

Room:301A

Time:May 26 14:15-14:45

Phycological perspective on evolution of life and earth environment

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Oxygenic photosynthesis first appeared in cyanobacteria (blue green algae) should have been a key evolutionary event that caused irreversible changes to earth's environment and determined fate of subsequent evolution of life. Evolution and diversification of algae (cyanobacteria and eukaryotic autotrophs excluding land plants) as primary producers resulted in increase of oxygen concentration in the ocean and atmosphere, which may have led to the evolution of eukaryotic organisms and eventually to the evolution of multicellular organisms. As compared with early ecosystem of prokaryotes, scale of primary production should have expanded millions times before the beginning of the Paleozoic era up to the level that can feed multicellular organisms in the ecosystem of Cambrian explosion. Since the Paleozoic, the green plants have been major producers on land. In contrast, primary producers changed to new groups of algae called secondary plants (algae evolved via multiple endosymbioses between heterotrophic eukaryotes and eukaryotic algae). Secondary plants have been playing significant roles in global ecosystem and carbon cycle since the Mesozoic.

Keywords: algae, oxygenic photosynthesis, eukaryotic organisms, endosymbioses, secondary plants, global ecosystem



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Time:May 26 14:45-15:00

Evolution of the photosynthetic life

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The plants (land plants and algae) play important roles in the evolution of earth environment through the oxygenic photosynthesis. The oxygenic photosynthesis was acquired only once by cyanobacteria, and transferred to eukaryotes via primary endosymbiosis. In addition, various plants such as diatoms originated via secondary and tertiary endosymbioses. The "plantize" by symbiosis are not only past events, but also progressive events. In addition, some plants lost photosynthetic ability. These complicated evolutionary events of plants generate and maintain the present environment of earth.

Keywords: endosymbiosis, algae

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BBG021-03

Room:301A

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Ongoing process of plastid acquisition in dinoflagellates

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Dinoflagellates, comprising about 2,000 species, exhibit remarkable diversity in terms of morphological and ecological properties. It is known that the dinoflagellates acquired their plastid via secondary endosymbiosis so that they are called secondary plants. However, about a half of known species lost their photosynthetic ability secondarily, and turned back to heterotrophic life forms. In the present-day ocean, photosynthetic dinoflagellates are important as primary producers together with diatoms and coccolithophorids, while heterotrophic ones play a significant role as primary consumers in microbial loop (or microbial food web).

It is known that some heterotrophic dinoflagellates again turned back to photosynthetic by acquiring new plastids via "tertiary endosymbioses". The tertiary endosymbioses are endosymbiotic evolutionary events between the secondary plants and dinoflagellates. Two types of tertiary endosymbioses are known: one is that the plastid is of haptophyte origin (e.g. *Karenia*) and the other diatom origin (e.g. *Durinskia*). The tertiary plastids appropriately work in the dinoflagellate cell as integrated organelles, and they are equally inherited to daughter cells, as are conventional plastids.

The tertiary endosymbiosis should begin by predating a secondary plant. Possible intermediate states between a simple predation and an established tertiary plastid are known in the dinoflagellates. Studies on such dinoflagellates would improve our understanding on evolutionary process of plastid acquisition. I will introduce six dinoflagellate species that would exhibit different evolutionary states of plastid acquisition. These dinoflagellates engulf cryptophytes from outside the cell and temporarily retain their chloroplasts (sometimes with their other organelles) within the cell. Such temporary plastids (symbionts) are called as "kleptochloroplasts (stolen chloroplasts)". Specificity between the host and symbiont and residual components of the symbionts are different among species, probably reflecting different states of evolution from preys to integrated plastids. Comparisons of these possibly different states of evolution would provide insights to understand the process of plastid acquisition, and future genome analyses on these dinoflagellates would improve our understanding further.

Dinoflagellates are an algal group that has prospered at least since the Mesozoic era onward. The dramatic changes on manners of nutrition may involve their prosperity. Dinoflagellate cysts remain as microfossils in sediments and are common targets of stratigraphy of the Mesozoic and Cenozoic.

Keywords: algae, dinoflagellate, tertiary endosymbiosis, kleptochloroplast



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Plasticity of eukaryotic genomes: The proteomes of dinoflagellate plastids as a case study

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There is no doubt that the endosymbioses of alpha-proteobacterium and cyanobacterium, which gave rise to mitochondria and plastids, respectively, had great impacts on eukaryotic genome evolution. As the extent organelle genomes are extremely reduced comparing to their free-living relatives, the vast majority of endosymbiont genes became dispensable for the lifestyle in a eukaryotic cell and were eventually discarded. On the other hand, the genes, which are essential for functions and maintenance of endosymbionts/organelles, were transferred to the host (eukaryotic) genome. According to the "gene flow" from the endosymbiot to host genomes (endosymbiotic gene transfer or EGT), bacterial genes – those with specific affinities to the bacterial homologs – encoded in eukaryotic genomes have been considered as the results of EGT. In this presentation, I discuss the origins of nucleus-encoded, plastid-targeted genes in dinoflagellates that experienced plastid exchange, and the putative plasticity of eukaryotic genomes: Eukaryotes most likely have the ability to integrate foreign genes not only from their endosymbionts, but also diverged organisms, which were involved in neither acquisition of mitochondrion nor plastid.

