Responses of chlorophyll a content for conchocelis phase of alaskan porphyra (bangiales,rhodophyta) species to environmental factors

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Abstract: Investigations were performed on variations of photosynthetic pigment in conchocelis of Alaskan Porphyra species, P. abbottae (Pa), P. pseudolanceolata (Pe), P. pseudolinearis (Pi) and P. torta (Pt), in response to environmental variables. Conchocelis were cultured under varying conditions of irradiance (0, 10, 40 and 160 µmol photons m⁻² s⁻¹), nutrient concentration (0, f/4, f/2 and f) for up to 60 days (with temperature 11°C and salinity 30ppt). Chlorophyll a (Chl a) content was measured by spectrophotometry. Results indicated that Chl content varied with different culture conditions and species. Photosynthetic pigment was significantly affected by irradiance, nutrient concentration and culture duration, including some interactions of major factors for different species. Light had the most obvious influence on pigment content. For all four species and culture conditions tested, the higher Chl a content (3.6-8.6 mg/g.dw) generally occurred at 0-10 µmol photons m⁻² s⁻¹ than at higher irradiances (≥40 µmol photons m⁻² s⁻¹) culture. For all culture conditions, Chl a content in conchocelis culture with no nutrients added was the lowest. Although there was some difference in Chl a content for cultures with f/2-f nutrient concentration, it was not statistically significant. ANOVA results showed that culture duration had influence on Chl a content of Pa, Pe and Pi species. However, pooled data analysis indicated there was no obvious difference in Chl content for four species of 10-60 day culture. There were significant differences in photosynthetic pigment content for different species. Pa and Pt produced much higher pigment content than the other two species responding to different environmental conditions. Maximal Chl. a content (8.6 mg/g.dw) for Pa occurred at 0 µmol photons m⁻² s⁻¹, f/2 nutrient concentration and 10 day culture duration. Pt contained the lowest pigment content for all culture conditions. Photosynthetic pigment remained relatively higher content under the complete darkness or the low irradiance continuously as long as 60 days for all tested species, which demonstrated the unique survival feature of Porphyra conchocelis. Variation patterns of pigment content, ecological significance and adaptation strategy to low or dark light conditions for microscopic conchocelis stage of Porphyra were discussed.

Keywords: Porphyra, Conchocelis, Photosynthetic Pigment, Chlorophyll A, Irradiance, Temperature, Nutrient, Environmental Factor, Alaska

1. Introduction

Among more than 440 species of marine algae in Alaskan waters, quite a few Porphyra have ecological significance and potential commercial value for successful utilization in mariculture [1, 2]. Porphyra is ranked the highest valued nearshore fishery. The food product often known as “nori” from Porphyra, sold in the form of dried and roasted sheets, is worth $1.4 billion per year and is one of the major aquaculture products produced in the world [3]. There is a growing worldwide market for this and other Porphyra products[4-9].

In the life cycle of Porphyra, two distinct phases are involved. One is the gametophyte, the macroscopic leafy thallus phase known as Porphyra. The other is the microscopic, filamentous sporophyte called the conchocelis stage, which generally lives inside shells in natural habitats.
Mariculture of all species of Porphyra requires artificial control of the life cycle to regulate the production of spores for seeding nets. The quality, quantity, and control of the conchocelis stage are important for successful Porphyra aquaculture [2, 15-16]. One potentially useful indicator of the quality of the conchocelis stage can be the photosynthetic pigment content [17-19]. Although there have been many studies dealing with the classification, morphology, life history, development, growth, desiccation tolerance and ecology regarding the gametophyte stage of Porphyra species from different geographic distributions [20-35], relatively few studies have investigated the physiology and biochemistry of the microscopic conchocelis stage[36, 37]. Chlorophyll a is the most important light-harvesting pigments in the chloroplasts of marine red algae. In photosynthetic process of red algae, accessory pigments (including phycobilins and carotenoids) absorb the particular wavelengths of light and transfer the light energy to chlorophyll a, which is responsible for converting all the absorbed light energy into the chemical energy in ATP and NADPH that are used in the synthesis of organic compounds from the carbon dioxide [38]. Therefore, harvesting pigments play the important roles in the utilization and absorption of light energy and are crucial in determining physiological responses of the Porphyra microscopic stage to the environmental change. Environmental factors may exert profound influences on important physiological processes and on the biochemical composition of the conchocelis filaments. Environmental factors should be examined to investigate their influences on important physiological processes and the biochemical composition of the Porphyra conchocelis stage and to produce high quality and quantity of conchocelis. To date, we have little information about how environmental factors affect the pigment content of the conchocelis stage of Porphyra. Studies are needed on the basic information how environmental factors affect the pigment content of the Porphyra microscopic stage. We report here on responses of photosynthetic pigment contents of Alaskan Porphyra conchocelis to the variations of multiple environmental variables.

