 accumulation in a unicellular model diazotroph, *Cyanothece* strain ATCC 51142. The study was carried out by varying Ni concentrations to 1, 10, or 100 nM in either relatively high or low dissolved Fe concentrations (100 and 400 nM) in a trace metal-defined culture medium. The H_2 accumulation and N_2 fixation rates were determined during late exponential growth periods, as well as the measurements of specific growth rate, cellular volume, and intracellular trace metal and major elemental quotas. The growth of the diazotroph was slightly limited in 1 nM dissolved Ni treatments, with specific growth rates to be 85% of the maximum growth rates obtained under relatively high Ni availability. We also repeatedly observed that Ni deficiency was closely related to H_2 accumulation rates, suggesting that Ni limitation decreases H_2 uptake and results in H_2 accumulation in the diazotrophic cyanobacteria. In brief, this study demonstrates the importance of Ni availability on influencing *Cyanothece*' s growth and the consumption or accumulation of H_2. Ni availability appears to be an important factor in regulating molecular hydrogen production of diazotrophic cyanobacteria.

A1-5

A primordial and reversible TCA cycle in a facultatively chemolithoautotrophic thermophile

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Inorganic carbon fixation is essential to sustain life on Earth, and the reductive tricarboxylic acid (rTCA) cycle is one of the most ancient carbon fixation metabolisms. A combination of enzymatic and multi-omics analyses of a deeply-branching chemolithotrophic *Thermosulfidibacter takaii* ABI70S6^{T} revealed an unprecedented reversible TCA cycle whose direction was controlled by the available carbon source (s). Under a chemolithoautotrophic condition, a rTCA cycle occurred with the reverse reaction of citrate synthase (CS) and not with the ATP-dependent citrate cleavage reactions that had been regarded essential for the conventional rTCA cycle. In the presence of acetate or succinate, different types of the bifurcated TCA cycles occurred. In the presence of both acetate and succinate, no carboxylation was observed. The recombinant CS presented kinetic features that adapt for citrate cleavage reaction as in the cases of ATP citrate lyase. In addition, phylogenetic analysis of citrate synthase domain suggests that ATP lyase and citryl-CoA lyase evolved from a lineage of citrate synthase. Phylometabolic evaluation suggests that the TCA cycle with reversible CS may represent an ancestral mode of the (r) TCA cycle and raises the possibilities of a chemolithomixotrophic origin of life.

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A1-6

Deep microbial ecosystems within Cretaceous igneous rocks

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Continental and oceanic crusts generally consist respectively of granitic and basaltic rocks overburdened with sediments. Deep microbial life is mainly habitable inside of rock fractures, which are vulnerable to drilling contamination from overlying soils and/or sediments. Thus, the existence of microbial life in the spatially vast crustal environment remains largely unknown. We investigated microbial communities in continental and oceanic crusts through deep underground facilities in Japan and Switzerland (Miznuami and Grimsel) and Integrated Ocean Drilling Program (IODP) Expedition 329 in the South Pacific Gyre (SPG).

Cretaceous granitic rock bodies at Mizunami and Grimsel were horizontally drilled from the underground facilities to avoid contamination from the surface. Microbial communities colonizing the horizontal boreholes were characterized by 16S rRNA gene sequence analysis. Interestingly, Nitrospirae and Chlorobi bacteria without cultivated relatives were commonly dominant at both sites, whereas site-specific microbial populations were also dominant: Parucubacteira, OP3 and ANME-2d were dominant at Mizunami; Novel bacteria at the phylum level were dominant at Grimsel.

We performed hydrogeochemical and metagenomic analyses and stable isotope labeling of methane and demonstrated that ANME-2d is mediating anaerobic methane oxidation coupled to sulfate reduction in the deep granitic environment.

Basaltic rock samples were obtained from 13.5-, 33.5- and 104-million-year-old basements overburdened with sediments nearly depleted in organic matter. The ultra-oligotrophic SPG environment promotes the extremely low activity of aerobic microbial communities in subseafloor sediments, which enables molecular oxygen to penetrate the ocean floor down to the basaltic basement. As open fractures were contaminated by fluorescence microspheres introduced from drilling fluid, mineral-filled fractures without the detection of fluorescence microspheres were investigated. Unexpectedly, nanoscale solid characterizations revealed that microbial cells were densely colonizing mineral-filled fractures in 104-million-year-old basaltic basement. 16S rRNA gene sequence analysis also revealed that dominant microbial populations shifted from chemolithotrophs to chemoorganotrophs and methanotrophs during the waning of crustal-fluid circulation.

