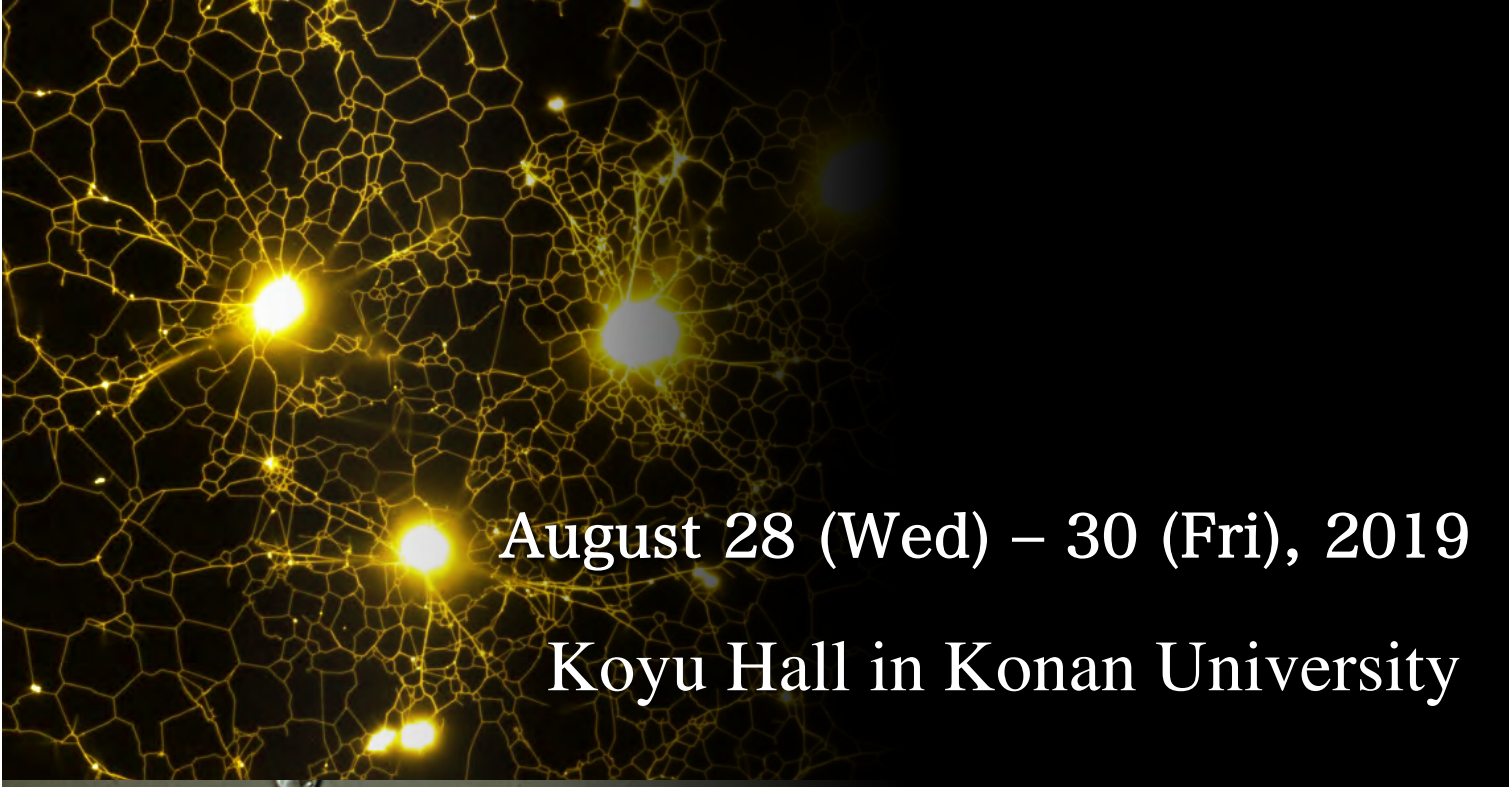



A microscopic image showing several elongated, branching protists with a central body and thin, thread-like extensions, set against a dark background.

First International Conference on Labyrinthulean Protists (ICoLP)

Program & Book of Abstract

A microscopic image showing a dense, interconnected network of thin, yellowish-brown filaments, possibly representing a protist colony or a network of cells, with several bright, glowing points of light.

August 28 (Wed) – 30 (Fri), 2019
Koyu Hall in Konan University

A microscopic image showing a cluster of small, round, translucent protists, possibly ciliates or flagellates, with some showing internal structures, set against a dark background.

KONAN GAKUEN 100th Anniversary
International Scientific Symposium Series
Sponsored by The Hirao Taro Foundation of
KONAN GAKUEN for Academic Research

First International Conference on Labyrinthulean Protists (ICoLP)

KONAN GAKUEN 100th Anniversary International Scientific Symposium Series
Sponsored by The Hirao Taro Foundation of KONAN GAKUEN for Academic Research

Period August 28 (Wed) – 30 (Fri), 2019

Venue Koyu Hall in Konan University

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Dr. Makoto Ito (Kyusyu University, Japan)
Dr. Masahiro Hayashi (Miyazaki University, Japan)
Dr. Tsunehiro Aki (Hiroshima University, Japan)
Dr. Jackie L. Collier (Stony Brook University, USA)

Co-organized by

Department of Biology and Institute for Integrative Neurobiology,
Konan University

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Welcome to the First International Conference on Labyrinthulean Protists (ICoLP)

Labyrinthulean protists are used as industrial microorganisms to produce high-value compounds such as polyunsaturated fatty acids and terpenes including carotenoids. They also play important roles in food webs and elemental cycles in marine ecosystems, and are involved in some diseases of marine plants and animals. Research on labyrinthulean protists therefore extends over a wide variety of academic and industrial fields, and so opportunities for information exchange among the diverse researchers working on these organisms are limited. Small symposia have been held several times in Japan since 2000. To reach a broader audience, we decided to hold the first international conference so that labyrinthulid researchers can gather together to promote future research and development on these interesting and useful microorganisms. This conference will also commemorate the 100th anniversary of Konan Gakuen in 2019. We are hoping to gather 200 participants from many countries to visit the land of Kobe beside the Seto Inland Sea, where many labyrinthulids live, for active discussions at keynote lectures, oral and poster presentations.

Invited Speakers

Seshagiri Raghukumar (Ex-Scientist of CSIR-National Institute of Oceanography, India)
Chandralata Raghukumar (Ex-Scientist of CSIR-National Institute of Oceanography, India)
Guangyi Wang (Tianjin University, China)
Xiao Qiu (University of Saskatchewan, Canada)
Jackie L. Collier (Stony Brook University, U.S.A.)
Varada Damare (Goa University, India)
Ning He (Xiemen University, China)
Javier del Campo (University of Miami, U.S.A.)
Brandon T. Hassett (University of Tromsø - The Arctic University of Norway, Norway)
Yoko Hamamoto (Konan University, Japan)
Daisuke Honda (Konan University, Japan)
Tsunehiro Aki (Hiroshima University, Japan)
Makoto Ito (Kyusyu University, Japan)

