Japan Geoscience Union Meeting 2011
(May 22-27 2011 at Makuhari, Chiba, Japan)

BBG021-P01 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

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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 Triassic[2]. 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 proposed[2]; 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, respectively[3]. 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.

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

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

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

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

Japan Geoscience Union Meeting 2011
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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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BBG021-P04 Room:Convention Hall Time:May 26 10:30-13:00

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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BBG021-P07 Room:Convention Hall Time:May 26 10:30-13:00

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 "picophytoplankton-clade", 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 photoheterotrophy. 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 ^{o} 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.

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<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