“BIOLOGY OF SYMBIOSIS” WORKSHOP

August 21st and 22nd, 2017 (Monday and Tuesday)

Lecture Hall (Koryuto Hall), Main Office Building, RIKEN Yokohama Campus

Meeting of "Biology of Symbiosis" project

August 21st and 22nd, 2017 (Monday and Tuesday)

Lecture Hall (Koryuto Hall), Main Office Building, RIKEN Yokohama Campus

Program August 21st

10:30-10:40 Opening remarks by Ken SHIRASU

10:40-11:00 **Ken SHIRASU** (CSRS, Plant Immunity Research Group), Chair

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

11:00-11:20 **Paul SCHULZE-LEFERT** (Max Planck Institute, Cologne)

"Rhizobial root microbiota membership predisposed convergent evolution of nitrogen-fixing symbiosis with legumes"

11:20-11:40 **Makoto HAYASHI** (CSRS, Plant Symbiosis Research Team)

"Underground symbiosis between plants and microbes: its significance and molecular evolution"

11:40-11:55 **Miki FUJITA** (CSRS, Gene Discovery Research Group)

"Development of "RIPPS", an automated system for evaluating plant environmental stress response"

12:00-12:55 Lunch

13:00-13:20 **Masahira HATTORI** (IMS, Laboratory for Microbiome)

"Long-read metagenomics of human gut microbiomes"

13:20-13:40 **Hiroshi OHNO** (IMS, Laboratory for Intestinal Ecosystem), Chair

"Characteristics of M cells and follicle-associated epithelium"

13:40-14:00 **Masayuki AMAGAI** (IMS, Skin homeostasis)

"Stratum corneum as niche for skin microbiome"

14:00-14:20 **Kenya HONDA** (IMS, Gut Homeostasis)

"Modulation of the immune system by the gut microbiota"

14:20-14:40 Break

14:40-15:00 **Sidonia FAGARASAN** (IMS, Laboratory for Mucosal Immunity), Chair

"Host-gut microbiota mutualism by IgA-glycan"

15:00-15:20 **Hidewaki NAKAGAWA** (IMS, Laboratory for Genome Sequencing Analysis)

"Cancer genome sequencing for pathogen and symbiosis in human cancers"

15:20-15:40 **Toru TAKUMI** (BSI, Laboratory for Mental Biology)

"The gut-brain axis in autism"

15:40-16:00 **Atsushi IRIKI** (BSI, Laboratory for Symbolic Cognitive Development)

"Primate model of "Mind-Body Interaction" through the mechanisms of gut-brain axis via microbiome."

16:00-16:20 Break

16:20-16:40 **Hiroyuki OSADA** (CSRS, Chemical Biology Research Group), Chair

"Activation of biosynthesis of tenuazonic acid in a plant pathogenic fungus"

16:40-17:00 **Katsuyuki SHIROGUCHI** (QBiC, Lab for Integrative Omics)

"Toward "The third generation" bacterial microbiota analysis"

17:00-17:15 **Hiroyoshi AOKI** (Center for Advanced Photonics, Ultrahigh Precision Optics Technology Team)

"Agarose Gel Microcapsule: Pico-liter-scale DNA Amplification Microvessel for Single Cell Genomics."

17:15-17:30 **Akiko MINODA** (CLST, Epigenome Technology Exploration Unit)

"Studying the effect of different microbiota at the cell type and subpopulations landscape level by single cell RNA-seq"

17:30-17:45 **Arnaud GERMOND** (QBiC, Laboratory for Comprehensive Bioimaging)

"Raman spectroscopy as a tool for cell-line discrimination and gene expression monitoring"

18:00- Get-together party with beverage

Program August 22nd

09:30-09:40 Opening remarks by Moriya OHKUMA

09:40-10:00 **Chikara FURUSAWA** (QBiC, Laboratory for Multiscale Biosystem Dynamics), Chair
"Analysis of phenotypic evolution and symbiosis using microbial laboratory evolution"

10:00-10:20 **Shunji TAKAHASHI** (CSRS, Natural Product Biosynthesis Unit)
"Unveiling Chemical Signal and Biosynthetic Mechanism of Reveromycin"

10:20-10:40 **Ryo NAKABAYASHI** (CSRS, Metabolomics Research Group)
"Metabolome analysis in the parasitism between Solanum lycopersicum and Cuscuta campestris"

10:40-11:00 **Shigehiro KURAKU** (CLST, Phyloinformatics Unit)
"Genome evolution and ecosystem of marine vertebrates"

11:00-11:15 Break

11:15-11:35 **Ryuhei NAKAMURA** (CSRS, Biofunctional Catalyst Research Team), Chair
"Energy Flow Organized by Benthic Ecosystems"

11:35-11:55 **Jun KIKUCHI** (CSRS, Environmental Metabolic Analysis Research Team)
"Marine and fishery product studies assisted by metabolic profiling with data science"

11:55-12:10 **Shigeharu MORIYA** (CSRS, Biomass research platform team)
"RNA-seq approaching to unlock the ecological functions of natural microbiome system"

12:15-13:05 PI Lunch

13:10-13:30 **Yuichi HONGO/Hirokazu KUWAHARA** (Tokyo Technology Institute, BRC Microbe Division)
"Functional genomics of multiple endo- and ectosymbionts of termite gut protists"

