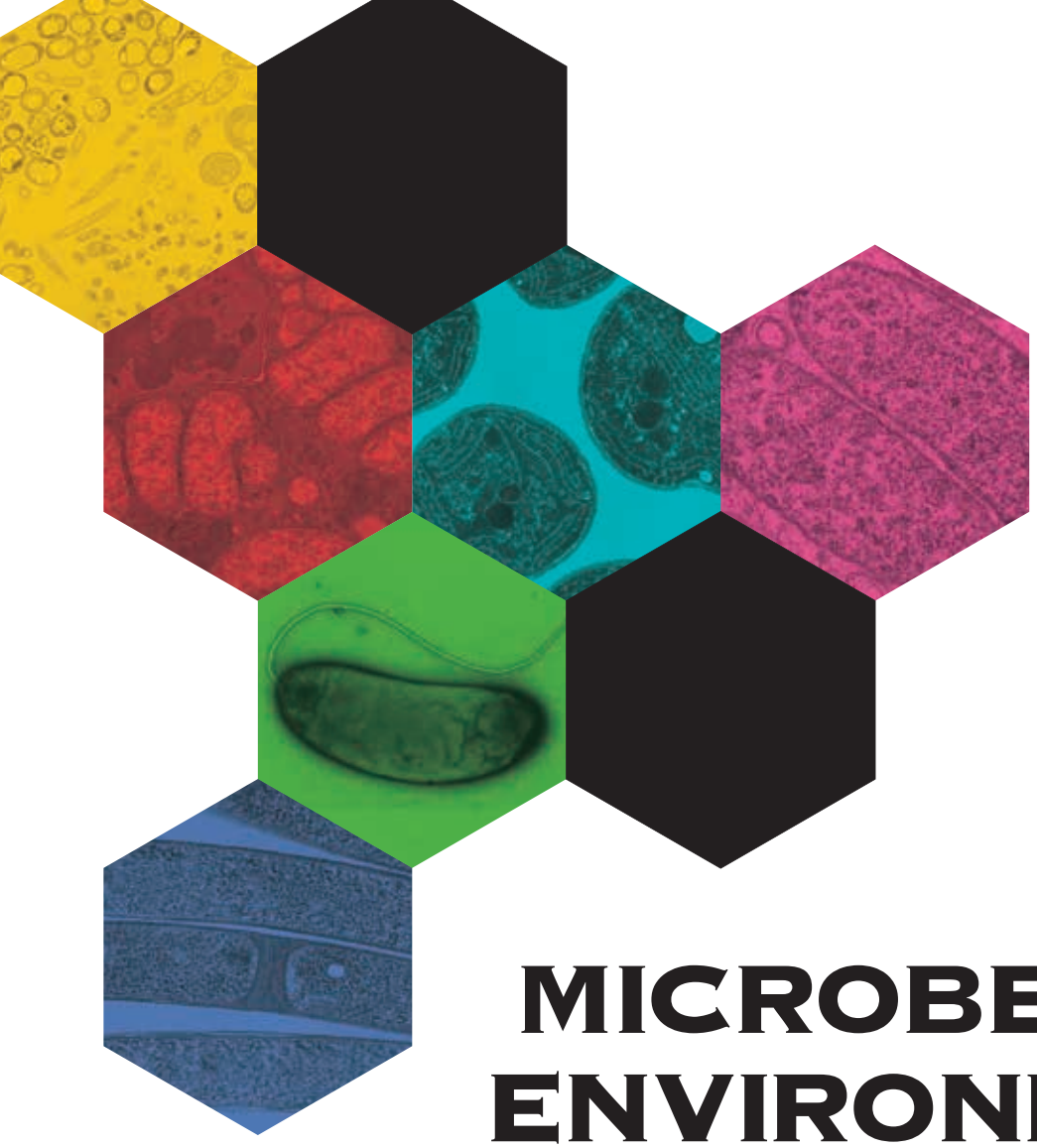
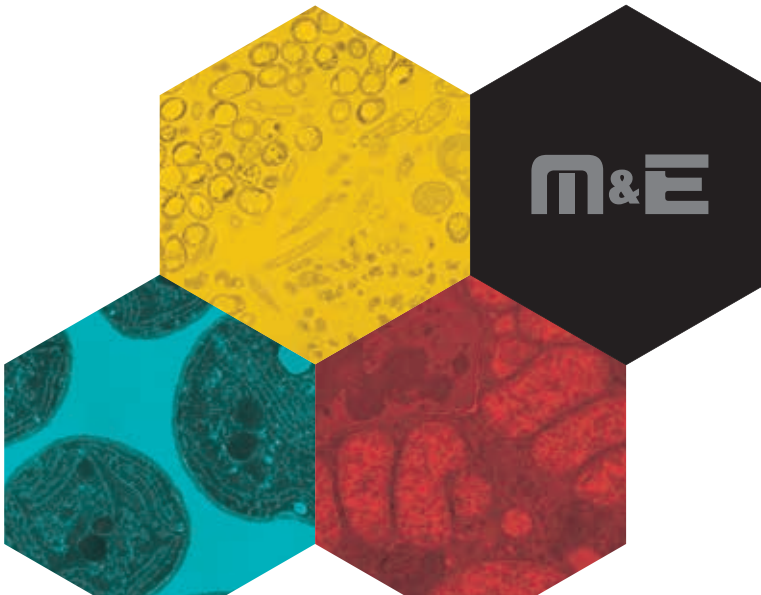


MICROBES AND ENVIRONMENTS

DIGEST



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August 2014

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Research Articles (Full-length Regular Papers and Short Communications)

Research articles can deal with cultured and non-cultured forms of any type of microorganism: Bacteria, archaea, fungi, yeasts, protozoa, algae and viruses. Topics including ecology of microorganisms in natural and engineered environments, microbial interactions and interrelations with other organisms (symbiosis and pathogenesis for plants, animals, and human), public health, pollution microbiology and microbial biocontrol are encouraged for publication. Articles on microbial physiology, molecular genetics, microbial genomics, microbial biotechnology, food microbiology, microbial systematics and microbiological methods are also considered for publication if they have an ecological perspective.

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- **Space Habitation and Microbiology: Status and Roadmap of Space Agencies** (in Vol.29, No.3)
- **A Novel Function of Controlled-Release Nitrogen Fertilizers** (in Vol.28, No.2)
- **Microbial Ecology Pushes Frontiers in Biotechnology** (in Vol.29, No.1)
- **Recent Trends in Microbial Inoculants in Agriculture** (in Vol.28, No.4)
- **Rediscovery of the Microbial World in Microbial Ecology** (in Vol.28, No.3)
- **Cultivating Yet-to-be Cultivated Microbes: The Challenge Continues** (in Vol.28, No.2)
- **Utilization of Phytic Acid by Cooperative Interaction in Rhizosphere** (in Vol.28, No.1)

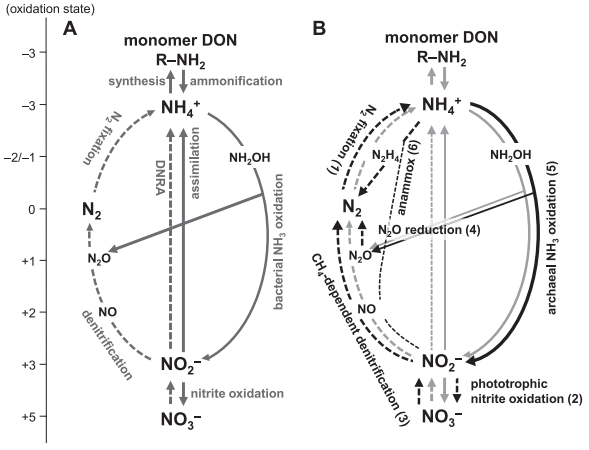
Signature Reviews

Microbes Environ. Vol. 29, No. 1, 4–16, 2014

Ecological Perspectives on Microbes Involved in N-Cycling

Kazuo Isobe, Nobuhito Ohte
The University of Tokyo

Nitrogen (N) cycles have been directly linked to the functional stability of ecosystems because N is an essential element for life. Furthermore, the supply of N to organisms regulates primary productivity in many natural ecosystems. Microbial communities have been shown to significantly contribute to N cycles because many N-cycling processes are microbially mediated. Only particular groups of microbes were implicated in N-cycling processes, such as nitrogen fixation, nitrification, and denitrification, until a few decades ago. However, recent advances in high-throughput sequencing technologies and sophisticated isolation techniques have enabled microbiologists to discover that N-cycling microbes are unexpectedly diverse in their functions and phylogenies. Therefore, elucidating the link between biogeochemical N-cycling processes and microbial community dynamics can provide a more mechanistic understanding of N cycles than the direct observation of N dynamics. In this review, we summarized recent findings that characterized the microbes governing novel N-cycling processes. We also discussed the ecological role of N-cycling microbial community dynamics, which is essential for advancing our understanding of the functional stability of ecosystems.

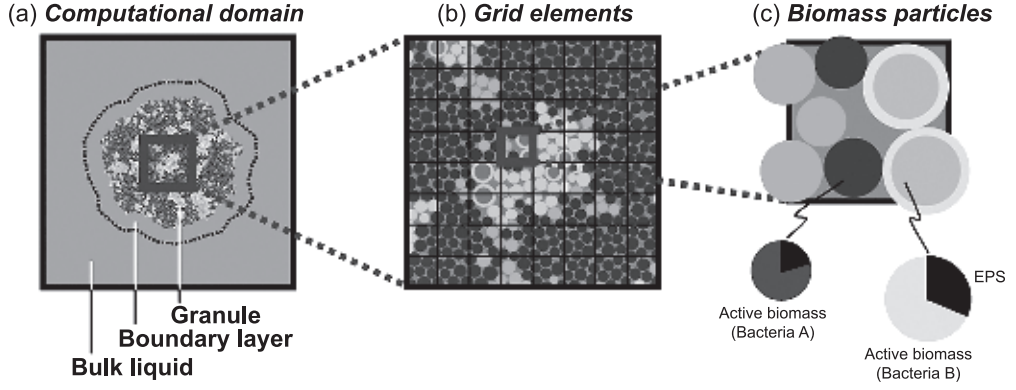


Microbes Environ. Vol. 28, No. 3, 285–294, 2013

Challenges for Complex Microbial Ecosystems: Combination of Experimental Approaches with Mathematical Modeling

Shin Haruta[1], Takehito Yoshida[2], Yoshiteru Aoi[3, 4], Kunihiko Kaneko[5], Hiroyuki Futamata[6]
1 Tokyo Metropolitan University; 2 University of Tokyo; 3 Hiroshima University; 4 Northeastern University; 5 University of Tokyo; 6 Shizuoka University

In the past couple of decades, molecular ecological techniques have been developed to elucidate microbial diversity and distribution in microbial ecosystems. Currently, modern techniques, represented by meta-omics and single cell observations, are revealing the incredible complexity of microbial ecosystems and the large degree of phenotypic variation. These studies propound that microbiological techniques are insufficient to untangle the complex microbial network. This minireview introduces the application of advanced mathematical approaches in combination with microbiological experiments to microbial ecological studies. These combinational approaches have successfully elucidated novel microbial behaviors that had not been recognized previously. Furthermore, the theoretical perspective also provides an understanding of the plasticity, robustness and stability of complex microbial ecosystems in nature.

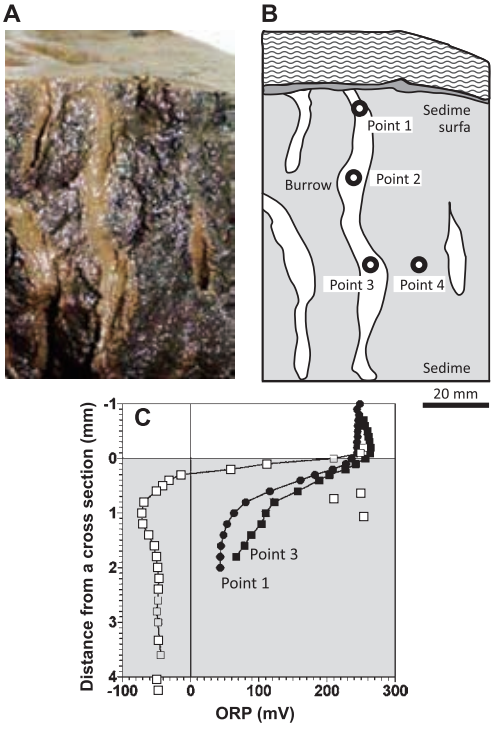


Microbes Environ. Vol. 28, No. 2, 166–179, 2013

Spatial and Temporal Oxygen Dynamics in Macrofaunal Burrows in Sediments: A Review of Analytical Tools and Observational Evidence

Hisashi Satoh, Satoshi Okabe
Hokkaido University

The availability of benthic O_2 plays a crucial role in benthic microbial communities and regulates many important biogeochemical processes. Burrowing activities of macrobenthos in the sediment significantly affect O_2 distribution and its spatial and temporal dynamics in burrows, followed by alterations of sediment microbiology. Consequently, numerous research groups have investigated O_2 dynamics in macrofaunal burrows. The introduction of powerful tools, such as microsensors and planar optodes, to sediment analysis has greatly enhanced our ability to measure O_2 dynamics in burrows at high spatial and temporal resolution with minimal disturbance of the physical structure of the sediment. In this review, we summarize recent studies of O_2-concentration measurements in burrows with O_2 microsensors and O_2 planar optodes. This manuscript mainly focuses on the fundamentals of O_2 microsensors and O_2 planar optodes, and their application in the direct measurement of the spatial and temporal dynamics of O_2 concentrations in burrows, which have not previously been reviewed, and will be a useful supplement to recent literature reviews on O_2 dynamics in macrofaunal burrows.

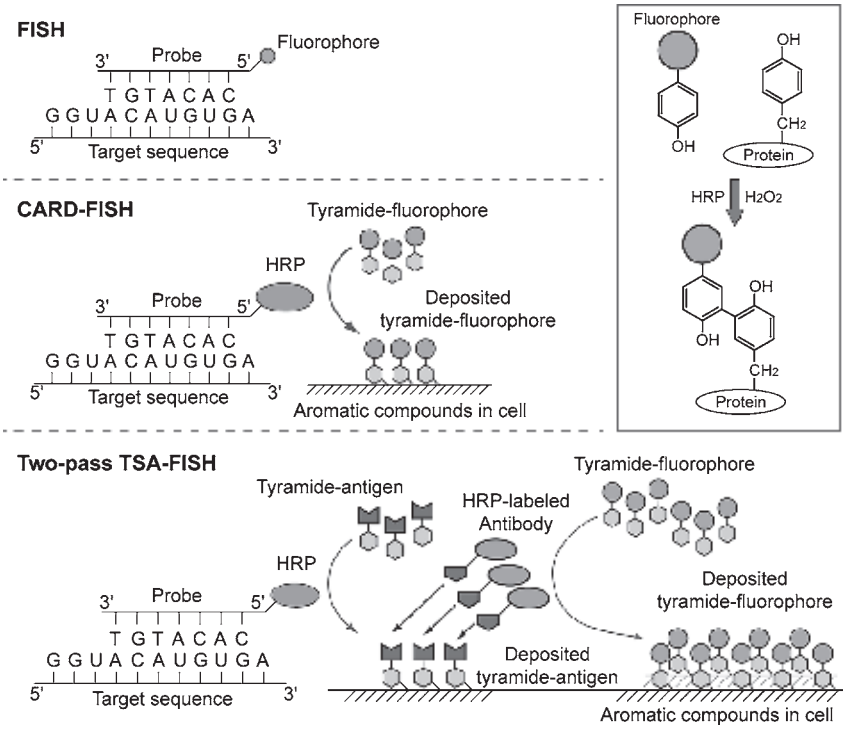


Microbes Environ. Vol. 28, No. 1, 3–12, 2013

CARD-FISH for Environmental Microorganisms: Technical Advancement and Future Applications

Kengo Kubota
Tohoku University

Fluorescence *in situ* hybridization (FISH) has become a standard technique in environmental microbiology. More than 20 years have passed since this technique was first described, and it is currently used for the detection of ribosomal RNA, messenger RNA, and functional genes encoded on chromosomes. This review focuses on the advancement and applications of FISH combined with catalyzed reporter deposition (CARD, also known as tyramide signal amplification or TSA), in the detection of environmental microorganisms. Significant methodological improvements have been made in CARD-FISH technology, including its combination with other techniques and instruments.

