Abstracts
JSME Symposium

S1-1

Generalist species drive microbial dispersion and evolution

ジェネラリストが駆動する微生物の分散と進化

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Microbes form fundamental bases of every Earth ecosystem. As their key survival strategies, some microbes adapt to broad ranges of environments, while others specialize to certain habitats. While ecological roles and properties of such ""generalists"" and ""specialists"" had been examined in individual ecosystems, general principles that govern their distribution patterns and evolutionary processes have not been characterized. Here, we thoroughly identified microbial generalists and specialists across 61 environments via meta-analysis of community sequencing data sets and reconstructed their evolutionary histories across diverse microbial groups using the Binary-State Speciation and Extinction (BiSSE) model. This revealed that generalist lineages possess 19-fold higher speciation rates and significant persistence advantage over specialists. Yet, we also detected three-fold more frequent generalist-to-specialist transformations than the reverse transformations. These results support a model of microbial evolution in which generalists play key roles in introducing new species and maintaining taxonomic diversity.

S1-2

Agroecosystem dynamics and fluctuating interaction networks of microbial communities

時間変動する微生物群集の相互作用ネットワークと農生態系の動態

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微生物群集は野外生態系において物質循環や他の生物の動態に影響を与えている。野外において微生物群集の動態や役割を理解するには二つのアプローチがある。一つは、単離培養やゲノム情報の解析により個々の微生物種の生態を解明し、多様な種で構成される野外微生物群集を再構成的に理解するボトムアップ型アプローチである。もう一つは、まず野外微生物群集の動態の全体を把握し、全体の挙動から系にとって重要な微生物を抽出するトップダウン型アプローチである。

本研究では後者のアプローチを用い、農生態系における微生物群集の役割の理解するため、水田のモニタリングを行った。滋賀県大津市の圃場に90 cm四方の小規模水田5つを設置し、2017年5月23日から122日間、1日1回のイネの草丈測定と水田水の採取・ろ過を行った。ろ過したフィルターからDNAを抽出し、原核生物を対象としたプライマーで16S rRNAを増幅しMiSeqシーケンシングを行った。この結果、122日×5プロット分の各変数の時系列データを得た。

優占的な原核生物の時系列データにEmpirical Dynamic Modeling (EDM) と呼ばれる非線形時系列解析を適用した。EDMは変数間の式を仮定せず「変動する相互作用」を捉えることが可能な柔軟な解析法である。その結果、水田の微生物群集の相互作用の数や強度が時間変動する様子が描かれた。特にイネの草丈成長が活発な6-7月に微生物群集内の相互作用強度の強くなる傾向が見られた。

さらに、イネの草丈成長への原核生物群集の貢献を調べるためイネ動態の予測モデルを構築した。イネの草丈成長を自己相関モデルで予測すると予測値と観測値の間の決定係数 (R^2) は0.40であった。イネの草丈成長のみを用いたEDMではR^2=0.54、イネと気温を用いたEDMではR^2=0.55であった。一方、イネと気温に微生物群集の情報を加えたEDMではR^2=0.66となり予測精度が改善された。このことは野外においてイネの成長に微生物群集が寄与していることを示唆している。微生物がイネに与える影響を精査すると、例えばChitinophagaceae科の細菌が正の効果、*Devosia*属の細菌が負の効果を与えていると推定された。現在、より詳細なデータ解析を進めている。今後は検出された微生物が実際にイネの成長を変えうるか、操作実験により確認したい。

S1-3

Microbial ecology and genome features

微生物の生態とゲノムの特徴

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Microorganisms have evolved in various environments, and their genome features can thus reflect not only their phylogeny but also their free-living or symbiotic lifestyle and ecological niches. Bioinformatics analyses of genome features such as oligonucleotide composition and codon usage have been conducted to predict hosts of mobile elements and to detect evidence of translational selection associated with growth rate and gene expression.

Plasmids are ubiquitous mobile elements that spread antibiotic resistance, virulence, and many other traits in bacterial communities. The oligonucleotide composition of a plasmid tends to be similar to that of their host chromosome, suggesting that plasmids acquire their hosts' compositional features over time. We found that narrow-host-range plasmids (e.g. IncF and IncI plasmids) have oligonucleotide compositions that are similar only to those of closely related bacteria, while broad-host-range plasmids (e.g. IncP and IncW plasmids) have oligonucleotide compositions that are similar to those of diverse bacteria or that are not similar to any of the sequenced bacteria. Our results suggest that plasmid host range can be inferred from their oligonucleotide compositions [1].

