Self-preservation of Polysaccharides Released from Spirulina Platensis Against Photo-oxidation Damage

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Abstract

Spirulina platensis **is a kind of prokaryotic cyanobacteria and has been used broadly in food and pharmaceutical industry. High light irradiance is one of the main limitations to the growth of S.** ***platensis*** **due to photo-oxidative damage, but the mechanisms by which S.** ***platensis*** **against this photodynamic damage have not been fully elucidated. In this paper, we observed that high light irradiance (8000 lux) would bleach the photosynthetic pigments and accelerate the aging of S.** ***platensis*** **cells. We hypothesized high light irradiance lead to phycobiliproteins losing balance between the generation and quenching of ROS, and that cause the photo-oxidative damage to S. platensis cells. Mover, S.** ***platensis*** **polysaccharide (PSP) supplement in the culture medium can obviously protect the photosynthetic pigments of S. platensis from photo-oxidative bleaching. Also, PSP treatment can significantly promote the grow rate of S.** ***platensis*** **when cultured in moderate light intense (5000 lux). These findings suggested that the polysaccharides released in S.** ***platensis*** **cells may involve in the mechanisms by which S.** ***platensis*** **self-preservatio against photo-oxidative damage. It shed a light on the potential value of PSP for S. platensis cultivation in tropical regions.**

Keywords

Spirulina platensis**, Phycobiliproteins, Polysaccharides, Photo-oxidative damage.**

1. Introduction

Spirulina platensis has been consumed by humans for thousands of years. This microalgae is abundant in proteins and other nutrients such as vitamins, essential amino acids, minerals, and unsaturated fatty acids [1]. Apart from its rich and balance in nutrients, *S. platensis* has lots of therapeutic properties such as antioxidative effects, immunomodulation, anticancer, antiviral infection and cholesterol regulatory. Thus, *S. platensis* has a broad potency of applications in functional food and pharmaceutical industry.

As a kind of blue-green alga, *S. platensis* has a high photosynthetic efficiency under the ideal growth conditions of temperature, light irradiance and pH value etc [2,3]. Currently, commercial *S. platensis* are mainly produced in areas with adequate light and heat condition, especially in tropical and subtropical regions [4,5]. One main problem associated with mass cultivation of *S. platensis* in these places is the growth inhibition to the algae cells induced by high light irradiance [6,7]. It has been reported that high light irradiance could induce photodynamic damage in *S. platensis* cells owing to the photo-bleaching of photosynthetic pigments by singlet oxygen[8], an effect that is similar to the inactivation of photosynthesis in higher plant cells owing to the photodestruction of the photosynthetic apparatus by a variety of reactive oxygen species (ROS) [6,8,9]. On the other hand, some functional or structural changes may have occurred in *S. platensis* under high light stress and result in a feedback of tolerance to photo-oxidative damage. This view has been clued by a report that the folding pattern of the filamentous cells was observed in a strain of chemical induced *S. platensis* mutant, which seems to provide an adaptation to the high irradiance by shielding the light intensity [10]. However, the mechanisms by which *S. platensis* protect itself against photo-oxidative damage during cultivation have not been fully elucidated.

S. platensis polysaccharides (PSP) possess many biological functions. The PSP yield was positively correlated with culturing light intensity. Herein, to investigate the role of PSP against photo-oxidation damage, we cultured *S. platensis* with high light (8000 lux) to induce bleaching of photosynthetic pigments and *S. platensis* cells aging as a photo-oxidation damage model, and evidenced that PSP added in the culture medium can obviously protect the photosynthetic pigments from photo-oxidative bleaching. Also, PSP treatment can significantly promote the grow rate of *S. platensis* when cultured in moderate light intense (5000 lux). Present data suggested that the polysaccharides released by *S. platensis* cells may involve in the mechanisms by which *S. platensis* itself against photo-oxidative damage. Our finding shed a light on the potential value of PSP for *S. platensis* cultivation in tropical regions.