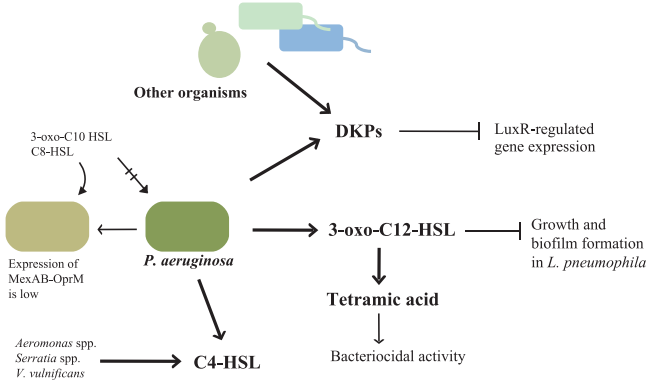


Microbes Environ. Vol. 28, No. 1, 13–24, 2013

Interspecies Interaction between *Pseudomonas aeruginosa* and Other Microorganisms

Yosuke Tashiro[1,†], Yutaka Yawata[2], Masanori Toyofuku[3], Hiroo Uchiyama[3], Nobuhiko Nomura[3]
1 Hokkaido University; 2 Massachusetts Institute of Technology; 3 University of Tsukuba; † Shizuoka University

Microbes interact with each other in multicellular communities and this interaction enables certain microorganisms to survive in various environments. *Pseudomonas aeruginosa* is a highly adaptable bacterium that ubiquitously inhabits diverse environments including soil, marine habitats, plants and animals. Behind this adaptivity, *P. aeruginosa* has abilities not only to outcompete others but also to communicate with each other to develop a multispecies community. In this review, we focus on how *P. aeruginosa* interacts with other microorganisms. *P. aeruginosa* secretes antimicrobial chemicals to compete and signal molecules to cooperate with other organisms. In other cases, it directly conveys antimicrobial enzymes to other bacteria using the Type VI secretion system (T6SS) or membrane vesicles (MVs). Quorum sensing is a central regulatory system used to exert their ability including antimicrobial effects and cooperation with other microbes. At least three quorum sensing systems are found in *P. aeruginosa*, Las, Rhl and *Pseudomonas* quinolone signal (PQS) systems. These quorum-sensing systems control the synthesis of extracellular antimicrobial chemicals as well as interaction with other organisms via T6SS or MVs. In addition, we explain the potential of microbial interaction analysis using several micro devices, which would bring fresh sensitivity to the study of interspecies interaction between *P. aeruginosa* and other organisms.

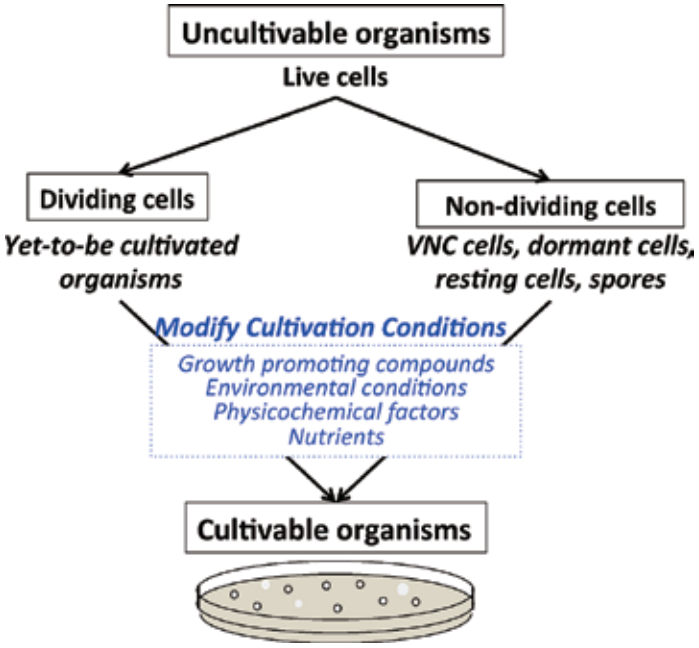


Microbes Environ. Vol. 27, No. 4, 356–366, 2012

Are Uncultivated Bacteria Really Uncultivable?

Indun Dewi Puspita[1], Yoichi Kamagata[1,2], Michiko Tanaka[1], Kozo Asano[1], Cindy H. Nakatsu[1,3]
1 Hokkaido University; 2 National Institute of Advanced Industrial Science and Technology (AIST);3 Purdue University

Many strategies have been used to increase the number of bacterial cells that can be grown from environmental samples but cultivation efficiency remains a challenge for microbial ecologists. The difficulty of cultivating a fraction of bacteria in environmental samples can be classified into two non-exclusive categories. Bacterial taxa with no cultivated representatives for which appropriate laboratory conditions necessary for growth are yet to be identified. The other class is cells in a non-dividing state (also known as dormant or viable but not culturable cells) that require the removal or addition of certain factors to re-initiate growth. A number of strategies, from simple to high throughput techniques, are reviewed that have been used to increase the cultivation efficiency of environmental samples. Some of the underlying mechanisms that contribute to the success of these cultivation strategies are described. Overall this review emphasizes the need of researchers to first understand the factors that are hindering cultivation to identify the best strategies to improve cultivation efficiency.

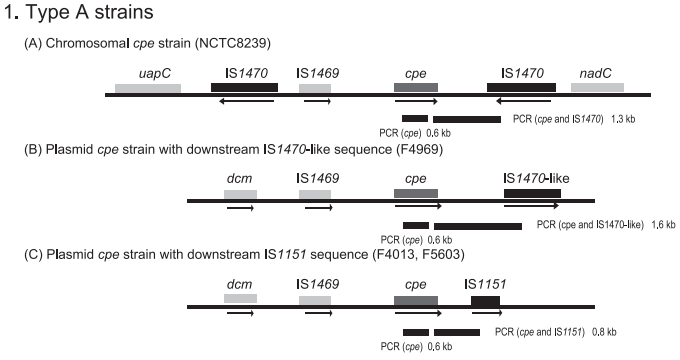


Microbes Environ. Vol. 27, No. 4, 343–349, 2012

Enterotoxigenic *Clostridium perfringens*: Detection and Identification

Kazuaki Miyamoto[1], Jihong Li[2], Bruce A. McClane[2]
1 Wakayama Medical University School of Medicine; 2 University of Pittsburgh School of Medicine

Recent advances in understanding the genetics of enterotoxigenic *Clostridium perfringens*, including whole genome sequencing of a chromosomal *cpe* strain and sequencing of several *cpe*-carrying large plasmids, have led to the development of molecular approaches to more precisely investigate isolates involved in human gastrointestinal diseases and isolates present in the environment. Sequence-based PCR genotyping of the *cpe* locus (*cpe* genotyping PCR assays) has provided new information about *cpe*-positive type A *C. perfringens* including: 1) Foodborne *C. perfringens* outbreaks can be caused not only by chromosomal *cpe* type A strains with extremely heat-resistant spores, but also less commonly by less heat-resistant spore-forming plasmid *cpe* type A strains; 2) Both chromosomal *cpe* and plasmid *cpe C. perfringens* type A strains can be found in retail foods, healthy human feces and the environment, such as in sewage; 3) Most environmental *cpe*-positive *C. perfringens* type A strains carry their *cpe* gene on plasmids. Moreover, recent studies indicated that the *cpe* loci of type C, D, and E strains differ from the *cpe* loci of type A strains and from the *cpe* loci of each other, indicating that the *cpe* loci of *C. perfringens* have remarkable diversity. Multi-locus sequence typing (MLST) indicated that the chromosomal *cpe* strains responsible for most food poisoning cases have distinct genetic characteristics that provide unique biological properties, such as the formation of highly heat-resistant spores. These and future advances should help elucidate the epidemiology of enterotoxigenic *C. perfringens* and also contribute to the prevention of *C. perfringens* food poisoning outbreaks and other CPE-associated human diseases.

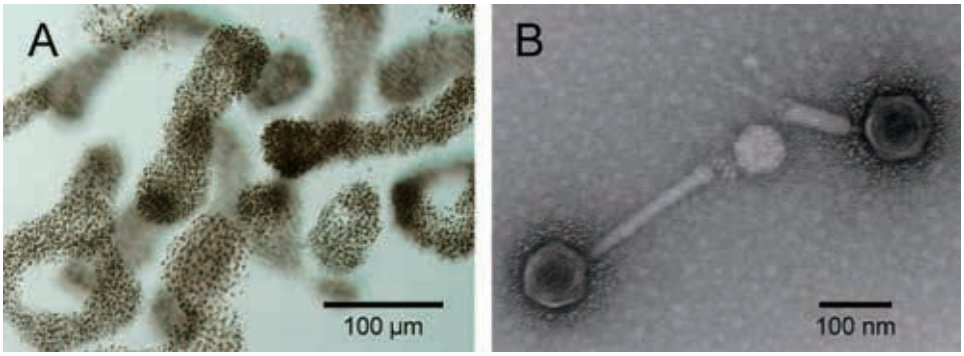


Microbes Environ. Vol. 27, No. 4, 350–355, 2012

Cyanophage Infection in the Bloom-Forming Cyanobacteria *Microcystis aeruginosa* in Surface Freshwater

Yukari Yoshida-Takashima[1], Mitsuhiro Yoshida[1], Hiroyuki Ogata[2], Keizo Nagasaki[3], Shingo Hiroishi[4], Takashi Yoshida[5]
1 Japan Agency for Marine-Earth Science and Technology (JAMSTEC); 2 Mediterranean Institute of Microbiology; 3 Fisheries Research Agency; 4 Fukui Prefectural University; 5 Graduate Kyoto University

Host-like genes are often found in viral genomes. To date, multiple host-like genes involved in photosynthesis and the pentose phosphate pathway have been found in phages of marine cyanobacteria *Synechococcus* and *Prochlorococcus*. These gene products are predicted to redirect host metabolism to deoxynucleotide biosynthesis for phage replication while maintaining photosynthesis. A cyanophage, Ma-LMM01, infecting the toxic cyanobacterium *Microcystis aeruginosa*, was isolated from a eutrophic freshwater lake and assigned as a member of a new lineage of the *Myoviridae* family. The genome encodes a host-like NblA. Cyanobacterial NblA is known to be involved in the degradation of the major light harvesting complex, the phycobilisomes. Ma-LMM01 *nblA* gene showed an early expression pattern and was highly transcribed during phage infection. We speculate that the co-option of *nblA* into *Microcystis* phages provides a significant fitness advantage to phages by preventing photoinhibition during infection and possibly represents an important part of the co-evolutionary interactions between cyanobacteria and their phages.



Microbes Environ. Vol. 27, No. 2, 111–121, 2012

Extraction of Bacterial RNA from Soil: Challenges and Solutions

Yong Wang, Masahito Hayatsu, Takeshi Fujii
Environmental Biofunction Division, National Institute for Agro-Environmental Sciences

Detection of bacterial gene expression in soil emerged in the early 1990s and provided information on bacterial responses in their original soil environments. As a key procedure in the detection, extraction of bacterial RNA from soil has attracted much interest, and many methods of soil RNA extraction have been reported in the past 20 years. In addition to various RT-PCR-based technologies, new technologies for gene expression analysis, such as microarrays and high-throughput sequencing technologies, have recently been applied to examine bacterial gene expression in soil. These technologies are driving improvements in RNA extraction protocols. In this mini-review, progress in the extraction of bacterial RNA from soil is summarized with emphasis on the major difficulties in the development of methodologies and corresponding strategies to overcome them.

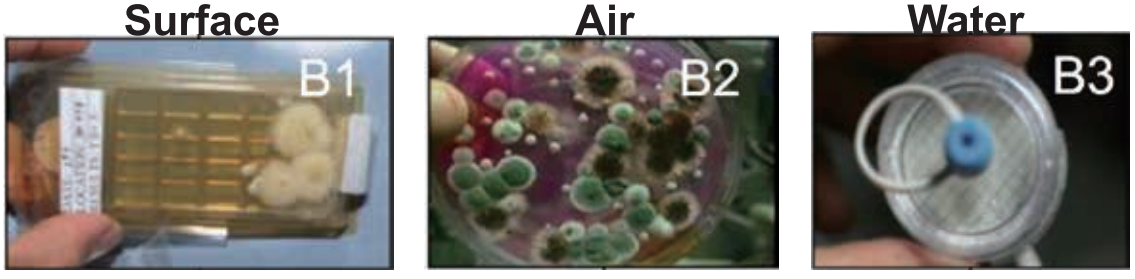
Kit	Manufacturer	Soil for processing	Lysis	Purification	Principle of purification
E.Z.N.A. Soil RNA Kit	Omega Bio-Tek (Norcross, GA, USA)	2 g	Bead beating	Single spin column	Adsorption
FastRNA Pro Soil-Direct Kit	MP-Biomedicals (Q-Biogene) (Solon, OH, USA)	0.5 g	Bead beating	Binding matrix	Adsorption
ISOIL for RNA	NIPPON GENE (Tokyo, Japan)	0.5 g	Bead beating	Precipitation	Information not publicly available
IT 1-2-3 Platinum PathTM Sample Purification kit	Idaho Technology (Salt Lake City, UT, USA)	0.5 g	Bead beating	Magnetic beads	Information not publicly available
RNA PowerSoil Total RNA Isolation Kit	MO BIO (Carlsbad, CA, USA)	2 g	Bead beating	Single gravity flow column	Adsorption
Soil Total RNA Purification Kit	Norgen (Thorold, ON, Canada)	0.5 g	Bead beating	Single spin column	Adsorption
ZR Soil/Fecal RNA MicroPrep	Zymo Research (Orange, CA, USA)	0.25 g	Bead beating	Multiple spin columns	Adsorption/gel filtration

Microbes Environ. Vol. 29, No. 3, in press, 2014

Microbial Monitoring of Crewed Habitats in Space -Current Status and Future Perspectives-

Nobuyasu Yamaguchi[1], Michael Roberts[2], Sarah Castro[3], Cherie Oubre[4], Koichi Makimura[5], Natalie Lays[6], Elisabeth Grohmann[7], Takashi Sugita[8], Tomoaki Ichijo[1], and Masao Nasu[1]
1 Osaka University; 2 NASA Kennedy Space Center; 3 NASA Johnson Space Center; 4 Wyle; 5 Teikyo University; 6 Belgian Nuclear Research Center SCK-CEN; 7 University Medical Centre Freiburg; 8 Meiji Pharmaceutical University

Previous space research conducted during short-term flight experiments and long-term environmental monitoring on board orbiting space stations suggests that the relationship between humans and microbes is altered in the crewed habitat in space. Both human physiology and microbial communities adapt to spaceflight. Microbial monitoring is critical to crew safety in long-duration space habitation and the sustained operation of life support systems on space transit vehicles, space stations, and surface habitats. To address this critical need, space agencies including NASA (National Aeronautics and Space Administration), ESA (European Space Agency), and JAXA (Japan Aerospace Exploration Agency) are working together to develop and implement specific measures to monitor, control, and counteract biological contamination in closed-environment systems. In this review, the current status of microbial monitoring conducted in the International Space Station (ISS) as well as the results of recent microbial spaceflight experiments have been summarized and future perspectives are discussed.