Together, we conclude that continental and oceanic crusts are spatiotemporally important microbial habitats that harbor microbial biomass equivalent to the overlying aquatic and sedimentary biospheres. we also indicate that methane, a previously unrecognized energy source for the deep biosphere, fuels microbial life at the planetary scale.

A2-1

Current Progress and Challenges in Microbial Source Tracking Based on Next-Generation-Sequencing

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In this presentation, two recently developed next-generation-sequencing (NGS) -based MST methods are discussed. One method defines specific operational taxonomic units (OTUs) for each fecal source, *e.g.*, humans and animals, and identifies OTUs shared between fecal and environmental microbial communities. The other method uses SourceTracker, a program using a Bayesian algorithm to determine which OTUs have contributed to an environmental community based on the composition of microbial communities in fecal sources. Emerging NGS-based MST tools offer a promising avenue to rapidly characterize fecal source contributions for water monitoring and remediation efforts. However, both methods require optimized sequence processing methodologies (*e.g.* quality filtering and clustering algorithms) and are influenced by primer selection for amplicon sequencing. Therefore, care must be taken when extrapolating data or combining datasets. Furthermore, traditional limitations of library-dependent MST methods, including differential decay of source material in environmental waters and spatiotemporal variation in source communities, remain to be fully characterized. Nevertheless, increasing use of these methods as well as expanding fecal taxon libraries representative of source communities will help improve the accuracy of these methods and provide promising tools for future MST investigations.

A2-2

Skin Microbiome: Fragile skin microbiomes in megacities are assembled by a predominantly niche-based process

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Given the higher incidence of skin diseases in more urbanized populations and their associations with the skin microbiome, we questioned how the skin microbiome differs depending on the degree of urbanization. Skin microbiomes of 231 healthy subjects in five large cities in China varied mainly depending on the city's environment and socioeconomic status. The differences among microbiomes could be explained by the predominantly niche-based assembly of the microbial communities, which was supported by a dominance test, β-null deviation, and edge-length abundance distribution. Networks among microbes in larger cities were more fragile, which may contribute to the higher incidence of skin diseases in more urbanized environments. These results suggest that microbial ecological theory can provide a framework for understanding crucial health-associated features of the human microbiome. We also investigated the difference in facial skin microbiomes depending on the age with young and older groups (n=73) to examine the relationship between skin microbiome and skin aging. Microbial community and predicted functional metagenome were significantly separated by age groups, and the skin microbiome of the older group was assembled heavily by niche-based process and had a more fragile network. Aging-associated skin microbiome can be suggested with the potential as a novel diagnostic and therapeutic target for skin aging and related diseases.

A2-3

Ecology of airborne fungi associated with respiratory allergy

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Respiratory allergies have been associated with exposure to allergenic fungal spores and the increasing prevalence of allergic disease has been associated with increasing urbanization. To know the spatiotemporal distributions of fungal spores and their seroepidemiology is conducive to the epidemiology and etiology of respiratory allergies. In this study, the concentrations of dominant fungi were investigated along an urbanization gradient through viable count and direct microscopic count. Airborne fungal spores increased along a vegetation gradient and their flora showed significant seasonal variation. We investigated the levels of fungal spores and culturable fungi in the houses of fungal allergic patients during inactive and exacerbation (active) stages. The exacerbation of allergic symptoms was not correlated with total fungal spore concentration or the indoor/outdoor ratio. Specific fungi, such as *Cladosporium oxysporum, C. cladosporioides,* and *Aspergillus niger*, were found to be significantly higher concentrations in the active stage than in the inactive stage. The exacerbation threshold levels of fungal concentration differed among species. Presumed allergenic spore concentration threshold levels were 100 CFU/m^3 for *C. oxysporum*, and 10 CFU/m^3 for *A. niger, Penicillium brevicompactum* and *P. oxalicum*. Seroepidemiology of the dominant fungal species confirmed their allergenicity. These results are useful in making a diagnosis and recommendations for environmental control for fungal allergic patients.

