Abstracts

Oral Presentations

O1-1

Non-invasive lipid productivity analysis by single-cell innate fluorescent signature

１細胞自家蛍光シグネチャー解析による油脂生産性の仮想的ラベリング

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微生物集団から有用微生物を探索するためには細胞の性質や能力を調べる必要があるが、その操作として従来は細胞破砕による細胞内物質の解析、標識遺伝子の導入による発現量の測定、目的物質の染色などが行われてきた。しかし、こうした従来の方法は細胞の破壊や生理状態の変化を伴うものであり、細胞の性質評価を非破壊的に行うことは困難であった。

そこで本研究では、一細胞ごとに取得した自家蛍光シグネチャーに基づいて油脂酵母*Lipomyces starkeyi*の油脂生産性が予測可能であるか検証した。細胞内の脂質やタンパク質などの分子はそれぞれ異なった自家蛍光を発しており、その集合体である細胞の自家蛍光シグネチャーは細胞の種類や生理状態を反映する。これまではコロニーや培養法など集合菌体の平均しか判別できなかったが、我々は１細胞レベルで自家蛍光シグネチャーを取得し細胞の性質を仮想的にラベリングするNIMI (Non-Invasive Meta Imaging) 法を最近開発した。これは、共焦点顕微鏡による細胞輪郭の認識および共焦点蛍光顕微鏡による蛍光の検出を組み合わせた観察により、細胞集団から１細胞ごとの自家蛍光シグネチャーを取得し、これを画像処理や機械学習を用いて解析することによって、細胞の種類や性質を迅速かつ非破壊的に予測する技術である。

本研究はNIMI法を用いて、油脂生産性の異なる*L. starkeyi*のＷＴおよび各種変異株から顕微鏡観察により１細胞ごとの自家蛍光シグネチャーを取得し、画像処理・機械学習によって細胞を識別できるか検討した。その結果、油脂生産性に応じた高精度なクラス分類が可能であった。

*L. starkeyi*など微生物細胞の油脂生産性を調べるためには、従来は菌体破砕・脂質抽出による脂質蓄積量の測定や、細胞ごとの脂肪球サイズの計測などの操作が必要あり、菌体へのダメージや多くの手間を要することが問題であった。一方、NIMI法を用いることで１細胞ごとの油脂生産性を効率良く予見的に調べることができ、非破壊検査であるため有用性の高い細胞をそのまま利用することができると考えられる。以上のように、NIMI法は従来の細胞解析方法が抱える問題のブレークスルー技術として、細胞集団から目的の性質を持つ細胞・微生物を自家蛍光シグネチャーに基づいて探索する革新的スクリーニング法の基盤となることが期待される。

O1-2

Molecular mechanism of methylotaxis in *Methylobacterium aquaticum* strain 22A

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Plants emit about 100 million tons of methanol annually into the atmosphere, and *Methylobacterium* species that can use methanol become dominant occupying 10-20% of the bacteria on the plant surface. They can promote growth of plants and are believed to be symbiotic bacteria beneficial to the plant. However, it is largely unknown what kind of chemical substances mediate the plant-bacteria recognition. We found that *M. aquaticum* strain 22A isolated from a moss, *Racomitrium japonicum*, shows chemotaxis to methanol (methylotaxis). The methylotaxis must be important for the bacteria to find plants to colonize, but its sensor and mechanism have never been characterized. The chemotaxis in bacteria is mediated by methyl-accepting chemotaxis protein (MCP) sensing the chemoattractant and multiple chemotaxis proteins controlling the flagellar rotation direction. There are 52 MCP genes in strain 22A genome. Based on RNA-Seq data, we chose several MCP candidate genes showing higher expression levels in methanol growth. We checked methylotaxis of the MCP gene deletion mutants, and finally we found that three genes (*mcp1*, *mcp2*, and *mcp3*) are involved in methylotaxis. The methylotaxis was completely lost in the triple gene mutant. But the mutant retained chemotaxis toward organic acids. These three MCPs function independently because methylotaxis was not lost in the single gene or double genes deletion mutants. Based on the prediction of MCP structures, Mcp1 was considered a cytoplasmic protein, which consists only of the MCP signal domain. Mcp2 contains a PAS domain that was responsible for energy taxis. Mcp3 has a typical MCP structure with a HAMP domain. The localization analysis of GFP-tagged MCPs in the cells showed that Mcp1 localizes in the cytoplasm, Mcp2 locates at a cell membrane or pole, and Mcp3 locates at the pole. Functional gene complementation could be done only for Mcp1. Methylotaxis in the presence of La showed that MCP2 is related to methanol metabolism since *Δmcp2* decreased methylotaxis in the presence of La. The triple gene deletion mutant gathered slower than wild type to the roots of rice and *Arabidopsis thaliana*. We concluded that methanol and methylotaxis are important for *Methylobacterium* species to establish symbiosis.

O1-3

Comparative metatranscriptomics reveals extracellular electron transfer pathways in electrogenic microbiomes conferring microbial adaptivity to surface redox potential changes

メタトランスクリプトーム解析によって解き明かす電気微生物の表面電極電位変化への応答

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Some microbes can capture energy through redox reactions with electron flow to solid-phase electron acceptors, such as metal-oxides or poised electrodes, via extracellular electron transfer (EET). While diverse oxide minerals, exhibiting different surface redox potentials, are widely distributed on Earth, little is known about how microbes sense and use the minerals. Here we show electrochemical, metabolic and transcriptional responses of EET-active microbial communities established on poised electrodes at three different redox potentials to changes in the surface redox potentials (as electron acceptors) and surrounding substrates (as electron donors). Combination of genome-centric stimulus-induced metatranscriptomics and metabolic pathway investigation revealed that nine *Geobacter/Pelobacter* microbes performed EET activity differently according to their preferable surface potentials and substrates. While the *Geobacter/Pelobacter* microbes coded numerous numbers of multi-heme c-type cytochromes (MH-cytCs) and conductive e-pili, wide variations in gene expression were seen in response to altering surrounding substrates and surface potentials, accelerating EET via poised electrode or limiting EET via an open circuit system. Especially, the open-circuit stimulus induced the most remarkable responses to adapt to the worse condition for the EET-active microbes; swimming away, seeking other electron acceptor, or inducing different outer-membrane MH-cytCs for tuning EET pathways. These flexible responses suggest that a wide variety of EET-active microbes utilizing diverse EET mechanisms may work together to provide such EET-active communities with an impressive ability to handle major changes in surface potential and carbon source availability.

O1-4

Algal polysaccharide degrading bacteria isolation and genetic background characterization

海藻多糖分解菌の単離およびその遺伝的バックグランドの関連性・特性

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Macroalgae constitutes of algal polysaccharides that are important resources for pharmaceutics, the food industry and bioethanol production. Thus, recent efforts to depolymerize these polysaccharides have promoted the isolation of numerous novel bacteria. However, understanding the traits of these strains have focused vastly on the function and structure of the depolymerizing enzymes. Primarily, much is still unclear on the importance of these strains within its habitat or with its residing host. In this work, working towards this goal, we elucidated some of the genetic features and characteristics of algal-associated bacteria by cell enrichment cultivation and metagenome analysis. Here, we report our recent discoveries focusing on bacteria that depolymerizes alginate. From our cell enrichment cultivation experiments, microbes were collected from the supernatant of fermented brown algae and feces of gastropods. Alginate-degrading bacteria were isolated by cultivation in marine agar plates supplemented with 1% alginate. Among the total 11 strains isolated, we were successful in isolating a novel alginate-degrading bacterium, *Falsirhodobacter* sp. alg1. Strain alg1 only harbors single homologs of each endo- and exotype alginate lyase which is rare among alginate-degrading bacteria and functional analysis of these lyases interestingly showed that they were extremely efficient in alginate depolymerization. Further genetic comparison with other bacterial strains showed that the alginolytic gene cluster of strain alg1 was highly conserved among terrestrial bacterial strains such as *Sphingomonas* sp. strain A1 and *Agrobacterium fabrum* str. C58. The discovery of strain alg1 indicates that algal polysaccharide degrading bacteria could serve as potential candidates for cross-lateral gene transfer and evolutionary studies. From our metagenomic approach, metagenome libraries were constructed from microbes isolated from fermented brown algae and screened for alginate lyase harboring clones. Annotating the genes and alginolytic gene clusters, we found a unique pectin/alginate degradation gene cluster that was completely identical to a recently reported *Halomonas* strain isolated from brown macroalgae in Canada. Although we were unable to determine the bacterial host of our identified gene cluster, the discovery of identical gene clusters from completely different geological regions strongly hinted that host-bacterial interactions may exist within macroalgae species. In summary, our different approaches to elucidate the genetic background and characteristics of algal-degrading bacteria realized the complexity and evolutionary aspects of algal polysaccharide microbes and its close relation to its residing macroalgae host. We hope that further understanding their genetic features would allow us to clarify the importance of these bacteria and subsequently promote other possible downstream applications.

O1-5

Phylogenomic analysis of catalase genes of lactic acid bacteria in the order *Lactobacillales*

乳酸菌（*Lactobacillales*）ゲノムにおける catalase の遺伝子分布と構造

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【背景】通性嫌気性である乳酸菌種は呼吸鎖を保持しておらず、一般的にはカタラーゼ（catalase）陰性であると考えられてきた。しかしながら近年、少数ながらも、カタラーゼ活性を保有する乳酸菌株の存在が報告されている。今回、*Lactobacillales*目に属する細菌種のゲノムよりカタラーゼ遺伝子を網羅的に検索し、系統分布、配列および構造特性について解析した。
【方法と結果】IMG (the integrated microbial genomes database) に登録されている*Lactobacillales*に属する細菌種のゲノム配列より、BLASTPを用いてカタラーゼ遺伝子を検索した。この結果、*Lactobacillus*属や*Enterococcus*属を中心に、ヘム依存性カタラーゼ遺伝子のホモログが高頻度で検出された。これらヘム依存性カタラーゼ遺伝子の配列中には、ヘム結合アミノ酸残基が高度に保存されていた。またSWISS-MODELによるタンパク質構造推定の結果、*Bacillus subtilis*のKatAなどと同様に、四量体を形成することが推察された。さらに*Enterococcus*属を中心とした乳酸菌種からは、マンガンカタラーゼのホモログが高頻度で検出された。これらは*Thermus thermophilus*等が保有する他のマンガンカタラーゼと同様に、六量体を形成することが推察された。
【考察】発酵食品の製造過程、および病原菌としての感染過程において、乳酸菌種は多大な酸化ストレスに曝露される。一般にカタラーゼ陰性と考えられてきた乳酸菌種であるが、実際には広範な系統においてカタラーゼが分布しており、活性酸素に対する防御機構として、巧妙な環境適応能力を獲得していることが推察された。

O1-6

Morphological and genomic characterization of a novel phagotrophic bacterium *Candidatus* "Uab amorphum"

新奇捕食性バクテリア *Candidatus* "Uab amorphum" の形態及びゲノム特性について

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真核生物と原核生物は、細胞サイズや複雑性、ゲノム構造において大きく異なる。祖先的な真核生物はその進化の過程で、細胞のサイズと複雑性の増大と内膜系や細胞小器官の獲得、アクチンやチューブリンからなる細胞骨格の発達が起こり、それらによって外界からの大型の粒子の取り込み（ファゴサイトーシス）が可能になったと考えられている。真核生物の初期進化についての仮説の中には、ファゴサイトーシスによるαプロテオバクテリアやシアノバクテリアの取り込みが、ミトコンドリアや葉緑体の獲得をもたらしたとするものもある。真核生物の誕生は生命進化において最も重要な出来事の一つである一方で、これまで真核生物と原核生物の中間に当たる生物が見つかってこなかったことから、その過程はほとんど明らかになってこなかった。

我々は2015年に、パラオ共和国の海水中から新奇バクテリア*Candidatus* “Uab amorphum”を発見した。これまでの研究から、本生物は大型で柔軟な細胞、複雑な繊維状の細胞骨格をもち、他のバクテリアや小型の真核生物を包み込んで捕食することが明らかとなっている。*Ca*. “Uab amorphum”は真核生物のファゴサイトーシスに似た捕食を行う初めての原核生物であり、進化的な重要性だけではなく、捕食者としての生態的な役割についても興味深い。分子系統解析では、本生物はバクテリアの一群であるPlanctomycetesに属することが明らかとなったが、16S rRNA遺伝子配列は最も近縁なバクテリアと比較しても20%以上の差異が見られた。また様々な環境由来の16S rRNA遺伝子配列が、本生物と単系統群を形成することも明らかとなり、これまで認識されてこなかったものの、本生物のような捕食性バクテリアが普遍的に存在していることを示唆している。ゲノム解析の結果、本生物は比較的大型の環状ゲノムをもち、少数の真核生物由来の遺伝子をもつ点や、核酸やアミノ酸の生合成経路において興味深い特徴が明らかになった。これらの結果に基づき、本発表では本生物の真核生物的な特徴の進化や、生態的な役割について議論したい。

O1-7

Molecular phylogenetic analysis of *Acanthamoeba castellanii medusavirus*

Acanthamoeba castellanii medusavirus の分子系統学的解析

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【目的】我々が日本の温泉水から分離したNCLDV（核細胞質性大型DNAウイルス）である*Acanthamoeba castellanii medusavirus*（*Medusavirus*）は、粒子の外径がおよそ250 nmの正二十面体ウイルスである。ゲノム解析の結果、これまでに知られているどのNCLDVにも属さない、新規のグループを形成するウイルスであることが明らかとなった。そこで本研究では、*Medusavirus*に特徴的な遺伝子について解析し、その生物学的意義を明らかにすることを目的とした。

【方法】GeneMarkを用い、*Medusavirus*ゲノムの遺伝子予測を行った。*Medusavirus*粒子からゲノムDNAをNucleoSpin® Tissue XSを用いて抽出した後、DNAをCy-3で蛍光標識し、*Medusavirus*感染細胞を経時的にメタノールで固定して、FISH法により*Medusavirus* DNAのアメーバ細胞内局在を検討した。また、精製した*Medusavirus*粒子からタンパク質を抽出し、LC-MS/MSを用いたプロテオーム解析を行った。現在、*Medusavirus*ヒストンタンパク質の精製を目的として、PCRにより*Medusavirus*ヒストン遺伝子を増幅し、pET21ベクターにクローニングした後、*Medusavirus*ヒストンタンパク質の大量発現を試みている。

【結果と考察】保有する遺伝子に関する興味深い特徴として、ほとんどのNCLDVが持つRNAポリメラーゼならびにトポイソメラーゼⅡ遺伝子を持たないこと、真核生物ヒストン遺伝子と相同性の高い遺伝子をフルセット（H1、H2A、H2B、H3、H4）持つことが明らかとなった。これまでNCLDVでは、*Marseilleviridae*がヒストン遺伝子を３～４種類持つことがわかっていたが、真核生物と同じ５種類のヒストン遺伝子を持つことが明らかとなったのは*Medusavirus*が初めてであり、また２種のヒストン遺伝子の融合遺伝子をもつ*Marseilleviridae*とは異なり、*Medusavirus*はこれら遺伝子をそれぞれ独立した遺伝子として保有していることが明らかとなった。*Medusavirus*粒子のプロテオーム解析の結果、５種のヒストン遺伝子のうち４種（H2A、H2B、H3、H4）のコアヒストンがタンパク質に翻訳されていることが明らかとなり、粒子内もしくは宿主細胞内で真核生物のヌクレオソーム構造と同じような構造を呈していることが示唆された。一方、FISH法を用いて、*Medusavirus* DNAの宿主細胞内での動態を解析したところ、感染後２時間までに宿主細胞の細胞核に局在し、感染後８時間にかけて細胞核内で複製することが明らかとなった。

【展望】今後は、*Medusavirus*ヒストンタンパク質の結晶化ならびに三次元構造の詳細な解析を行いつつ、粒子内でヌクレオソーム構造を形成しているか、宿主細胞核内での複製に*Medusavirus*ヒストンがどのような役割を果たしているかを解析していく予定である。

O1-8

[ASME] Skin biofilm-derived *Propionibacterium acnes* genotypes are unique to each individual

皮膚バイオフィルムを構成するアクネ菌遺伝子型は個人固有である

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The human skin surface harbors multiple species of bacteria and they form a highly complex biofilm. It has been reported that the skin microbiome profile is different in various body parts and relatively stable across long periods of time. In skin biofilm, gram-positive rod shape biofilm forming bacterium *Propionibacterium acnes* is one of the most abundant bacteria. A previous study showed that *P. acnes* accounts for 89 percent in the human pilosebaceous units. *P. acnes* prefers anaerobic conditions and is widely known as the pathogen that causes acne. *P. acnes* species is also known to have many variations of 16S rRNA gene genotypes. Although the skin microbiome profile of our body has been well studied, *P. acnes* composition in skin biofilm is still not fully understood. In this study, we looked into the difference of genotype composition of P. acnes in the skin biofilm of various body parts from 3 individuals as well as one's hand and his/her possession from 10 individuals. We also looked into the change of genotype composition across a long period (5 months). We developed a next generation sequencing based high-throughput *P. acnes* 16S rRNA gene genotyping method. Our study showed that *P. acnes* genotype composition unlike skin microbiome, differs between each individual but remains similar across different body parts. Furthermore, it is shown that *P. acnes* genotype composition is similar comparing one's skin and his/her possessions. Besides, our study showed that the similarity in *P. acnes* genotype composition is higher than skin microbiome across a long period of time. This suggests that the biofilm formation character of *P. acnes* may contribute to this phenomenon. In previous study, it is suggested that the skin microbiome that left on one's possession could act as a sort of "fingerprint" and be used for owner identification. Therefore, we considered that *P. acnes* 16S rRNA gene genotype may be used as a tool of owner identification and attempted owner identification by random forest machine learning. We were able to gain a high accuracy rate in owner identification using *P. acnes* genotype composition. Furthermore, the accuracy of using both *P. acnes* genotype composition and microbiome profile was even higher than that of using *P. acnes* genotype composition only. Therefore, it is considered that *P. acnes* genotype composition could be a useful tool in the field of owner identification.

