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紫球藻多糖浓度增加对其他逆境适应性的改变

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摘 要:紫球藻(*Porphyridium* sp.)是一种海水单细胞红藻,是多种天然产物的来源。在其培养繁殖过程中,能够合成藻胆蛋白、高不饱和脂肪酸、硫酸酯多糖等生物活性物质,具有广阔的应用前景。盐胁迫会导致紫球藻的结合态多糖浓度的增加,由此可能产生相应的耐盐性的提高,并有利于对其他逆境的适应。该项研究采用外加紫球藻多糖或采用盐逆境诱导紫球藻多糖的积累,然后解除盐逆境的胁迫的方法获得多糖含量有显著提高的紫球藻试材,再给与其他的逆境:如光抑制,低温处理,并测定主要的生理生化参数。试验结果表明,外加 0.05%紫球藻多糖的藻细胞光合效率,在光抑制条件下,低于不加多糖的对照,但在低温(4 ℃)时,高于对照。外加多糖对 PSⅡ没有显著影响。紫球藻在去盐后的 48 h 恢复培养时间内,多糖的含量以及光抑制和低温条件下的光合效率都逐渐恢复到对照的水平。但是,去盐恢复培养的紫球藻 PSⅡ效率在光抑制条件下却高于加盐及未加盐的两种对照,显示盐诱导的紫球藻多糖可能增加了 PSⅡ对光抑制的忍耐程度。

关键词:紫球藻;盐胁迫;多糖;光抑制;低温

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Modified responses of *Porphyridium* sp. cells with an increased polysaccharide level to stress

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Abstract: In this study, the responses of *Porphyridium* sp. cells with salt-elevated bound polysaccharide to the stress of high proton flux density (HPFD) or chilling temperature (4 ℃) was studied in order to evaluate possible biological roles of the induced polysaccharides under environmental stresses. Addition of 0.05% polysaccharides to the *Porphyridium* sp. culture caused the photosynthetic activity decreased under HPFD but increased under chilling temperature (4 ℃) compared to control (culture without external addition of polysaccharide). The PSⅡ efficiency (Fv/Fm) decline was not significantly affected with the exogenous polysaccharide. Within 48 h after removal of salt from the cultures, both photosynthetic activity of the salt pre-treated cells measured under HPFD and chilling temperature (4 ℃) and bound polysaccharide content returned to the levels of un-treated cells. However, the PSⅡ efficiency of the recovery cultures measured under HPFD still kept at a higher level, especially after 6 hours, compared with control and salt treated cultures, suggesting that the high polysaccharide content induced by the salt stress may enhance PSⅡ tolerance to high photon flux density.

Key words: *Porphyridium* sp.; salt stress; polysaccharide; photoinhibition; chilling temperature

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1 Introduction

Porphyridium sp. cells are attractive for bitotechnological purpose because they synthesize unique cell wall sulfated polysacchrides, accessory photosynthetic pigments (phycobilins and carotenoids), and essential fatty acids(Arad, 2000, 1999; Berge' *et al.*, 2002).

The wall-like capsules of sulphated polysaccharides around *Porphyridium* sp. cells has a high molecular mass of about $5\sim7\times10^6$ Da and is composed of about 10 different sugars, among which xylose, galactose and glucose are the main components. It also contains some minor sugars, protein, sulfate, and glucyronic acid. The latter two confers a negative charge on the polysaccharide. These polysaccharides are water soluble, highly viscous, and resistant to biodegradation (Arad, 2000). Ramus (1972) found that the capsules are thin in the logarithmic phase and become thicker in the stationary phase. Accordingly to Ramus (1972) the rate of production of the polysaccharide is higher than its rate of solubilization during the stationary phase. Several functions have been suggested for the capsular polysaccharides. The polysaccharides have already been shown to exert biological activity; it has been found that polysaccharides of *Porphyridium* sp. are a good antioxidant (Ramus, 1972) and exhibit antiviral activity against Herpes simplex viruses (HSV 1, 2) (Huheihel *et al.*, 2002) and Varicella zoster virus (Huheihel & Arad, 2001). Furthermore, they may provide mechanical protection for the cell and form impenetrable barriers to gases and water and protect the cells from desiccation. They may also create a buffer layer around the cells to protect them from extreme environmental conditions (Arad, 2000). In addition, the polysaccharide may also serve as an ion exchanger or an ion reservoir (Mariani *et al.*, 1985; Ritchie & Larkam, 1982). Therefore, it is reasonable to speculate that increased sulfated polysaccharides of *Porphyridium* sp. may play a role in cell protection against environmental stresses.