13:30-13:45 **Akiko FUJIWARA** (CSRS, Chemical Genomics Research Group)
"Elucidation of molecular mechanisms for multiple endosymbiotic systems and development of novel control technologies by the inhibition of endosymbiotic machinery in Bemisia tabaci, the agricultural pest"

13:45-14:00 **Seok-Won KIM** (IMS, Laboratory for Integrated Bioinformatics)
"Analysis of the onset mechanism of obesity and type 2 diabetes correlated with gut microbiota using data mining"

14:00-14:20 **Moriya OHKUMA** (BRC, Japan Collection of Microorganisms), Chair
"Single-cell genome analysis of termite gut symbionts"

14:20-14:30 Closing remarks by Hiroshi OHNO

List of Speakers on August 21st

Ken SHIRASU (CSRS, Plant Immunity Research Group)

"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 types of antibiotics. To investigate whether plants influence the root-associated actinomycetes for antibiotic production, we assayed the antibiotic activities of the strains isolated from *Arabidopsis*, the model plant. We show that the isolates altered their antibiotic activities in the presence of the *Arabidopsis* root extracts. In particular, *Streptosporangium* sp. AEG048, produced the red pigment when cultured with the root extracts but not with shoot extracts. Scanning electron microscopy (SEM) revealed that the hyphae of AEG048 extensively colonized the root surface, especially around the root hairs. To identify the secondary metabolite gene cluster(s) responsible for the red pigment production and its regulation in AEG048, we sequenced its genome using PacBio Single Molecule Real-Time (SMRT) sequencing. Based on antiSMASH analysis and phylogenetic analysis of the chain length factor of type II polyketide synthases (PKSs), we identified at least one type-II PKS that is predicted to synthesize the red pigment. The Arabidopsis-AEG048 pair and the genomic information on AEG048 provide a novel model system for studying of plant-actinomycete interactions. In addition, using bacterial culture collections from Max Plank Institute, their anti-Ralstonia activities were also altered by root extract, suggesting that plants control their associating microbes to produce anti-pathogenic compounds for self-protection.

Paul SCHULZE-LEFERT (Max Planck Institute, Cologne)

"Rhizobial root microbiota membership predisposed convergent evolution of nitrogen-fixing symbiosis with legumes"

Rhizobia are a paraphyletic group of soil-borne bacteria defined by their ability to induce nodule organogenesis in legume roots and fix atmospheric nitrogen for plant growth. In non-leguminous plants, species within the Rhizobiales order define a core lineage of the plant microbiota, suggesting alternative forms of interactions with plant hosts. We compared more than 1,300 whole-genome sequences of

Rhizobiales isolates, including microbiota members from non-legumes, and show that the set of genes required for nodulation and nitrogen fixation in legume symbiosis was acquired multiple independent times within each Rhizobiales sublineage. The majority of root-associated rhizobia colonize and promote root growth in the crucifer Arabidopsis without nitrogen fixation, indicating these are rhizobial traits of an ancestral root association. Thus, the capacity for nodulation and nitrogen fixation in legumes was likely acquired from a predisposed root association in multiple subsequent events, constituting an example of convergent evolution.

Makoto HAYASHI (CSRS, Plant Symbiosis Research Team)

"Underground symbiosis between plants and microbes: its significance and molecular evolution"

Land plants typically associate with soil-born microbes in order to obtain minerals that are essential for plant growth. There are two major types of root symbioses; one is association with arbuscular mycorrhizal fungi, the other is that with nitrogen-fixing bacteria. The latter symbiosis is limited to certain plant species, so called Nitrogen Fixing Clade (NFC). Since NFC is monophyletic, it is hypothesized that the most recent common ancestor had acquired the ability to evolve the mechanism involved in this association.

We are interested in how plants establish this intimate relationship, especially in development of root nodules that offer a milieu for nitrogen fixation. So far, molecular genetic analyses tell us the outline of plant components, from bacterial recognition to onset of nodule organogenesis: 3 membrane-bound receptor-like kinases, 5 nuclear membrane-associated proteins, and 5 nuclear proteins. Although most of them are also required for arbuscular mycorrhiza, a transcription factor called NIN is specific to nodulation. Since the ectopic expression of Nin confers nodule-like structures to roots, we believe NIN is the central player for nodule organogenesis. We hypothesize that the recruitment of NIN was the key for evolution of nodulation. Currently we are investigating how Nin expression is regulated, and how NIN regulates downstream gene expression.

Miki FUJITA (CSRS, Gene Discovery Research Group)

"Development of "RIPPS", an automated system for evaluating plant environmental stress response"

Plant health and fitness are greatly impacted by the rhizospheric microbiomes, as their interactions are co-adapted and co-evolved across time and space. Despite considerable evidence in the previous reports that beneficial or detrimental rhizospheric microbiomes can alter in plant growth, morphology, and mineral contents, the mechanism in which the reciprocal interactions affect plant health and fitness is largely unknown. To understand the effect of rhizospheric microbes on plant growth, development, and stress responses, phenotyping systems with strict regulation of environmental conditions and precise quantification of plant growth are expected to be useful. Recently we have developed such automatic phenotyping system that enables us to evaluate plant growth responses to a wide spectrum of environmental conditions. The system named RIPPS (RIKEN Plant Phenotyping System) can control individual soil moisture in continuously rotating 120 pots by a combination of automatic weighing and watering systems. RIPPS also can monitor each rosette size and expansion rate every two hours. We are also developing imaging techniques using various cameras to analyze plant water status. In this seminar, we will demonstrate the utility of the RIPPS in evaluating drought or salinity tolerance and water use efficiency.

