

Original Research

Effects of Concentrated Seawater on Photosynthetic Pigment Contents in *Porphyra haitanensis*

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Abstract

During the production of desalination plants, concentrated seawater is expelled to local marine environments, which may lead to hypersaline pollution. This study aimed to investigate the effects of concentrated seawater on *Porphyra haitanensis* by exposing *P. haitanensis* to salinity of 40‰, 36‰ and 30‰ (considered as the control) for 2 h, 6 h, 12 h, 24 h, 48 h and 96 h. The results showed that chlorophyll a content showed no significant change among different salinities, while carotenoid, phycocyanin and phycoerythrin contents increased in treatment with 40‰ compared with the control. Carotenoid, phycocyanin and phycoerythrin can all function as antioxidants, suggesting that hypersaline exposure might trigger oxidative stress in *P. haitanensis*. Overall, the present study suggests that hypersaline pollution caused by desalination plants raises significant ecological risks for local marine environments.

Keywords: *Porphyra haitanensis*, chlorophyll, carotenoid, phycocyanin, phycoerythrin

Introduction

Desalination of seawater is an effective way to alleviate freshwater shortages, but it will inevitably produce effluents with high salinity, high temperature and/or chemical pollutants [1, 2], which may negatively affect local environments and pose a threat to marine organisms (such as plankton, seagrass, sponges, shrimp and crabs, gastropods and bivalves) [3]. This issue has been of concerned since the early 1990s [4, 5].

During desalination of seawater, effluents with salinity higher than normal seawater (30‰) are produced and are generally expelled to the sea directly. The hypersaline effluents of brine can directly influence the physiological function of organisms, such as inhibiting photosynthesis and respiration, reducing chlorophyll content, changing the submicroscopic structure of chloroplast, and reducing the activity of enzymes, thus affecting their metabolism, growth, development and reproduction [6, 7]. For example, treatment with hypersaline water reduced species richness and density of planktons [8]. Some species, mainly the diatoms, showed a slight tolerance to hypersalinity, which was still lower in density than the normal salinity near

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desalination plants [9]. Liang et al. [10] revealed that relative growth rate and saturated fatty acid content decreased with the increase of salinity (from 18‰ to 38‰) in *Phaeodactylum tricornutum* Bohlin and *Nitzschia closterium* (Ehrenberg) W. Smith. Kou et al. found that the *Skeletonema costatum* (Greville) Cleve, *Chaetoceros curvisetus* Cleve and *Cyclotella cryptica* Reimann, Lewin and Guillard could survive at salinity of 48‰, but their growth rates and algal densities were lower than those at 32‰ [11]. Photosynthesis is a sensitive index in response to environmental changes. Compared with normal salinity, treatments with 39-43‰ reduced photosynthetic pigment contents and photosynthetic efficiency in the Mediterranean seagrass *Posidonia oceanica* (L.) Delile [12], treatments with salinity higher than 40‰ destroyed photosynthetic system and pigment in *Thalassia testudinum* König [13].

Porphyra haitanensis Chang et Zheng is a unique species in China, generally growing at intertidal and warm zones where tidal currents are smooth and nutrient salts are abundant [14]. It is widely applied in the food, textile, medicine and other industries. Thus, it is an important economic macroalgae species. In 2010, the yield of *P. haitanensis* in three main production provinces of China reached 82,800 tons, and its artificial cultivation area is still increasing [15]. Previous studies have shown that the growth and photosynthesis of *P. haitanensis* were inhibited by high temperature stress [16], strong sunlight [17] and lowered salinity [18]. To the best of our knowledge, no reports have been conducted to investigate the effects of hypersalinity on *P. haitanensis*.

In the present study, in order to investigate the effects of concentrated seawater on *P. haitanensis*, *P. haitanensis* was exposed to hypersaline solutions. Next, changes of chlorophyll a, carotenoid and phycobiliprotein contents were detected. The results would help us assess the ecological risk of desalination plants to local marine environments.

Materials and Methods

Sample Collection

P. haitanensis was collected from a wild population at Pingtan Island, Fujian Province, China (25°15'29"N, 119°28'26"E), rinsed with sterilized seawater, and then cultured in the laboratory at 24±1°C. The light cycle was 12 h:12 h (dark:light) with light intensity of 100 μmol photon/m²s. Afterward, spores were collected and then cultivated under the same conditions in 2-(4-Morpholino) ethanesulfonic acid (MES) medium as described by Baker et al. [19]. Healthy individuals in a similar size were chosen for the experiments.