2. Material and methods

2.1 Extraction and Purification of S. platensis Polysaccharide

S. platensis Polysaccharide (PSP) was extracted and purified by previous method with some modifications [11]. In brief, 50g of *S. platensis* dry powder was suspended in 500 ml ddH_2O in a glass beaker, and crude PSP was extracted by magnetic stirring for 8 h under 80℃. After centrifugation at 4000 r/min for 20min, the supernatant was concentrated to 1/5 of the original volume in reduced pressure. Adding 3 times (v/v) of 95% ethanol in the supernatant to precipitate total PSP at for 12 h , and the crude PSP was collected by centrifugation (4000 r/min) at 4℃ for 20 min. Proteins in the crude PSP were digested by neutral protease at 50 ℃ for 2.5h under pH 7.0, and then applied Sevag deproteinization method to further except proteins .The Sevag deproteinization progress was as follows: Adding 0.25 times volume of a mixed solvent of chloroform and isoamyl alcohol (mixing ratio is 3:1 in volume)into the crude PSP solution. After oscillating for 15 min, the underlayer organic solvents was discarded by centrifugation (4000r/min). After 5 repeats of above process, refined PSP were precipitated by 95% ethanol in the upper aqueous phase, and then washed by acetone for three times to remove pigments. The purity of PSP was evaluated up to 95% by H_2SO_4-Anthrone spectrophotometric analysis [12]. The PSP powder was made by freeze-drying and stored at -20℃ until use.

2.2 S. platensis Cultivation, Growth Evaluation and Pigments Determination

S. platensis in the logarithmic phase were inoculated into 250-ml flask containing 150 ml of Zarrouk medium with the initial OD560 about 0.2. And light intensities set three levels, which were1000 lux (low, L), 4000 lux (moderate, M) and 8000 lux (high, H), respectively. Cultivations were performed on a shaker in incubators at temperature of 30±2 ℃ and 12/12 hours illumination. All above treatments were set in 3 repeats. To evaluate the growth of *S. platensis* under different light intensities, OD560 value was monitored periodically every day to plot the growth curve. After two weeks cultivation, the biomasses of final cultures were measured by dry weight method, and the contents of photosynthetic pigments in algae cells were detected by methods provided previously [13].

2.3 Imaging of S. platensis Morphology and Cell Wall Structure

To observe the changes of *S. platensis* cells morphology caused by different light irradiance, images of algal cells were captured every day using optical microscope. After cultured for a week, the algae cell samples of the group 1000lux and 8000lux were collected and observe the cell-walls morphology using atomic force microscope (AFM) according to the method provided previously. The progress was as follows: Algal cells were harvest and washed with deionized water, and then suspended in ddH_2O and adjusted the cell concentration to about 1.0×10^4 /ml. Remove 20 μl sample to a clean glass slide surface. After dry in the air about 20min, using qualitative filter paper to absorb the excess dilution on the edges of the sample. After the samples were completely dried, they can be measured by AFM.

2.4 Observations of the anti-photooxidative ability of PSP to S.Platensis

S. platensis polysaccharide (PSP) was add to the culture medium to investigate its anti-photooxidative ability to S.Platensis (cultured in high light, 8000 Lux), and to investigate its impacts on *S. platensis*

cells growth and proliferation (cultured in moderate light, 5000 Lux). The PSP concentrations were 0, 30, 60, 100 mg/L respectively, and others cultured conditions were the same as above. All PSP concentrations experiments were set in 3 repeats.

2.5 Statistical Analyses

Data comparison of means was carried out using an unpaired Student's t test using GraphPad Prism version 4.0. All comparisons were considered significant at $P < 0.05$.

3. Results

3.1 High light irradiance inhibits the growth of S. platensis

When *S. platensis* was cultivated under different light intensities for two weeks, the growth of the alga was kept well with the light intensity ranging from 1000 to 4000 lux, but obviously inhibited under 8000 lux irradiance (Fig. 1 A). Consistently, the color of cultures turned from green to yellow gradually following the increase of the light irradiance (Fig. 1 B). After cultured for two weeks, the maximum algal biomass was harvested in 4000 lux group, and the cultivation biomass of 8000 lux irradiance group was significant higher than that of 1000 lux group ($P < 0.01$) (Fig. 1 C). Moreover, the photosynthetic pigment content of algal cells including chlorophyll a and b and total carotene were all decrease significantly as the light intense increase (Fig. 1 D).