Masahira HATTORI (IMS, Laboratory for Microbiome)

"Long-read metagenomics of human gut microbiomes"

Our team is working on metagenomics of human and other microbiomes to elucidate their ecological and biological features related to host's physiologies via microbe-host interaction.

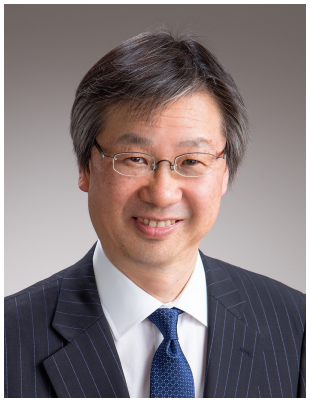
Metagenomics using large datasets of short-read (~300 bp) sequencing is the most popular approach to evaluate the human gut microbiome. However, short-read sequencing has several limitations in reconstruction of the whole microbial genomes including mobile genetic elements (MGEs) such as plasmids/phages and epigenetic base modifications. We applied single-molecule real-time (SMRT)

sequencing by PacBio to metagenomics of human gut microbiomes. We successfully obtained datasets with the average read-length of >9 kb by sequencing DNA templates prepared by the enzymatic lysis method, which was much longer than 2.7 kb previously published. The computational analysis identified many near-complete microbial genomes and complete circular sequences of putative MGEs, which included hitherto unknown species and MGEs. The comparison between the reconstructed and known genomes revealed large differences mostly due to indels of large genomic islands. We also found that some of the MGEs were highly prevalent among human gut microbiomes from different nations. Furthermore, the SMRT sequencing identified methylation motifs, many of which were novel. Thus, this study provides novel views of the human gut microbiome structure.

Hiroshi OHNO (IMS, Laboratory for Intestinal Ecosystem)

"Characteristics of M cells and follicle-associated epithelium"

Huge numbers of commensal bacteria inhabit our gut. However, we do not host this gut commensal microbiota unconditionally; rather we try to contain them by secreting mucus, antimicrobial molecules, and IgA. To evoke intestinal immune responses to produce IgA against commensal microbes, the gut-associated lymphoid tissue (GALT) needs antigenic microbial supply across the intestinal epithelial barrier. This microbial delivery across the epithelium is done by M cells, a subset of intestinal epithelial cells specialized for microbial uptake and delivery to the GALT. M cells exist scattered in the area of epithelium covering the GALT lymphoid follicles, called follicle-associated epithelium (FAE). We have identified the transcription factor Spi-B as a master regulator of M-cell differentiation, and bacterial uptake receptors on M cells, including GP2 and prion protein. Recently, we have also found that IL-22BP, an endogenous inhibitor of the cytokine IL-22 signaling, confers the characteristic of FAE that enables intestinal microbes easy access to the epithelial layer and hence aids the their uptake by M cells.



Masayuki AMAGAI (IMS, Skin homeostasis)

"Stratum corneum as niche for skin microbiome"

Enhanced cutaneous sensitization by skin barrier dysfunction has been extensively investigated as an initial important step for the onset of allergic disorders. Staphylococcus aureus-dominant dysbiosis in the skin plays an important pathogenic role for development of atopic dermatitis (AD). To understand how the dysbiosis is induced on the surface of stratum corneum (SC), we have been characterizing the normal nature of SC as niche for skin microbiome.

SC consists of stacked dead corneocytes which are generated from terminally-differentiated keratinocytes in stratum granulosum (SG). Visualization of SC by time-of-flight secondary-ion-mass-spectrometry (TOF-SIMS) demonstrated that SC has at least three layers of distinct functional properties; upper sponge-like layer which allows passive influx and efflux of ions, middle hydration layer, and lower barrier layer which is abrogated by filaggrin deficiency.

The pH of SC is considered to be acidic in general while its precise pH distribution and dynamic changes in different environments within SC layers remain to be clarified. Transgenic mice with a ratiometric pH biosensor with pH sensitive fluorescent protein, $Venus^{H148G}$, and pH insensitive protein, mCherry, were generated as a tool to visualize in vivo pH in SC and dissect a role of pH in SC homeostasis. Confocal microscopic analysis of living pH imaging demonstrated that SC has at least two distinct middle-acidic and upper-neutral layers with cross section view, rather than gradual pH changes across the layers. Furthermore, the upper-neutral layers showed mosaic patterns of uneven pH distribution by differentiated units with en face view. These findings indicated the dynamic and complex nature of SC in terms of pH regulation.

Uncovering the physiological mechanisms of SC homeostasis will lead us to develop a novel body surface tactics to conquer the dysbiosis of the skin.

Kenya HONDA (IMS, Gut Homeostasis)

"Modulation of the immune system by the gut microbiota"

The mammalian alimentary tract harbors hundreds of species of commensal microbes that critically influence a multitude of host physiological functions, including the immune system. Manipulation of the gut microbiota holds great promise for the treatment of inflammatory and allergic diseases. However, to date there are only a handful of examples of single species or defined communities of bacteria that can be used to activate and polarize distinct subsets of immune cells. By combining anaerobic culturing and gnotobiotic technique, together with the latest high-throughput sequencing technique, we have built a method to understand the role of individual components of commensal microbes, particularly their immunological attributes. Indeed, we have succeeded in isolation of human and mouse gut-associated commensal bacterial strains that specifically affect the development and function of Th17 cells, Treg cells, and Th1 cells.