O1-9

Metaepigenomic analysis reveals an unexplored diversity of DNA methylations in environmental prokaryotic community

メタエピゲノム解析が明らかにする環境細菌叢の DNA メチル化多様性

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ゲノム中の塩基のメチル基修飾（DNAメチル化）に代表されるエピジェネティクスは、ヒトを始めとする真核生物において遺伝子の発現制御などに深く関わっている一方で、細菌や古細菌においてもファージ感染に対する防衛機構 (制限修飾系) や遺伝子転写制御、DNA修復等の生理生態学的に重要な役割を担っている。近年の研究から、細菌・古細菌のほぼ全ての系統においてDNAメチル化が普遍的に起きていると考えられているが、未培養系統群においては勿論、培養可能な系統群であっても単離培養されていない微生物に対しては、実験的な制約の為にDNAメチル化の観測が困難であり、環境細菌群衆のDNAメチル化の普遍性や多様性は検証されて来なかった。そこで我々は、環境細菌叢を解明する上で有効な手段であるメタゲノム解析と、1分子シーケンシング技術（PacBioシーケンサー）によるメチル化観測技術を組み合わせ、難培養性微生物が優占する環境細菌叢のDNAメチル化を包括的に観測する「メタエピゲノム解析」を行ってきた。

本研究では、微生物生態学的な面白さや実験的な制約を考慮し、日本最大の湖である琵琶湖の水圏微生物を対象に、Circular Consensus Sequencing (CCS)の手法を用いたショットガンシーケンスを行い、環境細菌叢のDNAメチル化を検証した。表層5 mと深層65 mの水サンプルを採取し、シーケンスリードをアセンブリした結果、難培養性細菌種のドラフトゲノムを19株分得られ、その大半は難培養性で琵琶湖に優占している細菌種に由来していた。これらのゲノム中におけるDNAメチル化の検出を行ったところ、新規のものを含む複数のメチル化モチーフの検出に成功した。さらに各モチーフに対応するメチル化酵素遺伝子を推定し、新規の関係性を持つと予測された4組について大腸菌を用いた検証実験を行い、対応関係を実証した。一方でメチル化不検出であったゲノム中からはプロファージが多く検出され、制限修飾系がファージ感染の防衛に寄与していることが示唆された。本研究は、難培養性微生物が優占する環境微生物のDNAメチル化を世界に先駆けて検証した研究である。

O2-1

Glycogen Metabolism of Anammox Bacteria *Candidatus* Brocadia sinica: Comparison of Growing, Stationary, and Starvation Phase

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An anaerobic ammonium-oxidizing (anammox) bacterium, *Ca.* Brocadia sinica, is a chemolithoautotroph that obtains energy by oxidizing ammonia in the absence of oxygen and fixes CO_2 via the reductive acetyl-CoA pathway. Despite their environmental and engineering importance, nothing is known about their regulation and metabolism of glycogen, a source of carbon and energy storage. Here, we studied the glycogen metabolism in *Ca.* Brocadia sinica. *Ca*. B. sinica was continuously grown in a membrane bioreactor (MBR), in which actively growing, stationary, and starvation phase was developed intentionally by varying the substrate loading rate. Intracellular glycogen concentration was quantified during the entire cultivation period. The results revealed that glycogen was produced in the actively growing phase and utilized (from 2.3×10^{-2} to 4.3×10^{-4} mg glucose/mg biomass dw) in the starvation phase. After the starvation period, Ca. B. sinica was forced to grow actively again and the growth cycle was repeated. The same glycogen metabolic pattern was observed. The proteomic analysis was also performed under each growth phase and exhibited the expression of most of the enzymes involved in glycogen synthesis and degradation, which reflects the time course of the observed intracellular glycogen concentration. This finding provides a new insight of the intracellular organic carbon (glycogen) as an alternative carbon and energy source of anammox bacteria during starvation.

O2-2

Isolation Process and Genomic Analysis of Nitrite Oxidizer *Nitrotoga* sp. Provide Insights on Physiological Characteristics and Clues to Promote the Growth

分離培養プロセスとゲノム情報から推定する亜硝酸酸化細菌 *Nitrotoga* の生理学的性質および増殖促進条件

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[Introduction] The type species of genus *Nitrotoga* are nitrite-oxidizing bacteria (NOB), which is the key drivers in the second step of nitrification and biogeochemical nitrogen cycle. The recent cultivation-independent methods have revealed that *Nitrotoga* are widespread in cold terrestrial ecosystems and man-made habitats. Despite ecological and industrial significance of the cold-adapted nitrite oxidizers, no representative in pure culture and genome sequence was reported. This widens the gap between molecular-based studies and cultivation-dependent physiological experiments. Previously, we enriched *Nitrotoga* sp. AM1 after long-term incubation at low temperature and partially characterized its physiological properties. The aim of the current study is to obtain AM1 isolate and analyze the genome.

[Isolation] The physical enrichment process using a cell sorting system and antibiotics treatment drastically improve the purity of the AM1 enrichment culture. Slower growth rate of AM1 than some types of heterotrophs co-existing in the enrichment culture was obstacle for the isolation. Therefore, ammonia and pyruvate, which could stimulate the growth of some NOB in previous studies, were added into the mineral salts medium. Multiple cycles of sub-cultivation using the mixotrophic medium successfully resulted in isolating AM1 from the enrichment culture.

[Genome] High quality draft AM1 genome was reconstructed using paired-end and mate-pair reads. AM1 genome possessed the essential genes responsible for chemolithoautotrophic life style, glycolysis and tricarboxylic acid cycle. Interestingly, nitrite oxidoreductase, which is the key enzyme for nitrite oxidation by NOB, identified in the AM1 genome suggested that its active site faces the periplasmic space. Defense mechanisms against oxidative stress were also founded in AM1 genome.

[Physiological experiments with AM1 isolate] Genomic information indicated that AM1 could utilize ammonia as nitrogen source and pyruvate as energy source. To confirm whether ammonia and pyruvate promote the cell activity, the AM1 isolate was incubated in the mixotrophic medium. The nitrite oxidation rate increased by 1.2-1.6 fold in the presence of ammonia and pyruvate compared with control group. Another reason for the increase was that pyruvate likely decomposed hydrogen peroxide produced during growth on nitrite oxidation. We hypothesized that protection of AM1 cells against oxidative stress stimulate the cell activity. As expected, incubation with catalase from bovine liver strongly accelerated nitrite oxidation. This study based on the isolate and genome contributes to understand physiologically as-yet-unknown characteristics of *Nitrotoga*. Not only alternative nitrogen and carbon source but hydroxyl peroxide scavengers would facilitate to cultivate slow-growing NOB.

O2-3

Unveiling acetate- and CO_2-utilizing microbiota under methanogenic conditions in *Sasa*- - invaded wetland soils

笹侵食湿地土壌中でメタン生成菌と基質競合する未知微生物の網羅的同定

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【目的】メタン生成は嫌気有機物分解の最終反応であり、酢酸資化性または水素資化性のメタン生成菌が直接的に関与する。湿地は主要な大気メタン放出源のひとつであるが、笹に侵食された区域ではメタン生成が抑制される。ここでは酢酸および水素/二酸化炭素をめぐりメタン生成菌と基質競合する微生物の存在が強く示唆される。しかし、これらの反応を担う微生物の存在や多様性はほとんど分かっていない。本研究は、超高感度Stable Isotope Probing（SIP）によりメタン生成菌と基質競合する未知微生物の網羅的な同定を目的とした。
【方法・結果】北海道美唄湿原の笹侵食湿地土壌を試料に用い、実験①として^{13}C-酢酸、実験②として水素/^{13}C-重炭酸を基質として加え、それぞれ嫌気的に培養した。実験①では培養開始後$_{13}$C基質由来の$^{13}CH_4$と$^{13}CO_2$の生成および酢酸濃度の減少が観察された。培養2週間目の試料からRNAを抽出し、超高感度rRNA-SIPへと供した。その結果、^{13}C-酢酸を分解し取り込んだ微生物が36種検出された。嫌気酢酸酸化細菌として、脱ハロゲン化呼吸能を有する*Dehalogenimonas*属に近縁な細菌が1種検出され、また酢酸資化性メタン生成菌である*Methanosaeta*属古細菌2種も同定された。特筆すべきは、ここで同定された大部分を占める33種が既知微生物の16S rRNA遺伝子に対して低い配列相同性（77.8%－92.5%）を示したことである。実験②では培養開始後、$^{13}CH_4$の生成に伴って水素濃度が減少し、培養2週目には酢酸濃度の増加が観察された。上記と同様に培養2週間目の試料を超高感度rRNA-SIPに供した結果、全部で20種が^{13}Cを取り込んだ微生物として検出された。そのうち1種は酸耐性を示す*Clostridium*属細菌、4種は水素資化性メタン生成菌である*Methanoregula*属古細菌、1種は共生酸化反応を担う*Lentimicrobium*属に近縁な細菌であった。ここでも注目すべきは、その他に検出された14種が系統学的に新規な未培養細菌であったことである（配列相同性:78.4%－94.1%）。一方で、培養開始時の微生物群集構造を解析した結果、二つの実験で同定された微生物の相対存在量はいずれも0.67%以下の値を示した。これらの結果は、笹に侵食された湿地において希少な未培養微生物群がメタン生成菌と基質競合し、メタン放出の抑制に寄与するという事象を示している。

O2-4

Is the retinal-synthesizing gene (*blh*) essential for rhodopsin-containing bacteria?

レチナール生産遺伝子 (*blh*) はロドプシン保有細菌に必須か？

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ロドプシンはタンパク質部分の「オプシン」と発色団部分の「レチナール」色素からなる7回膜貫通型の光受容タンパク質である。海洋表層に生息する原核生物に広く分布し、その多くが光駆動型のイオン輸送体として働くことが知られている。レチナールは、環境中に広く存在するβカロテンからβ-carotene 15,15'-dioxygenase (*blh*)により生産される。これまで【βカロテン→(*blh*)→レチナール】と考えられてきたが、近年*blh*を持たないロドプシン保有細菌が数多く見つかってきた。そのためこれらの細菌は未知のレチナール生産経路を持つか、あるいは外部からの供給に依存すると考えられる。関連する先行研究では、ロドプシンのイオン輸送活性には外部からのレチナール添加が必要であると報告されているが、その一般化には至っていない。本研究ではこの疑問に答えることを目的として、先行研究で用いられた株に近縁である*Aurantimicrobium minutum* KNC^{T}を用いてイオン輸送活性等の解析を行った。

*A. minutum*は明条件・暗条件いずれの培養条件下においてもイオン輸送活性を示すと共に、レチナール分析の結果から、*A. minutum*の細胞にはレチナールが含まれることが示唆された。すなわち、*A. minutum*は*blh*を必要としない未知の経路によりレチナールを生産している可能性が示された。一方で、外部からのレチナール添加により、イオン輸送活性は上昇したことから、この株が生産できるレチナール量は細胞内のオプシン量に比べて、かなり少ないと考えられる。このレチナール添加による効果は、明条件の方が大きかったため、明条件ではレチナールと結合しているオプシンの割合が低く、暗条件ではその割合が高いことが示唆される。このことから、*A. minutum*は条件に応じて細胞内、外のいずれのレチナールも利用できる可能性が明らかとなり、この細菌がロドプシンを機能させるために*blh*は必須で無いことが示された。生成経路および生成条件の解明と*blh*を持たない他の株の検討を行っていく予定である。

O2-5

Characterization of *E.coli* drug efflux pump involved in bisphenol A resistance

大腸菌の薬剤排出ポンプがビスフェノール A 耐性に及ぼす影響

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【緒言】多剤耐性菌で院内感染を引き起こす*Stenotrophomonas maltophilia*は低濃度（0.3mM）のビスフェノールA（BPA）で増殖が阻害されるが、その詳細な増殖阻害機構は明らかになっておらず、その機構解明により新規微生物制御法の開発が期待できる。当研究室では本来BPA耐性である大腸菌が、薬剤排出ポンプの構成因子である外膜タンパク質をコードする*tolC*の欠損によってBPA感受性になることを見出した。そこで、*S. maltophilia*のBPA感受性の基礎的知見を得るため、大腸菌野生株及び*tolC*破壊株のBPA耐性と薬剤の排出活性の関係について検討した。

【実験方法】BPA耐性試験は0から0.3mMまで段階的にBPA濃度を調整したLB液体培地に前培養液を10μL接種し37℃で24時間振とう培養した後に濁度（OD_{660}）を測定した。薬剤の排出活性は菌体に蓄積されたローダミン6Gを蛍光測定により求めた。ローダミン6Gは蛍光物質であり、これを0.5mg/L含むLB液体培地に前培養液を10μL接種し37℃で3時間培養後集菌し、リン酸バッファーで2回洗浄した。菌体濁度が0.3になるよう同バッファーで希釈し分光蛍光光度計で蛍光強度を測定し（蛍光波長550nm、励起波長525nm）、上清の蛍光強度との差を菌体内に蓄積したローダミン6Gとした。

【結果、考察】ローダミン6Gの蓄積試験では大腸菌野生株に対し*tolC*破壊株が13.8倍大きな蛍光強度を示したことから、野生株ではTolCを有する薬剤排出ポンプでローダミン6Gを排出していると考えられた。またBPAの存在下でローダミン6G蓄積試験を行うことで、両物質の競合試験を行った。その結果BPAが存在しない場合と比較して0.8mM BPA共存下では1.1倍、1.2mM BPA共存下では3.2倍に蛍光強度が増加した。このことからローダミン6GとBPAの排出が競合していることが予想された。これらの結果から大腸菌野生株はTolCを有する薬剤排出ポンプで菌体内のBPAを排出することで耐性を得ていることが示唆された。