Venue

Koyu Hall in Konan University 甲南大学 甲友会館

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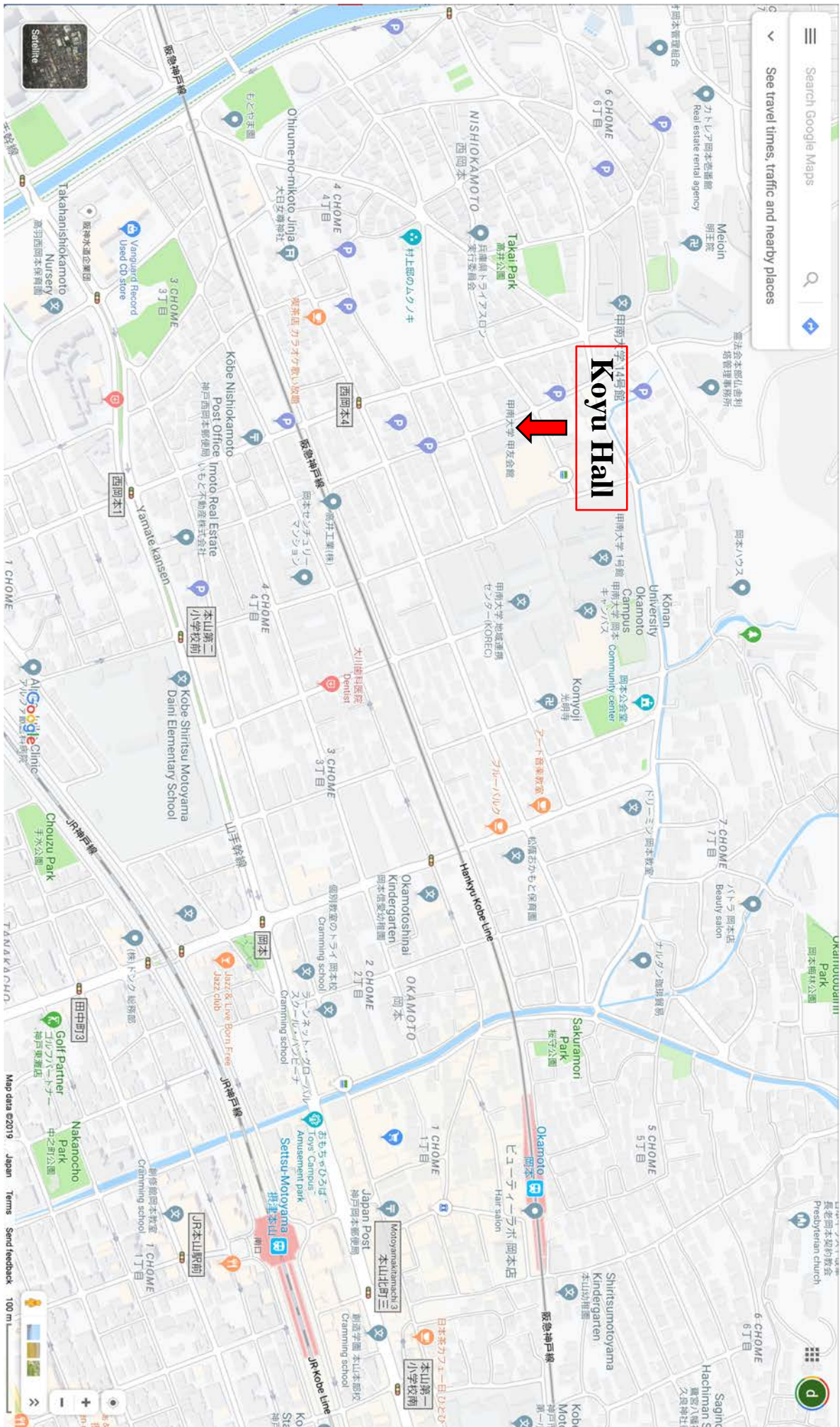
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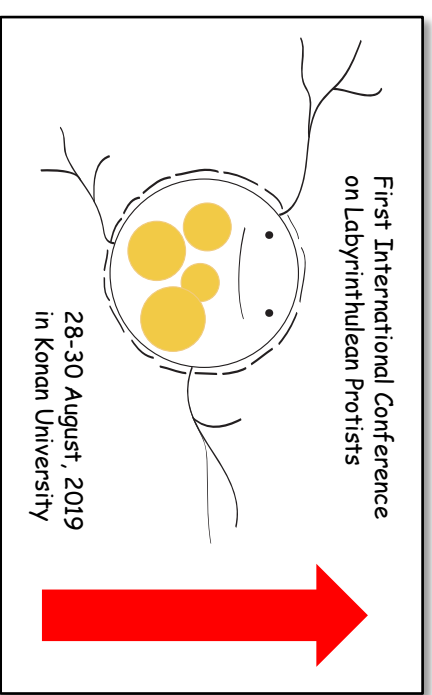
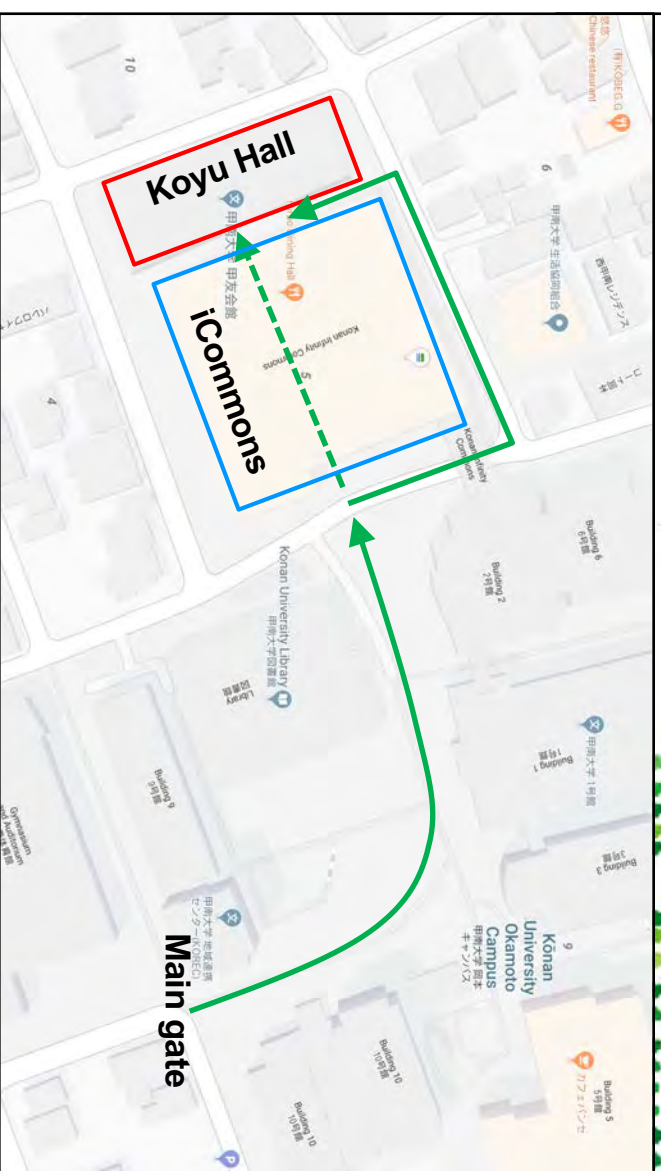
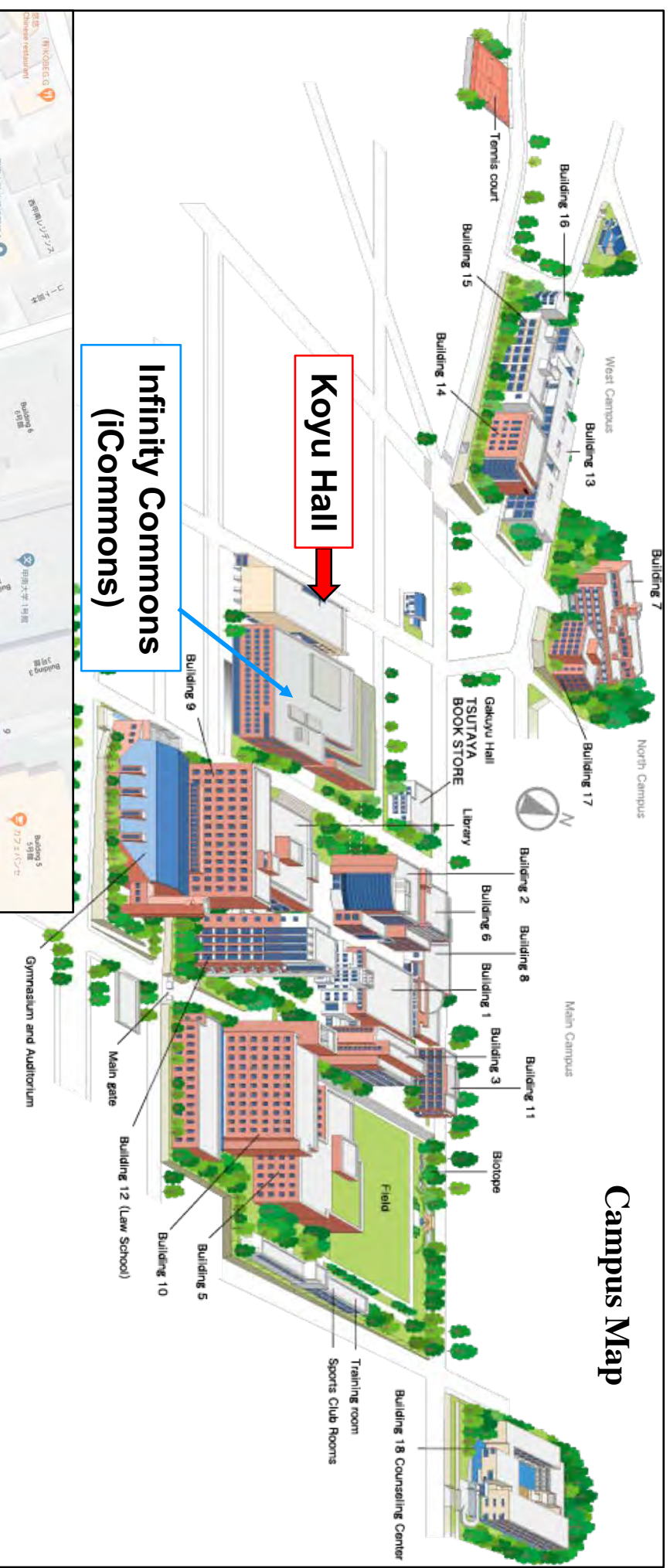
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28-30 August, 2019
in Konan University

Please find this mascot sign

Program of First International Conference on Labyrinthulean Protists (ICoLP)

Invited Papers & Contributed papers (Oral Presentations)

Wednesday, August 28

12:30 -	Registration at Koyu Hall
13:00 - 15:00	Tea Ceremony (Applicants only) at iCommons
15:15 - 15:30	Opening Remarks
15:30 - 16:10 Invited Paper 1	Seshagiri Raghukumar (Ex-Scientist of CSIR-National Institute of Oceanography, India) Eight decades and more: Emergence of Labyrinthulomycetes as major eukaryotic, marine osmotrophs
16:10 - 16:50 Invited Paper 2	Makoto Ito (Kyusyu University, Japan) LipoQuality of thraustochytrids: Synthesis and accumulation of DHA in thraustochytrids
17:00 - 19:00	Welcome Reception Poster Session 1 (Odd number)