2. Materials and Methods

2.1. Culture of Porphyra Conchocelis

Unialgal cultures of each Porphyra species (Porphyra abbottae Krishnamurthy - strain PaSGS01, P. pseudolanceolata Krishnamurthy - strain PeJB03, P. pseudolinearis Ueda - strain PiSC14 and P. torta Krishnamurthy - strain PtCH13a) were obtained from carpospore release. Mature blades of the gametophyte stage of each species were collected from the field. Blades were washed and scrubbed with sterile seawater to remove surface contamination. The cleaned blades were placed in sterile seawater in petri dishes for carpospore release. After 24-36 hours the blades were removed and the dishes incubated in Provasoli's enriched seawater [39] under 16L:8D photoperiod at 11°C. Conchocelis segments (around 110-250 µm ) of each species were placed in cell well plates (one piece per well) and incubated at 30 ppt salinity and 11°C (100-120 µmol photons m\(^{-2}\) s\(^{-1}\) irradiance) for the culture of pure genotype conchocelis, which were used for culture of bulk conchocelis materials for experiments. PES enriched seawater culture medium was used for conchocelis stage. Conchocelis were incubated at 11°C and 25 µmol photons m\(^{-2}\) s\(^{-1}\) irradiance with f/2 culture media.

2.2. Experimental Methods

Pigment experiments of conchocelis were conducted in the incubator which had been set at 11°C of temperature and illuminated with cool-white fluorescent lamps. Irradiance gradients were obtained by wrapping the culture containers with varying layers of white paper and determined using a Li-Cor Radiation Sensor (Li-190SB Quantum Sensor). The pH of the culture medium was adjusted to 7.8 - 8.0 (the ambient pH of the seawater in the inside waters of SE Alaska) using 6 M HCl or 6 M NaOH. The salinity of experimental seawater was set at 30ppt. Culture media were changed every 7 days. Long day (16L: 8D) photoperiods were used. Nutrients were added as an f culture medium concentration, which has a nitrogen concentration of 5.873 mmol. Therefore, nutrient levels of 0, f/4, f/2 and f concentrations represented 0.02, 1.468, 2.936 and 5.873 mmol of nitrogen concentration respectively (conchocelis at 0 nutrient concentration). Chlorophyll a content was measured and analyzed on the basis of its absorption peak value at the wavelength of maximum light absorption (670 nm) after the samples were ground and extracted by the organic solvent (acetone, 90%) with one drop of saturated MgCO\(_3\) added and centrifuged at 14,000g for 30 minutes. About 4-6 mg f.w. conchocelis was used for chlorophyll a measurements. For pigment experiments, different levels of three environmental factors were employed: nutrient levels of 0, f/4, f/2, f concentration; irradiances of 0, 10, 40, 160 µmol photons m\(^{-2}\) s\(^{-1}\); culture duration of 10, 20, 30, 60 days.

2.3. Procedure for Measurement and Analysis of Pigment Content

Porphyra conchocelis were grown in 200 ml flasks under the different culture conditions. After being incubated for 10, 20, 30, 60 days, conchocelis samples were rinsed with sterile seawater and ground at low temperature and low light. Chlorophyll a content was measured and analyzed on the basis of its absorption peak value at the wavelength of maximum light absorption (670 nm) after the samples were ground and extracted by the organic solvent (acetone, 90%) with one drop of saturated MgCO\(_3\) added and centrifuged at 14,000g for 30 minutes. About 4-6 mg f.w. conchocelis was used for chlorophyll a measurements. Four replicates of conchocelis samples from each combination of culture conditions were used for the pigment measurement and one corresponding sample was used for measurement of the
ratio of dry weight and fresh weight. Pigments were extracted at the low temperature and in the dark. Volume of the extracted pigment solution was set to 2 ml for the pigment measurement. Specific extinction coefficient used to calculate pigment amount in the red algal pigment extracts was obtained from O’hEocha [40]. Pigment absorbencies were determined using a Gilford spectrophotometer 250.

