

## Putative functions of kleptoplast in *Planoglabratella opercularis* (foraminifera)

Masashi Tsuchiya<sup>1\*</sup>, Seiji Miyawaki<sup>2</sup>, Yoshito Chikaraishi<sup>1</sup>, Kazumasa Oguri<sup>1</sup>, Akihiro Tame<sup>3</sup>, Katsuyuki Uematsu<sup>3</sup>, Hiroshi Miyake<sup>2</sup>, Tadashi Maruyama<sup>1</sup>, Naohiko Ohkouchi<sup>1</sup>

<sup>1</sup>Japan Agency for Marine-Earth Science and Technology, <sup>2</sup>Kitasato University, <sup>3</sup>Marine Works Japan Ltd.

A rocky-shore benthic foraminifera, *Planoglabratella opercularis*, constructs specific host-symbiont relationships that has chloroplast as kleptoplast. Host organisms may have some benefit from kleptoplast, such as organic matters, or amino acids. To understand the functions of kleptoplast, we conducted culture experiment, ultrastructural observations, oxygen micro-sensor observations and nitrogen stable isotope of amino acid analyses. The trophic position of individuals with or without kleptoplast, we measured stable isotopic composition of amino acid to understand whether their nutritional requirements come from kleptoplast or not. As a result, trophic position (TP) of the individual with kleptoplast shows 1.2. In contrast, TP of cultured individual specimens that digested kleptoplast shows 2.0. It is possible that *P. opercularis* behave as a primary producer, phyto-benthos, in nature.

Keywords: Kleptoplast, benthic foraminifera, nitrogen isotope of amino acid, oxygen micro-sensor, transmission electron microscope

## The effect of temperature on the composition of lipid biomarkers produced by *Chrysolita lamellosa*

Hideto Nakamura<sup>1\*</sup>, Ken Sawada<sup>1</sup>, Hiroya Araie<sup>2</sup>, Iwane Suzuki<sup>2</sup>, Yoshihiro Shiraiwa<sup>2</sup>

<sup>1</sup>Faculty of Science, Hokkaido University, <sup>2</sup>Graduate School of Life and Environmental Science, University of Tsukuba, <sup>3</sup>CREST, Japan Science and Technology Agency (JST)

Long chain alkenones are synthesized by several species of Haptophyte, and used for quantitative paleo-sea surface temperature reconstructions. Alkenones have also been found in many lakes around the world, although their origin is not clear. Recent phylogenetic study suggested that typical lake alkenones with high content of tetra-unsaturated compounds are possibly produced by *Chrysolita lamellosa*, *Isochrysis galbana* or their intimately-associated species. However, only two investigations hitherto reported the lipid composition for *C. lamellosa* as a function of culture temperature. Intraspecific variation in the physiological response are noted by culture experiments of *Emiliana huxleyi* strains (Conte et al., 1998), which is less understood in coastal/limnic species including *C. lamellosa*. Here, we report  $U^{K'}_{37}$  and  $U^K_{37}$  values for a *C. lamellosa* strain which no alkenone composition ever studied.

Keywords: Alkenone, Alkene, Haptophyte, *Chrysolita lamellosa*, UK'37, UK37

## The ecological role of green sulfur bacteria in the chemocline of Lake Suigetsu

Yumi Mori<sup>1\*</sup>, Ryuji Kondo<sup>1</sup>

<sup>1</sup>Department of Marine bioscience Fukui Prefectural University

Phototrophic sulfur bacteria are characterized by their oxidation of reduced sulfur compounds, which serve as electron donors during carbon fixation and anoxygenic photosynthetic growth in aquatic environments when the anoxic layers containing reduced sulfur compounds are exposed to light. Phototrophic sulfur bacteria often form dense blooms in the oxic-anoxic interfaces of stratified lakes. Furthermore, a high level of carbon fixation was detected at the oxic-anoxic interface in some meromictic lakes, indicating that phototrophic sulfur bacteria contribute significantly to primary production during the anaerobic carbon cycle. However, there is no evidence about in situ CO<sub>2</sub> fixation by phototrophic sulfur bacteria and the importance of CO<sub>2</sub> fixation by phototrophic sulfur bacteria in environment is still speculative. Lake Suigetsu is a meromictic lake, which is characterized by a permanent chemocline at a depth of 3-8 m that separates the oxic low salinity mixolimnion from the anoxic saline sulfidogenic monimolimnion. Green sulfur bacteria dominated at the chemocline of the Lake Suigetsu through the year. In this study, we evaluate the contribution of phototrophic sulfur bacteria to carbon fixation in the Lake Suigetsu.

