

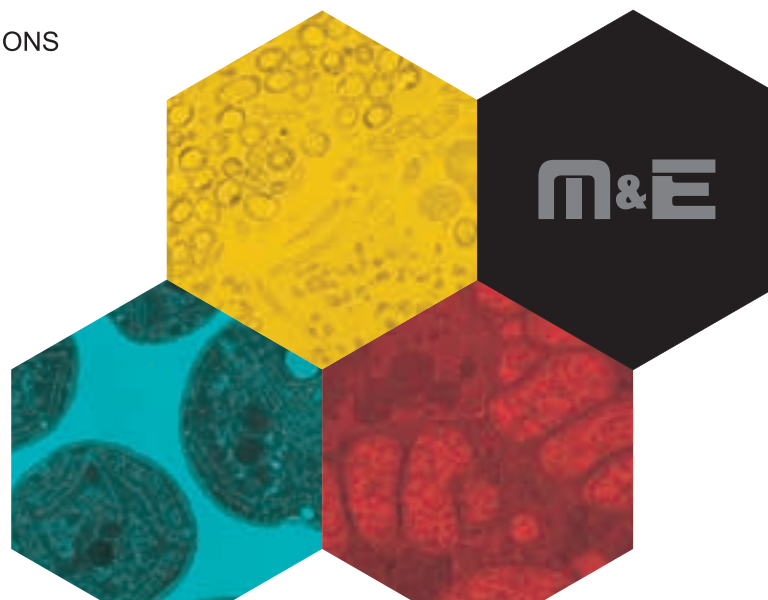


# **MICROBES AND ENVIRONMENTS**

# **DIGEST**

JAPANESE SOCIETY OF MICROBIAL ECOLOGY  
JAPANESE SOCIETY OF SOIL MICROBIOLOGY  
TAIWAN SOCIETY OF MICROBIAL ECOLOGY  
JAPANESE SOCIETY OF PLANT MICROBE INTERACTIONS

February 2016



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Microbes and Environments (M&E) is an international, peer-reviewed, open access journal devoted to the publication of original and review articles regarding all aspects of research on microbial ecology and environmental microbiology. It is published quarterly in a printed and an electronic version by Japanese Society of Microbial Ecology (JSME), Japanese Society of Soil Microbiology (JSSM), Taiwan Society of Microbial Ecology (TSME), and Japanese Society of Plant Microbe Interactions (JSPMI).

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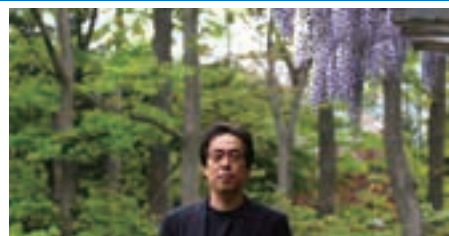
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- Molecular biology and biochemistry of microorganisms
- Molecular and genomic technologies in microbial ecology
- Methodology in general and environmental microbiology
- Interdisciplinary research areas for microbial ecology



## ***Message from Editor-in-Chief***



**Yoichi Kamagata**

National Institute of Advanced Industrial Science  
and Technology (AIST), Tsukuba, Japan

Microbes and Environments is now becoming one of the important journals for microbial ecology. The journal is being edited by four academic societies but the submission is open to all of the researchers including non-members. In fact, we are receiving manuscripts from non-members all over the world. Microbes and Environments is an open access and freely available online journal. Once the manuscript is accepted, it will be published immediately after proof corrections so that readers can see and read the paper in a timely fashion. Different from commercial journals, we are providing the publication opportunity with an affordable price. We look forward to receiving your high quality manuscripts.

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# RESEARCH PAPER AWARD 2014

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

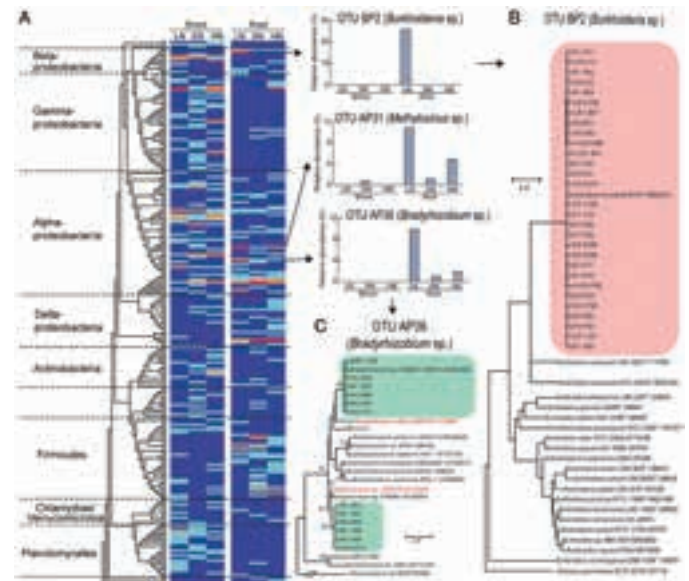
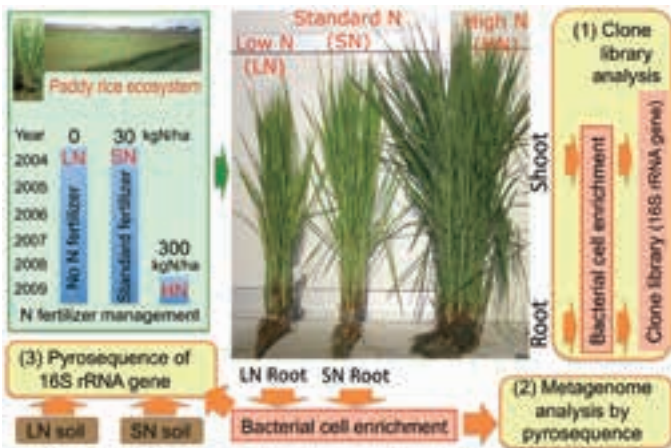
## Low Nitrogen Fertilization Adapts Rice Root Microbiome to Low Nutrient Environment by Changing Biogeochemical Functions

Seishi Ikeda<sup>1,2</sup>, Kazuhiro Sasaki<sup>1</sup>, Takashi Okubo<sup>1</sup>, Akifumu Yamashita<sup>1</sup>, Kimihiro Terasawa<sup>1</sup>, Zhihua Bao<sup>1</sup>, Dongyan Liu<sup>3</sup>, Takeshi Watanabe<sup>3</sup>, Jun Murase<sup>3</sup>, Susumu Asakawa<sup>3</sup>, Shima Eda<sup>1</sup>, Hisayuki Mitsui<sup>1</sup>, Tadashi Sato<sup>1</sup>, Kiwamu Minamisawa<sup>1</sup>.

<sup>1</sup>Graduate School of Life Sciences, Tohoku University, Japan; <sup>2</sup>Memuro Research Station, National Agricultural Research Center for Hokkaido Region, Japan; and <sup>3</sup>Soil Biology and Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Japan

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<sup>-1</sup>, 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 <sup>13</sup>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.



### Comment from Committee

To date, many studies of the microbial ecosystem in the rhizosphere have mainly focused on the biological interactions; namely, development, competition, inhibition, and promotion of a biological relationship between host plants and rhizosphere microbiome. However, the biogeochemical impact caused by rhizosphere microbiome remains obscure due to the absence of comprehensive study using combination of molecular biological and geochemical approaches. This study showed that suppression of nitrogen

fertilizers resulted in increased methane and sulfur oxidation in the rhizosphere and induced oxidative metabolism as well as nitrogen fixation. In contrast, loading of nitrogen fertilizers resulted in increased methane production in the rhizosphere, induced reductive metabolism, and inhibited nitrogen fixation. These results clearly indicated that the addition of nitrogen fertilizers had significant effects on not only rhizobial symbiosis but also biogeochemical cycle.

## MOST CITED PAPERS 2015

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

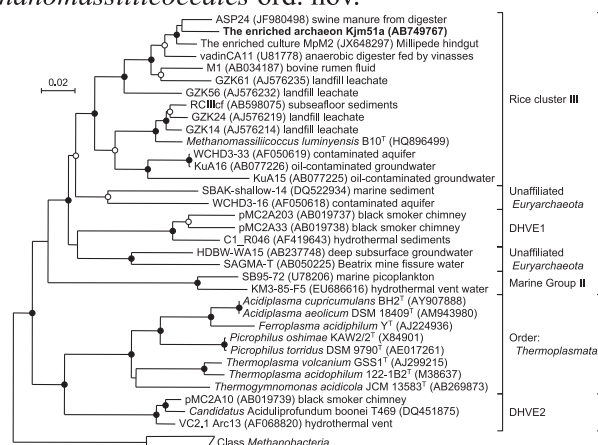
***Candidatus* Methanogranum caenicola: a Novel Methanogen from the Anaerobic Digested Sludge, and Proposal of *Methanomassiliicoccaceae* fam. nov. and *Methanomassiliicoccales* ord. nov., for a Methanogenic Lineage of the Class *Thermoplasmata***

Takao Iino<sup>1</sup>, Hideyuki Tamaki<sup>2</sup>, Satoshi Tamazawa<sup>2,3</sup>, Yoshiyuki Ueno<sup>4</sup>, Moriya Ohkuma<sup>1</sup>, Ken-ichiro Suzuki<sup>5</sup>, Yasuo Igarashi<sup>6</sup>, Shin Haruta<sup>7</sup>

<sup>1</sup>Japan Collection of Microorganisms, RIKEN BioResource Center, Japan; <sup>2</sup>National Institute of Advanced Industrial Science and Technology (AIST), Japan; <sup>3</sup>Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; <sup>4</sup>Kajima Technical Research Institute, Japan; <sup>5</sup>NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Japan; <sup>6</sup>Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan; and <sup>7</sup>Graduate School of Science and Engineering, Tokyo Metropolitan University, Japan

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<sup>T</sup> 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<sup>T</sup>, 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-bromoethanesulfonate 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. 22, No. 3, 232–242, 2007

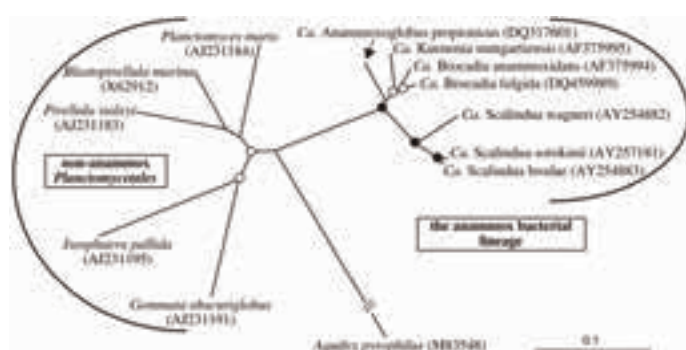
# Detection of Anammox Activity and Diversity of Anammox Bacteria-Related 16S rRNA Genes in Coastal Marine Sediment in Japan

Teruki Amano<sup>1</sup>, Ikuo Yoshinaga<sup>1</sup>, Kentaro Okada<sup>1</sup>, Takao Yamagishi<sup>2</sup>, Shingo Ueda<sup>3</sup>, Akira Obuchi<sup>2</sup>, Yoshihiko Sako<sup>1</sup>, Yuichi Suwa<sup>2</sup>

<sup>1</sup>Laboratory of Marine Microbiology, Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Japan; <sup>2</sup>Research Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology, Japan; <sup>3</sup>College of Bioresource Sciences, Nihon University, Japan

A first assessment of anammox activity, which is a unique N<sub>2</sub> emission process, was conducted in samples of coastal marine sediment from Japan with a <sup>15</sup>N tracer. The occurrence and diversity of bacteria possibly responsible for the anammox process were also evaluated by selective PCR-amplification of the 16S rRNA gene for known anammox bacteria. Anammox activity, detected by measuring <sup>14</sup>N<sup>15</sup>N gas production, was only found in samples collected at the intertidal sand bank located at the Yodo River estuary. In the Yodo River samples, 16S rRNA gene fragments affiliated with the known anammox bacterial lineage were also recovered, and the two major phylotypes were both "*Candidatus* Scalindua wagneri" relatives with 95% and 98% sequence similarity. Even from the other samples in which no recognizable anammox activity was detected, 16S rRNA gene fragments related to known anammox bacteria, but not to "*Ca. S. wagneri*", were detected. This is the first report

of anammox-mediated  $\text{N}_2$  emission in coastal marine environments in Japan. Notably, the PCR-based analysis allowed us to discover unexpected phylogenetic diversity of anammox bacteria-related 16S rRNA gene sequences. The selective PCR primer set developed in this study could be a powerful tool to unveil the ecology of anammox bacteria in natural environments.