Keywords: eukaryotes, genome evolution, endosymbiosis, dinoflagellates, plastids, proteome



Room:301A

Time:May 26 15:30-15:45

Dynamics of algal evolution represented by micropaleontological research

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0) Fossil research to know evolution

There is no method to know actual biological evolution without researching fossils, alt-hough most investigation has been held by phylogenic methods. Concerning diatom, there are evolutional studies of fossil species by Yanagisawa & Akiba and report about its valve-size reduction by Finkel et al. (2005) as examples. However, there is hardly any fossil study directly connecting with modern species and its ecology. Therefore, the author will introduce a taxonomic study of fossil diatom genus <u>Chaetoceros</u>, its evolutional event affected by geological and oceanic changes, and the influence to other marine organisms in this presentation. For suchlike studies, it is important to combine ecological information of living species and detail geological one of fossil, cross-cutting collaboration will be needed.

1) Importance of Chaetoceros resting spore studies

The marine diatom genus <u>Chaetoceros</u> is one of the most important taxa in present oceans, especially coastal upwelling regions (Hasle & Syvertsen, 1996). Under nutri-ent-rich conditions, most species reproduce rapidly and form long chains of thin-walled cells, but their valves are not preserved as fossil due to dissolution (Itakura, 2000). On the other hand, as nutrient supplies are depleted, most of them form thick-walled resting spores which sink to the sea floor where they await the return of favorable conditions (McQuoid & Hobson, 1996). The heavily silicified resting spore valves are preserved in sediment as fossils and abundantly occurred from near-shore sediments in association with other fossil diatom valves, therefore, they can provide useful information for recon-structing paleoproductivity and paleoenvironmental changes.

2) Chaetoceros Explosion Event across the Eocene/Oligocene boundary

The taxonomy of resting spores is less well understood because its vegetative frustules are rarely preserved with the spores and their valve structures are simple. No attention, therefore, has been paid to the significance of resting spores from a geological point of view, which contrast well with that the taxonomy and biostratigraphy of fossil dia-toms from Cenozoic sediments have been studied intensively in several oceans by using marine sedimentary successions collected by the DSDP, ODP and IODP.

Recently, a firm taxonomic basis of fossil resting spores formed in biostratigraphic and paleoceanographic research, using Eocene through the Recent samples (e.g. Suto, 2006). As the result, distinct resting spore event (<u>Chaetoceros</u> Explosion Event, CEE), including abrupt increasing of species richness and abundance, and reducing valve sizes was documented from the sediments collected in Norwegian Sea within a ~6 myr time interval across the Eocene/Oligocene boundary (Suto, 2006, Suto in prep.).

Based on evaluation of the ecologic differences between <u>Chaetoceros</u> and cyst-forming dinoflagellates, Suto (2006) indicated that i) the role of main primary producer might have switched from dinoflagellate in the Eocene to diatom, especially <u>Chaetoceros</u>, in the Oligocene; ii) the conditions in the Norwegian Sea changed from stable with a constant (annual) nutrient supply provided by upwelling in winter in the Eocene, to unstable with a sporadic supply of nutrients by increased vertical mixing in the Ocean after the development of Antarctic Circumpolar Current leading enhanced nutrient supply to the surface waters (Falkowski et al., 2004).

The CEE event was also recognized in the DSDP Holes 366 and 369A, eastern equa-torial Atlantic Ocean (Suto, in prep.), the event, therefore, might occur in all over the world oceans. Moreover, the evolution of the Mysticeti (baleen whales), which consumes a lot of copepods mainly eating diatoms, from the Archaeoceti (paleowhale) across the Eo-cene/Oligocene boundary, coincides with CEE. Consequently, CEE is likely to have en-hanced the evolution of whales (<u>Chaetoceros</u>-baleen whale co-evolution hypothe-sis)(Suto presented in AGU, 2007).

Keywords: diatoms, micropaleontology, paleoceanography, evolution, Eocene/Oligocene Boundary, algae

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BBG021-06

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Symbiosis between foraminifer and red algae.

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Large benthic foraminifera and some planktonic foraminifera have photosynthetic endosymbionts in their cytoplasm. These symbionts are cyanobacteria, diatoms, green algae, dinoflagellates, red algae and haptophytes. Each taxonomic group of the host foraminifera has a different taxonomic group of algal endosymbiont, furthermore there are few algal symbiont species. The unicellular red alga *Porphyridium purpureum* was identified as an endosymbiont of a Peneroplidaean foraminifer based on its color and ultrastructure (Lee 1990). Some symbionts can be isolated and maintained as clonal cultures. To evaluate the taxonomic and phylogenetic position of the *Porphyridium* symbionts, SSU rDNA sequences were done. All the *Porphyridium* symbionts were monophyletic and distinct from the clade of free-living *P. purpureum* collected from terrestrial habitats. Moreover, some physiological properties of the symbionts were also different from those of free-living isolates. These results suggested that symbionts of peneroplid foraminifers comprise an entity distinct from typical *P. purpureum*.

Phylogenetic analysis of the host foraminifera was performed using SSU rDNA sequences showed that all foraminifers having *Porphyridium* endosmbionts are monophyletic. Therefore I propose that the Porphyridium- foraminifer endosymbiosis arose only once.