In this study, we have identified and isolatedg-expressing CD8+ T cells in the intestine. IFNg+ CD8+ T cells were abundantly present in the intestine kept under specific-pathogen free conditions, whereas few IFNg+ CD8+ T cells were found in germ-free (GF) mice. To investigate whether the human gut microbiota was able to induce IFNg+ CD8+ T cells, GF mice were gavaged with stool samples collected from six healthy volunteers. IFNg+ CD8+ T cells were variably induced, and we selected a mouse that exhibited the highest frequency of IFNg+ CD8+ T cells. Cecal contents were collected from the selected mouse were inoculated into another GF mice, and treated with antibiotics in the drinking water in order to concentrate responsible microbes for IFNg+ CD8+ T cell induction. Ampicillin (Amp) treatment enhanced induction of colonic IFNg+ CD8+ T cells. Cecal contents were collected from Amp-treated mice and cultured in vitro. We succeeded in isolation of 26 strains, and selected 11 strains, which were correlated with the frequency of IFNg+ CD8+ T cells. The mixture of 11 strains sufficiently induced IFNg+ CD8+ T cells when colonized in GF mice. Our findings can be potentially applied to treatment/prevention of viral infection and cancer.

Sidonia FAGARASAN (IMS, Laboratory for Mucosal Immunity)

"Host-gut microbiota mutualism by IgA-glycan"

Immunoglobulin A (IgA) is the most abundant antibody isotype and a major regulator of symbiotic bacteria in the gut. We previously found that IgAs induced upon stimulation by symbiotic bacteria are coating bacteria but such coating contributes to retention rather than elimination of the bacteria. However, the mechanistic details of how IgAs add symbiosis remained largely unknown. Increasing number of experimental data indicate that the generation of gut IgAs is regulated by complex mechanisms. For example, while the majority of IgA are generated by reactions that are T cell-dependent, other IgAs can be induced in a T cell-independent manner. In addition, IgAs can be produced under normal and inflammatory conditions, yet whether they have different functional properties is not yet clear. We hypothesize that post-translational modification of secretory IgA might be regulated, at least in part, by glycan moiety of the antibody and such modifications may impact the symbiotic role of IgA. We are currently analyzing the impact of IgA-glycan on the function and composition of the gut microbiota. Here, we will discuss our recent findings about the role of IgA-glycosylation that hopefully will contribute to understanding of host-microbiota mutualism mediated by secretory IgAs in the gut.

Hidewaki NAKAGAWA (IMS, Laboratory for Genome Sequencing Analysis)

"Cancer genome sequencing for pathogen and symbiosis in human cancers"

Many types of cancers are asscoiated with virus and bactria infection. Hepatitis virus (HBV and HCV) infection is strongly associated with liver cancer, HPV with cervical and head&neck cancer, and H.pylori and EBV with gastric cancer. Some of virus infection can induce carcinogenesis through genomic alterations in the host.

We here examined whole genome seauencing (WGS) and RNA-seq data of 300 human liver cancers (Nat Genet 48:500-509, 2016), and found some pathogen and symbiosis in human liver (cancer) tissues in

addition to HBV and HCV. To detect HBV virus and its integration, we aligned unmapped reads to 79 different HBV genome sequences, and inferred genotypes of the infected HBV in each sample. 74 samples had ≥ 10 reads mapped to the HBV genomes. Of the 74 samples, nine patients were HBV negative by their serological tests, suggesting their "occult" HBV infection. Our analysis identified 223 HBV integration events into human genome. Furthermore, we performed ultra-deep sequencing targeting HBV in human liver tissues and human-hepatocyte chimeric mice with HBV infection, and characterized 1,684 HBV integration sites. To explore any potential of other pathogens and symbiosis, we performed *de novo* assembly of unmapped reads of RNA-seq data from 244 liver cancer and non-cancer liver samples. In addition to sequence contigs derived from HBV, HCV, and several bacteria, we detected some long contigs aligned to adeno-associated virus (AAV) in two liver cancer samples, which were validated to be integrated into human genome.

To make profiles of pathogen and symbiosis in human, we are now analyzing pathogens and symbiosis in human tissues (liver, digest organs, and prostate) by sequencing DNAs/RNAs from these human tissues.

Toru TAKUMI (BSI, Laboratory for Mental Biology)

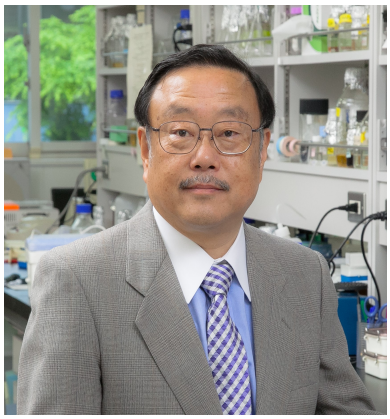
"The gut-brain axis in autism"

Autism spectrum disorder (ASD) is a childhood neuropsychiatric disorder and one of the most famous neurodevelopmental disorders. Besides major symptoms such as impaired social communication and repetitive behavior, gut and sleep problems are frequent comorbidity. The genetic contribution of the disease causes in ASD is higher than that in other psychiatric diseases, however, the genetic mechanism is not simple but complex as many other multi-factorial diseases and environmental causes are not excluded. On the other hand, the gut microbiota is essential in human health and its disturbance may be involved in various diseases. The gut is also known as a second brain and the gut-brain axis may also be involved in differ disorders including ASD. We are interested in the gut-brain axis in ASD and studying how this axis is regulated and contributes to the pathogenesis by using different mouse models of ASD.