# MOST CITED REVIEWS 2015

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

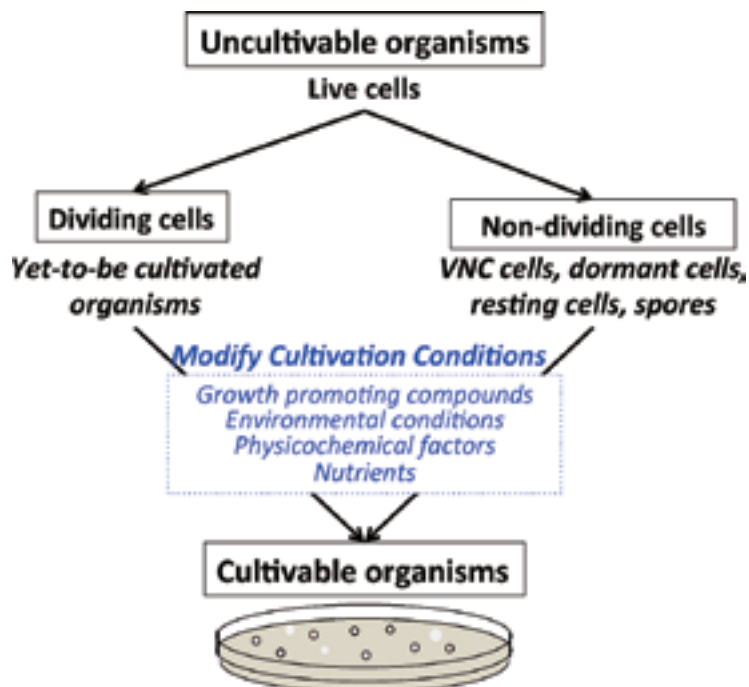
3-YEARS PERIOD

## Are Uncultivated Bacteria Really Uncultivable?

Indun Dewi Puspita<sup>1</sup>, Yoichi Kamagata<sup>1,2</sup>, Michiko Tanaka<sup>1</sup>, Kozo Asano<sup>1</sup>, Cindy H. Nakatsu<sup>1,3</sup>

<sup>1</sup>Graduate School of Agriculture, Hokkaido University, Japan; <sup>2</sup>Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan; and <sup>3</sup>Department of Agronomy, Purdue University, West Lafayette, Indiana 47907, USA

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. 23, No. 2, 118–127, 2008

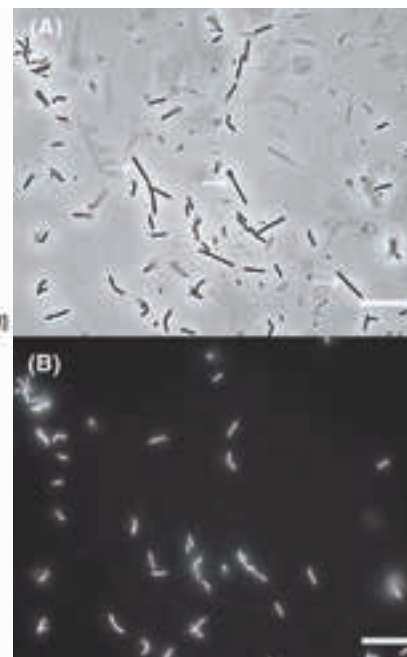
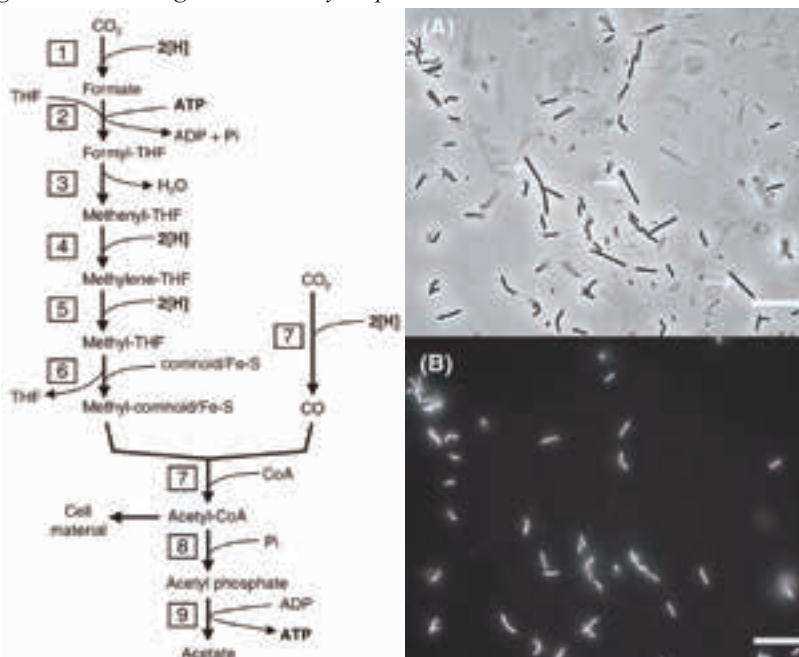
8-YEARS PERIOD

## Syntrophic Acetate-Oxidizing Microbes in Methanogenic Environments

Satoshi Hattori<sup>1</sup>

<sup>1</sup>Department of Bioresource Engineering, Faculty of Agriculture, Yamagata University, Japan

Acetate is one of the most important intermediates for methanogenesis in the anaerobic mineralization of organic materials. Methanogenic acetate degradation is carried out by either an aceticlastic reaction or an anaerobic acetate-oxidizing reaction. In contrast to the former reaction, the latter is energetically extremely unfavorable. However, the oxidation of acetate can occur with syntrophic interaction between certain bacteria and methanogenic archaea. The bacteria, namely syntrophic acetate-oxidizing bacteria, can oxidize acetate to produce hydrogen/ $\text{CO}_2$  only when their products are subsequently utilized by the hydrogen-scavenging methanogens. Surprisingly, some of these bacteria can also axenically grow on hydrogen/ $\text{CO}_2$  to produce acetate. This means that the bacteria can utilize both substrates and products reversibly. This review describes current studies of these curious and fascinating microbes.



SIGNATURE REVIEWS

Microbes Environ. Vol. 30, No. 2, 133–139, 2015

Biotechnological Aspects of Microbial Extracellular Electron Transfer

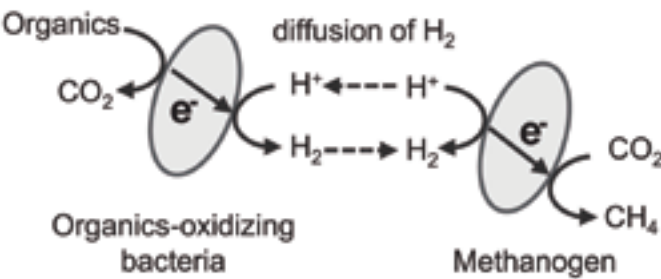
Souichiro Kato<sup>1,2,3</sup>

<sup>1</sup>Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan; <sup>2</sup>Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Japan; and <sup>3</sup>Research Center for Advanced Science and Technology, The University of Tokyo, Japan

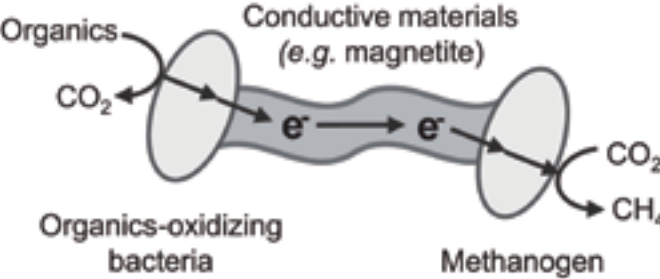
Extracellular electron transfer (EET) is a type of microbial respiration that enables electron transfer between microbial cells and extracellular solid materials, including naturally-occurring metal compounds and artificial electrodes. Microorganisms harboring EET abilities have received considerable attention for their various biotechnological applications, in addition to their contribution to global energy and material cycles. In this review, current knowledge on microbial EET and its application to

diverse biotechnologies, including the bioremediation of toxic metals, recovery of useful metals, biocorrosion, and microbial electrochemical systems (microbial fuel cells and microbial electrosynthesis), were introduced. Two potential biotechnologies based on microbial EET, namely the electrochemical control of microbial metabolism and electrochemical stimulation of microbial symbiotic reactions (electric syntrophy), were also discussed.

A. H<sub>2</sub>-dependent syntrophy



B. Electric syntrophy



Microbes Environ. Vol. 30, No. 2, 126–132, 2015

Effect of Probiotics/Prebiotics on Cattle Health and Productivity

Yutaka Uyeno<sup>1</sup>, Suguru Shigemori<sup>2,3</sup>, Takeshi Shimosato<sup>1,2</sup>

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Probiotics/prebiotics have the ability to modulate the balance and activities of the gastrointestinal (GI) microbiota, and are, thus, considered beneficial to the host animal and have been used as functional foods. Numerous factors, such as dietary and management constraints, have been shown to markedly affect the structure and activities of gut microbial communities in livestock animals. Previous studies reported the potential of probiotics and prebiotics in animal nutrition; however, their efficacies often vary and are inconsistent, possibly, in part, because the dynamics of the GI community have not been taken into consideration. Under stressed conditions, direct-fed microbials may be used to reduce the risk or severity of scours caused by disruption of the normal intestinal environment.

The observable benefits of prebiotics may also be minimal in generally healthy calves, in which the microbial community is relatively stable. However, probiotic yeast strains have been administered with the aim of improving rumen fermentation efficiency by modulating microbial fermentation pathways. This review mainly focused on the benefits of probiotics/prebiotics on the GI microbial ecosystem in ruminants, which is deeply involved in nutrition and health for the animal.

Table 1. Cattle GI microbial characteristics and relationships with host health and performance		
	Rumen	Large intestine
Major groups in microflora	Bacteroidetes Firmicutes Fibrobacter Archaea Protozoan species	in preweaned calves: Bacteroidetes Firmicutes Actinobacteria Bifidobacteria in weaned calves or older cattle: Bacteroidetes Firmicutes (including uncultured groups) Fibrobacter
Major microbial functions	Involved in host nutrition (digestion of fibrous plant material and anaerobic fermentation to short chain fatty acids, which can be used as an energy source by the host; microbial protein synthesis)	Immunological responses Digestion of polymers
Microbial dysfunctions	Overgrowth of lactate-producing bacteria, leading to a decrease in rumen pH and subsequent rumen acidosis Decrease in microbial activity by unbalanced nutrition, leading a decrease in feed protein efficacy	Pathogenesis by harmful bacteria, such as E. coli and Salmonella

# Recent Trends in Control Methods for Bacterial Wilt Diseases Caused by *Ralstonia solanacearum*

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<sup>1</sup>Research Center for Biology, Indonesian Institute of Sciences (LIPI), Indonesia; <sup>2</sup>Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Japan; and <sup>3</sup>Palangka Raya University Jl. Yos Sudarso, Center of Kalimantan, Palangka Raya, Indonesia

Previous studies have described the development of control methods against bacterial wilt diseases caused by *Ralstonia solanacearum*. This review focused on recent advances in control measures, such as biological, physical, chemical, cultural, and integral measures, as well as biocontrol efficacy and suppression mechanisms. Biological control agents (BCAs) have been dominated by bacteria (90%) and fungi (10%). Avirulent strains of *R. solanacearum*, *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp. are well-known BCAs. New or uncommon BCAs have also been identified such as *Acinetobacter* sp., *Burkholderia* sp., and *Paenibacillus* sp. Inoculation methods for BCAs affect biocontrol efficacy, such as pouring or drenching soil, dipping of roots, and seed coatings. The amendment of different organic matter, such as plant residue, animal waste,

and simple organic compounds, have frequently been reported to suppress bacterial wilt diseases. The combined application of BCAs and their substrates was shown to more effectively suppress bacterial wilt in the tomato. Suppression mechanisms are typically attributed to the antibacterial metabolites produced by BCAs or those present in natural products; however, the number of studies related to host resistance to the pathogen is increasing. Enhanced/modified soil microbial communities are also indirectly involved in disease suppression. New promising types of control measures include biological soil disinfection using substrates that release volatile compounds. This review described recent advances in different control measures. We focused on the importance of integrated pest management (IPM) for bacterial wilt diseases.

**Table 1.** Various biocontrol agents that have been tested in the field to control bacterial wilt diseases caused by *Ralstonia solanacearum* (2005–2014)

Microorganisms	Inoculation method and application rate	Mechanisms	BE (%)	Yield*	Ref
1. <i>Bacillus amyloliquefaciens</i> SQR-7 and SQR-101 and <i>B. methylotrophicus</i> SQR-29	Pouring, $6.8 \times 10^{10}$ cfu plant <sup>-1</sup> (SQR-7), $7.5 \times 10^{10}$ cfu plant <sup>-1</sup> (SQR-101), $8.2 \times 10^{10}$ cfu plant <sup>-1</sup> (SQR-7)	Production of indole acetic acid and siderophores	18–60% in tobacco	25–38%	143
2. <i>Ralstonia pickettii</i> QL-A6	Stem injection, 10 $\mu$ L of $10^7$ CFU mL <sup>-1</sup>	Competition	73% in the tomato	NA	125
3. <i>Pseudomonas monteilii</i> (A) + <i>Glomus fasciculatum</i> (B)	Stem cuttings were dipped in A ( $9.1 \times 10^6$ mL <sup>-1</sup> ), B (53 infective propagules) was added to each cutting, and A was then poured again	Increased plant nutrient uptake (N, P, K) and reduced the pathogen population	56–75% in herbs ( <i>Coleus forskohli</i> )	54%	111
4. <i>Brevibacillus brevis</i> L-25 + <i>Streptomyces roche</i> L-9 + organic fertilizer	Mixed with soil at a density of $7.3 \times 10^7$ (L-25) and $5.0 \times 10^5$ (L-9) cfu g <sup>-1</sup> of soil	Decreased root colonization by the pathogen	30–95% in tobacco	87–100%	76
5. <i>Bacillus amyloliquefaciens</i> + bio-organic fertilizer (BIO23) <i>B. subtilis</i> + bio-organic fertilizer (BIO36)	Mixed with soil at a density of $5.5 \times 10^6$ (BIO23) and $7.0 \times 10^6$ (BIO36) cfu g <sup>-1</sup> of soil	Plant growth promotion	58–66% in the potato	64–65%	26
6. <i>Bacillus</i> sp. (RCh6) <i>Pseudomonas mallei</i> (RBG4)	$3 \times 10^6$ cfu g <sup>-1</sup> (talc formulation). Seedlings were dipped in antagonist suspension (25 g talc formulation L <sup>-1</sup> ). Leftover suspension was poured around the root zone of the seedling (50 mL plant <sup>-1</sup> )	Production of inhibitory compounds and siderophores	81% in the eggplant	60–90%	103
7. <i>Trichoderma viride</i> (A), <i>B. subtilis</i> (B), <i>Azotobacter chroococcum</i> (C), <i>Glomus fasciculatum</i> (D), <i>P. fluorescens</i> (E)	D (53 infective propagules) was added to each stem cutting that was dipped in A ( $1.2 \times 10^6$ CFU mL <sup>-1</sup> ), B ( $1.8 \times 10^6$ CFU mL <sup>-1</sup> ), C ( $2.3 \times 10^7$ CFU mL <sup>-1</sup> ), and E ( $2.5 \times 10^8$ CFU mL <sup>-1</sup> ). A total of 5 mL of A, B, C, and E was then poured into 200 g soil.	Competition for nutrient uptake (NPK) and reduced <i>R. solanacearum</i> population	7–43% in herbs ( <i>Coleus forskohli</i> )	159–227%	110
8. <i>B. amyloliquefaciens</i> QL-5, QL-18 + organic fertilizer	Mixed with soil at a density of $1 \times 10^7$ (QL-5) or $1 \times 10^7$ (QL-18) cfu g <sup>-1</sup> of soil	Decreased root colonization by the pathogen	17–87% in the tomato	NA	124
9. <i>B. amyloliquefaciens</i> Bg-C31	Poured 10 mL of bacterial suspension plant <sup>-1</sup> (potato dextrose broth culture).	Production of antimicrobial proteins	60–80% in Capsicum	NA	44
10. <i>Acinetobacter</i> sp. Xa6, <i>Enterobacter</i> sp. Xy3	Poured 20 mL of the bacterial suspension ( $1 \times 10^6$ cells mL <sup>-1</sup> ) plant <sup>-1</sup> or seedling roots were soaked in the bacterial suspension.	Rhizocompetence and root colonization	57–67% in the tomato	32–41%	130
11. <i>B. vallismortis</i> ExTN-1	Bacterial suspension was mixed into an organic fertilizer ( $10^6$ cfu mL <sup>-1</sup> ) and poured onto soil.	Induction of systemic resistance	48–49% in the tomato	17%	119
12. <i>Glomus mosseae</i>	A total of 30 g of the inoculum (650–700 spores of <i>G. mosseae</i> 100 g <sup>-1</sup> soil) was added to a planting hole.	Competition for nutrients and decreased pathogen population	25% in the tomato	16%	113