Saline Treatment

The general salinity at the sampling location was 30‰ and salinity of seawater near the local desalination plant was up to 40‰. In order to investigate tolerance to hypersalinity, *P. haitanensis* thalli were treated with salinities of 36‰ and 40‰. Salinity of 30‰ was also included as the control. Experiments were conducted in Erlenmeyer flasks containing 500 mL of media and each treatment included 20 individuals. After 2 h, 6 h, 12 h, 24 h, 48 h and 96 h, three individuals were collected, cut into pieces and pooled as one sample to measure contents of chlorophyll a, carotenoid and phycobiliproteins (PBPs). Each treatment was repeated three times independently.

Measurements of Chlorophyll Contents

For each treatment, 100 mg of pooled sample was homogenized in methanol using a mortar and pestle and then extracted at 4°C in the dark overnight. After centrifuging at 10,000 rpm for 15 min, the supernatant was transferred to measure absorbance at 750 nm, 665 nm, 652 nm, 510 nm and 480 nm using a UV-visible light spectrophotometer (VARIAN Cary50). The chlorophyll a (Chl-a) and carotenoid (Car) contents were calculated using the following equations [20]:

$$\text{Chl-a } (\mu\text{g/g}) = 16.29 \times (\text{OD}_{665} - \text{OD}_{750}) - 8.54 \times (\text{OD}_{652} - \text{OD}_{750})$$

$$\text{Car } (\mu\text{g/g}) = 7.6 \times [(\text{OD}_{480} - \text{OD}_{750}) - 8.54 \times (\text{OD}_{510} - \text{OD}_{750})]$$

...where OD means the absorbance at the corresponding wavelength (nm).

Measurements of PBP Contents

For each treatment, 100 mg of sample was homogenized in phosphate buffer (0.1 M, pH = 6.5), extracted at 4°C overnight, and then centrifuged at 10,000 rpm for 15 min. The supernatant was then collected to measure the absorbance at 564 nm, 455 nm, 592 nm, 618 nm and 645 nm using a UV-visible light spectrophotometer (VARIAN Cary50). Contents of phycoerythrin (PE) and phycocyanin (PC) were calculated by the following equations [21]:

$$\text{PE (mg/g)} = [(\text{OD}_{564} - \text{OD}_{592}) - 0.2 \times (\text{OD}_{455} - \text{OD}_{592})] \times 0.12 \times V / \text{FW}$$

$$\text{PC (mg/g)} = [(\text{OD}_{618} - \text{OD}_{645}) - 0.2 \times (\text{OD}_{592} - \text{OD}_{645})] \times 0.15 \times V / \text{FW}$$

...where OD means the absorbance at the corresponding wavelength (nm), V is the final volume of phosphate

buffer, and FW represents fresh weight of sample used for extraction.

Statistical Analyses

The results are expressed as mean values±standard deviation (SD). Data were analyzed using SPSS 17.0 statistical software (SPSS Inc., Chicago, USA) by one-way analysis of variance (ANOVA), followed by *Duncan's* multiple comparison test to determine significant differences among treatments and the control. $P<0.05$ was considered statistically significant.

Results and Discussion

Salinity is an important environmental factor that affects the physiological activities of macroalgae. Maintenance of saline concentration is crucial for proper cell functioning, ion regulation, membrane potential, osmotic balance, and metabolic activity. Generally, certain macroalgae has appropriate salinity range, and extremely high or low salinity will cause stress on its physiological process. Previous studies have shown that the growth and photosynthesis of *Chlamydomonas reinhardtii* Dangeard [22], *Ulva fasciata* Delile [23] and *Dunaliella* [24] were significantly inhibited under saline stress. At the same time, after long-term adaptation, macroalgae can protect themselves from the negative stress caused by salinity through regulating physiological processes, such as induction of an antioxidant system [25, 26].