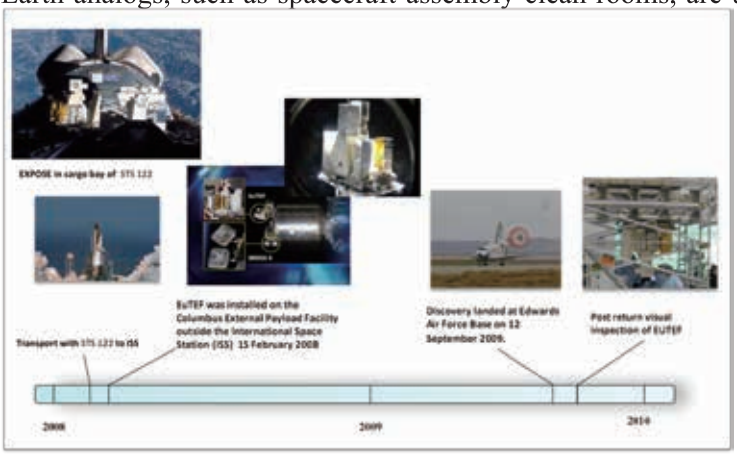


Microbes Environ. Vol. 29, No. 3, in press, 2014

Microbial Existence in Controlled Habitats and Their Resistance to Space Conditions

Kasthuri Venkateswaran[1], Myron T. La Duc[1], and Gerda Horneck[2]
1 California Institute of Technology, Jet Propulsion Laboratory; 2 Institute of Aerospace Medicine, DLR (German Aerospace Center)

The National Research Council (NRC) has recently recognized the International Space Station (ISS) as uniquely suitable for furthering the study of microbial species in closed habitats. Answering the NRC's call for the study, in particular, of uncommon microbial species in the ISS, and/or of those that have significantly increased or decreased in number, space microbiologists have begun capitalizing on the maturity, speed, and cost-effectiveness of molecular/genomic microbiological technologies to elucidate changes in microbial populations in the ISS and other closed habitats. Since investigators can only collect samples infrequently from the ISS itself due to logistical reasons, Earth analogs, such as spacecraft-assembly clean rooms, are used and extensively characterized for the presence of microbes. Microbiologists identify the predominant, problematic, and extremophilic microbial species in these closed habitats and use the ISS as a testbed to study their resistance to extreme extraterrestrial environmental conditions. Investigators monitor the microbes exposed to the real space conditions in order to track their genomic changes in response to the selective pressures present in outer space (external to the spacecraft) and the spaceflight (in the interior of the spacecraft). In this review, we discussed the presence of microbes in space research-related closed habitats and the resistance of some microbial species to the extreme environmental conditions of space.

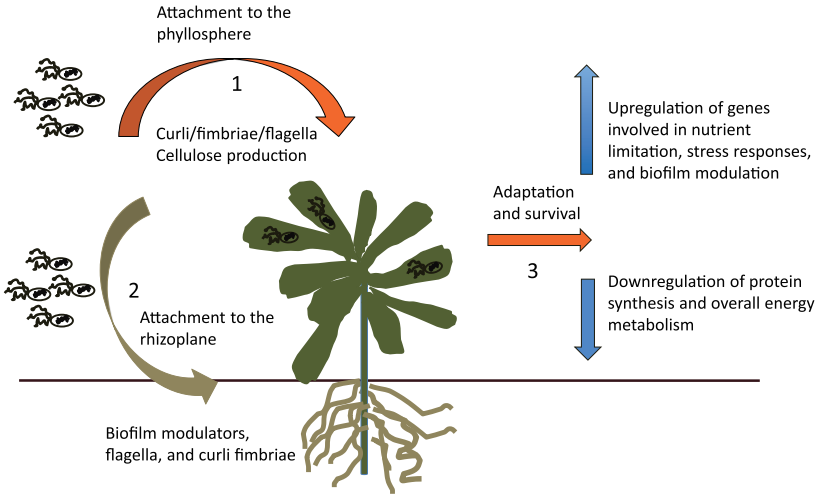


Microbes Environ. Vol. 29, No. 2, 123–135, 2014

Enteric Pathogen-Plant Interactions: Molecular Connections Leading to Colonization and Growth and Implications for Food Safety

Betsy M. Martínez-Vaz[1], Ryan C. Fink[2], Francisco Diez-Gonzalez[2], Michael J. Sadowsky[3, 4]
1 Hamline University; 2) 3) 4) University of Minnesota

Leafy green vegetables have been identified as a source of foodborne illnesses worldwide over the past decade. Human enteric pathogens, such as *Escherichia coli* O157:H7 and *Salmonella*, have been implicated in numerous food poisoning outbreaks associated with the consumption of fresh produce. An understanding of the mechanisms responsible for the establishment of pathogenic bacteria in or on vegetable plants is critical for understanding and ameliorating this problem as well as ensuring the safety of our food supply. While previous studies have described the growth and survival of enteric pathogens in the environment and also the risk factors associated with the contamination of vegetables, the molecular events involved in the colonization of fresh produce by enteric pathogens are just beginning to be elucidated. This review summarizes recent findings on the interactions of several bacterial pathogens with leafy green vegetables. Changes in gene expression linked to the bacterial attachment and colonization of plant structures are discussed in light of their relevance to plant-microbe interactions. We propose a mechanism for the establishment and association of enteric pathogens with plants and discuss potential strategies to address the problem of foodborne illness linked to the consumption of leafy green vegetables.



Signature Research Articles

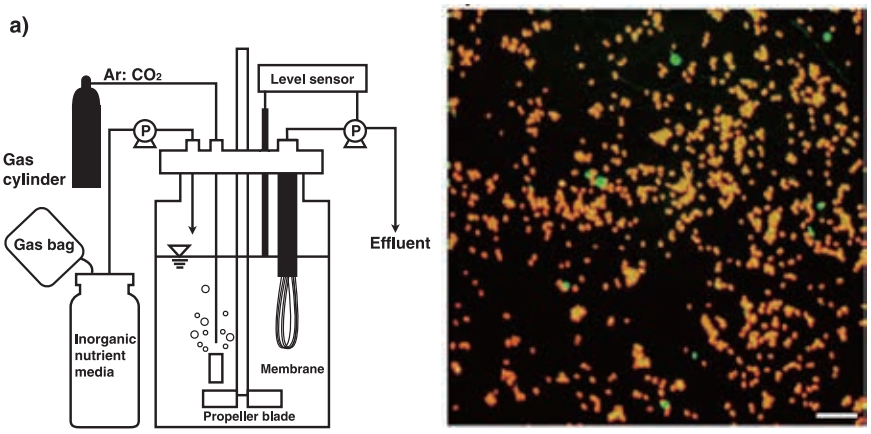
Cultivation / isolation

Microbes Environ. Vol. 28, No. 4, 436–443, 2013

Cultivation of Planktonic Anaerobic Ammonium Oxidation (Anammox) Bacteria Using Membrane Bioreactor

Mamoru Oshiki[1], Takanori Awata[2], Tomonori Kindaichi[2], Hisashi Satoh[1], Satoshi Okabe[1]
1 Hokkaido University; 2 Hiroshima University

Enrichment cultures of anaerobic ammonium oxidation (anammox) bacteria as planktonic cell suspensions are essential for studying their ecophysiology and biochemistry, while their cultivation is still laborious. The present study aimed to cultivate two phylogenetically distinct anammox bacteria, "*Candidatus* Brocadia sinica" and "*Ca*. Scalindua sp." in the form of planktonic cells using membrane bioreactors (MBRs). The MBRs were continuously operated for more than 250 d with nitrogen loading rates of 0.48–1.02 and 0.004–0.09 kgN m^{-3} d^{-1} for "*Ca*. Brocadia sinica" and "*Ca*. Scalindua sp.", respectively. Planktonic anammox bacterial cells were successfully enriched (>90%) in the MBRs, which was confirmed by fluorescence *in-situ* hybridization and 16S rRNA gene sequencing analysis. The decay rate and half-saturation constant for NO_2^- of "*Ca*. Brocadia sinica" were determined to be 0.0029–0.0081 d^{-1} and 0.47 mgN L^{-1}, respectively, using enriched planktonic cells. The present study demonstrated that MBR enables the culture of planktonic anammox bacterial cells, which are suitable for studying their ecophysiology and biochemistry.

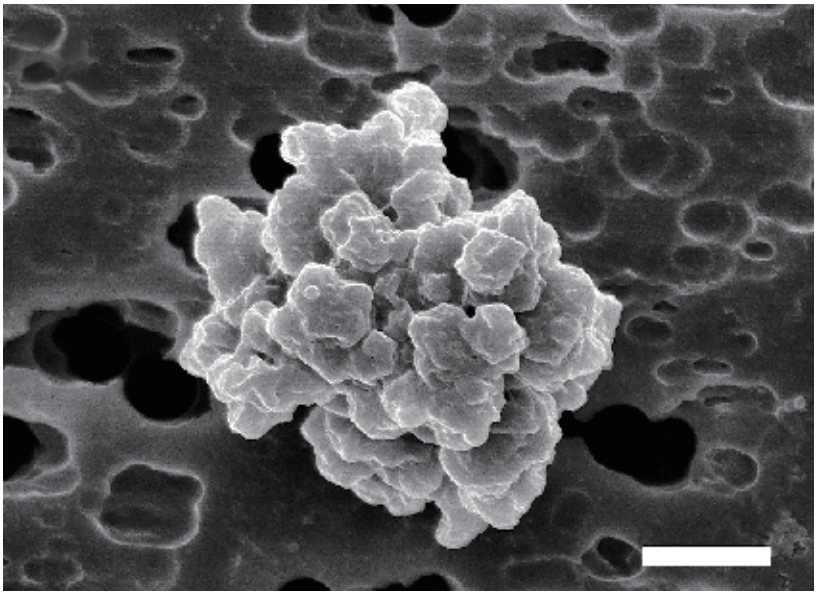


Microbes Environ. Vol. 28, No. 3, 346–353, 2013

Isolation of *Nitrospira* belonging to Sublineage II from a Wastewater Treatment Plant

Norisuke Ushiki[1], Hirotsugu Fujitani[1], Yoshiteru Aoi[2, 3], Satoshi Tsuneda[1]
1 Waseda University; 2 Hiroshima University; 3 Northeastern University

Nitrite oxidation is a key step in nitrogen removal in biological wastewater treatment plants. Recently, two phylogenetically different *Nitrospira* (sublineages I and II) have been recognized as the numerically dominant nitrite-oxidizing bacteria in wastewater treatment plants. However, *Nitrospira* sublineage II inhabiting activated sludge was not isolated and its detailed properties were unclear. In this study, we developed a new method for the isolation of *Nitrospira* forming micro-colonies using a cell sorter. We obtained a novel pure strain "*Nitrospira japonica*" from the activated sludge. Subsequently, phylogenetic and physiological analyses revealed that *Nitrospira japonica* belongs to sublineage II and grew in medium containing formate. This method has the potential to isolate other uncultured microorganisms forming micro-colonies.

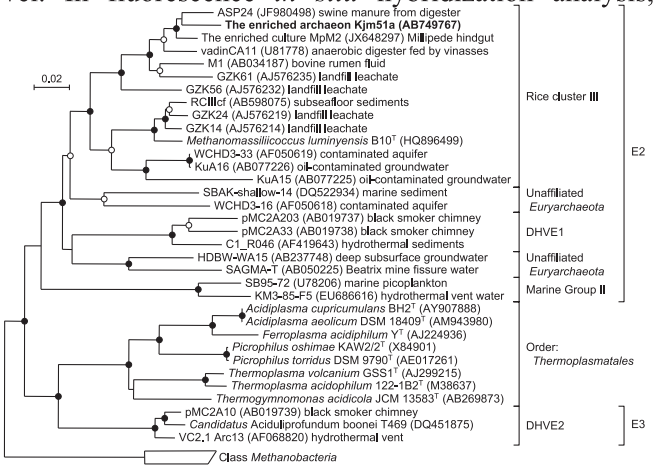


Microbes Environ. Vol. 28, No. 2, 244–250, 2013

Candidatus Methanogranum caenicola: a Novel Methanogen from the Anaerobic Digested Sludge, and Proposal of *Methanomassiliicoccaceae* fam. nov. and *Methanomassiliicoccales* ord. nov., for a Methanogenic Lineage of the Class *Thermoplasmata*

Takao Iino[1], Hideyuki Tamaki[2], Satoshi Tamazawa[2, 3], Yoshiyuki Ueno[4], Moriya Ohkuma[1], Ken-ichiro Suzuki[5], Yasuo Igarashi[6], Shin Haruta[7]
1 RIKEN BioResource Center; 2 National Institute of Advanced Industrial Science and Technology (AIST); 3 University of Tsukuba; 4 Kajima Technical Research Institute; 5 National Institute of Technology and Evaluation (NITE); 6 The University of Tokyo; 7 Tokyo Metropolitan University

The class *Thermoplasmata* harbors huge uncultured archaeal lineages at the order level, so-called Groups E2 and E3. A novel archaeon Kjm51a affiliated with Group E2 was enriched from anaerobic sludge in the present study. Clone library analysis of the archaeal 16S rRNA and mcrA genes confirmed a unique archaeal population in the enrichment culture. The 16S rRNA gene-based phylogeny revealed that the enriched archaeon Kjm51a formed a distinct cluster within Group E2 in the class *Thermoplasmata* together with *Methanomassiliicoccus luminyensis* B10^T and environmental clone sequences derived from anaerobic digesters, bovine rumen, and landfill leachate. Archaeon Kjm51a showed 87.7% 16S rRNA gene sequence identity to the closest cultured species, *M. luminyensis* B10^T, indicating that archaeon Kjm51a might be phylogenetically novel at least at the genus level. In fluorescence *in situ* hybridization analysis, archaeon Kjm51a was observed as coccoid cells completely corresponding to the archaeal cells detected, although bacterial rod cells still coexisted. The growth of archaeon Kjm51a was dependent on the presence of methanol and yeast extract, and hydrogen and methane were produced in the enrichment culture. The addition of 2-bromo ethanesulfonate to the enrichment culture completely inhibited methane production and increased hydrogen concentration, which suggested that archaeon Kjm51a is a methanol-reducing hydrogenotrophic methanogen. Taken together, we propose the provisional taxonomic assignment, named *Candidatus* Methanogranum caenicola, for the enriched archaeon Kjm51a belonging to Group E2. We also propose to place the methanogenic lineage of the class *Thermoplasmata* in a novel order, *Methanomassiliicoccales* ord. nov.

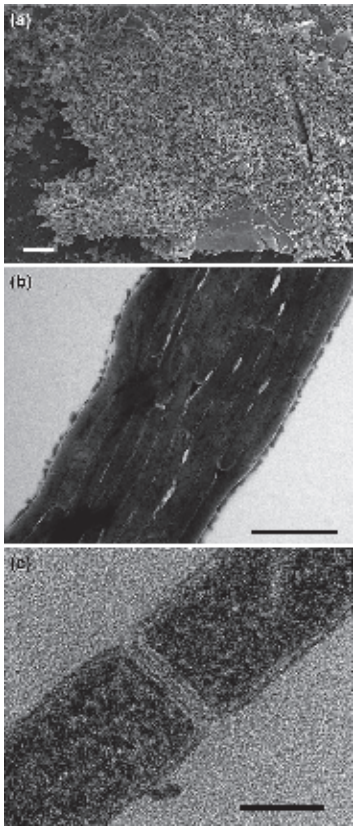


Microbes Environ. Vol. 28, No. 2, 228–235, 2013

Isolation and Characterization of a Thermophilic, Obligately Anaerobic and Heterotrophic Marine *Chloroflexi* Bacterium from a *Chloroflexi*-dominated Microbial Community Associated with a Japanese Shallow Hydrothermal System, and Proposal for *Thermomarinilinea lacunofontalis* gen. nov., sp. nov.