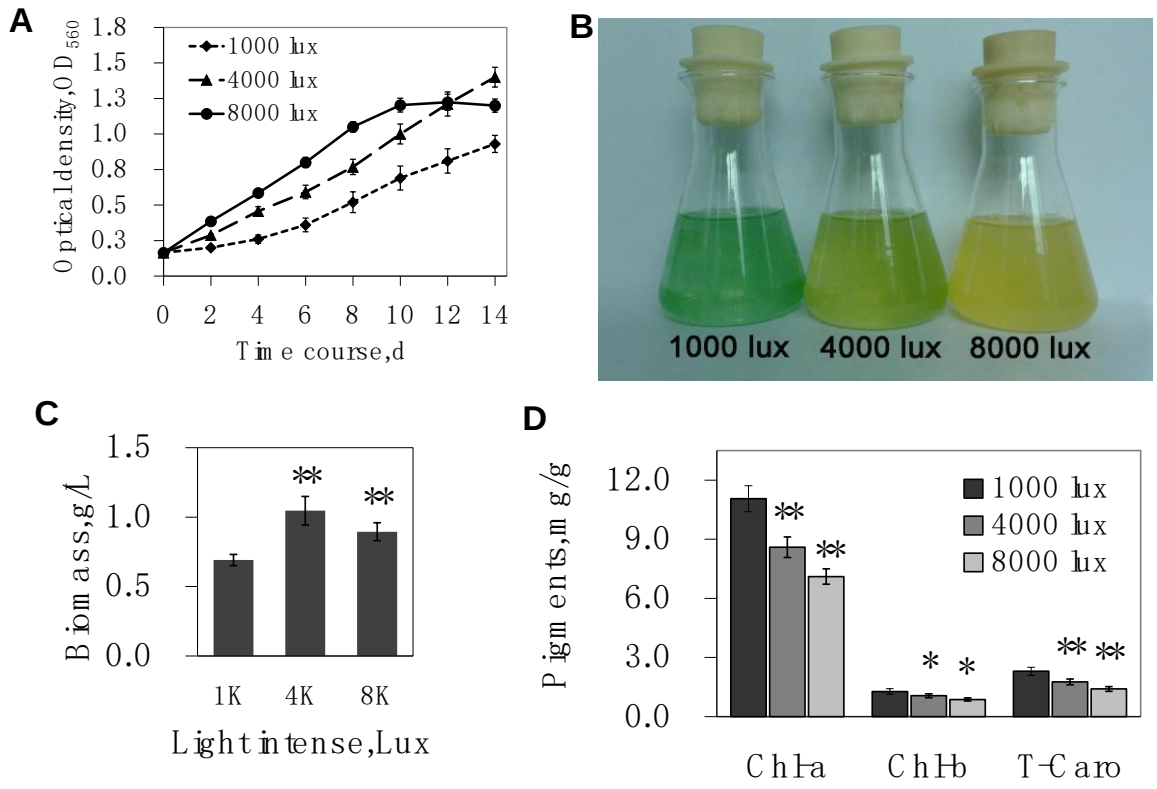

Fig. 1: High light irradiance inhibit the growth of *S. platensis*. (A) Growth curve. (B) Algae color. (C) Biomass. (D) Pigments content.

Our present data suggested that the growth of *S. platensis* was inhibited by high light irradiance which mediated the photo-oxidative damage. Photo-oxidative damage is a catholic phenomenon among blue-green alga [6,9]. It has been proposed that the bleach of photosynthesis pigments might be caused by singlet oxygen generation in photosynthesis system when *S. platensis* was exposed by high light over the photosynthesis saturating light intensity (1995). Interestingly, it was found that the phycobiliproteins, the main light-harvesting proteins of *S. platensis*, not only could generated a

variety of reactive oxygen species (ROS) exposed to visual light [20], but also could quench the ROS even stronger than the well-known antioxidants molecules such as Trolox, ascorbic acid, and reduced glutathione [21,22]. Previously, we also found that C-phycocyanin, a primary component of phycobiliproteins, could scavenge ROS in vitro highly associated with its aggregation state al, . Thus, combined with our results, we hypothesized that as light irradiance increase, it arise difficulty for the phycobiliproteins to balance the generation and quenching of ROS, and as a result cause oxidative bleaching of photosynthesis pigments.