Changes of Chl-a Content under Hypersaline Stress

Photosynthesis, the primary step of energy production, is inhibited by saline stress through affecting chlorophyll content. Hypersaline stress may increase activity of chlorophyllase and destroy the membrane structure of chloroplast, which should promote degradation of chlorophyll and reduce chlorophyll content [27]. Thus, chlorophyll content has been proposed as one of the biochemical indicators of saline tolerance in plants [28]. In the present study, Chl-a content was not affected in response to treatments with 36‰ and 40‰ during the 96 h exposure period, compared with the control (Fig. 1). These results suggested that exposure to hypersaline seawater might not affect Chl-a content in *P. haitanensis*.

Changes of Car Content under Hypersaline Stress

In response to biotic and abiotic stress, carotenoids play an important role as a precursor in signaling during plant development and/or resistance [29]. Carotenoids

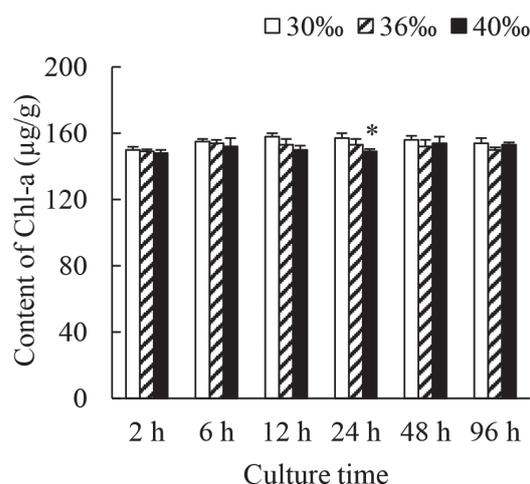


Fig. 1. Effects of concentrated seawater on chlorophyll-a (Chl-a) content of *P. haitanensis*; data represent mean±SD. *significant difference from the control ($P<0.05$)

are capable of scavenging reactive oxygen (ROS) as antioxidants, protecting membranes from oxidative damage in algae. In the present study, Car content was significantly higher in treatment with 40‰ than those in treatments with 30‰ and 36‰ after exposure for 6 h to 96 h ($P<0.05$). No significant difference was detected between treatments with 36‰ and 30‰ ($P>0.05$). Car content in 40‰ treatment increased gradually from 2 h to 48 h, and stabilized after 48 h. After 96 h, the Car content in 40‰ treatment increased by 26.77% and 25.75%, compared with that in 30‰ and 36‰ treatments, respectively (Fig. 2). These results were similar to previous reports that increased saline-elevated Car content in *Spirulina fusiformis* Voronikhin [30] and *Botryococcus braunii* Kutz [31], suggesting that hypersalinity might induce oxidative

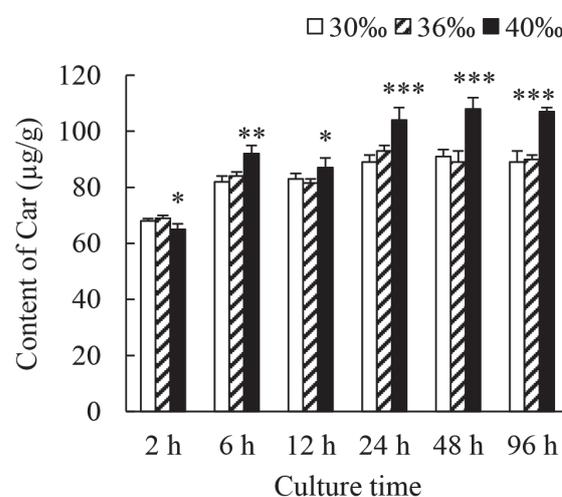


Fig. 2. Effects of concentrated seawater on carotenoid (Car) content of *P. haitanensis*; data represent mean±SD. *significant difference from the control ($P<0.05$)

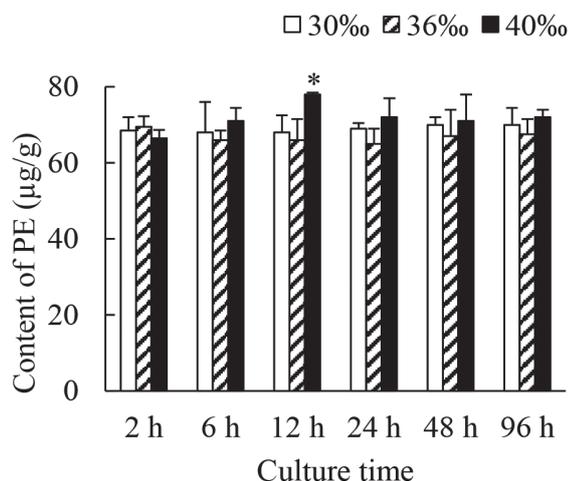


Fig. 3. Effects of concentrated seawater on phycoerythrin (PE) content of *P. haitanensis*; data represent mean \pm SD. *significant difference from the control ($P<0.05$)