Thursday, August 29

09:20 - 10:00 Invited Paper 3	Guangyi Wang (Tianjin University, China) Significant role of Labyrinthulomycetes protists in the ecological and biogeochemical processes of coastal and pelagic waters
10:00 - 10:40 Invited Paper 4	Yoko Hamamoto (Konan University, Japan) Studies on ecological role and effect of labyrinthulids in marine environment
10:40 - 11:00	Tea Break
11:00 - 11:40 Invited Paper 5	Varada Damare (Goa University, India) Relation of aplanochytrids with the zooplankton in the marine environment
11:40 - 12:05 Contributed paper 1	Chandralata Raghukumar (Ex Scientist: National Institute of Oceanography, India) Occurrence of thraustochytrids in an extreme marine niche
12:05 - 12:30 Contributed paper 2	Yousuke Taoka (University of Miyazaki, Japan) In vitro analysis of gastrointestinal tolerance and isolation of thraustochytrids from Japanese littleneck clam (<i>Ruditapes philippinarum</i>)
12:30 - 13:30	Lunch
13:30 - 14:10 Invited Paper 6	Jackie L. Collier (Stony Brook University, U. S. A.) Insight into the ecology of the thraustochytrid QPX, an opportunistic pathogen of hard clams
14:10 - 14:50 Invited Paper 7	Javier del Campo (University of Miami, U. S. A.) Unveiling the ecological role of novel labyrinthulid diversity. A call to action
14:50 - 15:30 Invited Paper 8	Brandon T. Hassett (University of Tromsø - The Arctic University of Norway, Norway) Arctic marine thraustochytrids: culturing and metabarcoding reveals novel diversity
15:30 - 15:55 Contributed paper 3	Ryosuke Nakai (National Institute of Advanced Industrial Science and Technology, Japan) Unexpected distribution of labyrinthulomycetes in Antarctic freshwater habitats
16:00 - 16:50	Poster Session 2 (Even number)
17:00 - 18:00	Transfer to the Banquet Hall
18:00 - 20:30	Conference Banquet at Sakabayashi

Friday, August 30

09:20 - 10:00 Invited Paper 9	Xiao Qiu (University of Saskatchewan, Canada) Biosynthesis and assembly of very long chain polyunsaturated fatty acids in <i>Thraustochytrium</i>
10:00 - 10:40 Invited Paper 10	Ning He (Xiemen University, China) Studies on the polyketide synthase pathway and omega-3 polyunsaturated fatty acids synthesis in <i>Schizochytrium</i>
10:40 - 11:00	Tea Break
11:00 - 11:25 Contributed paper 4	Evgeniya Kiseleva (AJINOMOTO CO., INC., Japan) <i>Aurantiochytrium</i> sp.1 AJ7869 strain, a novel production platform for functional lipids, others than DHA
11:25 - 11:50 Contributed paper 5	Tonje M. B. Heggeset (SINTEF Industry, Norway) Genome annotation and transcriptome analysis of the lipid- and DHA-accumulating <i>Aurantiochytrium</i> sp. T66
11:50 - 12:15 Contributed paper 6	Inga Marie Aasen (SINTEF Industry, Norway) Fatty acid synthesis in <i>Aurantiochytrium</i> sp., revealed by analyses of growth and production kinetics and global proteomes
12:15 - 13:15	Lunch
13:15 - 13:40 Contributed paper 7	Nozomu Okino (Kyushu University, Japan) Visualization of endoplasmic reticulum and mitochondria in <i>Aurantiochytrium limacinum</i> ATCC MYA-1381
13:40 - 14:05 Contributed paper 8	Yohei Ishibashi (Kyushu University, Japan) A novel thraustochytrid lipase/phospholipase with unique positional specificity contributes to microbial competition and fatty acid acquisition from the environment
14:05 - 14:45 Invited Paper 11	Tsunehiro Aki (Hiroshima University, Japan) Gas-to-lipids bioprocessing by acetogens and thraustochytrids
14:45 - 15:00	Closing Ceremony

Contributed papers (Poster Presentations)

Odd number: Wednesday August 28, 17:00 –

Even number: Thursday August 29, 16:00 –

Contributed paper 9	Charose Marie Ting Perez (Hiroshima University, Japan) Metabolite profile analysis of <i>Aurantiochytrium limacinum</i> SR21 grown on acetate-based medium for biotechnological applications
Contributed paper 10	Jackie L. Collier (University, Stony Brook, USA) Characterization of carotenoid knockout mutants in <i>Aurantiochytrium limacinum</i>
Contributed paper 11	Jiaqian Li (Tianjin University, China) Labyrinthulomycetes protist communities in oxygen minimum zones (OMZs) of the bay of Bengal (the Indian Ocean)
Contributed paper 12	Minato Kasari (Kyushu University, Japan) Distribution of a novel sphingolipid, Ceramide Glyoxylic Ethanolamine (CGE), in thraustochytrids
Contributed paper 13	Toshiki Miyaoka (Konan University, Japan) Search for nutrients required for growth of the strains of “difficult-to-culture” aplanochytrids
Contributed paper 14	Ayako Matsuda (University of Miyazaki, Japan) Metabolic profiling of xylose assimilating thraustochytrid <i>Aurantiochytrium</i> sp.
Contributed paper 15	Yuki Kubo (Kyoto University, Japan) Overexpression of the carotenoid synthesis gene, <i>CarS</i> , in <i>Aurantiochytrium limacinum</i>
Contributed paper 16	Tomi Morimoto (Konan University, Japan) Examination of quantitative PCR to quantify the abundance of oblongichytrids in marine environment
Contributed paper 17	Yui Takeuchi (University of Miyazaki, Japan) Characterization of novel eicosapentaenoic acid producing thraustochytrid
Contributed paper 18	Haruka Umazume (Konan University, Japan) Examination of the condition in the gene transfer into <i>Parietichytrium</i> cells by electroporation (Labyrinthulea, Stramenopiles)

Contributed paper 19	Mai Shiroy (Kyoto University, Japan) Research on a dehydratase gene which is responsible for DHA synthesis in <i>Aurantiochytrium limacinum</i>
Contributed paper 20	Ryu Tsukasaki (University of Miyazaki, Japan) Utilization of plant biomass wastes (soybean whey and molasses) for cultivation of thraustochytrids
Contributed paper 21	Yoshitake Takao (Fukui Prefectural University, Japan) Genomic analysis of <i>Sicyoidochytrium minutum</i> DNA virus (SmDNAV)
Contributed paper 22	Haruka Nakamura (Konan University, Japan) Examination of the condition of CARD-FISH for specific detection of the aplanochytrid cells

Invited Papers (Keynote Lecture)

Eight decades and more: Emergence of Labyrinthulomycetes as major eukaryotic, marine osmotrophs

Seshagiri Raghukumar

Myko Tech Pvt. Ltd., 313 Vainguinnim Valley, Dona Paula, Goa – 403004, India

More than 150 years have elapsed since the first Labyrinthulomycete, *Labyrinthula* was described by Leon Cienkowski and over 80 years since F.K. Sparrow discovered the first thraustochytrid, followed by those of Y. Kobayashi and M. Ookubo. My talk traces research of early, eminent mycologists, particularly during the 1950's to the 70's that has led to our present understanding on the ecology of Labyrinthulomycetes and the continuing relevance of their work to the future. The discovery of many novel species by Solomon Goldstein stimulated interest in their biodiversity. Nearly 50 species are currently known and many novel species are being detected using metagenomics. Physiological studies carried out by Goldstein and Helen S. Vishniac provided an insight to their habitat preference and role in the sea. Extending the studies of Willy Hoehnke, Alwin Gaertner not only described the life cycles of many novel thraustochytrids, but also developed quantitative techniques that led to the recognition that Labyrinthulomycetes are abundant in water column from the surface to the deep sea, as well as in sediments. Novel methods to estimate their biomass, such as epifluorescence microscopy, FISH, quantitative pCR and flow cytometry are presently being developed and used. It is now clear that Labyrinthulomycetes often rival bacteria in terms of biomass in the oceans. While focus has been on their occurrence in the water column, their importance in coastal sediments, as well as deep sea waters and sediments has not been adequately addressed. Annemarie Ulken reported the common occurrence of thraustochytrids in mangrove sediments. Their important role in mangrove detrital dynamics has subsequently been firmly established. It also now appears possible that thraustochytrids play a role in microaerophilic or anaerobic mangrove sediments, as well as in polluted waters. Ultrastructure of ectoplasmic net elements and sargenetogenosome by David Porter, Frank O. Perkins and Steve Moss have been important in understanding the mechanism by which Labyrinthulomycetes obtain their nutrition in nature. Guenther Bahnweg and F.K. Sparrow extended our knowledge of these organisms to the Antarctic and clearly showed that psychrophilic thraustochytrids occur in such cold waters. They also described *Aplanochytrium*, species of which now seem to particularly play an important role in the water column. The discovery of viruses in a thraustochytrid by F.Y. Kazama and K.L. Schornstein has been confirmed in recent years by Daisuke Honda and his colleagues. An early report of a thraustochytrid oyster parasite has been followed by the discovery of a number of invertebrate parasites, as well as possible commensals and mutualists. It now appears that the Labyrinthulomycetes are the most significant eukaryotic osmotrophs in the sea. In this, they appear to play a role equivalent to that of the mycetaen fungi on land. The future challenge is to unravel their role in energy transfers in marine biogeochemical pathways and their importance in the food web.

LipoQuality of thraustochytrids: Synthesis and accumulation of DHA in thraustochytrids

Makoto Ito

Department of Bioscience and Biotechnology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan

LipoQuality is the synthetic word indicating the Quality of Lipids of cells, tissues and organisms, which may reflect the evolutionary process including environments. In this symposium, the author will report and discuss the LipoQuality of thraustochytrids, which are single-cell marine protists capable of accumulating a large amount of docosahexaenoic acid (DHA, 22:6n-3) in their lipid droplets. We first developed a versatile transformation system applicable to thraustochytrids (1) and analyzed the global lipid metabolism of thraustochytrids. The author will talk about how thraustochytrids synthesize DHA (2), and how the DHA is incorporated into glycerolipids in thraustochytrids (3,4). They accumulate DHA in lipid droplets, mainly as constituents(s) of triacylglycerol (TG). We identified the thraustochytrid-specific lipid droplet protein (TLDP1) that regulates the TG accumulation and lipid droplet morphology in thraustochytrids (5). Finally, the author will discuss about the biological significance of DHA synthesis in thraustochytrids using DHA-null mutants of *Thraustochytrium aureum*.