Synonymous codon usage varies both between organisms and among genes within a genome, and can reflect a balance between genome-wide and strand-specific mutational biases and translational selection acting on highly expressed genes. Various statistical methods have been proposed to identify determinants of synonymous codon usage and to predict highly expressed or foreign genes. However, previous methods may be affected by biases (e.g. amino acid usage and codon degeneracy) masking the effect of synonymous codon usage. Therefore, we proposed alternative methods to avoid these biases, and applied these methods to microbial genomes, transcriptomes (RNA-Seq), and metagenomes [2-4].

References

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S1-4

Microbial ecosystem revealed by multi-lakes' comparative environmental genomics

複数湖の比較環境ゲノム解析から紐解く微生物の生き様

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アンプリコンシーケンス、ショットガンメタゲノム等の環境ゲノム解析技術の発展と普及により、環境中の微生物の多数派であった未培養系統を対象とした研究が容易になり、生態系における微生物の位置づけと生き様の包括的・網羅的な理解が進みつつある。水域の微生物生態学は海洋の研究がリードしてきたが、淡水湖沼においても知見の蓄積・整理が進められ、海洋とは異なる独自の微生物多様性が明らかになってきた。特に世界中の湖沼の表水層で普遍的に優占する細菌系統(acI, acIV, LD12, LD28など)については高い関心が向けられ、その単離への挑戦とともに、環境ゲノム解析をはじめとした培養非依存的なアプローチによる生理・生態の解明が精力的に進められている。

その中で演者は、琵琶湖・洞爺湖・中禅寺湖・本栖湖といった大水深（>50 m）の淡水湖をフィールドとして研究を行っている。これまでの研究で、これら大水深淡水湖の好気的深水層（水温躍層以下、水深10～30 m以深の有酸素の水層）において、既知の表水層の優占系統とは門レベルで異なる独自の細菌系統群が優占することが明らかとなり、深海と同様、淡水湖においても深水層では表水層と異なる微生物生態系が駆動していることが示された(Okazaki et al., 2017, ISME J)。しかし、彼らの生理・生態・進化的な背景についてはほぼ未解明であり、「何を食べ、誰に食われているのか？」「なぜ『淡水湖の有酸素深水層』という限定された環境でのみ、しかも地理的に隔絶された複数の湖で共通して優占しているのか？」という問いは残されたままである。

本発表では、その問いに答えるべく現在進めている、大水深淡水湖の大規模環境ゲノム解析について紹介する。特に、国内外の複数湖の網羅的調査により実現した、微生物の系統地理的な背景に焦点を当てた研究をとり上げ、従来のpartial SSU rRNAアンプリコンシーケンスでは成しえなかった高解像度な比較解析によって、一部の細菌系統で地理的な距離に応じて明確な遺伝的差異が見られる結果（＝Everything is everywhere ではない）が得られたことを紹介する。

さらに、この環境ゲノムデータを活用して進めている他の研究の状況も報告しながら、「大水深淡水湖」という研究系ならではの利点やアプローチを整理し、本研究が「湖の微生物生態学」という枠を超え、微生物学・生態学のより一般的な課題の解決に貢献できる可能性について論じたい。

S1-5

Exploring core microbiomes for designing ecosystems

コア共生微生物を見出し、生態系を設計する

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陸上植物は4億5000年前に陸上進出した最初期の頃から、菌根菌をはじめとする微生物たちに依存した戦略の下、「共生体」として進化してきた。現在においても、農地生態系であれ、自然生態系であれ、植物は多様な細菌・真菌類と関わり合って生存している。しかし、植物ゲノムと共生・環境微生物群集との関連性について、人類はまだその一部しか知り得ていない。 土壌中のリンや窒素の効率的供給や、病原性真菌・卵菌・節足動物・線虫からの保護など、微生物が植物にもたらす機能は計り知れない。次世代シーケンサーの登場以降、こうした微生物たちへの関心が一層高まっているが、微生物たちの極めて高い多様性に阻まれて、共生系の包括的理解からは未だほど遠い状態である。植物とその共生微生物で構成される系を読み解く際、宿主と共生者間の相互作用だけではなく、無数の共生微生物同士の相互作用ネットワークについて理解することが求められる。個々の微生物と宿主の関係を積み上げただけでは共生系全体の動態は予測できないため、従来とは異なる研究の戦略が必要とされるに至っている。 本発表では、植物－共生微生物ネットワーク（*Nature Communications* 5:5273; *Science Advances* 1:e1500291）や共生微生物間ネットワーク（*J. Royal Soc. Interface* 13:20151097）に関して、膨大な生物群集データをいかに解析していけるのか解説する。また、微生物群集の中で「コア」として働いている微生物を探索する手法（*Nature Ecology & Evolution* 1:0024）について、次世代シーケンスデータをもとにした解析法を概説する。生態学、微生物学、情報学を融合した先には、持続可能な農業生態系の設計や自然生態系の再生を可能とする微生物叢制御技術が見えてくるであろう（*Nature Plants* 10.1038/s41477-018-0139-4）。構成的アプローチで新たな科学領域をいかに創生できるのか、議論したい。