The identity of active CO<sub>2</sub>-fixing bacteria in the chemocline was assessed by DNA-stable isotope probing. The water at the chemocline was incubated with <sup>13</sup>C-labelled sodium bicarbonate and under light or dark condition. The community composition of active CO<sub>2</sub>-fixing bacteria was revealed by analysis of <sup>13</sup>C-labelled DNA fractions. The diversity of 16S rRNA gene was analyzed using clone libraries. And productivity was measured in light or dark conditions by <sup>14</sup>C method.

Chemotrophic carbon fixation accounted for about 80% of the carbon fixation rate in the chemocline. This indicates the contribution of chemotrophic bacteria to carbon fixation was larger than phototrophic bacteria in the chemocline. Clone sequences related to sulfide-oxidizing *Thiomicrospira* and sulfur-reducing *Thioreductor* were frequently recovered from <sup>13</sup>C-DNA fraction library under dark condition, suggesting that these bacteria assimilate CO<sub>2</sub> using sulfur compounds in the water in the dark. Most of 16S rDNA sequences amplified from <sup>13</sup>C-DNA under light condition were related to the genera *Chlorobium*. This indicated green sulfur bacteria assimilate CO<sub>2</sub> in the light. And sulfur-disproportionating *Desulfocapsa* also recovered from <sup>13</sup>C-DNA fraction library under light condition. Although *Desulfocapsa* grow chemolithotrophically, clones related to *Desulfocapsa* did not detect from in dark incubation. In light condition, green sulfur bacteria also main bacteria and they accumulate elemental sulfur on its cell surface coupled with photosynthesis. We speculated *Desulfocapsa* use sulfur deposited on green sulfur bacteria as energy source for CO<sub>2</sub> fixation.

This study indicated that green sulfur bacteria fix carbon in the chemocline. And chemolithotrophic bacteria also play a significant role in the anaerobic CO<sub>2</sub> fixation in the chemocline of Lake Suigetsu. Our results suggest new ecological role of green sulfur bacteria serving energy for chemotrophic bacterial CO<sub>2</sub> fixation.

Keywords: meromictic lake, CO<sub>2</sub> fixation, green sulfur bacteria, stable isotoping method

## Steroid analysis in culture samples of Parmales: Search for Parmales biomarker

Chisato Kanou<sup>1</sup>, Ken Sawada<sup>1\*</sup>, Akira Kuwata<sup>2</sup>, Shinya Yoshikawa<sup>3</sup>, Mutsuo Ichinomiya<sup>4</sup>

<sup>1</sup>Faculty of Science, Hokkaido University, <sup>2</sup>Tohoku National Fisheries Res. Inst., <sup>3</sup>Fukui Prefectural University, <sup>4</sup>Prefectural University of Kumamoto

Palmales is picoplankton that has siliceous tests, and may be closely related to diatom, which is a main important primary producer in the Cenozoic ocean. There have been no reports for siliceous fossil of Parmales. It is known to well preserve siliceous diatom fossil in ancient sediment, and however, such fossil is frequently lost through its dissolution by diagenesis during post-deposition. Therefore, very small siliceous tests of Parmales must be easily dissolved by diagenesis, and it cannot evaluate the timing of first appearance and reconstruct productivity of Parmales by using its siliceous fossil. Thus, we clarified the Parmales biomarkers and their compositions, and these biomarkers are used as molecular fossils for giving understanding evolution processes and historical variations of productivity of this alga. In the present study, we try to search lipid biomarkers, especially steroid, of the Parmales, and to give understanding for taxonomic variability for steroid composition and concentration.

We use culture strains of *Triparma laevis*, *Triparma laevis f. longispina* and *Triparma strigata* for analysis of lipid biomarker. Wet culture samples were extracted with methanol/ dichloromethane, and the extracts were fractionated by silica gel chromatography. Polar fraction was silylated by BSTFA before analyses using GC/MS (Sawada and Shiraiwa, 2004, *Phytochem.* 65, 1299).

We can identify C<sub>21:6</sub> n-alkene, C<sub>20:5</sub> and C<sub>22:6</sub> n-alkenoic acids as well as C<sub>27</sub>-C<sub>29</sub> sterols as Parmales biomarkers. These lipids have been detected from diatom cultures as reported previously (e.g. Rampen et al., 2010, *Limnol. Oceanogr.* 55, 91). In particular, *T. laevis* strain is found to be characterized by overwhelmingly abundance of C<sub>29</sub> beta-sitosterol. However, C<sub>28</sub> sterol as ostreasterol is more abundant rather than C<sub>29</sub> sterols in *T. strigata*. These results indicate that there is possibly interspecies variability in sterol composition within *Triparma* genus. In addition, we can detect a number of unknown polar compounds with higher molecular weight. These unknown compounds may have potential as specific *Triparma* biomarkers.