BE: biological control efficacy, NA: not applicable, Yield\*: increase in yield

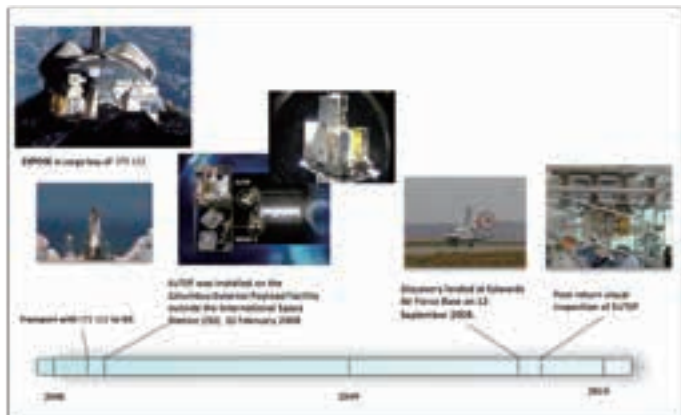
## Microbial Existence in Controlled Habitats and Their Resistance to Space Conditions

Kasthuri Venkateswaran<sup>1</sup>, Myron T. La Duc<sup>1</sup>, and Gerda Horneck<sup>2</sup>

<sup>1</sup>*Biotechnology and Planetary Protection Group, California Institute of Technology, Jet Propulsion Laboratory, Pasadena, CA 91109, USA; and* <sup>2</sup>*Institute of Aerospace Medicine, DLR (German Aerospace Center), D 51170, Köln, Germany*

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.



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

Nobuyasu Yamaguchi<sup>1</sup>, Michael Roberts<sup>2</sup>, Sarah Castro<sup>3</sup>, Cherie Oubre<sup>4</sup>, Koichi Makimura<sup>5</sup>, Natalie Lays<sup>6</sup>, Elisabeth Grohmann<sup>7</sup>, Takashi Sugita<sup>8</sup>, Tomoaki Ichijo<sup>1</sup>, and Masao Nasu<sup>1</sup>

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<sup>3</sup>*Biomedical Research and Environmental Sciences Division, NASA Johnson Space Center, Houston, TX, USA; <sup>4</sup>Science, Technology & Engineering Group, Wyle, Texas, USA; <sup>5</sup>Laboratory of Space and Environmental Medicine, Graduate School of Medicine, Teikyo University, Japan; <sup>6</sup>Molecular and Cellular Microbiology, Belgian Nuclear Research Center SCK-CEN, Mol, Belgium;*

<sup>7</sup>*Division of Infectious Diseases, University Medical Centre Freiburg, Germany; and <sup>8</sup>Department of Microbiology, Meiji Pharmaceutical University, Japan*

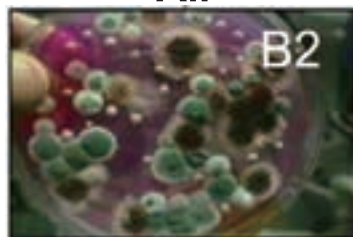
Previous space research conducted during short-term flight experiments and long-term environmental monitoring on board orbiting space stations suggests that the relationship between humans and microbes is altered in the crewed habitat in space. Both human physiology and microbial communities adapt to 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.

### Surface



### Air



### Water



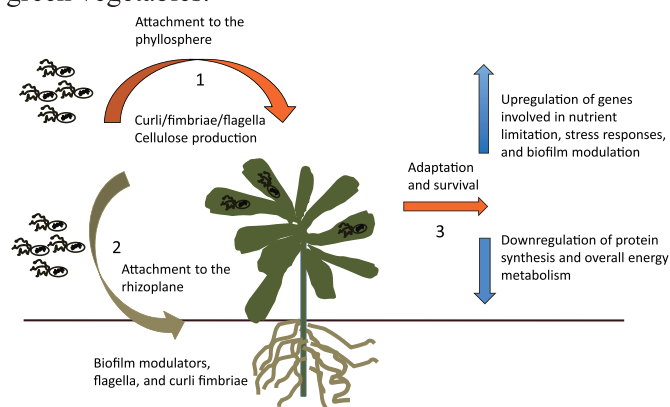
# Enteric Pathogen-Plant Interactions: Molecular Connections Leading to Colonization and Growth and Implications for Food Safety

Betsy M. Martínez-Vaz<sup>1</sup>, Ryan C. Fink<sup>2</sup>, Francisco Diez-Gonzalez<sup>2</sup>, Michael J. Sadowsky<sup>3,4</sup>

<sup>1</sup>Department of Biology, Hamline University, MN, USA; <sup>2</sup>Department of Food Science and Nutrition, University of Minnesota, MN, USA; <sup>3</sup>Biotechnology Institute, University of Minnesota, MN, USA; and <sup>4</sup>Department of Soil, Water and Climate, University of Minnesota, MN, USA

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.



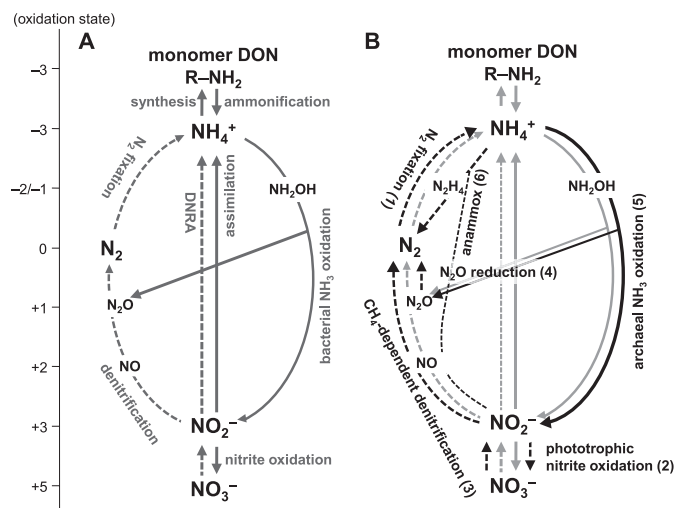
# Ecological Perspectives on Microbes Involved in N-Cycling

Kazuo Isobe<sup>1</sup>, Nobuhito Ohte<sup>2</sup>

<sup>1</sup>Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan; and <sup>2</sup>Department of Forest Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan

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.



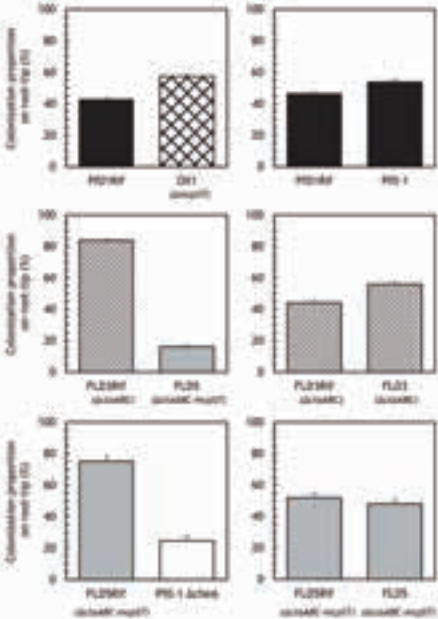
PLANT-MICROBE INTERACTION

Microbes Environ. Vol. 29, No. 4, 413–419, 2014

Identification of *Pseudomonas fluorescens* Chemotaxis Sensory Proteins for Malate, Succinate, and Fumarate, and Their Involvement in Root Colonization

Shota Oku<sup>1</sup>, Ayaka Komatsu<sup>1</sup>, Yutaka Nakashimada<sup>1</sup>, Takahisa Tajima<sup>1</sup>, Junichi Kato<sup>1</sup>  
<sup>1</sup>Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Japan.

*Pseudomonas fluorescens* Pf0-1 exhibited chemotactic responses to L-malate, succinate, and fumarate. We constructed a plasmid library of 37 methyl-accepting chemotaxis protein (MCP) genes of *P. fluorescens* Pf0-1. To identify a MCP for L-malate, the plasmid library was screened using the PA2652 mutant of *Pseudomonas aeruginosa* PAO1, a mutant defective in chemotaxis to L-malate. The introduction of Pfl01\_0728 and Pfl01\_3768 genes restored the ability of the PA2652 mutant to respond to L-malate. The introduction of Pfl01\_0728 and Pfl01\_3768 genes restored the ability of the PA2652 mutant to respond to L-malate. The Pfl01\_0728 and Pfl01\_3768 double mutant of *P. fluorescens* Pf0-1 showed no response to L-malate or succinate, while the Pfl01\_0728 single mutant did not respond to fumarate. These results indicated that Pfl01\_0728 and Pfl01\_3768 were the major MCPs for L-malate and succinate, and Pfl01\_0728 was also a major MCP for fumarate. The Pfl01\_0728 and Pfl01\_3768 double mutant unexpectedly exhibited stronger responses toward the tomato root exudate and amino acids such as proline, asparagine, methionine, and phenylalanine than those of the wild-type strain. The *ctaA*, *ctaB*, *ctaC* (genes of the major MCPs for amino acids), Pfl01\_0728, and Pfl01\_3768 quintuple mutant of *P. fluorescens* Pf0-1 was less competitive than the *ctaA ctaB ctaC* triple mutant in competitive root colonization, suggesting that chemotaxis to L-malate, succinate, and/or fumarate was involved in tomato root colonization by *P. fluorescens* Pf0-1.



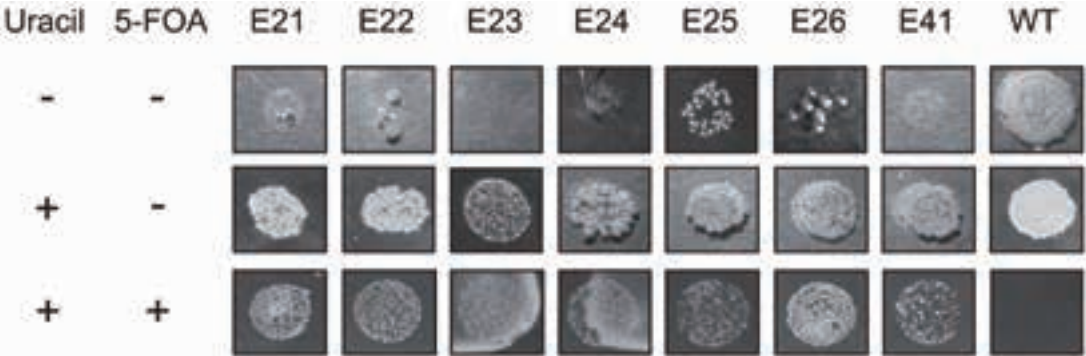
Microbes Environ. Vol. 29, No. 1, 31–37, 2014

Isolation of Mutants of the Nitrogen-Fixing Actinomycete *Frankia*

Kentaro Kakoi<sup>1</sup>, Masatoshi Yamaura<sup>1</sup>, Toshihito Kamiharai<sup>1</sup>, Daiki Tamari<sup>2</sup>, Mikiko Abe<sup>1</sup>, Toshiki Uchiumi<sup>1</sup>, Ken-Ichi Kucho<sup>1</sup>  
<sup>1</sup>Graduate School of Science and Engineering, Kagoshima University, Japan; and <sup>2</sup>Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, Japan

*Frankia* is a nitrogen (N)-fixing multicellular actinomycete which establishes root-nodule symbiosis with actinorhizal plants. Several aspects of *Frankia* N fixation and symbiosis are distinct, but genes involved in the specific features are largely unknown because of the lack of an efficient mutant screening method. In this study, we isolated mutants of *Frankia* sp. strain CcI3 using hyphae fragments mutagenized by chemical mutagens. Firstly, we isolated uracil auxotrophs as gain-of-function mutants resistant to 5-fluoroorotic acid (5-FOA). We obtained seven 5-FOA resistant mutants, all of which required uracil for growth. Five strains carried a frame shift mutation in orotidine-5'-phosphate decarboxylase gene and two carried an amino acid substitution in the orotate

phosphoribosyltransferase gene. Secondly, we isolated mutants showing loss-of-function phenotypes. Mutagenized hyphae were fragmented by ultrasound and allowed to multiply at their tips. Hyphae were fragmented again and short fragments were enriched by filtration through 5 μm pores filters. Next-generation and Sanger sequencing revealed that colonies formed from the short hyphae fragments consisted of cells with an identical genotype. From the mutagenized colony population, we isolated three pigmentation mutants and a mutant with reduced N-fixation activity. These results indicate that our procedure is useful for the isolation of loss-of-function mutants using hyphae of *Frankia*.