A2-4

Role of bacterial type VI secretion system in rhizobium-legume mutualistic symbiosis

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Bacterial type VI secretion system (T6SS) has been considered the armed force of bacteria because it can deliver toxin effectors to prokaryotic or eukaryotic cells for survival and fitness. It is found in more than a quarter of the sequenced Gram-negative bacteria, including many pathogens and symbionts. Although many legume symbiotic rhizobacteria encode T6SS in their genomes, the biological function of T6SS in these bacteria is still unclear. To elucidate this issue, we used *Azorhizobium caulinodans* ORS571 and its symbiotic host *Sesbania rostrata* as our research model to elucidate the role of T6SS in mutualism. We analyzed the biological function of azorhizobial T6SS in both free-living state and symbiotic state to unveil whether T6SS of symbionts participate in pathogenicity, anti-prokaryotic and/or anti-eukaryotic activity. Contradictory to what have been demonstrated in other animal and plant pathogens, the T6SS of A. *caulinodans* does not use its T6SS during antagonistic interactions with neighboring prokaryotic or eukaryotic competitors under tested conditions. Under symbiotic condition, we found that although the deficient of T6SS had no adverse effects on symbiotic effectiveness (i.e number and size of nodule, as well as biological nitrogen activity), T6SS could confer A. *caulinodans* with better symbiotic competitiveness (i.e higher nodule occupancy rate). It suggests that the T6SS of A. *caulinodans* could serve as a signal to be recognized by the legume host plants to prioritize for establishing a symbiotic relationship with the bacteria.

A2-5

Challenging the dogma of bacterial membrane vesicle formation

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Bacterial membrane vesicles (MVs) , which size range from 20 to 400nm in diameter, play important role in diverse biological processes, including horizontal gene transfer, virulence, phage decoy and cell-to-cell communication. They are abundant in natural environments and have certain impact in the carbon cycling of the marine ecosystem. Despite their biological importance, the mechanisms of MV biogenesis is not fully understood. MVs were traditionally thought to be produced through the blebbing of the outer membrane in Gram-negative cells and are often referred to as outer membrane vesicles (OMVs) . Blebbling of the outer membrane is initiated either by intercalation of molecules into the outer leaflet of the membrane or the imbalance of membrane and peptidoglycan (PG) synthesis and are thought to happen without affecting the cell viability. More recent findings show that Gram-positive cells, that do not possess the outer membrane, also produce vesicles, which mechanism remained largely unknown. By using live cell imaging techniques, we recently found that, in addition to the canonical blebbing model, MVs are formed through explosive cell lysis in*Pseudomonas aeruginosa* (Turnbull and Toyofuku et al. *Nature Comm.*2016) .During explosive cell lysis, the PG is degraded by endolysin which is a well conserved enzyme that is typically involved in the release of dsDNA phage from cells. PG degradation leads to the cell explosion that give rise to shattered membrane fragments. These membrane fragments round up and form MVs. Since endolysin is conserved among bacteria, we further investigated the role of this enzyme in MV formation of a Gram-positive bacterium, *Bacillus subtilis.*MV formation was also trigged by endolysin in *B. subtilis* but the detailed mechanism differed from that of *P. aeruginosa.*In *B. subtilis,* cells did not explode but endolysin created holes in the cell wall through which membrane protruded and formed MVs. During this process, named bubbling cell death, the remaining membrane also rounded up and produced vesicles inside the cell (Toyofuku et al, *Nature Comm.*2017) . Hence, our results show that endolysin can trigger MV formation in structurally distinct bacteria through different mechanisms. Our results propose a new model in bacterial MV formation where cell lysis or cell death play important roles. Given the abundance of phage in natural environments, endolysin may be a major trigger in MV formation.