The following formula was used for the estimation of pigment content in conchocelis samples on the basis of the absorbancies of the pigment extracts at specified wavelengths and its corresponding specific extinction coefficient:

\[
\text{Chlorophyll } a (\text{mg g.dw}^{-1}) = \frac{19.8 A_{670}}{\text{sample amount (mg.dw)}}
\]

2.4. Statistical Analyses of the Experimental Data

The factorial effects (including potential factor interactions) were analyzed by using a three-way model I ANOVA (pigment content as a function of light, nutrient, culture duration) and S-Plus 4.5 for windows [41]. The Newman-Keuls multiple comparison test [42] was performed to identify which tested factors were important in controlling pigment content of Porphyra conchocelis.

| Source of variation | df | Sum of squares | Mean square | F        |
|---------------------|----|----------------|-------------|----------|
| **P. abbottae**     |    |                |             |          |
| Nutrient            | c  | 39.3185        | 13.1062     | 18.203** |
| Light               | b  | 832.2614       | 277.4205    | 385.302**|
| Duration            | c  | 109.0687       | 36.3562     | 50.494** |
| Nc x Light          | 9  | 36.9991        | 4.1110      | 5.710**  |
| Nc x Day            | 9  | 22.0478        | 2.4498      | 3.402**  |
| Light x Day         | 9  | 26.5725        | 2.9525      | 4.101**  |
| Nc x Light x Day    | 27 | 21.1674        | 0.7840      | 1.089    |
| Residuals           | 192| 138.2416       | 0.7200      |          |
| **P. pseudolinearis** |   |                |             |          |
| Nutrient            | c  | 34.7435        | 11.5812     | 18.994** |
| Light               | b  | 42.5447        | 14.1816     | 23.258** |
| Duration            | c  | 33.3750        | 11.1250     | 18.246** |
| Nc x Light          | 9  | 6.0534         | 0.6726      | 1.103    |
| Nc x Day            | 9  | 4.0953         | 0.4550      | 0.746    |
| Light x Day         | 9  | 34.1028        | 3.7892      | 6.214**  |
| Nc x Light x Day    | 27 | 18.3748        | 0.6805      | 1.116    |
| Residuals           | 192| 117.0698       | 0.6097      |          |
| **P. torta**        |    |                |             |          |
| Nutrient            | c  | 77.7924        | 25.9308     | 27.179** |
| Light               | b  | 318.3588       | 106.1196    | 111.227**|
| Duration            | c  | 24.0444        | 8.0148      | 8.401**  |
| Nc x Light          | 9  | 50.9408        | 5.6601      | 5.932**  |
| Nc x Day            | 9  | 33.4742        | 3.7194      | 3.898**  |
| Light x Day         | 9  | 32.3669        | 3.5963      | 3.769**  |
| Nc x Light x Day    | 27 | 45.1706        | 1.6750      | 1.754*   |
| Residuals           | 192| 183.1837       | 0.9541      |          |

3. Results

3.1. Variations Chlorophyll A Content of P. abbottae Responding To Environmental Variables

Chlorophyll a contents of the conchocelis of P. abbottae were significantly influenced by all three factors, including most of the interactions on chlorophyll a content (Table 1). Conchocelis cultures with no nutrients added generally

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Table 1. ANOVA table for chlorophyll a content of the conchocelis of four Porphyra species at combinations of nutrient concentration (Nc), irradiance (Light) and culture duration (day). At 0, f/4, f/2, f; b 0, 10, 40, 160 µmol photons m−2 s−1; c 10, 20, 30, 60 days. (*P<0.05; **P<0.01)
had significantly lower pigment contents for longer culture duration (20-60 days), but not for 10-day culture. At high irradiances, cultures with no nutrients added had lowest pigment contents (Fig.1).