Takuro Nunoura[1], Miho Hirai[1], Masayuki Miyazaki[1], Hiromi Kazama[1], Hiroko Makita[1], Hisako Hirayama[1], Yasuo Furushima[2], Hiroyuki Yamamoto[2], Hiroyuki Imachi[1], Ken Takai[1]
1, 2 Japan Agency for Marine-Earth Science & Technology (JAMSTEC)

A novel marine thermophilic and heterotrophic *Anaerolineae* bacterium in the phylum *Chloroflexi*, strain SW7^T, was isolated from an in situ colonization system deployed in the main hydrothermal vent of the Taketomi submarine hot spring field located on the southern part of Yaeyama Archipelago, Japan. The microbial community associated with the hydrothermal vent was predominated by thermophilic heterotrophs such as *Thermococcaceae* and *Anaerolineae*, and the next dominant population was thermophilic sulfur oxidizers. Both aerobic and anaerobic hydrogenotrophs including methanogens were detected as minor populations. During the culture-dependent viable count analysis in this study, an *Anaerolineae* strain SW7^T was isolated from an enrichment culture at a high dilution rate. Strain SW7^T was an obligately anaerobic heterotroph that grew with fermentation and had non-motile thin rods 3.5–16.5 μm in length and 0.2 μm in width constituting multicellular filaments. Growth was observed between 37–65°C (optimum 60°C), pH 5.5–7.3 (optimum pH 6.0), and 0.5–3.5% (w/v) NaCl concentration (optimum 1.0%). Based on the physiological and phylogenetic features of a new isolate, we propose a new species representing a novel genus *Thermomarinilinea*: the type strain of *Thermomarinilinea lacunofontalis* sp. nov., is SW7^T (=JCM15506^T=KCTC5908^T).



Metagenomics

Microbes Environ. Vol. 28, No. 1, 120–127, 2013

Metagenomic Analysis of the Rhizosphere Soil Microbiome with Respect to Phytic Acid Utilization

Yusuke Unno, Takuro Shinano
NARO Hokkaido Agricultural Research Center

	Relative abundance (%)		Ratio
	NF	F	F/NF
Outer membrane protein	0.05	0.27	5.42
Citrate synthase (si) (EC 2.3.3.1)	0.05	0.20	4.07
Leucyl-tRNA synthetase (EC 6.1.1.4)	0.05	0.20	4.07
putative integral membrane protein	0.05	0.20	4.07
Transcriptional regulator, TetR family	0.05	0.20	4.07
Glycosyltransferase (EC 2.4.1.-)	0.12	0.47	3.80
Alkaline phosphatase (EC 3.1.3.1)	0.07	0.27	3.62
ABC-type transport systems	0.10	0.33	3.39
Beta-galactosidase (EC 3.2.1.23)	0.25	0.07	−3.69
3-oxoacyl-[acyl-carrier protein] reductase	0.32	0.07	−4.79

Subsystems showing >3-fold ratio.

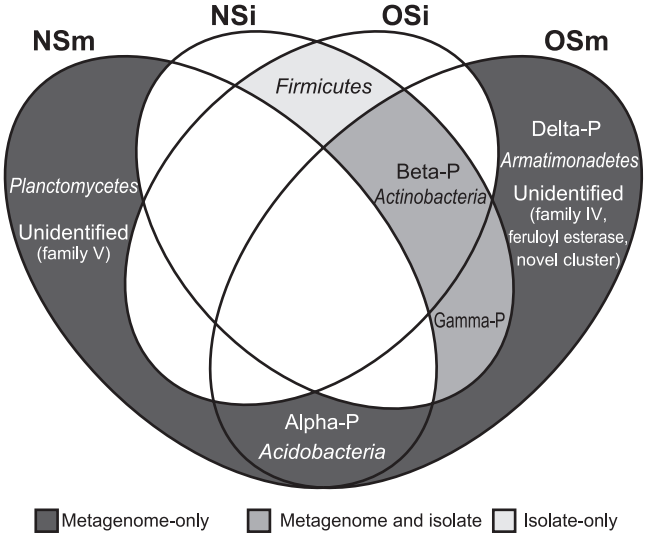
While phytic acid is a major form of organic phosphate in many soils, plant utilization of phytic acid is normally limited; however, culture trials of *Lotus japonicus* using experimental field soil that had been managed without phosphate fertilizer for over 90 years showed significant usage of phytic acid applied to soil for growth and flowering and differences in the degree of growth, even in the same culture pot. To understand the key metabolic processes involved in soil phytic acid utilization, we analyzed rhizosphere soil microbial communities using molecular ecological approaches. Although molecular fingerprint analysis revealed changes in the rhizosphere soil microbial communities from bulk soil microbial community, no clear relationship between the microbiome composition and flowering status that might be related to phytic acid utilization of *L. japonicus* could be determined. However, metagenomic analysis revealed changes in the relative abundance of the classes *Bacteroidetes*, *Betaproteobacteria*, *Chlorobi*, *Dehalococcoidetes* and *Methanobacteria*, which include strains that potentially promote plant growth and phytic acid utilization, and some gene clusters relating to phytic acid utilization, such as alkaline phosphatase and citrate synthase, with the phytic acid utilization status of the plant. This study highlights phylogenetic and metabolic features of the microbial community of the *L. japonicus* rhizosphere and provides a basic understanding of how rhizosphere microbial communities affect the phytic acid status in soil.

Microbes Environ. Vol. 29, No. 2, 154–161, 2014

The Combination of Functional Metagenomics and an Oil-Fed Enrichment Strategy Revealed the Phylogenetic Diversity of Lipolytic Bacteria Overlooked by the Cultivation-Based Method

Takashi Narihiro[1, 2], Aya Suzuki[1, 3], Kazuaki Yoshimune[1], Tomoyuki Hori[1], Tamotsu Hoshino[1], Isao Yumoto[1, 3], Atsushi Yokota[4], Nobutada Kimura[2], Yoichi Kamagata[1, 2, 3, 4]
1, 2 National Institute of Advanced Industrial Science and Technology (AIST); 3, 4 Hokkaido University

Metagenomic screening and conventional cultivation have been used to exploit microbial lipolytic enzymes in nature.We used an indigenous forest soil (NS) and oil-fed enriched soil (OS) as microbial and genetic resources. Thirty-four strains (17 each) of lipolytic bacteria were isolated from the NS and OS microcosms. These isolates were classified into the (sub)phyla *Betaproteobacteria*, *Gammaproteobacteria, Firmicutes,* and *Actinobacteria*, all of which are known to be the main microbial resources of commercially available lipolytic enzymes. Seven and 39 lipolytic enzymes were successfully retrieved from the metagenomic libraries of the NS and OS microcosms, respectively. The screening efficiency (a ratio of positive lipolytic clones to the total number of environmental clones) was markedly higher in the OS microcosm than in the NS microcosm. Moreover, metagenomic clones encoding the lipolytic enzymes associated with *Alphaproteobacteria, Deltaproteobacteria, Acidobacteria, Armatimonadetes*, and Planctomycetes and hithertouncultivated microbes were recovered from these libraries. The results of the present study indicate that functional metagenomics can be effectively used to capture as yet undiscovered lipolytic enzymes that have eluded the cultivationbased method, and these combined approaches may be able to provide an overview of lipolytic organisms potentially present in nature.

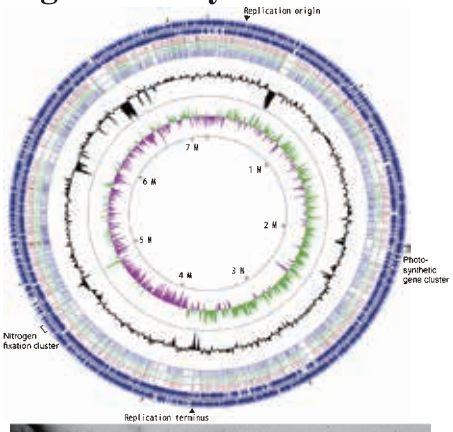


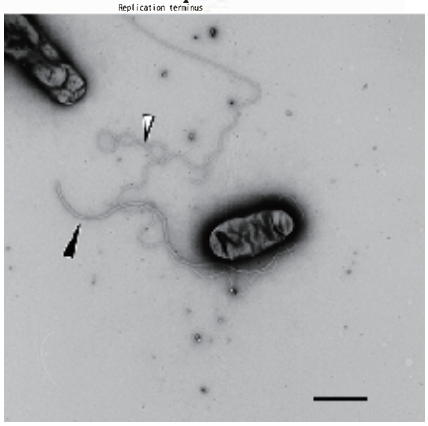
Genomics

Microbes Environ. Vol. 27, No. 3, 306–315, 2012

Complete Genome Sequence of *Bradyrhizobium* sp. S23321: Insights into Symbiosis Evolution in Soil Oligotrophs

Takashi Okubo[1], Takahiro Tsukui[1], Hiroko Maita[1, 2], Shinobu Okamoto[3], Kiwamu Minamisawa[1], and other 44 authours
1 *Tohoku University;* 2 *Kazusa DNA Research Institute;*
3 Research Organization of Information and Systems (ROIS)





Bradyrhizobium sp. S23321 is an oligotrophic bacterium isolated from paddy field soil. Although S23321 is phylogenetically close to *Bradyrhizobium japonicum* USDA110, a legume symbiont, it is unable to induce root nodules in siratro, a legume often used for testing Nod factor-dependent nodulation. The genome of S23321 is a single circular chromosome, 7,231,841 bp in length, with an average GC content of 64.3%. The genome contains 6,898 potential protein-encoding genes, one set of rRNA genes, and 45 tRNA genes. Comparison of the genome structure between S23321 and USDA110 showed strong colinearity; however, the symbiosis islands present in USDA110 were absent in S23321, whose genome lacked a chaperonin gene cluster (*groELS3*) for symbiosis regulation found in USDA110. A comparison of sequences around the tRNA-Val gene strongly suggested that S23321 contains an ancestral-type genome that precedes the acquisition of a symbiosis island by horizontal gene transfer. Although S23321 contains a *nif* (nitrogen fixation) gene cluster, the organization, homology, and phylogeny of the genes in this cluster were more similar to those of photosynthetic bradyrhizobia ORS278 and BTAi1 than to those on the symbiosis island of USDA110. In addition, we found genes encoding a complete photosynthetic system, many ABC transporters for amino acids and oligopeptides, two types (polar and lateral) of flagella, multiple respiratory chains, and a system for lignin monomer catabolism in the S23321 genome. These features suggest that S23321 is able to adapt to a wide range of environments, probably including low-nutrient conditions, with multiple survival strategies in soil and rhizosphere.

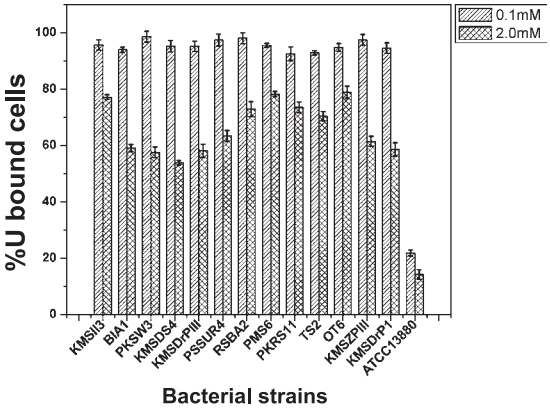
Bioremediation / waste treatment

Microbes Environ. Vol. 28, No. 1, 33–41, 2013

Uranium (U)-Tolerant Bacterial Diversity from U Ore Deposit of Domiasiat in North-East India and Its Prospective Utilisation in Bioremediation

Rakshak Kumar[1], Macmillan Nongkhlaw[1], Celin Acharya[2], Santa Ram Joshi[1]
1 North-Eastern Hill University; 2 Bhabha Atomic Research Centre

Uranium (U)-tolerant aerobic chemo-heterotrophic bacteria were isolated from the sub-surface soils of U-rich deposits in Domiasiat, North East India. The bacterial community explored at molecular level by amplified ribosomal DNA restriction analysis (ARDRA) resulted in 51 distinct phylotypes. Bacterial community assemblages at the U mining site with the concentration of U ranging from 20 to 100 ppm, were found to be most diverse. Representative bacteria analysed by 16S rRNA gene sequencing were affiliated to *Firmicutes* (51%), *Gammaproteobacteria* (26%), *Actinobacteria* (11%), *Bacteroidetes* (10%) and *Betaproteobacteria* (2%). Representative strains removed more than 90% and 53% of U from 100 µM and 2 mM uranyl nitrate solutions, respectively, at pH 3.5 within 10 min of exposure and the activity was retained until 24 h. Overall, 76% of characterized isolates possessed phosphatase enzyme and 53% had P_{IB}-type ATPase genes. This study generated baseline information on the diverse indigenous U-tolerant bacteria which could serve as an indicator to estimate the environmental impact expected to be caused by mining in the future. Also, these natural isolates efficient in uranium binding and harbouring phosphatase enzyme and metal-transporting genes could possibly play a vital role in the bioremediation of metal-/radionuclide -contaminated environments.

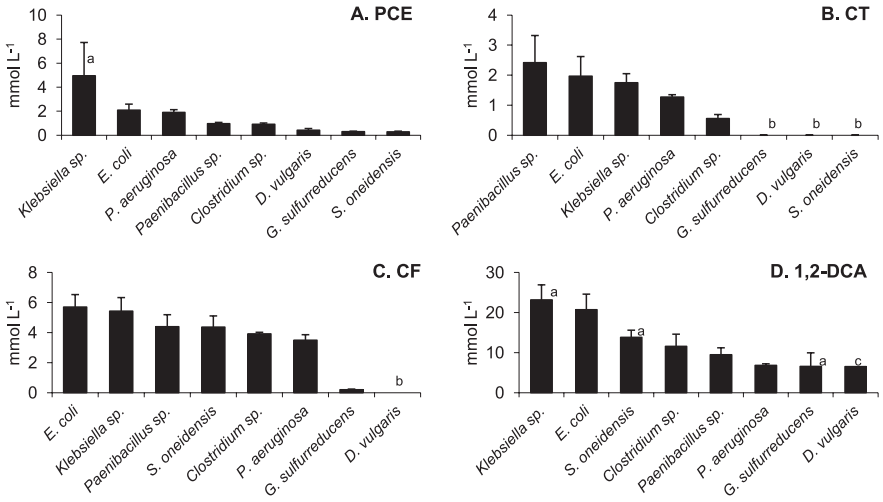


Microbes Environ. Vol. 29, No. 1, 23–30, 2014

Tolerance of Anaerobic Bacteria to Chlorinated Solvents

Joanna C. Koenig[1], Kathrin D. Groissmeier[2], Mike J. Manefield[1]
1 University of New South Wales; 2 Helmholtz Institute of Groundwater Ecology

The aim of this research was to evaluate the effects of four chlorinated aliphatic hydrocarbons (CAHs), perchloroethene (PCE), carbon tetrachloride (CT), chloroform (CF) and 1,2-dichloroethane (1,2-DCA), on the growth of eight anaerobic bacteria: four fermentative species (*Escherichia coli, Klebsiella* sp., *Clostridium* sp. and *Paenibacillus* sp.) and four respiring species (*Pseudomonas aeruginosa, Geobacter sulfurreducens, Shewanella oneidensis* and *Desulfovibrio vulgaris*). Effective concentrations of solvents which inhibited growth rates by 50% (EC_{50}) were determined. The octanol-water partition coefficient or log $P_{o/w}$ of a CAH proved a generally satisfactory measure of its toxicity. Most species tolerated approximately 3-fold and 10-fold higher concentrations of the two relatively more polar CAHs CF and 1,2-DCA, respectively, than the two relatively less polar compounds PCE and CT. EC_{50} values correlated well with growth rates observed in solvent-free cultures, with fast-growing organisms displaying higher tolerance levels. Overall, fermentative bacteria were more tolerant to CAHs than respiring species, with iron- and sulfate-reducing bacteria in particular appearing highly sensitive to CAHs. These data extend the current understanding of the impact of CAHs on a range of anaerobic bacteria, which will benefit the field of bioremediation.