A2-6

Genomic insight into the predominance of candidate phylum Atribacteria JS1 lineage in marine sediments

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Candidate phylum Atribacteria JS1 lineage is one of the predominant bacterial groups in anoxic subseafloor environments, especially in organic-rich or gas hydrate-containing sediments. However, due to the lack of axenic culture representatives, metabolic potential and biogeochemical role of this phylum have remained elusive. Here, we examined the microbial communities of marine sediments of the Ross Sea, Antarctica, and found candidate phylum Atribacteria lineage JS1 was the most abundant candidate phylum accounting for 9.8-40.8% of the bacterial communities with a single dominant operational taxonomic unit (OTU). To elucidate the metabolic potential and ecological function of this species, we applied a single-cell genomic approach and obtained 18 single-cell amplified genomes presumably from a single species that was consistent with the dominant OTU throughout the sediment. The composite genome constructed by co-assembly showed the highest genome coverage, 90%, among available Atribacteria JS1 genomes. Metabolic reconstruction suggested fermentative potential using various substrates and syntrophic acetate oxidation with hydrogen or formate scavenging methanogens. This metabolic versatility supports the predominance of Atribacteria JS1 in anoxic environments expanding our knowledge of the ecological function of this uncultivated group.

A2-7

Uncovering the bacterial community assembly pattern and novel populations in Baltic Sea surface waters

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The Baltic Sea represents a good model ecosystem for the study of microbial ecology due to its various environmental gradients. Previous studies have documented temporal variations in the phylogenetic structure of planktonic bacterial communities with a primary focus on populations selective under specific environmental conditions (e.g., summer vs. winter) . This work hypothesized that planktonic communities harbor core populations that highly frequently occur over the seasons, although the majority are transient. To test the hypothesis, we examined the extensive bacterial community dynamics in Baltic Sea surfaces waters. Our results revealed several core bacterial populations that highly frequently occur (> 80% of temporal occurrence) . Although they represented < 2% of the total OTUs, they accounted for substantial relative abundance (> 25% of the total) over four seasons. An occurrence-abundance modeling approach was employed to predict the microbial community successional pattern. The employed model could explain well the quantitative relationship between the temporal occurrence frequency and overall relative abundance over the seasons. Further, this study uncovered a key core-bacterial population, phylogenetically affiliated to SAR11 Subclade IIIa. Unexpectedly, the population was closely related to those inhabiting a mesosaline lacustrine ecosystem on the Tibetan Plateau, rather than other marine/coastal IIIa members. We further documented spatiotemporal patterns of the newly-identified population in Baltic Sea planktonic waters. Overall, our results provide new insights into the predictive pattern of microbial community succession over the seasons and advance understanding of the core microbial populations in the Baltic Sea planktonic ecosystem.

A2-8

Plant microbial fuel cell for remediation of hexavalent chromium contaminated soil and treating mechanisms

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The plant microbial fuel cell (PMFC) is a novel technology that integrates plants, microbes and electrochemical elements together to create renewable energy. However, research is lacking regarding the application of the PMFC system to remediate metal-contaminated soil. In this study, we evaluate the potential of PMFC using different plants and various electrode materials to investigate their electricity generation and Cr (VI) removal under different soil Cr (VI) concentrations. We build PMFC based on Chinese pennisetum and common reeds with carbon felt and graphite carbon felt as the electrodes and subjected it to the following Cr (VI) concentrations within the greenhouse: 0 mg/kg, 50 mg/kg, 200 mg/kg and 500 mg/kg. By examining the different concentrations, we evaluate the best treating performance. Furthermore, we explore mechanisms of PMFC, adopting Chinese pennisetum and common reeds' ability to uptake Cr (VI) , as well as using MFC systems to reduce Cr (VI) to Cr (III) and precipitate Cr (III) on the electrode. We hope that such integrated mechanisms can enhance Cr (VI) removal in contaminated soil and produce energy. According to the results of this experiment, PMFC can turn acid topsoil into neutral topsoil. Furthermore, the maximum removal of Cr (VI) in soil is 99% for 96 days. The ratios of organically bound and residual Cr states in PMFC systems are higher than when not treating soil. After 53 days, PMFC systems with graphite carbon felt could achieve a maximum average output voltage of 469.21 mV under 1KΩ external resistance. According to the actual contaminated soil experiment, PMFC could remove 67.07% Cr (VI) from contaminated soil and produce 64.70 mV output voltage. The passive diffusive gradients in the thin-films (DGT) technique is a kind of in-situ and non-destructive tool for determining elements. After applying PMFC for soil remediation, we adopted DGT with a N-methyl-D-glutamine functional resin to determine the Cr (VI) concentrations in both solid and liquid phases and found that Cr (VI) concentrations are nearly 0 mg/L in the liquid phase. These results indicate the bioelectrochemical process is the major mechanism for Cr (VI) removals. Furthermore, redox reactions in PMFC promote Cr (VI) precipitates on the electrode. Furthermore, Cr-reducing microorganisms and plant uptake improve Cr (VI) and total Cr removal. Finally, through both direct and indirect benefits, PMFC possesses multiple functions for soil remediation: treating pollutants, no secondary pollution, greening, utilizing sunlight energy, generating green energy, and ecological conservation.