Atsushi IRIKI (BSI, Laboratory for Symbolic Cognitive Development)

"Primate model of "Mind-Body Interaction" through the mechanisms of gut-brain axis via microbiome."

States of human's conscious mind has impacts on bodily states, such as social stress-induced weakening of immune reactions and alteration in metabolic regulatory organ, but no animal models reflecting more highly developed brain function for such mind-body interactions exists to the date. Marmoset Wasting Syndrome (MWS, lethal dysfunction of immune system suspected) is a common problem in captivity among colonies across the world, with unknown mechanisms and thus no treatment established. Social and mental stress in the captivity has been highly suspected, as no evidence of this symptom observed in the wild habitat. Occasionally, our preliminary fecal microbiota transplantation (FMT) have in part attenuated the more sever form of symptoms. Therefore, MWS could be an ideal primate model for studying human mind-body interactions, with most likely implication of understanding microbiome mechanisms.



Hiroyuki OSADA (CSRS, Chemical Biology Research Group)

"Activation of biosynthesis of tenuazonic acid in a plant pathogenic fungus"

Plant pathogenic fungi produce a wide variety of small molecules which contain unique and complex structures. However, most biosynthetic gene clusters are not working in the laboratory condition. Moreover, the relationship between pathogenicity and the small molecules remains unclear. To activate the silent gene clusters in fungi, successful approaches such as epigenetic regulation, promoter exchange, and heterologous expression have been reported in these days.

Tenuazonic acid (TeA) is a well-known mycotoxin produced by various plant pathogenic fungi, but its biosynthetic gene has been unknown to date. Thus, we undertook this study to know the biosynthetic mechanism of TeA in *Pyricularia oryzae* (Rice blast fungus). *P. oryzae* did not produce TeA under the laboratory condition. However, the production of TeA was induced by the disruption of OSM1 gene which is involved in the response to environmental signals. Interestingly, the addition of 1% DMSO also induced TeA production in *P. oryzae*. TeA is synthesized from isoleucine and acetoacetyl-coenzyme A by TeA

synthetase 1 (TAS1). TAS1 is a unique non-ribosomal peptide synthetase and polyketide synthase (NRPS-PKS) hybrid enzyme.

Katsuyuki SHIROGUCHI (QBiC, Lab for Integrative Omics)

“Toward "The third generation" bacterial microbiota analysis”

We are developing a novel high-throughput cell-number quantification method which counts the number of bacteria at single-cell level based on single-base identification in 16S rRNA sequences. Using this method, we try to understand the cell-cell network, and to identify "master" bacteria which may affect the cell-number distribution in a bacteria community. Eventually, we would like to control the cell-number distribution using, e.g., the "master" bacteria.

Hiroyoshi AOKI (Center for Advanced Photonics, Ultrahigh Precision Optics Technology Team)

“Agarose Gel Microcapsule: Pico-liter-scale DNA Amplification Microvessel for Single Cell Genomics.”

Whole genome amplification (WGA) revealed a world of diverse and complex yet-uncultured microorganisms (YUM) in symbiotic microbiomes. Single cell genomics of YUM provided us physiological functions of the individual YUM. However, the single cell WGA had some technical problems: DNA contamination and amplification bias in next generation sequencing (NGS). To overcome the technical issues, we developed agarose gel microcapsule (AGM) for the single cell genomics, which was consisted of an alginate-sol core and an agarose-gel shell. In a closed microtube, the AGM was easily prepared by gelating emulsion of the alginate gel–the agarose sol with cooling, and then the alginate-gel core was solated with EDTA. The agarose gel shell prevented the external DNA contamination whereas it allowed to diffuse low molecular WGA reagents. In the AGM, the alginate core embedded single cell and worked as a separable pL-scale reaction microvessel for WGA. Since the amplification bias was suppressed in quite low reaction volume, the alginate core would be effective for prevention of the amplification bias.

Thus, the AGM provided the pL-scale DNA amplification microvessel, which was able to isolated single cell for NGS. The AGM will accelerate the microbiome network analysis by single YUM genomics.

Akiko MINODA (CLST, Epigenome Technology Exploration Unit)
"Studying the effect of different microbiota at the cell type and subpopulations landscape level by single cell RNA-seq"

Different environments give different signals to the host that may result in epigenetic changes. These changes in turn may result in different distribution of cell types or subpopulations. In this project, we are interested in determining how the host plant is affected in the presence of different microbiota, for example at the roots. To address this question, we will utilize single cell transcriptome technology, which is a powerful tool in identifying cell types and subpopulations in tissues that are constituted from multiple cell types. I will present our ongoing efforts on single cell analyses with mouse tissues, where we have been able to identify novel subpopluations.
Lastly, we hope to carry out and integrate additional omics analyses such as ATAC-seq (open chromatin regions) both at the single cell and bulk levels to further understand the effect of different microbiomes at the chromatin level.