S2-1

Single-cell analysis to understand the behavior of bacterial cells

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In the majority of past biological studies, the behavior of bacterial cells was understood by the group-average, rather than the behavioral characteristics of individual cells. The recent technological developments in live-cell imaging and high-throughput data analysis allow us to investigate bacterial physiology at the single-cell level. Microfluidics, the technology used to grow cells at the micro-scale, also provides the advantage for this purpose. In this talk, how these techniques can be useful to find previously unseen aspects of bacterial physiology will be discussed with two examples. Cell size control in bacteria: It has been a long-standing question how bacteria control the timing of division and therefore maintain cell size homeostasis. We performed single-cell analysis in two model organisms, Caulobacter crescentus and Escherichia coli and found that these organisms maintain size homeostasis by elongating constant amount between two successive divisions. Collective migration of chemotactic bacteria: Individual cells in a clonal population are known to display phenotypic diversity, yet they are able to perform group behavior. How do individuals coordinate their diversity to achieve the collective behavior? We used bacterial chemotaxis as a model system to address this question. Single-cell tracking experiments in microfluidic devices and simulation analysis indicated that spontaneous spatial organization, which arises from phenotypes of the single cells, resolves conflicts between individuality and collective migration.

S2-2

Miniscule space designing for microbial cultivation

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One of the most important observations in microbiology is that the vast majority of microorganisms from most environments resist cultivation. This is a significant impediment for both academic and applied microbiology, necessitating innovations in cultivation technologies. Thus, developing a new cultivation method has a huge potential and possibility to overcome obstructed situations which have depended heavily on conventional method. Several recently advanced methodologies offer a promise to close the gap between the high richness of environmental species and low number of their cultivable representatives. However, the number of innovative cultivation method is extremely limited so far and thus, it is still significant to develop a new cultivation method. Microscale technologies such as micro-fabrication technologies, micro-engineered mechanical systems (MEMS) methods, are promising to revolutionize microbial cultivation.

In this talk, several challenges for application of microscale technologies to microbial cultivation will be presented as follows.

1) Microbial-trap device A new cultivation device has been developed based on new concept exploiting nano- and micro-fabrication technology. The device enables isolating pure culture from heterogeneous populations, performing several steps necessary to cultivate pure culture automatically. This device consists of multiple chambers for growth filled with medium, and a narrow channel (submicron size width and height) connecting to outside environment. The first cell attracted by leaked medium fits inside the constriction, begins to divide, blocks the entrance to the food chamber, and prevents other cells from entering. The cell can continue to grow and divide, thus populating the growth chamber with a single species.

2) Illuminating growth controlling network in nature A new microbial cultivation platform has been developed. This method provides extremely high inoculum cell density (>10^7 cells/ml) but keep individuality of each growth unit (single cells and micro-colonies). Hydrogel particles (10-30 μm in diameter) entrapping single cells with medium softly aggregated in oil (GMD-oil cultivation) is the key structure of this method. As a result of comparative experiment on colony formation using environmmental samples, the cultivation efficiency showed approximately 10 times higher than the condition without microbial interactions. This suggested that the enhancing microbial interactions facilitate microbial resuscitation from dormancy. Accordingly, inter-species microbial interactions are significant factor for growth triggering in a microbial community. This suggests that microbial network controls growth of specific microbial type in nature, and it might be a major reason for microbial unculturability.