References

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2. Matsuda T, *et al.* Analysis of delta12-fatty acid desaturase function revealed that two distinct pathways are active for the synthesis of PUFAs in *T. aureum* ATCC 34304. *J Lipid Res*, 53, 1210-1222, 2012.
3. Abe E, *et al.* Novel lysophospholipid acyltransferase PLAT1 of *Aurantiochytrium limacinum* F26-b responsible for generation of palmitate-docosahexaenoate-phosphatidyl choline and phosphatidylethanolamine. *PLOS ONE* 9:e102377, 2014.
4. Nutahara E, *et al.* The glycerol-3-phosphate acyltransferase PLAT2 functions in the generation of DHA-rich glycerolipids in *Aurantiochytrium limacinum* F26-b. *PLOS ONE* 14:e0211164, 2019.
5. Watanabe T, *et al.* Regulation of TG accumulation and lipid droplet morphology by the novel TLDP1 in *Aurantiochytrium limacinum* F26-b. *J Lipid Res*, 58, 2334-2346, 2017.

Significant roles of Labyrinthulomycetes protists in the ecological and biogeochemical processes of coastal and pelagic waters

Guangyi Wang^{1,2}

¹ School of Environmental Science & Engineering, Tianjin University, China

² Center for Marine Environmental Ecology, Tianjin University, China

Heterotrophic microbes dominate the secondary production and biogeochemical cycles in the world's oceans. Although heterotrophic bacteria and archaea have been shown to play key roles in these processes, the function of their eukaryotic counterparts (e.g., marine fungi and heterotrophic protists) remain largely unknown in the vast majority of ocean provinces. To that end, this talk will discuss results of our recent studies on Labyrinthulomycetes protists in the coastal waters of China and North Carolina (USA) as well as in the pelagic waters of the South China Sea and the Indian Ocean. In the coastal water, they can dominate the biomass of heterotrophic microbial communities and display interesting complementary dynamic changes in the biomass of heterotrophic microbes. Particularly, some groups of these protists display interesting temporal patterns with complicated interactions with specific bacterial groups in the coastal waters. Although the abundance of these protists in the pelagic waters is slightly less than that in coastal ecosystems, their relative abundance can be more dominant in the secondary production of heterotrophic communities in the deep waters. Clearly, Labyrinthulomycetes protists can be a key component of heterotrophic microbial communities with unique ecological and biogeochemical roles in both coastal and pelagic oceans.

Studies on ecological role and effect of labyrinthulids in marine environment (Labyrinthulea, Stramenopiles)

Yoko Hamamoto^{1,2}, Takanori Shono¹, Ryosuke Nakai³, Mayumi Ueda⁴, Satoshi Nagai⁵, and Daisuke Honda^{1,2},

¹Department of Biology, Faculty of Science and Engineering, Konan University, Kobe, Hyogo, Japan.

² Institute for Integrative Neurobiology, Konan University, Kobe, Hyogo, Japan

³ National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan

⁴ Research Institute of Environment, Agriculture and Fisheries, Osaka, Japan

⁵ National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, Yokohama, Kanazawa, Japan

Thraustochytrids inhabit the ocean all over the world and they have been recognized as important eukaryotic decomposers in the marine ecosystem. Our recent studies revealed a newly recognized pathway in the grazing food chain in the marine ecosystem for thraustochytrids, especially in *Aplanochytrium*. For example, *Aplanochytrium* strains obtain nutrients by consuming living diatoms (Hamamoto & Honda, 2019, PLoS ONE 14: e0208941). Also, *Aplanochytrium* is detected in the gut of zooplankton copepods, suggesting undetected predator–prey relationships in marine ecosystems (Hirai, Hamamoto et al., 2018, Plankton & Benthos Research, 13: 75–82). Here, we investigate their biomass to understand the ecological impact of *Aplanochytrium* and also thraustochytrids. First, we collected the environmental sample at Osaka Bay, Japan and cell number of *Aplanochytrium* in the seawater were estimated by quantitative PCR (qPCR) method. The averages of cell number were 2,100 cells l⁻¹ and 13,000 cells l⁻¹ in the inner and middle part of Osaka Bay respectively. We next evaluated Illumina MiSeq-based metagenomic analyses using these samples. In this result, *Aplanochytrium* were particularly abundant among the taxa identified as thraustochytrids, accounting at the inner and middle part of Osaka Bay, 77.1% (min. 0.0, max. 100.0%) and 67.8% (min. 0.0, max. 100.0%) respectively. Finally, we considered the carbon flow among major components of food web in Osaka Bay to assess the trophic roles of thraustochytrids. Based on the biomass of thraustochytrids, it was estimated thraustochytrids graze 6.1% and 19.0% of the carbon produced by phytoplankton which is consumed by the predator, on the other hand, they also supply 3.6% and 16.2% of the carbon consumed by the microzooplankton (e.g., ciliates) and 1.8% and 6.3% of the carbon consumed by the herbivorous net-zooplankton (e.g., copepods) in the inner and middle part respectively. The previous studies on copepod diets showed that the copepods consumed heterotrophic dinoflagellates (41%), ciliates (36%), diatoms (15%), nanoflagellates (6%), other microalgae (3%) (Yang et al., 2009, Journal of Plankton Research, 31: 647–659). From these results, we speculate that thraustochytrids may also supply almost equal amount of the carbon to copepods which is known for nanoflagellates.

Relation of Labyrinthulomycetes protists, the aplanochytrids, with zooplankton in the marine environment

Varada S. Damare^{1,2}

¹ Biological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Goa 403004, India

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Labyrinthulomycetes protists form an essential component of marine microbial biota. The ectoplasmic net elements produced by these organisms help them not only to secrete various degradative enzymes but also for anchoring to various substrates or particulate matter in pelagic waters. They, therefore, form a part of marine aggregates and attract attention of the predators feeding on aggregates. Zooplankton are sloppy feeders in marine waters, and they are known to consume phytoplankton and bacteria. The occurrence of Labyrinthulomycetes protists in the guts of the mesozooplankton (2-20 mm size range) was revealed by in situ hybridization studies using probes specific for the aplanochytrids. The aplanochytrids are the dominant Labyrinthulomycetes in the oceanic water column, mainly associated with the zooplankton in coastal waters too. The protists were also found in faecal pellets of the zooplankton. Dilution experiments revealed microzooplankton (20-200 μm size range) grazing on aplanochytrids. Labyrinthulomycetes protists produce polyunsaturated fatty acids (PUFAs) that provide nourishment to the zooplankton for their development and reproduction. The Labyrinthulomycetes, therefore form an important part of the marine microbial loop, forming the so-called 'mycoloop', transferring nutrients back to the classical food chain. In addition to this, the protists-fed zooplankton such as rotifers increase the nutritional status of the fish when fed to them in aquaculture. All these organisms harbor on marine aggregates, which are also called hotspots of microbial activity. The aplanochytrids gain significance in marine environment as the aggregates eventually settle to the bottom, thus transferring the particulate organic matter there and serving as carbon sink.

Insight into the ecology of the thraustochytrid Quahog Parasite Unknown (QPX), an opportunistic parasite of hard clams *Mercenaria mercenaria*

Sabrina Geraci-Yee, Bassem Allam, and Jackie L. Collier

School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY, USA

Only a few labyrinthulomycetes are known to be pathogens of marine plants or animals, and the best studied of those infecting animals is Quahog Parasite Unknown (QPX), an opportunistic pathogen of the hard clam, *Mercenaria mercenaria*. To better understand the ecology of QPX, we surveyed the distribution and abundance of QPX and total labyrinthulomycetes over 3 years throughout the marine district of New York, USA. To assess total labyrinthulomycetes we used a quantitative PCR (qPCR) assay based on LABY-A and LABY-Y. To assess QPX we used an existing specific qPCR assay for clam samples, and also created a specific nested qPCR (nqPCR) assay because two rounds of PCR were required to detect and quantify the single thraustochytrid genus in environmental samples. Sequencing of PCR products confirmed the specificity of all three assays. We examined surface seawater, bottom seawater, settleable aggregates, and sediment samples as well as clam tissue and pallial fluid samples. Labyrinthulomycetes were always detected, and sometimes we observed ‘thraustochytrid spikes’ (Ueda et al. 2015) and QPX spikes. QPX was detected in about half of the samples, of all types, and contributed a small fraction of the total labyrinthulomycete community, usually less than 1%. The presence and abundance of QPX in the environment were not related to the prevalence or intensity of QPX detected in clam tissues. QPX was frequently detected in clam pallial fluid, and we detected an intriguing inverse relationship between the abundance of QPX in pallial fluid vs in tissue of individual clams.

Invited Paper #7

Unveiling the ecological role of novel labyrinthulid diversity. A call to action.

Javier del Campo

Rosenstiel School of Marine and Atmospheric Science – University of Miami, USA

Labyrinthulids have a diverse lifestyle in the range of heterotrophy. They can be saprophytic or phagotrophic, they can be free living or parasites, they can be gliders or they can be free-swimmers, etc. This plasticity of life strategies allows them to occupy a wide range of ecological niches. Recently, they have been revealed by high-throughput environmental metabarcoding studies as very diverse, abundant, and ubiquitous. Metabarcoding data are somehow limited in order to define their ecological functions, and despite their potential environmental relevance little is known about the role played by most of this newly described diversity. Thus, we need to increase our culturing and genome sequencing efforts in order to have a better understanding of their biology. The labyrinthulid community traditional expertise of using isolation and culturing methods to describe new species establishes the perfect foundation to succeed in this endeavor.