**Fig 1.** *P. abbottae* (Pa). Chlorophyll a content of the conchocelis as a function of irradiance, nutrient concentration (♦, 0; ■, f/4; △ f/2; ○, f) and culture duration. Error bars are ± S.E

Chlorophyll a contents of *P. abbottae* also varied with different light environments. Cultures in the darkness or at a low irradiance (10 μmol photons m⁻² s⁻¹) had higher pigment contents. Irradiances of 40-160 μmol photons m⁻² s⁻¹ resulted in a remarkable decline in pigment content. The favorable light environment for the production of pigments was a dark environment or a low irradiance which caused the highest content of chlorophyll a.

Pigment contents did not obviously decrease with culture duration of 10-60 days, although there was a slight variation for a long duration of culture (Fig.1, Fig.5).

The mean maximal chlorophyll a content (8.2 mg g.dw⁻¹) was achieved at 0 μmol photons m⁻² s⁻¹, f/4- f/2 nutrient concentration and 10-20 day culture duration.

### 3.2. Variations Chlorophyll A Content of *P. Pseudolanceolata* Responding To Environmental Variables

Chlorophyll a content was influenced by all three factors with the only one interaction (i.e., interaction between irradiance and culture duration, Table1). Similarly, conchocelis cultures with no nutrients added generally had low pigment contents for 10-60 day culture duration. There was no a significant difference in chlorophyll a content between different light environments, excepting significantly higher content occurring in the dark environment for 60 day of culture (Fig.2).

Nutrients between f/4 and f concentrations did not significantly affect pigment contents of *P. pseudolanceolata*, with the pooled mean of chlorophyll a contents being 3.0-3.3 mg g.dw⁻¹. However, cultures with no nutrient added had significantly lower pigment contents than those with nutrients added (Fig.2, Fig.5).

Basically, pigment contents of *P. pseudolanceolata* did not decline with culture duration. The highest chlorophyll a was achieved at 0 μmol photons m⁻² s⁻¹, f nutrient concentration and 20 day culture duration for this species (Fig.2).

### 3.3. Variations Chlorophyll A Content of *P. Pseudolinearis* Responding To Environmental Variables

Pigment contents of the conchocelis of *P. pseudolinearis* were influenced by all three factors, including all
interactions between these factors (Table 1). Conchocelis cultures with no nutrients added generally had lower pigment contents, particularly for longer culture duration.

Statistical test did not indicated that chlorophyll a content of *P. pseudolinearis* decreased with culture duration, although there was a slight declining with a longer culture period (Fig. 5).

The peak chlorophyll a contents (8.6 mg g dw$^{-1}$) was achieved at 0 µmol photons m$^{-2}$ s$^{-1}$, f/2 nutrient concentration and 10-day culture duration and 0 µmol photons m$^{-2}$ s$^{-1}$, f/2 nutrient concentration and 10-day culture duration.

**Fig 5.** Comparison of pooled chlorophyll a (Chl a) content of Porphyra conchocelis for each parameter tested. Error bars are ± S.E. Different letters above the bars indicate significant difference ($P<0.01$) based on multiple comparisons using the Newman-Keuls test. Letter comparisons are relevant within a species (for left figures) and relevant between species (for right figures). Units of parameters tested are: irradiance (µmol photons m$^{-2}$ s$^{-1}$), nutrient concentration (expressed as the f fraction) and culture duration (day).
3.4. Variations Chlorophyll A Content of P. Torta Responding To Environmental Variables

Among three factors, nutrient and light affected chlorophyll a content of the conchocelis of \textit{P. torta} but culture duration did not affect chlorophyll a content of this species. There were no any interactions occurring among factors (Table 1).

Conchocelis cultures with no nutrients added generally remained same pigment content as the cultures with nutrients added, except for cultures under dark environments. At higher irradiances, the cultures with no nutrients added generally had little variation in the pigment contents (Fig. 4).

Unlike the other three species, the conchocelis of \textit{P. torta} produced more pigments under the dark environment than under the light environment. Cultures under the light environment had little variation in the pigment contents (Fig.4).