O2-6

[ASME] Exogenous addition of biosurfactants to disrupt *Pseudomonas aeruginosa* PAO1 biofilms

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Pseudomonas aeruginosa is a Gram-negative bacterium found ubiquitously throughout the environment. It has been used extensively as a model biofilm forming organism and for its ability to rapidly acquire resistance to chemotherapeutic agents. Simple hand washing with soap and water is effective in killing and removing many potentially harmful bacteria due to the surfactants, amphiphilic surface-active substances. These molecules lyse cells and change the wettability of liquids on surfaces thereby enabling the washing away of bacteria; importantly, surfactants do not apply selective pressure on bacteria. Our research focuses on the application of sophorolipid (SLL) biosurfactant as an antibiofilm agent on *P. aeruginosa* PAO1. Biosurfactants are surfactant synthesized by living organisms and is made from 16-18 carbon fatty acid tail and sophorose. Biosurfactants have gained interest because they are typically more eco-friendly than chemically synthesized surfactants and because they are biosynthesized may have novel functions. To characterize the effect of SLLs on PAO1 growth, we first test its bactericidal effects. We find that addition of SLL does not affect the growth rate of PAO1 in shaking culture, both as measured by the optical density and by counting colony forming units. Interestingly, in shaking culture PAO1 treated with 1 wt% SLL shows a 95% decrease in pyocyanin production as compared to an untreated sample, which is a major PAO1 virulence factor. SLL does not seem to be bactericidal but seems to decrease virulence factors suggesting that it may affect intracellular signaling. To understand the effect of SLL on biofilms we examine the early stages of surface attachment in microfluidic channels. We find that SLL strongly inhibits PAO1 surface attachment to glass surfaces at low concentrations. At higher concentrations, nearly all bacteria are washed out of the device without being killed. To explore these effects further, we tested the effect of SLL on mature PAO1 biofilms grown in microchannels. We find that low concentrations of SLLs can catastrophically disrupt pre-formed PAO1 biofilms, which was unexpected since it suggests that SLLs can weaken the surface links and breakup the internal crosslinks of the extracellular matrix in WT PAO1. This biosurfactant has interesting properties since it is able to suppress attachment, disrupt mature biofilms without killing the bacteria and down regulate major virulence factor. Understanding the balance to control bacterial communication and biofilm formation is an important nuanced view of biofilm formation that we are trying to develop.

O2-7

Microbiome in awamori moromi (mash) affecting the flavours and its application of awamori production

泡盛の芳香に影響を与えるもろみ中の微生物群と泡盛製造への応用

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泡盛はインディカ米を原料とする沖縄の蒸留酒で、黒麹菌と泡盛酵母が関与し、これを特徴付ける芳香成分の一つにバニリン(V)がある。これは原料米中のフェルラ酸(FA)がフェノール酸脱炭酸酵素(Phenolic Acid Decarboxylase；PAD)により4-ビニルグアヤコール(4-VG)へ変換され酸化により生成する。従って泡盛の芳香生成におけるPADの重要性が示唆されるが、本酵素活性がどの微生物由来なのか？は明らかではない。黒麹菌はクエン酸を生産しもろみ中の雑菌混入を防ぐが、乳酸菌はクエン酸存在下でも生き延びる。これは泡盛の発酵系は黒麹菌と酵母のみではなく、第3の微生物が関与する複合的な発酵システムと捉えることができる。本研究では、この泡盛もろみを複合微生物生態系と捉え、その微生物多様性について解析すると共に、泡盛もろみより分離した乳酸菌の役割を評価し、PADがどの微生物由来か？を明らかにし、泡盛芳香改良のヒントを得ることを目的とした。

まず始めに泡盛もろみ中のNGS（16SrDNA）解析を行い、もろみマイクロビオーム(モロミクス)解析を行った。次にもろみから乳酸菌の分離を試み、グラム染色及び糖資化性試験、16SrDNA解析を行った。また黒麹菌及び泡盛酵母ゲノム配列を用いてPAD遺伝子を探索した。さらに乳酸菌添加と未添加の泡盛もろみを作成し、FA・4-VG・V濃度を測定した。さらにPAD遺伝子誘導条件の検討も行った。上記解析により分離された乳酸菌は*Lactobacillus plantarum*を示し、ゲノム上にPAD遺伝子が見いだされ、もろみ中での4-VG変換の寄与が示唆された。黒麹菌にもPAD遺伝子が見いだされたが、泡盛酵母にはコードされていなかった。従ってもろみ中でのFから4-VG変換への寄与は、黒麹菌及び乳酸菌の両者が関与している可能性があり、乳酸菌添加・未添加もろみのFA、4-VG、V濃度を比較したところ、添加もろみ中の4-VG濃度の増加が確認された。これは、乳酸菌が関与する複合的な発酵系による、泡盛芳香生成への寄与を示唆する。現在、PADの発現誘導メカニズムなど詳細な解析を行っている。また、泡盛の芳香を豊かにする一つの候補としてカプロン酸エチル（吟醸香）にも着目し、本物質高生産泡盛酵母の育種も同時に行い泡盛芳香改善の多様性技術の確立も試みている。さらにこれら発酵過程のモデル化を通したシュミレーションを目指すことで「ゲノム育種を基盤としたデザイン発酵技術(genome-breeding based designed fementation)」研究もスタートさせた。本研究発表では、上記結果とこれからの将来展望も併せて議論したい。

O2-8

Visualization of temporal dynamics of single-cell innate fluorescence signature

一細胞自家蛍光シグネチャーの時間的ダイナミクスの可視化

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微生物細胞を構成する分子は環境に合わせてダイナミックに変動する。例えば、増殖過程では栄養の枯渇や代謝産物の蓄積に対応して代謝系や細胞内の化学組成が大きく変化する。しかし、伝統的な細胞分析手法では染色や細胞破砕、抽出など侵襲的な処理を必要とするために、こうした生理状態の変化をリアルタイムで可視化することは困難だった。一方で細胞の自家蛍光パターンは細胞内の生体分子が発する自家蛍光の総和であり、細胞内の代謝ネットワーク再構成や化学組成変化を反映して大きく変化する。従来、細胞自家蛍光の分析はコロニーや培養液といった細胞集団の平均値でしか分析できなかったが、我々は最近、一細胞の自家蛍光を分析する手法NIMI(Non-Invasive Meta Imaging)法を開発した。本研究では、この新規手法を利用することで共焦点顕微鏡の視野下で細菌細胞を培養しながら、一細胞の自家蛍光パターンが増殖フェイズと共に大きく変化していくダイナミクスをリアルタイムで可視化した。マイクロ流体デバイス中に保持したグラム陰性細菌の一細胞ごとの分裂速度と自家蛍光パターンの変化を追跡した結果、特に細胞の分裂速度が低下する時期に、定常期の細胞を特徴づける長波長の自家蛍光成分が急速に現れることが記録された。さらに、自家蛍光パターンの特徴の違いから、生育段階を判別することも可能であった。このことから自家蛍光パターンの分析を通じて、細胞が環境の変化に応答するタイムスケールやその度合い（magnitude）を定量的に分析できる可能性が示唆された。自家蛍光は細胞に生得的に備わっているものであり、今回の実験でも細胞には後天的な染色や遺伝子導入といった処理は行なっていない。つまり本研究の結果は、intactな細胞が周囲と相互作用する様子を捉え、実環境中での微生物の生理状態をリアルタイムで解析する手法の青写真を示すものである。

O2-9

[ASME] Analysis on the single-cell metabolic pathway of electrogenic bacteria with nanoscale secondary ion mass spectrometry

高分解能二次イオン質量分析を用いた単一細胞の炭素・窒素同化速度比に基づく代謝経路解析

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Rapid development of analytical methods enabled to examine metabolic features of a single cell and revealed unexpected diversity within a microbial population [1,2]. Those single cell analyzing techniques, however, has not widely succeeded in capturing metabolic pathway and activity of a single microbial cell at the same time due to the insufficient sensitivity. This problem is even more critical for the cells with low metabolic activity, though the importance of oligotrophic bacteria for geochemical cycles was identified [3]. Nanoscale secondary ion mass spectrometry (NanoSIMS) is one of the most promising methods for simultaneous analysis on microbial activity and metabolic pathway at single-cell level due to its quite high sensitivity [2]. However, it cannot provide the information about various metabolites in the cells but only the isotopic composition of elements such as carbon and nitrogen because soluble metabolites are not preserved in sample preparation procedure. Therefore, NanoSIMS has not been adopted to the analysis of microbial metabolic pathways, although it was applied to the analysis on metabolic activity in environmental [4] and laboratory microbes [1,2]. Here, we show that the assimilation ratio of carbon to nitrogen quantified by NanoSIMS reflects the metabolic pathway in individual cells of *Shewanella oneidensis* MR-1. After 24 hours of electrochemical incubation on the surface of electrode poised at +0.4 V vs standard hydrogen electrode in the presence of [1-^{13}C]lactate as an electron donor and $^{15}NH_4^+$, the plot of $^{13}C/C_{total}$ over $^{15}N/N_{total}$ in individual cells gathered around a single linear line despite their variable metabolic rate. The slope of this linear line reflected the alteration of metabolic pathway induced by mutation and control of substrate concentration, suggesting that the ratio of assimilated ^{13}C to ^{15}N in single cells stands for their intracellular metabolic pathway. Thus, double isotope labeling of substrates for anabolic reaction allows the measurement of single cell metabolic pathway and activity at the same time. We confirmed that this methodology was also applicable to microbes respiring soluble electron acceptors. We will discuss how our method can be applied to analysis on environmental samples by combining with in situ hybridization techniques.

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O3-1

Metatranscriptomics reveals ecology of chemosynthetic ectosymbiosis of the deep-sea squat lobster, *Shinkaia crosnieri*

深海性甲殻類の外部共生菌叢の生態をメタトランスクリプトーム解析で解明する

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深海の熱水噴出孔周辺に密集して生息する動物の多くは、化学合成細菌と共生関係にある。例えば、沖縄トラフの熱水噴出域に優占種として生息する無脊椎動物ゴエモンコシオリエビは、体表面に付着する化学合成細菌（外部共生菌）を経口摂取することにより、主要な栄養源として利用している。この外部共生菌叢ではGammaproteobacteriaに属するMethylococcales、ThiotrichalesおよびEpsilonproteobacteriaに属するSulfurovumが優占し、メタン酸化や硫黄酸化を行っていることがFISHや同位体実験などからわかっている。しかしながら、これらの細菌は単離培養が難しく、外部共生菌叢の生態の全体像に関する知見は乏しい。その解明のためにはメタトランスクリプトーム解析が有効だが、深海研究においてはサンプルを船上に回収するまでに環境変化やストレスが生じ、遺伝子発現プロファイルがRNA固定処理前に変化してしまうこと、特にRNAの半減期が短い細菌の解析においては実際の生態を反映したデータが得られないことが問題点として挙げられる。

そこで本研究では、深海でサンプリングした直後にRNA固定処理を行う装置「in situ RNA固定システム」を用いたゴエモンコシオリエビ外部共生菌叢のメタトランスクリプトーム解析を行うことで、実際の深海環境における外部共生菌叢の生態の全体像を明らかにすると同時に、in situ RNA固定法の有効性を遺伝子発現プロファイル解析によって検証することを目的とした。その結果、各分類群に由来すると推定される転写産物を検出したとともに、各分類群が持つ硫黄酸化、メタン酸化、炭酸固定などの代謝パスウェイの推定を行い、ゴエモンコシオリエビ外部共生菌叢の生態の全体像を明らかにした。さらに、船上に回収してからRNA固定を行ったサンプルの発現プロファイルとの比較を行ったところ、エネルギー生産や転写翻訳など、ほぼ全ての機能カテゴリーに関わる遺伝子群で転写産物の発現量に変動が認められた。こうした発現量の変動は分類群によって増減傾向が有意に異なっていたことから、in situ RNA固定を用いたメタトランスクリプトーム解析により、実際の環境における微生物叢の代謝活性の全体像をより正確に観測できることが示唆された。

O3-2

Microbial community structure in gastrointestinal tracts of wood-eating crab

木喰いガニの消化管微生物群集構造解析

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【背景・目的】
石川県能登半島をはじめ，海岸に面した水辺林では，アカテガニやクロベンケイガニといった陸ガニの生息がよく観察される。これらの陸ガニは，落ち葉や木片などのリグノセルロース系バイオマスを食料とすることが知られる。本研究では，陸ガニのバイオマス分解システムを明らかにし，バイオマス利用技術への応用をはかることを目的としている。　われわれはこれまでに，陸ガニ消化管の粗酵素液のリグニン分解活性およびセルラーゼ活性について報告してきた（裏ら, 2016； 知田ら, 2017)。本発表では, リグノセルロース分解に関わる消化管微生物を探索するべく，次世代シーケンサーによる群集構造解析に取り組んだ。

【方法・結果】
加賀地方や能登地方の水辺林に生息するアカテガニを採集し，個別に飼育した。個体ごとにフンを採取し，Mshandete et al. (2005) の方法に準じてセルラーゼおよびキシラナーゼを含むバイオマス分解酵素活性を測定した。酵素活性を確認したフンからDNAを抽出し，16S rDNAのV3-V4領域を増幅した後，次世代シーケンサーMiSeqにより配列情報を得て，QIIME softwareにより解析した。アサイン（97% sequence similarity）された全taxaの代表配列は，Blast検索によりその最近縁種を決定した。当日は，これらの解析結果について報告する予定である。

O3-3

[ASME] Land snail *Macrochlamys hippocastaneum* has *Mycoplasma*-dominated gut microbiota surrounded by chitinous peritrophic matrix-like layer

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Macrochlamys hippocastaneum is a land snail species commonly encountered in agricultural farms. This species of snails enter dormant state to reduce water loss at times of increased temperature or reduced humidity. They can sustain long-term dormancy lasting for weeks to months. During dormancy, keeping gut microbiota under control to prevent infection is required for the hosts to survive. In this study we monitored changes in both host body and the gut microbiota during desiccation-induced dormancy. Both histological examination of gut tissue and NGS-based gut microbiota characterization did not reveal differences between active and dormant animals. Gut community was dominated by Tenericutes, Proteobacteria, and Bacteroidetes. A structure similar to the chitin-based peritrophic matrix of insect (peritrophic matrix-like layer, PML) was found coating the gut content, and preventing direct contact of gut contents with epithelial cells. The PML was seen in both active and dormant snails, and remained intact at 28 and 56 days post dormancy (dpd). Sections of snail gut and feces were stained with fluorescein-WGA to detect chitin. The results showed a clear lining of chitin around gut contents, separating them from the gut epithelium. We hypothesize that this PML keeps gut microbes from invading gut tissue, and therefore enables the snails to maintain long-term survival without feeding during dormancy. Surprisingly, *Mycoplasma* was detected at 0 and 28 dpd, with high relative abundance of 20.7-73.2%. *Mycoplasma* species were detected in all 8 local *M. hippocastaneum* populations we examined, and were detected in feces excreted from the same individuals continuously for 4 weeks, indicating that the association of *Mycoplasma* and *M. hippocastaneum* is a stable and generalized condition.

