Frontiers in Rhizosphere Research

November 20th, 2018

RIKEN Yokohama Lecture Hall

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iSYM

Frontiers in Rhizosphere Research
Date: November 20th, 2018 (Tuesday) Venue: RIKEN Yokohama, Lecture Hall

Program

10:30-10:35 Opening remarks

Chair:Shuta Asai

10:35-11:10 **Stephane Hacquard**

(Multitrophic Plant-Microbe Interactions, Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research)

"Microbial Interkingdom Interactions in Roots Promote *Arabidopsis* Survival"

11:10-11:45 **Yasunori Ichihashi**

(Plant-Microbe Symbiosis Research and Development Team, RIKEN BRC)

"Soil solarization-induced organic nitrogen increase agricultural crop yield"

11:45-12:10 **Arno Germond**

(Laboratory for Comprehensive Bioimaging, RIKEN BDR)

"Toward predicting gene expression and metabolism from label free spectral imaging."

12:10-13:00 Lunch

Chair:Yuji Ishigaki

13:00-13:35 **Hirokazu Toju**

(Center for Ecological Research, Kyoto University)

"Core microbiomes for designing sustainable ecosystems"

13:35-14:10 **Ruben Garrido-Oter**

(Integrative Bioinformatics, Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research)

"Association studies of plant-associated microbial genomes"

14:10-14:45 **Shuta Asai**

(Plant Immunity Research Group, RIKEN CSRS)

"Field-Patho-Genomics －for establishment of soil diagnostics－"

14:45-15:15 Coffee Break

Chair: Yasunori Ichihashi

15:15-15:50 **Ryohei Thomas Nakano**

(Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research)

"Ternary interaction assays revealed modular traits of root microbiota that dictate host growth and immune status"

15:50-16:25 **Masahito Hosokawa**

(Research Institute for Science and Engineering, Waseda University)

"Obtaining high-quality draft genomes from uncultured microbes by single-cell genome sequencing with droplet microfluidics"

16:25-17:00 **Yuji Ishigaki**

(Plant Immunity Research Group, RIKEN CSRS)

"Rhizosphere interactions regulate secondary metabolite production of root-associated microbes"

17:00-17:05 Closing remarks

Abstracts

Stephane Hacquard
(Multitrophic Plant-Microbe Interactions, Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research)

Microbial Interkingdom Interactions in Roots Promote *Arabidopsis* Survival

Roots of healthy plants are inhabited by soil-derived bacteria, fungi, and oomycetes that have evolved independently in distinct kingdoms of life. How these microorganisms interact and to what extent those interactions affect plant health are poorly understood. We examined root-associated microbial communities from three *Arabidopsis thaliana* populations and detected mostly negative correlations between bacteria and filamentous microbial eukaryotes. We established microbial culture collections for reconstitution experiments using germ-free *A. thaliana*. In plants inoculated with mono- or multi-kingdom synthetic microbial consortia, we observed a profound impact of the bacterial root microbiota on fungal and oomycetal community structure and diversity. We demonstrate that the bacterial microbiota is essential for plant survival and protection against root-derived filamentous eukaryotes. Deconvolution of 2,862 binary bacterial-fungal interactions *ex situ*, combined with community perturbation experiments *in planta*, indicate that biocontrol activity of bacterial root commensals is a redundant trait that maintains microbial inter-kingdom balance for plant health.

Yasunori Ichihashi
(Plant-Microbe Symbiosis Research and Development Team, RIKEN BRC)

Soil solarization-induced organic nitrogen increase agricultural crop yield

Organic agriculture is of increasing importance in global food and ecosystem security. Soil solarization, one of environmentally friendly organic farming method, is the disinfestation of soil borne plant pathogens. Even in the absence of major pathogens, soil solarization has an unexpected beneficial effect to improve plant growth, but the underling mechanisms have been unclear. Here we utilized an agricultural filed of Japanese mustard spinach (Brassica rapa var. perviridis) using several farming methods including soil solarization, together with an integrated omics approach. We show that soil solarization assembles and maintains the specific rhizosphere microbiota despite soil microbiota homogenized during plant cultivation. Our integrated omics data suggests that the accumulation of several rhizosphere microbes as well as organic nitrogen correlate with the crop yield. Using a germ-free plant system, we identified that an organic nitrogen alanine was directly absorbed into plant body to increase their biomass as much as inorganic nitrogen does. This suggests a possible mechanism for how soil solarization induces complex material circulation between rhizosphere microbiota and soil metabolites to increase crop yield. Our findings provide new insight into the theory of plant nutrition underling the organic agriculture, potentially leading a sustainable innovation in agriculture.