Arctic marine thraustochytrids: culturing and metabarcoding reveals novel diversity

Brandon T. Hassett and Marti A. Arumi

UiT – The Arctic University of Norway, Department of Arctic and Marine Biology, Tromsø, Norway

High-latitude warming is modifying Arctic marine ecosystem structure and ushering in novel biological interactions as southerly species migrant north and heterotrophic metabolic rates increase. The Labyrinthulea are heterotrophic eukaryotic stramenopiles that have been characterized as important degraders of marine detritus and selectively as parasites of photosynthetic organisms. Throughout the Arctic Ocean, the Labyrinthulea have been detected in general molecular surveys of eukaryotic diversity and found in association with algae. However, this diversity has not been characterized and the corresponding ecology has not been explored. In an effort to fill this gap of knowledge, we analyzed rDNA metabarcode data from across the Arctic Ocean and conducted culturing surveys using pine pollen and axenic diatom cultures. These culturing efforts have yielded several thraustochytrid isolates with low molecular identity, including the recently described saprotroph *Labyrinthulochytrium arktikum* and a new diatom parasite with ~90% 18s rRNA identity to any organism in GenBank. To supplement this culturing-based assessment of Labyrinthulea diversity, metabarcoding was conducted across the Arctic Ocean in sea ice, open ocean, and under-ice sediment. These efforts have revealed that the Labyrinthulea are widely distributed throughout the Arctic marine realm and generally exist in low quantities in the open ocean, but are more abundant in coastal marine ecosystems. Warming temperatures and regionally increasing export of permafrost suggest that more carbon might flow through saprotrophic thraustochytrids in the future Arctic Ocean, with unknown consequences on algal bloom dynamics.

Biosynthesis and assembly of very long chain polyunsaturated fatty acids in *Thraustochytrium*

Xiao Qiu

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Thraustochytrium is a marine protist that can accumulate a large amount of very long chain polyunsaturated fatty acids (VLCPUFAs) such as docosahexaenoic acid (DHA, 22:6-n3), a nutritionally important omega-3 fatty acid for humans and animals. Our interest in this protist went back about 20 years ago when little was known about the biosynthesis of DHA. We discovered the first delta-4 desaturase in the aerobic pathway for the biosynthesis of DHA in *Thraustochytrium*, which opened the opportunity to implement the aerobic pathway to produce DHA in oilseed plants. Recently, we found that the aerobic pathway for the biosynthesis of DHA in *Thraustochytrium* was actually partial with missing a few desaturation steps, and the anaerobic pathway catalyzed by a PUFA synthase, a mega-enzyme with three subunits each with multiple catalytic domains, was solely responsible for the biosynthesis of DHA. Dissecting the embedded catalytic domains of the PUFA synthase and expressing them as standalone enzymes in *E. coli* and *Arabidopsis thaliana* showed they could functionally replace the corresponding discrete enzymes of Type II fatty acid synthases for the synthesis of long chain fatty acids. Very recently, we demonstrated that freshly synthesized DHA was initially incorporated into phosphatidylcholine (PC), and then mobilized to triacylglycerol (TAG) for storage via diacylglycerol (DAG) as the intermediate in *Thraustochytrium*. Elucidating the biosynthesis and assembly of VLCPUFAs would be instrumental in improving the production of VLCPUFAs in native microorganisms and designing new strategies for effective metabolic engineering of these fatty acids in heterologous systems, particularly oilseed crops.

Studies on the polyketide synthase pathway and omega-3 polyunsaturated fatty acids synthesis in *Schizochytrium*

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Polyunsaturated fatty acids (PUFAs), especially ω -3 docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been widely used in food, medical service, health care and other fields. However, the mechanism of PUFAs synthesis by polyketide synthase (PKS) pathway is still unclear, which limited further development of PUFAs production. Malonyl-CoA: ACP transacylase (MAT) was overexpressed in *Schizochytrium* ATCC1381. Lipids and PUFAs yields in MAT overexpressing strain were increased by 10.1 and 24.5%, respectively. The total lipids yield was further increased to 110.5 g/L with fed-batch fermentation, which was 39.6% higher than the wild strain. DHA and EPA yields were enhanced to 47.39 g/L and 1.65 g/L, respectively. Dehydrogenase (DH)-disrupted and chain length factor (CLF)-disrupted strains had significant decreases (65.85 and 84.24%) in PUFAs yield, while the saturated fatty acids proportion in lipids was slightly increased. Meanwhile, the disruption of CLF decreased C-22 PUFA proportion by 57.51% without positive effect on C-20 PUFAs accumulation while DH-disrupted mutant decreased the production of each PUFA. The knockdown of ketoacyl reductase (KR) and ketoacyl synthase (KS) decreased the ratio of DPA and DHA and increased the content of saturated fatty acids. MAT, ER and DH genes were speculated to play a role in the synthesis of lipids in both PKS and FAS pathways while KR, KS and CLF are essential in PKS pathway.

Gas-to-lipids bioprocessing by acetogens and thraustochytrids

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Research and development is being promoted as a pressing issue worldwide on the reduction and reuse of greenhouse gases. We have previously established composite fermentation systems for producing useful lipids from various unutilized biomass using two kinds of microorganisms having different metabolic systems. These outcomes led us to find the condition where the oleaginous microorganism, *Aurantiochytrium* sp., shows high assimilability of organic acids, especially acetic acid. We therefore focused on acetogens, which release acetic acid as the main product of their metabolism, to examine its combination in lipid fermentation. Acetic acid fermentation with *Acetobacterium woodii* was performed using carbon dioxide as a substrate, and the culture broth was successfully utilized by *Aurantiochytrium* sp. for efficient production of triacylglycerols. Furthermore, the metabolic profile of lipid fermentation was subjected to the metabolome analysis to extract improvement factors for optimization of this new gas-to-lipids bioprocess.

Contributed Papers (Oral Presentation)

Contributed Paper (Oral Presentation) #1

Occurrence of thraustochytrids in an extreme marine niche

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Stramenopiles have been reported from hydrothermal vents. We isolated thraustochytrids from the shallow water hydrothermal vent of the D. João de Castro Sea mount, Azores in the Atlantic Ocean. The 1000 m high D. João de Castro seamount lies in the middle of Azores Archipelago (Portugal) on the hyperslow-spreading Terceira rift in Atlantic. Hydrothermal vents are found on top of this seamount, at about 25 m below surface. The vent sites are surrounded by two distinct color zonation, white and yellow. The white zone is characterized by high content of H₂S, CH₄, H₂, Pb and Co and temperature of ca. 37°C. The yellow zone is characterized by high concentrations of Fe, Ba and Mn and a temperature of ca. 60°C. Species diversity of benthic diatoms was marginally higher in the white zone. A higher numbers of heterotrophic bacteria were isolated from the yellow zone. Almost equal numbers of thraustochytrids were isolated from both these zones. One of the thraustochytrid isolates, *Ulkenia visurgensis* from this site showed better growth and protease production in the presence of Fe, Mn and Pb suggesting its adaptation to survive in metal-enriched waters of hydrothermal vent. The protease enzyme was halophilic and showed maximum activity at alkaline pH and 40 to 50 °C temperature. The shallow water vent (23 m at the deepest) was rich in algal growth and thus algal detritus. Thraustochytrids in such vent sites may play a role in degradation of algal detritus and enrichment of waters with dissolved organic matter.

Contributed Paper (Oral Presentation) #2

***In vitro* analysis of gastrointestinal tolerance and isolation of thraustochytrids from Japanese littleneck clam (*Ruditapes philippinarum*)**

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Thraustochytrids are widely distributed in marine environment, and isolated from various samples, such as seawater, sediments, dead leaves, coral and seaweed but there is very little information about isolation from digestive tract in aquatic animals. Previously, we observed that the tolerance of thraustochytrids against gastric juices is depending on genus based on *in vitro* assay, in short, genera *Aurantiochytrium* and *Ulkenia* had higher tolerance and genus *Oblongichytrium* had lower tolerance. In this study, we evaluated the tolerance of thraustochytrids isolated from marine environment to artificial gastrointestinal juices *in vitro*. *Ulkenia* sp. strain Nob9 and *Oblongichytrium* sp. Strains TakIII and Aoi14 isolated from seashore area in Miyazaki, Japan were exposed to artificial acidic buffer, gastric juices and intestinal juices. Results showed that *Oblongichytrium* sp. was sensitive to lower pH (less than pH 3.0) as compared with *Ulkenia* sp. We reared Japanese littleneck clam *R. philippinarum* in artificial seawater for two weeks, and dissected the digestive tract out. The digestive tract samples were homogenized in a 0.01M phosphate buffer saline and directly inoculated on B1 agar plate medium containing antibiotics, penicillin, streptomycin and amphotericin. After incubation, thraustochytrids-like colonies on agar plates were randomly picked up, and phylogenetic analysis using 18S rDNA gene sequence was performed. Results showed that 4 strains and 7 strains were identified to genus *Aurantiochytrium* and *Siyoidochytrium*, respectively. In this study, thraustochytrids were firstly isolated from digestive tracts in *R. philippinarum*.

Contributed Paper (Oral Presentation) #3

Unexpected distribution of labyrinthulomycetes in Antarctic freshwater habitats

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Labyrinthulomycetes are heterotrophic straminipilan protists and occur in detectable amounts mostly in the marine habitats. They possess the unique ability to produce long-chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) and to degrade a wide variety of organic substrates by means of extracellular enzymes. While the phylogenetic diversity and distribution of labyrinthulomycetes in marine environments has been well documented, information pertaining to their freshwater lineage remains limited. In addition to the several lineages already documented from the freshwater environments (e.g. species of the genera *Diplophrys* and *Fibrophrys*), our previous clone library surveys reported a baseline existence of unseen freshwater lineages inhabiting aquatic mosses in an Antarctic lake (Nakai *et al.*, *Polar Biol.*, 2012; Nakai *et al.*, Chapter 2 in *The Ecological Role of Micro-organisms in the Antarctic Environment*, 2019). In the present study, we examined moss specimens and lake water samples collected from the freshwater lakes in the vicinity of Syowa Station during the 56th Japanese Antarctic Research Expedition (JARE56). Using the 18S rRNA gene amplicon sequencing, we detected several phylotypes belonging to labyrinthulomycete groups. These phylotypes showed very low sequence similarity with known cultured labyrinthulomycetes, and they formed an independent cluster on the phylogenetic tree. This implies that uncultured labyrinthulomycetes with high phylogenetic novelty are distributed in the studied freshwater habitats.