Keywords: Parmales, biomarker, culture, steroid, evolution of diatom, chemotaxonomy

## Chlorophyll detoxification catabolism associated with protistan phycophagy and evolution of phototrophic symbiosis

Yuichiro Kashiya<sup>1\*</sup>, Akiko Yokoyama<sup>2</sup>, Hitoshi Tamiaki<sup>3</sup>

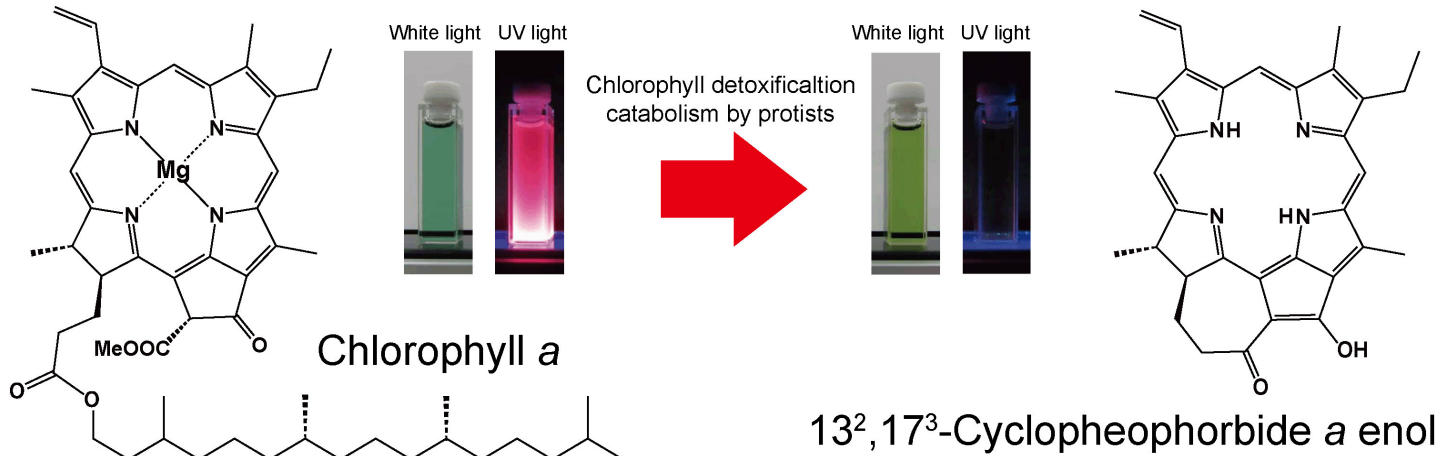
<sup>1</sup>JST PRESTO; Ritsumeikan Univ., <sup>2</sup>Life Environ. Sci., Univ. Tsukuba, <sup>3</sup>Grad. Sch. Life Sciences, Ritsumeikan Univ.

Chlorophylls are highly phototoxic and thus potentially problematic during their biosynthesis, organization, and degradative processes [1]. We have recently reported that metabolic conversion of chlorophyll to 13<sup>2</sup>,17<sup>3</sup>-cyclopheophorbide enol ("cyclo-enol") is a major detoxification mechanism for phycophagic protists (i.e., unicellular eukaryotes feeding on algae).[2] Significantly, a cyclo-enol is completely non-fluorescent and proven to be non-photosensitive in spite of their intact cyclic tetrapyrrole structure exhibiting green color in a solution. We cultured a series of phycophagic protists feeding on uniclonal algae and identified cyclo-enols as a sole major chlorophyll derivative presenting in extracts of the cultures. In addition, we demonstrated in microscopic observations of phycotrophic protists a quick disappearance of the autofluorescence of chlorophylls in the chloroplasts of ingested algae in an early stage of their digestion in phagocytosis, suggesting very rapid and nonradiative quenching of the presumable chlorophyll degradative product therein. We also infer that the cyclo-enol catabolism would be significant for the evolutions of algae that possess chloroplasts originating in secondary symbionts.

### References

- [1] Scheer, H. Proc. Natl. Acad. Sci. USA 2012. 109, 17311.  
[2] Kashiya, Y.; Yokoyama, A. et al. Proc. Natl. Acad. Sci. USA 2012. 109, 17328.