O3-4

Single spore analysis reveals diverse host-parasite relationships between phytoplankton and fungi

Single spore PCR 法による植物プランクトンと菌類の多様な宿主寄生者関係の解明

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Molecular analyses of environmental DNA samples have revealed an unexpectedly large diversity of undescribed fungi, so called “dark matter fungi” (DMF). DMF are ubiquitous, but have not been cultured. DMF are likely to be abundant across the entire fungal tree, but are particularly common on the early diverging branches of the fungal tree of life, many of which presumably represent zoosporic fungi thriving in aquatic ecosystems. In this talk, we focus on the recently discovered zoosporic fungal lineages, i.e. Cryptomycota (Rozellomycota) and Chytridiomycota, and especially on those which parasitize phytoplankton in lakes. We applied a genomic single-spore method in addition to isolation-culturing to identify fungi infecting various phytoplankton species in Lake Inba (Japan) and Lake Stechlin (Germany).
In Lake Inba, dominant diatoms (Aulacoseira granulata, A. ambigua) were infected by diverse fungi. These parasitic fungi were affiliated not only to Chytridiomycota (chytrids), but also to Cryptomycota and Aphelida. Some chytrids formed novel phylogenetic lineages. Host specificity depended on the fungal clade, i.e. some were specific to A. granulata, while others could infect both host species. The degree of host specificity was also confirmed by cross infection experiments using 4 chytrids and 3 potential host diatoms (A. granulata, A. ambigua, Synedra sp.). 2 chytrids could infect all 4 diatoms, while other 2 chytrids can infect only their own host diatoms.
In Lake Stechlin, diverse phytoplankton including diatoms, green algae and cyanobacteria were found to be infected by different fungi. They mainly belonged to Chytridiomycota, and some formed novel clades. Most fungi were specialists and specific to a single host species. Some fungi displayed a more generalist behavior and could infect various host species, though host range never crossed taxonomic boundaries between diatoms and green algae. Sometimes, host species turned out to be a species complex and were composed out of several (pseudo)-cryptic species. Using several fungal cultures, novel clades were confirmed by morphological observations, such as the morphology of thallus, style of zoospore discharge, sexual reproductions and zoospore ultrastructures. We could propose new species, genus, or even order for the parasitic chytrids. Further studies will surely discover more host parasite combinations, but careful interpretations are necessary when host and parasite species were identified.

O3-5

Comparative genome analysis of two endosymbiotic *Treponema* species of cellulolytic protists in the termite gut

シロアリ腸内に共生する原生生物の *Treponema* 属細胞内共生細菌の比較ゲノム解析

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The gut of wood-feeding termites harbors a complex microbial community, which comprises protists, bacteria and archaea. A typical feature of this community is species-specific endosymbiotic and ectosymbiotic associations of bacteria with the gut protists. The cellulolytic protist species in the genus *Eucomonympha* hosts an endosymbiotic bacterial species, "*Candidatus* Treponema intracellularis" , which has abilities of reductive acetogenesis from H_2 plus CO_2 and nitrogen fixation. The single-cell genomics of "*Ca*. Treponema intracellularis" suggested that its acquisition by *Eucomonympha* protists was a more recent event because the estimated genome size of the endosymbiont is not severely reduced and there are more than 100 transposable elements in the genome. Here, we analyzed the genome of another closely related endosymbiotic species, "*Cadidatus* Treponema teratonymphae" , of the protist species in the genus *Teranympha*. We obtained its draft genome by hybrid assembly of the three single-cell samples, and the estimated completeness of the genome was 88%. The genome of "*Ca*. Treponema teratonymphae" is small, has several transposable elements and many pseudogenes. We are currently doing comparative analyses of the two endosymbionts' genomes in order to evaluate the functional divergence and evolution of the endosymbiosis.

O3-6

Single cell transcriptome analyses of the symbiotic protists in termite gut

シングルセルに基づくシロアリ腸内原生生物のトランスクリプトーム解析

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Wood-feeding termites harbor a complex symbiotic system in their gut comprising various species of bacteria, archaea, and unicellular eukaryotes (protists). The microbiome is thought to contribute to the symbioses through digesting recalcitrant wood taken by termite and supplying nitrogen compound. However, the function of most of individual microbial species remains unknown due to their formidable unculturability. Recent advance in single-cell genomics indicated that some bacterial species in termite gut play roles in nitrogen fixation, acetogenesis, and lignocellulose digestion. In contrast, the sequence data of each protist species are still scarce although the metatranscriptomic analyses of whole gut content found the genes for a series of Carbon Active enZYmes (CAZYs), suggesting their involvement in wood digestion.

Coptotermes formosanus and *Reticulitermes speratus* are common termite species inhabiting in Japan. Both of them belong to the same family, Rhinotermitidae, but they retain the distinct fauna of the symbiotic protists; *C. formosanus* harbors 3 parabasalid species while *R. speratus* not only contains the different set of parabasalids and also several oxymonad species. To reveal the role of these symbionts, single-cell transcriptome techniques were applied for them with single or a small number of isolated cells. As a result, we successfully obtained transcriptomic data of the all protists species in *C. formosanus* and some of those in *R. speratus*. Although we have not yet collected the data of all species in *R. speratus*, considering the population structure, our data is considered to cover a vast majority of the protist community. Our preliminary comparative analysis of the transcriptomes showed the different expression pattern of CAZYs among species. This trend seemed more significant in *C. formosanus* symbionts, suggesting their division of roles for lignocellulose digestion. During the analysis, we also noticed that one of the protist species in *C. formosanus*, *Holomastigotoides mirabile*, can be separated into two close, but distinct species. The presence of the two *Holomastigotoides* species in *C. formosanus* was further confirmed by *in situ* hybridizations, breaking the common belief over almost a century. In this presentation, we will report the diversity, function, and evolutionary history of the protist symbionts in the termite guts.

O3-7

Endosymbiotic interaction in anaerobic ciliates with methanogens and bacteria

嫌気性繊毛虫におけるメタン菌、バクテリアとの共生関係

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The presence of endosymbiotic microorganisms, methanogenic archaea and bacteria, within the cells of anaerobic ciliated protozoa has been reported by mainly microscopic observation. These tripartite symbioses are unique relationships that each partner is from three different domains, i.e. Eukaryote, Achaea, and Bacteria. However, due to the difficulty of culturing anaerobic ciliates in laboratory, the details of the symbiotic interaction are still unknown. In our laboratory, an anaerobic ciliate, *Trimyema compressum* isolated from small-scale sewage treatment reactors, has been successfully maintained in a defined medium with food bacteria over 20 years. Based on this monoxenic culture system for *T. compressum*, we recently tried to culture other anaerobic ciliates and succeeded obtaining one stably cultured strain of a small anaerobic scuticociliate, named strain GW7, from the sludge sampled in a large-scale sewage treatment reactor in Okinawa prefecture, Japan. The strain GW7 has been maintained for almost three years in our laboratory. By transmission electron microscopic observation and fluorescent *in situ* hybridization with domain-specific probes, we demonstrated that strain GW7 possessed both methanogenic archaeal and bacterial symbionts in its cytoplasm. These endosymbionts were independently associated with hydrogenosomes, which are organelle producing hydrogen and ATP in anaerobic condition. The following clone analyses targeting prokaryotic 16S rRNA genes, fluorescent *in situ* hybridization with endosymbiont-specific probes, and molecular phylogenetic analyses revealed phylogenetic affiliation and intracellular localization of these endosymbionts. In the presentation, we will show the results of phylogenetic analyses and compare the symbiosis in strain GW7 with those in other anaerobic ciliates including *T. compressum*.

O3-8

Analysis of gene functions related to quorum sensing in *Roseomonas* sp. TAS13 isolated from an activated sludge

活性汚泥由来 *Roseomonas* sp. TAS13 株の Quorum sensing 関連遺伝子の機能解析

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Quorum Sensing (QS) in gram negative bacteria is an intercellular signaling system using mainly *N*-acylhomoserine lactone (AHL) for regulation of gene expression including virulence factor production and biofilm formation. AHL-degrading bacteria exist in natural environments and several AHL hydrolases have been identified from these bacteria. In this study of novel AHL-degrading bacteria *Roseomonas* sp. TAS13, the AHL degradation characteristics and identification of important genes responsible for AHL degradation were performed. AHLs with an acyl side chain from C6 to C12 except for *N*-dodecanoyl-L-homoserine lactone (C12-HSL) were completely degraded by *Roseomonas* sp. TAS13. Among the predicted genes contained in the genome of *Roseomonas* sp. TAS13, 11 candidate genes were focused based on functional classification of acylase/amidase group. Some genes were cloned and expressed as MBP-tag fusion proteins in *Escherichia coli*, and their AHL degradation activity were analyzed. The cloned three hydrolases showed 99%, 99% and 84% amino acid sequence homology to Penicillin acylase, Glutamyl-tRNA amidotransferase subunit A (GatA) and Acylamidase of other *Roseomonas* bacteria in the GenBank database, respectively. All of these enzymes hydrolyze *N*-hexanoyl-L-homoserine lactone (C6-HSL), but showed different substrate specificities for AHL with an acyl side chain length of C10 or higher. Interestingly, it has been suggested that the multiple hydrolases are responsible for degrading activity to AHLs with different length of acyl side chain in *Roseomonas* sp. TAS13. We constructed the genomic DNA library of *Roseomonas* sp. TAS13 and used a functional screening method to identify the genes involved in each AHL degradation.

O3-9

Analyses of Adapting Processes for Growth Repressing Effects by *Pseudomonas* sp. strain C8

Pseudomonas sp. C8 株の増殖抑制物質に対する適応プロセスの解析

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It is challenging for microbial ecologists to understand forming mechanisms of microbial ecosystems including interactions between microbes. *Pseudomonas* sp. strain C8 is isolated as one of general environmental microbes, and strain C8 exhibits the growth repressing effects to several kinds of bacteria belonged to different genera. When the soil-born cultures are enriched with the supernatant of strain C8 under batch condition, the bacterial community structure is different from that without the supernatant. The effect is not to kill bacterial cells but to repress growth temporarily, and the growth is then recovered. The adapting processes and the mechanism of action were analyzed using KEIO collection of *Escherichia coli* BW25113 single-gene deletion mutants. The growth of wild type strain BW25113 was repressed by addition of the supernatant from strain C8. Glucose added as sole carbon and energy source was hardly consumed till 16 h incubation. An oxygen-consumption rate per protein (OCR) of culture at 6 h incubation was quit low. The OCR at 14 h incubation was approximately 5-fold higher than that at 6 h incubation and was similar level to that at 24 h incubation when the growth was recovered and glucose was quickly consumed. Of 3285 mutants in KEIO collection, 11 mutants exhibited tolerance to the repressing effects, and mutants were categorized into three groups; deletions of transporters (*Δyec*C, *Δcvr*A, and *Δyad*H), enzyme which involved in pentose phosphate pathway (*Δrpe*, *Δepd*, *Δpab*C, and *Δgua*D), and unknown functions (*Δygg*P, *Δsse*B, *Δyge*N, and *Δrem*). The lag time of 11 mutants was approximately 2- to 5-folds shorter than that of the wild type, suggesting that these mutants already made bypass to avoid the deleted pathway, resulting in shot time for adaptation. The wild type was enriched with the supernatant under transferred batch conditions. When the cells were transferred in a fresh medium with the supernatant at end of logarithmic phase, the lag time shortened from 30 h to 9 h and the growth rate constant increased from 0.2 h^{-1} to 0.4 h^{-1}. On the other hand, when the cells were transferred at stationary phase, the lag time and the growth rate constant changed to be longer and shorter, respectively. These results suggested that the mechanism of action might inhibits the part of the pentose phosphate pathway and that it is important for adaptation whether microorganisms are capable of making bypass to compensate inhibited pathways.

O4-1

Deep-sea geochemist meets microbial ecologist

深海の化学組成を調べて微生物生態を想像する

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大洋深海の化学組成は，ある時空間区分においてほぼ一様な変化を呈する。しかし海底と水塊との間で活発な物質循環が起こる領域においては，この限りではなく，メートルからキロメートルのスケールで不均一が認められる。たとえば海底熱水噴出口近傍の水塊においては，噴出した熱水に由来する局所的な化学組成の異常が認められる。これを熱水プルームと呼ぶ。熱水プルームの化学組成が熱水と海水の混合のみにより支配されているならば，各成分の相対存在比は一定であることが期待される。しかし実際は，熱水海水混合比とは別に，各成分の相対比が変動している。特に生元素成分の変動は，水塊中での微生物活動によって説明できる。また,超深海海溝域の水塊においても,微生物活動によって説明できる化学組成の変化が観測される。

こうして地球化学者は，化学組成の変化のみを観測して「微生物活動によって説明できる」と軽々に言う。しかしこれは，微生物群集組成やその活性を直接調べる微生物生態学が存在するにもかかわらず，それを利用することなく，都合の良い微生物活動を想像しているに過ぎない。もしかすると，これと逆のことが微生物生態学でも行われているかもしれない。それはつまり，微生物生態を調べた結果として「このような微生物活動が起こっている」という説明を与えておきながら，そうであれば必ず生じているはずの化学組成の変化について観測によって確認していない，ということである。

「この世界で一体何が起こっているのかを科学的に描写する」という営みにおいて，地球化学と微生物生態学は同じ立場にある。可能な限り多面的な観測を実施し，その結果を整合的に説明することを目指すべきであり，それは多分野協働に対する心のハードルを少し下げるだけですぐに実現できるであろう。

O4-2

Vertical profiles of chemical state of inorganic sulfur and sulfur-oxidizing bacteria in launched marine sediment by tsunami

海洋由来津波堆積物内の無機硫黄形態及び硫黄酸化細菌の鉛直プロファイル

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【背景】津波が打ち上げた海底堆積物に関する我々の研究によって、堆積物表層で起こる微生物応答の先駆けとして、硫黄酸化細菌が重要であることが示された。硫黄酸化細菌の機能を把握するためには、堆積物内の硫黄の形態を明らかにすることが重要である。そこで本研究では、海洋由来津波堆積物における無機硫黄化学種と硫黄酸化細菌の鉛直的な分布を調査した。【方法】宮城県東松島市大曲に打ち上げられた海底堆積物を、2012年7月に採取した。この堆積物は採取時までほぼ撹乱されずに残されており、赤褐色を呈した表層 (深さ0-2mm) 以外の下層部は一様に黒色であった。コアサンプラーを用いて堆積物を採取した後、深さ0-2 mm、2-10 mm、10-20 mm、20-40 mmの４つの深度に切り分けてその後の分析に用いた。堆積物内の硫酸イオン濃度を測定し、さらに、無機硫黄化合物を分画定量する独自に改良した実験手法を用いて、堆積物に含まれる酸揮発性硫化物（AVS:主にFeS)、クロム還元性硫化物（CRS：主にFeS2)、単体硫黄（ES）を定量した。微生物群集構造の解明のため、堆積物からDNAを採取した後、16S rRNA遺伝子を対象とした次世代シークエンサー解析を行った。【結果】0-2 mmの層には還元型硫黄はほとんど含まれておらず､2mm以下の層ではCRS が主要な無機硫黄化学種であった｡2-10 mmの層と10-20 mmの層の間では、ESのみが含有量の有意な違いを示し、2-10 mmの層の含有量は10-20 mmの層の含有量の２倍であった。2-10 mmの深さでは、硫黄酸化細菌であるEpsilonproteobacteria網の*Sulfurimonas denitrificans*に近縁なOTU (operational taxonomic unit)が群集全体の25.6%を占めた。これらの結果は、津波由来海底堆積物の表層下で優占した硫黄酸化細菌により、還元型硫黄が単体硫黄に酸化された可能性を示している。さらに、硫黄不均化細菌である*Desulfocapsa sulfexigens*も2-10 mmの深さで比較的高い相対存在量を示した。以上から、海洋由来津波堆積物の表層以下の硫黄循環に対して、硫黄酸化細菌、硫黄不均化細菌が重要な役割を果たし、単体硫黄が鍵となる硫黄種であったことが示唆された。

O4-3

[ASME] Syntrophic association between sulfur disproportionating bacterium and anoxygenic photosynthetic bacterium, *Chloroflexus aggregnas*

硫黄不均化菌と酸素非発生型光合成細菌 *Chloroflexus aggregans* の共生関係

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Chloroflexus aggregans is a thermophilic filamentous anoxygenic photosynthetic bacterium and widely distributed in slightly alkaline sulfidic hot springs. This bacterium grows well photoheterotrophically under anaerobic conditions in the light. The genome analysis indicated that *C. aggregans* has ability to fix carbon dioxide and oxidize sulfide to elemental sulfur, and our previous study reported that anaerobic sulfide oxidation by *C. aggregans*-dominated microbial community was stimulated by carbon dioxide. However, the photoautotrophic growth of *C. aggregans* has not been shown with sulfide as a sole electron source. In this study, we examined the effects of a sulfur disproportionating bacterium on the sulfide-dependent autotrophic growth of *C. aggregans* through interspecies exchange of sulfur compounds.

