Japan Geoscience Union Meeting 2012
(May 20-25 2012 at Makuhari, Chiba, Japan)

MIS28-01 Room:301A Time:May 20 15:33-15:54

Light-induced transcriptional responses of proteorhodopsin-containing marine bacteria

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Proteorhodopsin (PR) is a photoprotein that functions as a light-driven proton pump in diverse marine Bacteria and Archaea. Recent studies have suggested that PR may enhance both growth rate and yield in some flavobacteria when grown under nutrient limiting conditions in the light. The direct involvement of PR, and the metabolic details enabling light-stimulated growth, however, remain uncertain.

Here, we surveyed transcriptional and growth responses of a PR-containing marine flavobacterium during carbon-limited growth in the light and the dark. As previously reported (1), Dokdonia strain MED134 exhibited light-enhanced growth rates and cell yields under low carbon growth conditions. Inhibition of retinal biosynthesis abolished the light-stimulated growth response, supporting a direct role for retinal-bound PR in light enhanced growth. Among protein-coding transcripts, both PR and retinal biosynthetic enzymes showed significant upregulation in the light. Other light-associated proteins, including bacterial cryptochrome and DNA photolyase, were also expressed at significantly higher levels in the light. Membrane transporters for Na+/phosphate and Na+/alanine symporters, and the Na+-translocating NADH-quinone oxidoreductase (NQR) linked electron transport chain, were also significantly upregulated in the light (2).

Culture experiments using a specific inhibitor of Na+-translocating NQR indicated that sodium pumping via NQR is a critical metabolic process in the light-stimulated growth of MED134. In total, the results suggested the importance of both the PR-enabled, light-driven proton gradient, as well as the generation of a Na+ ion gradient, as essential components for light-enhanced growth in these flavobacteria (2).

1. Gomez-Consarnau et al., Nature 445: 210-213, 2007.
2. Kimura et al., The ISME Journal 5: 1641-1651, 2011.

Keywords: marine bacteria, proteorhodopsin, proton pump, photoheterotrophy, transcriptomics

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Photoheterotrophy of marine aerobic anoxygenic phototrophic bacteria

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Aerobic anoxygenic phototrophic bacteria (AAnPB) containing the photosynthetic pigment bacteriochlorophyll (BChl) *a* can grow phototrophically and/or heterotrophically. 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. Although it has become clear the ubiquitous distributions of AAnPB in the upper oceans with their high spatiotemporal variations, what controls their population dynamics is still an open question. Based on the intriguing AAnPB ecophysiological characteristics, there is a hypothesis that their photoheterotrophy could be beneficial in nutrient-poor environments such as oligotrophic oceanic waters. However, this hypothesis has not yet been experimentally verified well. Therefore, we investigated the photosynthetic responses of a coastal *Roseobacter* strain OBYS 0001 of marine AAnPB to an organic substrate limitation. In the batch cultures at 20^{o}C, the growth curve and cellular BChl *a* concentration of the substrate-limited strain grown in 1/100 ZoBell 2216E medium kept constant, while those in the undiluted medium increased with time. Variable BChl *a* fluorescence measurements revealed that changes over time in the functional absorption cross-section (sigma) of the photosystem for the strain were little distinct between the two conditions. However, the maximum photochemical quantum efficiencies (F_v/F_m) of the photosystem under the substrate-limited condition were significantly higher than those in the substrate-rich circumstance. These results suggested that AAnPB can enhance their photosynthetic activity with increasing the photochemical conversion efficiency without changing their antenna size under organic substrate limitations. In this presentation, we would like to emphasize the significance of photoheterotrophy for AAnPB in the oceans using our latest results and the past literatures.

Keywords: aerobic anoxygenic phototrophic bacteria, bacteriochlorophyll, photoheterotrophy, variable fluorescence

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Characterization of photosynthetic apparatuses from a new aerobic chlorophototroph discovered in microbial mats.

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Most anoxygenic chlorophototrophs are strict anaerobes and can only grow and perform light-energy capture and conversion under anoxic conditions. The recently discovered thermophilic acidobacterium ”*Candidatus* Chloracidobacterium thermophilum” is the first aerobic chlorophototrophic bacterium that has a type-I, homodimeric reaction center (RC). This organism and its type-I RCs were initially detected by the occurrence of pscA gene sequences, which encode the core subunit of the RC complex, in metagenomic sequence data derived from hot spring microbial mats.

Here, we report the isolation and biochemical characterization of the type-I RC and light-harvesting complexes from *Ca*. C. thermophilum. The RC complex comprised two polypeptides: the reaction center core protein, PscA, and a 22-kDa carotenoid-binding protein. The light-induced difference spectra of the isolated RC showed maximal bleaching at 840 nm, which is attributed to the special pair and which we denote as P840. The RC was photoactive even in the presence of oxygen. In combination with the spectroscopic measurements, HPLC and MS analyses revealed that the RC complex contained bacteriochlorophyll (BChl)-a, chlorophyll-a and Zn-containing BChl-a molecules. The possible functions of the Zn-BChl-a molecules and the carotenoid-binding protein will be discussed. Light-harvesting complexes, chlorosomes, were also isolated from *Ca*. C. thermophilum. Although *Ca*. C. thermophilum is an aerobe, energy transfer among the BChls in these chlorosomes was very strongly quenched in the presence of oxygen, as measured by quenching of fluorescence emission. Spectroscopic, biochemical, and structural analyses showed that the chlorosomes of *Ca*. C. thermophilum possess a number of unique features but also share some properties with the chlorosomes found in anaerobic photosynthetic bacteria of other phyla.