Arno Germond
(Laboratory for Comprehensive Bioimaging, RIKEN BDR)

Toward predicting gene expression and metabolism from label free spectral imaging.

Gene expression and metabolic information are invaluable sources of information for biological and biomedical investigations. Here we report a quantitative method to integrate transcriptomic data with the label-free spectral data of living cells. Our multivariate analysis enables the prediction of gene expression from spectral data of unknown cells while highlighting overrepresented metabolic shifts with a cell-line specificity. *Escherichia coli* cell lines exhibiting antibiotic resistance are used as a case study.

Hirokazu Toju
(Center for Ecological Research, Kyoto University)

Core microbiomes for designing sustainable ecosystems

In an era of ecosystem degradation and climate change, maximizing microbial functions in natural and agricultural ecosystems has become a prerequisite for the future of humanity. However, managing species-rich communities of plant-associated microbiomes remains a major challenge. Informatics now allows us to identify members and characteristics of “core microbiomes”, which may be deployed to organize otherwise uncontrollable dynamics of resident microbiomes. After the emergence of high-throughput sequencers, my colleagues and I have uncovered root microbiome structures of hundreds of plants species across various types of ecosystems. We then found that diverse taxonomic groups of endophytic fungi ubiquitously interacted with plant communities, potentially playing crucial, but often overlooked, roles at the ecosystem level. Based on the patterns found in microbe-plant and microbe-microbe networks, we have begun to design optimal core microbiomes for managing agroecosystems with high resource-efficiency and stress-resistance. I will discuss how interdisciplinary research will advance our ability for managing sustainable agroecosystems and that for restoring degraded ecosystems.

Ruben Garrido-Oter
(Integrative Bioinformatics, Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research)

Association studies of plant-associated microbial genomes

Determining functional and co-evolutionary relationships between genes based on sequence alone is a crucial step in integrating large genomic datasets and understanding how bacterial populations and communities adapt to diverse environments. Here, we propose a phylogenetic approach for determining clusters of co-evolving genes and their network organization by modeling gene gain and loss as a continuous process along the branches of the species tree. Our method accounts for uncertainty in the reconstruction of the ancestral states as well as in the inference of the species tree and robustly identifies clusters of co-evolving genes that significantly enrich for functional categories
and pathways and which are relevant for adaptation to diverse environments. We demonstrate its ability to detect biologically meaningful gene family interactions by analyzing a total of 2,737 bacterial genomes, including diverse populations of multiple plant-associated and symbiotic Rhizobia and a phylogenetically wide collection of bacterial genomes representative of the Arabidopsis thaliana leaf and root microbiota.

Shuta Asai
(Plant Immunity Research Group, RIKEN CSRS)

Field-Patho-Genomics　– for establishment of soil diagnostics –

Plant disease results only if all of three essential factors (susceptible host plant, pathogen, and favorable environment for disease) occur simultaneously. Certain pathogens can infect only specific plant species, so-called host specificity. Therefore, soilborne disease can be avoided if the host specificity of pathogens living in the planting soil can be specified. *Fusarium oxysporum* species complex consists of ubiquitous soil inhabiting fungi that can infect and cause disease in over 120 different plant species, which has been classified into more than 120 lineages (forma specialis; f. sp.). Since mechanisms for determining host specificity in *F. oxysporum* are largely unknown, it is difficult to evaluate which lineage of *F. oxysporum* inhabits the soil. Here, we report and discuss the methods to specify *F. oxysporum* lineages living in the soil (soil diagnostics) and to predict the occurrence of Fusarium disease based on the field environmental data (disease prediction model).