Contributed Paper (Oral Presentation) #4

***Aurantiochytrium* sp.1 AJ7869 strain, a novel production platform for functional lipids, others than DHA**

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Thraustochytrids are well known as a ω 3-fatty acids producer, accumulating large amount of DHA in lipid droplets. To clarify whether *Thraustochytrids* can be used as a platform for sterol production, the following study was conducted. *Aurantiochytrium* sp.1 sterol-rich AJ7869 strain, accumulating 4.1% of total sterols/DCW, was isolated from the nature. Modified versatile transformation system for *Thraustochytrids* was developed for this strain. We found out, that disruption of 24-dehydrocholesterol reductase gene led to 24-methyl-sterols (stigmasterol and brassicasterol) accumulation and animal sterols disappearance. Otherwise, disruption of sterol 24-C-methyltransferase gene led to animal sterols (cholesterol) accumulation and terminated plant and fungal sterols biosynthesis. Thus, we showed, that AJ7869 strain can be used as a potential producer of both, plant and fungal sterols, or animal sterols. To confirm whether accumulation of a target sterol can be increased by breeding technologies, we constructed a cholesterol producer strain from AJ7869 parent strain, accumulating 1.3% cholesterol/DCW. Expression of 7-dehydrocholesterol reductase and 24-dehydrocholesterol reductase genes, disruption of 24-C-methyltransferase and C-22 desaturase genes led to cholesterol/DCW increase from 1.3% to 3.1%. Also we showed, that disruption of 7-dehydrocholesterol reductase gene led to pro-vitamin D3 accumulation. These results allow to conclude, the AJ7869 strain might be used as a platform for various sterols production.

Contributed Paper (Oral Presentation) #5

Genome annotation and transcriptome analysis of the lipid- and DHA-accumulating *Aurantiochytrium* sp. T66

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Thraustochytrids of the genera *Schizochytrium* and *Aurantiochytrium* accumulate oils rich in the essential, marine n3 fatty acid docosahexaenoic acid (DHA). As part of studies of the DHA-production in *Aurantiochytrium* sp. T66 (ATCC® PRA-276™), the 43 Mbp draft genome was annotated based on ab-initio prediction combined with RNA-Seq mapping. The genome encodes 11683 putative genes, of which functional information could be assigned to 9090. T66 show high sequence similarity to *Aurantiochytrium* sp. S-3 (ATCC® 26185™), despite the publicly available genome record of S-3 covering only 18.1 Mbp.

A global transcriptome analysis was carried out with the aim to provide more knowledge about factors that affect the DHA-productivities and the contributions of the two enzyme systems used for fatty acid synthesis in thraustochytrids, fatty acid synthetase (FAS) and Polyunsaturated fatty acid (PUFA) synthase. Batch cultivation with nitrogen starvation, which is well-known to initiate lipid accumulation in oleaginous organisms, were compared to cultivation with nitrogen in excess, where lipid accumulation was obtained by oxygen limitation. A significant up-regulation of FAS under N-deficient conditions was found, while the PUFA-synthase genes were only marginally upregulated. Neither of them was upregulated under O₂-limitation where nitrogen was in excess, suggesting that N-starvation mainly affects the FAS and may be less important for the PUFA-synthase. The transcriptome analysis also revealed responses likely to be related to the generation of reducing power (NADPH) for fatty acid synthesis.

Contributed Paper (Oral Presentation) #6

Fatty acid synthesis in *Aurantiochytrium* sp., revealed by analyses of growth and production kinetics and global proteomes

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Thraustochytrids have become important industrial production organisms for the essential, n3 fatty acid docosahexaenoic acid (DHA). They are the only microorganisms that produce long-chain polyunsaturated fatty acids (PUFA) as part of a triacylglycerol oil. Yet, the biosynthesis of DHA in thraustochytrids is not fully understood, in particular which factors that control the distribution between the two pathways used for fatty acids synthesis, fatty acid synthase (FAS) and PUFA-synthase, and how to obtain high DHA productivities.

When *Aurantiochytrium* sp. T66 was cultivated on a defined medium using glycerol or glucose as carbon source and glutamic acid or ammonium as nitrogen source, the fatty acid production rates decreased some hours after nitrogen (N) exhaustion. Proteome analyses showed that the amounts of the majority of the enzymes related to the core metabolism (glycolysis and TCA-cycle) were not affected by N-exhaustion, while the amounts of FAS and PUFA-synthase dropped rapidly after N-exhaustion. By feeding N in limiting concentrations, the enzyme concentrations were maintained at higher levels for a longer period, and the overall fatty acid productivities increased. Complete fatty acid profiles were acquired as a function of time at the different cultivation conditions. DHA and palmitic acid (C16:0) were the dominating fatty acids. Myristic acid (C14:0) and the C16 and C18 monounsaturated fatty acids increased rapidly immediately after N-exhaustion in batch fermentations. When N was supplied continuously, the content of these three fatty acids was even higher, confirming that the availability of N affects both the enzyme concentrations and the fatty acid profiles.

Contributed Paper (Oral Presentation) #7

Visualization of endoplasmic reticulum and mitochondria in *Aurantiochytrium limacinum* ATCC MYA-1381

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Thraustochytrids, single cell marine eukaryotes, produce large amounts of polyunsaturated fatty acids such as docosahexaenoic acid. In the present study, we report the visualization of endoplasmic reticulum (ER) and mitochondria in a type strain of the thraustochytrid, *Aurantiochytrium limacinum* ATCC MYA-1381, using the enhanced green fluorescent protein (EGFP) with specific targeting/retaining signals. We first expressed the *egfp* gene in *A. limacinum*. The fluorescent signals of free EGFP were mainly detected in the cytosol and nucleus, indicating that free EGFP was similarly expressed in mammalian cell lines. We next expressed the *egfp* gene with ER targeting/retaining signals from *A. limacinum* calreticulin or BiP/GRP78 in the thraustochytrid, resulting in the distribution of EGFP signals at the perinuclear region and near lipid droplets. ER-Tracker Red, an authentic fluorescent probe for the visualization of ER in mammalian cells, also stained the same region. Expression of the *egfp* gene with the mitochondria targeting signal from *A. limacinum* cytochrome *c* oxidase resulted in the localization of EGFP near the inner leaflet of the plasma membrane. The distribution of EGFP signals coincided with that of MitoTracker Red CMXRos, which is used to visualize mitochondria in eukaryotes. The ER and mitochondria of *A. limacinum* were visualized for the first time by EGFP with thraustochytrid organelle-specific targeting/retaining signals. These results will contribute to investigations of the intracellular localization of proteins expressed in ER and mitochondria as well as analyses of these organelles in thraustochytrids.

Ref: Okino, N. *et al*, Mar. Biotechnol. 20(2):182-192 (2018)

Contributed Paper (Oral Presentation) #8

A novel thraustochytrid lipase/phospholipase with unique positional specificity contributes to microbial competition and fatty acid acquisition from the environment

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Thraustochytrids are heterotrophic marine protists that are considered as important decomposers in the marine ecosystem; however, how they digest and uptake lipid nutrients from the environment is largely unknown. Genomic clustering analysis using thraustochytrid draft genome databases revealed that novel putative proteins with a Lipase_3 domain are commonly present in thraustochytrids, including *Aurantiochytrium limacinum*. After heterologous expression in *Escherichia coli* and His tag-based purification, protein ID: 145138 was identified as a lipase/phospholipase capable of hydrolyzing triacylglycerol (TG) and phosphatidylcholine (PC). 145138 was secreted into the culture medium, and deletion of the 145138 gene in *A. limacinum* reduced the degradation of extracellular TG and PC. Fatty acids generated by the action of 145138 were reused for the biosynthesis of PC and TG, and *A. limacinum* was able to survive even in medium containing TG as a sole source of carbon. 145138 hydrolyzed all the acyl-ester linkages of TG; however, the enzyme showed strict positional specificity toward PC and phosphatidylethanolamine (PE), generating 2-acyl lysophospholipids. The 2-acyl lysophospholipids showed very strong antimicrobial activity compared with 1-acyl lysophospholipids. These results suggested that 145138 is a bifunctional enzyme that contributes to the acquisition of fatty acids from the environment after the digestion of TG and phospholipids, as well as to generate antimicrobial lysophospholipids that are beneficial for competition with bacteria over lipid nutrients in the marine environment.

Contributed Papers (Poster Presentation)

Contributed Paper (Poster Presentation) #9

Metabolite profile analysis of *Aurantiochytrium limacinum* SR21 grown on acetate-based medium for biotechnological applications

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Thraustochytrids, genus *Aurantiochytrium*, are excellent microbial catalyst to produce lipid products for various biotechnological applications. In order to expand their application range, it is desirable to be able to convert diverse resources to lipids. Therefore, lipid production system using *Aurantiochytrium* species from unutilized biomass such as food waste and marine macroalgae has been constructed. In this study, acetate, which can be easily generated from various resources by acetogenic microorganisms, proved to have a potential as a substrate of *Aurantiochytrium limacinum* SR21. Specific growth rates (μ) of the strain SR21 in 3% acetate (APY)-or glucose(GPY)-based medium were 0.55 and 0.98 h⁻¹, respectively, while maximum yield of total fatty acid were 4.8 g/L at 48 h and 6.8 g/L at 30 h, respectively. Metabolome analysis was performed to comprehensively elucidate metabolic fluctuations caused by acetate assimilation to understand the difference in growth profile between two substrates. Results indicated that cells grown in GPY showed significant activity in glycolysis, pentose phosphate pathway and amino acids metabolism while cells grown in APY showed significant activity in glyoxylate cycle, and mevalonate pathway. The phenotype of the strain SR21 was interpreted based on results of metabolome analysis and possible targets for optimization of lipid productivity were theorized.