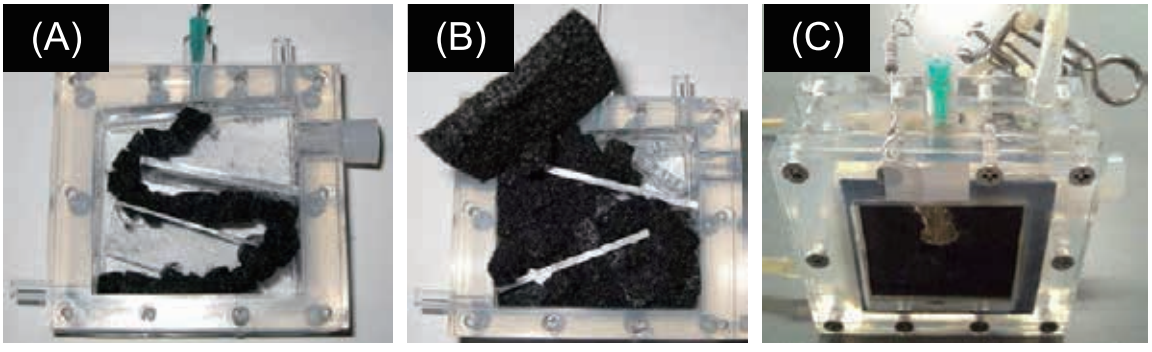


Microbes Environ. Vol. 29, No. 2, 145–153, 2014

Dynamics of Different Bacterial Communities Are Capable of Generating Sustainable Electricity from Microbial Fuel Cells with Organic Waste

Shuji Yamamoto, Kei Suzuki, Yoko Araki, Hiroki Mochihara, Tetsuya Hosokawa, Hiroko Kubota, Yusuke Chiba, Owen Rubaba, Yosuke Tashiro, Hiroyuki Futamata
Shizuoka University

The relationship between the bacterial communities in anolyte and anode biofilms and the electrochemical properties of microbial fuel cells (MFCs) was investigated when a complex organic waste-decomposing solution was continuously supplied to MFCs as an electron donor. The current density increased gradually and was maintained at approximately 100 to 150 mA m^{-2}. Polarization curve analyses revealed that the maximum power density was 7.4 W m^{-3} with an internal resistance of 110 Ω. Bacterial community structures in the organic waste-decomposing solution and MFCs differed from each other. Clonal analyses targeting 16S rRNA genes indicated that bacterial communities in the biofilms on MFCs developed to specific communities dominated by novel *Geobacter*. Multidimensional scaling analyses based on DGGE profiles revealed that bacterial communities in the organic waste-decomposing solution fluctuated and had no dynamic equilibrium. Bacterial communities on the anolyte in MFCs had a dynamic equilibrium with fluctuations, while those of the biofilm converged to the *Geobacter*-dominated structure. These bacterial community dynamics of MFCs differed from those of control-MFCs under open circuit conditions. These results suggested that bacterial communities in the anolyte and biofilm have a gentle symbiotic system through electron flow, which resulted in the advance of current density from complex organic waste.



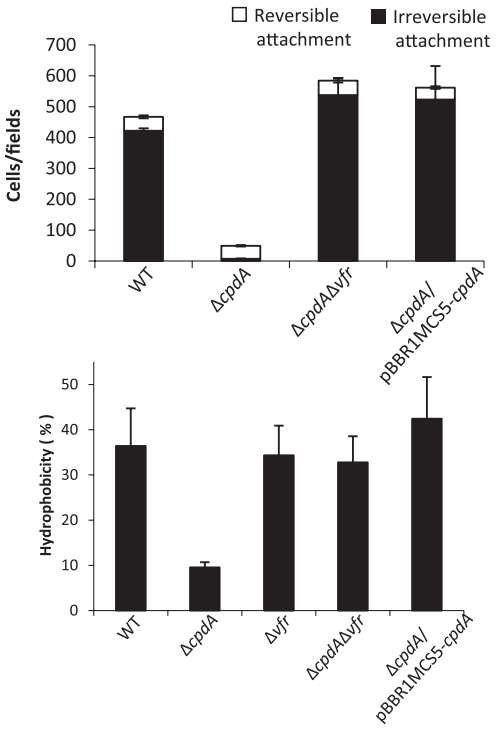
Microbial physiology

Microbes Environ. Vol. 29, No. 1, 104–106, 2014

cAMP Signaling Affects Irreversible Attachment During Biofilm Formation by *Pseudomonas aeruginosa* PAO1

Kaori Ono, Rie Oka, Masanori Toyofuku, Ayane Sakaguchi, Masakaze Hamada, Shiomi Yoshida, Nobuhiko Nomura
University of Tsukuba

Pseudomonas aeruginosa responds to environmental changes and regulates its life cycle from planktonic to biofilm modes of growth. The control of cell attachment to surfaces is one of the critical processes that determine this transition. Environmental signals are typically relayed to the cytoplasm by second messenger systems. We here demonstrated that the second messenger, cAMP, regulated the attachment of cells. Our results suggest cAMP inhibited the transition from reversible to irreversible attachment. Further analyses revealed that cell surface hydrophobicity, one of the key factors in cell attachment, was altered by cAMP.



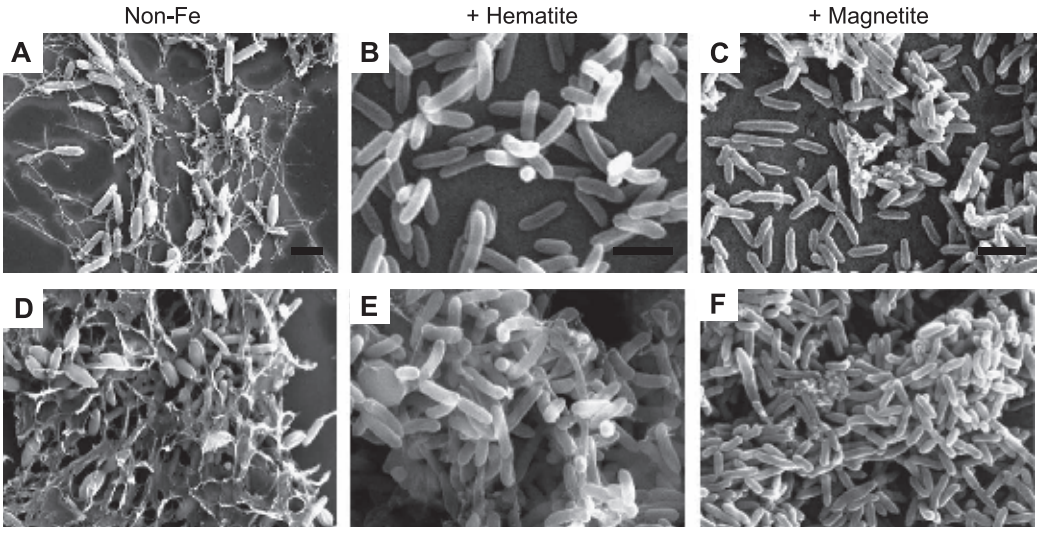
Microbes Environ. Vol. 28, No. 1, 141–148, 2013

Iron-Oxide Minerals Affect Extracellular Electron-Transfer Paths of *Geobacter* spp.

Souichiro Kato[1], Kazuhito Hashimoto[1, 2, 3], Kazuya Watanabe[1, 2, 4]
1 Hashimoto Light Energy Conversion Project, ERATO, JST;
2, 3 The University of Tokyo; 4 Tokyo University of Pharmacy and Life Sciences

2013 M&E Award paper

Some bacteria utilize (semi)conductive iron-oxide minerals as conduits for extracellular electron transfer (EET) to distant, insoluble electron acceptors. A previous study demonstrated that microbe/mineral conductive networks are constructed in soil ecosystems, in which *Geobacter* spp. share dominant populations. In order to examine how (semi)conductive iron-oxide minerals affect EET paths of *Geobacter* spp., the present study grew five representative *Geobacter* strains on electrodes as the sole electron acceptors in the absence or presence of (semi)conductive iron oxides. It was found that iron-oxide minerals enhanced current generation by three *Geobacter* strains, while no effect was observed in another strain. *Geobacter sulfurreducens* was the only strain that generated substantial amounts of currents both in the presence and absence of the iron oxides. Microscopic, electrochemical and transcriptomic analyses of *G. sulfurreducens* disclosed that this strain constructed two distinct types of EET path; in the absence of iron-oxide minerals, bacterial biofilms rich in extracellular polymeric substances were constructed, while composite networks made of mineral particles and microbial cells (without polymeric substances) were developed in the presence of iron oxides. It was also found that uncharacterized c-type cytochromes were up -regulated in the presence of iron oxides that were different from those found in conductive biofilms. These results suggest the possibility that natural (semi) conductive minerals confer energetic and ecological advantages on *Geobacter*, facilitating their growth and survival in the natural environment.



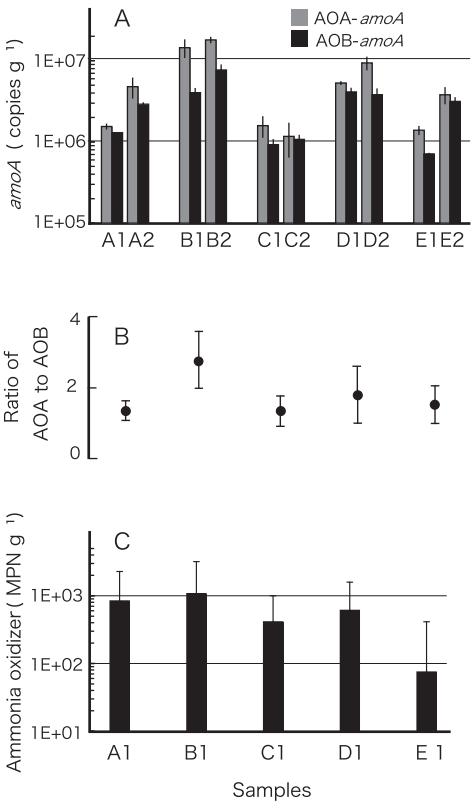
N-cycling microbes

Microbes Environ. Vol. 27, No. 1, 61–66, 2012

Distribution of Ammonia-Oxidizing Archaea and Bacteria in the Surface Sediments of Matsushima Bay in Relation to Environmental Variables

Tomoko Sakami
Fisheries Research Agency

Ammonia oxidization is the first and a rate-limiting step of nitrification, which is often a critical process in nitrogen removal from estuarine and coastal environments. To clarify the correlation of environmental conditions with the distribution of ammonia oxidizers in organic matter-rich coastal sediments, ammonia-oxidizing archaea (AOA) and bacteria (AOB) ammonia monooxygenase alpha subunit gene (*amoA*) abundance was determined in sediments of Matsushima Bay located in northeast Japan. The AOA and AOB *amoA* copy numbers ranged from 1.1×10^6 to 1.7×10^7 and from 7.1×10^5 to 7.6×10^6 copies g^{-1} sediment, respectively. AOA and AOB amoA abundance was negatively correlated with dissolved oxygen levels in the bottom water. AOA amoA abundance was also correlated with total phosphorus levels in the sediments. On the other hand, no significant relationship was observed between the *amoA* abundance and ammonium, organic matter (ignition loss), or acid volatile sulfide-sulfur levels in the sediments. These results show the heterogeneous distribution of ammonia oxidizers by the difference in environmental conditions within the bay. Moreover, AOA *amoA* diversity was relatively low in the area of high AOA *amoA* abundance, suggesting the variability of AOA community composition.

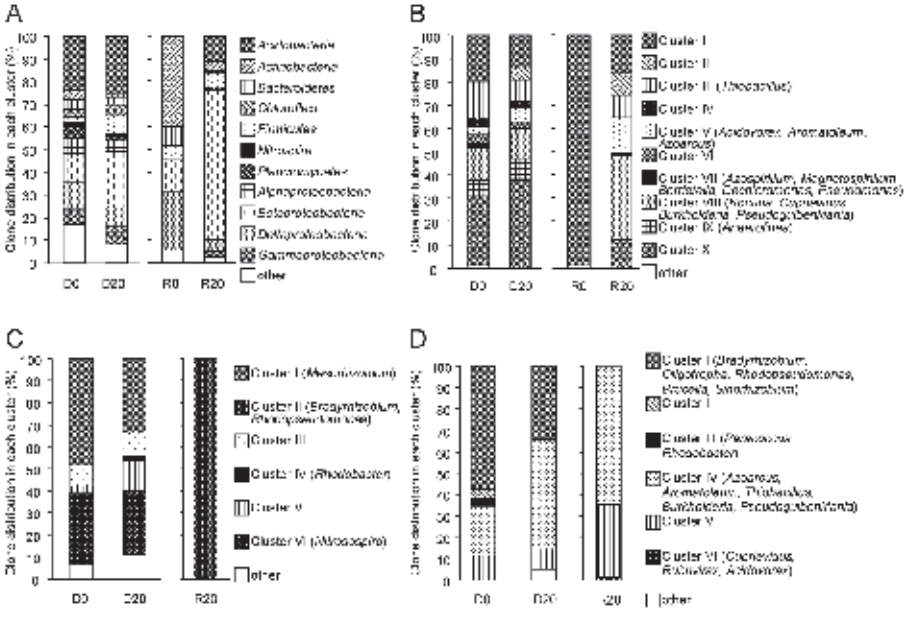


Microbes Environ. Vol. 27, No. 4, 456–461, 2012

Identification of Active Denitrifiers in Rice Paddy Soil by DNA- and RNA-Based Analyses

Megumi Yoshida, Satoshi Ishii†, Daichi Fujii, Shigeto Otsuka, Keishi Senoo
The University of Tokyo; † Hokkaido University

Denitrification occurs markedly in rice paddy fields; however, few microbes that are actively involved in denitrification in these environments have been identified. In this study, we used a laboratory soil microcosm system in which denitrification activity was enhanced. DNA and RNA were extracted from soil at six time points after enhancing denitrification activity, and quantitative PCR and clone library analyses were performed targeting the 16S rRNA gene and denitrification functional genes (*nirS, nirK* and *nosZ*) to clarify which microbes are actively involved in denitrification in rice paddy soil. Based on the quantitative PCR results, transcription levels of the functional genes agreed with the denitrification activity, although gene abundance did not change at the DNA level. Diverse denitrifiers were detected in clone library analysis, but comparative analysis suggested that only some of the putative denitrifiers, especially those belonging to the orders *Neisseriales, Rhodocyclales* and *Burkholderiales*, were actively involved in denitrification in rice paddy soil.

