

PHOTOSYNTHETIC RESPONSES AND CHLOROPLAST VARIATIONS TO ENVIRONMENTAL CONDITIONS IN FOLIOSE GREEN SEAWEEDS

(海産葉状緑藻における環境条件への光合成反応と葉緑体の変異)

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Chapter 1. General Introduction

Background of the study

Seaweeds play an important role in the marine ecosystem as primary producers and are widely used as food for human consumption, produced mainly by cultivation. In addition, ecologically they provide a source of food to higher organisms; shelter and nursing grounds for higher organisms; nutrient retention and cyclin; substrate stabilizer; carbon dioxide sink and others. Furthermore, economically they are used as food supplements, fertilizer, and extracts as food additives for the food industry, cosmetics, pharmaceutical, biotechnology and industrial purposes. Seaweeds can be collected from the natural or wild stock but due to the increasing demands for raw materials and derived products it is now increasingly cultivated commercially.

Global production of seaweeds significantly increasing over time indicating the increasing demand for these resources (Buchholz *et al.* 2012). The demand for seaweeds have an estimated total annual value to US\$ 5.5-6.0 billion that ranges from food for human consumption; extracted substances i.e., agar, alginates, carrageenan; fertilizers, animal feed additives and others (McHugh 2003). These commercially important seaweed species in the world ranges from green seaweeds i.e., *Monostroma* spp., *Ulva* spp., *Caulerpa* spp.; brown seaweeds i.e., *Undaria pinnatifida*, *Saccharina japonica*; and red

seaweeds i.e., *Gracilaria* spp., *Eucheuma* spp., *Kappaphycus alvarezii*, *Porphyra* spp.

With the increasing global demand for seaweeds, depletion of these resources from over-exploitation of natural or wild stock and cultivated species; destruction of natural habitat; anthropogenic and even natural calamities is inevitable. Thus a sound and science-based cultivation could be one of the solutions to augment the increasing demand for raw materials from seaweeds. In addition, the need to increase the competitiveness of selecting a high quality and stable cultivars or seedstock is necessary to increase the production and sustainable harvest. Furthermore, understanding of the basic physiological mechanisms of seaweed growth is important not only for considering the effects of the environmental conditions on seaweed production but also for the selection of suitable strain for commercial cultivation.

Seaweeds are photo-auxotrophic organisms and their growth is highly dependent on their photosynthetic activities. In order for seaweeds to grow, they need inputs of carbon dioxide and water with light energy in a chemical process by which seaweeds used these resources to produced oxygen and carbohydrates as their building blocks in the process called photosynthesis. This process is carried out on a specialized intracellular organelle called chloroplast which consists of a photosynthetic pigment called chlorophyll that captures the light energy and convert it to chemical energy. The diversity of chloroplast, the organelle for photosynthesis, is widely known for physiological and biochemical processes as well as its morphology, i.e. the shape, number per cell and intracellular distribution are quite different among taxa. Therefore, seaweeds would potentially be suitable organisms for studying the relationship of chloroplast morphology and photosynthetic activity, but only a few studies have been published so far. This is probably due to the difficulty in finding suitable taxa for a precise and comparative

measurements of the photosynthesis due to the wide variation in thallus morphology and its plasticity even in a single species.

Outline of the study

Species with leaf-like (foliose) thallus with constant thickness are known in many taxa of seaweeds including commercially important species. Using such species, photosynthetic measurements could be advantageously made by giving uniform and constant illumination. Moreover, some foliose green seaweeds of Ulvaceae and Monostromataceae are known to have distinct localization of a single parietal chloroplast. From these, I did the present study to determine the relationship between the chloroplast position and photosynthesis using the foliose green seaweeds by three separate studies. Firstly, the dependence of growth on photosynthesis was investigated using *Monostroma nitidum* under various environmental condition controlled in the laboratory. This is to understand the fundamental information on the physiological significance of photosynthesis in the growth of this commercially important species. Secondly, I made comparative studies of the photosynthesis of *M. angicava* and *Protomonostroma undulatum*, that coexist in the intertidal area but distinct in chloroplast localization. This is to determine the effect of different chloroplast position, which is genetically determined, on the photosynthesis. Thirdly, the effect of chloroplast position caused by movement according to the photoperiod on the photosynthesis in *Ulva conglobata* was investigated. This is to understand how the photosynthesis is dependent on chloroplast position with in a species.