Contributed Paper (Poster Presentation) #10

Characterization of carotenoid knockout mutants in *Aurantiochytrium limacinum*

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Some labyrinthulomycetes have the ability to synthesize carotenoids, which is uncommon among heterotrophic eukaryotes. We have found that the trifunctional gene *crtIBY*, which encodes the first three carotenogenesis-specific reactions (phytoene synthase, phytoene desaturase, lycopene cyclase) in thraustochytrids, was acquired by horizontal gene transfer from bacterial sources. However, the selective forces that favor the acquisition and maintenance of carotenoid biosynthesis in some, but not all, labyrinthulomycetes are unknown. One hypothesis is that carotenoids may protect the large amounts of essential omega-3 polyunsaturated fatty acids stored in lipid droplets from oxidative damage. To evaluate this hypothesis and understand carotenoid function in thraustochytrid physiological ecology, we have produced mutants of *Aurantiochytrium limacinum* ATCC MYA-1381 in which *crtIBY* has been interrupted by double homologous recombination with a construct containing a zeocin resistance (*shble*) expression cassette. As predicted, the *crtIBY* knockout mutants lack the carotenoid pigmentation found in the wild-type. While the carotenoid knockout mutants don't exhibit any clear growth defects, we have detected differences between the wild-type and knockout strains in lipid accumulation in late growth and stationary phase, and in sensitivity to oxidative stress caused by treatment with hydrogen peroxide. Complementation with the wild-type gene to confirm that these phenotypes are due to the knockout is in progress, as are studies of gene expression related to redox stress response under a variety of growth conditions.

Contributed Paper (Poster Presentation) #11

Labyrinthulomycetes protist communities in oxygen minimum zones (OMZs) of the bay of Bengal (the Indian Ocean)

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Dissolved oxygen (DO) plays a key role in shaping the structure and function of marine ecosystems. Below the sunlight-zone (>200 m), heterotrophic microbial communities can deplete DO much more quickly through respiration than it is replenished by phytoplankton and other physical processes. Oxygen minimum zones (OMZs) have been commonly defined as those oceanic regions containing oxygen level below low nanomolar concentrations. The OMZ communities are considered to be featured with the dominant microbial anaerobic activities. However, the structure and diversity of heterotrophic microbial communities in OMZs remain largely unknown.

In this study, we investigate the diversity of bacterioplankton and Labyrinthulomycetes (fungus-like protists) in the water columns of OMZs in the Bay of Bengal (the Indian Ocean). Nutrient analyses revealed interesting vertical profiles of nitrate, nitrite, total nitrogen (TN), total phosphate (TP) and Silicate with their much lower concentrations detected in the water samples of the OMZ stations than those of normal oxygen zone (NOZ). The abundance of bacterioplankton and Labyrinthulomycetes is generally lower in OMZs than NOZs. High-throughput sequencing analyses revealed intriguing community structures of bacterioplankton and Labyrinthulomycetes with considerable fraction of novel OTUs detected in OMZs. This study represents the first report of eukaryotic heterotrophic microbial communities in OMZs.

Contributed Paper (Poster Presentation) #12

Distribution of a novel sphingolipid, ceramide glyoxylic ethanolamine (CGE), in thraustochytrids

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Sphingolipids, a class of lipids containing a sphingoid base, are ubiquitous cellular membrane components of all eukaryotes and some prokaryotes. Since thraustochytrids have the characteristic of producing large quantities of DHA-containing glycerolipids, special attention has been paid to glycerolipids and their metabolism so far. However, the composition and metabolism of sphingolipids are largely unknown in thraustochytrids. In this study, we analyzed the sphingolipid profile of different genera of thraustochytrids, *Aurantiochytrium*, *Thraustochytrium*, *Schizochytrium*, *Aplanochytrium* and *Parietichytrium* with LC-ESI MS/MS. Sphingolipid profile of *Thraustochytrium* is completely different from that of *Aurantiochytrium*; that is, the former possesses ceramide phosphoethanolamine (CPE), a phosphosphingolipid composed of a ceramide and phosphoethanolamine, as a major complex sphingolipid, while the latter does not contain known sphingolipids such as CPE or glycosphingolipids. Instead, *Aurantiochytrium* possesses a novel sphingolipid showing unknown fragmentation pattern in MS/MS analysis. NMR and high-resolution mass spectrometry analyses revealed that the novel sphingolipid, tentatively designated as Ceramide Glyoxylic Ethanolamine (CGE), has a novel head group containing amino and carboxyl groups, which is bound to ceramide via acetal structure. CGE is distributed in not only *Aurantiochytrium*, but also in *Thraustochytrium*, *Schizochytrium*, and *Aplanochytrium*, although the content is quite different depending on the genera. At present, CGE has not been found in mammals, fishes, algae, and other stramenopiles except thraustochytrids, suggesting that CGE is the thraustochytrid-specific sphingolipid.

Contributed Paper (Poster Presentation) #13

Search for nutrients required for growth of the strains of “difficult-to-culture” aplanochytrids

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Many of the thraustochytrids, especially *Aurantiochytrium* strains, grow well in standard media for heterotrophs including glucose and yeast extract. However, the most strains of genus *Aplanochytrium* are hard to grow in these media. Recently, it was reported that *Aplanochytrium* cells grew well by obtaining nutrients from living diatoms in two-membered culture examination. This result suggests that *Aplanochytrium* cells potentially have the ability of well-growth but they cannot grow in the standard media because the unknown materials obtaining from living diatoms is the limiting factor. In this study, we searched for essential nutrients required for growth of the strains of *Aplanochytrium*. First, the proliferation test was examined using PM plates (BIOLOG, Hayward, USA) containing nutrients such as sugars, amino acids, nucleoside, and vitamins in 96-well plates. The *Aplanochytrium* cells appeared to be proliferating, for example, in the wells of PM plates containing nicotinic acid and quinolinic acid, but re-examination on a larger culture scale did not show particularly greater growth. While, it was investigated whether there was anything in the food that could enhance the growth of the cells. The highest cell growth was observed in the culture with the medium including the glucose, yeast extract, minerals and a liquid prepared by soaking the dried shrimps in Milli-Q water. It has been reported that the aplanochytrids detected in the guts of the copepods and the other zooplankton. Enhancing the cell growth by adding crustacean extracts may be related to the association of aplanochytrids with crustaceans found in marine environments.

Contributed Paper (Poster Presentation) #14

Metabolic profiling of xylose assimilating thraustochytrid *Aurantiochytrium* sp.

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Thraustochytrids remarkably accumulate lipids in the cells. Their lipids rich in fatty acids are expected utilization for biodiesel fuel. As a cheap carbon source for cultivation of thraustochytrids, lignocellulose containing 30-40% of pentose (mainly xylose) is promising recently. For microbial production of the biodiesel fuel from lignocellulosic biomass by thraustochytrids, xylose assimilating thraustochytrids have been isolated. In the present study, to make clear a mechanism of lipid synthesis of the isolates, the major metabolites of central metabolism in the cells of the isolate cultivated in the medium containing glucose or xylose as a sole carbon source were analyzed by GC-MS and LC-MS/MS. Moreover, the cells cultivated in the nitrogen sufficient medium or nitrogen deficient medium were compared, respectively. As a result, the metabolites of TCA cycle were decreased in the cells cultivated in the medium containing glucose and deficient nitrogen. It was suggested that the carbon incorporated in the cells were used for lipid synthesis. The contents of total lipids and fatty acids in the cells were remarkably increased. However, the metabolites of TCA cycle were not decreased in the cells cultivated in the medium containing xylose. And the cellular lipids were not changed drastically in the cells. Therefore, it was suggested that the differences of the carbon source and nitrogen deficiency affected on the metabolic carbon flow in the isolate. These results showed that the optimization of carbon flow and coenzyme balance made the biofuel production from lignocellulosic biomass much more efficient in the future.

Contributed Paper (Poster Presentation) #15

Overexpression of the carotenoid synthesis gene, *CarS*, in *Aurantiochytrium limacinum*

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Labyrinthuleans are heterotrophic and eukaryotic microorganisms that inhabit marine and brackish water environments, and are known to accumulate large amounts of ω 3 fatty acids such as docosahexaenoic acid (DHA) that have anti-inflammatory effect. *Aurantiochytrium limacinum*, which is a kind of labyrinthuleans, can accumulate not only ω 3 fatty acids but also a small amount of antioxidant carotenoids such as astaxanthin. Therefore, *A. limacinum* is thought to be the potential source of both DHA and carotenoids for health food and feed. In this study, by using genetic engineering, we aim to improve the carotenoid synthesis ability of *A. limacinum*, and to create a strain which can accumulate large amounts of both DHA and carotenoids. To achieve this goal, we first screened an endogenous carotenoid synthesis gene *CarS* homolog, which is identified in closely related species *Schizochytrium* sp. and encodes a multifunctional carotene synthase containing phytoene synthase, phytoene dehydrogenase, and lycopene β -cyclase, in the *A. limacinum* genome. After that, we amplified the corresponding region of *CarS* from the *A. limacinum* genome by PCR, ligated it to a protein expression vector for *A. limacinum*, and then transformed *A. limacinum*. After screening with antibiotics G418, carotenoids were extracted from the transformants and quantified by HPLC. As compared with the wild strain, carotenoids accumulation in the transformants was enriched, suggesting that putative *CarS* had an ability to synthesize carotenoids, and overexpressing it resulted in increasing carotenoid accumulation in the cell.