Arnaud GERMOND (QBiC, Laboratory for Comprehensive Bioimaging)
"Raman spectroscopy as a tool for cell-line discrimination and gene expression monitoring"

Raman spectroscopy is a laser microscopy technique that characterizes the vibration of molecule, allowing to monitor the chemical fingerprint of a given biological entity in a fast, non-labelling and non-destructive manner. Single a Raman spectrum has been proved to be a faithful signature of the metabolic state of living cells (1-2), since it holds information about the major cellular compounds (protein, RNA, DNA, cytochrome, aromatic compounds, pigments, …). Multivariate statistical analyses of Raman spectra allow to perform cell-line discrimination and classification with very low error

rates. In RIKEN, I built an automated Raman spectroscopy platform to discriminate cell lines (strains) of human cells and microorganisms with a single-cell resolution. Previous studies showed the possibility to monitor the effects of antibiotic drugs in term of exposure and concentration. No study, however, has tried to identify strains according to their acquired resistance or to the mechanism of antibiotic resistance (ribosomal RNA, cell-wall,…). My recent work demonstrates the ability of Raman spectroscopy to identify the population of *E. coli* strains with acquired antibiotic resistance, and also to classify strains by the molecular mechanism of their respective antibiotic resistance. Three individual experiments were performed to show the results are reproducible. Finally, in my most recent work, I built a predictive model to monitor the gene expression based on the sole Raman spectral information. Major biological functions of *E. coli* populations were successfully predicted. We envision that our approach will find a broad range of applications in non-invasive biological and biomedical research, ranged from diagnosis to monitoring of gene expression at single-cell level.

References

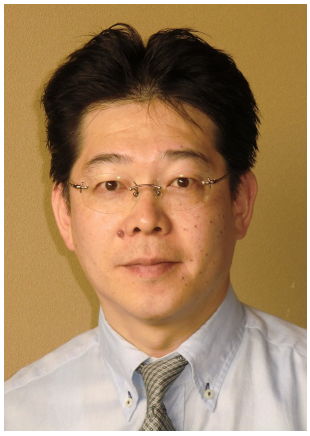
1. *Visualizing the appearance and disappearance of the attractor of differentiation using Raman spectral imaging*. Taro Ichimura, Liang-da Chiu, Katsumasa Fujita, Hiroaki Machiyama, Satoshi Kawata, Tomonobu M. Watanabe, Hideaki Fujita. Scientific Reports, doi:10.1038/srep11358 (Jun. 2015)
2. *Visualizing Cell State Transition Using Raman Spectroscopy.* Taro Ichimura, Liang-da Chiu, Katsumasa Fujita, Satoshi Kawata, Tomonobu M. Watanabe, Toshio Yanagida, Hideaki Fujita. PLoS One, 9(1), e84478 (Jan. 2014)

List of Speakers on August 22nd

Chikara FURUSAWA (QBiC, Laboratory for Multiscale Biosystem Dynamics)

"Analysis of phenotypic evolution and symbiosis using microbial laboratory evolution"

In the process of symbiotic formation, organisms change their phenotype adaptively each other in a flexible and robust manner. Here, to understand the nature of robust adaptive evolution and possible changes of phenotype-genotype mappings, we studied laboratory evolution of microorganisms under various conditions. In the first project, we analyzed evolutionary dynamics of *E. coli* under 96 different stress conditions including addition of acids, metals, surfactants, antibiotics, and so on, and the changes of phenotypes and genotypes were quantified. The results of these comprehensive analyses suggested that the expression changes were restricted to a low-dimensional dynamics, while diverse genomic changes can contribute to similar phenotypic changes. In the second project, to understand the contribution of expansion of genotype space to evolutionary dynamics, we performed laboratory evolution of cells obtained by protoplast fusion. We obtained actively growing protoplast cells, called L-form cells, from various microorganism species. Then, cells after protoplast fusion were cultured under various stress conditions, to obtain stably fused cells. The preliminary results showed that after laboratory evolution of fused cells, a mixture of ancestor phenotypes, i.e., acquisition of resistances to two different stresses was observed, suggesting that intra-cellular symbiosis contributed to the evolution. We expect that the analyses of evolutionary constraints and evolvability of microorganisms will contribute to capturing potentiality of symbiotic formation among microorganisms.



Shunji TAKAHASHI (CSRS, Natural Product Biosynthesis Unit)

"Unveiling Chemical Signal and Biosynthetic Mechanism of Reveromycin"

RM-A is a polyketide natural product isolated from *Streptomyces*. Microorganisms utilize a variety of chemical signals. *Streptomyces*, a soil-dwelling gram-positive bacteria, utilize autoregulators to produce a variety of specialized metabolites (SMs). Chemical signals derived from extra-species/environmental stimuli also induce SM production in *Streptomyces* species. Improved production of bioactive SMs has been a long-standing goal. SM production by *Actinomycetes* has been explored by modifying the components of the culture medium. By testing various culture media, we found that reveromycin (RM) production by *Streptomyces reveromyceticus* SN-593 was enhanced by adding tomato juice to the culture medium. This observation led us to speculate that naturally existing extracellular chemicals can activate secondary metabolism. Based on the screening a natural product library, we found that β-carboline chemical signalling enhanced RM production by activating the LuxR family transcriptional regulator in the RM gene cluster.