Keywords: Foraminifer, Microalgae, Red algae, symbiosis, Peneroplidae, Porphyridium



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Time:May 26 16:00-16:15

Environmental factors that enhance or collapse foraminifer-microalgal symbiosis

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Algal symbionts-bearing large benthic foraminifers are known as prolific primary and carbonate producers in coral-reef associated environments at the present and geological times. Understanding the mechanism of foraminifer-microalgal symbiosis is necessary to clarify their responses to secular variations in the Earth system, paleoceanographic changes, and future global environmental changes. Here I reviewed environmental factors that enhance or collapse the foraminifer-microalgal symbiosis. Negative factors which reduce net primary production of algal symbionts and the growth of a foraminiferal host include extremely high and low temperature, low pH, stagnant water motions and high nutrient concentrations. On the other hand, positive factors which enhance net primary production and calcification are poorly known. Our new culturing results indicated that higher pCO₂ enhanced the calcification of symbiont-bearing hyaline taxa due to enhanced photosynthetic activity of the symbionts under high pCO₂ seawater. Future culturing experiments under unrealistic seawater chemistry will give new insights into the mechanisms of foraminiferal-microalgal symbiosis.



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Time:May 26 16:30-16:45

Establishment and breakdown of symbiosis between corals and zooxanthellae

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Enormous numbers of symbiotic dinoflagellates (zooxanthella or genus Symbiodinium) are engaged in scleractinian corals in tropical seas. They are seemingly mutualistic; Corals rely on the organic materials from the symbionts, and the symbionts do on the inorganic nutrients and carbonates from the corals. This close relationship has been passed down unbroken from Triassic and been firmly grounded on their evolutions. However, they might be rather independent.

Coral harbors symbiont intracellularly. Most of the coral species acquire symbionts not maternally but from environments. In this case, they must at least select the symbionts from other suspending particles in seawater, and keep them in their cells. How? Lively discussions have been done on this topic so far, and as yet not been brought to a conclusion. We consider this event is involved in some chemical recognition by a lectin, a carbohydrate-recognizing protein. Lectins from corals bind to the free-swimming zooxanthellae, and other non-symbiotic microalgae, but then, reverse reactions occurred; soon the zooxanthella deformed into spherical form and lost mobility as it were arrested, but still maintained active growth. On the other hand, non-symbiotic algae bust or aggregated and non-growthable anymore. Recently, Dr. M. Jimbo, Kitasato University, found that artificially removal of surface carbohydrate structure on the zooxanthella significantly retarded the establishment of symbiosis with corals. In this connection we may add that zooxanthellae in free-swimming form possess flagella and a well-developed eye-spot. But after symbiosis with animals, they lose these organella. Of course they may not need foot and eye in animals, that would be, but it seems host-directed mechanisms might control the failure of these formations in the symbionts, and if it is, this should be another arresting mechanism by corals.

Another particular interest to the symbiont acquisition by corals would be a presence of the symbiont-sources in the environment. According to our previous analysis by using quantitative PCR, at maximum several hundred thousand cells were existing in 1 L of seawater at a coral reef. Subsequent analyzes of environmental DNA clones and culture strains isolated from ambient waters or sands revealed; zooxanthellae never engaging to the animal-bearing lineage were in the environment, and contrarily those exactly much to the subsidiary engagement to animals were also. We still do not know the symbiotic implication of the former group (it should be very interesting when considering the symbiont evolution), but can suspect the latter group might be derived from ambient corals.

Next we performed an experiment to know whether corals actually release symbionts to the water. Healthy corals in an aquarium indeed discharged the symbionts at accurate periodicity. Corals in field also discharge them to a bottle attached to the branch. Based on the qPCR quantifications, ca. 6000 cells/h were released from just 1 cm2 surface of the coral, and interestingly, a certain type of genetic clade (clade C) was alternatively discharged ahead of another clade (clade D) which is recognized as thermally tough clade. This phenomenon might be interpreted as a survival strategy of corals suffering to rapid environmental changes, such as global warming.

Symbionts may thus be arrested by corals and eventually dismissed for corals' reason. Their relationship is mutualistic as a result, but corals are somehow selfish.

Keywords: Symbiodinium, coral, zzooxanthella



Room:301A

Time:May 26 16:45-17:00

Mass extinction of photosynthetic organisms and environmental perturbation caused by an impact at the K/Pg boundary

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We review geoogic records of the mass extinction at the K/Pg boundary and the hypotheses of the environmental perturbation caused by the impact. We also try to assess the infulence of the impact on photosynthetic organisms and its ecosystem.

Keywords: impact, mass extinction, K/Pg boundary, environmental perturbation

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BBG021-10

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Paradigm shift of primary producers of the Oceans

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Phototrophs carry out the light-induced electron flow, and synthesize organic compounds using the resulted proton-electrochemical potential gradient across the membrane. Ecosystem depends its energy on the organic compounds produced by phototrophs. The phototrophs are, therefore, the starting point of energy flow into the ecosystem. Marine ecosystem takes about a half of global primary production accounting for ca. 50 gigatons of carbon per year. For a long time, it was believed that diatom and flagellated phytoplankton were responsible for the marine primary production. However, recent improvements and developments of HPLC detection technique for photosynthetic pigments, molecular detection technique for microbes, flowcytometry, metagenome analysis and so on revealed following discoveries;

1) Picocyanobacteria including Synechococcus and Prochlorococcus are widely and significantly distribute over the ocean surface (1-3).