3.2 High light irradiance accelerate the aging of S. platensis cells

Observing Spirulina platensis cells morphology cultured in three lever light intense. We observed that the *S. platensis* cultured in high light (8000 lux) showed a coarser algal body, a large spiral degree, trended to clustered, as well as alga color turned to yellow. These changes in algal filaments morphology may contribute to shielding the light intensity, and also may indicated the aging of alga cells. Relatively, the *S. platensis* cultured in mild light (1000 lux) had a fine algal body, low helicity, and well in dispersion and floating, as well as the alga color was bright green. These may mean alga cells kept new ecological (data are not provided). The results of AFM scans show that the wrinkles in *S. platensis* cell surface increased significantly when the light irradiance increase from 1000 lux to 8000 lux (Fig. 2). The increase of wrinkles in cell surface may indicate the aging of *S. platensis* cells. In conclusion, we suppose that the ROS generated by high light stress could bleach photosynthesis pigments as well as could damage the cell walls and many other cell structures. And as a result accelerating the aging of *S. platensis* cells.

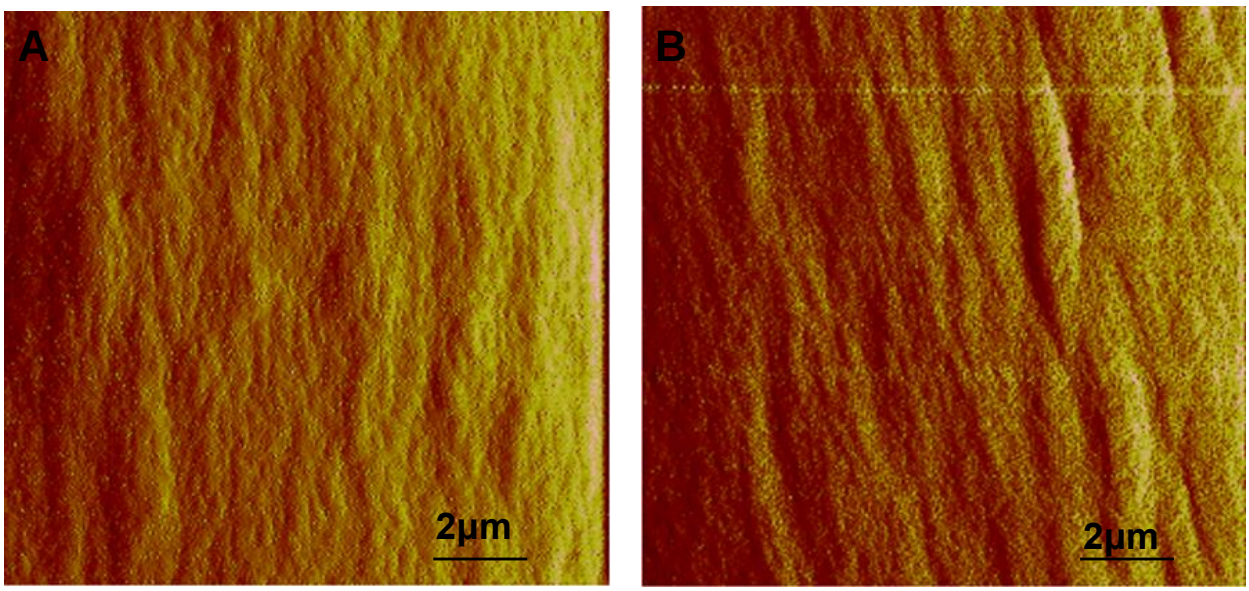

Fig. 2: High light irradiance damage the cell-wall of *S. platensis*. (A) Mild light-induced. (B) High light-induced.

3.3 S. platensis polysaccharides protect photosynthetic pigments from photo-oxidation bleaching

To investigate the anti-photooxidative activity of PSP to *S. platensis*, we cultured *S. platensis* with different concentrations of PSP in high light intensity (8000 lux). After cultured for one week, the content of chlorophyll a, chlorophyll b and total carotenoids of alga cells in the groups cultured with PSP are significantly higher than the controlled group without PSP. And as the PSP concentration increase the protective effect are more obvious (Fig.3 A). This result confirms that PSP can effectively protect the algae cells pigments from photo-oxidation bleaching. The color of alga cells of each group after cultured for a week are shown in Fig.3 B. This photo provide a visual evidence of the protective effect of PSP. *S. platensis* polysaccharide has been confirmed by many vivo and vitro studies that it

have free radical scavenging activity and antioxidant ability [14,15]. Therefore, we suppose the reason PSP protecting photosynthetic pigments from photo-oxidation bleaching is that PSP can help in quenching those active oxygen species generated by photosynthetic system. The polysaccharides distributed in the *S. platensis* cells may help the phycobiliproteins balance the generation and quenching of ROS.