C. aggregans strain NA9-6, newly isolated from Nakabusa hot springs, grew well photoautotrophically with hydrogen, but not with sulfide. *Caldimicrobium thiodismutans*, a sulfur disproportionating bacterium which has been isolated from Nakabusa hot springs, is known to utilize thiosulfate, elemental sulfur and sulfite as electron donors and also electron acceptors for cellular metabolism. *C. aggregans* and *C. thiodismutans* were co-cultivated in the presence of bicarbonate as a sole carbon source and thiosulfate as an electron source. The optical density of the culture was increased from 0.03 to 0.2 after 13 days cultivation. No growth of *C. aggregans* was observed under the conditions without *C. thiodismutans* in the light and with it in the dark. During the co-cultivation in the light, the consumption of thiosulfate and the accumulation of sulfate were observed. Increase in the cell numbers of both species were also observed microscopically.

These results indicate that *C. aggregans* grows using sulfide continuously supplied by the sulfur disproportionating bacterium under the autotrophic conditions. *C. aggregans* likely works as a sulfide scavenger for the *C. thiodismutans*. This study shows mutualistic relationships between sulfide-oxidizing anoxygenic photosynthetic bacterium and the sulfur disproportionating bacterium via dissimilatory sulfur metabolites under autotrophic conditions.

O4-4

Microbial adaptive evolution to the extreme geochemistry occurring at the serpentinization systems

蛇紋岩水系に見られる極限環境への微生物の適応的進化

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Serpentinization is an aqueous alteration process in which low-silica ultramafic rocks (for example, olivines and pyroxenes) are hydrolyzed with water into serpentinite, brucite, magnetite and other minerals. During these reactions, abundant hydrogen gas is produced. Serpentinization also releases hydroxide ion which issues highly alkaline fluids (usually above pH 11). In the highly alkaline fluid, carbonate is the dominant form of inorganic carbon which can precipitate out of solution as carbonate minerals with the divalent cations, such as Ca^{2+} and Mg^{2+} in the serpentinite fluids. While reductants (fuel) are abundant in these systems, corresponding oxidants and available carbons are severely limited, which restrain the range of potential microbial metabolisms. Here we present the unique physiological and genomic features of microbes inhabiting at the oxic/anoxic interfaces in serpentinized systems, where oxidants and carbon dioxide must be supplied from the air beside the reduced compounds from the deep. I further show the evidence of potential adaptive evolution to the local geochemistry of the various serpentinized systems. Three-related microorganisms named genus Serpentinomonas were from highly alkaline (pH 11.6) serpentinizing springs at The Cedars, California. All three strains are obligate alkaliphiles with an optimum for growth at pH 11 and are capable of autotrophic growth with hydrogen, calcium carbonate and oxygen. The features fit well to the alkaline and calcium-rich environments represented by the terrestrial serpentinizing ecosystems, thus the organisms are considered to be adapted to the geochemical setting. Although the closely-related Serpentinomonas strains have been detected globally in all of the studied highly-alkaline serpentinized waters, the chemistry of water discharging from different serpentinizing sites are considerably different especially in the concentrations of methane, hydrogen and sulfate/sulfide. Since microbial community composition and metabolic activities rely on the geochemistry of the habitat, geochemical differences of respective serpentinization sites must affect to the selection of associated microbial taxa and the metabolic activities. Comparative physiological and genomic studies of the Serpentinomonas strains isolated from the two different serpentinization sites, The Cedars and the Cabeço de Vide in Central Portugal, illustrated the evidence of potential adaptive evolution to the geochemistry of the site. Namely, genomic constitution of the Serpentinomonas strains were changed toward having advantages in the respective geochemistry of the springs where the strains were originally isolated.

O4-5

Crude oil biodegradation and methane production in a high-temperature oil field

深部地下高温油層環境における原油分解とメタン生成

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Previous geochemical studies suggest the formation of methane in oil reservoirs in association with oil biodegradation. However, the microbial methanogenic processes in high-temperature oil fields are still poorly understood. To better understand the formation process of the natural gas deposit and to evaluate the current state of the reservoir, we performed geochemical and molecular genetic analyses together with incubation experiments. The formation water, crude oil, and gas samples were obtained from commercial oil producing wells in the prefectures of Yamagata and Akita, Japan. To measure methanogenic potential, formation water samples were collected in N_2-filled sterilized glass bottles. Carbon isotopic composition of gas components and saturated hydrocarbon composition of crude oil were determined by GC-C-IRMS and GC-FID, respectively. Phylogenetic analysis of archaeal and bacterial communities in the formation water was conducted based on 16S rRNA gene clone libraries constructed using archaeal- and bacterial-specific primers. The number of culturable methanogens were determined by serial dilution method. The result of geochemical analyses showed the predominance of isoprenoid over straight-chain alkanes, indicating that the oil has been partially biodegraded. The NaCl concentration in the formation water was almost the same as that in general seawater, whereas the sulfate concentration was extremely low. Such a characteristic was commonly observed in formation waters from oil and gas fields in Japan. We successfully isolated the methylotrophic methanogen *Methermicoccus shengliensis* strain AmaM from the Yamagata sample with methanol. This methanogen was later found to also use methoxlated aromatic compounds as substrates (Mayumi et al., Science, 2016). Serial dilution method revealed that the abundance of the same methanogen was about 10 times higher than the total of hydrogenotrophic methanogens in the Akita samples. The type strain of *Methermicoccus shengliensis* (ZC-1), isolated from petroleum reservoir in Shengli oilfield (Cheng et al., IJSEM, 2007), was recently reported to also predominate in the formation water from the same oil field, accounting for more than 70% of the total archaea (Liang et al. Front. Microbiol. 2017). The high percentage of *Methermicoccus methanogens* in high-temperature biodegraded oil reservoirs suggests their contribution to the process of methanogenic oil degradation, which should be investigated in future studies.

O4-6

[ASME] Enrichment and Function Study of A High-temperature Methanogenic *n*-alkane Degrading Microbial Community

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It has been well documented that various methanogenic communities can produce methane by degrading crude oil alkanes. Among them, *Smithella* spp. have always been proved to utilize *n*-alkanes under mesophilic, methanogenic condition. However, the diversity and ecophysiological function of anaerobic microbial community involved in n-alkane degradation at high temperature were rarely documented. Here, we enrich a high-temperature (45℃) methanogenic alkane-degrading culture H553, from Heiyoushan oil-contaminated soil of China. During over 2200 days incubation, the culture grow with mixture of hexadecane, docosane and triacontane, and single compound of hexadecane, with the maximum methane producing rate raising from 1.38 μmol/day/mL mixed alkane (hexadecane, docosane, triacontane) at the first stage, to 4.5 μmol/day/mL hexadecane during the third transfer incubation. Amplicon sequencing analysis of 16S rRNA gene revealed that the bacterial community was mainly composed of *Coprothermobacter* (40.3%), *Anaerobaculum* (10.6%), *unclassified Firmicutes* (8.2%), *unclassified Synergistaceae* (7.7%), *Aminicenantes genera incertae sedis* (6.5%), *Petrotoga* (2.1%), and *unclassified Clostridia* (1.5%). *Methanothrix* (57.0%) and *Methanothermobacter* (36.0%) were dominant phylotypes in the archaeal domain. Furthermore, a total of 26 genome bins (GB) with above 80% completeness was reconstructed using a genome-based metagenomic technique. These GBs can be clustered into three functional groups based on their carbon metabolic pathways: fermentation bacteria group is the biggest functional group contains 15 GBs, such as *Coprothermobacter*, *Anaerobaculum*, *unclassified Firmicutes*, *unclassified Synergistaceae*, et al., which are the main bacteria composition of H553. This group is able to degrade biomass, such as fatty acid, sugar and amino acid; VFA-degrading bacteria group, which contains *unclassified Anaerolineaceae*, *unclassified Synergistaceae*, *unclassified Anaerolineaceae*, *unclassified Chloroflexi* and *Tepidanaerobacter*, mainly produces acetate, carbon dioxide by degrading VFA; Methanogenic archaea group contains two types of methanogenic archaea, *Methanothrix* and *Methanothermobater*. Although an assA-like gene was identified from metagenomic sequences, no assA-like gene were identified from GBs. Thus, we infer that the H553 culture may be methanogenic degraded alkane by following processes: first, alkanes were initially degraded by an unknow alkane-degrading bacteria through fumarate addition reaction or alternative pathways; then, biomass in the culture was transformed to acetate, hydrogen and carbon dioxide by fermentation bacteria and VFA-degrading bacteria; finally, methanogenic archaea produced methane by using these productions. Our study expands the view of high-temperature methanogenic alkane-degrading microbial community structure and their function. It will help us make a better understanding of transform subsurface residual crude oil to biogas in the future.

O4-7

[ASME] Interspecies electron transfer driving syntrophy in mesophilic and thermophilic propionate-degrading anaerobic chemostats

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Propionate is one of the major intermediates in the anaerobic decomposition of organic matter in anaerobic wastewater and solid-waste treatment systems. Under methanogenic conditions, propionate is decomposed through syntrophic interaction between propionate-oxidizing bacteria and methanogens. Interspecies electron transfer is a key process in such syntrophic consortia. Those syntrophic partners cooperate by transferring electrons from one species to the other using H_2, formate, acetate or other pathways, and maintaining H_2 at low levels, so that the overall reactions are thermodynamically more favorable. Temperature is an important environmental and industrial regulator, yet its effect on syntrophic propionate oxidation has been poorly understood. In the present study, using metagenomics, we investigated the methanogenic consortia inside mesophilic (37 ° C) and thermophilic (55 ° C), propionate-degrading anaerobic chemostats, and identified the interspecies interactions that define composition and dynamics within syntrophic communities that play a key role in the global carbon cycle. Our results revealed that *Syntrophobacter* spp., *Pelotomaculum* spp., *Syntrophomonas* spp. and *Smithella* spp. were dominant syntrophs through methyl-malonyl-coenzyme A (CoA) pathway and beta-oxidation to H_2/CO_2 and acetate in mesophilic chemostat. Hydrogenotrophic *Methanoculleus* and aceticlastic *Methanosaeta* acted as methanogenic partners at 37° C. While *Pelotomaculum* spp., and *Peptococcaceae* acted as the main propionate oxidizers in thermophilic chemostat, and *Methanoculleus*, *Methanothermobacter*, *Methanosarcina* and *Methanomassiliicoccus* dominated the methanogenic community. We proposed the metabolic interspecies interactions in mesophilic and thermophilic chemostats and illustrated the electron transfers that driving syntrophy in communities of anaerobic bacteria and archaea. Collectively, temperature markedly influenced the activity and community structure of syntrophic guilds degrading propionate. The propionate-degrading consortium consists of multiple syntrophic interactions beyond the standard H_2-producing syntroph-methanogen partnership that may serve to improve the community stability.

O4-8

[ASME] Hydrogen production using hybrid MEC with TiO2 photoanode

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Conventional microbial electrolysis cell(c-MEC) needs higher hydrogen production rate to achieve practical application. We propose a novel hybrid microbial electrolysis cell(h-MEC) coupled TiO2 photoanode with c-MEC. Through coupling TiO2 nanotubes(TNT) arrays with bioanode, h-MEC achieve increased H2 evolution rate and enhanced power output. The h-MEC exhibits power density of 1451.02 mW m-2 and H2 evolution rate of 26.1 μmol h-1, comparing c-MEC displays 1180.17 mW m-2 and 21.4 μmol h-1. This result shows that photogenerated electrons from TNT arrays enhances about 20 % for H2 production and power density. This hybrid system suggests the new way for enhancing electrons supply to improve hydrogen production.

O4-9

Effect of lactate and riboflavin on arsenic-mobilizing microbial communities

ヒ素可溶化微生物群に及ぼす乳酸とリボフラビンの影響

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Arsenic (As) is one of the most common toxic contaminants in soils. In most cases, soil replacement and solidification methods are utilized for the treatment of soils contaminated with metals including As, but these techniques are often expensive and labor intensive. Our previous studies suggested that microbial reductive dissolution of As might be a cost-effective alternative for remediation of As-contaminated soil as an extraction technique. The microbial As dissolution occurs mainly through two processes, i.e., arsenate (As(V)) reduction to mobile arsenite (As(III)) and reductive dissolution of Fe(III) minerals. However, important components of the microbial community related to these processes during As extraction are largely unknown. In this study, we conducted microcosm experiments consisting of As(V)-laden Al or Fe(III) precipitates and microbial communities obtained from a pristine soil. High-throughput Illumina sequencing of 16S rRNA genes was applied to monitor the shift of microorganisms in the experiments. In both Al and Fe(III) precipitates, lactate addition markedly enhanced As dissolution compared to unamended controls. In the presence of lactate, riboflavin addition further promoted As dissolution from As(V)-laden Fe(III), but it inhibited As dissolution from As(V)-laden Al. During the incubation, relative abundance of the phylum Firmicutes remarkably increased in all treatments. At the lower taxonomic level, the relative frequencies of operational taxonomic unit (OUT) from the family *Sporomusaceae*, known as fermentative Fe(III) reducers, and the genus *Desulfitobacterium*, known as a dissimilatory As(V) reducer, became the dominant members in the presence of lactate. In contrast, in the absence of lactate, the relative frequencies of *Sporomusaceae*-related OTU remained in negligible level, although *Desulfitobacterium*-related OTU predominated. Interestingly, a *Sporomusaceae*-enriched culture obtained from the lactate-amended microcosm rapidly reduced As(V) as well as Fe(III), and released As from both As(V)-laden (hydr)oxides in the presence of lactate. These results suggested an important role of fermenting bacteria in the extraction of As from contaminated soils. Our findings also highlight unexpected role of *Sporomusaceae* bacteria within the phylum Firmicutes in biogeochemical As cycling.

O4-10

Enrichment of bacterial community that precipitates antimony in water phase

水相のアンチモンを不溶化させる細菌群集の集積

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Antimony (Sb), the 51th element on periodic table, is a metal resource used as a material for production of semiconductors, alloys, pigments, etc. Sb is also known to be highly toxic, therefore environmental pollution caused by such industrial activities has been concerned. Recently, bacterial activity that precipitates Sb in water phase by reducing Sb(V) to Sb(III) under anaerobic condition has been reported, suggesting a possibility for development of biological Sb removal technology. However, studies on biological removal of Sb are still limited, and its mechanism has not been revealed in detail. This study aimed to enrich and characterize bacterial community that precipitates Sb in water phase.

A sediment sample obtained from brackish tideland of the Yodo River was inoculated into 20 mL of mineral salt medium containing 0.8 or 1 mM of $K[Sb(OH_6)]$, 2 mM of $MgSO_4$, and 5 mM of sodium lactate in a 50-mL serum bottle tightly sealed with butyl rubber septum and aluminum crimp in an anaerobic chamber. The 1% volume of the culture after 7- or 10-day incubation at 28℃ was transferred to fresh medium for the next cultivation. This operation was repeated to establish stable enrichment culture precipitating Sb. The concentration of soluble Sb was periodically analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometer. The structure and elemental composition of the precipitates were analyzed using Transmission Electron Microscopy equipped with Energy Dispersive X-ray spectrometry (TEM-EDX). Bacterial community was analyzed by Terminal-Restriction Fragment Length Polymorphism (T-RFLP) and next-generation sequencing (NGS) targeting amplicons of 16S ribosomal RNA gene.