Light is the ultimate source of energy that drives photosynthesis. However, excess light can also be harmful to the photosynthetic apparatus. The efficiency of photosynthesis can be significantly reduced when cells are exposed to high light intensity, particularly under adverse environmental conditions (Barber & Andersson, 1992; Kyle, 1987). Photoinhibition is defined as a reduction in photosynthetic efficiency due to damage caused by photon flux densities above that photosynthesis is saturated (Torzillo & Vonshak, 1998; Vonshak *et al.*, 1996). A general mechanism of photoinhibition can be summarized as follows: high irradiance causes a marked increase in the turnover rate of D_1 protein, which is linked to the repair PS Ⅱ after it has been damaged by photoinhibitory irradiation. Photoinhibition is controlled by the balance between damage to D1 protein and recovery of D1 protein (Anderson *et al.*, 1997; Baroli & Melis, 1996; Tyystjarvi & Aro, 1996). For both phytoplankton and terrestrial plants, photodamage to PS Ⅱ reaction centers can be detected with high sensitivity from changes in variable chlorophyll fluorescence (Baker *et al.*, 1994; Björkman, 1987a, b; Long *et al.*, 1994; Neale, 1987). The ease with which variable fluorescence measurements can be made has led to their common usage as a tool to diagnose for photoinhibition, although the consequence of PS Ⅱ inactivation on photosynthetic electron flow remains controversial (Anastasios, 1999).

The most striking characteristic of red algae is the presence of supplementary external antennae, the phycobilisomes (PBSs) in addition to all the functional elements needed for trapping and trasducing light energy into chemical energy as in higher plants (Schirmer *et al.*, 1985, 1986) Consequently, in red algae energy is transferred, via phycoerythrin-> phycocyanin-> allophycocyanin to PSII reaction centers through chromophores differently positioned along the transfer channel of PBS rods(Talarico, 1999).

In this study, we reported the effect of the increased content of bound polysaccharides of *Porphyridium* sp. cells on the photosynthetic activity as well as the modified responses of those *Porphyridium* sp. cells with increased polysaccharides to HPFD and chilling temperature (4 ℃).

2 Materials and Methods

2.1 Preparation of *Porphyridium* sp. cultures with salt elevated polysaccharides and their recovery cultivation after salt removal

Porphyridium sp. cultures all with 5 mg/L chlorophyll as an initial cell concentration in 100 mL of artificial seawater (Jones *et al.*, 1963) with or without 1.5 mol/L NaCl were kept in 250 mL shaken Erlenmeyer flasks and incubated at 24～25 ℃. Air enriched with 1% CO_2 was sparged into the incubators. A bank of fluorescent lamps was used as illumination source giving photon flux densities (PFD) in the range of 100～110 μmol m^{-2} s^{-1}. *Porphyridium* sp. cells grown in ASW+1.5 mol/L NaCl at optimal condition (25 ℃, light intensity of 100～110 μmol$m^{-2}$$s^{-1}$) were harvested at log phase (8～10 μg/mL of chlorophyll concentration), washed twice with ASW (50 mL×2), and then resuspended in fresh medium ASW. Cells were incubated in a shaker for 6, 12, 48 h respectively. Meanwhile non-salt treated cells were used as control. Then the cultures were exposed to HPFD (high photon flux density) or chilling temperature as described before (Xiaocan&Vonshak, 2003).

2.2 Variable chlorophyll fluorescence

PS Ⅱ activity was determined following the variable fluorescence parameters of Fo and Fm and calculating the ratio of (Fm-Fo)/Fm = Fv/Fm (Torzill & Vonshak, 1994). Algal samples were incubated in the dark for 5 min to allow for full dark adaptation. Measurements were performed using the Plant Efficiency Analyzer (Hansatech, UK).