## The Tomato Wilt Fungus *Fusarium oxysporum* f. sp. *lycopersici* Shares Common Ancestors with Nonpathogenic *F. oxysporum* isolated from Wild Tomatoes in the Peruvian Andes

Keigo Inami<sup>1</sup>, Takeshi Kashiwa<sup>1</sup>, Masato Kawabe<sup>2</sup>, Akiko Onokubo-Okabe<sup>1</sup>, Nobuko Ishikawa<sup>1</sup>, Enrique Rodríguez Pérez<sup>3</sup>, Takuo Hozumi<sup>4</sup>, Liliana Aragón Caballero<sup>5</sup>, Fatima Cáceres de Baldarrago<sup>6</sup>, Mauricio Jiménez Roco<sup>7</sup>, Khalid A. Madadi<sup>8</sup>, Tobin L. Peever<sup>9</sup>, Tohru Teraoka<sup>1</sup>, Motoichiro Kodama<sup>10</sup>, Tsutomu Arie<sup>1</sup>

<sup>1</sup>Graduate school of Agriculture, Tokyo University of Agriculture and Technology, Japan; <sup>2</sup>National Agricultural Research Center (NARC), Japan; <sup>3</sup>Department of Plant Breeding, Chapingo Autonomous University, Mexico; <sup>4</sup>Interdisciplinary Research Center for Environment and Rural Service, Chapingo Autonomous University, Mexico; <sup>5</sup>Department of Phytopathology, National Agrarian University—La Molina, Peru; <sup>6</sup>Department of Biology, National University of San Agustín, Peru; <sup>7</sup>Tarapacá University, a la Casilla 6-D, Chile; <sup>8</sup>Kabul University, Afghanistan; <sup>9</sup>Department of Plant Pathology, Washington State University, USA; and <sup>10</sup>Department of Agriculture, Tottori University, Japan

*Fusarium oxysporum* is an ascomycetous fungus that is well-known as a soilborne plant pathogen. In addition, a large population of nonpathogenic *F. oxysporum* (NPF) inhabits various environmental niches, including the phytosphere. To obtain an insight into the origin of plant pathogenic *F. oxysporum*, we focused on the tomato (*Solanum lycopersicum*) and its pathogenic *F. oxysporum* f. sp. *lycopersici* (FOL). We collected *F. oxysporum* from wild and transition *Solanum* spp. and modern cultivars of tomato in Chile, Ecuador, Peru, Mexico, Afghanistan, Italy, and Japan, evaluated the fungal isolates for pathogenicity, VCG, mating type, and distribution of *SIX* genes related to the pathogenicity of FOL, and constructed phylogenies based on ribosomal DNA intergenic spacer sequences. All *F. oxysporum* isolates sampled were genetically more diverse than FOL. They were not pathogenic to the tomato and did not carry *SIX* genes. Certain NPF isolates including those from wild *Solanum* spp. in Peru were grouped in FOL clades, whereas most of the NPF isolates were not. Our results suggested that the population of NPF isolates in FOL clades gave rise to FOL by gaining pathogenicity.

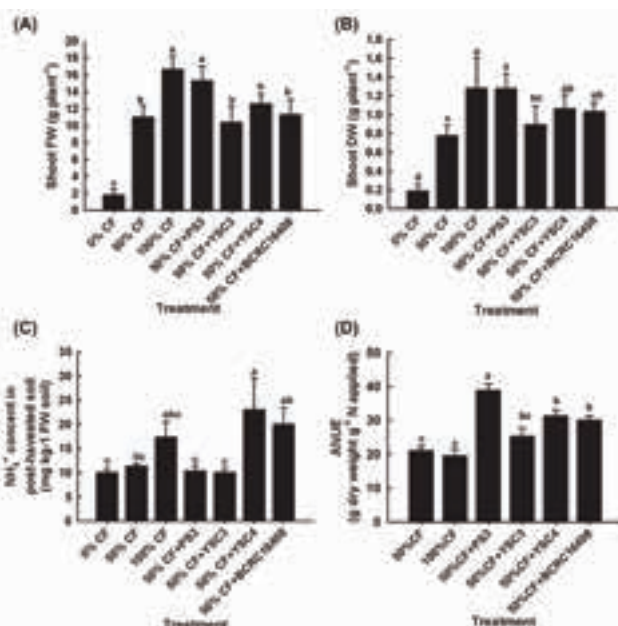


## Promoting Effects of a Single *Rhodopseudomonas palustris* Inoculant on Plant Growth by *Brassica rapa chinensis* under Low Fertilizer Input

Wai-Tak Wong<sup>1</sup>, Ching-Han Tseng<sup>2</sup>, Shu-Hua Hsu<sup>2</sup>, Huu-Sheng Lur<sup>1</sup>, Chia-Wei Mo<sup>2</sup>, Chu-Ning Huang<sup>3</sup>, Shu-Chiung Hsu<sup>4</sup>, Kung-Ta Lee<sup>5</sup>, Chi-Te Liu<sup>2,6</sup>

<sup>1</sup>Department of Agronomy, National Taiwan University, Taiwan; <sup>2</sup>Institute of Biotechnology, National Taiwan University, Taiwan; <sup>3</sup>Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan; <sup>4</sup>Great Victory Chemical Industry Co., Ltd. Taiwan; <sup>5</sup>Department of Biochemical Science and Technology, National Taiwan University, Taiwan; and <sup>6</sup>Agricultural Biotechnology Research Center, Academia Sinica, Taiwan

Several *Rhodopseudomonas palustris* strains have been isolated from rice paddy fields in Taiwan by combining the Wino-gradsky column method and molecular marker detection. These isolates were initially screened by employing seed germination and seedling vigor assays to evaluate their potential as inoculants. To fulfill the demand in the present farming system for reducing the application of chemical fertilizers, we assessed the plant growth-promoting effects of the *R. palustris* YSC3, YSC4, and PS3 inoculants on *Brassica rapa chinensis* (Chinese cabbage) cultivated under a half quantity of fertilizer. The results obtained showed that supplementation with approximately  $4.0 \times 10^6$  CFU g<sup>-1</sup> soil of the PS3 inoculant at half the amount of fertilizer consistently produced the same plant growth potential as 100% fertility, and also increased the nitrogen use efficiency of the applied fertilizer nutrients. Furthermore, we noted that the plant growth-promotion rate elicited by PS3 was markedly higher with old seeds than with new seeds, suggesting it has the potential to boost the development of seedlings that were germinated from carry-over seeds of poor quality. These beneficial traits suggest that the PS3 isolate may serve as a potential PGPR inoculant for integrated nutrient management in agriculture.



# Metagenomic Analysis of the Bacterial Community Associated with the Taproot of Sugar Beet

Hirohito Tsurumaru<sup>1</sup>, Takashi Okubo<sup>1</sup>, Kazuyuki Okazaki<sup>2</sup>, Megumi Hashimoto<sup>1</sup>, Kaori Kakizaki<sup>1</sup>, Eiko Hanzawa<sup>1</sup>, Hiroyuki Takahashi<sup>2</sup>, Noriyuki Asanome<sup>3</sup>, Fukuyo Tanaka<sup>4</sup>, Yasuyo Sekiyama<sup>5</sup>, Seishi Ikeda<sup>2</sup>, Kiwamu Minamisawa<sup>1</sup>

<sup>1</sup>Graduate School of Life Science, Tohoku University, Japan; <sup>2</sup>Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Japan; <sup>3</sup>Yamagata Integrated Agricultural Research Center, Yamagata, Japan; <sup>4</sup>Agricultural Research Center, National Agriculture and Food Research Organization, Japan; and <sup>5</sup>National Food Research Institute, National Agriculture and Food Research Organization, Japan

We analyzed a metagenome of the bacterial community associated with the taproot of sugar beet (*Beta vulgaris* L.) in order to investigate the genes involved in plant growth-promoting traits (PGPTs), namely 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA), N<sub>2</sub> fixation, phosphate solubilization, pyrroloquinoline quinone, siderophores, and plant disease suppression as well as methanol, sucrose, and betaine utilization. The most frequently detected gene among the PGPT categories encoded β-1,3-glucanase (18 per 10<sup>5</sup> reads), which plays a role in the suppression of plant diseases. Genes involved in phosphate solubilization (e.g., for quinoprotein glucose dehydrogenase), methanol utilization (e.g., for methanol dehydrogenase), siderophore production (e.g. isochorismate pyruvate lyase), and ACC deaminase were also abundant. These results suggested that such PGPTs are crucially involved in supporting the growth of sugar beet. In contrast, genes for IAA production

(*iaaM* and *ipdC*) were less abundant (~1 per 10<sup>5</sup> reads). N<sub>2</sub> fixation genes (*nifHDK*) were not detected; bacterial N<sub>2</sub>-fixing activity was not observed in the <sup>15</sup>N<sub>2</sub>-feeding experiment. An analysis of nitrogen metabolism suggested that the sugar beet microbiome mainly utilized ammonium and nitroalkane as nitrogen sources. Thus, N<sub>2</sub> fixation and IAA production did not appear to contribute to sugar beet growth. Taxonomic assignment of this metagenome revealed the high abundance of *Mesorhizobium*, *Bradyrhizobium*, and *Streptomyces*, suggesting that these genera have ecologically important roles in the taproot of sugar beet. *Bradyrhizobium*-assigned reads in particular were found in almost all categories of dominant PGPTs with high abundance. The present study revealed the characteristic functional genes in the taproot-associated microbiome of sugar beet, and suggest the opportunity to select sugar beet growth-promoting bacteria.

Table 2. Detection frequency of potential functional genes in the bacterial community of sugar beet taproot

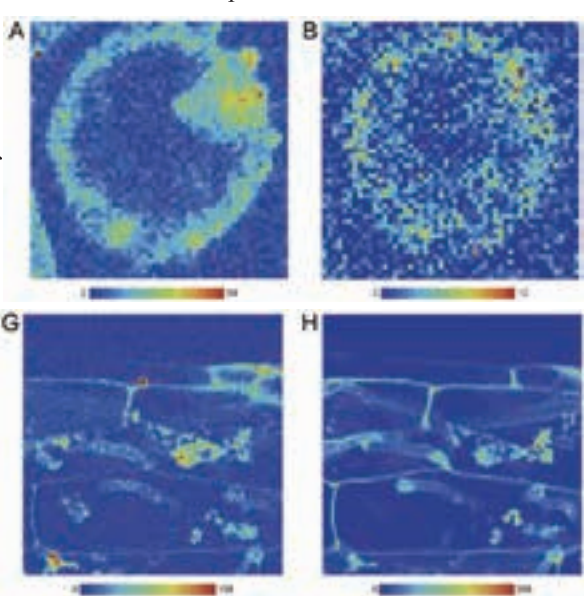
	ID of KEGG	Gene length <sup>a</sup>		Frequency per 10 <sup>5</sup> reads <sup>b</sup>
		ID of UniRef	length (kbp)	
ACC deaminase	K01505	UniRef50_A5EJ46	1.020	9±6
IAA				1±0.5
tryptophan 2-monooxygenase ( <i>iaaM</i> )	K00466	UniRef50_A6W7Y1	1.707	0.7±0.5
indolepyruvate decarboxylase ( <i>ipdC</i> )	K04103	UniRef50_J0BL41	1.755	0.8±0.6
N <sub>2</sub> fixation <sup>c</sup>				ND
nitrogenase iron protein ( <i>nifH</i> )	K02588	UniRef50_O07641	0.984	ND
nitrogenase molybdenum-iron protein alpha chain ( <i>nifD</i> )	K02586	UniRef50_P19066	1.515	ND
nitrogenase molybdenum-iron protein beta chain ( <i>nifK</i> )	K02591	UniRef50_P25314	1.566	ND
nitrogenase delta subunit ( <i>nifG</i> )	K00531	UniRef50_Q68940	0.351	ND
PQQ biosynthesis protein ( <i>pqqC</i> ) <sup>d</sup>		UniRef50_Q6F9J1	0.768	3±0.4

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

Keiichiro Nayuki<sup>1,2</sup>, Baodong Chen<sup>3</sup>, Ryo Ohtomo<sup>3</sup>, Yukari Kuga<sup>1</sup>

<sup>1</sup>Graduate School of Integrated Arts and Sciences, Hiroshima University, Japan; <sup>2</sup>Graduate School of Agriculture, Shinshu University, Japan; and <sup>3</sup>Nasu Research Station, NARO Institute of Livestock and Grassland Science, Japan

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.



# MICROBIAL PHYSIOLOGY

Microbes Environ. Vol. 29, No. 2, 162–167, 2014

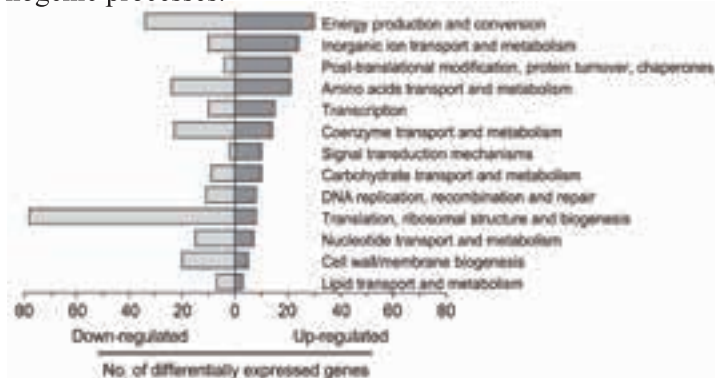
## Physiological and Transcriptomic Analyses of the Thermophilic, Aceticlastic Methanogen *Methanosaeta thermophila* Responding to Ammonia Stress

Souichiro Kato<sup>1,2,3</sup>, Konomi Sasaki<sup>1,4</sup>, Kazuya Watanabe<sup>3,5</sup>, Isao Yumoto<sup>1</sup>, Yoichi Kamagata<sup>1,2</sup>

<sup>1</sup>Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan; <sup>2</sup>Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Japan; <sup>3</sup>Research Center for Advanced Science and Technology, The University of Tokyo, Japan; and <sup>4</sup>Hokkaido High-Technology College, Japan, <sup>5</sup>School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Japan

The inhibitory effects of ammonia on two different degradation pathways of methanogenic acetate were evaluated using a pure culture (*Methanosaeta thermophila* strain P<sub>T</sub>) and defined co-culture (*Methanothermobacter thermautotrophicus* strain TM and *Thermacetogenium phaeum* strain PB), which represented aceticlastic and syntrophic methanogenesis, respectively. Growth experiments with high concentrations of ammonia clearly demonstrated that sensitivity to ammonia stress was markedly higher in *M. thermophila* P<sub>T</sub> than in the syntrophic co-culture. *M. thermophila* P<sub>T</sub> also exhibited higher sensitivity to high pH stress, which indicated that an inability to maintain pH homeostasis is an underlying cause of ammonia inhibition. Methanogenesis was inhibited in the resting cells of *M. thermophila* P<sub>T</sub> with moderate concentrations of ammonia, suggesting that the inhibition of enzymes involved in methanogenesis may be one of the major factors responsible for ammonia toxicity. Transcriptomic analysis revealed a broad range of disturbances in *M. thermophila* P<sub>T</sub> cells under ammonia stress conditions, including protein denaturation,

oxidative stress, and intracellular cation imbalances. The results of the present study clearly demonstrated that syntrophic acetate degradation dominated over aceticlastic methanogenesis under ammonia stress conditions, which is consistent with the findings of previous studies on complex microbial community systems. Our results also imply that the co-existence of multiple metabolic pathways and their different sensitivities to stress factors confer resiliency on methanogenic processes.