stress and the accumulation of carotenoids. In contrast, increased salinity from 45 g/L to 55 g/L inhibited content of Car and algal growth of *Nannochloropsis oculata* (Droop) Hibberd [32]. Different tolerance among species and tested salinities might explain this inconsistency [33].

Changes of PBP Contents under Hypersaline Stress

PBPs, hydrophilic pigment proteins with fluorescent characteristics, are ubiquitous in red algae and cyanobacteria. Many reports have indicated that PBPs possessed outstanding biological activities, such as antioxidant, hepato-protective and anti-inflammatory activities [34, 35]. Thus, PBPs were applied in the food, pharmaceutical, and cosmetic industries [36]. *P. haitanensis* is always considered as a healthy food

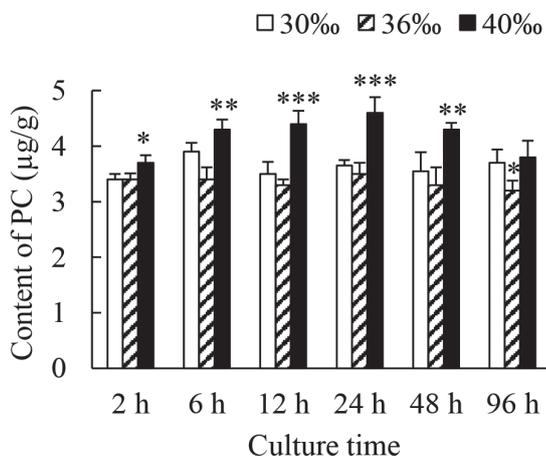


Fig. 4. Effects of concentrated seawater on phycocyanin (PC) content of *P. haitanensis*; data represent mean \pm SD. *significant difference from the control ($P<0.05$)

because of abundant contents of bioactive substances such as PBPs [37], which can be divided into three subclasses according to the maximal absorbance, including PE, PC and allophycocyanin [38]. PE and PC were the main components of PBPs extracted from *P. haitanensis*.

In the present study, PE content in 40‰ treatment increased after 2 h, reached the maximum value at 12 h and then decreased (Fig. 3). At 12 h, PE content in treatment with 40‰ was 11.83% and 14.78% higher than that in the 30‰ and 36‰ treatments, respectively ($P<0.05$). No significant difference in PE content was detected between treatments with 36‰ and 30‰ during the entire experiment.

Fig. 4 shows the changes of PC content under different salinities. There was no significant difference between treatments with 36‰ and 30‰ except at 96 h. In comparison, content of PC in 40‰ treatment was significantly higher than those in treatments with 30‰ and 36‰ from 2 h to 48 h ($P<0.05$).

Environmental factors have remarkable effects on PBP composition of *P. haitanensis*. Previous studies have shown that contents of PE and PC decreased with increased salinity in *Spirulina platensis* Geitler [33], possibly because the highly saline stress could inhibit their synthesis or promote their decomposition. Similarly, lower concentrations of salt (10 mM NaCl) resulted in an increase of PBP content in cyanobacterium, while further increases in the saline level resulted in a gradual decline of levels of PBPs and the inhibition of algal growth [39]. The negative effects of high salinity on PBP levels might be attributed to excess absorption of Na^+ , which induced the decomposition of PBPs [40, 41]. Differently, the present study indicated that treatment with 40‰ increased levels of both PC and PE, compared with the control. These results were similar to changes of carotenoid content, which might be related to the oxidative stress caused by hypersalinity. As previously reported, saline stress significantly induced the production of reactive oxygen species [42], and PBPs are effective antioxidants against reactive oxygen species [43].

Conclusions

Overall, the present study revealed that short-term exposure to hypersaline seawater did not significantly affect chlorophyll-a content, but induced oxidative stress in *P. haitanensis*. Pollution of concentrated seawater from desalination plants raised serious ecological risks to local environments.

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Conflict of Interest

The authors declare no conflict of interest.

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