A2-9

Microbial community composition and functional capacity in a terrestrial ferruginous, sulfate-depleted mud volcano

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Terrestrial mud volcanoes (MVs) are an important natural source of methane emission. The role of microbial processes in methane cycling and organic transformation in such environments remains largely unexplored. In this study, we aim to uncover functional potentials and community assemblages across geochemical transitions in a ferruginous, sulfate-depleted MV of eastern Taiwan. Geochemical profiles combined with 16S rRNA gene abundances indicated that anaerobic oxidation of methane (AOM) mediated by ANME-2a group coincided with iron/manganese reduction by Desulfuromonadales at shallow depths deprived of sulfate. The activity of AOM was stimulated either by methane alone or by methane and a range of electron acceptors, such as sulfate, ferrihydrite, and artificial humic acid. Metagenomic analyses revealed that functional genes for AOM and metal reduction were more abundant at shallow intervals. In particular, genes encoding pili expression and electron transport through multi-heme cytoc

A2-10

Nitrogen and oxygen isotope effect of anaerobic ammonium oxidation

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Both nitrogen (N) and oxygen (O) isotopes of fixed nitrogen compounds are fractionated during their microbial production and consumption processes comprising the global marine N cycle. Thus, measurements of nitrogen ($^{15}N/^{14}N$) and oxygen ($^{18}O/^{16}O$) isotope ratios of fixed nitrogen compounds have long been used as biogeochemical stable isotopic tracers to estimate the global marine N budget. To fully exploit these tracers, the N and O isotope effects ($^{15}\varepsilon$ and $^{18}\varepsilon$) associated with the respective nitrogen transformation processes must be known. However, the N and O isotope effects of anaerobic ammonium oxidation (anammox), one of the major fixed N sinks, are not yet measured. Here we report the dual N and O isotope effects associated with anammox by three different anammox bacteria '*Ca.* Scalindua japonica', '*Ca.* Jettenia caeni', and '*Ca.* Brocadia sinica' for the first time. '*Ca.* Scalindua japonica' is a marine anammox species, which is one of main players of the oceanic fixed N sinks. In this study, the N and O isotope effects were measured using enrichment cultures grown in continuous membrane bioreactors (MBRs), in which more realistic oceanic conditions (namely low substrate concentrations were stably maintained with high reproducibility). In the case of NO_2^- oxidation to NO_3^-, all three species showed inverse kinetic isotope effects ($^{15}\varepsilon_{NO2\rightarrow NO3}$ = -30.1‰ to -45.3‰). For the conversion of NH_4^+ to N_2, the N isotope effects of all three species were consistent ($^{15}\varepsilon_{NH4 \rightarrow N_2}$ = 30.9‰ to 32.7‰). This is probably because this reaction is mediated through the same enzymes such as hydrazine synthase (*hzs*) and hydrazine dehydrogenase (*hdh*) in all anammox bacteria species. In contrast, N isotope effects for NO_2^- reduction to N_2 were significantly different among three species ($^{15}\varepsilon_{NO2\rightarrow N2}$ = 5.9‰ to 29.3‰), which was likely depending on the type of nitrite reductase (copper-containing nitrite reductase (Cu-NIR) and cytochrome cd_1-containing nitrite reductase (Fe-NIR)). Furthermore, all three anammox strains yielded distribution of $\delta^{18}O$ and $\delta^{15}N$ of nitrite and nitrate significantly deviated from the previously reported distribution for nitrate reduction by denitrifying bacteria. Observing $\delta^{18}O$ and $\delta^{15}N$ of nitrite and nitrate could be a valuable signature to distinguish between anammox and denitrification. The unique kinetic isotope effect for anammox should help to better understand its role in the nitrogen cycling in ocean.