S2-3

Probing phytoplankton-bacteria interactions with *in situ* microtechnology

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Marine microorganisms frequently inhabit environments that are heterogeneous down to the microscale and have evolved strategies to cope with resource patchiness. However, because of the difficulty of *in situ* measurements at appropriately small spatio-temporal scales, our understanding of these strategies is largely derived from laboratory-based experiments. Here we show how recent progress in the development of *in situ* microfluidic techniques and low input sequencing enables fine-scale examination of microbial interactions at scales relevant to individual microorganisms. The *In Situ* Chemotaxis Assay (ISCA) and the Millifluidic *In Situ* Enrichment (MISE) are a set of instruments designed to mimic microscale nutrient hotspots. Via an automated system, MISE wells, containing natural communities from the deployment site, are amended with a chemical treatment and injected with RNA preservative at predetermined time points. With this design the MISE enables interrogation of whole-community, rapid transcriptional responses to resource encounter. The ISCA is a deployable microdevice that, upon deployment, generates chemical microplumes, which microbes can respond to by swimming into the device. Leveraging recent advances in low input metagenomics it is then possible to examine the identity and functional capacity of responsive microbes. Combining chemical profiling techniques with ISCA and MISE deployments containing phytoplankton extracts and key osmolytes we are beginning to assemble a better understanding of phycosphere interactions. Here we present method validation experiments and preliminary field results, which demonstrate the power of this instrument suite to delve into the mechanisms underlying phytoplankton-bacteria interactions.

S2-4

Integrating microfluidics, robotics and noninvasive cell analysis tehcnologies: Toward controlled ecophysiological studies at single-cell resolution

マイクロ流体テクノロジー、ロボティクス、非破壊細胞分析の統合：１細胞解像度のライブ生態学に向けて

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The advent of new microscale technologies such as microfluidics and most recently microfluidic robotics offers unprecedented opportunities to manipulate environmental conditions over length scales relevant to bacterial transport and on time scales short enough to resolve bacterial responses to rapid shift in a surrounding condition. These approaches are enabling controlled ecological studies of environmental bacteria that account for fundamental characteristics of natural microbial habitats, and are shedding light on the ecological principals underpinning the dynamic microbial consortiums that are the fundamental driver of global element cycles. On the other hand,　emerging noninvasive technologies are revolutionizing our notion of cell analysis, by allowing a microbiologist to interogate live and intac cell about its physiological status and taxonomy without affecting cellular physiology and integrity. Here, I argue the great potential of combining novel microscale technologies and noninvasive analysis technologies to expand the scope of controlled ecological studies of live and intact environmental microbes, to bring about new insights into the complex microbial life in the nature.

S3-1

Considering plant-microbe interactions at the cellular level ~a journey to understand a thousand microbes begins with a single microbe~

植物ー細菌相互作用を細胞レベルで考える　～千種の微生物も一種から～

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植物とその周辺微生物は様々な相互作用をしている。それら相互作用の分子機構に関して理解が進んでいる例として、植物と病原菌や根粒菌との相互作用が挙げられる。例えば、植物―病原菌相互作用においては、遺伝学や生化学を中心とした数多くの解析により、病原体認識から防御応答に至るおおよその分子機構が理解されつつある。その結果、病原体分子パターンを認識して誘導されるパターン誘導免疫（PTI）や、病原体エフェクターを認識することで誘導されるエフェクター誘導免疫（ETI）といった概念が生まれ、それらに必要な植物ホルモンであるサリチル酸（SA）の重要性が広く認知されるようになってきた。しかし､本来､感染は局所的に起きる現象であり、植物免疫も時空間的に制御されているはずであるが、その分子機構はほとんどわかっていない。そこで私は、プロモーターレポーター植物とライブイメージングを用いて、植物防御応答の時空間的動態を可視化することで植物免疫システムの全貌に迫ろうとしている。この系を用いて、ETI時には、SAと相互拮抗関係にあるジャスモン酸（JA）シグナル系が感染部位の外側細胞群で活性化することで、感染細胞周辺でのみSAが高蓄積し、感染部位での細胞死領域とそれをリング状に取り囲むSA依存性防御領域が形成されることを見出した。ETI誘導時に感染局所で生じる同心円状のSA/JA活性二層構造からなる免疫反応の場は、空間的SA-JA拮抗作用によるSA防御応答の限定に加え、細胞死領域に誘引される腐生菌や虫害などの二次被害に対してJA層が防御しているとも考えられ、非常に機能的な構造であるとも言える。