2.3 Addition of the exogenous polysaccharide into non-stressed *Porphyridium* sp. cultures

When algal cells reached at log phase of growth were harvested and resuspended in fresh medium ASW with the addition of 20 mmol/L $NaHCO_3$ to final chlorophyll contents 5 μg/mL, 0.05% polysaccharides isolated from *Porphyridium* (kindly provided by Prof. Arad, Ben-Gurion University of Negev, Israel) were added. The same cultures without addition of the external polysaccharides used as control.

2.4 Measurement of bound polysaccharide concentrations

The bound and water soluble polysaccharide of *Porphyridium* sp. cells were separated and determined according to Dubois with the following modifications: the culture (5 mL) was centrifuged at 10 000 rpm for 10 min. The pellets were used to determine the bound polysaccharide content. The pellets was washed with 7 mL of phosphate buffer, pH7.5, and then centrifuged again. The supernatant was then discarded, and 10 mL of 1 N H_2SO_4 was added to the pellet. The mixture was kept in the boiling water for 1 h. Then, 25 mL of the mixture and 975 mL distilled water were taken, 1 mL 5% phenol and 5 mL 98% sulphuric acid were added and mixed well. After 30 min, the concentration of bound polysaccharide was determined OD at 490 nm. A calibration curve was constructed with a series of concentrations of D-galactose.

3 Results

3.1 Stress-modified responses of *Porphyridium* sp. cells with increased polysaccharide level

In order to evaluate the role of the elevated polysaccharide content in salt-stressed *Porphyridium* sp. cells two experimental approaches were employed. One was to induce high polysaccharide production by exposing the cells to stress followed by a recovery time that will restore the metabolic activity of the cells. Another was to add external polysaccharide to non-stressed *Porphyridium* sp. cells assuming that we can somehow produce a similar condition.

3.1.1 Non-stressed *Porphyridium* sp. culture with

the addition of external polysaccharides

Polysaccharides isolated from *Porphyridium* were added to a culture before exposing it to the HPFD stress. With the addition of 0.05% external polysaccharide to the *Porphyridium* sp. culture the decline in the photosynthetic activity measured under HPFD with time intervals proceeded faster and reached to a slightly lower level as compared to the culture without the addition of polysaccharide (Fig. 1:A). Interestingly the PSⅡ efficiency (Fv/Fm) decline was not significantly affected (Fig. 1: B).

However, when *Porphyridium* sp. culture with the addition of 0.05% exogenous polysaccharides was exposed to low temperature (4 ℃) the decline in oxygen evolution rate was slightly higher than the control (Fig. 2:A) whereas there was no significant difference in the PSⅡ efficiency (Fv/Fm)(Fig. 2:B) between the cells with or without the addition of external polysaccharide.

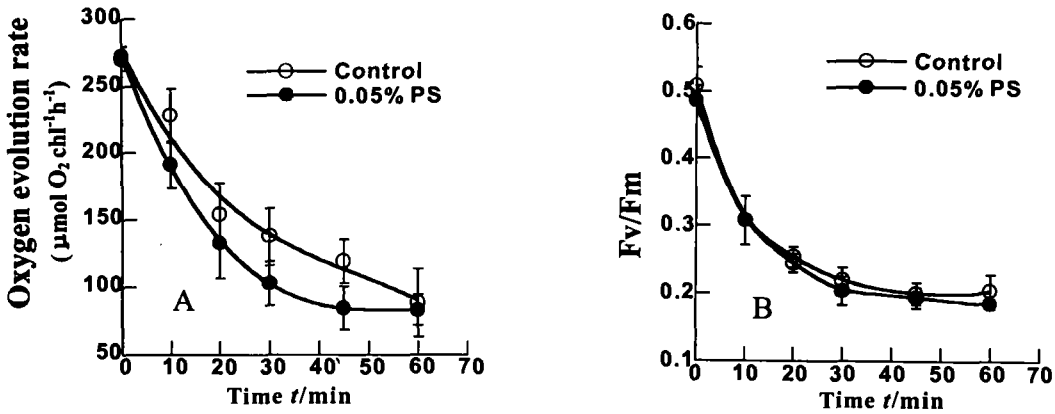

Fig. 1　External polysaccharide on the oxygen evolution rate

(A) and maximal photochemical efficiency of PSⅡ (Fv/Fm); (B) of *Porphyridium* sp. cells under HPFD.