During the enrichment, the efficiency of Sb removal was gradually improved, and the generation of orange-colored precipitate was increased. The precipitate obtained in the 14th batch culture was analyzed by TEM-EDX and identified as Sb_2S_3, suggesting that simultaneous reductions of Sb(V) and S(VI) occurred to form Sb(III) and S(-II), respectively, in the culture. T-RFLP analysis confirmed that stable bacterial community was established after the 112th batch. The NGS analysis of the 122nd batch culture revealed that the enrichment culture mostly consisted of 3 operational taxonomic units belonged to family *Desulfovibrionaceae* (75.4%), genus *Pseudomonas* (18.5%) and genus *Sedimentibacter* (5.5%). The results suggest that S(-II) production by *Desulfovibrionaceae* and spontaneous Sb(V) reduction coupled with S(-II) oxidation mainly contributed to the formation of Sb_2S_3 precipitate in the enrichment culture. In addition, *Sedimentibacter*, which was previously reported as an arsenic reducer, might also have reduced Sb(V) as a terminal electron acceptor of anaerobic respiration to produce Sb(III).

O4-11

Metabolic strategy of predatory bacteria in a complex microbiome

微生物コミュニティにおける捕食性細菌の代謝戦略

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捕食－被食関係は最も一般的な生物間相互作用であり、既往の多くの研究によって生態系の構造に強く影響することがわかっている。微生物生態系においては原生動物や後生動物等の真核生物が捕食者として振る舞うことが一般的だが、細菌の中にも微生物捕食能を有する種が存在し、それらが自然界に広く分布することがわかってきた。一方で、それら捕食性細菌が微生物群集構造に与える影響や、彼らの微生物コミュニティ中での生存戦略－特に代謝戦略－については未解明の部分が大きい。本研究では水処理微生物コミュニティ（活性汚泥）を対象に、次世代シークエンサーを用いた微生物群集構造解析、*de novo* assemblyに基づくメタトランスクリプトーム解析（RNA-seq)、CE-TOFMSによるメタボローム解析を行い、飢餓条件および富栄養条件における捕食性細菌群の挙動の解明に挑んだ。

活性汚泥を富栄養条件で培養すると少数の従属栄養性細菌が顕著に優占化したが、栄養源を加えない飢餓条件におくと活性汚泥全体のバイオマス量が低下し（12日間で22%減少)、*Lysobacter*属、*Myxococcales*目、*Sphingobacteriales*目の捕食性細菌および近縁種が優占化した。RNA-seqの結果からは、脂肪酸やタンパク質などの生体高分子の分解酵素や、その分解物を用いてTCA回路等の中央代謝を駆動する代謝系が高活性化していることが示された。興味深いことに種々のアミノ酸の中でもグルタミン酸を代謝する酵素が特に高発現しており、グルタミン酸からTCA回路構成物質である2-オキソグルタル酸と呼吸基質であるNADHを生成する経路が活性化していることがわかった。このことはメタボローム解析の結果からも支持されており、富栄養条件では非検出あった2-オキソグルタル酸が飢餓条件では細胞内に蓄積されていた。メタボロームデータにおいて飢餓条件に特徴的だった点として、プリンおよびピリミジン塩基が蓄積していたことが挙げられる。このことから、飢餓条件では核酸を分解することで糖（リボース）を獲得・利用していたと考えられる。RNA-seqの結果も統合すると、飢餓条件では転写・翻訳・増殖等の活性を落とす一方、核酸も含めた生体高分子を分解・資化する代謝系が高活性化しており、菌体や細胞残渣等の限られた基質を有効利用する、捕食性細菌の巧みな代謝戦略が明らかになった。

O5-1

To use light or to avoid it? Light-adaptation strategies in marine Flavobacteria

光を使うか、それとも避けるか？海洋性フラボバクテリアの光適応戦略

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Proteorhodopsin (PR) is a light-mediated proton pump that is found in diverse bacteria and archaea species and is widespread in marine microbial ecosystems. To date, many studies have suggested the advantage of PR for microorganisms in sunlit environments. The ecophysiological significance of PR is still not fully understood however, including the drivers of PR gene gain, retention, and loss in different marine microbial species. To explore this question we sequenced 21 marine Flavobacteriia genomes of polyphyletic origin, which encompassed both PR-possessing as well as PR-lacking strains. Here, we show that the possession or alternatively the lack of PR genes reflects one of two fundamental adaptive strategies in marine bacteria. Specifically, while PR-possessing bacteria utilize light energy (“solar-panel strategy”), PR-lacking bacteria exclusively possess UV-screening pigment synthesis genes to avoid UV damage and would adapt to microaerobic environment (“parasol strategy”), which also helps explain why PR-possessing bacteria have smaller genomes than those of PR-lacking bacteria. Collectively, our results highlight the different strategies of dealing with light, DNA repair, and oxygen availability that relate to the presence or absence of PR phototrophy. In the future research, we will perform culture experiments in order to verify these hypotheses developed in this study.

O5-2

Ecological effects of labyrinthulean protists in the marine environment estimated from their biomass

原生生物ラビリンチュラ類の現存量から推定された海洋生態系における影響力

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海洋の原生生物であるラビリンチュラ類は，熱帯から極域，表層，深海まで，普遍的に生息する生物群であり，セルラーゼなどを分泌することから，デトリタスなどに対する分解者であると認識されてきた。しかしながら発表者らは，*Aplanochytrium* 属が生きている珪藻から積極的に栄養摂取する“捕食者”としての役割を持つことを見出し，系統群ごとに生態学的な戦略を異にしていることを示唆した。さらに，世界中の海洋サンプルの18S rDNAを網羅的に決定したTARA Oceansのデータを解析した結果，ラビリンチュラ類の登録配列数が珪藻類の約10%に達すること，また，*Aplanochytrium* 属の登録配列数が全ラビリンチュラ類の約22%を占めることが明らかになった。そこで，*Aplanochytrium* 属を中心とした主要な系統群の現存量を把握し，その上で生態系におけるラビリンチュラ類の物質循環に対する寄与を明らかにすることを目指している。　本研究では，18S rDNA領域に対する特異的プライマーを用いた定量PCRによって，大阪湾から採取したサンプルから，*Aplanochytrium* 属の現存量を推定した。この値から試算された生産速度は，瀬戸内海における微小動物プランクトンの生産速度の約半分になった（Uye & Shimazu, 1997)。*Aplanochytrium* 属のみでこのような高い値を示したことは，ラビリンチュラ類全体の影響力を考える上で興味深い結果である。また，*Aplanochytrium* 属は外質ネットと呼ばれる仮足状の構造によって，珪藻などの基質に付着し，集合体を形成するため，より大型の動物プランクトンや小型の魚類に直接に捕食されている可能性がある。カイアシ類の消化管の内容物に対する次世代シーケンサーによる網羅的解析において，単細胞真核生物の中で読まれた配列数を比較したところ，珪藻の*Thalassiosira* 属の10%に次いで，2番目が3.5%の*Aplanochytrium* 属であったと報告されており，この仮説を支持している（Hirai, Hamamoto et al. in Plankton & Benthos Res, in press)。今後，より継続的な現存量調査や，培養実験による転送効率の測定などから，ラビリンチュラ類の生態学的な影響力を明らかにしていきたい。

O5-3

Viruses of eukaryotic plankton: insight into their diversity, host range and role in carbon export

真核生物プランクトンのウイルス：その多様性、宿主域、そして炭素輸送における役割

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Viruses are abundant, diverse and an essential component of marine ecosystems. While most efforts to characterize their diversity and ecology have been focused on bacteriophages, current molecular sequence data derived from marine samples indicate that the diversity of eukaryotic viruses is vast and largely unexplored; with virtually no knowledge on the role that the various lineages fulfill in the environment.

We leveraged meta-omics data derived from viral-to-metazoan size-fractionated water samples, collected in the sunlit layer of dozens worldwide distributed oceanic stations to picture out the phylogenetic diversity of eukaryotic viruses in the sea, gain insight into their host range and sort out lineages with detectable impact on carbon export.

Marker genes-based phylogenies unveil the vast diversity of DNA viruses belonging to the family *Mimiviridae* and RNA viruses of the order *Picornavirales*. Co-occurrence analysis of viral marker genes and 18S rDNA barcode gives insight into their potential hosts. Finally, we used viral marker gene abundance profiles and an indirect measurement of carbon export at sampling sites to build models predictive of carbon export. These models highlight key eukaryotic viruses driving carbon export, in particular, *Prasinovirus* infecting small green algae and an RNA virus infecting *Labyrinthulomycetes*.

This work may serve as a compass to orientate future targeted studies aiming at characterizing the diversity and ecological roles of the eukaryotic virioplankton in the sea.

O5-4

Metagenomic insights into the microbial life in the oligotrophic Pacific Ocean

メタゲノムから見た太平洋貧栄養海域における微生物群集の生態

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The tropical and subtropical ocean except for upwelling region is less productive area than the coastal and the high latitude areas. Especially, the surface water of the southeastern Pacific is extremely oligotrophic, where the clearest seawater in the world is found, and nutrient concentrations are below the limit of detection by standard analytical methods. One of the questions is how microbes adapt to such a lean environment. Particle association may be one of the important adaptation strategies to nutrient limited seawater environments, since particulate organic matter (POM) is a hotspot of microbial activities. Here we report comparative metagenomics of microbial communities differentiating particle-associated (PA) and free-living (FL) assemblages in the tropical and subtropical Pacific Ocean. Seawater samples were collected from the surface at 9 stations located in the North Pacific Subtropical Gyre (NPSG), the South Pacific Subtropical Gyre (SPSG), and an Equatorial Region (EQ) during the cruises of R/V Hakuho-Maru in 2011 and 2012. MiSeq 250 bp paired-end sequences was performed to have about 10-20 million reads per sample. KEGG orthology was assigned to each predicted gene. We evaluated metabolic potential of PA and FL bacterial communities by using the MAPLE system, an automatic system for mapping genes to the KEGG modules. Metagenomes as well as community structures of PA and FL bacteria showed distinctive repertories of functional gene sets. The similarity percentage analysis suggested that greater abundance of some transporters (amino acids, sugar, iron complex, phosphate and metals) largely contributed to the functional dissimilarity between PA and FL bacterial communities. Comparison between regions also suggested that transporters contributed the difference between regions. Especially, the urea transport system was enriched in the subtropical region. Metagenome mapping to *Prochlorococcus* suggested again that the urea transporter system was more abundant in the NPSG and SPSG than the EQ and also the cyanate transporter and the cyanate hydratase were more abundant in the SPSG than the other regions. Surface seawater in a subtropical gyre is extremely oligotrophic with depletion of nitrate and nitrite, which might require the use of alternative sources of nitrogen such as urea and cyanate. These results implied that the distribution and dynamics of alternative nitrogen sources especially urea and cyanate should be the key to understand ecosystem dynamics in the subtropical Pacific Ocean.

O5-5

[ASME] Biodiversity of the coral-killing sponge *Terpios hoshinota*-associated Bacteria in the western Pacific Ocean

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Terpios hoshinota is a coral-killing, encrusting sponge and enables to cause massive mortality of stony corals. This study collected T. hoshinota samples from different reefs across the western Pacific Ocean in the past 10 years. Close attention is paid to cyanobacterial species in the sponge because they are frequently observed in microscopic studies. Moreover, an in-situ light shading experiment has shown discontinued expansion of *T. hoshinota* suggested cyanobacteria played an important role in sponge growth (Soong et al., 2009). This work focuses to answer following questions: (1) is there any geographic variation in *T. hoshinota*-associated bacterial community composition from all study sites? And (2) does dominant cyanobacteria species in T. hoshinota the same from all study sites? The sponge-associated bacteria were identified by 16S rRNA amplicon sequencing. The statistics analysis suggests that no geographic variation were detected in the samples. The dominated cyanobacteria were similar in all the samples suggesting an intimate symbiosis between the sponge and its cyanobacteria. This work provides a more complete picture of bacterial community associated with the sponge and serves as a fundamental data for the sponge studies, particularly in the ecological relationship of the sponge and their bacterial symbionts.

O5-6

Transcriptome analysis of immune response in the coral *Acropora digitifera* against infection of pathogenic bacterium *Vibrio coralliilyticus*

病原細菌 *Vibrio coralliilyticus* に対するサンゴ *Acropora digitifera* 免疫応答のトランスクリプトーム解析

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Vibrio coralliilyticus causes several coral diseases including bacterial bleaching, tissue lysis, and white syndrome [1,2]. Although a diverse virulence repertoire of *V. coralliilyticus* revealed by genomic and proteomic analyses [3], the mechanisms of immune response in coral is poorly known. In this study, in order to elucidate the mechanisms of coral immune response against *V. coralliilyticus*, we performed transcriptome analysis of the reef-building coral *Acropora digitifera* after bacterial challenge. During mass spawning in Okinawa, Japan, in June 2017, the gametes of *A. digitifera* were collected. Embryos were developed to obtain planula larvae, and these larvae were kept at 25 ℃ in a bucket containing 0.22 μm filtered seawater. Metamorphosis into juvenile polyps was induced with peptide Hym248. After bacterial challenge experiment with *V. coralliilyticus*, total RNA was extracted from juvenile polyps. Transcriptome of juvenile polyps before and after *V. coralliilyticus* infection were analyzed using the Illumina Hiseq4000 platform. Comparison of gene expression levels between uninfected control and 60 min post-infection revealed differentially expressed genes (DEGs) (FDR $<$ 0.01; absolute log2-fold change $>$ 1). At this time, a total of 3052 genes of the *A. digitifera* were differentially expressed: 1497 were up-regulated and 1555 were down-regulated compared to the uninfected control. Among them, a total of 1809 genes were annotated by BLASTP search against the SWISS-PROT database and filtering the best hits (E-value $<$ 10-5). Based on these results, we investigated the mechanisms of immune response in *A. digitifera* against *V. coralliilyticus*. [1] Y. Ben-Haim et al., *Appl. Environ. Microbiol.*, 69, 4236-4242 (2003). [2] B. Ushijima et al., *Appl. Environ. Microbiol.*, 80, 2102-2109 (2014). [3] E. O. Santos et al., *ISME J.*, 5, 1471-1483 (2011).

O5-7

[ASME] Preliminary Results of the Stagnant Water Microbiome Project [SWaMP]

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Standing water containers are an important breeding site for many insects. Mosquitoes of the genus *Aedes* are particularly fond of containers, including the vectors of Dengue and Chikungunya viruses. Plant based containers and natural stone containers differ from artificial containers in their attractiveness to different mosquito species for unknown reasons. We hypothesize that the different microbial biodiversity of natural and artificial containers may be a factor. To test this hypothesis, we are inventorying the insect and microbial diversity of natural and artificial container waters in southern Taiwan. We gather data on container type and material. Bacterial and eukaryote diversity is measured with 16S and 18S rDNA metagenomics and with direct culturing, with cultured microbes to be later used to test hypothesized insect-microbe interactions in the lab. Mosquito larvae are collected from containers and identified to species. The goal is to map the ecology of mosquito-microbe interactions in containers to explain why different species prefer different containers. We present the results of our first collection of over 80 containers from Kaohsiung, Tainan, and Pingtung counties and the correlations between container, microbial diversity, and the presence of *Aedes aegypti, A. albopictus,* and other genera of mosquito.