A2-11

Nitrogen-fixing bacteria and nitrogenase activity in thermophilic chemosynthetic microbial communities at Nakabusa hot springs

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Nitrogen-fixation in thermophilic microbial communities at ≥70℃ is poorly understood. Previously, we detected nitrogenase activity in chemolithoautotrophic microbial mats at ≥70℃ in Nakabusa hot springs. In this study, we examined candidate diazotrophs in the communities using molecular-based analysis and isolated diazotrophic community members.

Amplicon sequence analyses based on the *nifH* gene were performed to identify candidate diazotrophs. High relative abundance of NifH operational taxonomic units could be assigned to three distinct taxa; (1) the sulfate-reducing bacterium, *Thermodesulfovibrio yellowstonii* (91.2% amino acid [AA] sequence identity) in the phylum *Nitrospira*, (2) the sulfur/hydrogen-oxidizing bacteria, *Thermocrinis albus* and *Hydrogenobacter thermophilus* in the phylum *Aquificae* (91.5% - 95.6% AA sequence identity) , and (3) the fermentative bacterium, *Caldicellulosiruptor kronotskyensis* (100% AA sequence identity) in the phylum *Firmicutes.* All of these were previously shown to contain NifH genes. However, the nitrogen-fixing ability had not been demonstrated yet.

Two strains, strain 1-6 and 2-18, were successfully isolated under nitrogen-fixing conditions from microbial mats at 70-77℃. Their deduced NifH sequences were 100% identical to one of the environmental clone sequences in the phylum *Aquificae*, showing 96.5% and 97.4% AA sequence identity with that from *H. thermophilus.* Phylogenetic analysis based on 16S rRNA gene placed both isolates in the genus of *Hydrogenobacter* in the phylum *Aquificae*. 16S rRNA gene sequence identity value of <98% to the closest related type species indicates strain 2-18 to represent a novel species in the genus.

Nitrogenase activity in both isolated strains was determined and confirmed by acetylene reduction assays at 70℃. Both strains grew under microaerobic conditions using CO_2 as sole carbon source and N_2 as sole nitrogen source with thiosulfate or H_2 as electron donor at 70℃. The two strains showed slightly different O_2 optima for growth. Strain 1-6 grew better in 10% vol. O_2 atmosphere compared to 5%; whereas strain 2-18 showed growth at 5% vol. O_2 but not at 10%. To our knowledge, this is the first report of active nitrogenase activity in bacteria at 70℃ and also in the phylum *Aquificae*.

Our results showing nitrogenase activity in isolates and distribution of diverse candidate diazotrophs suggest that nitrogen-fixation in these thermophilic environments takes place in different ecological niches by diverse bacterial members with different requirements for redox states, oxygen concentrations, and electron donors. Nitrogen-fixation may support the biomass production in the communities under the shortage of nitrogen compounds in these oligotrophic, thermophilic environments.

A3-1

Syntrophic association between exoelctrogenic bacteria and methanogenic archaea via granular activated carbon in anaerobic digestion

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Anaerobic digestion is an anaerobic process for treating organic waste with the production of methane gas. This process is dependent on interspecies electron transfer (IET) for successful performance. Hydrogen, a fermentation product, is an electron carrier mediating the IET for methane production. Thermodynamically, hydrogen is produced only when it is maintained at low levels by continuously consuming by hydrogen utilizing methanogenic archaea. This condition necessarily results in slow methane production in anaerobic digestion. Transferring electrons directly to methanogens is thus suggested to be a route accelerating methane production that can cope with IET via hydrogen. Recently, several experimental evidences have been reported for the possibility (i.e. direct interspecies electron transfer (DIET)) when conductive materials such as granular activated carbon were supplemented in anaerobic reactors. Conductive materials accept electrons released by exoelectrogenic bacteria to transfer them to methanogenic archaea. Nevertheless, it has not been clearly explained for the syntrophic association between them especially in the reactors harboring mixed populations, although several candidates were suggested to be involved in DIET. Here, we operated the reactors supplemented with granular activated carbon. The reactors were initially seeded with anaerobic sludge of a wastewater treatment plant. Bacterial and archaeal 16S rDNA sequences were retrieved in the course of operation. Syntrophic association among bacterial and archaeal species from the reactors was investigated using network analyses based on operational taxonomic units deduced from the sequences. The analyses demonstrated that specific putative exoelectrogenic bacteria (e.g. Geobacter species) significantly co-occurred with specific methanogenic archaea (e.g. Methanosarcina and Methanosaeta species) . These results suggest that granular activated carbon stimulated DIET by enriching specific microorganisms and associating them syntrophically in the reactors. Furthermore, these results would be useful for better understanding DIET and for analyzing the reactors promoting DIET.