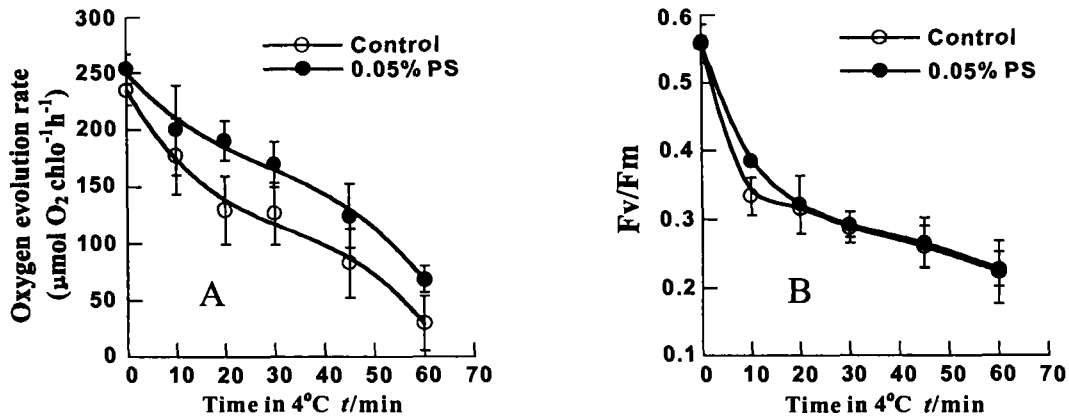

Fig. 2　External polysaccharide on the oxygen evolution rate

(A) and maximal photochemical efficiency of PSⅡ (Fv/Fm); (B) of *Porphyridium* sp. cells under chilling temperature (4 ℃).

3.1.2 Changes of polysaccharide content and photosynthetic parameters of *Porphyridium* sp. cells recovered from salt stress

In order to study the modified responses of salt treated cells with relatively high content bound polysaccharide to light and chilling temperature stresses, recovery experiments were designed as described in the part of materials and methods. Bound polysaccharide concentration, oxygen evolution rate and PSⅡ efficiency were determined.

Increased bound polysaccharide concentration and reduced photosynthetic activity and PSⅡ effi-

ciency of salt grown cultures recovered to the levels of control cultures within 48 h after removing salt stress. It should be noted that PSⅡ efficiency recovered faster than the oxygen evolution rate and polysaccharide content (Table 4).

3.1.3 Responses of salt pretreated *Porphyridium* sp. with increased endogenous polysaccharide to photoinhibition and chilling temperature (4 ℃)

Salinity stress causes *Porphyridium* sp. cells to accumulate relatively high levels of bound polysaccharides. After removal of the salt stress, the

Table 4 Time-course changes of bound polysaccharide and photosynthetic activity of salt adapted *Porphyridium* sp. cells after removing of salinity stress

Time (h)	Bound polysaccharide (μg/μg chl)	Oxygen evolution rate (μmol O_2 h^{-1} chl^{-1})	Fv/Fm
0	75±3 a*	177 a	0.45 b
6	45±2 b	200 b	0.52 a
12	40±2 b	200 b	0.53 a
48	25±3 c	254 c	0.54 a
Control	24±3 c	252 c	0.51 a

* the values with the same letter in the same column have no significant difference, p=0.05.

