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Effects of Light Quality on Growth and Physiological Characteristics of *Neopyropia yezoensis* Free Living Conchocelis

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Abstract [**Objectives**] To study the differences of growth rate, morphology, ultrastructure, pigment content and antioxidant enzyme activity of free-living conchocelis of cultivated type of *Neopyropia yezoensis* under different light qualities (white, red, blue, and green light). [**Methods**] The study was carried out through light quality design and culture, growth rate determination, microstructure and ultrastructure observation, chlorophyll a content and carotenoid content determination, phycobiliprotein content determination, malondialdehyde (MDA) content determination, superoxide dismutase (SOD) activity determination. [**Results**] After 21 d of culture, the specific growth rate (SGR) and chlorophyll a content of free-living conchocelis of *N. yezoensis* were significantly increased by white light (WL), followed by red light (RL) and green light (GL), and they were the lowest under blue light (BL). Compared with the WL group, the BL group had the highest content of phycoerythrin (PE), and the RL and GL groups had the highest content of phycocyanin (PC). The algal body of WL group was normal black brown, and the cell wall was the thickest. In RL and GL groups, the algal bodies were green, and their diameters and cell wall thicknesses were similar to those in WL group. In BL group, the algal body was bright red, the diameter was the smallest, the cell wall was the thinnest, and the ultrastructure showed that the number of plastoglobulus on the thylakoid was the largest. After BL irradiation, the highest MDA content and the lowest SOD activity were observed. The results revealed that WL is the most beneficial to the growth of free-living conchocelis, followed by RL and GL, while BL has adverse effects. [**Conclusions**] This study explored the most suitable light quality conditions for the propagation of free-living conchocelis. It is expected to provide germplasm guarantee for the production and seedling of *N. yezoensis*.

Key words *Neopyropia yezoensis*, Free-living conchocelis, Light quality, Growth, Physiological characteristics

1 Introduction

Neopyropia yezoensis[1] is an important economic seaweeds in China, with an annual output value of more than 5 billion yuan, and is widely cultivated in the coastal areas of Jiangsu and Shandong provinces[2]. *N. yezoensis* has a heterotypic life cycle, and it is composed of free-living conchocelis (sporophyte) and thallus (gametophyte)[3]. Free-living conchocelis can be stored for a long time under low temperature and low light conditions, and can grow rapidly once transferred to a suitable culture environment. Taking advantage of this characteristic, free-living conchocelis is often used as an ideal material for preservation and rapid amplification of laver germplasm[4]. At present, free-living conchocelis inoculation method is used in production, and free-living conchocelis is crushed and sprayed on the shell to form conchocelis[5]. This method has the advantages of simple operation, high efficiency, stable germplasm traits and good seedling collection effect, and more and more laver seedling factories adopt this method[6]. With the increasing demand of free-living conchocelis, it is of great significance to study the efficient culture technology of free-living conchocelis.

Light is an important ecological factor regulating the growth and development of algae, and the characteristics of light include light intensity, photoperiod and light quality[7]. Light quality, as one of the most important characteristics of light, can affect the photosynthesis, growth, development and morphogenesis of algae[8]. Different seaweeds have different requirements for light quality. Wang *et al.*[9] stated that the photosynthetic rate of conchocelis of *Pyropia haitanensis* under blue light (BL) was lower than that under white light (WL) and green light (GL); Kim *et al.*[10] found that the growth rate of *Pyropia dentata* conchocelis under red light (RL) + blue light (BL) or BL alone was significantly greater than that under RL and WL; the thalli of *Pyropia leucosticta* has low photosystem II (PSII) efficiency and carbon accumulation under BL, resulting in a lower growth rate than under WL and GL[11]; compared with RL, the slower growth rate of thalli of *Porphyra umbilicalis* under BL was attributed to low light absorption efficiency and photosynthetic efficiency[12]; *Meristotheca papulosa* shows high photosynthesis and growth rate under GL[13]. These indicate that RL, BL and GL have special effects on the regulation of algae growth and photosynthesis.