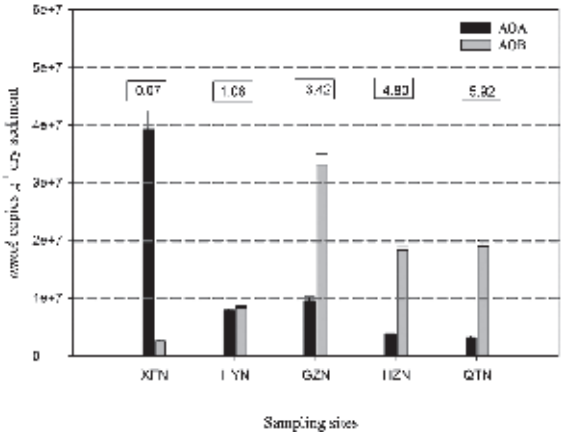


Microbes Environ. Vol. 28, No. 4, 457–465, 2013

Distribution and Abundance of Archaeal and Bacterial Ammonia Oxidizers in the Sediments of the Dongjiang River, a Drinking Water Supply for Hong Kong

Wei Sun[1, 2, 4], Chunyu Xia[2, 4], Meiying Xu[2, 4], Jun Guo[2, 4], Aijie Wang[3], Guoping Sun[2, 4]
1 South China University of Technology; 2 Guangdong Institute of Microbiology; 3 Harbin Institute of Technology; 4 State Key Laboratory of Applied Microbiology

Ammonia-oxidizing archaea (AOA) and bacteria (AOB) play important roles in nitrification. However, limited information about the characteristics of AOA and AOB in the river ecosystem is available. The distribution and abundance of AOA and AOB in the sediments of the Dongjiang River, a drinking water source for Hong Kong, were investigated by clone library analysis and quantitative real-time PCR. Phylogenetic analysis showed that Group 1.1b- and Group 1.1b-associated sequences of AOA predominated in sediments with comparatively high carbon and nitrogen contents (*e.g.* total carbon (TC) >13 g kg^{-1} sediment, NH_4^+-N >144 mg kg^{-1} sediment), while Group 1.1a- and Group 1.1a-associated sequences were dominant in sediments with opposite conditions (e.g. TC <4 g kg^{-1} sediment, NH_4^+-N <93 mg kg^{-1} sediment). Although *Nitrosomonas*- and *Nitrosospira*-related sequences of AOB were detected in the sediments, nearly 70% of the sequences fell into the *Nitrosomonas*-like B cluster, suggesting similar sediment AOB communities along the river. Higher abundance of AOB than AOA was observed in almost all of the sediments in the Dongjiang River, while significant correlations were only detected between the distribution of AOA and the sediment pH and TC, which suggested that AOA responded more sensitively than AOB to variations of environmental factors. These results extend our knowledge about the environmental responses of ammonia oxidizers in the river ecosystem.

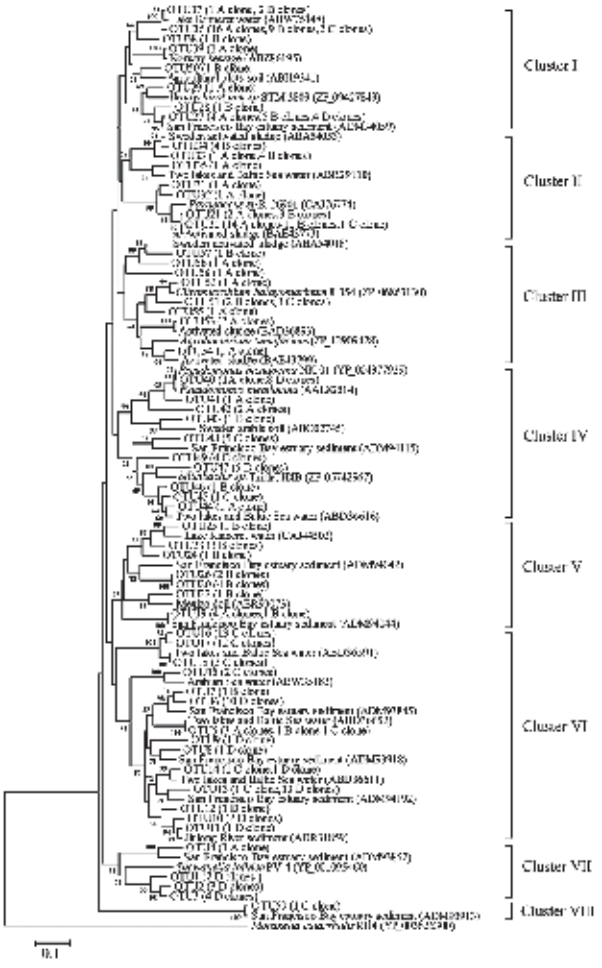


Microbes Environ. Vol. 29, No. 1, 107–110, 2014

Diversity and Distribution of *nirK*-Harboring Denitrifying Bacteria in the Water Column in the Yellow River Estuary

Jing Li[1], Guangshan Wei[1], Ningxin Wang[2], Zheng Gao[1]
1,2 Shandong Agricultural University

We investigated the diversity and community composition of denitrifying bacteria in surface water from the Yellow River estuary. Our results indicated that the diversity of the denitrifying community in freshwater based on the *nirK* gene was higher than that in seawater. Furthermore, phylogenetic analysis suggested that the bacteria community could be distributed into eight clusters (Clusters I to VIII). Redundancy analysis (RDA) revealed that community compositions were related to multiple environment factors, such as salinity and nitrate concentration. The results of the present study have provided a novel insight into the denitrifying community in water columns in estuaries.



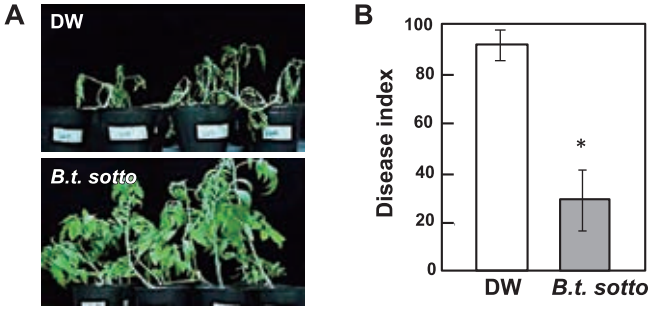
Plant-microbe interaction

Microbes Environ. Vol. 28, No. 1, 128–134, 2013

Bacillus thuringiensis Suppresses Bacterial wilt Disease Caused by *Ralstonia solanacearum* with Systemic Induction of Defense-Related Gene Expression in Tomato

Mitsuro Hyakumachi[1], Mitsuyoshi Nishimura[1], Tatsuyuki Arakawa[1], Shinichiro Asano[2], Shigenobu Yoshida[3], Seiya Tsushima[3], Hideki Takahashi[4]
1 Gifu University; 2 Hokkaido University; 3 National Institute for Agro-Environmental Sciences; 4 Tohoku University

Bacillus thuringiensis is a naturally abundant Gram-positive bacterium and a well-known, effective bio-insecticide. Recently, *B. thuringiensis* has attracted considerable attention as a potential biological control agent for the suppression of plant diseases. In this study, the bacterial wilt disease-suppressing activity of *B. thuringiensis* was examined in tomato plants. Treatment of tomato roots with *B. thuringiensis* culture followed by challenge inoculation with *Ralstonia solanacearum* suppressed the development of wilt symptoms to less than one third of the control. This disease suppression in tomato plants was reproduced by pretreating their roots with a cell-free filtrate (CF) that had been fractionated from *B. thuringiensis* culture by centrifugation and filtration. In tomato plants challenge-inoculated with *R. solanacearum* after pretreatment with CF, the growth of *R. solanacearum* in stem tissues clearly decreased, and expression of defense-related genes such as PR-1, acidic chitinase, and β-1,3-glucanase was induced in stem and leaf tissues. Furthermore, the stem tissues of tomato plants with their roots were pretreated with CF exhibited resistance against direct inoculation with *R. solanacearum*. Taken together, these results suggest that treatment of tomato roots with the CF of *B. thuringiensis* systemically suppresses bacterial wilt through systemic activation of the plant defense system.

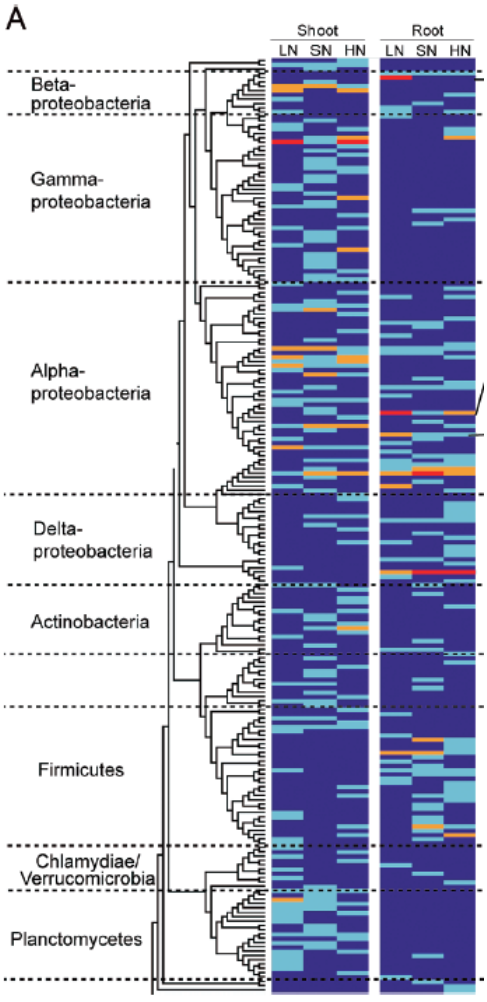


Microbes Environ. Vol. 29, No. 1, 50–59, 2014

Low Nitrogen Fertilization Adapts Rice Root Microbiome to Low Nutrient Environment by Changing Biogeochemical Functions

Seishi Ikeda[1, 2], Kazuhiro Sasaki[1], Kiwamu Minamisawa[1], and other 11 authors
1 Tohoku University; 2 National Agricultural Research Center for Hokkaido Region

Reduced fertilizer usage is one of the objectives of field management in the pursuit of sustainable agriculture. Here, we report on shifts of bacterial communities in paddy rice ecosystems with low (LN), standard (SN), and high (HN) levels of N fertilizer application (0, 30, and 300 kg N ha^{-1}, respectively). The LN field had received no N fertilizer for 5 years prior to the experiment. The LN and HN plants showed a 50% decrease and a 60% increase in biomass compared with the SN plant biomass, respectively. Analyses of 16S rRNA genes suggested shifts of bacterial communities between the LN and SN root microbiomes, which were statistically confirmed by metagenome analyses. The relative abundances of *Burkholderia, Bradyrhizobium* and *Methylosinus* were significantly increased in root microbiome of the LN field relative to the SN field. Conversely, the abundance of methanogenic archaea was reduced in the LN field relative to the SN field. The functional genes for methane oxidation (*pmo* and *mmo*) and plant association (*acdS* and *iaaMH*) were significantly abundant in the LN root microbiome. Quantitative PCR of *pmoA/mcrA* genes and a ^{13}C methane experiment provided evidence of more active methane oxidation in the rice roots of the LN field. In addition, functional genes for the metabolism of N, S, Fe, and aromatic compounds were more abundant in the LN root microbiome. These results suggest that low-N-fertilizer management is an important factor in shaping the microbial community structure containing key microbes for plant associations and biogeochemical processes in paddy rice ecosystems.

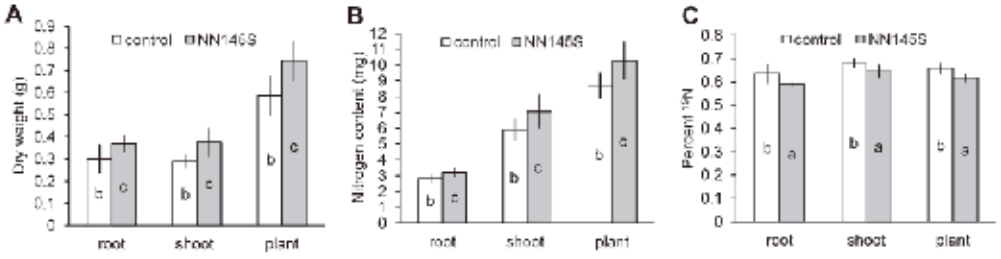


Microbes Environ. Vol. 27, No. 4, 391–1398, 2012

Plant Growth-Promoting Nitrogen-Fixing Enterobacteria Are in Association with Sugarcane Plants Growing in Guangxi, China

Li Lin[1, 2, 3], Zhengyi Li[4], Chunjin Hu[2], Xincheng Zhang[1], Siping Chang[4], Litao Yang[1, 3], Yangrui Li[1, 2, 3], Qianli An[4]
1 Guangxi University; 2 Guangxi Academy of Agricultural Sciences;
3 Chinese Academy of Agricultural Sciences; 4 Zhejiang University

The current nitrogen fertilization for sugarcane production in Guangxi, the major sugarcane-producing area in China, is very high. We aim to reduce nitrogen fertilization and improve sugarcane production in Guangxi with the help of indigenous sugarcane-associated nitrogen-fixing bacteria. We initially obtained 196 fast-growing bacterial isolates associated with the main sugarcane cultivar ROC22 plants in fields using a nitrogen-deficient minimal medium and screened out 43 nitrogen-fixing isolates. Analysis of 16S rRNA gene sequences revealed that 42 of the 43 nitrogen-fixing isolates were affiliated with the genera *Enterobacter* and *Klebsiella*. Most of the nitrogen-fixing enterobacteria possessed two other plant growth-promoting activities of IAA production, siderophore production and phosphate solubilization. Two *Enterobacter* spp. strains of NN145S and NN143E isolated from rhizosphere soil and surface-sterilized roots, respectively, of the same ROC22 plant were used to inoculate micropropagated sugarcane plantlets. Both strains increased the biomass and nitrogen content of the sugarcane seedlings grown with nitrogen fertilization equivalent to 180 kg urea ha^{-1}, the recommended nitrogen fertilization for ROC22 cane crops at the seedling stage. ^{15}N isotope dilution assays demonstrated that biological nitrogen fixation contributed to plant growth promotion. These results suggested that indigenous nitrogen-fixing enterobacteria have the potential to fix N_2 associated with sugarcane plants grown in fields in Guangxi and to improve sugarcane production.

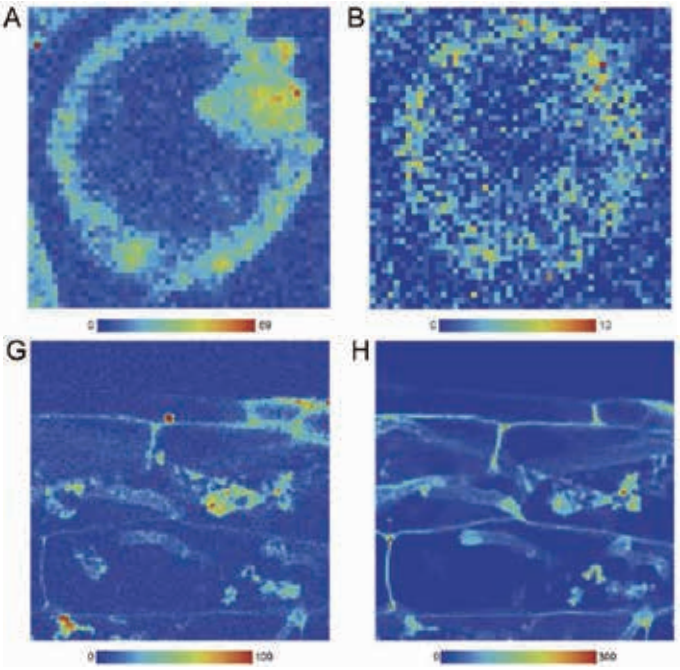


Microbes Environ. Vol. 29, No. 1, 60–64, 2014

Cellular Imaging of Cadmium in Resin Sections of Arbuscular Mycorrhizas Using Synchrotron Micro X-ray Fluorescence

Keiichiro Nayuki[1, 2], Baodong Chen[3], Ryo Ohtomo[3], Yukari Kuga[1]
1 Hiroshima University; 2 Shinshu University; 3 NARO Institute of Livestock and Grassland Science

Arbuscular mycorrhizal (AM) fungi function as extended roots and take an active part in plant acquisition of nutrients and also soil pollutants, such as heavy metals. The objective of this study was to establish a method to observe the localization of cadmium (Cd) Kα at subcellular levels using X-ray fluorescence (XRF) imaging with a synchrotron irradiation microbeam in resin-embedded sections of mycorrhizas. To evaluate the methodology, distributions of Cd in high-pressure-frozen *Lotus japonicus—Rhizophagus irregularis* mycorrhizal roots were compared between two treatments; Cd was exposed either to the roots or to the extraradical hyphae. Results showed that, in the latter treatment, Cd was restricted to fungal structures, whereas in the former, Cd was detected in cell walls of the two organisms. Plunge-frozen extraradical mycelium of *Gigaspora margarita* exposed to Cd showed high signals of Cd in the cell walls and vacuoles, and low in the cytoplasm. With selective staining and elemental mapping by electron-dispersive X-ray spectrometry (EDS), a positive correlation between distributions of Cd and P was revealed in the vacuole, which suggested polyP as a counter ion of Cd. These results indicated that there was no Cd relocation in rapidly frozen resin-embedded materials, therefore supporting the usefulness of this methodology.