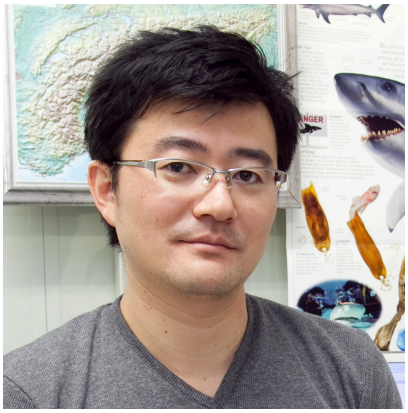
We also analysed biosynthetic mechanism of RM. The biosynthesis includes 2-alkylmalonyl-CoA formation, polyketide truncation, spiroacetal formation, hydroxylation of spiroacetal structure, and subsequent succinylation. Among these, we focused on the mechanism of succinylation of tertiary alcohol in the last step of biosynthesis. By chemical synthesis approach, the succinylation required high pressure (1.5 Gpa). However, *S. reveromyceticus* SN-593 performs the reaction under normal culture condition. Therefore, it is of interest to understand the mechanism. We found that three enzyme (RevK, RevL, and RevM) were required to catalyse succinylation in the presence of ATP, fumaric acid, NADH, and RM-T1. To understand the mode of reaction we performed crystal structure analysis.

Ryo NAKABAYASHI (CSRS, Metabolomics Research Group)

"Metabolome analysis in the parasitism between Solanum lycopersicum and Cuscuta campestris"

It is suggested that plants exert specialized metabolites (previously called secondary metabolites) to defend against parasitic plants, but little is known so far. Here, metabolome analysis using liquid chromatography-mass spectrometry was performed to understand specialized metabolites in tomato (*Solanum lycopersicum*) induced by *Cuscuta campestris*. The comparative analysis of the metabolome in *Cuscuta*, tomatoes, and safflower showed that tomato-specific metabolites induced by *Cuscuta*. Computational metabolome annotation found that dehydrophenolamides including kukoamine A are highly

induced by *Cuscuta* as well as phenolics and glycoalkaloids. The accumulation of the dehydrophenolamides decreased in *jai1* mutant, which lacks the jasmonic acid (JA) signaling, revealed that the biosynthesis of the dehydrophenolamides are under the JA signaling. A discriminate analysis between tomatoes that killed *Cucuta* and ones which healthfully grew *Cuscuta* showed that the metabolites are associated with the defense to *Cuscuta*.



Shigehiro KURAKU (CLST, Phyloinformatics Unit)

"Genome evolution and ecosystem of marine vertebrates"

Phyloinformatics Unit in RIKEN Kobe was initially launched as a DNA sequencing core facility of Center for Developmental Biology (CDB) and later in 2014 fused into Center for Life Science Technologies (CLST). Its current activities range from original technical development for next-generation sequencing and genome informatics to technical support for sequencing data production for whole RIKEN. Based on experience of participating multiple international consortia for sequencing and analyzing vertebrate genomes, it now autonomously conducts *de novo* genome sequencing and analysis of several early vertebrates including elasmobranch sharks whose genome sizes exceed those of many other vertebrates. In particular, our ongoing analyses focus on evolution of protein-coding gene repertoire responsible for embryonic development, endocrine control of homeostasis, and physiology. In the near future, the scope of the unit will include monitoring ecosystems of marine organisms based on environmental DNA analysis and host-parasite interaction in collaboration with local aquaria. This presentation will cover some of the unit's past achievements, overview of ongoing projects, and the near future perspectives.

Ryuhei NAKAMURA (CSRS, Biofunctional Catalyst Research Team)

"Energy Flow Organized by Benthic Ecosystems"

As represented by three key energy metabolisms in biology (i.e. photosynthesis, respiration, and fermentation), life can be regarded as the material organized by energy flow. Like individual organisms, highly complicated ecosystems stand on the sophisticated mechanisms of energy flow generated by the intricate chain of various metabolic interaction between bacteria and animals. If the modest biological and physical disturbance to healthy ecosystems occurs, it may return to original status, as opposed to becoming very different ecosystems. Such an ability is called energy homeostasis and is a key feature for ecosystem to recover original energy flow state from excess amount of energy input by human activity. Therefore, understanding the mechanisms how ecosystems maintain energy flow is of fundament importance to keep ecosystems healthy and enable sustainable harvest and production from the ecosystems.

To deepen our understanding of energy homeostasis, we have applied the knowledge and technique developed in the field of electromicrobiology to coastal benthic ecosystems. In this presentation, I will introduce a novel electrochemical system to monitor, control, and optimize microbial metabolisms in the reconstituted benthic ecosystem and discuss how benthos and microbes cooperatively maintain energy homeostasis.

Jun KIKUCHI (CSRS, Environmental Metabolic Analysis Research Team)

"Marine and fishery product studies assisted by metabolic profiling with data science"

Data-driven approaches were applied to investigate the temporal and spatial changes of natural fishes and environmental waters in Japan(1-6). To do this, NMR-based metabolic profiling, ICP-OES-based ionomic profiling, as well as NGS-based microbial profiling were employed to obtain the "big-data" from natural ecosystems (7,8). I will also introduce the first achievement of collaborative research with Fishery Research Agency, for the trans-omics approach to characterize fish nutritional biorhythm in Leopard coral groupers (9).

References

1) Yoshida et al. (2014) *Sci. Rep*. 4, 7005
2) Asakura et al. (2014) *PeerJ* 2, e556 (2014).
3) Misawa et al. (2016) *Anal. Chem*. 88, 6130-6134.
4) Asakura et al. (submitted).
5) Wei et al. (submitted).
6) Uchimiya et al. (submitted).
7) Kikuchi et al. (invited) *Prog. NMR Spect.*
8) Kikuchi & Yamada (invited) *Analyst*
9) Mekuchi et al. (in press) *Sci. Rep*.