O5-8

Distribution and predicted origin(s) of macrolide resistance genes *mef*(C)-*mph*(G) in Taiwan waters

台湾北部の河川におけるマクロライド耐性遺伝子 *mef*(C)-*mph*(G) の分布と発生源の推定

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[Background] Antibiotic resistance among environmental bacterial communities has been paid much attention so far. Most of environmental bacteria are non-culturable. Hence, spreading of antibiotic resistance genes among environmental bacteria has still been obscure by ordinary approaches. In our previous study, it was found that the novel macrolide resistance genes, *mef*(C)-*mph*(G), were carried by two types of plasmids and chromosomal integrative conjugative element (ICE) in environmental isolates. We should know the information that gene distribution in whole environmental bacterial community including non-culturable bacteria to clarify the dynamics of antibiotic resistance in environment. [Aims] This study aimed to reveal vector diversity for *mef*(C)-*mph*(G) in natural bacterial communities.[Methods] We collected pig manure leachate, river and coastal waters of Taiwan. Bacteria were captured on 0.2　μ m pore-size-filters by filtration. Additionally, isolates were obtained from agar plate supplemented with erythromycin (16 μg/mL). Whole DNA was extracted from filters and pooled-colonies. We quantitated *mef*(C)-*mph*(G) and *traI*coded on plasmids pAQU1 and IncA/C and SXT/R391 family ICE (SRI) using quantitative PCR. Non-metric multidimensional scaling analysis from all data showed the profile of *mef*(C)-*mph*(G) and tra I relationship in various environments.[Results]*mef*(C)-*mph*(G) were detected in both filter and colony DNAs from pig farm and river, whereas only in filter-DNA from marine fish farm. This suggests that these genes are possessed by non-culturable assemblage in seawater. *mef*(C)-*mph*(G) was not detected in more upstream than pig farm. Pig farm might be a source of this gene set. *traI*was detected in allareas; however, no positive correlation was observed with *mef*(C)-*mph*(G). This suggests *mef*(C)-*mph*(G) are carried by other mobile genetic elements than the plasmids and SRI targeted in this study. [Conclusions] This study showed *mef*(C)-*mph*(G) were widely disseminated in aquatic environment. Non-culturable bacteria possess this gene set in the sea. Pig farm might be one of sources of *mef*(C)-*mph*(G). Various mobile genetic elements would play a role to spread *mef*(C)-*mph*(G).

O5-9

Ecological and hygenical roles of bacteria selection at a blackish water area

汽水域の微生物選択作用が担う生態学的および衛生的役割

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河川水と海水が交わり潮汐や波浪等で塩濃度が変化する汽水域には豊富な栄養が存在する。塩濃度は微生物の機能や細胞構造に影響を与え、塩濃度変化が細菌数を減少させることが知られている。我々は絶えず塩濃度変化が生じる汽水域に着目し、塩濃度変化が細胞を破壊し溶菌させ、細胞内の核酸やタンパク質のような高分子有機物が放出されることが汽水域の栄養供給源の一つになり得ると考えた。同時に、汽水域での塩濃度変化は、下水処理放流水等に含まれる大腸菌を減少させる可能性や、細胞構造と細胞径の違いから微生物系統分類群ごとに選択的に溶菌させることも考えられる。しかしながら、汽水域の塩濃度変化が担う生態学的・衛生的役割は明らかではない。そこで、本研究では塩濃度変化が細菌に及ぼす影響について汽水域に存在する主要な微生物系統分類群ごとに調査した。その結果、我々は、汽水域の塩濃度変化により細菌数は減少し、塩濃度変化は細菌を溶菌させること、溶菌特性は微生物系統群ごとに異なることを明らかにした。これにより、塩濃度変化による溶菌現象は、河川から海洋にかけて微生物群集構造が大きく変化する要因であることが示唆された。また、大腸菌や大腸菌群も塩濃度変化により溶菌し、汽水域の塩濃度変化は糞便性細菌を減少させる衛生的役割を担うといえる。溶菌前後の窒素およびリン濃度を評価した結果、溶菌により汽水域の窒素の約1%、リンの約9%が供給されていると推定され、塩濃度変化による溶菌は汽水域の重要な栄養供給源になっていることが示された。このように汽水域における塩濃度変化がもたらす細菌の溶菌現象を理解することは汽水域の環境管理の観点から重要であるといえる。

O5-10

Characterization and Producing Process of Bio-minerals Produced by Sulfate-Reducing Bacteria

硫酸還元細菌の生成するバイオミネラルの特性と生成プロセスの解析

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硫酸還元細菌は嫌気環境下における代表的な微生物であり、硫黄、炭素および鉄等の地球科学的物質循環に深く関わっている。これまでに当研究室では、汽水湖底泥を接種源とした微生物燃料電池の負極バイオフィルムから分離した*Desulfovibrio* sp. HK-II株およびHK-IV株が、Fe^{3+}存在下および硫酸呼吸時に、バイオミネラルとしてMackinawite（FeS）を生産することを明らかにした。生成されたMackinawiteは蓄電性を示し、また、系統学的に両菌株は極めて近縁であるにも関わらず、HK-IV株が生成した蓄電鉱物の充放電容量はHK-IIの約2倍であった。そこで本研究では、Mackinawiteの物質科学的特性と蓄電能の違いを生み出す微生物の特性を明らかにすることを目的としてゲノム比較を実施した。

HK-II株およびHK-IV株のゲノムサイズはそれぞれ、3.92 Mbpおよび3.97 Mbpであり、Average Nucleotide Identity（ANI）解析の結果、ゲノム間の相同性は99.91%であった。HK-II株由来Mackinawiteは壁構造を有する数十μmサイズの箔片である一方、HK-IV株由来のMackinawiteは数十μmサイズの箔片上に球体が観察された。微生物由来のMackinawiteは数日掛けて生成されるのに対し、化学合成では一瞬にして生成され形態および充放電容量は異なった。そのため、Mackinawite生成に関与すると推定される硫化水素の生成速度が影響していると考えられた。そこで硫酸還元に関わる代謝プロセスを遺伝的に解析した結果、両菌株とも電子伝達鎖、硫酸還元経路、亜硫酸還元経路と既存の異化的硫酸還元プロセスを有していた。硫酸還元遺伝子*sat*、*aprAB*、亜硫酸還元遺伝子*dsrAB*、電子伝達鎖遺伝子*qmoABC*、*tplc3*、*qrcABCD*、*dsrMKJOP*とそれらの周辺領域、さらに制御タンパク遺伝子*rex*を比較したところ、99%以上一致していた。しかし、HK-IV株に特有の遺伝子領域が少なくとも4ヶ所見出された。その内の1つに亜硫酸排出に関わるタンパクTauEをコードする遺伝子が見出された。TauE遺伝子はゲノム全体ではHK-II株およびHK-IV株にそれぞれ10および11コピー存在し、それらの多様性が確認され、硫化水素生成速度の差が影響していることが示唆された。現在、上記関連遺伝子の発現量比較を進めている。

O5-11

Dispersal of microbes to the deep subseafloor biosphere to the hydrosphere through mud volcanoes

海底下深部生命圏から海水中への泥火山を通した微生物の拡散

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Submarine mud volcanoes (SMVs) are formed by muddy sediments and breccias extruded to the seafloor from a source in the deep subseafloor. From the SMVs, hydrocarbon gasses including methane and deep-sourced fluids discharge into the overlying seawater. Regarding to the characteristic, “a window to the subsurface” is often used as a metaphor for SMVs. However, interaction between subseafloor biosphere between the overlying hydrosphere through SMVs remain largely unknown. In the present study, we investigated the microbial communities in sediment and overlying seawater at two SMVs located on the Ryukyu Trench off Tanegashima Island, southern Japan. The microbial community structure in both SMVs are different from that in the overlying seawater and well-stratified Pacific margin sediments, inferring the deep origin of microbial communities in SMVs. The comparative analysis of microbial communities in the overlying water column and sediment in SMVs indicates an OTU affiliated with *Atribacteira* is commonly found in all the samples. We designed a new oligonucleotide probe targeting *Atribacteria* and applied it for CARD-FISH. The enumeration of Atribacteria by CARD-FISH and its relative abundances in sequence libraries consistently indicate that more *Atribacteira* are found in methane plumes above the two SMVs compare to the surrounding waters. This observation suggests that microbial cells in subseafloor sediments are dispersed as “deep-biosphere seeds” into the ocean. The findings of this study provide insights for the microbial transmigration between the deep subseafloor biosphere and the hydrosphere.

O6-1

[ASME] Identification and isolation of a keystone species in the rhizosphere microbiome of tomato resistant to bacterial wilt

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Bacterial wilt is a severe plant disease caused by the soil-borne bacterium *Ralstonia solanacearum*. Although plant disease resistance is known to be mediated by its own immune system, plant-associated microorganisms may play an important role. We initiated a whole metagenomic analysis of the rhizosphere communities of two tomato cultivars, Hawaii 7996 and Moneymaker, that are either resistant or susceptible to bacterial wilt, respectively. Taxonomic analysis of the 16S rDNA reads, which have been extracted from the whole metagenome data, revealed that the proportion of *Flavobacteriia* is higher in the rhizosphere of Hawaii 7996 than in the rhizosphere of Moneymaker, whereas the proportion of *Betaproteobacteria* is higher in Moneymaker than in Hawaii 7996. Through phylogenetic binning, we were able to reconstruct the genome of a novel uncultured *Flavobacteriaceae* bacterium, designated TRM1, from the metagenomic sequences of Hawaii 7996. Based on the genome information, we successfully isolated the corresponding bacterium that contributes to the disease resistance. Our study illuminates that microbiome structures of the rhizospheres are distinct between the two cultivars and underscores the pivotal role that the native microbiome plays in protecting plants from infection.[Financial support from the Strategic Initiative for Microbiomes in Agriculture and Food, the National Research Foundation (NRF-2014M3C9A33068822 and NRF-2011-0017670), the Next-Generation BioGreen 21 Program (PJ008201), and BK21 PLUS]

O6-2

Traits of *Burkholderia kururiensis*, an important diazotrophic endophyte inhabited the root of a rice line, pLIA-1 derived from a cross between *Oryza longistaminata* and *O. sativa* ssp. *japonica*

アフリカイネ *Oryza longistaminata* と *O. sativa* ssp. *japonica* の交雑後代系統イネ pLIA-1 根に棲息する窒素固定エンドファイト *Burkholderia kururiensis* の性質と挙動

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A hybrid paddy rice pLIA-1 (potential low-input adaptable rice line-1), derived from a cross between African paddy *Oryza longistaminata* and *O. sativa* ssp. *japonica* cv Taichung-65, is highly adaptable to a long-period non-fertilized paddy field. Despite of the nitrogen-poor condition, this hybrid rice maintains a high productivity of rice grains in the non-fertilized paddy field. To know how pLIA-1 acquires nitrogen, we investigated eubacterial communities in the roots of the paddy rice cultivated there using 16S rRNA gene-targeted PCR-DGGE (denaturing-gradient gel electrophoresis) and high-throughput sequencing technology. The root-associating bacteria in the paddy rice were compared to those of pLIA-1 grown in fertilized paddy field. The high-throughput analysis showed that *Burkholderia kururiensis* often occupied more than 10% of copies as an abundant eubacterium in the roots at the booting and panicle-maturing stages of the paddy plants grown in the non-fertilized paddy field. Although *B. kururiensis* was obviously the dominant root-associating bacterium, its isolation using a normal screening method on potato-dextrose agar or Winogradsky’s agar plate had been unsuccessful despite of our efforts for 3 years. We eventually succeeded in isolation of the target bacterium on a gellan gum plate of modified Winogradsky’s medium, using a highly diluted inoculant (5-10 colonies per a ø 9 cm plate). After the isolation and further investigation of its physiological traits, we found the reason why isolation of *B. kururiensis* was difficult. A high plating density of varying colonies (approximately 100-400 of colonies per a ø 9 cm plate) exposed *B. kururiensis* to high competition with other saprotrophic bacteria. Indeed, under such a high dense condition, colonies of B. kururiensis clearly suppressed its colony development. Particularly, *Burkholderia vietnamiensis*, a free-living diazotroph, severely inhibited growth of the co-cultured *B. kururiensis*. It is likely that *B. kururiensis* is highly adapted to the root aerenchyma of pLIA-1 to avoid competition with saprotrophic rhizobacteria. In a gas-chromatography vial, *B. kururiensis* showed significant acetylene reduction, which was particularly active when the bacterial cells were inoculated to rice seedlings growing on sand bed. In the non-fertilized paddy field, overwintering *B. kururiensis* survived in the root residues buried in the soil, whereas this bacterium was rarely found in the seeds or panicles of pLIA-1. Hence, it was suggested that the semi-symbiotic nitrogen-fixer is transmitted vertically and horizontally to transplanted paddy plants via rice root residues in soil of the paddy field. Interface between *B. kururiensis* and paddy rice pLIA-1 should be further focused.

O6-3

Illumina-based analysis of Rhizosphere and Endosphere Bacterial Communities related to Halophytes *Glaux maritima* and *Salicornia europaea*

塩生植物 ウミミドリおよびアッケシソウの根圏・内生細菌相の解析と比較

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Root-associated microbial community structures are very important in the adaptation of halophytes in coastal ecosystems. However, few reports have described bacterial communities related to halophytes, and the distribution patterns of these bacteria in different plants have been rarely compared. In this report, we mainly studied the diversity and community structure of rhizosphere (Rh) and root endosphere (En) bacteria related to two kinds of halophytes, *Glaux maritima* and *Salicornia europaea*. We collected Rh, root (R), and bulk (Bl) control soil samples, and sequenced the V3-V4 region of the bacterial 16S rRNA gene using the Illumina MiSeq platform to identify bacterial communities originating from different plant species. Among samples related to *G. maritima*, the bacterial richness and diversity in Rh were higher than those in R, but lower than those of the Bl. Meanwhile Bl, Rh and R from *S. europaea* had similar bacterial richness and diversity. The numbers of operational taxonomic units exclusive to the R, Rh and Bl were 181, 366, and 924 in *G. maritima* and 126, 416, and 596 in *S. europaea*, respectively, implying habitat-specific patterns in each halophyte. In total, 35 phyla and 566 genera were identified. The dominant phyla across all samples were *Proteobacteria* and *Bacteroidetes*. *Actinobacteria* in the R from *G. maritima* have extremely high abundance. The beneficial genera of bacteria were enriched in R and Rh from two halophytes. *Rhizobium*, *Actinoplanes*, and *Marinomonas* significantly enriched in *G. maritima*, while *Sulfurimonas* and *Coleofasciculus* exhibited relatively higher abundances in *S. europaea*. Principal coordinate analysis demonstrated significant differences in microbiota composition associated with plant species and sample types. These results strongly indicate that there were clear differences in bacterial community diversity and structure between *G. maritima* and *S. europaea*. This study is the first report to characterize root microbiome of *G. maritima* and compare diversity and community structure of Rh and root En bacteria in between *G. maritima* and *S. europaea*.