2) Bacteriochlorophyll a-containing aerobic anoxygenic photosynthetic bacteria are widely and significantly distribute over the ocean surface (4).

3) Proteorhodopsin-containing bacteria are widely and significantly distribute over the ocean surface (5).

4) Eukaryotic picophytoplankton are widely and significantly distribute over the ocean surface (6,7).

5) Diverse of chlorophylls could be detected in the sediment core samples (8).

These discoveries suggest that the starting point of energy flow in the marine ecosystem is not only the microalgae such as diatom and flagellated phytoplankton but also diverse phototrophs.

In this presentation, I would like to discuss the paradigm shift of the understanding of marine primary producers which had been treated as black box.

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Keywords: photosynthesis, cyanobacteria, microalgae, bacteriochlorophyll, proteorhodopsin, solar radiation



Room:301A

Time:May 26 17:30-17:45

Biodiversity of eukaryotic picoplankton

Masanobu Kawachi^{1*}

¹National Institute for Environmental Stu

not yet

Keywords: picoplakton, biodiversity, phylogeney



Room:301A

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Viral impacts on algal blooms: quantitative and qualitative effects

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Recently, an increasing number of viruses infectious to bloom-forming microalgae are isolated and characterized. In this paper, significance of their ecological roles is mainly introduced. HcRNAV is a polyhedral virus infecting the bivalve-killing dinoflagellate Heterocapsa circularisquama, which harbors a linear 4.4-kb ssRNA genome. In the RdRp sequence tree, this virus fell into a phylogenetic clade which is distant from any other virus ever reported so far; just recently approved as the typical species of a new genus "Dinornavirus" by the International Committee on Taxonomy of Viruses (ICTV). Based on a field survey for more than six years in Ago Bay, Japan, an intimate ecological relationship between the host and the virus was elucidated. HcRNAV population is composed of multiple types which differ in intraspecies host specificity; i.e., differing in strain-specific infectivity among them; on the other hand, the host is composed of distinctive types differing in virus sensitivity spectra. Thus, virus infection can change clonal composition of the host population. Results of genome comparison supported the hypothesis that the nanostructural difference on the viral surface among these types is crucial for determining the specificity. We assume that the type variations of H. circularisquama and its viruses are more complicated than we suspect in natural environments. The host algal population dynamics is apparently affected by HcRNAV infection; viral impact on the algal population is considered to be not only quantitative (on biomass) but also qualitative (on clonal composition).

Keywords: virus, microalgae, algal bloom, aquatic ecology



Room:301A

Time:May 26 18:00-18:15

Responses of phytoplankton and heterotrophs in open oceans to nutrient supply

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Many elements are required for the photosynthesis and growth of phytoplankton. However, Liebig's law of the minimum states only one element limits the growth of organisms at any given time. Here we briefly introduce the behavior of limited nutrients for phytoplankton in the sea, and the responses of not only phytoplankton, but also heterotrophs in open waters, especially the western North Pacific, to nutrient additions. In the subarctic western North Pacific, ample amount of nutrients are supplied from deeper layers to the surface through winter mixing. From spring to summer, phytoplankton growth is stimulated by increases in solar radiation and stratification of the water column. However, levels of chlorophyll (Chl) a, an indicator of phytoplankton biomass, in this region during summer are lower than expected from surface nitrate concentrations. Hence, the subarctic western North Pacific has recently been recognized as one of the HNLC (high nitrate, low Chl) regions, where iron (Fe) availability in seawater is often very low, because of low Fe solubility in seawater and low Fe supplies from land. To verify Fe deficiency in phytoplankton assemblages in this region, two in situ Fe fertilization experiments, SEEDS and SEEDS-II, were carried out in summers of 2001 and 2004, respectively. The large chain-forming centric diatom Chaetoceros debilis dramatically bloomed after Fe enrichment during SEEDS, while small phytoflagellates such as green algae and cryptophytes flourished with a small magnitude during SEEDS-II. The causes of the different responses of phytoplankton between the two experiments could be due to: 1) high grazing pressure of mesozooplankton during SEEDS-II, and 2) a dilution of bioavailable Fe by deeper mixed layer in SEEDS-II, resulting in continued Fe deficiency in large diatoms. Increases in bacterial productivity were also observed even in SEEDS-II, although apparent dissolved organic carbon concentrations little changed. From the results of the two in situ Fe enrichment experiments, it has become evident that Fe availability controls the ecosystems and biogeochemical processes in the subarctic western North Pacific. On the other hand, in the tropical and subtropical western North Pacific, permanent pycnocline has well developed throughout the year, resulting in the depletion of surface nitrate. In such conditions, pico-sized autotrophic cells, which have advantage in nutrient uptake because of their high surface area to volume ratios, become dominant in the phytoplankton assemblages. Recently, it has been pointed out that intensity of tropical cyclones including typhoon may have increased. During 1997-2007, total number of typhoon was 170, and 63% out of them induced substantial increases in Chl a level as estimated from satellite remote sensing. As a typical example, in fall 2003, Chl a concentrations increased by ca. 7 times after the passage of Typhoon Ketsana, which probably caused a strong upwelling in the sea. Relatively high concentrations of the Chl a continued for approximately 1 month. However, it is still unclear which phytoplankton species became dominant after typhoon passage. Effects of the phytoplankton assemblages increased by typhoon on the ecosystems and biogeochemical processes are also uncertain. Therefore, we conducted on-deck incubation experiments using the mixture of surface and subsurface waters in the areas on September 2007 and 2008. As common results of the experiments, levels of specific chemotaxonomic carotenoids probably derived from diatoms and chrysophytes increased after incubation. Our microscope observations also revealed that micro-sized diatoms such as *Pseudo-nitzchia seriata* complex increased. These results suggest that the enhancement of typhoon intensity may increase number of the blooms mainly consisted of diatoms in the tropical and subtropical North Pacific.