Similar to \textit{P. pseudolinearis}, a decrease in pigment contents of \textit{P. torta} with a longer culture was not statistically significant, although a slight declining tendency was observed (Fig.4, Fig.5).

The maximal pigment content was achieved at 0 µmol photons m$^{-2}$ s$^{-1}$, f nutrient concentration and 30-day culture duration.

3.5. The Effect Difference between Species Based On Pooled Pigment Content Data

Comparison of pooled pigment contents of four species of \textit{Porphyra} for each parameter tested (for comparison of effect difference between species) was shown in the right column of Fig.5. Results from those pooled data analyses showed that conchocelis of \textit{Pa} and \textit{Pi} contained significantly higher Chl. a contents than the other two species for the comparison of all levels of three factors.
pigment contents showed the lowest Chl. a content occurred with no nutrient added for all four species of *Porphyra*, and much higher Chl. a content was observed with nutrients added excepting Pt.

Fig 3. *P.pseudolinearis* (Pi). Chlorophyll a content of the conchocelis as a function of irradiance, nutrient concentration (♦, 0; ■, f/4; △, f/2; ○, f) and culture duration. Error bars are ± S.E.

Pooled pigment contents also showed much higher Chl. a content appeared at complete darkness and the low irradiance for all four species of *Porphyra* (Fig.5). There was an obvious decrease in Chl. a content at higher irradiances (≥40 µmol photons m⁻²s⁻¹).

Pooled pigment contents indicated, basically there was no remarkable decline in Chl. a content of four species of *Porphyra* for all culture duration (10-60 days) excepting 10 day of culture and other culture duration for Pa.

The pooled pigment content data suggested that Pa and Pi could be potentially more suitable species for *Porphyra* cultivation based on their capacities to reserve more photosynthetic pigments responding to environmental variables.

Light had the most obvious influence on pigment content. For all four species, the higher Chl. a content (3.6-8.1 mg/g.dw) generally occurred at 0-10 µmol photons m⁻²s⁻¹, f/2-f nutrient concentration and 10 day culture duration. Higher irradiances (≥40 µmol photons m⁻²s⁻¹), low nutrients and longer culture duration generally caused a decline of photosynthetic pigment content.

4. Discussions

It was reported that the spectral absorbances of *P. abbottae* gametophytes from Washington State increased in low light and high nutrient levels and *P. abbottae* blades grown under different conditions contained 4.7-7.2 mg of Chl. a g dw⁻¹ if a conventional conversion coefficient 10 was used for the ratio of fresh and dry weight of red algae. Compared with our experimental results, it appeared that both sporophytic and gametophytic stages of *P. abbottae* have similar chlorophyll a content range [43].

From our results, pigment levels were found to vary inversely with the amount of available light, which was higher at the low irradiance and had a significant decline at the higher irradiance. This is an interesting and worth-discussed phenomenon, e.g., what are its implications and significances in physiological, ecological and biological aspects for *Porphyra* conchocelis? Here are
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In red algal cells, the photosynthetic pigments are associated closely with proteins in the thylakoid membranes of chloroplasts to form light-harvesting complexes. Occurrence of photosynthetic activity must rely upon pigment-protein complexes, which structurally are biological macro-molecules and needs some time for their synthesis in plant cells. Unlike sun plants or other plants which can, more or less, obtain regular light, cryptic Porphyra sporophytes have relatively very few chances to access the light. Therefore, as an adaptation mechanism, one possible reason that they still maintain the higher content of photosynthetic pigments under the low light or complete darkness is in order to catch and utilize light, i.e. their photosynthetic pigments are ready for light harvesting at any time when light becomes available. Thus, these characteristics could likely be owned by the benthic red algae. This could be interpreted as increasing pigments to maximize numbers of the photons collected.

Because photosynthetic pigments are most essential for plants to perform photosynthetic process, variations of pigment contents likely determine the growth, development, physiological responses and the survival of plants [43-46]. Our experimental findings showed that photosynthetic pigments of the conchocelis for four Porphyra species are significantly influenced by environmental factors such as irradiance, nutrient concentration and culture duration, including some interactions among these factors. Therefore, pigment contents of the conchocelis appear to be sensitive to the environmental change and could be used to indicate physiological responses of sporophytic stage of Porphyra.