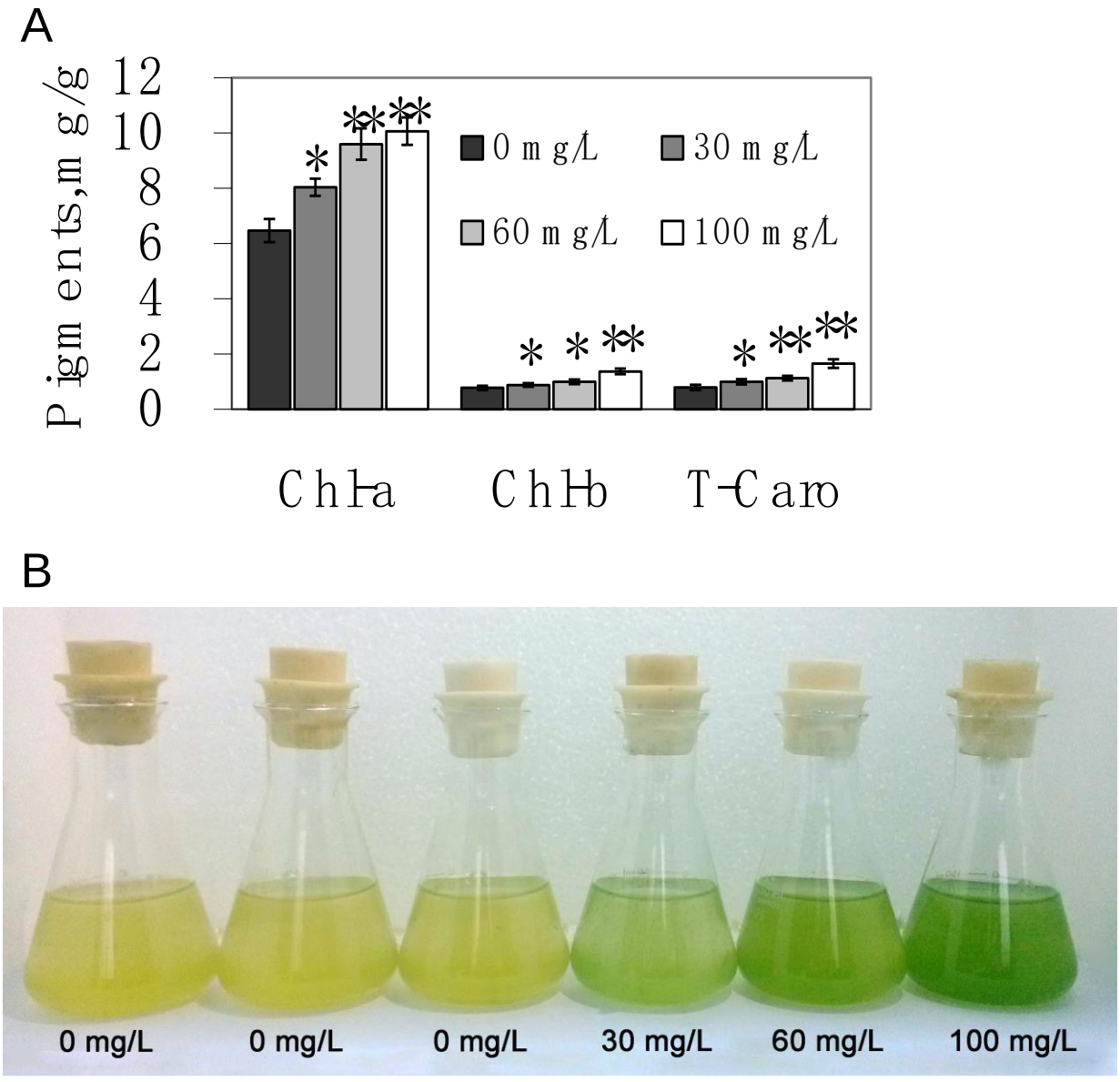

Fig.3: PSP protect photosynthetic pigments from photo-oxidation bleaching. (A) Pigments content of algae cells. (B) Algae color.