At present, the research on the growth and development of free-living conchocelis of *N. yezoensis* mainly focuses on the environmental factors such as light intensity, photoperiod, temperature and nutrients[14-20], but there are few reports on the effects of light quality on free-living conchocelis of *N. yezoensis*. In this paper, we studied the growth and physiological characteristics of free-living conchocelis of *N. yezoensis* under different light quality conditions, to provide a theoretical basis for scientific propagation of conchocelis.

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2 Materials and methods

2.1 Experimental materials *N. yezoensis* was collected from the sea area of algae on the culture raft in Lianyungang of Jiangsu Province (34°55′25″ N, 119°12′13″ E). The healthy and mature conchocelis were washed in sterilized seawater, the surface of the conchocelis was brushed with a sterilized cotton swab to remove miscellaneous algae and other attachments, the mature conchocelis sporangium was cut, and the conchocelis sporangium was placed in the sterilized seawater for culture until the free-living conchocelis were obtained. The culture conditions were as follows: temperature (20 ± 1) ℃, light intensity 20 – 40 μmol/ (m^2 · s), photoperiod 12 L: 12 D, and salinity 26.

2.2 Light quality design and culture The free-living conchocelis of *N. yezoensis* with the same weight (0.1 ± 0.02) g was transferred into a 1 000 mL conical flask and cultured in light incubators (MGZ-120B-3, Shanghai) with different light qualities (WL, RL, BL, and GL). All light quality luminous sources were LED array (25 cm × 52 cm) light source boards (customized by Shanghai Binglin Electronics Co., Ltd.) with a power of 24 W. The main technical parameters of each light quality were measured with a remote spectral scintillation illuminometer (SFIM-300, Hangzhou) (Table 1). Four parallel groups were set for each light quality, with WL as the control group. The culture conditions of each group were light intensity (20 ± 0.2) μmol/ (m^2 · s), temperature (18 ± 1) ℃, photoperiod 12 L: 12 D, and salinity 26. The nutrient solution Provassoli's Enriched Seawater (PES)[21] was replaced every 7 d, and the culture time was 21 d.

2.3 Determination of specific growth rate (SPR) After 21 d of culture, the initial fresh weight (W_0) and the fresh weight (W_t) after 21 d of culture of free-living conchocelis of *N. yezoensis* under different light qualities were measured. The SPR can be calculated using the Equation (1):

$$SGR = [(W_t/W_0)^{1/t} - 1] \times 100\% \quad (1)$$

where t denotes the culture days, W_0 is the initial fresh weight, and W_t is the fresh weight after t days of culture.

Table 1 Major technical parameters of different light qualities

Light quality	Wavelength range // nm	Peak wavelength // nm	Dominant wavelength // nm	Light intensity μmol/(m^2 · s)
WL	440 – 700	456	580	20.14
RL	640 – 680	663	642	20.20
BL	440 – 480	461	464	20.02
GL	500 – 560	525	534	20.12

2.4 Microscopic and ultrastructural observation Morphological observation and microphotography of free-living conchocelis under different light qualities were carried out using a Nikon-Eclipse Ni optical microscope (Nikon, Japan) at 21 d of culture. The diameter of algae was measured by NIS-Elements D software. The conchocelis were fixed in 2.5% glutaraldehyde under different light qualities and sent to Wuhan MISP Biotechnology Co., Ltd. Samples were dehydrated by ethanol gradient and then treated by propylene oxide, embedded by resin gradient infiltration, and the embedded blocks were semi-thin positioned and ultrathin sectioned by ultramicrotome. After staining, the ultrastructure of the tissues was observed by transmission electron microscopy (HITACHI, Japan).

2.5 Determination of chlorophyll a and carotenoid content After 21 d of culture, 0.1 g of conchocelis were weighed and placed in 100% methanol solution in the dark, and then ground with a tissue grinder (JXFSTPRP, Jingxin Technology, Shanghai, China) for 3 min at 4 ℃ in the dark for 24 h. The supernatant was centrifuged (10 000 r/min, 15 min), and the absorbance values at 480, 510, 652, 665 and 750 nm were measured by Genesys 180 (Thermo Scientific, USA) spectrophotometer. Chlorophyll a content and carotenoid content were calculated according to the formula proposed by Porra[22] and Parsons[23]:

$$\text{Chlorophyll } a \text{ content (mg/g)} = \frac{16.29 \times [(OD_{665} - OD_{750}) - 8.54 \times (OD_{652} - OD_{750})]}{1\,000 \times FV} \times V \quad (2)$$

$$\text{Carotenoid content (mg/g)} = \frac{7.6 \times [(OD_{480} - OD_{750}) - 1.49 \times (OD_{510} - OD_{750})]}{1\,000 \times FW} \times V \quad (3)$$

where OD represents the optical density, V represents the extraction volume (mL), and FW represents the fresh weight of the algae (g).