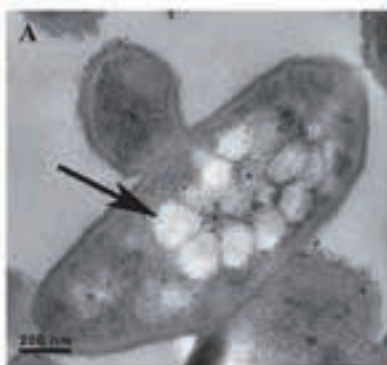
Microbes Environ. Vol. 30, No. 1, 76–85, 2015

## Bioconversion of Styrene to Poly(hydroxyalkanoate) (PHA) by the New Bacterial Strain *Pseudomonas putida* NBUS12

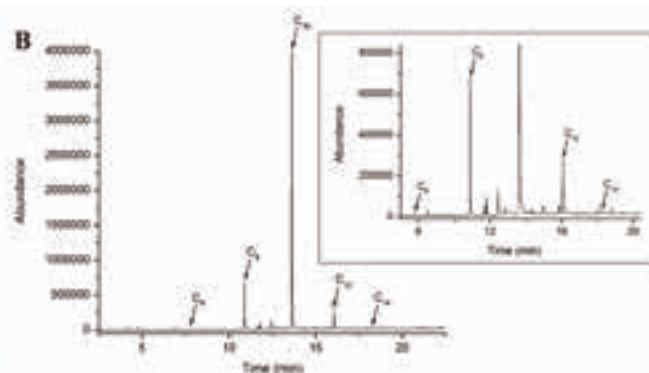
Giin-Yu Amy Tan<sup>1,2</sup>, Chia-Lung Chen<sup>1</sup>, Liya Ge<sup>1</sup>, Ling Li<sup>1</sup>, Swee Ngin Tan<sup>3</sup>, Jing-Yuan Wang<sup>1,2</sup>

<sup>1</sup>Residues and Resource Reclamation Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, Singapore; <sup>2</sup>Division of Environmental and Water Resources Engineering, School of Civil and Environmental Engineering, Nanyang Technological University, Singapore; and <sup>3</sup>Natural Sciences and Science Education Academic Group, Nanyang Technological University, Singapore

Styrene is a toxic pollutant commonly found in waste effluents from plastic processing industries. We herein identified and characterized microorganisms for bioconversion of the organic eco-pollutant styrene into a valuable biopolymer medium-chain-length poly(hydroxyalkanoate) (mcl-PHA). Twelve newly-isolated styrene-degrading *Pseudomonads* were obtained and partial *phaC* genes were detected by PCR in these isolates. These isolates assimilated styrene to produce mcl-PHA, forming PHA contents between 0.05±0.00 and 23.10±3.25% cell dry mass (% CDM). The best-performing isolate was identified as *Pseudomonas putida* NBUS12. A genetic analysis of 16S rDNA and *phaZ* genes revealed *P. putida* NBUS12 as a genetically-distinct strain from existing phenotypically-similar bacterial strains.



This bacterium achieved a final biomass of 1.28±0.10 g L<sup>-1</sup> and PHA content of 32.49±2.40% CDM. The extracted polymer was mainly comprised of 3-hydroxyhexanoate (C<sub>6</sub>), 3-hydroxyoctanoate (C<sub>8</sub>), 3-hydroxydecanoate (C<sub>10</sub>), 3-hydroxydodecanoate (C<sub>12</sub>), and 3-hydroxytetradecanoate (C<sub>14</sub>) monomers at a ratio of 2:42:1257:17:1. These results collectively suggested that *P. putida* NBUS12 is a promising candidate for the biotechnological conversion of styrene into mcl-PHA.



## GUT ENVIRONMENT

Microbes Environ. Vol. 30, No. 3, 235–244, 2014

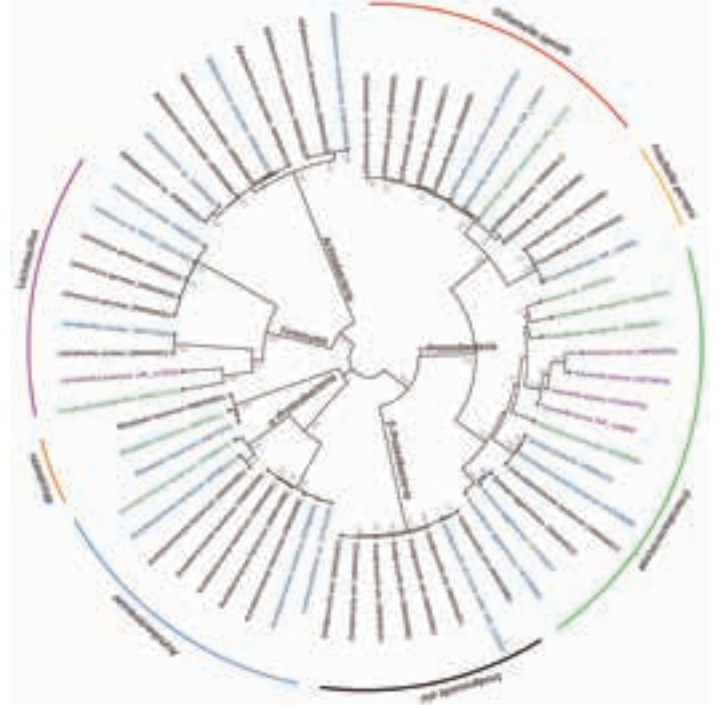
### Shifts in the Midgut/Pyloric Microbiota Composition within a Honey Bee Apiary throughout a Season

Jane Ludvigsen<sup>1</sup>, Anbjørg Rangberg<sup>1</sup>, Ekaterina Avershina<sup>1</sup>, Monika Sekelja<sup>1,2</sup>, Claus Kreibich<sup>1</sup>, Gro Amdam<sup>1,3</sup>, Knut Rudi<sup>1</sup>

<sup>1</sup>Norwegian University of Life Sciences, Chemistry, Biotechnology and Food science department, Norway; <sup>2</sup>Genetic Analysis Norway; and <sup>3</sup>School of Life Sciences, Arizona State University, USA

Honey bees (*Apis mellifera*) are prominent crop pollinators and are, thus, important for effective food production. The honey bee gut microbiota is mainly host specific, with only a few species being shared with other insects. It currently remains unclear how environmental/dietary conditions affect the microbiota within a honey bee population over time. Therefore, the aim of the present study was to characterize the composition of the midgut/pyloric microbiota of a honey bee apiary throughout a season. The rationale for investigating the midgut/pyloric microbiota is its dynamic nature. Monthly sampling of a demographic homogenous population of bees was performed between May and October, with concordant recording of the honey bee diet. Mixed Sanger- and Illumina 16S rRNA gene sequencing in combination with a quantitative PCR analysis were used to determine the bacterial composition. A marked increase in  $\alpha$ -diversity was detected between May and June. Furthermore, we found that four distinct phylotypes belonging to the *Proteobacteria* dominated the microbiota, and these displayed major shifts throughout the season. *Gilliamella apicola* dominated the composition early on, and *Snodgrassella alvi* began to dominate when the other bacteria declined to an absolute low in October. *In vitro* co-culturing revealed that *G. apicola* suppressed *S. alvi*. No shift was detected in the composition of the

microbiota under stable environment/dietary conditions between November and February. Therefore, environmental/dietary changes may trigger the shifts observed in the honey bee midgut/pyloric microbiota throughout a season.



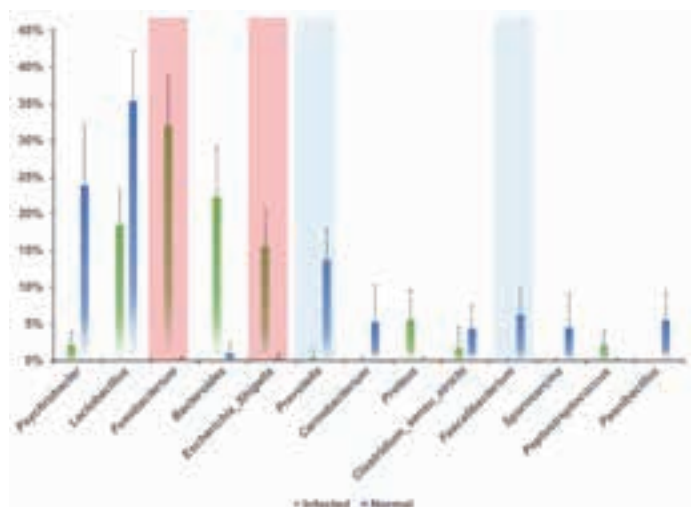
Microbes Environ. Vol. 30, No. 3, 284–287, 2015

### Changes in the Swine Gut Microbiota in Response to Porcine Epidemic Diarrhea Infection

Hyeon-Woo Koh<sup>1</sup>, Myun Soo Kim<sup>2</sup>, Jong-Soo Lee<sup>3</sup>, Hongik Kim<sup>2</sup>, Soo-Je Park<sup>1</sup>

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The gastrointestinal tract of mammals is a complex ecosystem with distinct environments and comprises hundreds of different types of bacterial cells. The gut microbiota may play a critical role in the gut health of the host. We herein attempted to identify a microbiota shift that may be affected by porcine epidemic diarrhea (PED). We observed significant differences in microbiota between the control and PED virus (PEDV)-infected groups at both the phylum and genus level. Most commensal bacteria (i.e. *Psychrobacter*, *Prevotella*, and *Faecalibacterium*) in the healthy gastrointestinal tract were decreased due to dysbiosis induced by PEDV infection.



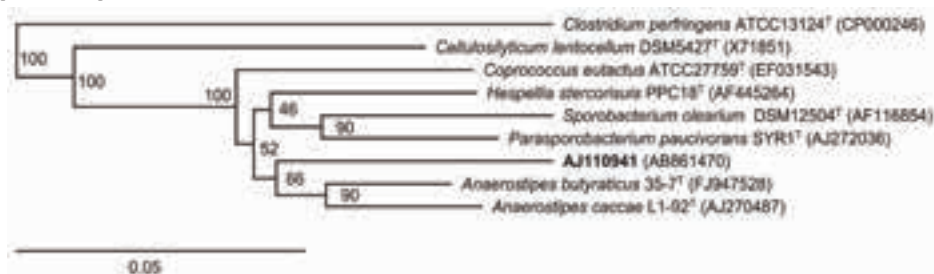
## Intestinal Colonization by a *Lachnospiraceae* Bacterium Contributes to the Development of Diabetes in Obese Mice

Keishi Kameyama<sup>1</sup>, Kikuji Itoh<sup>2</sup>

<sup>1</sup>Institute for Innovation, Ajinomoto Co., Inc., Japan; and <sup>2</sup>Department of Veterinary Public Health, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan

The aim of the present study was to identify bacteria that may contribute to the onset of metabolic dysfunctions. We isolated and identified a candidate bacterium belonging to *Lachnospiraceae* (strain AJ110941) in the feces of hyperglycemic obese mice. The colonization of germ-free *ob/ob* mice by AJ110941 induced significant increases in fasting blood glucose levels as well as liver

and mesenteric adipose tissue weights, and decreases in plasma insulin levels and HOMA- $\beta$  values. These results indicated that the specific gut commensal bacterium AJ110941 influenced the development of obesity and diabetes in *ob/ob* mice with genetic susceptibility for obesity.



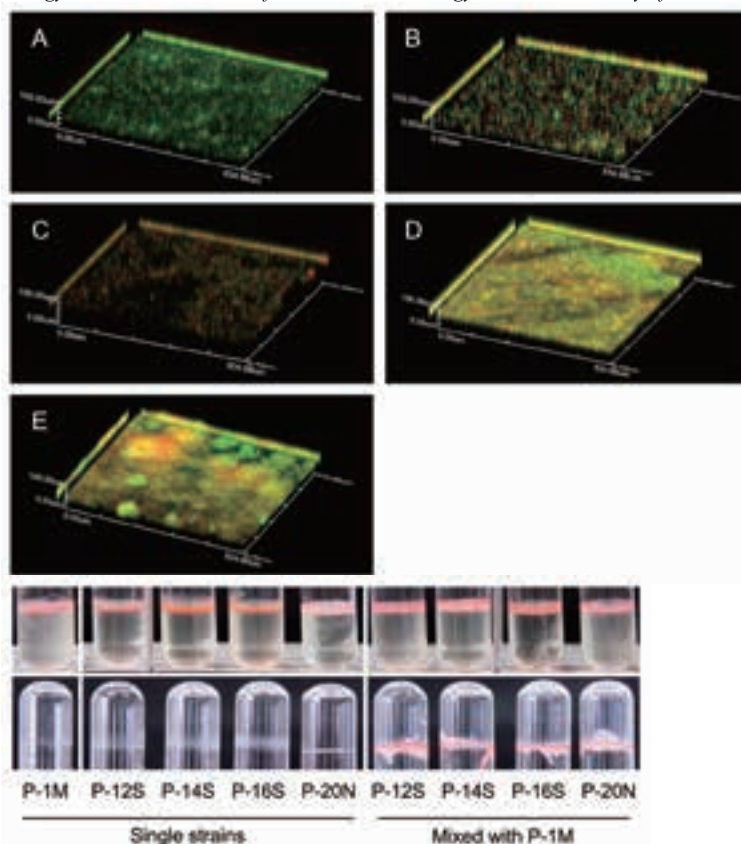
## BIOFILM

## Evaluation of Intraspecies Interactions in Biofilm Formation by *Methylobacterium* Species Isolated from Pink-Pigmented Household Biofilms

Fang-Fang Xu<sup>1,2,3</sup>, Tomohiro Morohoshi<sup>1,3</sup>, Wen-Zhao Wang<sup>1,2</sup>, Yuka Yamaguchi<sup>1</sup>, Yan Liang<sup>2</sup>, Tsukasa Ikeda<sup>1,3</sup>

<sup>1</sup>Department of Material and Environmental Chemistry, Graduate School of Engineering, Utsunomiya University, Japan; <sup>2</sup>Laboratory for Food Safety and Environmental Technology, Institutes of Biomedicine and Biotechnology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, China; and <sup>3</sup>JST, CREST, Japan

Concern regarding household biofilms has grown due to their widespread existence and potential to threaten human health by serving as pathogen reservoirs. Previous studies identified *Methylobacterium* as one of the dominant genera found in household pink slime. In the present study, we examined the mechanisms underlying biofilm formation by using the bacterial consortium found in household pink slime. A clone library analysis revealed that *Methylobacterium* was the predominant genus in household pink slime. In addition, 16 out of 21 pink-pigmented bacterial isolates were assigned to the genus *Methylobacterium*. Although all of the *Methylobacterium* isolates formed low-level biofilms, the amount of the biofilms formed by *Methylobacterium* sp. P-1M and P-18S was significantly increased by co-culturing with other *Methylobacterium* strains that belonged to a specific phylogenetic group. The single-species biofilm was easily washed from the glass surface, whereas the dual-species biofilm strongly adhered after washing. A confocal laser scanning microscopy analysis showed that the dual-species biofilms were significantly thicker and tighter than the single-species biofilms.