Keywords: limited nutrients, in situ iron fertilization experiments, typhoon disturbance



Room:301A

Time:May 26 18:15-18:30

Dynamics and phototrophy of microbial communities in the ocean

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Possible role of phototrophy by heterotrohic bacterial populations on significant energy supply to oligotrophic ocean ecosystems has been recently suggested. Their ubiquitous distribution and unexpected abundance in surface seawater environments have been reportedly emphasized. Oxygenic photosynthetic bacteria, cyanobacteria, are one of the major primary producers in the ocean, whereas anoxygenic photosynthetic bacteria are rather minor in oxidized ocean environments because they are basically anaerobes and require reduced coumpounds as an electoron donners. Aerobic anoxygenic photosynthetic bacteria possessing an ability of photosynthetic light reactions under an aerobic condition were firstly isolated in 1979 by Shiba and colleagues. In 2000, Kolber and his colleagues reported that these photosynthetic bacteria widely distributed in oceanic surface seawaters and accounted for 11 % of total bacteria and 5-10 % of total chlorophyll a concentrations. Also, a wide distribution of some bacteria possessing light-dependent proton pump to generate ATP has been recently suggested. Some bacteria inhabiting marine surface waters possess rhodopsin-retinal complex to absorb light and carry proton ions across a cell membrane. This rhodopsin was just discovered ten years ago and named proteorhodopsin to distinguish from bacteriorhodopsin of archaeal homologous proteins. Both culture-dependent and -independent works indicated a ubiquitous distribution of proteorhodopsin-possessing bacteria in surface seawater environments. In this presentation, we would like to discuss spatiotemporal dynamics of these light-utilizing heterotrophic bacterial populations and possible effect of light and organic matter supply on their growth and survival in marine environments.

Keywords: marine bacteria, photosynthetic bacteria, rhodopsin, photoheterotrophy



Room:Convention Hall

Time:May 26 10:30-13:00

Origin and molecular evolution of endosymbiotic cyanobacteria seen in rhopalodiacean diatoms

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Members of the diatom family Rhopalodiaceae possess cyanobacterium-like structures termed spheroid bodies, as well as the typical plastid, in their cells. Although the precise function of the spheroid bodies in the diatom cells remains unclear, photosynthesis is unlikely to occur in the spheroid bodies as they are devoid of chlorophyll autofluorescence and only possess degenerate thylakoid membrane. Rather, the spheroid bodies are proposed to carry out nitrogen fixation for the host cells, since nitrogen-fixing capacity was observed in *R. gibba*, one of spheroid-body-bearing diatoms. In addition, past studies showed that the spheroid bodies cannot survive outside host cells, implying that they are well integrated into the host cell system. Understanding of the organelle acquisition mechanism through endosymbiosis is one of major issue for trace evolution of eukaryotes and, in this matter, in-depth investigations on the spheroid bodies in rhopalodiacean diatoms may provide key insights. However, because most of past studies for spheroid body have been done with only *R. gibba*, origin and evolutionary process of spheroid bodies in Rhopalodiaceae still remain unclear.

In this study, firstly we amplified the small subunit ribosomal DNA sequences from both host and spheroid bodies in three rhopalodiacean diatom species. Phylogenetic analyses considering these new sequences clearly indicate that the spheroid bodies were acquired by a common ancestor of rhopalodiacean diatoms and have been retained during host speciation. Then we detected nucleotide sequence of the nitrogen-fixation gene cluster from spheroid bodies of *Epithemia turgida*, and compared it with corresponding region of *R. gibba* which has been already reported. Two sequences shared most of gene eliminations and pseudogenizations that likely occurred along with the genome reduction, but also certain difference has been found. This implies that major genome mutations have occurred before the split of present rhopalodiacean diversity and then independent evolutions accompanying host speciation have been going in spheroid body genomes.

Keywords: Endosymbiosis, Cyanobacteria, Nitrogen fixation, Diatom



Room:Convention Hall

Time:May 26 10:30-13:00

Understanding evolution and rise of algae with secondary red plastids in the sea

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(1) Background: Why is the land green and the ocean red? ¹; When and how?