2.6 Determination of phycobiliprotein content After 21 d of culture, 0.1 g of thallus was weighed, added with phosphate buffer (0.1 mol/L, pH = 6.8), placed in a tissue grinder for grinding for 3 min, and stored at 4 ℃ in the dark for 24 h. The supernatant was taken by centrifugation (10 000 r/min, 20 min). According to Beer's formula[24], the absorbance of the supernatant was measured at 455, 564, 592, 618 and 645 nm.

$$\text{Phycoerythrin (PE) content (mg/g)} = \frac{[(OD_{564} - OD_{592}) - 0.20 \times (OD_{455} - OD_{592})]}{FV} \times 0.12 \times V \quad (4)$$

$$\text{Phycocyanin (PC) content (mg/g)} = \frac{[(OD_{618} - OD_{645}) - 0.51 \times (OD_{592} - OD_{645})]}{FW} \times 0.15 \times V \quad (5)$$

2.7 Determination of superoxide dismutase (SOD) and malondialdehyde (MDA) The activity of SOD was determined by xanthine oxidase method and T-SOD kit (Nanjing Jiancheng Bioengineering Institute). Phosphate buffer (0.1 mol/L, pH = 7.2) was added to the algal tissue, and the homogenate was prepared by mechanical homogenization in ice-water bath. Centrifuged at 4 000 r/min for 10 min, and carefully suck the supernatant to obtain the crude extract. The absorbance value was measured at 550 nm with a microplate reader (MULTISKAN FC, Thermo Scientific, USA), and the total SOD content was calculated. The malondial-

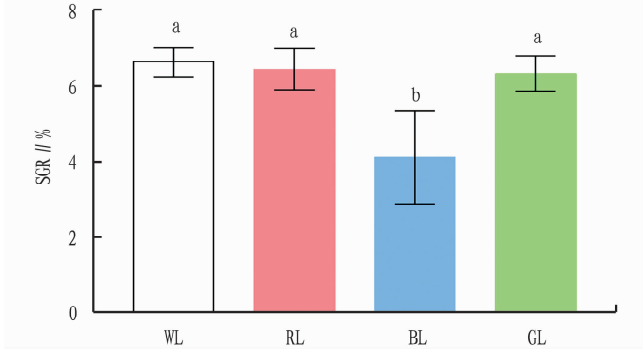
dehyde (MDA) content was determined by a plant MDA test kit (Nanjing Jiancheng Bioengineering Institute). The preparation method of algae homogenate and crude extract was the same as above. The absorbance of supernatant was measured at 530 nm, and the MDA content was calculated according to the formula in the instructions.

2.8 Statistical analysis SPSS 19.0 software was used for data processing and statistical analysis. Graph Pad Prism 7.0 software was used to plot. One-way analysis of variance was used to test the significant level of difference between groups, and the significant level was set as $P<0.05$.

3 Results and analysis

3.1 Effects of light quality on the specific growth rate (SGR) of *N. yezoensis* free-living conchocelis The free-living conchocelis of *N. yezoensis* were cultured under different light qualities for 21 d. As shown in Fig. 1, the algae SGR of WL group was the highest, about 6.59%, followed by RL and GL groups. The SGR of BL group was the lowest, about 4.12%, which was significantly lower than that of other light quality groups ($P<0.05$). The results showed that compared with WL, RL and GL, BL was not conducive to the growth of free-living conchocelis of *N. yezoensis*.