Ryohei Thomas Nakano
(Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research)

Ternary interaction assays revealed modular traits of root microbiota that dictate host growth and immune status

Plant-associated microorganisms, constituting the plant microbiota, are capable of manipulating host physiology, including host growth, immunity, and abiotic stress tolerance. Despite decades of efforts, however, application of these beneficial capacities to the crops growing in natural field remains challenging, likely due to the interference by the other microbes that are already present in the field and in the plant niche. We recently found a root growth promotion (RGP) activity that is conserved among root-associated Rhizobiales species and that stimulation of host immunity disables this RGP capacity. Remarkably, we recorded a strong RGP activity even under immune-activating conditions when plants were co-inoculated with two bacterial strains, one with RGP activity and another strain that is able to suppress host immune response. Both isolates are unable to promote root growth under immune-stimulative conditions, thereby demonstrating the emergent properties of root microbiota. This further delineates the importance of ternary or higher-order interaction assays to elucidate the community-level functional governance of host-associated microbial communities. We are currently investigating the molecular mechanisms by which Rhizobiales isolates promote host root growth and root-associated bacteria suppress host immunity, toward ultimate understanding of their impacts on plant fitness and nutrition, as well as their evolutionary trajectories.

Masahito Hosokawa
(Research Institute for Science and Engineering, Waseda University)

Obtaining high-quality draft genomes from uncultured microbes by single-cell genome sequencing with droplet microfluidics

Since more than 99% of environmental bacteria are uncultivable, single-cell genomics is the powerful tool for understanding their diversity and function without cultivation. For genome sequencing of single microbial cells, whole genome amplification (WGA) is required because the quantity of DNA contained in a single cell is quite small. However, conventional WGA usually resulted in uneven genome sequence coverage because of amplification bias, background amplification of contaminating DNA, and formation of chimeric sequences. Thus, to maximize the quality and throughput of single-cell sequencing, there is a great demand for novel techniques, which enable massively parallel reactions with high accuracy. Recently, we have developed droplet-based microfluidics for massively parallel single-cell genomics (Nishikawa et al, *PLoS ONE*, 2015, Hosokawa et al, *Sci. Rep.*, 2017), to overcome the limitations of conventional techniques. In addition, we have also developed the sequencing analysis pipeline, termed ccSAG (Cleaning and Co-assembly of single amplified genomes) (Kogawa et al, *Sci. Rep.*, 2018), for *de novo* assembly of single-cell amplified genome to eliminate artefact sequences and contaminants. Our results demonstrated that microfluidic-generated droplets showed a potential for contamination-free reaction and enhancing sequencing coverage from single microbial cells isolated from various environments. These advantages will greatly reduce the cost and labor investment required for single-cell genomics study of environmental microbial cells. The integrated workflow will enable the comparative genomic analyses of uncultured microbes at single-cell levels, as well as genetic and functional investigation of microbial dark matters

Yuji Ishigaki
(Plant Immunity Research Group, RIKEN CSRS)

Rhizosphere interactions regulate secondary metabolite production of root-associated microbes

Many plant roots-associated actinomycetes are known antagonists toward plant pathogens, often by producing various type of antibiotics. To investigate whether plants influence the root-associated actinomycetes for antibiotic production, we assayed the antibiotic activities of the strains isolated from plant roots. The isolates altered their antibiotic activities in the presence of the Arabidopsis root extracts. In particular, *Streptosporangium* sp., produced the red pigment when cultured with the root extracts but not with shoot extracts. Interestingly, the red pigment production was not triggered by the family *Solanaceae* roots. Scanning electron microscopy (SEM) revealed that the hyphae extensively colonized the Arabidopsis root surface, especially around the root hairs. Genome sequencing and antiSMASH analysis identified at least one type-II polyketide synthases (PKSs) that is predicted to synthesize the red pigment. Disruption of genes encoding a ketosynthase and a chain length factor in one of the type-II PKS clusters abolished the red pigment production, suggesting the only one type-II PKS cluster is responsible for the production of the red pigment. In addition, overexpression of a transcriptional activator in the cluster resulted in increased the production of the red pigment. The Arabidopsis-*Streptosporangium* pair and the genomic information of the bacterium provide a novel model system for studying of plant-actinomycete interactions.