私のグループでは現在、このSA/JA二層構造の形成機構とその意義に迫るだけでなく、病原細菌を可視化することで植物－病原細菌相互作用における両者の時空間的な細胞応答動態を１細胞レベルで理解することを目指している。病原体の可視化技術は、そもそも分子レベルでの知見に比べて貧弱な植物病原細菌の感染挙動に関する基礎的な理解を深めるためにも非常に有用である。細菌が植物のどこでどのように定着・局在し、それに対し、植物組織が感染部位でどのような応答をするのかを正しく理解することは、微生物叢網羅解析の先においても私たち植物―微生物相互作用研究者がいつかは相対する重要課題の一つではないだろうか。我々の可視化による相互作用研究を紹介して議論したい。

S3-2

An approach to stipulate the rhizosphere: analysis of the dynamics of isoflavones in soybean rhizosphere

代謝物の動態解析から根圏を規定できるか？ ~ ダイズ根圏でのイソフラボンを例として ~

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Rhizosphere is small region around the roots, defined as the area affected by plant roots, where plants and millions of microbes have various interactions. Recent evidence supports that rhizosphere microbes have intense activity and are important for plant health and growth, and for development of sustainable agriculture. For example, microbes called plant-growth-promoting rhizobacteria affect plant growth either directly or indirectly, and mycorrhiza and rhizobia provide phosphorous and nitrogen, respectively. It has been shown that plants accommodate rhizosphere microbes by providing platform and nutrients. Around 10-40 % of photosynthates are shown to be secreted into the rhizosphere. These metabolites include sugars, aminoacids, mucigel and plants specialized metabolites (secondary metabolite). Plant specialized metabolites have functions in rhizosphere interaction among adaptation to both biotic and abiotic stresses; for example, symbiosis with bacteria and fungi, inhibition of the growth of neighbor plants, and inhibition of pathogen. Despite of the accumulation evidence that plant specialized metabolites are important in the interaction between plants and microbes in the rhizosphere, there still remain largely unknown at the molecular level how these metabolite functions in the rhizosphere. The aim of our research is to elucidate the dynamics and function of plant specialized metabolites in the rhizosphere in order to obtain the clear picture of the rhizosphere. We used isoflavone as a model to analyze the rhizosphere dynamics. Isoflavones are a class of flavonoid predominantly found in legume plants, and act as a signal molecule to induce the expression of nod genes of rhizobia. It has also recently be shown that isoflavones modulate the rhizosphere microbial communities. We analyzed the secretion of isoflavones into the rhizosphere both in hydroponic culture and in field condition, and found that isoflavone secretion is higher at the vegetative stages than at the reproductive stages. The dynamics of isoflavones were simulated via the analysis of isoflavone decomposition and distribution and then validated using the rhizobox. In addition, changes of bacterial communities were analyzed under artificial rhizosphere condition. An approach to stipulate the rhizosphere will be discussed.

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S3-3

Pathogenic R-body-production in legume symbiont *Azorhizobium caulinodans*

セスバニア根粒菌の宿主殺傷能　ー巨大構造体 R-body を生産する意義ー

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R-bodies are insoluble large polymers consisting of small proteins encoded by *reb* genes and are coiled into cylindrical structures in bacterial cells. They were first observed in *Caedibacter* species, which are obligate bacterial endosymbionts of paramecia. The paramecia that harbor the R-body-producing *Caedibacter* cells release the bacterial cells through their cytopyge. Subsequently, the paramecia without the endosymbiont ingest the released bacteria and die. This phenomenon is called the 'killer trait' of paramecia. Besides *Caedibacter* species, R-bodies have also been observed in a few free-living bacteria, but the significance of R-body production in these bacteria is still unknown. Recent advances in genome sequencing technologies revealed that many Gram-negative bacteria possess *reb* genes, and interestingly, many of them are animal and plant pathogens.