Falkowski et al. (2004)² and others have drawn attention to both bio- and geo-scientists on important observations regarding the algal evolution; they pointed that the major algal clades flourishing in the modern ocean appeared only in the early Mesozoic in fossil records, which had took over the place of green algae who was the only major algae known from the Paleozoic. With limited geologic evidences, it has been suggested that the oceanic photosynthetic production was chiefly dominated by green algae in Paleozoic, from which land plants were originated and diversified. The "ecological reset" of oceanic phototrophs apparently took place in the early Mesozoic as body fossils of dinoflagellates, coccolithoforids, and diatoms, occur in and after the late Triassic². These three major taxa are algae with the plastid that derived as secondary symbionts of red algae. Two potential explanations on this turnover event of oceanic algae was proposed²; each attributed to (a) physiological advantage and (b) biochemical advantage of those lineages with secondary red plastid. They claimed that the latter explains better adaptation of the red photobiochemical machinery to the metal compositions of the modern ocean relative to that of the green algae, after possible drastic alteration of oceanic chemistry beyond the end-Permian mass extinction event. However, their argument failed to explain why those with red plastids as well as red algae had not succeeded before the turnover event nontheless that the secondary symbiotic events are predicted as much older than the P-Tr boundary from genomic studies (regarded as Neoproterozoic events).

(2) Seeking for a methodology elucidating the trajectory of algal evolution in the Phanerozoic

In the present study, I propose use of molecular fossils, fossil porphyrins in particular, to trace the timing and pattern/pace of the green-red algal turnover event in the ocean. All green algae produces chlorophyll *b* as their unique photopigment. On the other hand, almost all known lineages of algae with secondary red plastids produce a variety of chlorophylls *c*. All known primary symbiotic red algae, as well as all other phototrophs, do not produce any chlorophyll *c*, making those photopigments of reliable biomarkers of the algae with secondary red plastids only. Significantly, chemo-taphonomic considerations of chlorophyll *b* and chlorophylls *c* suggested that each leaves certain fossil porphyrins, diagenetic products of chlorophylls, with unique chemical structures, respectively³. Thus, we can identify the evidences of both chlorophyll *b* and chlorophylls *c* productions in the past by analyses of fossil porphyrins extracted from sedimentary rocks (occuring as old as the Proterozoic). I also introduce a new potential method to identify trace of chlorophylls *c* even from relatively matured rocks. Recent advancements of studies on modern oceanic algae suggested more complex evolutionary history of algae than as discussed in ref. 1; it has been revealed that picophytoplankton of green algae is still major producers, whereas a much wider variety of non-fossilizable algae with secondary red plastids, such as pico-haptophytes, are found besides traditional primary producers with mineralized tissue. Such an organic geochemical approach are expected to provide better resolutions on the issue of rise of the algae with secondary red plastid as well as decline of green algae, particularly of earlier Proterozoic where identifiable body fossils of algae were scarce, hence contributing understanding of the algal evolution.

¹Falkowski PG et al. (2004b) In: Therstein H & Young JR (eds) Coccolithophores, Elseveir, pp 429-453.

²Falkowski PG et al. (2004a) *Science* **305**, 354-360.

³Kashiyama Y (2010) Res. Org. Geochem. 26, 39-71.

Keywords: chlorophyll-c, red algae, secondary symbiosis, plastid, macroevolution, fossil porphyrin



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Chloroplast acquisition in Virgulinella fragilis (foraminifera)

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Both bacteria and kleptoplasts exist in *Virgulinella fragilis*, thought to be allowing *V. fragilis* to survive in dysoxic environments. *V. fragilis* kept a same kind of delta-proteobacteria, closely related to *Desulfobacterium*, distribute at the host foraminiferal cell surface. Desulfobacterium uses dissolved for the heterotrophic oxidation of organic matter. These bacteria may therefore use organic material provided by the host for carbon oxidation. Kleptoplasts in host individuals of different investigated areas differ in origin of diatom species. From the expected four membranes around single kleptoplasts, we can only find double membranes. This strategy may have a role in the interaction between the cellular substrates and the kleptoplasts.

Keywords: Benthic foraminifera, Virgulinella fragilis, Kleptoplast, symbiotic bacteria, symbiosis, evolution



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Relationship between coastal benthic foraminifera and its symbiotic algae

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Benthic foraminifers were collected from coastal area of Japan Sea to investigate the relationship between coastal benthic foraminifera and its symbiotic algae. Collected benthic foraminifers were cultured with sea water and seaweed in the constant temperature water tank set to 20 degrees. Living benthic foraminifers were washed with sterilized sea water, and its test was broken in order to isolate its protoplasm. The isolated protoplasm was washed with sterilized sea water, and transferred into test tube filled with a culture medium. The test tubes were put into the incubator set to 20 degrees and 12 hour light, 12 hour dark for several weeks.

The diatom *Cylindrotheca closterium* grew from *Amphistegina*, *Quinqueloculina*, and *Ammonia beccarii*, , but mainly grew from *A. beccarii*. On the other hand, the diatom *Nitzschia* sp. and *Amphora* sp. grew characteristically from *Amphistegina* and *Glabratella*, respectively. It may be that the fed algae remained in the foraminiferal protoplasm, but there is a preference between foraminifera and its intracellular algae.