Publich health

Microbes Environ. Vol. 28, No. 2, 187–194, 2013

Identification of Human and Animal Fecal Contamination after Rainfall in the Han River, Korea

Ji Young Kim[1], Heetae Lee[1], Jung Eun Lee[1], Myung-Sub Chung[2], Gwang Pyo Ko[1]
1 Seoul National University; 2 Chung-Ang University

We investigated the effect of rainfall on the levels and sources of microbial contamination in the Han River, Korea. Thirty-four samples were collected at two sampling sites located upstream and downstream in the river from July 2010 to February 2011. Various fecal indicator microorganisms, including total coliform, fecal coliform, *Escherichia coli, Enterococcus* spp., somatic and male-specific (F+) coliphage, and four major enteric viruses were analyzed. Rainfall was positively correlated with the levels of fecal coliform and norovirus at both sampling sites. Additionally, rainfall was positively correlated with the levels of total coliform, *E. coli, Enterococcus* spp., and F+ coliphage at the upstream site. To identify the source of fecal contamination, microbial source tracking (MST) was conducted using both male-specific (F+) RNA coliphage and the *Enterococcus faecium esp* gene as previously described. Our results clearly indicated that the majority of fecal contamination at the downstream Han River site was from a human source. At the upstream sampling site, contamination from human fecal matter was very limited; however, fecal contamination from non-point animal sources increased following rainfall. In conclusion, our data suggest that rainfall significantly affects the level and source of fecal contamination in the Han River, Korea.

	Rainfall	Total coliform	Fecal coliform	*E. coli*	*Enterococcus* spp.	Somatic coliphage	F+ coliphage
S1 (upstream)	Before rainfall	8,900 (±12,091)[a]	245 (±332)	95 (±152)	17 (±28)	15 (±20)	2 (±4)
	After rainfall	16,330 (±16,271)	1,060 (±1,218)	286 (±349)	377 (±486)	18 (±13)	18 (±39)
S2 (downstream)	Before rainfall	170,607 (±119,344)	16,498 (±13,363)	15,915 (±13,983)	2,301 (±3,802)	2,371 (±4,451)	890 (±763)
	After rainfall	1,247,207 (±1,085,081)	168,490 (±187,709)	139,081 (±142,842)	25,542 (±46,696)	3,308 (±4,738)	4,195 (±6,139)

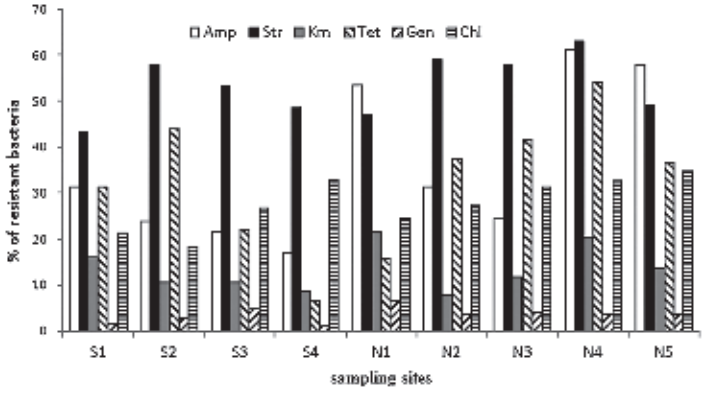
The levels of fecal indicator microorganisms were calculated by MPN or PFU 100 mL^{-1}.
[a] Mean (±standard deviation)

Microbes Environ. Vol. 28, No. 4, 479–486, 2013

Occurrence and Distribution of Antibiotic-resistant Bacteria and Transfer of Resistance Genes in Lake Taihu

Qian Yin, Dongmei Yue, Yuke Peng, Ying Liu, Lin Xiao
Nanjing University

The overuse of antibiotics has accelerated antibiotic resistance in the natural environment, especially fresh water, generating a potential risk for public health around the world. In this study, antibiotic resistance in Lake Taihu was investigated and this was the first thorough data obtained through culture-dependent methods. High percentages of resistance to streptomycin and ampicillin among bacterial isolates were detected, followed by tetracycline and chloramphenicol. Especially high levels of ampicillin resistance in the western and northern regions were illustrated. Bacterial identification of the isolates selected for further study indicated the prevalence of some opportunistic pathogens and 62.0% of the 78 isolates exhibited multiple antibiotic resistance. The presence of ESBLs genes was in the following sequence: $bla_{TEM} > bla_{SHV} > bla_{CTMX}$ and 38.5% of the isolates had a class I integrase gene. Of all tested strains, 80.8% were able to transfer antibiotic resistance through conjugation. We also concluded that some new families of human-associated ESBLs and AmpC genes can be found in natural environmental isolates. The prevalence of antibiotic resistance and the dissemination of transferable antibiotic resistance in bacterial isolates (especially in opportunistic pathogens) was alarming and clearly indicated the urgency of realizing the health risks of antibiotic resistance to human and animal populations who are dependent on Lake Taihu for water consumption.



Microbes Environ. Vol. 29, No. 2, 136–144, 2014

Characterization of Multi-antibiotic-resistant *Escherichia coli* Isolated from Beef Cattle in Japan

Shiori Yamamoto[1], Motoki Nakano[1], Wataru Kitagawa[1, 2], Michiko Tanaka[1], Teruo Sone[1], Katsuya Hirai[3], Kozo Asano[1]
1 Hokkaido University; 2 National Institute of Advanced Industrial Science and Technology (AIST); 3 Tenshi College

The emergence of multiple-antibiotic-resistance bacteria is increasing, which is a particular concern on livestock farms. We previously isolated 1,347 antimicrobial-resistant (AMR) *Escherichia coli* strains from the feces of beef cattle on 14 Japanese farms. In the present study, the genetic backgrounds and phylogenetic relationships of 45 AMR isolates were characterized by the chromosome phylotype, AMR phenotype, AMR genotype, and plasmid type. These isolates were classified into five chromosome phylotypes, which were closely linked to the farms from which they were isolated, suggesting that each farm had its own *E. coli* phylotype. AMR phenotype and plasmid type analyses yielded 8 and 14 types, all of which were associated with the chromosomal phylotype and, thus, to the original farms. AMR genotype analysis revealed more variety, with 16 types, indicating both inter- and intra-farm diversity. Different phylotype isolates from the same farm shared highly similar plasmid types, which indicated that plasmids with AMR genes could be transferred between phylotypes, thereby generating multi-antibiotic-resistant microorganisms. This ecological study demonstrated that the chromosome phylotype was strongly corre lated with the farm from which they were isolated, while the AMR phenotype, genotype, and plasmid type were generally corre lated with the chromosome phylotype and farm source.

Incompatibility types[a]	Number of isolate detected	Resistance genes detected[b]	Sizes (kbp)	Number of different genotype of plasmid
FIB	39	bla_{TEM}, bla_{CTX-M}, bla_{CMY}, *strA, strB, aphA1, aphAI-IAB, aacC2, tetA, tetB, tetC, catI, dhfrI, dhfrVII, dfrA12*	51–144	22
F	37	bla_{TEM}, bla_{CTX-M}, bla_{CMY}, *strA, strB, aphA1, aphAI-IAB, aacC2, tetA, tetB, tetC, dhfrI, dhfrXIII, dfrA12*	46–144	24
FIA	16	bla_{TEM}, bla_{CTX-M}, *strA, strB, aphA1, aphAI-IAB, aacC2, tetB, catI, dhfrVII*	55–127	10
Y	16	*dfrA12*	67–68	5
N	14	bla_{CTX-M}, *tetA, tetB, dhfrXIII, dfrA12*	106–109	4
A/C	8	bla_{TEM}, bla_{CMY}, *strA, strB, tetA, tetC, floR, dhfrI*	155–181	7
I1	5	bla_{TEM}, *strA, strB, aphA1, aphAI-IAB*	51–53	3
P	1	Not detected	51	1
Others	45	bla_{CTX-M}, bla_{CMY}, *strA, strB, aphAI-IAB, tetA, tetC, floR, dfrA12*	2–774	45

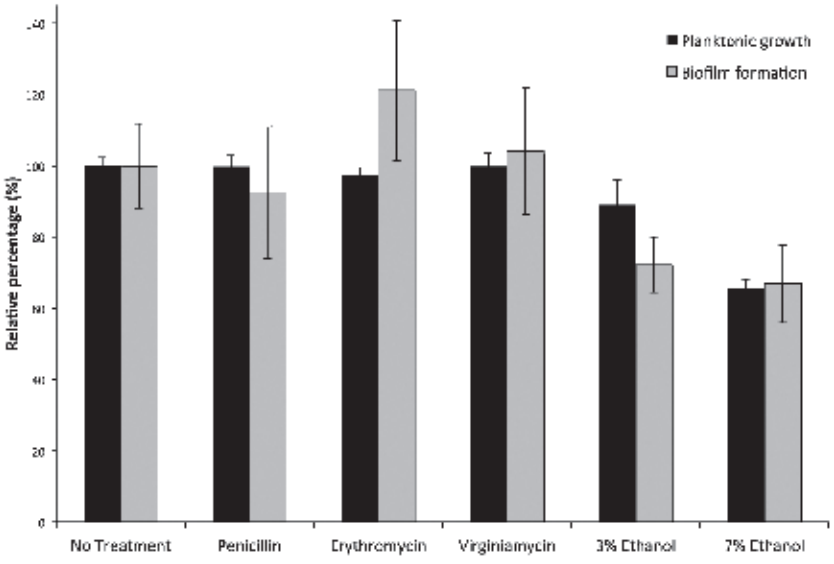
[a] Incompatibility types of HI1, HI2, X, L/M, W, FIC, T, FIIAs, K and B/O were not detected.
[b] bla_{TEM}, bla_{CTX-M} and bla_{CMY}, beta-lactams resistance genes; *strA* and *strB*, DSM resistance gene; *aacC2*, GM resistance gene; *aphA1* and *aphAI-IAB*, KM resistance gene; *tetA*, *tetB* and *tetC*, OTC resistance gene; *catI* and *floR*, CP resistance gene; *dhfrI*, *dhfrVII*, *dhfrXIII* and *dfrA12*, TMP resistance gene.

Microbes Environ. Vol. 29, No. 3, in press, 2014

A Multiple Antibiotic-Resistant *Enterobacter cloacae* Strain Isolated from a Bioethanol Fermentation Facility

Colin A. Murphree[1], Qing Li[1], E. Patrick Heist[2], Luke A. Moe[1]
1 University of Kentucky; 2 Ferm Solutions, Inc.

An *Enterobacter cloacae* strain (*E. cloacae* F3S3) that was collected as part of a project to assess antibiotic resistance among bacteria isolated from bioethanol fermentation facilities demonstrated high levels of resistance to antibiotics added prophylactically to bioethanol fermentors. PCR assays revealed the presence of canonical genes encoding resistance to penicillin (*ampC*) and erythromycin (*ermG*). Assays measuring biofilm formation under antibiotic stress indicated that erythromycin induced biofilm formation in *E. cloacae* F3S3. Planktonic growth and biofilm formation were observed at a high ethanol content, indicating *E. cloacae* F3S3 can persist in a bioethanol fermentor under the highly variable environmental conditions found in fermentors.

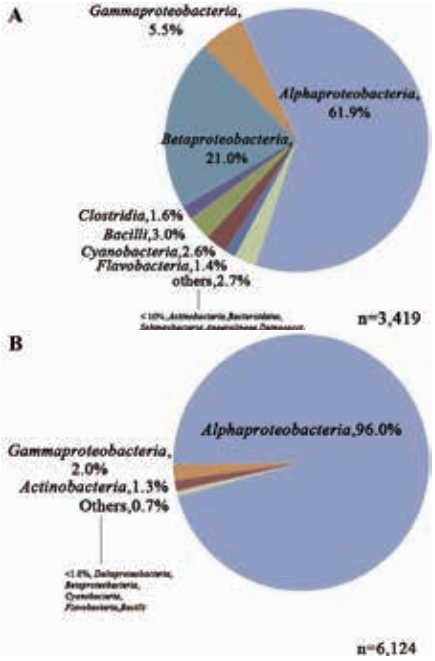


Environmental microbiology

Microbes Environ. Vol. 27, No. 4, 443–448, 2012

Molecular Analysis of Bacterial Communities in Biofilms of a Drinking Water Clearwell

Minglu Zhang[1], Wenjun Liu[1], Xuebiao Nie[1], Cuiping Li[1], Junnong Gu[2], Can Zhang[1, 3]
1 Tsinghua University; 2 Water Quality Monitoring Center, Beijing Waterworks Group; 3 Academy of Military Medical Sciences



Microbial community structures in biofilms of a clearwell in a drinking water supply system in Beijing, China were examined by clone library, terminal restriction fragment length polymorphism (T-RFLP) and 454 pyrosequencing of the amplified 16S rRNA gene. Six biofilm samples (designated R1-R6) collected from six locations (upper and lower sites of the inlet, middle and outlet) of the clearwell revealed similar bacterial patterns by T-RFLP analysis. With respect to the dominant groups, the phylotypes detected by clone library and T-RFLP generally matched each other. A total of 9,543 reads were obtained from samples located at the lower inlet and the lower outlet sites by pyrosequencing. The bacterial diversity of the two samples was compared at phylum and genus levels. *Alphaproteobacteria* dominated the communities in both samples and the genus of *Sphingomonas* constituted 75.1%–99.6% of this phylum. A high level of *Sphingomonas* sp. was first observed in the drinking water biofilms with 0.6–1.0 mg L^{-1} of chlorine residual. Disinfectant-resistant microorganisms deserve special attention in drinking water management. This study provides novel insights into the microbial populations in drinking water systems and highlights the important role of *Sphingomonas* species in biofilm formation.