Chapter 2. General Methodology

To answer the objectives of the study, three (3) independent studies were done and used common methods for determining the photosynthetic characteristics by light absorption spectra, photosynthesis-irradiance (*P-I*) curve and Pulse Amplitude Modulation (PAM) fluorometry as described in Saco *et al.* (2018). In addition, I made some improvements in the methodology of photosynthetic measurements and related techniques. The photosynthesis was measured by oxygen evolution using a commercial chamber with homemade devices and a Clark-type oxygen electrode that allows the shortened incubation period and the decreased medium volume and therefore the thallus area. The light was irradiated perpendicularly and uniformly to the thallus so that the light condition was reproducible among measurements. PAM chlorophyll fluorometry was used for further physiological characterization of photosynthesis. In addition to quantification of extracted chlorophylls, thallus absorbance was also measured by spectrophotometry to know the actual light absorbance by the chlorophylls in situ.

Chapter 3. "Characterization of photosynthesis and growth of *Monostroma nitidum* (Ulvophyceae) from Kochi, Japan"

Monostroma nitidum Wittrock is a green benthic seaweed consist entirely of a single-layered blade like thallus with single parietal chloroplast that mostly inhabits the middle to the upper intertidal area which usually growing on rocky substrate and/or epiphytic to larger seaweeds. This species typically grows from late autumn and end in mid-spring attaining maturity in March to May (Segi & Kita 1968; Ohno 1993; Bast 2011). In Japan, the species has been successfully cultivated in commercial scale and being part of the staple food time immemorial (Nisizawa *et al.* 1987 and Bast 2011, respectively). The cultivation of the species (both *M. nitidum* and *M. latissimum* Wittrock) are mostly

done in shallow, calm, and inner bay districts under fully lighted places (Segi & Kita 1968; Ohno 1993).

Earlier studies were done by Maegawa (1980); Maegawa and Aruga (1974, 1993) provide detailed information on the photosynthetic and growth characteristics of the cultivated population of *M. latissimum* under ambient and natural conditions. Population photosynthesis showed to be more correlated with cultivation period coinciding with the aging of the fronds than water temperature variations and on various weather types. Highest photosynthetic activity was observed during the earlier cultivation period and fine days; while lowest during later cultivation period and rainy days. The species was shown to have relatively higher saturating irradiance and no photoinhibitory responses beyond $1,850 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, which reflects adaptation to high light levels (Maegawa and Aruga 1974, 1983). In addition, no significant effect was observed in the population growth rate when tested on the different weather types (Maegawa 1980).

Although previous studies showed that photosynthesis and growth of the species were affected by cultivation and weather types but the effect of the environmental conditions on the photosynthesis and growth have not been fully understood due to the use of natural and ambient conditions that cannot be controlled artificially. In addition, previous reports also did not mention the relationship between photosynthesis and growth of the species. Because it is by understanding the basic mechanisms controlling primary production is important to achieve efficient cultivation in which photosynthesis is one of the most important factors that affect algal growth. Although photosynthesis in seaweeds is affected by different environmental conditions i.e., salinity and nutrients the irradiance and temperature are among the important parameters that drive photosynthesis and affects molecular activities and metabolism, respectively, and subsequently their growth (Lobban

et al. 1985; Lobban & Harrison 1994).

The present study underwent to examine the photosynthesis and growth in the commercially important and edible green seaweed, *Monostroma nitidum* (Ulvophyceae) from a naturally occurring population in the intertidal area, in the laboratory. The photosynthesis did not differ significantly under various temperatures that might reflect the growing season of the species from autumn to mid-spring. In parallel, the growth rate (cultured in 5 and 10 days) was same under various temperatures but decreases at 25°C (cultured in 15 days) suggesting that prolonged exposure to higher temperature might have an adverse effect. Similarly, the maximum quantum yield decreases as temperature increases suggesting some physiological stress on photosystem II (PSII) at a higher temperature. Contrary, the light compensation point, saturating irradiance, initial slope, and efficiency of light absorption by PSII increases as temperature increases. This suggests that the species optimized the photosynthesis to low and high light conditions that might reflect the growing season of the species characterized by irradiance limitation in winter and higher irradiance in spring. No photoinhibitory responses indicating tolerance to higher irradiance. In parallel, the growth rate significantly increases as irradiance increases that might indicate higher growth rate response at higher irradiance. Overall, the photosynthetic responses were in parallel to the growth rate response of *M. nitidum*. Thus the fundamental information on the photosynthetic characteristics can be used to improve its cultivation techniques.