3.2 Effects of different light quality on morphology and ultrastructure of *N. yezoensis* free-living conchocelis Under different light qualities, the morphology of *N. yezoensis* free-living conchocelis changed significantly. As shown in Fig. 2, in WL group, the appearance of the algal body was normal black brown; under the optical microscope, the algal conchocelis were thick, with a diameter of about (5.40 ± 0.91) μm, and the pigment bodies were fully distributed around the cells. In RL group, the color of the algal body changed to light green; the diameter was about (5.17 ± 0.96) μm, and the pigment bodies were light green and distributed in the cells in a diffuse manner. In BL group, the algal body was bright red; the algal conchocelis were thin, about (4.88 ± 0.84) μm in diameter, and the pigment bodies were red and arranged in the cells in a broken band close to the cell membrane. In GL group, the algal body was green in color, with a diameter of about (5.17 ± 0.77) μm, and the pigment bodies were green and intermittently distributed in the cells.



NOTE Different letters indicate significant difference between groups ($P<0.05$). The same below.

Fig. 1 SGR of *Neopyropia yezoensis* free-living conchocelis under different light qualities

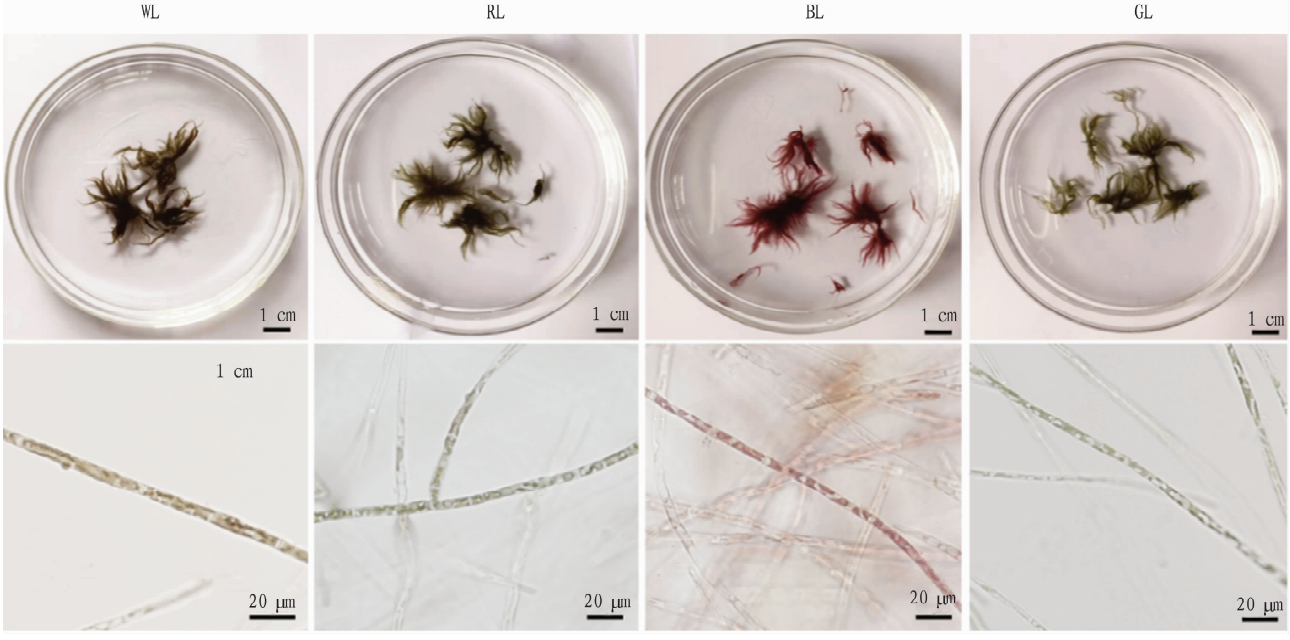
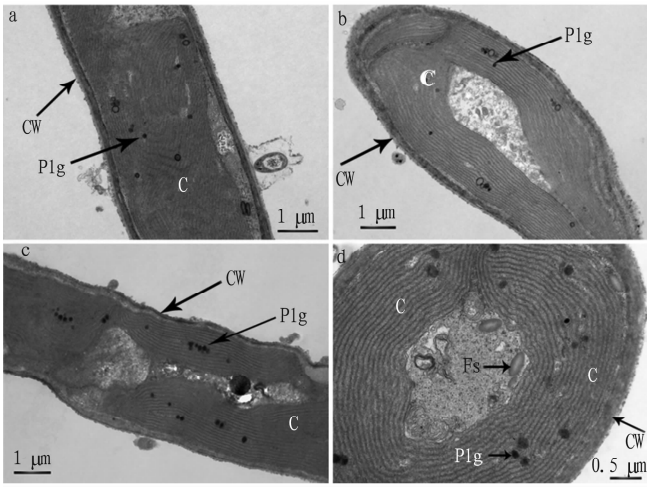