Keywords: benthic foraminifera, symbiotic algae, diatom



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Kleptoplastidy in the benthic foraminifera *Planoglabratella opercularis* (d'Orbigny)

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The aim of this study is to clarify the mechanism of endosymbiosis of *P. opercularis*, we conducted culture experiment, ultrastructural observation by using transmission electron microscope (TEM), and molecular phylogenetic analyses of both host foraminiferal small subunit (SSU) ribosomal RNA (rRNA) and chloroplastid 16S rRNA. Chloroplasts were existed inside the foraminferal cell as kleptoplast that originate diatom species belonging to *Bacillariophyceae*. The culture experiments suggest that host foraminifers gain chloroplast only from diatom cells.



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Diversity of symbiotic algae in Radiolaria

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Members of Acantharea, Polycystinea, and Phaeodarea are holo planktonic protists that are widely distributed in tropical, subtropical, and even polar marine environments. Many researchers use the conventional term Radiolaria to include these three classes. Recent molecular studies (e.g., Polet et al., 2004; Yuasa et al., 2005; Kunitomo et al., 2006) based on small-subunit ribosomal DNA (18S rDNA) sequences have resolved that these three classes branch off within the Rhizaria.

Various types of algae occur as intracellular symbionts in the polycystine Radiolaria; dinoflagellates, prasinophytes, and prymnesiophytes (e.g., Anderson, 1976). The acquisitions of the photo-symbionts have may have had their survival under low nutrient condition in the geologic time. Although dinoflagellates, prasinophytes, and haptophytes have been identified as endosymbionts of radiolarians by ultrastructural and molecular studies (e.g., Anderson, 1983; Gast and Caron, 1996), the accurate taxonomic affiliation of these symbionts has not been clarified by the lack of diagnostic morphological features, such as theca or flagella, during the symbiotic state. In this study, we report some new findings on molecular phylogeny and fine-structural studies of symbiotic algae in the polycystine radiolarians.

Keywords: Radiolaria, symbiosis, algae, Polycystinea, ultrastructure, 18S rDNA



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Genetic diversity and community dynamics of *Synechococcus* spp. in the northern basin of Lake Biwa

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Synechococcus is a unicellular cyanobacterium and its cell size is from 0.8 to 2.0 micrometers in diameter. Comparably sized photosynthetic planktons, including Synechococcus, other picocyanobacteria and picoeukaryotic algae, are called as picophytoplanktons (0.2-2.0 micrometers) and they are known to be the important primary producers in various aquatic ecosystems. Picophytoplanktons in lakes are mainly comprised of Synechococcus spp., which are assigned to the "picophytoplankton-clade" (sensu Urbach et al. 1998) in molecular phylogenetic trees. In the northern basin of Lake Biwa, it was reported that the abundance of picocyanobacteria reached to $10^5 - 10^6$ cells/ml level, and the chlorophyll abundance of them made up about 45% of total chlorophyll in summer (Nagata 1986). Although the abundance of Synechococcus in Lake Biwa is seasonally changing, it's always more than 10^3 cells/ml. So, they are thought to significantly affect the material cycles and the ecosystems in Lake Biwa. Three strains of Synechococcus spp. (clones Pink, Green and Brown) have been isolated from Lake Biwa (Maeda et al. 1992), and they were thought to be major components of picophytoplanktons in the lake. However, it's difficult to make out the differences of them by microscopy because of these small and simple shaped cells. So the diversity and community dynamics of Synechococcus in Lake Biwa were unclear. In this study, we investigated the genetic diversity of Synechococcus spp., and analyzed vertical distribution and seasonal changing of their community structures in the northern basin of Lake Biwa by using a molecular method.

We monthly collected water at a point in Lake Biwa (35°22'44"N, 136°5'43"E), which is near to the deepest point of the lake, from April 2009 to March 2010. Water samples were collected every 10 meters in depth by using the Niskin bottle from surface to 90 m in depth. One litter of water from each sample was filtered with GF/F glass-fiber filter (25 mm in diameter), and we prepared total DNAs from them. By using these DNAs as template, PCR were performed by using the unicellular cyanobacteria specific primer set (GC-CYA353F/CYA781R(b)), followed by denaturing gradient gel electrophoresis (DGGE). Bands were excised from DGGE gels and the base sequences of these bands were determined. These sequences were phylogenetically compared to known sequences derived from *Synechococcus* spp.

All of the obtained base sequences, which were derived from *Synechococcus* spp., were assigned to the "picophytoplanktonclade", and they were devided into 14 phylogenetic groups. The phylotypes, which were identical to or closely related to the clone Pink, were detected from samples collected in April to August 2009, and in January to March 2010, and they were thought to be the major components of picocyanobacteria in those months, because of the density of DGGE bands. On the other hand, the phylotype closely related to the isolates Green and Brown was only detected from the 0-10 m samples collected in August 2009. Therefore, it was suggested that this phylotype affected to the increase of picocyanobacteria in summer. In other season, the phylotypes, which had not been discovered in Lake Biwa, were mainly detected. During the months of June to September, the compositions of phylotypes varied with depth; single or two phylotypes were dominant in surface layer (0-20 m), but some other phylotypes were dominant in deeper layer. On the contrary, community structures of *Synechococcus* spp. were almost same from surface to bottom in other months. From these results, it was revealed that there were many phylotypes of *Synechococcus*, which were phylogenetically different from already known ones, in Lake Biwa. Additionally, it was also revealed that not only the abundance of *Synechococcus* cells, but also the dominant phylotypes and community structure of them were seasonally changing in Lake Biwa.