Chapter 4. "Chloroplast position and photosynthetic characteristics in two monostromatic species, *M. angicava* and *P. undulatum*, having a shared ecological niche"

In Ulvophyceae, the genera *Gayralia*, *Monostroma*, *Protomonostroma*, and *Ulvaria* are foliose green algae that are closely related, as inferred from their molecular phylogeny; they are commonly found in marine and estuarine intertidal areas (Bast 2015). They share similar features of gross morphology, having a thallus with a single layer of cells and a single parietal chloroplast per cell, but are distinctly different in their thallus ontogeny and life history (Lobban & Wynne 1981; Brodie *et al.* 2007; Bast 2012). In addition, the shape of the chloroplast among filamentous to tubular genera of Ulvophyceae varies from stellate in *Blidingia*, reticulated in *Chaetomorpha*, parietal in *Percusaria*, girdle-shaped in *Ulothrix*, and perforated in *Urospora* and others (Graham & Wilcox 2000; Brodie *et al.* 2007).

Monostroma angicava Kjellman and *Protomonostroma undulatum* (Wittrock) Vinogradova are both monostromatic and benthic green algae that grow in the intertidal areas of Muroran, Hokkaido, Japan. Field observations show that these species coexist on the same rocks and on other seaweeds, like *Gloiopeltis furcata* (Postels & Ruprecht) J. Agardh. They grow abundantly from March to May and usually disappear by the end of June (Tatewaki 1969). Tatewaki (1969) observed the differences in the development of their fronds; *M. angicava* was observed to give-off erect saccate fronds that develop into the expanded monostromatic membrane, whereas, the erect filaments of *P. undulatum* directly developed into the expanded monostromatic membrane.

Moreover, the surface view of both the species captured in the micrographs of Tatewaki (1969) revealed a degree of variation in their appearance. In *M. angicava*, the cells appeared to have a single parietal chloroplast localized uniformly along the thallus, whereas the cells of *P. undulatum* had a single chloroplast and seemed to have an uneven appearance and its position seemed to be different among the cells (Tatewaki, 1969). This

indicates the possibility that the intracellular chloroplast localization is different between the two species.

In addition, Hori (1973) observed a more complex ultrastructure of the pyrenoid in *M. angicava* showing matrix material divided into 6-12 compartments by the intrusion of many interpyrenoidal bands of a single thylakoid, than in *P. undulatum* that showed 6 compartments lacking thylakoid elements within the matrix. The same author mentioned that it would be interesting to correlate these differences with their metabolic function in photosynthesis. Because the two closely-related species that coexist on the same habitat are distinct in their chloroplast distribution pattern, these two species might also have distinct photosynthetic characteristics that might provide insights on their coexistence in the intertidal area. We examined the relationship between the chloroplast location and the photosynthetic performance by microscopic observations of the chloroplast location from the surface and in sectional views and determined the content of photosynthetic pigments as well as the various parameters of photosynthesis.

Similarly, results show that both species have a single chloroplast with one pyrenoid and the parietal chloroplast is located on the periphery of the thallus in both species, although the location of the chloroplast differs in the two. In *M. angicava*, the chloroplast was observed to be arranged on one-side of the thallus surface, whereas, in *P. undulatum*, it was dispersed and randomly located on either side of the thallus or on the lateral face. The density of chlorophylls assessed from the absorption spectra of the thallus and its solvent extract was higher in *M. angicava*, which appeared dark-green in color, than in the light-green colored *P. undulatum*. The photosynthesis per thallus area was higher in *M. angicava*, whereas, per total chlorophyll content was higher in *P. undulatum*. Both species showed the similar efficiency of photosynthesis at light-limiting conditions.

The efficiency of light absorbed by photosystem II (PSII) in *P. undulatum* was higher than *M. angicava*, whereas the photoprotective mechanisms were higher in *M. angicava*. This indicates that more energy is utilized in *M. angicava* to protect its PSII due to the chloroplast position, which has more direct exposure to light and, therefore, lowers the efficiency of light absorbed by PSII. The higher density of chlorophylls in *M. angicava* could explain higher photosynthesis per thallus area, whereas, higher efficiency of light absorbed by PSII in *P. undulatum* could explain higher photosynthesis per total chlorophyll content. The differences in light absorption efficiency and quantum efficiency of PSII might be an important ecological strategy in these two species for their coexistence in the intertidal area.

Chapter 5. "Chloroplast translocation and photosynthetic characteristics in *U. conglobata*."