A3-2

The selective enrichment of syntrophs, methanogens and exoelectrogens on granular activated carbon in stage anaerobic fluidized bed membrane bioreactors (SAF-MBRs)

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Stage anaerobic fluidized bed membrane bioreactors (SAF-MBRs), using granular activated carbon (GAC) as fluidizing media, are developed as energy-positive technology for wastewater treatment. The addition of GAC was originally intended as a membrane scouring agent and as a carrier for promoting biomass growth and retention. From an engineering perspective, SAF-MBRs have advantages over conventional MBRs in terms of their reduced membrane fouling and high solid retention time (SRT). This study analyzed the microbial composition of a SAF-MBR and found that the presence of GAC also had significant impacts on the SAF-MBR's microbial composition. In a typical anaerobic digestion process, syntrophs and methanogens are key players involved in the final conversion of volatile fatty acids (VFAs) to methane. This is facilitated by the mediated interspecies electron transfer (MIET) between syntrophs and methanogens. The addition of GAC however, led to the co-enrichment of exoelectrogen alongside syntrophs and methanogens on the GAC surface. The conductive property of GAC enables direct interspecies electron transfer (DIET) to occur between exoelectrogen and methanogens. The co-occurrence of MIET and DIET has implications on the dynamics between syntrophs and methanogens, and the thermodynamics of methanogenesis.

A3-3

Isolation of *Methanoculleus* species from deep-sea potential gas hydrate bearing area and their comparative genomic analyses

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Two novel methanoarchaeal species, *Methanoculleus taiwanensis* CYW4^T and *M. sediminis* S3Fa^T, were isolated from deep-sea core sediment samples at Deformation Front and Mud Volcano 4 (MV4) , respectively, at offshore southwestern Taiwan, where was intensively investigated by Taiwan gas hydrate research team and both geophysical and geochemical data indicated this is gas hydrate bearing area. In addition, the strain S3Fa^T is closely related to *M. submarinus* and *M.* sp. strain MH98A with 99 % similarity, which were isolated from deep sea methane hydrate-bearing sediments. Evidences here shed dim light that *Methanoculleus* species may play a major role in methane hydrate habitats. To compare the metabolic and physiological features of *Methanoculleus* species isolated from various methanogenic ecosystems including methane hydrate habitats, comparative genomic analyses of nine *Methanoculleus* species were performed. Through comparative genomic analyses, 2-3 coding genes for trehalose synthase were found in all nine *Methanoculleus* genomes, which were not detected in other methanogens and are therefore suggested as a signature of genus *Methanoculleus* among methane-producing archaea. In addition, the structural genes adjacent to trehalose synthase genes are comprised of the signaling module of Per-Arnt-Sim (PAS) domain-containing proteins, Hsp20 family proteins, arabinose efflux permeases and multiple surface proteins with fasciclin-like (FAS) repeat. This indicates that trehalose synthase gene clusters in *Methanoculleus* may play roles as a compatible solute in response to various stresses and regulate carbon storage and modification of surface proteins through accumulation of trehalose. The non-gas hydrate-associated *Methanoculleus* strains harbor carbon-monoxide dehydrogenase (*cooS/acsA*) genes, which are important for the conversion of acetate to methane at the step of CO oxidation/CO_2 reduction in acetoclastic methanogens and further implies that these strains may be able to utilize CO for methanogenesis in their natural habitats. The profile of Cluster of Orthologous Groups (COGs) categories showed that the genome of *M. taiwanensis* CYW4^T contains a significantly high abundance of COGs in the category of 'signal transduction mechanisms' , which indicates that this species may have additional sensing modules to monitor environmental changes. In addition, both genomes of *M. bourgensis* strains MS2^T and MAB1 harbor highly abundant transposase genes, which may be disseminated from microbial communities in their sewage treatment plant and biogas reactors, which are breeding grounds for antibiotic resistance. Through comparative genomic analyses, we gained insight into understanding the life of strictly anaerobic methane-producing archaea in various habitats, especially in methane-based deep-sea ecosystems.