Fig. 2 Morphological observations of *Neopyropia yezoensis* free-living conchocelis under different light qualities

The effects of different light quality on the ultrastructure of *N. yezoensis* free-living conchocelis were shown in Fig. 3. In WL group, the longitudinal section showed a bilayer structure of the cell wall with a thickness of (0.267 ± 0.069) μm. The pigment bodies were mostly long and banded, the thylakoids were densely arranged in parallel, and the plastoglobulus was distributed everywhere in the pigment body (Fig. 3a). In RL group, the cell wall thickness was about (0.257 ± 0.056) μm, the pigment

bodies were continuously arranged close to the cell membrane, and the plastoglobulus was sporadically distributed in the pigment bodies (Fig. 3b). In BL group, the longitudinal section showed that the cell wall structure was significantly thinner, and the thickness was only (0.174 ± 0.088) μm, and the plastoglobulus appeared in clusters of two or five or six between thylakoids, and the number was significantly higher than that in other light qualities (Fig. 3c). In GL group, the cross section showed that the algal cells were nearly round, and the cell wall thickness was about (0.241 ± 0.054) μm. The pigment bodies were filled around the cell cavity, the thylakoids were dense, and many plastoglobulus were distributed. The red algal starch was oval and attached to the edge of the pigment bodies (Fig. 3d).



NOTE CW denotes cell wall, C refers to pigment body, Plg represents plastoglobulus, and Fs is red algae starch.

Fig. 3 Ultrastructural observations of *Neopyropia yezoensis* free-living conchocelis under WL (a), RL (b), BL (c), and GL (d)

3.3 Effects of different light qualities on the contents of photosynthetic pigments and phycobiliprotein in *N. yezoensis* free-living conchocelis Fig. 4 shows that photosynthetic pigment content of *N. yezoensis* conchocelis under different light quality treatments. Under different light quality treatments, the chlorophyll a content of WL group was the highest, which was about 1.238 mg/g. Followed by GL and RL groups. BL group was the lowest, only 0.777 mg/g ($P < 0.05$). The content of carotenoid varied from 0.341 mg/g to 0.464 mg/g, which was consistent with the trend of chlorophyll a. The content of WL group was the highest, but there was no significant difference between WL group and other light quality groups ($P > 0.05$).

Fig. 5 shows the phycobiliprotein content in *N. yezoensis* conchocelis under different light qualities. The content of phycoerythrin (PE) in BL group was the highest, about 4.97 mg/g, followed by WL group. The content of PE in RL and GL groups was lower, about 2.51 mg/g, which was only 0.5 times of that in BL group ($P < 0.05$). BL was beneficial to the accumulation of PE, while RL and GL were not. However, RL and GL were beneficial to the synthesis of phycocyanin (PC). The highest PC content (about 3.90 mg/g) was found in RL and GL, followed by BL, and the lowest PC content was found in WL ($P < 0.05$).

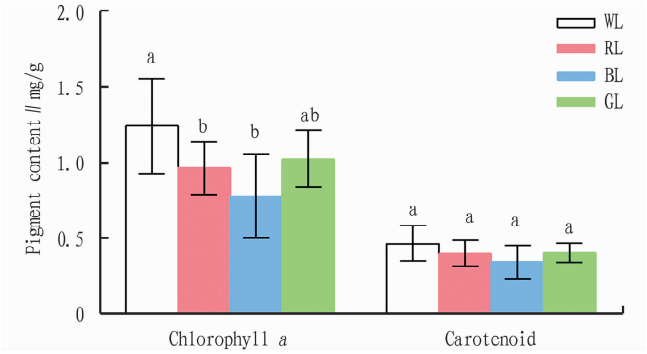


Fig. 4 Chlorophyll *a* and carotenoid contents of *Neopyropia yezoensis* free-living conchocelis under different light qualities