Shigeharu MORIYA (CSRS, Biomass research platform team)

"RNA-seq approaching to unlock the ecological functions of natural microbiome system"

The current expansion of next-generation high-throughput sequencing has promoted various applications for the biological sciences, one of which is microbial ecology analysis. Because most natural microbial community members are uncultivable, such a meta-genomic and/or meta-transcriptomic analysis is one of the few ways to access information regarding the community structures of environmental microbial communities and the genetic activities in these communities.

Here, we developed a quantitative analysis pipeline for both microbial community structure and gene expression. Mapping-based analysis is designed with ordinary transcriptome analysis tools, a public small subunit RNA gene database and self-assembled contigs as a structural gene reference database. This new pipeline, All RNA Information sequencing (ARIseq), can be applied to any environmental samples.

We performed this analysis on samples from an acidic mine drainage biofilm ecosystem and confirmed estimates of its quantity by quantitative PCR. In addition to identifying an iron oxidation bacterial-based community that has been reported previously, the results of this analysis suggest that target ecosystems include a specific diatom community that may have an important role in this environment.

Also, we will report about progress and future plan of research about termite symbiotic system by using of meta- or single cell- transcriptome analysis.

Yuichi HONGO/Hirokazu KUWAHARA (Tokyo Technology Institute, BRC Microbe Division)

“Functional genomics of multiple endo- and ectosymbionts of termite gut protists”

Termites harbor symbiotic protists and prokaryotes in their hindgut, which are unique to termites. Certain prokaryotic species are specifically associated with the protist cells: this multilayer symbiosis is a prominent feature of the termite gut ecosystem. We have been investigating the functions of such unculturable endo- or ectosymbiotic prokaryotes of the cellulolytic gut protists by analyzing their genome sequences. We will present the newest results from the genome analysis of cytoplasmic, nucleoplasmic, and surface-embedded bacterial symbionts of the *Trichonympha* protists in the termite gut.

Akiko FUJIWARA (CSRS, Chemical Genomics Research Group)

“Elucidation of molecular mechanisms for multiple endosymbiotic systems and development of novel control technologies by the inhibition of endosymbiotic machinery in Bemisia tabaci, the agricultural pest”

Bemisia tabaci is a pest insect on agricultural crops worldwide. They are highly resistant to major insecticides. Therefore, novel pest control methods that are effective and environmental-friendly are needed. *B. tabaci* harbors endosymbiotic bacteria. The symbionts are critical to survival and reproduction of host, because they supply host with essential nutrients. The aim of this study is to elucidate the molecular mechanisms and develop the novel control technologies by targeting endosymbiotic machinery. In our previous studies, several candidate genes related to the symbiotic systems were discovered. We’ve analyzed for localization and function of symbiotic factors in preparatory for screening for inhibitors of symbiotic mechanism by chemical biology high throughput methods. In this workshop, a summary of present research is presented.

Seok-Won KIM (IMS, Laboratory for Integrated Bioinformatics)

"Analysis of the onset mechanism of obesity and type 2 diabetes correlated with gut microbiota using data mining"

Recently, many studies have reported that disorders in the gastrointestinal tract are not only associated with intestinal diseases such as colitis or colon cancer but also connected to obesity or metabolic diseases like type 2 diabetes and arteriosclerosis, and connected to systemic diseases such as allergic rhinitis and autoimmune diseases. Therefore, it is considered that understanding and proper controlling intestinal environment changes related to obesity or a disease such as type 2 diabetes can lead to a better prevention, diagnosis, and development of therapy. We collect clinical information such as lifestyle, gut microbiota, metabolic functions, and genetic polymorphisms from patients' medical checkup, and data from animal experiments using disease model mice. These data are integrated and analyzed transversely to reveal the correlation between obesity or type 2 diabetes and shape of gut microbiota. From understanding the relations between onset mechanism of lifestyle diseases and gut microbiota change, our goal is to establish methods to prevent the diseases. This initial study allows us to develop the platform for analysis and to perform a proof of concept with model cases of type 2 diabetes. Later, it is expected to apply the same workflow to other diseases related to an imbalance of gut microbiota.

Moriya OHKUMA (BRC, Japan Collection of Microorganisms)

"Single-cell genome analysis of termite gut symbionts"

Functional roles of individual microbial symbionts and their interactions among members of symbiotic microbial communities are important for understanding the symbioses. Unfortunately, most symbionts are formidably difficult to culture and usually comprise a complex community, which resist standard microbiological and molecular biological studies. We are investigating termite-gut microbial symbionts

particularly through single-cell genome analyses. There are several species-specific associations between gut cellulolytic protists and bacteria belonging to phyla Spirochaetes, Bacteroidetes, and others. The associations involve both intracellular endosymbiosis and cell-surface attached ectosymbioses. The associated bacteria comprise a majority of the gut community and play roles in the host nutrition. We also investigate the gut microbial community of a termite species that typically lack gut cellulolytic protists and thus bacteria are responsible for lignocellulose digestion. In addition to studies on mechanisms for efficient digestion and metabolisms of the symbionts, comparative studies of various symbionts are also beneficial to understand their coevolution.