A3-4

Novel energy conservation strategies and behavior of *Pelotomaculum schinkii* driving syntrophic propionate catabolism

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Syntrophic propionate degradation is one of the most thermodynamically constrained microbial catabolism and requires complex energy conservation. Although several studies reveal biochemical strategies at such syntrophic organisms' disposal, the specific mechanisms and behavior revolving around the energy metabolism of propionate degradation remain unclear. Genome sequencing of syntrophic propionate degraders (*Pelotomaculum schinkii* HH, *P. propionicum* MGP, and *Pelotomaculum* sp. FP) was performed. Further comparative genomics of syntrophic propionate-degrading genera (*Pelotomaculum* and *Syntrophobacter*) reveals ubiquitous energy metabolisms necessary for propionate metabolism. Through investigation of the first transcriptome for an obligate syntrophic propionate degrader (*P. schinkii* strain HH) in co-culture with *Methanospirillum hungatei* JF-1, we further demonstrate that strain HH conserves energy through formate generation (i.e., electron confurcation or reverse electron transport) and novel electrogenic extrusion of syntrophic byproducts (i.e., formate or acetate) . In contrast with current understanding of syntrophy, strain HH depends on interspecies electron transfer via formate rather than hydrogen and does not use conventional energy metabolism associated with syntrophy (i.e., CoA transferase, Fix, and Rnf) . We also found that strain HH and the partner methanogen may also interact through both flagellar contact and complementary amino acid exchange. These findings provide a new model for syntrophic energy acquisition and interactions.

A3-5

Smithella propionica LYP uses a novel fourth pathway for syntrophic propionate degradation

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Propionate is a common product of odd-chain fatty acid, amino acid, and sugar decomposition and can be toxic at high concentrations. To prevent propionate accumulation and toxicity, all domains of life encode propionate-catabolizing pathways. So far, three pathways have been characterized (*i.e.*, methylmalonyl-CoA pathway, acrylyl-CoA pathway, and 2-methylcitrate cycle) , but work by de Bok *et al*. (2001) showed that *Smithella propionica* LYP employs an uncharacterized fourth pathway. Although stable isotope analyses revealed that two propionate molecules are condensed and oxidatively split into three acetate molecules, the detailed biochemistry of this unique pathway remains to be uncovered. To elucidate the gene systems involved in propionate metabolism, we sequenced the genome of the type strain *Smithella propionica* LYP and performed transcriptome analysis on a propionate-degrading co-culture of LYP with *Methanobacterium formicicum*. By combining gene expression, in-depth protein phylogeny, and comparative protein 3D modeling, we successfully identified the genes and enzymes responsible for the unusual transformation of propionate to acetate. Notably, *S. propionica* LYP uses a novel 2-methyl-3-oxo-valeryl-CoA mutase and 4-hydroxycaproyl-CoA dehydratase that are phylogenetically and likely functionally unique from any known homologs. In methanogenic environments where *Smithella* inhabits, the conditions are thermodynamically restricting and propionate decomposition requires “syntrophic” electron transfer between a propionate oxidizer and electron-receiving partner. Inspection of LYP’ s genome reveals an unusual set of enzymes for transferring reducing power from propionate oxidation to H^+ and CO_2 respiration. For example, LYP does not depend on conventional energy conservation mechanisms (*e.g.*, electron-confurcating hydrogenase) that all other syntrophic organisms encode. The results suggest that the novel propionate degradation pathway is mechanistically and energetically distinct from known propionate degradation pathways, which has significant ecological implications for syntrophy in methanogenic ecosystems.