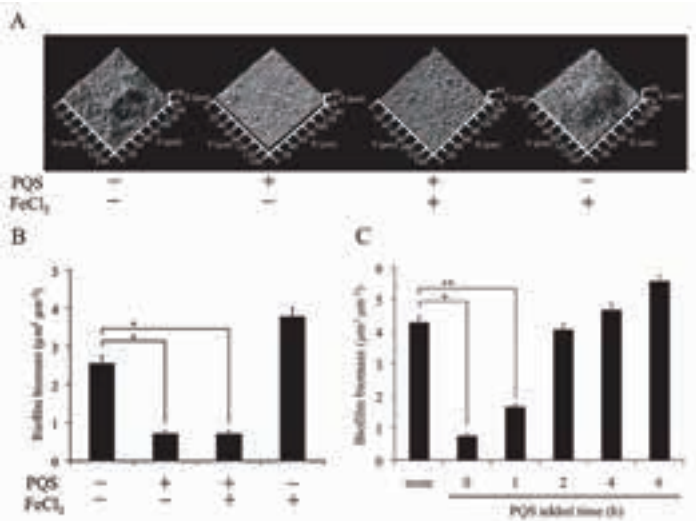


The *Pseudomonas* Quinolone Signal Inhibits Biofilm Development of *Streptococcus mutans*

Tomohiro Inaba<sup>1</sup>, Hiromu Oura<sup>1</sup>, Kana Morinaga<sup>1</sup>, Masanori Toyofuku<sup>1</sup>, Nobuhiko Nomura<sup>1</sup>

<sup>1</sup>Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan

Bacteria often thrive in natural environments through a sessile mode of growth, known as the biofilm. Biofilms are well-structured communities and their formation is tightly regulated. However, the mechanisms by which interspecies interactions alter the formation of biofilms have not yet been elucidated in detail. We herein demonstrated that a quorum-sensing signal in *Pseudomonas aeruginosa* (the *Pseudomonas* quinolone signal; PQS) inhibited biofilm formation by *Streptococcus mutans*. Although the PQS did not affect cell growth, biofilm formation was markedly inhibited. Our results revealed a unique role for this multifunctional PQS and also indicated its application in the development of prophylactic agents against caries-causing *S. mutans*.



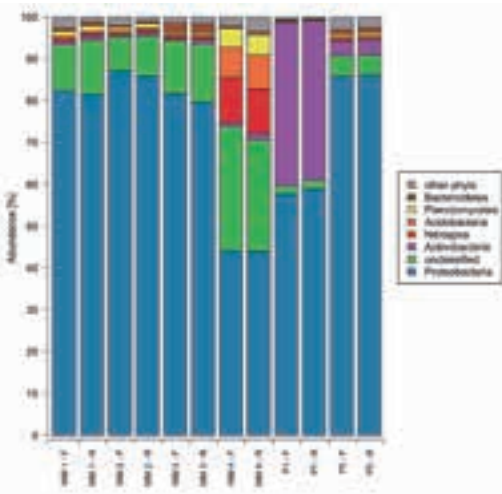
Bacterial Community Analysis of Drinking Water Biofilms in Southern Sweden

Katharina Lührig<sup>1,2</sup>, Björn Canbäck<sup>3</sup>, Catherine J. Paul<sup>1,4</sup>, Tomas Johansson<sup>3</sup>, Kenneth M. Persson<sup>2,4</sup>, Peter Rådström<sup>1</sup>

<sup>1</sup>Applied Microbiology, Department of Chemistry, Lund University, Sweden; <sup>2</sup>Sydvatten AB, Sweden; <sup>3</sup>Microbial Ecology Group, Department of Biology, Lund University, Sweden; and <sup>4</sup>Water Resources Engineering, Department of Building and Environmental Technology, Lund University, Sweden

Next-generation sequencing of the V1–V2 and V3 variable regions of the 16S rRNA gene generated a total of 674,116 reads that described six distinct bacterial biofilm communities from both water meters and pipes. A high degree of reproducibility was demonstrated for the experimental and analytical work-flow by analyzing the communities present in parallel water meters, the rare occurrence of biological replicates within a working drinking water distribution system. The communities observed in water meters from households that did not complain about their drinking water were defined by sequences representing *Proteobacteria* (82–87%), with 22–40% of all sequences being classified as *Sphingomonadaceae*. However, a water meter biofilm community from a household with consumer reports of red water and flowing water containing elevated levels of iron and

manganese had fewer sequences representing *Proteobacteria* (44%); only 0.6% of all sequences were classified as *Sphingomonadaceae*; and, in contrast to the other water meter communities, markedly more sequences represented *Nitrospira* and *Pedomicrobium*. The biofilm communities in pipes were distinct from those in water meters, and contained sequences that were identified as *Mycobacterium*, *Nocardia*, *Desulfovibrio*, and *Sulfuricurvum*. The approach employed in the present study resolved the bacterial diversity present in these biofilm communities as well as the differences that occurred in biofilms within a single distribution system, and suggests that next-generation sequencing of 16S rRNA amplicons can show changes in bacterial biofilm communities associated with different water qualities.



Sample	OTU based (greenhousehouse)		OTU and sequence based	
	Venn Diagram	Shared OTUs	Venn Diagram	Shared sequences
WM 1 + WM 2		160		16,804
WM 3 + WM 4		114		11,170
WM 5 + WM 6		110		10,818
WM 7 + WM 8		100		9,111
PI + PI		100		9,800
PI + WM 1		110		9,762
PI + WM 2		100		9,000

# WASTEWATER TREATMENT

Microbes Environ. Vol. 29, No. 4, 353–362, 2014

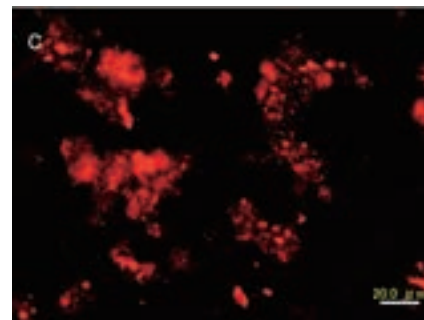
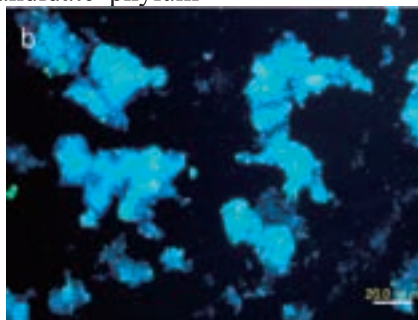
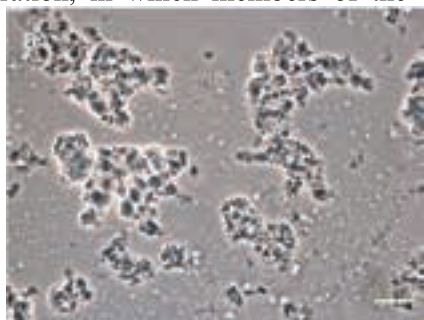
## Bacteria of the Candidate Phylum TM7 are Prevalent in Acidophilic Nitrifying Sequencing-Batch Reactors

Akiko Hanada<sup>1</sup>, Takashi Kurogi<sup>1</sup>, Nguyen Minh Giang<sup>1</sup>, Takeshi Yamada<sup>1</sup>, Yuki Kamimoto<sup>2</sup>, Yoshiaki Kiso<sup>1</sup>, Akira Hiraishi<sup>1</sup>

<sup>1</sup>Department of Environmental and Life Sciences, Toyohashi University of Technology, Japan; and <sup>2</sup>EcoTopia Science Institute, Nagoya University, Japan

Laboratory-scale acidophilic nitrifying sequencing-batch reactors (ANSBRs) were constructed by seeding with sewage-activated sludge and cultivating with ammonium-containing acidic mineral medium (pH 4.0) with or without a trace amount of yeast extract. In every batch cycle, the pH varied between 2.7 and 4.0, and ammonium was completely converted to nitrate. Attempts to detect nitrifying functional genes in the fully acclimated ANSBRs by PCR with previously designed primers mostly gave negative results. 16S rRNA gene-targeted PCR and a subsequent denaturing gradient gel electrophoresis analysis revealed that a marked change occurred in the bacterial community during the overall period of operation, in which members of the candidate phylum

TM7 and the class *Gammaproteobacteria* became predominant at the fully acclimated stage. This result was fully supported by a 16S rRNA gene clone library analysis, as the major phylogenetic groups of clones detected (>5% of the total) were TM7 (33%), *Gammaproteobacteria* (37%), *Actinobacteria* (10%), and *Alphaproteobacteria* (8%). Fluorescence in situ hybridization with specific probes also demonstrated the prevalence of TM7 bacteria and *Gammaproteobacteria*. These results suggest that previously unknown nitrifying microorganisms may play a major role in ANSBRs; however, the ecophysiological significance of the TM7 bacteria predominating in this process remains unclear.



Microbes Environ. Vol. 29, No. 3, 261–268, 2014

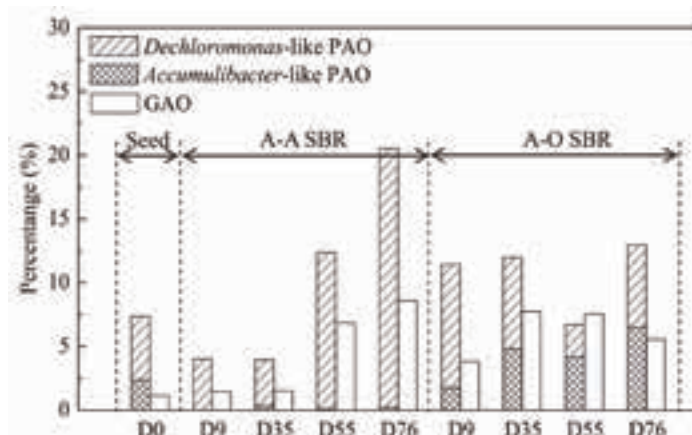
## A Comparative Study of the Bacterial Community in Denitrifying and Traditional Enhanced Biological Phosphorus Removal Processes

Xiao-Mei Lv<sup>1,2</sup>, Ming-Fei Shao<sup>1,2,3</sup>, Chao-Lin Li<sup>1,2</sup>, Ji Li<sup>1,2,3</sup>, Xin-lei Gao<sup>1,2</sup>, Fei-Yun Sun<sup>1,2,3</sup>

<sup>1</sup>Harbin Institute of Technology Shenzhen Graduate School, China; <sup>2</sup>Shenzhen Key Laboratory of Water Resource Utilization and Environmental Pollution Control, China; and <sup>3</sup>Shenzhen Public Technological Service Platform for Urban Waste Energy Regeneration, China

Denitrifying phosphorus removal is an attractive wastewater treatment process due to its reduced carbon source demand and sludge minimization potential. Two lab-scale sequencing batch reactors (SBRs) were operated in alternating anaerobic-anoxic (A-A) or anaerobic-oxic (A-O) conditions to achieve denitrifying enhanced biological phosphate removal (EBPR) and traditional EBPR. No significant differences were observed in phosphorus removal efficiencies between A-A SBR and A-O SBR, with phosphorus removal rates being 87.9% and 89.0% respectively. The community structures in denitrifying and traditional EBPR processes were evaluated by high-throughput sequencing of the PCR-amplified partial 16S rRNA genes from each sludge. The results obtained showed that the bacterial community was more diverse in A-O sludge than in A-A sludge. Taxonomy and  $\beta$ -diversity analyses indicated that a significant shift occurred in the dominant microbial community in A-A sludge compared with the seed sludge during the whole acclimation phase, while a slight fluctuation was observed in the abundance

of the major taxonomies in A-O sludge. One *Dechloromonas*-related OTU outside the 4 known *Candidatus* “*Accumulibacter*” clades was detected as the main OTU in A-A sludge at the stationary operation, while *Candidatus* “*Accumulibacter*” dominated in A-O sludge.



## METHODOLOGY

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### A Comprehensive, Automatically Updated Fungal ITS Sequence Dataset for Reference-Based Chimera Control in Environmental Sequencing Efforts

R. Henrik Nilsson<sup>1</sup>, Leho Tedersoo<sup>2</sup>, Martin Ryberg<sup>3</sup>, Erik Kristiansson<sup>4</sup>, Martin Hartmann<sup>5,6</sup>, Martin Unterseher<sup>7</sup>, Teresita M. Porter<sup>8</sup>, Johan Bengtsson-Palme<sup>9</sup>, Donald M. Walker<sup>10</sup>, Filipe de Sousa<sup>1</sup>, Hannes Andres Gamper<sup>11</sup>, Ellen Larsson<sup>1</sup>, Karl-Henrik Larsson<sup>12</sup>, Urmas Kõljalg<sup>2,13</sup>, Robert C. Edgar<sup>14</sup>, Kessy Abarenkov<sup>13</sup>

<sup>1</sup>Department of Biological and Environmental Sciences, University of Gothenburg, Sweden; <sup>2</sup>Institute of Ecology and Earth Sciences, University of Tartu, Estonia; <sup>3</sup>Department of Organismal Biology, Uppsala University, Sweden; <sup>4</sup>Department of Mathematical Statistics, Chalmers University of Technology, Sweden; <sup>5</sup>Forest Soils and Biogeochemistry, Swiss Federal Research Institute WSL, Switzerland; <sup>6</sup>Molecular Ecology, Institute for Sustainability Sciences, Agroscope, Switzerland; <sup>7</sup>Ernst-Moritz-Arndt University, Institute of Botany and Landscape Ecology, Germany; <sup>8</sup>Department of Biology, McMaster University, Canada; <sup>9</sup>Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Sweden; <sup>10</sup>Department of Natural Sciences, The University of Findlay, USA; <sup>11</sup>Group of Plant Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, Switzerland; <sup>12</sup>Natural History Museum, Norway; <sup>13</sup>Natural History Museum, University of Tartu, Estonia; and <sup>14</sup>Tiburon, USA

The nuclear ribosomal internal transcribed spacer (ITS) region is the most commonly chosen genetic marker for the molecular identification of fungi in environmental sequencing and molecular ecology studies. Several analytical issues complicate such efforts, one of which is the formation of chimeric—artificially joined—DNA sequences during PCR amplification or sequence assembly. Several software tools are currently available for chimera detection, but rely to various degrees on the presence of a chimera-free reference dataset for optimal performance. However, no such dataset is available for use with the fungal ITS region. This study introduces a comprehensive, automatically updated reference dataset for fungal ITS sequences based on the UNITE database for the molecular identification of fungi. This dataset supports chimera detection throughout the fungal kingdom and for full-length ITS sequences as well as partial (ITS1 or ITS2 only) datasets. The performance of the dataset on a large set of artificial chimeras was above 99.5%, and we subsequently used the dataset to remove nearly 1,000 compromised fungal ITS sequences from public circulation. The dataset is available at <http://unite.ut.ee/repository.php> and is subject to web-based third-party curation.