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Distribution and diversity of chlorophyll *d* containing cyanobacteria

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Almost all oxygenic photosynthetic organisms (land plants, algae and cyanobacteria) contain chlorophyll (Chl) *a* as their major pigment, and utilize visible light (400-700 nm in wavelength) for photosynthesis. This spectral range of radiation is called as photosyntherically active radiation (PAR), which has been thought to be necessary for oxygenic photosynthesis. *Acaryochloris* is a genus in the Cyanobacteria, which contains chlorophyll (Chl) *d* as the predominant pigment. They can utilize far-red light (700-750 nm) in addition to photosynthetically active radiation (PAR: 400-700 nm) for oxygenic photosynthesis. To reveal the ecological significances of this organism and the Chl *d*-based photosynthesis, the basic ecological studies on *Acaryochloris* were required. In this study, we aimed to investigate the distribution, diversity and amounts of *Acaryochloris* spp. using molecular biological methods.

The polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to detect epiphytic *Acaryochloris* cells from macroalgae. However, *Acaryochloris* cells were hardly detected from most samples of didemnid ascidian and sponge which were collected from the coasts of the Republic of Palau, because of predominant cyanobacterial symbionts existing in these invertebrates. Then, I designed and used the *Acaryochloris*-selective primer set to detect *Acaryochloris* cells and analyzed phylogenetic diversity of them. By using this selective detection method, many phylotypes of *Acaryochloris* were detected in invertebrate samples collected from Palau and macroalgae collected from the coast of Japan and South Africa. Moreover, *Acaryochloris* phylotypes were also detected from pebbles, sands and seawater samples collected from Japanese coast. These results revealed that *Acaryochloris* spp. could randomly attach to various substrates, and they were widely distributed from tropical to subarctic region. It was also revealed that many and diverged phylotypes of *Acaryochloris* were distributed in environments. Phylogenetic analyses demonstrated that these phylotypes were diverged into three phylogenetic subgroups which were different one another at least in species level.

The detection and quantification methods for Chl *d* from environmental samples were also developed by employing high performance liquid chromatography, and I determined the amounts of Chl *d* extracted from macroalgal samples. As a result, certain amounts of Chl *d* were detected from most macroalgae, and the ratio of Chl *d* to Chl *a* was approximately 1% on average.

In conclusion, it was revealed that certain amounts of *Acaryochloris* spp. were globally distributed around the coastal environments, and the ratio of Chl *d*/Chl *a* reached up to approximately 1% in the seaweed beds. It suggested that Chl *d* could contribute about 1% of the primary production in seaweed bed area, which has been neglected from the estimation of carbon cycle at the coastal environments.

Keywords: chlorophyll *d*, cyanobacteria

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Isolation and characterization of Parmales and its dynamics in the Oyashio region, western North Pacific

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The order Parmales (Heterokonta) is a group of marine phytoplankton species with small solitary cells that are generally 2 to 5 micro-meter in diameter and surrounded by 5 to 8 silica plates. Since diatoms, which are the most successful groups of marine phytoplankton, are also composed of silica cell walls, Parmales has a possible close association with diatoms. Parmales may play a key role in the diatom evolution, to acquire silica synthesis and unique life cycle of diatoms. However, the relationship between Parmales and diatoms is unknown because there is no phylogenetic study due to lack of Parmales culture.

We successfully isolated and characterized a Parmales species, *Triparma laevis*, in the Oyashio region, the western North Pacific for the first time with the aid of a fluorescent silicon tracer. Molecular phylogenetic analyses of SSU rDNA and *rbc*L gene showed that our strain was within the bolidophycean clade of autotrophic naked flagellates and a sister group of diatoms. This indicates a close evolutionary relationship between Parmales, Bolidophyceae and diatoms. We can hypothesize that parmalean and bolidophycean algae or their common ancestor have a life cycle switching between silicified non-flagellated and naked flagellate stages. This hypothetical life cycle is similar to centric diatoms. Centric diatom species also have a silicified vegetative stage and produce naked flagellated male gametes (sperms).

It is important to elucidate similarity and difference between Parmales and diatoms for understanding the evolutionary relationship between these two algae. However, there is little ecological information about Parmales, such as seasonal variations and life cycles, while it is known that Parmales mainly distributes in polar and sub-polar waters. We investigated the seasonal variations of abundance and species composition of the Parmales assemblages in the Oyashio regions.

Samplings were conducted in the Oyashio region on March, May, July and October 2009. The samples for quantifying abundance of the Parmales assemblage were collected from seven depths at 0-100 m. On March and May, the water column was vertically mixed and water temperature was less than 10 degree centigrade at almost stations. Abundance of the Parmales assemblage was high at the stations where water temperature was less than 10 degree centigrade. On the other hands, the vertical stratification was evident between 10-40 m on July and October. Parmales was absent above pycnocline, being abundant in the subsurface layer of 30-50 m where water temperature was lower than 10 degree centigrade. Abundance of the Parmales assemblage on July and October was lower than that on March and May, as the decline of the abundance over pycnocline. Among the Parmales assemblage, *T. laevis* was almost dominant and the seasonal variation of the species composition was small. This characterized the Parmales assemblage compared to the diatom assemblage with clear seasonal succession. Our results showed that the Parmales assemblage, mainly composed of *T. laevis*, would grow in the surface layer in winter-spring and maintain its population under pycnocline in summer-winter. The population under pycnocline would play a role as the initial cells, so-called seed population, for the vegetative growth.

Keywords: Parmales, diatoms, bolidophytes, pico-phytoplankton, Oyashio region