Azorhizobium caulinodans ORS571 is a mutualistic microsymbiont of a tropical legume, *Sesbania rostrata*, which forms nitrogen-fixing nodules on the stems and roots. A series of studies of ours in recent years revealed that *A. caulinodans* could potentially kill the host plant cells by producing R-bodies. This bacterium carries a *reb* operon containing four *reb* genes. The expression of the *reb* operon is usually suppressed by some transcription factors such as PraR, both in free-living and symbiotic states. *A. caulinodans* mutants highly expressing *reb* operon, such as a *praR* mutant, are toxic to the plant host cells because of its R-body production. In the stem nodules formed by these mutants, two distinct abnormal interaction patterns between the bacteria and the host cells are observed. The plant host cells kill the bacteria in the first interaction, while the bacteria kill the plant host cells in the second interaction. These observations suggest that pathogenicity conferred by an R-body might be universal in bacteria possessing *reb* genes, not only in *Caedibacter* species. Furthermore, we provide the first insight into the molecular mechanism underlying the expression of R-body production in response to environmental factors. The *reb* operon is highly expressed at low temperatures and that 2-oxoglutarate induces the expression of the *reb* operon by inhibiting PraR binding to the *reb* promoter.

Unlike obligate endosymbionts of paramecia, *A. caulinodans* can be cultured in vitro and genetic manipulation techniques have been established in this bacterium, warranting further use of *A. caulinodans* as a model for studies of R-body/*reb* genes.

S3-4

Transmission of plant-associated microbes through soil and seed

土壌を介した植物共生微生物の伝播 ～共生・病原微生物のソースは土壌か種子か～

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マイクロバイオーム研究により、生物の様々な器官で微生物が共生していることが明らかになった。植物においても、これまでよく知られていた根粒菌や菌根菌等の有用微生物、または病原微生物以外にも様々な微生物種が共生し、それらが植物の生育や病害虫抵抗性などの表現形質に影響することが分かってきた。しかしながら、これらの共生微生物がいつ、どこから宿主植物に感染し、ライフサイクルを循環させているのかは不明な点が多い。一般的には土壌中の微生物のうち、植物に共生関係を許される微生物種が種子から発芽した根を通して感染し、共生微生物叢を形成していくと考えられているようである。一方、植物の種子には既に特定の微生物種が包括されており、植物の世代を越えて垂直的に共生微生物が伝搬される可能性もあげられている。本トピックでは国内外の研究事例とともに植物の共生微生物叢構築における土壌と種子の関わりについて話し合いたい。

S3-5

Plant microbiome: Can we find key interactions between plant and microbe from holobiome?

植物マイクロバイオーム研究：ホロビオームから鍵となる新規相互作用や微生物を見出せるか

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Plant environments provide a diversity of ecological niches for microorganisms including rhizobia, plant growth-promoting microbes, vast neutral microbes and pathogens. Among them, rhizobia have been extensively studied for their dynamics in genome structures, polyphasic interactions with host plants, and biogeochemical functions as representative plant-associated microbes. Thus, I would like to introduce our two topics of rhizobia including nitrogen transformation in legume rhizosphere and strain-specific nodulation restrictions (incompatibility between host legume and rhizobia and subsequent genome dynamics in rhizobia), which indicate the dynamic evolution of rhizobial genomes with host plants and environments. Plant microbiomes is recently paid the most attention to address the community-level functions. Our microbiome studies on paddy rice root revealed CH4 oxidation by methanotrophs is a driving force in shaping bacterial communities in rice roots grown in CH4-rich environments. A hypothesis was proposed for the interplay between rice plant genes, root microbiomes, and their biogeochemical functions (Minamisawa et al. 2016). New experimental approaches have been recently developed for plant microbiome research: synthetic engineering approach to plant microbial communities in gnotobiotic systems (Bai et al. 2015) and informatics approach to identification of “core microbes” (Toju et al. 2018). Integration of these new approaches with conventional techniques and knowledge may provide new insights of plant microbiomes and their application to agriculture. For such integrations, I want to propose several approaches: (1) determinations and utilization of plant microbiome to maximize specific functions such as nitrogen fixation, seed production and pathogen protection; (2) Transplantation of plant microbiomes with phenotype changes. For example, the functional expression of nitrogen fixation in bacteria generally requires optimum (low) oxygen concentrations and appropriate electron donors (Yoneyama et al. 2018), which may be generated by transient oxygen respiration and carbon metabolism in plant microbiome including non-diazotrophs along with plant growth stages. If so, plant microbiomes and plant genotypes are important cues for the expression of nitrogen fixation in key-diazotrophic bacteria in plants. The failure in simple inoculation experiments of single diaoztroph to plant seedlings would suggest the significance to evaluate plant microbiome to support the expression of nitrogen fixation in key-diazotrophic bacteria in different stages of the target plant. Thereafter, the knowledge could lead the idea for inoculation of plant microbiomes to maximize nitrogen fixation towards agricultural productions.