The chloroplast movement is a physiological strategy among higher plants and algae that provide efficiency for photosynthesis and protection against excessive light. The chloroplast strategically moves to the area of weak light for absorbing any available light allowing efficient photosynthesis and strategically move away from the area of strong light to avoid damage caused by absorption of excess light (Wada 2013; Kataoka 2015). Similarly in the brown alga, *Dictyota dichotoma* (Hudson) Lamouroux the chloroplasts occupy the cell walls perpendicular to the light direction at low light intensity, occupy the side walls parallel to the light direction at high light and occupy the anticlinal and inner periclinal cell walls at dark but the photosynthetic activities were independent of the chloroplast arrangement (Nultsch *et al.* 1981).

Contrary, the single parietal chloroplast in the two-layered green algae, *Ulva* spp.,

moves on the surface of the cells at day time and on the side walls of the cells at night time and show circadian rhythmic responses (Britz 1975a; Britz & Briggs 1976; Britz *et al.* 1976; Nultsch & Pfau 1979; Nultsch *et al.* 1981). This phenomenon is microtubules-mediated movement revealed by their colchicine sensitivity (Britz 1975b; Britz & Briggs 1983). When the chloroplast covers the periphery of the thallus surface characterized by higher light absorbance or low light transmittance, the photosynthetic activity was higher and when the chloroplast is along the side wall or away from the thallus surface exhibiting low light absorbance or high light transmittance, the photosynthetic activity was lowered (Britz 1975a, b; Britz & Briggs 1976; Britz *et al.* 1976). Moreover, Britz and Briggs (1983) showed a linear correlation between thallus transmittance (436 nm) and the computed orientation index of chloroplast position in *Ulva* spp. But Nultsch *et al.* (1981) found out that the photosynthetic activity in *U. lactuca* was not parallel on the chloroplast position but on pre-irradiation.

Since then, there has not yet been a decisive conclusion on the dependency of photosynthetic activities to the chloroplast position in *Ulva* spp. This study further investigated the dependency of the photosynthetic characteristics to the chloroplast position in the foliose green alga, *U. conglobata* Kjellman. In order to facilitate statistical analysis to quantitatively determine the dependency of the photosynthetic responses to the chloroplast position, the chloroplast position must be quantitatively described. Previous observations showed that the chloroplast position was in parallel with their light absorbance and therefore, the peak thallus absorbance was used to evaluate the chloroplast position quantitatively verify by direct microscopy in the present study. The photosynthetic characteristics were measured from photosynthesis-irradiance (*P-I*) curve using oxygen evolution technique and PAM fluorometry. Both photosynthetic

characteristics and chloroplast position were determined at the various time under normal photoperiod. In addition, the effect of the inhibition of the chloroplast movement by colchicine was also investigated to examine the involvement of a circadian rhythm in the diurnal changes of photosynthesis.

The physiological changes according to the chloroplast position showed that the light absorbance of the thallus was higher in the thallus with chloroplast along the surface than along the side wall and, therefore, the thallus absorbance ($A_{680\text{nm}}$) was considered to evaluate the chloroplast position quantitatively in the present study. Some photosynthetic parameters were linearly correlated with thallus absorbance, i.e., responses are highest at 3 to 9 h after beginning of the light period (12 h), lowest at 3 to 9 after the beginning of dark period, and intermediate at 10 to 15 h after beginning of light or dark period ($r^2 = 0.76 - 0.95$). The results from inhibitory experiments of the chloroplast movement also showed the dependence of photosynthetic parameters on the chloroplast positions.

Chapter 6. General Discussion and Perspective

The present study demonstrated the close relationship between photosynthesis and growth in *M. niditum* in the laboratory under the controlled conditions. This would strengthen the necessity of understanding the mechanism controlling photosynthesis in order to achieve an efficient and maximal growth yield. Furthermore, both *M. angicava* and *P. undulatum*, and *U. conglobata* studies indicated that the photosynthetic features were affected by different chloroplast position that provides significant fundamental information relating to their efficient photosynthetic performance and ecological responses. The contrasting results obtained from the Study 2 and 3 suggest that the photosynthetic activity is highly dependent on the chloroplast position in the same cell

probably through the changes in the effectiveness of light absorption. Moreover, the photosynthesis could also be controlled strongly by genetically determined, intrinsic mechanism among different species. In addition, the chloroplast positions among these intertidal foliose green seaweeds could be their adaptive mechanism to protect their chloroplast from excessive light for absorption and conserving energy for other related metabolic processes. This implies that photosynthesis is dependent not simply on the light absorbance by chlorophylls, but also on some intrinsic mechanism that would be genetically determined, extrinsic factors i.e., photoperiod and functionality of chloroplast position. Therefore, the precise measurement of photosynthesis would be necessary to understand the physiological mechanisms of the photosynthesis of seaweed species wherein the methodologies that have been devised and improved for precision and efficiency would also be helpful in other species.

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