Keywords: cyanobacteria, picoplankton, Synechococcus, Lake Biwa, community dynamics



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Analysis of Genetic Diversity of Phytoplankton in Lake Biwa using Molecular Biological Technique

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Seasonal variation of phytoplankton in Lake Biwa was investigated by denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified fragments of 16S rDNA from April 2009 to March 2010.

Water samples were collected at a pelagic site (water depth >90 m) of the north basin of Lake Biwa. Samples were collected at every 10 m depths. After extracting all DNA from the samples, 16S rDNA fragments were amplified using primers GC-341F/CYA781R, and the PCR product was analyzed by DGGE. The DNA sequences of DGGE bands were searched BLAST and constructed phylogenetic trees to estimate related species.

From December to April, diatom and cryptophyceae were mainly detected, and from May to November, cyanobacteria were dominant. In addition, unknown species were detected such as *Radiocystis* sp. and *Acaryochloris* sp., and various unknown genotypes were found in *Synechococcus* sp. By using this method, unknown species and diversity can be detected in Lake Biwa.

Keywords: phytoplankton, diversity, seasonal variation, PCR-DGGE



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Photosynthetic characteristics of marine aerobic anoxygenic phototrophic bacteria Roseobacter and Erythrobacter strains

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<INTRODUCTION> Aerobic Anoxygenic Phototrophic bacteria (AAnPB) containing the photosynthetic pigment bacteriochlorophyll (BChl) *a* can grow using both phototrophy and heterotrophy (Yurkov and Csotonyi 2009). Therefore, their metabolic performance is called <u>photoheterotrophy</u>. Recently, AAnPB and other photoheterotrophs including proteorhodopsin-containing bacteria and cyanobacterium *Prochlorococcus* have been classified into a new functional group in terms of energy acquisition (Beja and Suzuki 2008; Cottrell and Kirchman 2009).

From the ecological standpoint, Kolber et al. (2001) reported AAnPB comprised at least 11% of total bacterial abundance in the upper open ocean. Thereafter, it has become clear that AAnPB are widely distributed and their spatio-temporal changes are large in the upper oceans (e.g. Schwalbach and Fuhrman 2005; Lami et al. 2007). However, what controls their population dynamics is still an open question. One of the main reasons is that less is still known about the physiological characteristics of AAnPB. For example, the contribution of photosynthesis to their growth has seldom been quantified (Yurkov and Csotonyi 2003). Koblizek et al. (2003) determined the biochemical and physiological characteristics of several *Erythrobacter* strains in terms of 16S rRNA and *pufM* gene sequences, growth rates, in vivo absorption and fluorescence excitation spectra, and pigment composition. More recently, Koblizek et al. (2010) revealed the photosynthetic properties of AAnPB belonging to *Roseobacter* clade (strain COL2P). However, those parameters for the other AAnPB have not yet been reported. Moreover, the differences in photosynthetic characteristics between *Roseobacter* and *Erythrobacter* remain unclear.

<PURPOSE> The purpose of this study is to clarify similarity and dissimilarity in photosynthetic characteristics of the two AAnPB genera *Roseobacter* and *Erythrobacter*.

<MATERIALS AND METHODS> Here we isolated coastal marine AAnPB bacteria belonging to the genus *Roseobacter* (strain OBYS 0001) and characterized physiological and biochemical properties, especially in photosynthesis, and compared them to those of the *Erythrobacter* longus type strain (NBRC 14126). Both strains were cultured at 20 ° C in ZoBell 2216E medium, the below 4 parameters were determined in each growth condition. 1. Growth rate by epifluorescence microscopy, 2. Photosynthetic activities by FIRe fluorometry, 3. Pigmentation by HPLC, 4. Absorption and fluorescence excitation properties by spectrophotometry and spectrofluorometry, respectively.

<RESULTS AND DISCUSSION> Growth curves of the two strains represented similar patterns. Cellular bacteriochlorophyll *a* concentrations of the strains showed maxima in stationary growth conditions. In vivo fluorescence excitation/optical density spectra between 470 and 600 nm for OBYS 0001 represented higher values than NBRC 14126. Variable fluorescence measurements revealed that the functional absorption cross-section (sigma PSII) of photosystem II for OBYS 0001 was significantly higher than that for NBRC 14126 under green excitation. These results suggest that *Roseobacter* can capture green light more efficiently than *Erythrobacter* for photosynthesis. On the other hand, the photochemical quantum efficiencies (F_v/F_m) of photosystem II for OBYS 0001 were consistently lower than those for NBRC 14126. A relationship between the growth rate and F_v/F_m was significant for OBYS 0001, but that was not found for NBRC 14126. These results suggested that F_v/F_m for AAnPB could not be simply used for a proxy of growth rate, and the uncertainty was probably caused by their heterotrophy.

Keywords: aerobic anoxygenic phototrophic bacteria, *Roseobacter*, *Erythrobacter*, variable fluorescence, absorption spectrum, fluorescence excitation spectrum



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Environmental pH effect on living foraminifera

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no english abstract

Keywords: foraminifera, pH, culture experiment