3.4 Spirulina polysaccharides promote the grow rate of S. platensis

To investigate the growth and proliferation effect of PSP to *S. platensis*, we cultured *S. platensis* in a moderate light intensity (5000 lux) with different concentrations of PSP. The time-growth curves were draw and showed in Figure 4A. According to the time-growth curves, calculating the specific growth rate (μ) of each group using the formula $\mu = 1/t \times Ln (Xt/Xo)$ (t is the culture time, Xt is the biomass of day t, Xo is the initial biomass)[16-19]and the results is shown in Fig. 4 B. Date show that *S. platensis* in experimental group with PSP have a higher growth rate, and as the PSP concentration increase, the growth rate is higher. This result confirms that PSP could promote the grow rate of *S.*

platensis. We speculate that the PSP supplement in the culture medium has created a reductive atmosphere for alga cells and thus helping alga cells keep new ecological. This result provides a further evidence that *S. platensis* polysaccharide has great anti-photooxidative activity to *S. platensis*.

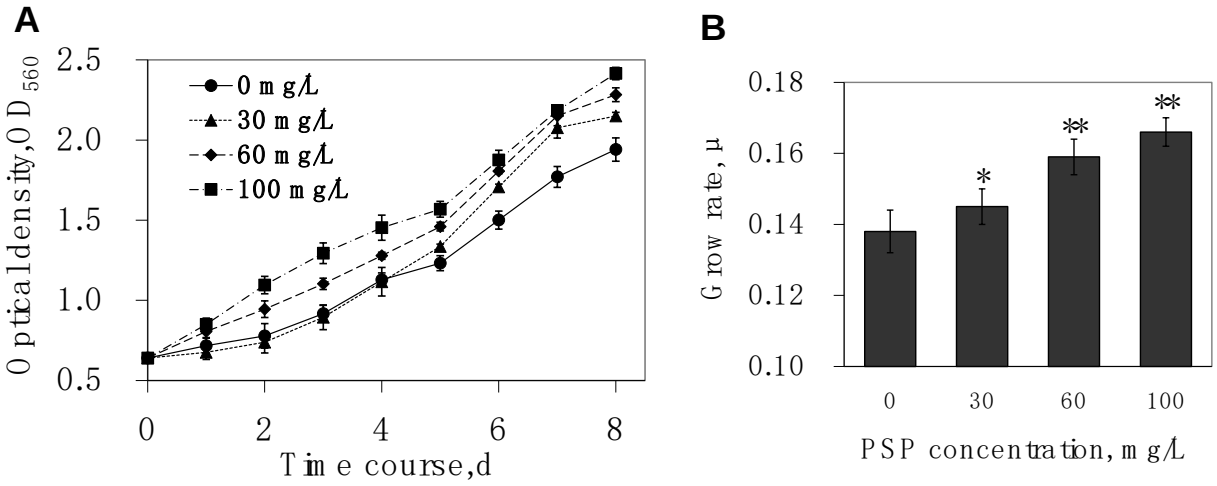

Fig.4: *S. platensis* polysaccharides promote the grow rate of *S. platensis*. (A) Growth curve o. (B) Growth rate (μ).

4. Conclusion

Photo-oxidative damage induced by irradiance stress is a main limiting factor for the growth of blue-green alga. Frequently, people can observe that the blue-green algae blooms in ponds would suddenly and rapidly disintegrate with exposure of strong sunshine irradiance [20]. This raises the question of limitation to the microalgae farming in open systems, especially in tropics countries/areas and in summer season [21-24]. In this paper, we report that *S. platensis* had significant response under high light stress, including photosynthetic pigments bleaching, alga cell aging, and the inhibition of cell growth and proliferation [25,26]. We hypothesized that high light irradiance lead to phycobiliproteins losing balance at the generation and quenching of ROS, and as a result cause a series of oxidative damage to the alga cells.

Polysaccharide is a major component of *S. platensis*, accounting for 6% of cell dry weight. *S. platensis* polysaccharide mainly store in the cytoplasm and the cell wall, and it can also be found in the culture medium [27]. It is report that high light stress can increase the secretion of extracellular polysaccharides [28]. Here, we report that the *S. platensis* polysaccharides added into the culture medium can effectively protect the photosynthetic pigments from photo-oxidation bleaching, and PSP can also significantly improve the growth rate *S. platensis*. Therefore, we confirm that *S. platensis* polysaccharides has great anti-photooxidative activity to *S. platensis*. The polysaccharide distributed within and outside the *S. platensis* cells is a defense mechanism resisted to photo-oxidative damage.

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