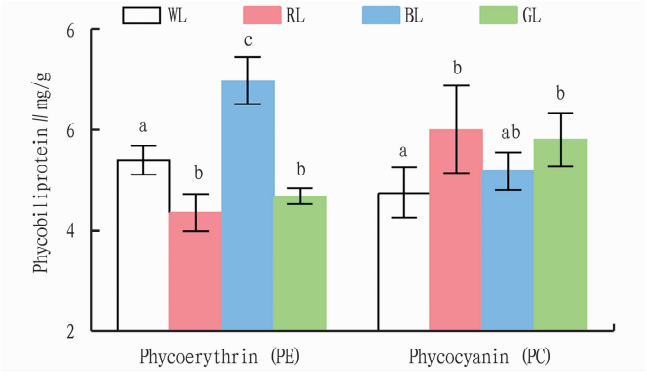


Fig. 5 Phycoerythrin and phycocyanin contents of *Neopyropia yezoensis* free-living conchocelis under different light qualities

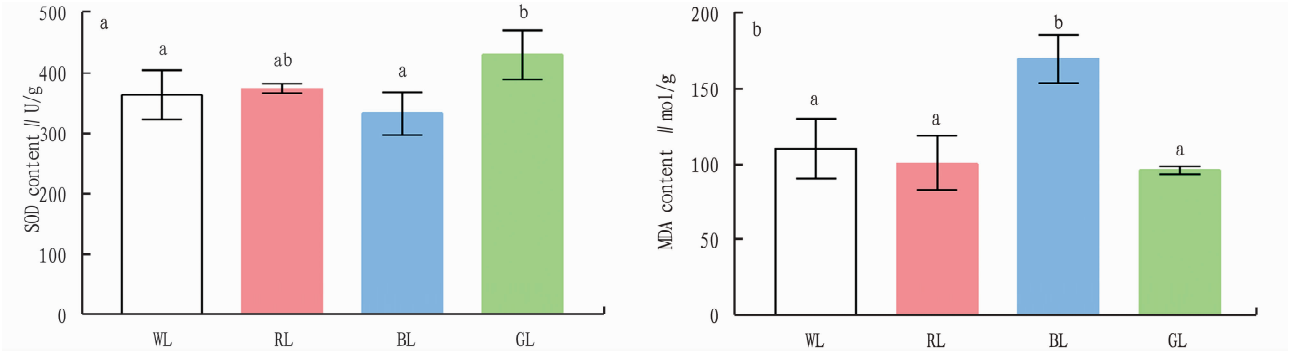


Fig. 6 SOD and MDA contents of *Neopyropia yezoensis* free-living conchocelis under different light qualities

3.4 Effects of different light quality on the content of SOD and MDA in *N. yezoensis* free-living conchocelis The level of SOD activity reflects the body's ability to scavenge oxygen free radicals[25], while the level of MDA content can indirectly reflect the degree of cell damage[26]. SOD enzyme activity detection showed (Fig. 6a) that SOD enzyme activity was the lowest in BL group and the highest in GL group ($P<0.05$). The content of MDA in BL group was significantly higher than that in other light quality groups ($P<0.05$), but there was no significant difference among white, red and GL groups ($P>0.05$).

4 Discussion

Light quality plays an important role in the regulation of algal growth and development[8]. In this study, the specific growth rate of *N. yezoensis* conchocelis was the highest under WL, followed by GL and RL, and the lowest under BL. The specific growth rate of algae was consistent with the content of chlorophyll a, indicating that WL was more conducive to the growth of algae and the accumulation of pigments. Compared with monochromatic light, WL has a wide wavelength range, including various wavelength signals and energies, which can well meet the needs of algae growth[27]. In this experiment, the growth rate of BL was significantly lower than that of WL and GL, which was consistent with the results of algae studies such as *Porphyra haitanensis* conchocelis[9], *Porphyra umbilicata* Thallus[12] and *Gracilaria birdiae*[28]. The lower growth rate under BL may be related to the lower photosynthesis caused by the lower chlorophyll a content[12].