Contributed Paper (Poster Presentation) #16

Examination of quantitative PCR to quantify the abundance of oblongichytrids in marine environment

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The labyrinthuleans have been recognized as ubiquitous and abundant heterotrophs in the marine and brackish water. Recent studies suggested that each phylogenetic group has different ecological roles such as decomposers and predators, so that the quantitative PCR (qPCR) method is suitable for quantifying the abundance of each phylogenetic group of labyrinthuleans. In this study, we focused on the phylogenetic group including the genus *Oblongichytrium* because the oblongichytrids and aplanochytrids are shown as the major groups of labyrinthuleans in the marine environment by the metagenomic studies (e.g., TARA ocean project) and monitoring study by the culturing method. For establishment of the method for quantifying the abundance of oblongichytrids by qPCR, we continue to work on the design for the specific primers of oblongichytrids, preparation for the standard samples of calibration curve, and estimation of the copy number of 18S rDNA gene sequence per a cell. By combining this study with the already established qPCR method for measuring aplanochytrids, it should be possible to understand the ecological impact of the labyrinthuleans.

Contributed Paper (Poster Presentation) #17

Characterization of novel eicosapentaenoic acid producing thraustochytrid

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Thraustochytrids are known as marine heterotrophic unicellular protists that remarkably accumulate lipids in the cells. Their lipids are rich in polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA). On the other hand, although their lipids contain DHA and n-6 docosapentaenoic acid as PUFA, only trace amount of eicosapentaenoic acid (EPA) is contained. Actually EPA is purified from sardine oil and has been utilized as a medicine for arteriosclerosis and hyperlipidemia. In the present study, a novel thraustochytrid producing EPA was isolated and its characterization was investigated. The novel thraustochytrids were isolated by a pine pollen baiting method. After isolation of novel thraustochytrids, their fatty acid compositions and contents were determined by GC analysis of their total lipids. The phylogenetic analyses based on the 18S rRNA gene sequences were also carried out for an identification of the isolates.

As a result of screening, only the strain SEK704 contained significant amount of EPA. This thraustochytrid can grow only on the medium containing glycerol as a carbon source. The growth and productivity of the strain was greatly affected by the condition of the culture such as agitation or aeration. The finding of the EPA producing thraustochytrid and its characterization would be quite profitable for microbial production of EPA.

Contributed Paper (Poster Presentation) #18

Examination of the condition in the gene transfer into *Parietichytrium* cells by electroporation (Labyrinthulea, Stramenopiles)

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The strains of genus *Aurantiochytrium* have been used in applied molecular biology and industrial production of PUFAs, carotenoids and so on because of their high growth ability and high gene transfer efficiency by electroporation or *Agrobacterium*-mediated plant transformation. While, *Parietichytrium* is also important for high PUFA productivity by the different synthetic pathway of the fatty acids, but the gene transfer has been not successful except particle bombardment method with relatively low efficiency. In this study, we examined the condition in the gene transfer into *Parietichytrium* cells by electroporation. First, we attempted gene transfer with multiple pulses that square electric poring pulses for forming pores in cell membrane were applied, followed by multiple transferring pulses, for delivering the target molecules into cells. In general, it is known that transformation efficiency tends to increase under pulse condition that survival rate decrease rapidly. Then as the result of examination of the survival rates under the wide voltage condition (300–2,400 V), the survival rates in *Parietichytrium* cells decrease rapidly at relatively low voltage conditions. However, no transformants have obtained in this condition yet. It has continued the examination of conditions in low-voltage conditions such as using the pulses with voltage decay.

Contributed Paper (Poster Presentation) #19

Research on a dehydratase gene which is responsible for DHA synthesis in *Aurantiochytrium limacinum*

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Omega-3 polyunsaturated fatty acids (PUFAs), such as DHA and EPA are known to show beneficial effects on our health. It has been reported that PUFAs reduce blood lipid levels, the risk of cardiovascular disease, the symptoms of rheumatoid arthritis, and the risk of ALS. *Aurantiochytrium limacinum*, a Labyrinthulean, has received great attention because of its high accumulation of PUFAs, especially docosahexaenoic acid (DHA). Previous works have revealed that *Thraustochytrium* sp., a related species of *A. limacinum* relies on both a $\Delta 4$ -desaturation-dependent pathway and a PKS-like pathway to synthesize DHA. PKS-like pathway is catalyzed by PUFA-synthases, which comprise three multifunctional genes *PKS-A*, *-B*, and *-C*. According to previous studies, overexpression of the dehydratase gene in *Schizochytrium limacinum* is predicted to improve the ability of DHA synthesis in silico analysis, and the reduced amount of biomass is also smaller than when overexpressing other genes. In this study, to accelerate the accumulation of DHA, we have tried to overexpress dehydratase (*DH*) region which is located on the *PKS-C*. *PKS-C* of *A. limacinum* was searched using the amino acid sequence of *Thraustochytrium* sp. as a query, and then *DH* region was deduced. The corresponding region was amplified by PCR and ligated to a protein expression vector for *A. limacinum*. After screening with antibiotic G418, we obtained 24 transformant colonies. These transformants had 5-folds higher DHA accumulation than that of wild strain at most, suggesting that the overexpression of the *DH* region of *PKS-C* could improve the DHA content in *A. limacinum*.

Contributed Paper (Poster Presentation) #20

Utilization of plant biomass wastes (soybean whey and molasses) for cultivation of thraustochytrids

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For the production of biofuels and functional lipids, thraustochytrids has the advantage of being remarkably high growth and lipid accumulation. Since thraustochytrids is, however, heterotrophic oleaginous microorganisms, it is required to consume a large amount of carbon sources for the mass cultivation and the production of biofuels and functional lipids. As for the cheap carbon sources, plant biomass wastes have been one of the candidates in the near future. Although saccharified cellulosic and lignocellulosic plant biomass are investigated as carbon sources for biofuel production, the cost of saccharification is still high for the actual utilization. On the other hand, molasses and soybean whey are also plant biomass wastes and consist of disaccharide and/or oligosaccharides. Although it was reported that the activity of sucrase or α -glucosidase were detected in the supernatant of the culture of some thraustochytrids, the activities were very weak, and thraustochytrids could not assimilate as a sole carbon source actually. As these plant biomass wastes are smaller molecules than cellulosic and lignocellulosic biomass, these are much easier hydrolyzed to monosaccharides. In this study, utilization of soybean whey and molasses as carbon sources for the cultivation of thraustochytrids were investigated.

As a results, molasses and soybean whey were easily hydrolyzed to monosaccharides such as glucose, fructose, galactose and the others. These hydrolysates could be assimilated as carbon sources for thraustochytrids. These results suggest that the utilization of molasses or soybean whey provided cheap carbon sources for mass cultivation of thraustochytrids

Contributed Paper (Poster Presentation) #21

Genomic analysis of *Sicyoidochytrium minutum* DNA virus (SmDNAV)

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Viruses are highly abundant in marine environments and are recognized as important factors in controlling bacterial and algal biomass, in nutrient cycling, and in maintaining the bio-diversity of bacteria and microalgae. Until today, many viruses infecting marine microalgae and bacteria have been isolated and characterized. As for Thraustochytrids, we reported two distinct viruses and their ecological dynamics: AuRNAV (*Aurantiochytrium* RNA virus: reported as SssRNAV) and SmDNAV (*Sicyoidochytrium minutum* DNA virus), previously.

Recently, we succeeded in isolating new viral strains infecting *Sicyoidochytrium* sp. FPUMEL09M18 from Obama bay, Japan Sea. On the basis of morphological features, host range and genome type, we identified them as the SmDNAV. In this study, we determined and compared full-genome sequences of five SmDNAV strains (SmDNAV 001^T, Ns-Ss, Os-Ss, Ks-Ss, Nm-Ss).

The genomes of SmDNAV strains were dsDNA sized in 234,007 ~ 250,349 bp which included nucleotide compositions of 50.3 ~ 51.63 % G+C and 129 ~ 175 protein coding sequences (CDSs), respectively. However, only ca. 10% of CDSs had been annotated with functional product predictions on the basis of sequence similarity or protein domain matches. Fewer CDSs were found to be homologous to known viral sequences, those of predicted function included those encoding virion packaging ATPase, lambda-type exonuclease, zinc finger/DNA-binding protein, deoxynucleoside monophosphate kinase. From the results of comparative analysis among SmDNAV strain genomes, many insertions, deletions and single short inversion were found in strain Nm-Ss.

Contributed Paper (Poster Presentation) #22

Examination of the condition of CARD-FISH for specific detection of the aplanochytrid cells

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The labyrinthuleans have been detected as ubiquitous and abundant heterotrophs in the marine and brackish water. Recent studies suggested that each phylogenetic group has different ecological roles such as decomposers and predators, so that the catalyzed reporter deposition – fluorescence *in situ* hybridization (CARD-FISH) method is effective to stain each phylogenetic group of labyrinthuleans specifically. In this study, we focused on the phylogenetic group including the genus *Aplanochytrium* because the aplanochytrids is one of the major groups in the marine environment by the metagenomic studies and it was revealed that the aplanochytrid cells intake the nutrients from the important primary producer diatoms. Initially specific fluorescence of aplanochytrids was not confirmed, but non-specific fluorescence was observed in microalgae and zooplankton. After the examination of the condition of the hybridization temperature and endogenous peroxidase inactivation, specific fluorescence of the aplanochytrids was successively confirmed. The observation of the environmental samples with this method will provide the important information for understanding the role and impact of the aplanochytrids in the marine ecosystem.

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