Overall, the conchocelis of all species of Porphyra tested produced and maintained higher content of photosynthetic pigments in the dark environment or at a low irradiance (10 µmol photons m⁻² s⁻¹). Such a unique physiological trait, which derived likely from the historical acclimation and adaptation to environments and the process of natural selection, could possess particularly important biological implication for them to survive and persist in the habitats with very limited light source and could also partly explain the cause why these indigenous Porphyra species could exist and persist in high-altitude habitats (like in Alaska) with very limited light available. Variation and magnitude of pigment contents of the conchocelis vary considerably from species to species in response to varying environments. Our research findings showed that Porphyra conchocelis had the peculiar adaptability to the dark environment or a low irradiance.

Many studies indicated that nutrients, especially nitrogen could affect both growth and development of marine algae [47-55]. Our experimental results also indicated that nutrients are very important for the sporophytic stage of Porphyra. Under culture conditions, conchocelis grown in

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**Fig 4.** P. torta (Pt). Chlorophyll a content of the conchocelis as a function of irradiance, nutrient concentration (♦, 0; ■, f/4; △, f/2; ○, f) and culture duration. Error bars are ± S.E.
Porphyra play a crucial role in sustaining all life activities in plant pigment contents. Culture duration also should be taken place with immense seasonal fluctuations and are related to the seasonal occurrence, which could result in the variations of growth and abundance of marine algae [43, 47, 55]. In natural habitats, since Porphyra sporophytes occur mainly during the period of summer season for the most of species, without doubt, their occurrences during this season would encounter the limiting nitrogen availability. For instance, in Alaska, a drastic decline of nutrient concentration usually occurs during the late spring and summer as the result of frequent phytoplanton blooms. Hence, shortage of nutrient supply during this period would exert a potentially negative effect on the growth, development and survival of natural populations of Porphyra sporophytes.

Sufficient nutrient supply is necessary to promote higher pigment content for Porphyra conchocelis. However, different species exhibited differences in nutrient requirements. For example, higher nutrient concentration (f concentration) might be needed for P. pseudolanceolata. For the other three species, intermediate nutrient concentrations (f4-f2) were basically sufficient for high pigment contents. Culture duration also should be taken into consideration for Porphyra conchocelis to produce more pigments. P. abbotae tended to synthesize significantly less photosynthetic pigments with prolonged culture duration, in contrast to the other three species having a relative constancy in pigment production throughout the entire period of culture.

Our findings have indicated the environmental variables could exert significant influences on chlorophyll a content for three factors tested. Particularly the presence of interactions could have important implications for the interpretation of the variations of chlorophyll a content (Table1). It is observed that interactions occurred in almost all environmental variables for Pa and Pi species, this implied that more flexible variations of chlorophyll a content existed responding to environmental variables for these two species. Therefore it is more possible to produce the conchocelis cultures with healthy and higher chlorophyll a content for these two species through modifying those environmental variables. In contrast, there is no interaction occurring while simultaneous existence of environmental variables tested for Pt species. This suggested that Pt could maintain stable chlorophyll a content in spite of the changes in environmental variables. This species appeared there is less potential prospect in Porphyra culture for another reason of its lowest chlorophyll a content.

Since plants rely on various photosynthetic pigments to perform photosynthetic process, photosynthetic pigments play a crucial role in sustaining all life activities in plant cells. Conchocelis is critical to successful mariculture of Porphyra. Possibility of conchospore maturation and release, to a great extent, rests on whether or not the best cultures of the conchocelis are grown. On the other hand, the importance of marine algae as sources of functional ingredients has been well recognized due to their valuable health beneficial effects. Various bioactives isolated from marine algae have also attracted increasing attention in the fields of food, cosmetic and pharmacology [56-65]. The optimal culture conditions at which the highest production of photosynthetic pigments occurred could not only provide high quality and healthy conchocelis for successful maricultivation of these indigenous Porphyra species but also other potentially high value accessory products such as phycoerythrin, phycocyanin and carotenoids which possess potential active biological functions serving as antioxidants for exploiting and utilizing phycological resources[66-69].

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