The study of ultrastructure is helpful to evaluate the effect of light quality on the cell structure of laver conchocelis[9]. In this study the cell wall of WL, RL and GL groups was thicker, while the cell wall of BL group was the thinnest, which was consistent with the results of algal diameter observed by optical microscope. Thicker cell walls indicate that algae produce more polysaccharides, which can provide a stable internal environment for the synthesis of cell contents[29]. The number of plastoglobulus in thylakoids of BL was the highest among the other groups. Tsekos *et al.*[30] also found that there were more plastoglobulus in the algal bodies of *Porphyra leucosticta* grown under BL than under RL and GL. Plastoglobulus is a complex of lipids, proteins, plastoquinones, plastophenols, and other metabolites[31]. In higher plants such as cucumber (*Cucumis sativus*)[32], Norway spruce (*Picea abies*)[33] and Chinese chestnut (*Castanea mollissima*)[34], it was found that the increase in the number of plastoglobulus indicated the degradation of thylakoid membrane, which was generally related to leaf senescence, indicating that the thylakoid membrane system of *N. yezoensis* conchocelis in BL group might be damaged. Phycobiliprotein is a light-harvesting antennae associated with photosystem II in algae, which transfers light energy to chlorophyll for photosynthesis[35].

In this study, the effect of different light quality on PE content of *N. yezoensis* conchocelis showed a higher effect under BL treatment, while PC content showed a higher effect under RL and GL treatment. A large number of studies have shown that BL and GL can increase the PE or PC content of seaweed, such as *Porphyra haitanensis* thallus[36], *Porphyra leucosticta* thallus[11], *Gracilaria birdiae* sporophyte[28], *Chondrus crispus*[37], and *Brassica celerifolia*[13], *etc.* The increase in PE and PC content in *N. yezoensis* conchocelis can be attributed to the adaptation to BL, RL, and GL spectra. Takahashi[38] found in the thallus of *N. yezoensis* that after RL or BL irradiation, the thallus could change its color by adjusting the content of PC or PE. In this study, we also found the similar phenomenon that the initial color of the *N. yezoensis* conchocelis is dark brown. After irradiation of RL and GL, the algae turned green due to the increase of PC content, and after irradiation of BL, the algae turned bright red due to the significant increase of PE content. Therefore, the pigment content of *N. yezoensis* can be regulated by different wavelength treatments in the long-period expansion culture of thalli. When plants encounter stress, it will induce the increase of active oxygen *in vivo*, and the high concentration of active oxygen will promote the peroxidation of polyunsaturated fatty acids (PUFA) in membrane lipids into MDA, thereby destroying the integrity of membrane structure[39]. SOD can scavenge reactive oxygen species and protect cells from damage[40]. The level of SOD activity reflects the body's ability to scavenge oxygen free radicals[25]. MDA content reflects the severity of lipid peroxidation and cell membrane system attacked by free radicals[26]. In this experiment, the activity of superoxide dismutase in *N. yezoensis* conchocelis under BL was the lowest, while the content of MDA was significantly higher than that of other light quality groups, which indicated that the *N. yezoensis* conchocelis were stressed and the cell membrane was damaged under BL irradiation. This is possibly because compared with RL and GL with longer wavelength under the same light intensity, BL with shorter wavelength has higher light irradiation energy[41], and the stronger energy causes high-energy damage to cells[27], accordingly leading to physiological stress of algae.

5 Conclusions

In this experiment, the free-living conchocelis of *N. yezoensis* under WL was normal black and brown, with the thickest cell wall, the highest specific growth rate and chlorophyll a content. Under RL and GL, the algal body was green, the content of phycocyanin (PC) was higher, the thickness of cell wall was similar to that under WL, and the specific growth rate and pigment content were second only to those under WL. Under BL, the algal body was bright red, the content of phycoerythrin (PE) was the highest, the cell wall was the thinnest, the number of plastoglobulus on thylakoid was the highest, and the specific growth rate and pigment content were the lowest. Compared with WL group, the SOD activity was the lowest in BL group and the highest in GL group. The MDA content was the highest in BL group, and lower in RL and GL groups. In conclusion, WL is the most beneficial to the growth of algae, followed by RL and GL, and BL has adverse effects. This study explored the most suitable light quality conditions for propagation of *N. yezoensis* free-living conchocelis. It is expected to provide germplasm protection for the production and seedling of laver.

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