Keywords: phototoxicity of chlorophyll, protists, phototrophic symbiosis, evolution of secondary algae, cyclopheophorbide enol



## Spatio-temporal relationship between chlorophyll derivatives and eukaryotic microorganisms in a coastal water.

Akiko Yokoyama<sup>1\*</sup>, Yuichiro Kashiyama<sup>2</sup>, Shigeharu Moriya<sup>3</sup>, Hitoshi Tamiaki<sup>4</sup>, Isao Inouye<sup>1</sup>

<sup>1</sup>Fac. Life Environ. Sci., University of Tsukuba, <sup>2</sup>JST PREST, <sup>3</sup>ASI, RIKEN, <sup>4</sup>Grad. Sch., Life Sci., Ritsumeikan University

Chlorophylls(Chls) are essential components of photosynthetic organism (algae), which include Chls-*a*, *b*, *c*, *d*, and *f*. The composition of the photosynthetic pigments including Chls as well as carotenoids and phycobiliproteins can be used as the taxonomic character or the biomarker to distinguish the dominant species in the aquatic ecosystems. While various Chl metabolites are known, their sources in the nature are not clear. Recently, ubiquitous occurrence of cyclophorbide *a* enol (cPPB-*a*E) is reported, and its producers, herbivorous protists, were elucidated. Therefore, we inspected that cPPB-*a*E can be able to be the biomarker to detect the feeding activity of protists. To understand the spacial-temporal relationships between the Chl derivatives and microorganisms, pigment analysis by HPLC, calculation of the cells and quantitative analysis using the environmental sequencing were performed.

The results demonstrated that quantity of the Chls and microorganisms were co-related. Chl-*a* was an extremely abundant pigment and much detected in shallow water. A quantitative trend of the cPPB-*a*E was similar to Chl-*a*, but the quantity in deep water in mid summer to early winter was much larger than shallow water. Even though a considerable amount of Chl-*a* was detected, cPPB-*a*E in winter was less abundant than in summer. Those trend shown in cPPB-*a*E was consistent with the abundances of the heterotrophic protists indicated by the environmental sequences.

Keywords: Chlorophyll derivatives, Cyclophorbide *a* enol, Protist, Algae

## Distribution of chlorophyll *f* within hot spring microbial mat

Satoshi Ohkubo<sup>1\*</sup>, Hideaki Miyashita<sup>1</sup>

<sup>1</sup>Grad. Sch. of Human Environ. Stud., Kyoto Univ.

Chlorophyll (Chl) *f* is a recently discovered photosynthetic pigment, which absorbs far-red (FR) light (700-750 nm) in vivo. The distribution and role of Chl *f* in natural environments were still unclear. We have isolated Chl *f*-containing cyanobacteria from various habitats by cultivation using FR-LED as their sole light sources. These cyanobacteria produced Chl *f* only when the cells were grown under FR-LED. Therefore, we hypothesized that Chl *f* was distributed only in certain environments where FR light mainly existed, and it contributed to the oxygenic photosynthesis at those habitats. We thought that the inner layer of microbial mat was one of such environments, because photosynthetically active radiation (PAR 400-700 nm) was absorbed by phototrophs in surface layer. In this study, we aimed to reveal the vertical distribution of Chl *f* and the light environment within hot spring microbial mats.

We collected 20 microbial mat samples at 6 hot springs in Nagano and Gifu prefectures in Japan. Chl *f* was detected from 5 samples of them. Vertical profiles of Chl *f* and downward spectral irradiance within microbial mats were measured by using HPLC and fiber optic spectrophotometer, respectively. Community structure analysis in mats was also performed by PCR-DGGE to reveal the vertical distribution of Chl *f*-producing cyanobacteria. In this poster, we discuss the adaptive significance of Chl *f* in microbial mats.

Keywords: chlorophyll *f*, cyanobacteria, microbial mat

## Spatio-temporal dynamics of chlorophylls and chlorophyll-derived catabolites in Lake Biwa

Yuichiro Kashiya<sup>1\*</sup>, Kanako Ishikawa<sup>2</sup>, Hideaki Miyashita<sup>3</sup>

<sup>1</sup>JST PRESTO, <sup>2</sup>LBERI, <sup>3</sup>Human Environ., Kyoto Univ.

Chlorophylls in aquatic samples have been regarded as important biomarker for phototrophic microbes such as cyanobacteria and algae. Chlorophyll *a* (Chl-*a*) in particular has been treated as a proxy for photosynthetic production in oceans and lakes. Recently, Kashiya, Yokoyama et al. (2012) [1] reported that <sup>13</sup>C<sup>2</sup>,<sup>17</sup>O<sup>3</sup>-cyclophorbide *a* enol (cPPB-*a*E), a pigment derived from Chl-*a*, occurs ubiquitously from most of aquatic environments. cPPB-*a*E comprises 7-16% of total Chl-*a* derivatives in euphotic water column and 51% in the surface sediment at the center of Lake Biwa. We herein report monthly changes in pigment concentrations of vertical water column profile to discuss on year-around variations in activities of phototrophic and phycophagic microbes in Lake Biwa.

### References

[1] Kashiya, Y.; Yokoyama, A.; Kinoshita, Y.; Shoji, S. et al. Proc. Natl. Acad. Sci. USA 2012. 109, 17328.

Keywords: Lake Biwa, Protists, cycloenls, algae, microbial loop