Microbes Environ. Vol. 29, No. 3, 286–295, 2014

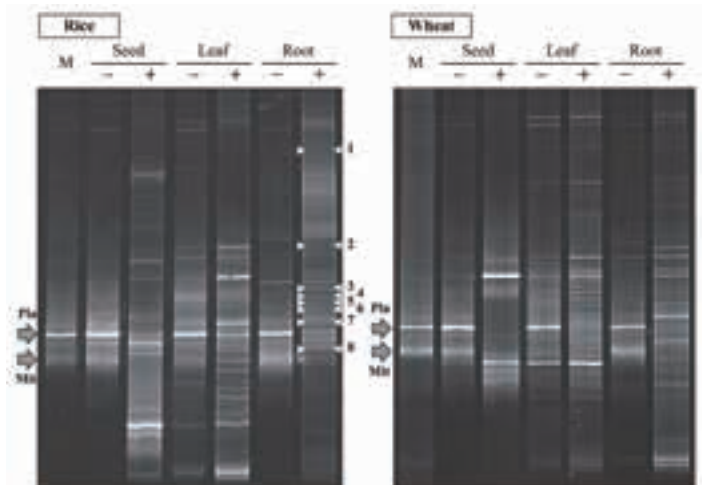
### Application of Locked Nucleic Acid (LNA) Oligonucleotide–PCR Clamping Technique to Selectively PCR Amplify the SSU rRNA Genes of Bacteria in Investigating the Plant-Associated Community Structures

Makoto Ikenaga<sup>1</sup>, Masao Sakai<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, Kagoshima University, Japan

The simultaneous extraction of plant organelle (mitochondria and plastid) genes during the DNA extraction step is a major limitation in investigating the community structures of bacteria associated with plants because organelle SSU rRNA genes are easily amplified by PCR using primer sets that are specific to bacteria. To inhibit the amplification of organelle genes, the locked nucleic acid (LNA) oligonucleotide–PCR clamping technique was applied to selectively amplify bacterial SSU rRNA genes by PCR. LNA oligonucleotides, the sequences of which were complementary to mitochondria and plastid genes, were designed by overlapping a few bases with the annealing position of the bacterial primer and converting DNA bases into LNA bases specific to mitochondria and plastids at the shifted region from the 3' end of the primer-binding position. PCR with LNA oligonucleotides selectively amplified the bacterial genes while inhibited that of organelle genes. Denaturing gradient gel electrophoresis (DGGE) analysis revealed that conventional amplification without LNA oligonucleotides predominantly generated DGGE bands from mitochondria and plastid genes with few bacterial bands. In contrast, additional bacterial bands were detected in DGGE patterns, the amplicons of which were prepared using LNA oligonucleotides. These results indicated that the detection of bacterial genes had been screened by the excessive amplification of the organelle genes. Sequencing of the bands newly detected by using LNA oligonucleotides

revealed that their similarity to the known isolated bacteria was low, suggesting the potential to detect novel bacteria. Thus, application of the LNA oligonucleotide–PCR clamping technique was considered effective for the selective amplification of bacterial genes from extracted DNA containing plant organelle genes.



# ENVIRONMENTAL MICROBIOLOGY

Microbes Environ. Vol. 30, No. 3, 262–269, 2015

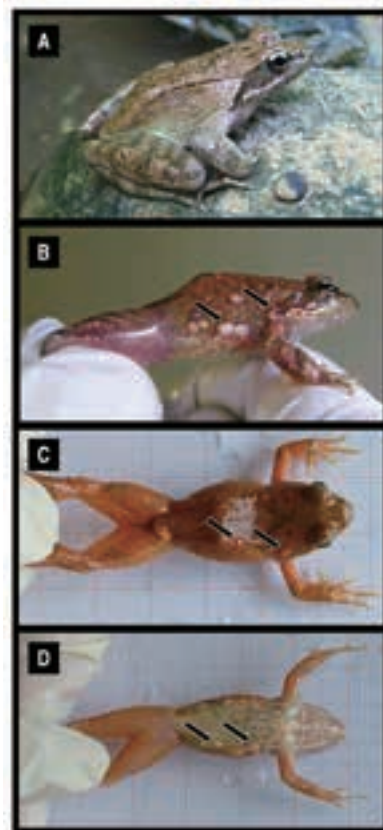
## Characterization of the Skin Microbiota in Italian Stream Frogs (*Rana italica*) Infected and Uninfected by a Cutaneous Parasitic Disease

Ermanno Federici<sup>1</sup>, Roberta Rossi<sup>1</sup>, Laura Fidati<sup>1</sup>, Romina Paracucchi<sup>1</sup>, Silvia Scargetta<sup>1</sup>, Elena Montalbani<sup>1</sup>, Andrea Franzetti<sup>2</sup>, Gianandrea La Porta<sup>1</sup>, Anna Fagotti<sup>1</sup>, Francesca Simonceli<sup>1</sup>, Giovanni Cenci<sup>1</sup>, Ines Di Rosa<sup>1</sup>

<sup>1</sup>Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy;

<sup>2</sup>Department of Earth and Environmental Sciences, University of Milano-Bicocca, Italy

In human and wildlife populations, the natural microbiota plays an important role in health maintenance and the prevention of emerging infectious diseases. In amphibians, infectious diseases have been closely associated with population decline and extinction worldwide. Skin symbiont communities have been suggested as one of the factors driving the different susceptibilities of amphibians to diseases. The activity of the skin microbiota of amphibians against fungal pathogens, such as *Batrachochytrium dendrobatidis*, has been examined extensively, whereas its protective role towards the cutaneous infectious diseases caused by *Amphibiocystidium parasites* has not yet been elucidated in detail. In the present study, we investigated, for the first time, the cutaneous microbiota of the Italian stream frog (*Rana italica*) and characterized the microbial assemblages of frogs uninfected and infected by *Amphibiocystidium* using the Illumina next-generation sequencing of 16S rRNA gene fragments. A total of 629 different OTUs belonging to 16 different phyla were detected. Bacterial populations shared by all individuals represented only one fifth of all OTUs and were dominated by a small number of OTUs. Statistical analyses based on Bray-Curtis distances showed that uninfected and infected specimens had distinct cutaneous bacterial community structures. Phylotypes belonging to the genera *Janthinobacterium*, *Pseudomonas*, and *Flavobacterium* were more abundant, and sometimes almost exclusively present, in uninfected than in infected specimens. These bacterial populations, known to exhibit antifungal activity in amphibians, may also play a role in protection against cutaneous infectious diseases caused by *Amphibiocystidium* parasites.



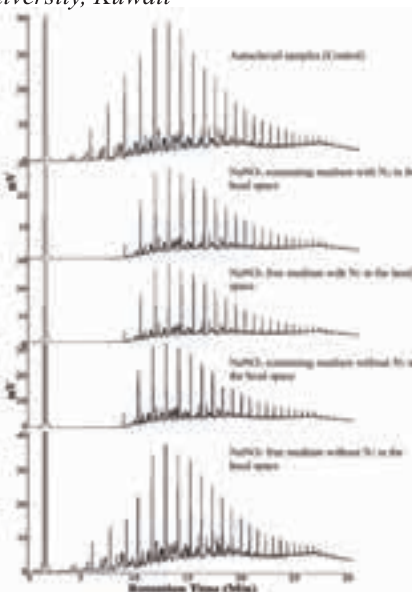
Microbes Environ. Vol. 30, No. 1, 70–75, 2015

## Most Hydrocarbonoclastic Bacteria in the Total Environment are Diazotrophic, which Highlights Their Value in the Bioremediation of Hydrocarbon Contaminants

Narjes Dashti<sup>1</sup>, Nedaa Ali<sup>1</sup>, Mohamed Eliyas<sup>1</sup>, Majida Khanafer<sup>1</sup>, Naser A. Sorkhoh<sup>1</sup>, Samir S. Radwan<sup>1</sup>

<sup>1</sup>Microbiology Program, Department of Biological Sciences, Faculty of Science, Kuwait University, Kuwait

Eighty-two out of the 100 hydrocarbonoclastic bacterial species that have been already isolated from oil-contaminated Kuwaiti sites, characterized by 16S rRNA nucleotide sequencing, and preserved in our private culture collection, grew successfully in a mineral medium free of any nitrogenous compounds with oil vapor as the sole carbon source. Fifteen out of these 82 species were selected for further study based on the predominance of most of the isolates in their specific sites. All of these species tested positive for nitrogenase using the acetylene reduction reaction. They belonged to the genera *Agrobacterium*, *Sphingomonas*, and *Pseudomonas* from oily desert soil and *Nesiotobacter*, *Nitratireductor*, *Acinetobacter*, *Alcanivorax*, *Arthrobacter*, *Marinobacter*, *Pseudoalteromonas*, *Vibrio*, *Diatzia*, *Mycobacterium*, and *Microbacterium* from the Arabian/Persian Gulf water body. A PCR-DGGE-based sequencing analysis of *nifH* genes revealed the common occurrence of the corresponding genes among all the strains tested. The tested species also grew well and consumed crude oil effectively in  $\text{NaNO}_3$ -containing medium with and without nitrogen gas in the top space. On the other hand, these bacteria only grew and consumed crude oil in the  $\text{NaNO}_3$ -free medium when the top space gas contained nitrogen. We concluded that most hydrocarbonoclastic bacteria are diazotrophic, which allows for their wide distribution in the total environment. Therefore, these bacteria are useful for the cost-effective, environmentally friendly bioremediation of hydrocarbon contaminants.



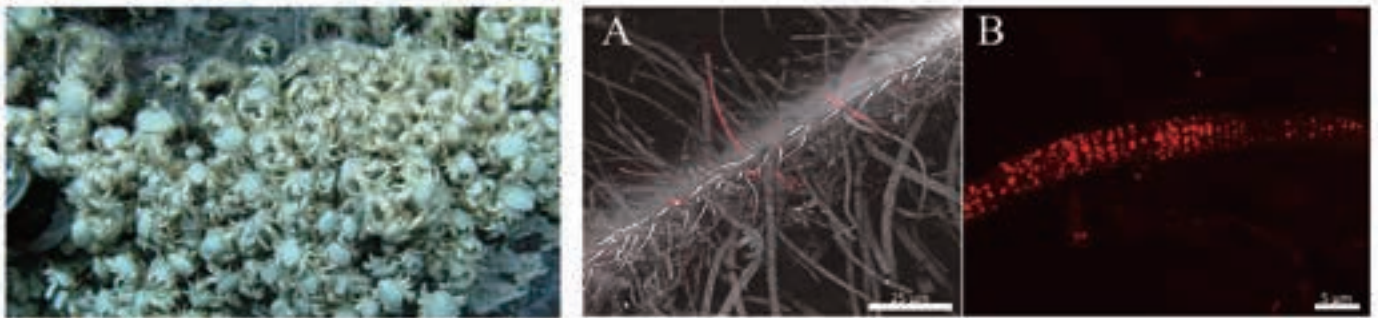
## Effects of Hemagglutination Activity in the Serum of a Deep-Sea Vent Endemic Crab, *Shinkaia Crosnieri*, on Non-Symbiotic and Symbiotic Bacteria

So Fujiyoshi<sup>1</sup>, Hiroaki Taten<sup>2</sup>, Tomoo Watsuji<sup>3</sup>, Hideyuki Yamaguchi<sup>4</sup>, Daisuke Fukushima<sup>4</sup>, Sayaka Mino<sup>5</sup>, Makoto Sugimura<sup>6</sup>, Tomoo Sawabe<sup>5</sup>, Ken Takai<sup>3</sup>, Shigeki Sawayama<sup>1</sup>, Satoshi Nakagawa<sup>1,3</sup>

<sup>1</sup>Laboratory of Marine Environmental Microbiology, Graduate School of Agriculture, Kyoto University, Japan; <sup>2</sup>Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Japan; <sup>3</sup>Subsurface Geobiology and Advanced Research project (SUGAR), Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Japan; <sup>4</sup>Leica Microsystems K.K. Osaka Sales Office Shogyo Japan; <sup>5</sup>Laboratory of Microbiology, Faculty of Fisheries Sciences, Japan; and <sup>6</sup>Enoshima Aquarium, Japan

In deep-sea hydrothermal environments, most invertebrates associate with dense populations of symbiotic microorganisms in order to obtain nutrition. The molecular interactions between deep-sea animals and environmental microbes, including their symbionts, have not yet been elucidated in detail. Hemagglutinins/lectins, which are carbohydrate-binding proteins, have recently been reported to play important roles in a wide array of biological processes, including the recognition and control of non-self materials. We herein assessed hemagglutination activity in the serum of a deep-sea vent endemic crab, *Shinkaia crosnieri*, which harbors chemosynthetic

epibionts on its plumose setae. Horse and rabbit erythrocytes were agglutinated using this serum (opt. pH 7.5 and opt. temperature 15°C). Agglutinating activity was inhibited by eight kinds of sugars and several divalent cations, did not require any divalent metal ions, and remained detectable even after heating the serum at 100°C for 30 min. By using fluorescently labeled serum, we demonstrated that deep-sea crab serum components bound to the epibionts even in the presence of sugars. This study represents the first immunological assessment of a deep-sea vent endemic crab and demonstrated the possibility of a non-lectin-mediated symbiont-host interaction.