O6-4

Phylogeny and physiological characteristics of a novel *Bacteroidetes* bacterium KFE18 promoting microalgae growth

微細藻類の成長を促進する新規 *Bacteroidetes* 門細菌 KFE18 株の系統と生理特性

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【背景と目的】藻類の付着・共生微生物には*Proteobacteria*門、*Bacteroidetes*門、*Cyanobacteia*門など多様な微生物が存在している。中でも*Bacteroidetes*門細菌は微細藻類の付着微生物において優占系統群であるため、その生理生態機能の解明は微細藻類と微生物の相互作用を理解する上で重要な課題の一つである。当グループでは、水生植物から単離した微生物をモデル植物であるウキクサに接種・共培養することで植物－微生物相互作用の実態解明を試みてきた。本研究ではその方法を利用して野生ウキクサから単離した*Bacteroidetes*門新規細菌KFE18株をコウキクサと微細藻類ユーグレナに接種し、共生的機能を解明することを目的とした。

【実験方法】北海道内の池から野生コウキクサを採取し、超音波破砕を行った後、低栄養寒天培地に接種して植物体に付着する細菌の培養を行った。次に、寒天培地から分離した単離株について16S rRNA遺伝子配列に基づく分子系統解析を行った。また、これら単離株を培養し、無菌コウキクサおよび無菌ユーグレナに接種した。コウキクサは葉状体数、ユーグレナはクロロフィル(Chl)含量でその成長促進効果を評価した。このとき、対照実験系として無菌系(control)と既知のPGPB(Plant Growth Promoting Bacteria)接種系を設定して比較した。

【結果と考察】野生コウキクサから付着・共生微生物としてKFE18株の単離に成功した。分子系統解析の結果、KFE18株は最近縁種*Chryseolinea serpens* RYG^{T}との相同性が 95%以下を示す系統的に新規な*Bacteroidetes*門細菌であった。コウキクサ－KFE18共培養系では、最終葉状体数がcontrol比2倍以上であった。また、ユーグレナ－KFE18共培養系では、最終Chl含量はcontrol比2倍以上となり、KFE18株はMGPB(Microalgal Growth Promoting Bacteria)であることがわかった。既知の成長促進微生物の系統群とは大きく異なっている新規細菌KFE18株の発見によって、水生植物と微細藻類の両方に作用する何らかの新しい成長促進メカニズムの存在が期待される。

O6-5

[ASME] Denitrification is lower in *Bradyrhizboum japonicum* than in *B. diazoefficiens* due to impaired nitrate reductase activity

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Members of the genus *Bradyrhizobium* are able to denitrify when oxygen is limiting. Denitrification is the sequential reduction of $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ mediated by periplasmic nitrate reductase (Nap), nitrite reductase, nitric oxide reductase and nitrous oxide reductase (encoded by *napA, nirK, norCB* and *nosZ* genes), respectively. Generally, *Bradyrhizobium diazoefficiens* (*nos*+) possesses complete denitrification pathway, whereas, *Bradyrhizobium japonicum* (*nos*-) lacks the *nos* gene cluster and has an incomplete process from nitrate to nitrous oxide. In an initial analysis, anaerobic growth with NO_3^- as the electron acceptor was significantly lower in *B. japonicum* than in *B. diazoefficiens*, but it was not explained by the absence of *nos* genes in *B. japonicum*. Under anaerobic NO_3^--respiring conditions, *B. japonicum* was less capable of reducing NO_3^- to N_2O than *B. diazoefficiens*. Nap activity was markedly lower in *B. japonicum* than in *B. diazoefficiens*, indicating that the reason for the limited growth in *B. japonicum* is a low NO_3^- due to an impaired Nap activity. *napA* expression in *B. japonicum* and *B. diazoefficiens* was not significantly different, indicating that the reason for impaired Nap activity may rely on posttranscriptional events. Accordingly, the growth of *B. japonicum* USDA 6 overexpressing *napEDABC* genes was similar to that of the wild-type USDA 6, further suggesting that the low performance of USDA 6 in NO_3^- reduction is independent of the transcript level. Haem-staining of NapC revealed that *B. japonicum* produced a very low amount of Nap compared to *B. diazoefficiens*. This suggested that impaired Nap activity in *B. japonicum* is the result of a low amount of Nap protein. It has been suggested that *B. diazoefficiens* (*nos+*) is predominant in Gleysol soils, rich in low-oxygen conditions, whereas *B. japonicum* (*nos*-) is predominant in Andosols, rich in aerobic environments. The growth of *B. diazoefficieins* USDA 110 lacking *nosZ* gene (*nosZ*-) and *B. japonicum* USDA 6 wild-type cohabiting the same culture environment under a NO_3^- -respiring condition indicated that USDA 110 *nosZ*- possessed higher competitiveness than USDA 6. Taken together, these results indicate that the capacity in NO_3^- reduction may be the main factor that drives the distribution of bradyrhizobia in soybean fields in Japan.

O6-6

Genetic and biochemical diversity for *N*-acylhomoserine lactone biosynthesis in plant pathogen *Pectobacterium carotovorum*

植物病原菌 *Pectobacterium carotovorum* におけるアシル化ホモセリンラクトン生合成系の多様性

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*Pectobacterium carotovorum*は、様々な農作物に感染して組織の軟化を引き起こし、強い悪臭を伴いながら腐敗させる軟腐病の原因菌として知られている。多くの植物病原菌において、アシル化ホモセリンラクトン（AHL）をシグナル物質とした細胞間情報伝達機構Quorum Sensingを用い、細胞密度依存的に病原性因子の発現を制御する機構が知られている。*P. carotovorum*においても、AHLを介したQuorum Sensingにより、主要な病原性因子であるペクチナーゼの発現が制御されることが広く知られている。軟腐病防除技術の開発のためには、*P. carotovorum*におけるAHL生合成系を詳細に解析する必要があると考えられる。本研究では、農業生物資源ジーンバンクに保存されている*P. carotovorum*を中心に、生産するAHLの構造解析を行うとともに、AHL合成遺伝子（*expI*）の多様性を明らかにすることを目的とした。 農業生物資源ジーンバンクに保存されている数百株の*P. carotovorum* subsp. *carotovorum*とNBRCより入手した2株の*P. carotovorum* subsp. *carotovorum*を用い、培養上清から抽出したAHLの構造をLC-MS/MSにより解析したところ、主なAHLとして3-oxo-C6-HSLを主に生産するグループIと、3-oxo-C8-HSLを生産するグループIIに分類可能であることが明らかとなった。さらに、これらの菌株の*expI*をPCRによりクローニングし、翻訳後のアミノ酸配列を決定したところ、グループI及びIIに属する細菌間で、ExpIのアミノ酸配列が高い相同性を示すことが明らかとなった。以上より、*P. carotovorum* subsp. *carotovorum*においては、AHL合成系を指標として2つのサブグループに分類可能であることが明らかとなった。

O6-7

Solidification of soil using microbial function

微生物機能を利用した土壌固化技術の開発

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《背景》微生物機能による鉱物化反応（バイオミネラリゼーションあるいはバイオグラウト）を利用した地盤改良技術は、ここ10年の間に大学・研究機関を中心に研究開発が進められている（川﨑, 2015)。地盤改良に微生物機能を利用するメリットはCO_2排出量削減、地下水および周辺地盤環境の保全、コスト低減などがあげられ、環境調和型施工技術の開発につながると考えられる。一方で、先行研究のほとんどが尿素分解菌（*Sporosarcina pasteurii*）の機能を利用しており、尿素分解によるアンモニア発生のために悪臭の問題が懸念されている。他に硝酸還元菌（*Halomonas halodenitrificans*）の利用も考えられているが、反応速度が低く、地盤改良に求められる強度の均一性に課題がある。川﨑ら（川﨑, 2015）は入手および取扱いが容易であるドライイーストを利用したが、鉱物化に最適なpH条件を整えるために高価な試薬を必要としたため、現場適用に難があると考えられた。
《目的》簡易な地盤改良施工法あるいは簡易舗装法への適用を想定した微生物機能を利用した土壌固化技術の開発を目的として、安価な資材を利用した土壌固化実験を実施した。
《方法》ドライイーストあるいは土壌微生物群を利用した。固化対象は山砂とした。pH調整剤および2価金属イオン供給源として市販されている肥料を用いた。資材および微生物を混合し水道水を満たしたものを供試体とした。30℃の恒温室で2週間養生し、経時的に簡易土壌硬度計、土壌pH計、Caイオンメータを用いて供試体の測定を行った。
《結果》ドライイーストを利用した場合、数日のうちにかなりの強度を発現した。一部は一般的なコンクリート強度を超えた。強度発現に連動して供試体内のCaイオン濃度が減少しており、炭酸カルシウムの生成が強度発現に寄与していると考えられた。土壌微生物群を利用した場合にも数日のうちに固化現象は見られたが、地盤改良で求められるような強度には達しなかった。ドライイーストおよび市販肥料を用いて土壌固化が可能であることが分かった。本法が実用できれば、大型重機等や大量のセメントを使用することなく地盤改良や簡易的な舗装が可能となり、環境調和型施工技術の開発が期待できる。

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O6-8

Isolation and taxonomic classification of novel ktedonobacterial strains from a soil in Mt. zao and “Tengu-no-mugimeshi”

蔵王山の土壌と「天狗の麦飯」から新しいクテドノバクテリアの分離および系統分類

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【背景及び目的】近年、創薬微生物「放線菌」からの新規抗生物質の発見頻度は顕著に減少しているなか、多剤耐性菌は日々拡大を続けており、「放線菌」に代わる創薬微生物資源の開拓が急務となっている。クテドノバクテリア（綱）は2007年に初めて創設された現在2目3科4属7種から成る知見の乏しい系統であり、多くは当研究グループにより発見・創設された。これらは放線菌とは系統が大きく離れているが共通する形態を示し、種々の生物活性を示すなど、まさに放線菌と類似した特徴を有している。

近年、宮下らは、群馬県や長野県の標高の高い火山地帯に棲息し、食べられる土として知られる「天狗の麦飯」を解析し、クテドノバクテリアが優占菌の1つであることを報告した[1)]。そこで本研究では、群馬県の湯ノ丸山及び角間山に棲息する「天狗の麦飯」（褐色～橙色の微生物の塊）及び類似環境の蔵王山の土壌から新規クテドノバクテリアの分離と系統分類を行うこととした。

【結果】群馬県湯ノ丸高原の「天狗の麦飯」から分離された16株を分子系統解析した結果、*Ktedonobacterales*目のクラスター内に4つのクレードを形成した。各クレードから任意に選抜したUno3, Uno11, Uno16, Uno17株は最近縁の*Dictyobacter aurantiacus* S27^{T}株との相同性が91.3, 96.4, 95.5, 95.0%であり、各々種あるいは属レベルで区別できる事が強く示唆された。これら4株は既報株と同様菌糸を形成し、コロニーの色はライトグレー～橙色と多様であり、「天狗の麦飯」本体の色と質感が似ていた。それらの系統分類学的試験の結果からUno3株は*Ktedonobacteraceae*科に新属･新種として、その他の3株は*Dictyobacter*属の新種として提唱中である。また角間山の「天狗の麦飯」及び蔵王山の土壌から分離した*Ktedonobacteria*綱に属する中温性細菌6株の系統解析の結果も併せて報告する。

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O6-9

An incubation experiment examining the carbon dynamics during the thawing of a frozen soil core collected at a black spruce forest, Interior Alaska

アラスカ内陸部、黒トウヒ林で採取した凍結土壌コアの融解過程における炭素代謝の培養実験

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The carbon dynamic during the thawing of frozen soils is an important process determining the annual greenhouse gas (GHG) balance in northern ecosystems. In this study, an incubation experiment was conducted for a frozen soil core collected in a black spruce forest of Interior Alaska. The core from ground surface to upper permafrost in 90 cm depth were vertically grouped into three layers (top, middle, and bottom layers) and incubated for 3 weeks, with the measurement of carbon dioxide (CO_2) and methane (CH_4) fluxes. During the incubation, temperature was weekly changed from 0 to 5 then 10 ℃. Net CO_2 release was 1.5 to 19.2-fold greater at 5 ℃ than at 0 ℃ in most soils of top and middle layers, while the release at 10 ℃ was reduced in some top and middle layer soils. Net CH_4 release was the greatest in bottom layer soils incubated at 0 ℃, while net CH_4 release was concurrently observed with net CH_4 absorption at soils of upper two layers. At 5 and 10 ℃, net CH_4 release was reduced, then net CH_4 uptake was observed in top and middle layer soils. Both net uptake and release of CH_4 were reduced by the addition of a chemical inhibitor (e.g. 2-bromo-ethane sulfonate) for anaerobic methanotrophic and methanogenic activity. The genomic information of bacterial and archaeal community gradually changed along the depth, while the overall microbial community less responded to the temperature rising. Thus, this study presented that soil GHG flux in a northern lowland forest responded sensitively and diversely to the soil thawing, while the overall bacterial and archaeal community was insensitive to the soil thawing.

O6-10

[ASME] Molecular diversity of arbuscular mycorrhizal fungi along pH gradients from different habitats in Hungary

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Optimal composition of beneficial soil microbiota seems to be a prerequisite for plant survival, functioning and diversity, therefore it is of high importance to understand subterranean relationships to protect natural habitats and possibly apply soil biological services in agriculture. Arbuscular mycorrhizal (AM) fungi are one of the key groups of root symbiotic organisms having a basal role in nutrient exchange and stress alleviation of their hosts. Their presence in natural soils is highly dependent on edaphic and climatic conditions and the structure and successional state of vegetation, representing a crucial link between plants and soil. Study of extreme environments may give new insights into the functioning of AM relationships under stress conditions threatening the state of soils globally due to climate change, e.g. drought, salinity or flooding/anoxia. In our study, molecular (PCR-RFLP of partial 18S rDNA or 5.8S-ITS2) diversity of AM fungi and root colonization were characterized in two separate habitats, both of which included a wide range of sampled soils/substrates with highly different pH regimes. Three locations of floating islands having different pH (3.74 - 6.62), trophity, geomorphology and vegetation were studied in different parts of Hungary, as a first report of AM diversity in this type of habitat. Floating islands are pseudo-dry lands composed by littoral macrophyte vegetation bending down on water and forming interwoven rhizomes and roots collecting debris and litter, which may be followed by a succession of herbal and woody species. In a second study, four vegetation types (from a saline bare spot to alkali fescue grass steppe) of a saline solonchak grasslandwith a pH (7.11 - 10.58) and salinity gradientwere investigated from central Hungary. From both type of habitats, several AM fungal phylotypes were identified belonging to Glomeraceae, Claroideoglomeraceae, Acaulosporaceae and Paraglomeraceae groups, the diversity of which were comparable to previous studies. Very few of the phylotypes were however found exclusively in these extreme environments supporting the view that arbuscular mycorrhizae are widely distributed across plant species and habitats. Our results suggest that AM fungi are substantial players influencing plant community structure and ecosystem processes also in these extreme habitats. They represent a potential “warehouse” of beneficial functional diversity to improve sustainability and resilience in agriculture with a stronger focus on soil biology.

O6-11

[ASME] Fates of Antibiotic Resistance Genes in Cattle Manure after Aerobic Composting and the Resistome Dissemination in Agricultural Soils

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The increasing prevalence of antibiotic resistance genes (ARGs) in environment has become a global public health concern. Livestock manure is recognized as a rich reservoir for antibiotics residues, antibiotic-resistance bacteria and ARGs, because more than 70% of global antibiotics are consumed for livestock. Composting has been suggested as a potential strategy to eliminate antibiotic residues and pathogens in livestock manure before land application. However, the impacts of composting on ARGs in livestock manure and the resistome tracing after land application are not well understood. In this study, the fates of ARGs in cattle manure after aerobic composting was investigated, and the effects of manure and compost application to agricultural soils on resistome profile was compared by constructing laboratory microcosms. The high-throughput quantitative PCR array detected a total of 144 ARGs across all the soil, manure and compost samples, with Macrolide-Lincosamide-treptogramin B, aminoglycoside, multidrug, tetracycline, and β -lactam resistance as the most dominant types. Composting significantly reduced the diversity and relative abundance of ARGs and mobile genetic elements (MGEs) in the cattle manure. In the 120-day microcosm incubation, the diversity and abundance of ARGs in manure-treated soils were significantly higher than those in compost-treated soils at the beginning of the experiment. The level of antibiotic resistance rapidly declined over time in all manure and compost-treated soils, coupled with similar temporal patterns of manure- and compost-derived bacterial communities as revealed by SourceTracker analysis. The network analysis revealed more intensive interactions/associations among ARGs and MGEs in manure-treated soils than in compost-treated soils, suggesting that mobility potential of ARGs was lower in soils amended with compost. These results provide evidence that aerobic composting of cattle manure may be an effective approach to relieve the risk of antibiotic resistance propagation before land application.