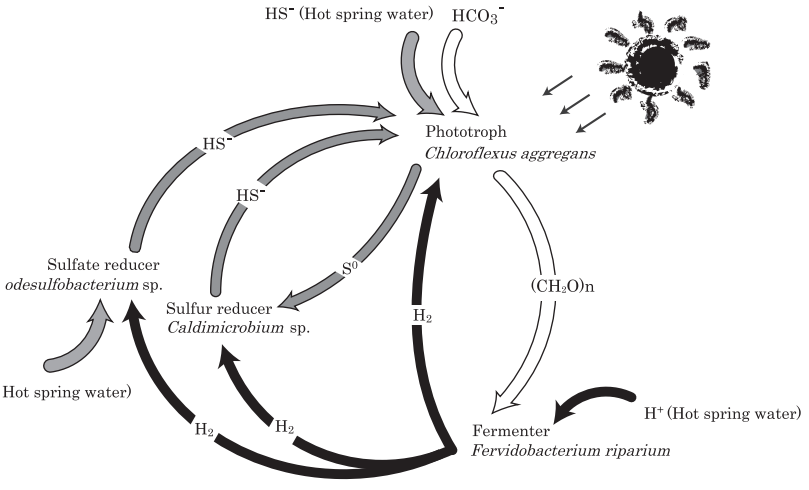
Microbes Environ. Vol. 27, No. 3, 293–299, 2012

Production and Consumption of Hydrogen in Hot Spring Microbial Mats Dominated by a Filamentous Anoxygenic Photosynthetic Bacterium

2012 M&E Award paper

Hiroyo Otaki, R. Craig Everroad, Katsumi Matsuura, Shin Haruta
Tokyo Metropolitan University

Microbial mats containing the filamentous anoxygenic photosynthetic bacterium *Chloroflexus aggregans* develop at Nakabusa hot spring in Japan. Under anaerobic conditions in these mats, interspecies interaction between sulfate-reducing bacteria as sulfide producers and *C. aggregans* as a sulfide consumer has been proposed to constitute a sulfur cycle; however, the electron donor utilized for microbial sulfide production at Nakabusa remains to be identified. In order to determine this electron donor and its source, *ex situ* experimental incubation of mats was explored. In the presence of molybdate, which inhibits biological sulfate reduction, hydrogen gas was released from mat samples, indicating that this hydrogen is normally consumed as an electron donor by sulfate-reducing bacteria. Hydrogen production decreased under illumination, indicating that *C. aggregans* also functions as a hydrogen consumer. Small amounts of hydrogen may have also been consumed for sulfur reduction. Clone library analysis of 16S rRNA genes amplified from the mats indicated the existence of several species of hydrogen-producing fermentative bacteria. Among them, the most dominant fermenter, *Fervidobacterium* sp., was successfully isolated. This isolate produced hydrogen through the fermentation of organic carbon. Dispersion of microbial cells in the mats resulted in hydrogen production without the addition of molybdate, suggesting that simultaneous production and consumption of hydrogen in the mats requires dense packing of cells. We propose a cyclic electron flow within the microbial mats, *i.e.*, electron flow occurs through three elements: S (elemental sulfur, sulfide, sulfate), C (carbon dioxide, organic carbon) and H (di-hydrogen, protons).

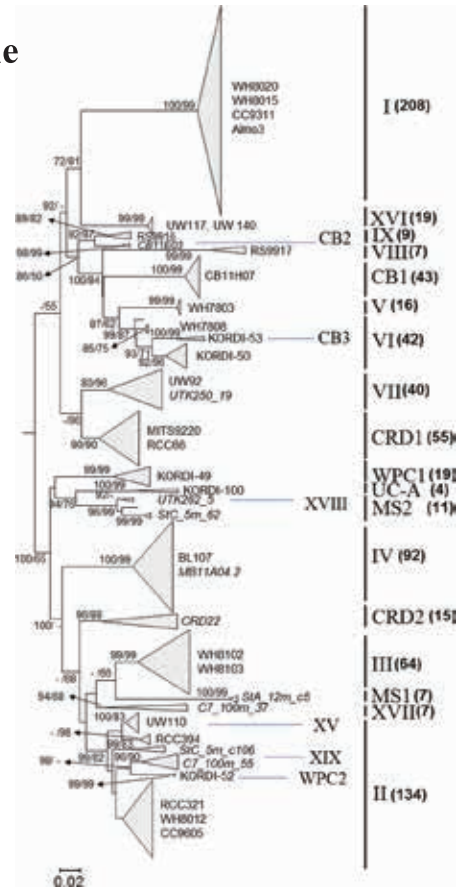


Microbes Environ. Vol. 29, No. 1, 17–22, 2014

Application of Pyrosequencing Method for Investigating the Diversity of *Synechococcus* Subcluster 5.1 in Open Ocean

Dong Han Choi[1], Jae Hoon noH[2], Jung-Hyun Lee[1]
1, 2 Korea Institute of Ocean Science and Technology

Synechococcus are distributed throughout the world's oceans and are composed of diverse genetic lineages. However, as they are much less abundant than *Prochlorococcus* in oligotrophic open oceans, their in-depth genetic diversity cannot be investigated using commonly used primers targeting both *Prochlorococcus* and *Synechococcus*. Thus, in this study, we designed a primer specific to the 16S–23S rRNA internal transcribed spacer (ITS) of the *Synechococcus* subcluster 5.1. Using the primer, we could selectively amplify *Synechococcus* sequences in oligotrophic seawater samples. Further, we showed that a barcoded amplicon pyrosequencing method could be applicable to investigate *Synechococcus* diversity using sequences retrieved in GenBank and obtained from environmental samples. Allowing sequence analyses of a large number of samples, this high-throughput method would be useful to study global biodiversity and biogeographic patterns of *Synechococcus* in marine environments.

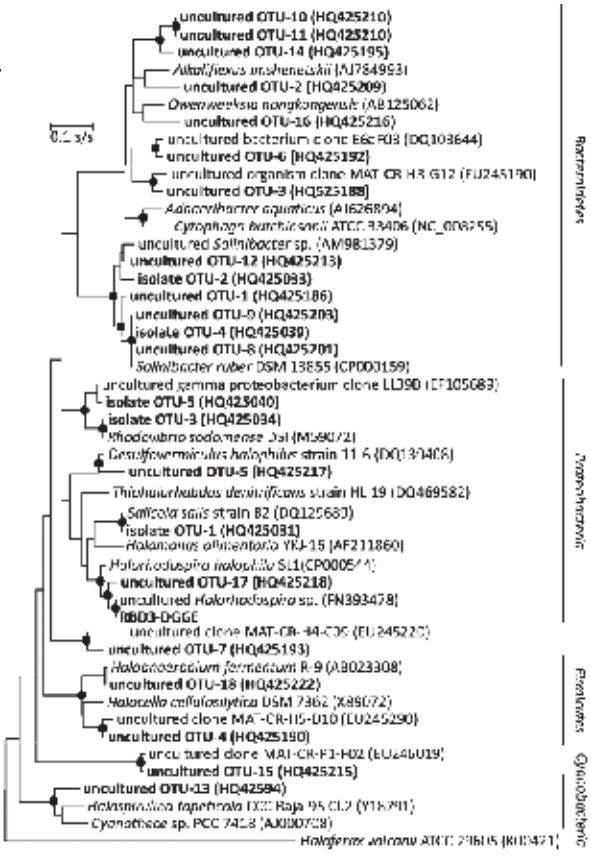


Microbes Environ. Vol. 27, No. 1, 87–93, 2012

Prokaryotic Diversity in Aran-Bidgol Salt Lake, the Largest Hypersaline Playa in Iran

Ali Makhdoumi-Kakhki[1], Mohammad Ali Amoozegar[1],
Bahram Kazemi[2], Lejla PaiC[3], Antonio Ventosa[4]
1 University of Tehran; 2) Shaheed Beheshti University of Medical Sciences;
3 University of Ljubljana; 4 University of Sevilla

Prokaryotic diversity in Aran-Bidgol salt lake, a thalasohaline lake in Iran, was studied by fluorescence *in situ* hybridization (FISH), cultivation techniques, denaturing gradient gel electrophoresis (DGGE) of PCR-amplified fragments of 16S rRNA genes and 16S rRNA gene clone library analysis. Viable counts obtained (2.5-4 × 10^6 cells mL^{-1}) were similar to total cell abundance in the lake determined by DAPI direct count (3-4×10^7 cells mL^{-1}). The proportion of Bacteria to Archaea in the community detectable by FISH was unexpectedly high and ranged between 1:3 and 1:2. We analyzed 101 archaeal isolates and found that most belonged to the genera *Halorubrum* (55%) and *Haloarcula* (18%). Eleven bacterial isolates obtained in pure culture were affiliated with the genera *Salinibacter* (18.7%), *Salicola* (18.7%) and *Rhodovibrio* (35.3%). Analysis of inserts of 100 clones from the eight 16S rRNA clone libraries constructed revealed 37 OTUs. The majority (63%) of these sequences were not related to any previously identified taxa. Within this sampling effort we most frequently retrieved phylotypes related to *Halorhabdus* (16% of archaeal sequences obtained) and *Salinibacter* (36% of bacterial sequences obtained). Other prokaryotic groups that were abundant included representatives of *Haloquadratum*, the anaerobic genera *Halanaerobium* and *Halocella*, purple sulfur bacteria of the genus *Halorhodospira* and *Cyanobacteria*.

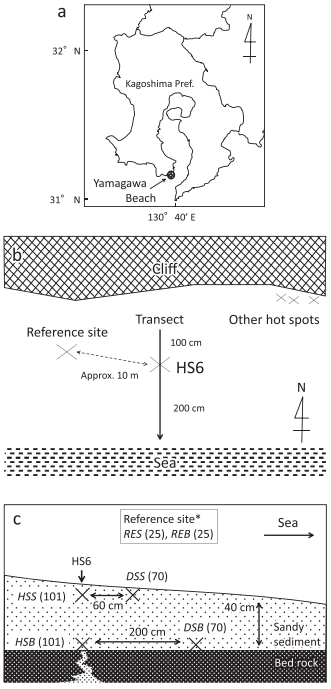


Microbes Environ. Vol. 28, No. 4, 405–413, 2013

Bacterial and Archaeal Diversity in an Iron-Rich Coastal Hydrothermal Field in Yamagawa, Kagoshima, Japan

Satoshi Kawaichi, Norihiro Ito, Takashi Yoshida, Yoshihiko Sako
Kyoto University

Physicochemical characteristics and archaeal and bacterial community structures in an iron-rich coastal hydrothermal field, where the temperature of the most active hot spot reaches above 100°C, were investigated to obtain fundamental information on microbes inhabiting a coastal hydrothermal field. The environmental settings of the coastal hydrothermal field were similar in some degree to those of deep-sea hydrothermal environments because of its emission of H_2, CO_2, and sulfide from the bottom of the hot spot. The results of clone analyses based on the 16S rRNA gene led us to speculate the presence of a chemo-synthetic microbial ecosystem, where chemolithoautotrophic thermophiles, primarily the bacterial order *Aquificales*, function as primary producers using H_2 or sulfur compounds as their energy source and CO_2 as their carbon source, and the organic compounds synthesized by them support the growth of chemoheterotrophic thermophiles, such as members of the order *Thermales* and the family *Desulfurococcaceae*. In addition, the dominance of members of the bacterial genus *Herbaspirillum* in the high temperature bottom layer led us to speculate the temporal formation of mesophilic zones where they can also function as primary producing or nitrogen-fixing bacteria.

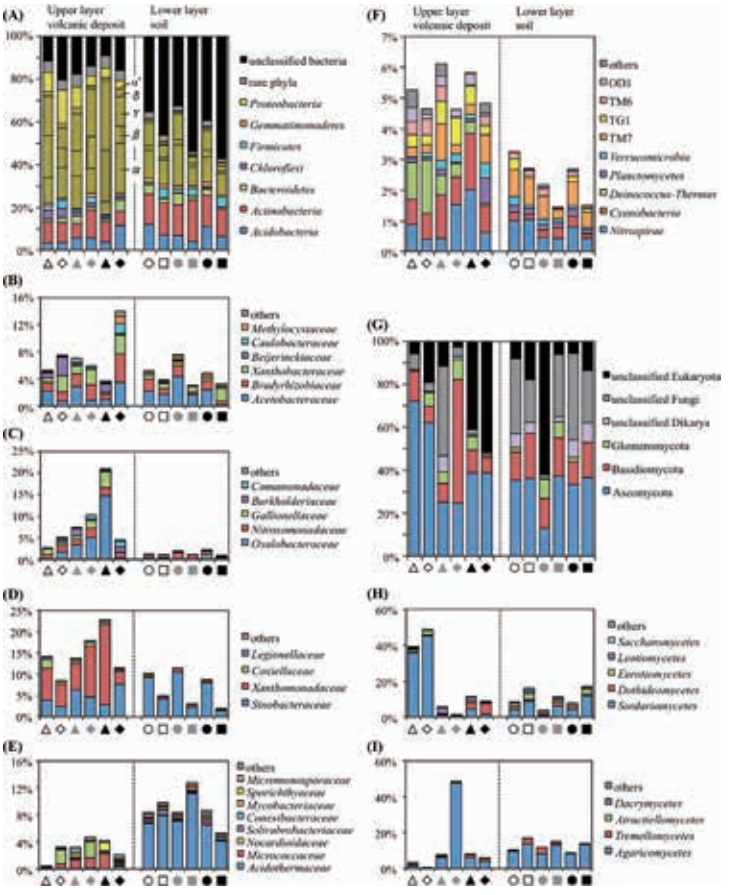


Microbes Environ. Vol. 29, No. 1, 38–49, 2014

Characterization of Early Microbial Communities on Volcanic Deposits along a Vegetation Gradient on the Island of Miyake, Japan

Yong Guo[1, 2], Reiko Fujimura[2], Yoshinori Sato[3], Wataru Suda[4], Seok-won Kim[4], Kenshiro Oshima[4], Masahira Hattori[4], Takashi Kamijo[5], Kazuhiko Narisawa[1, 2], Hiroyuki Ohta[1, 2]
1 Tokyo University of Agriculture and Technology; 2 Ibaraki University
3 National Research Institute for Cultural Properties; 4 The University of Tokyo; 5 University of Tsukuba

The 2000 eruption of Mount Oyama on the island of Miyake (Miyake-jima) created a unique opportunity to study the early ecosystem development on newly exposed terrestrial substrates. In this study, bacterial and fungal communities on 9- and 11-year-old volcanic deposits at poorly to fully vegetation-recovered sites in Miyake-jima, Japan, were characterized by conventional culture-based methods and pyrosequencing of 16S rRNA and 18S rRNA genes. Despite the differences in the vegetation cover, the upper volcanic deposit layer samples displayed low among-site variation for chemical properties (pH, total organic carbon, and total nitrogen) and microbial population densities (total direct count and culturable count). Statistical analyses of pyrosequencing data revealed that the microbial communities of volcanic deposit samples were phylogenetically diverse, in spite of very low-carbon environmental conditions, and their diversity was comparable to that in the lower soil layer (buried soil) samples. Comparing with the microbial communities in buried soil, the volcanic deposit communities were characterized by the presence of *Betaproteobacteria* and *Gammaproteobacteria* as the main bacterial class, *Deinococcus-Thermus* as the minor bacterial phyla, and *Ascomycota* as the major fungal phyla. Multivariate analysis revealed that several bacterial families and fungal classes correlated positively or negatively with plant species.



Microbes and Environments
Instructions for Authors

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1. Saito, A., S. Ikeda, H. Ezura, and K. Minamisawa. 2007. Microbial community analysis of the phytosphere using culture-independent methodologies. Microbes Environ. 22:93–105.
2. Katsuyama, C., N. Kondo, Y. Suwa, T. Yamagishi, M. Itoh, N. Ohte, H. Kimura, K. Nagaosa, and K. Kato. 11 November 2008. Denitrification activity and relevant bacteria revealed by nitrite reductase gene fragments in soil of temperate mixed forest. Microbes Environ. doi:10.1264/jsme2.ME08541.
3. Sambrook, J., and D.W. Russell. 2001. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, New York.
4. Hiraishi, A. 1989. Isoprenoid quinone profiles for identifying and classifying microorganisms in the environment, p. 663-668. *In* T. Hattori, Y. Ishida, Y. Maruyama, R. Y. Morita, and A. Uchida (ed.), Recent Advances in Microbial Ecology. Japan Scientific Societies Press, Tokyo.

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