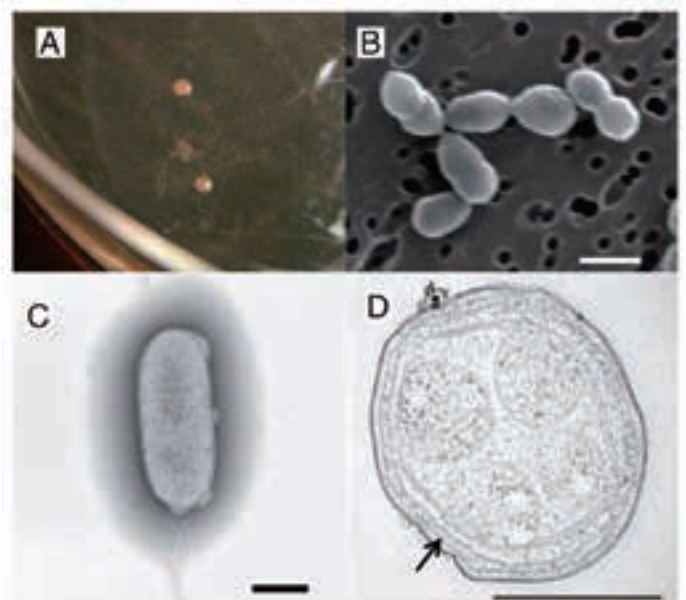


## *Nitrosomonas stercoris* sp. nov., a Chemoautotrophic Ammonia-Oxidizing Bacterium Tolerant of High Ammonium Isolated from Composted Cattle Manure

Tatsunori Nakagawa<sup>1</sup>, Reiji Takahashi<sup>1</sup>

<sup>1</sup>College of Bioresource Sciences, Nihon University, Japan

Among ammonia-oxidizing bacteria, *Nitrosomonas eutropha*-like microbes are distributed in strongly eutrophic environments such as wastewater treatment plants and animal manure. In the present study, we isolated an ammonia-oxidizing bacterium tolerant of high ammonium levels, designated strain KYUHI-S<sup>T</sup>, from composted cattle manure. Unlike the other known *Nitrosomonas* species, this isolate grew at 1,000 mM ammonium. Phylogenetic analyses based on 16S rRNA and *amoA* genes indicated that the isolate belonged to the genus *Nitrosomonas* and formed a unique cluster with the uncultured ammonia oxidizers found in wastewater systems and animal manure composts, suggesting that these ammonia oxidizers contributed to removing higher concentrations of ammonia in strongly eutrophic environments. Based on the physiological and phylogenetic data presented here, we propose and call for the validation of the provisional taxonomic assignment *Nitrosomonas stercoris*, with strain KYUHI-S as the type strain (type strain KYUHI-S<sup>T</sup> = NBRC 110753<sup>T</sup> = ATCC BAA-2718<sup>T</sup>).



# SYMBIOSYS

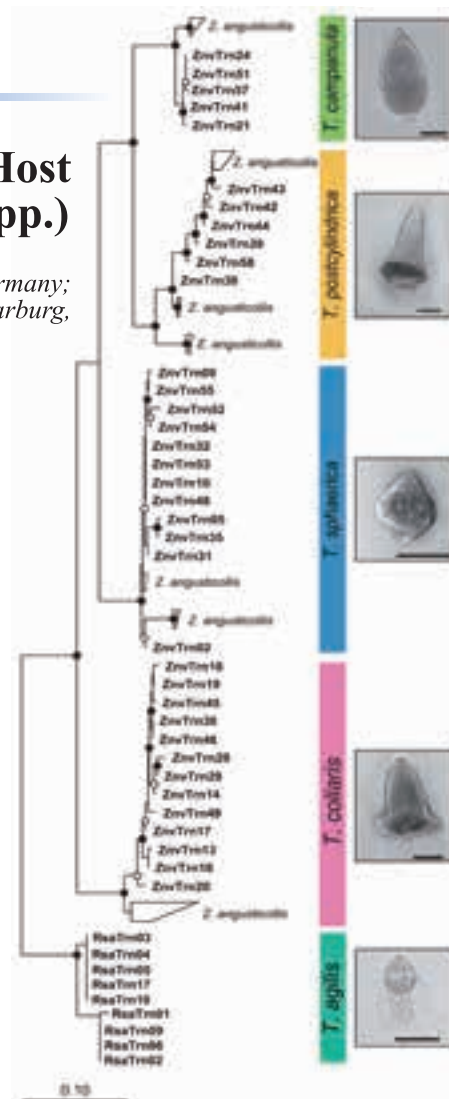
Microbes Environ. Vol. 30, No. 1, 92–98, 2015

## Population Structure of Endomicrobia in Single Host Cells of Termite Gut Flagellates (*Trichonympha* spp.)

Hao Zheng<sup>1</sup>, Carsten Dietrich<sup>1</sup>, Claire L. Thompson<sup>2</sup>, Katja Meuser<sup>1</sup>, Andreas Brune<sup>1,2</sup>

<sup>1</sup>Department of Biogeochemistry, Max Planck Institute for Terrestrial Microbiology, Germany; and <sup>2</sup>LOEWE Center for Synthetic Microbiology, SYNMIKRO, Philipps-Universität Marburg, Germany

The gut microbiota of many phylogenetically lower termites is dominated by the cellulolytic flagellates of the genus *Trichonympha*, which are consistently associated with bacterial symbionts. In the case of Endomicrobia, an unusual lineage of endosymbionts of the *Elusimicrobia* phylum that is also present in other gut flagellates, previous studies have documented strict host specificity, leading to the cospeciation of “*Candidatus* Endomicrobium trichonymphae” with their respective flagellate hosts. However, it currently remains unclear whether one *Trichonympha* species is capable of harboring more than one Endomicrobia phylotype. In the present study, we selected single *Trichonympha* cells from the guts of *Zootermopsis nevadensis* and *Reticulitermes santonensis* and characterized their Endomicrobia populations based on internal transcribed spacer (ITS) region sequences. We found that each host cell harbored a homogeneous population of symbionts that were specific to their respective host species, but phylogenetically distinct between each host lineage, corroborating cospeciation being caused by vertical inheritance. The experimental design of the present study also allowed for the identification of an unexpectedly large amount of tag-switching between samples, which indicated that any high-resolution analysis of microbial community structures using the pyrosequencing technique has to be interpreted with great caution.



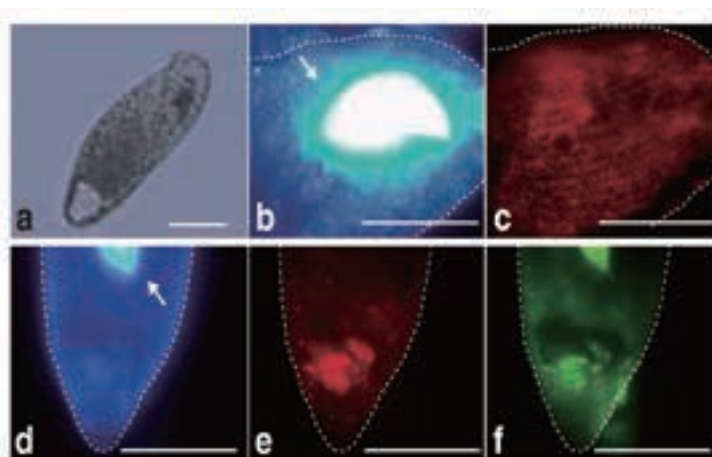
Microbes Environ. Vol. 30, No. 3, 221–227, 2015

## Identification and Detection of Prokaryotic Symbionts in the Ciliate *Metopus* from Anaerobic Granular Sludge

Yuga Hirakata<sup>1,2</sup>, Mamoru Oshiki<sup>1</sup>, Kyohei Kuroda<sup>3</sup>, Masashi Hatamoto<sup>3</sup>, Kengo Kubota<sup>4</sup>, Takashi Yamaguchi<sup>2</sup>, Hideki Harada<sup>5</sup>, Nobuo Araki<sup>1</sup>

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The aim of the present study was to investigate the prokaryotic community structure of the anaerobic ciliate, *Metopus* sp. using rRNA sequencing, fluorescence *in situ* hybridization (FISH), and transmission electron microscopy (TEM). *Metopus* sp. was physically separated from anaerobic granular sludge in a domestic wastewater treatment plant and anoxically cultivated for 7 d. 16S rRNA gene sequences from the prokaryotes *Methanoregula boonei* and *Clostridium aminobutyricum* were abundantly detected in *Metopus* ciliates. The FISH analysis using the oligonucleotide probes Mg1200b and Cla568 demonstrated that these prokaryotes were localized within *Metopus* cells. These results identify *M. boonei*- and *C. aminobutyricum*-like prokaryotes as novel endosymbionts of *Metopus* ciliates.



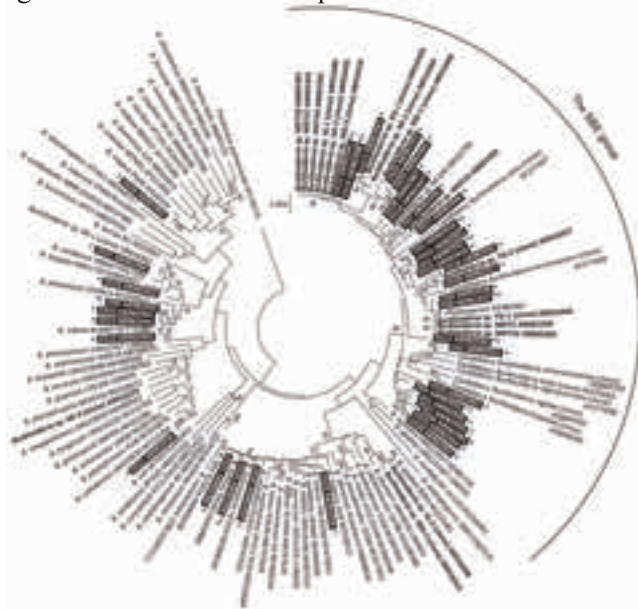
## Insecticide-Degrading *Burkholderia* Symbionts of the Stinkbug Naturally Occupy Various Environments of Sugarcane Fields in a Southeast Island of Japan

Kanako Tago<sup>1</sup>, Takashi Okubo<sup>1</sup>, Hideomi Itoh<sup>2</sup>, Yoshitomo Kikuchi<sup>2</sup>, Tomoyuki Hori<sup>3</sup>, Yuya Sato<sup>3</sup>, Atsushi Nagayama<sup>4</sup>, Kentaro Hayashi<sup>1</sup>, Seishi Ikeda<sup>5</sup>, Masahito Hayatsu<sup>1</sup>

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The stinkbug *Cavelerius saccharivorus*, which harbors *Burkholderia* species capable of degrading the organophosphorus insecticide, fenitrothion, has been identified on a Japanese island in farmers' sugarcane fields that have been exposed to fenitrothion. A clearer understanding of the ecology of the symbiotic fenitrothion degraders of *Burkholderia* species in a free-living environment is vital for advancing our knowledge on the establishment of degrader-stinkbug symbiosis. In the present study, we analyzed the composition and abundance of degraders in sugarcane fields on the island. Degraders were recovered from field samples without an enrichment culture procedure. Degrader densities in the furrow soil in fields varied due to differences in insecticide treatment histories. Over 99% of the 659 isolated degraders belonged to the genus *Burkholderia*. The strains related to the stinkbug symbiotic group predominated among the degraders, indicating a selection for this group in response to fenitrothion. Degraders were also isolated from sugarcane stems, leaves, and rhizosphere in fields that were continuously exposed to fenitrothion. Their density was lower in the plant sections than in the rhizosphere. A phylogenetic analysis of 16S rRNA gene sequences demonstrated that most of the degraders from the plants and rhizosphere clustered with the stinkbug symbiotic group, and some were identical to the midgut symbionts of *C. saccharivorus* collected from the same field. Our results confirmed that

plants and the rhizosphere constituted environmental reservoirs for stinkbug symbiotic degraders. To the best of our knowledge, this is the first study to investigate the composition and abundance of the symbiotic fenitrothion degraders of *Burkholderia* species in farmers' fields.



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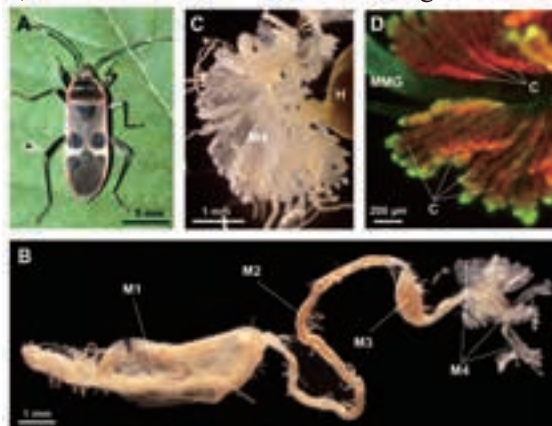
## *Burkholderia* of Plant-Beneficial Group are Symbiotically Associated with Bordered Plant Bugs (Heteroptera: Pyrrhocoroidea: Largidae)

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A number of phytophagous stinkbugs (order Heteroptera: infraorder Pentatomomorpha) harbor symbiotic bacteria in a specific midgut region composed of numerous crypts. Among the five superfamilies of the infraorder Pentatomomorpha, most members of the Coreoidea and Lygaeoidea are associated with a specific group of the genus *Burkholderia*, called the “stinkbug-associated beneficial and environmental (SBE)” group, which is not vertically transmitted, but acquired from the environment every host generation. A recent study reported that, in addition to these two stinkbug groups, the family Largidae of the superfamily Pyrrhocoroidea also possesses a *Burkholderia* symbiont. Despite this recent finding, the phylogenetic position and biological nature of *Burkholderia* associated with Largidae remains unclear. Based on the combined results of fluorescence *in situ* hybridization, cloning analysis, Illumina deep sequencing, and egg inspections by diagnostic PCR, we herein demonstrate that the largid species are consistently associated with the “plant-associated beneficial and environmental (PBE)” group of *Burkholderia*, which are phylogenetically distinct from the SBE group, and that they maintain symbiosis through the environmental acquisition of the bacteria. Since the

superfamilies Coreoidea, Lygaeoidea, and Pyrrhocoroidea are monophyletic in the infraorder Pentatomomorpha, it is plausible that the symbiotic association with *Burkholderia* evolved at the common ancestor of the three superfamilies. However, the results of this study strongly suggest that a dynamic transition from the PBE to SBE group, or *vice versa*, occurred in the course of stinkbug evolution.



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