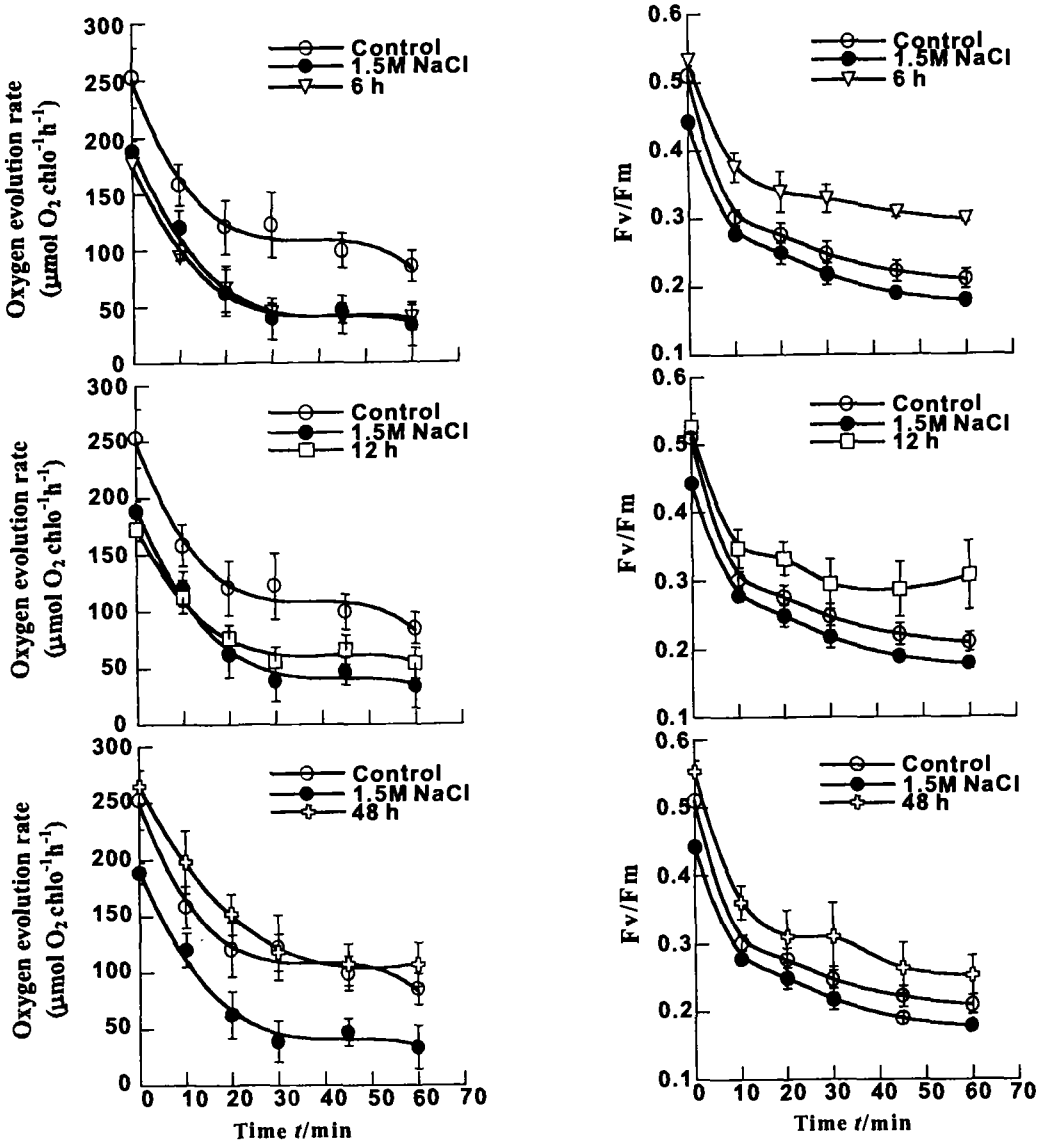

Fig. 3 Effect of HPFD on PSⅡ efficiency (Fv/Fm) and oxygen evolution rate of *Porphyridium* sp. cells with or without (as control) 1.5 M NaCl recovered for 6, 12, 48 h after salt was removed

decline of photosynthetic activity of the salt pretreated cells under photoinhibition stress gradually returned to the level of control cells. But the PS Ⅱ efficiency was kept at a higher level, especially after 6 h, compared with control and salt treated cultures, suggesting that the high polysaccharide content induced by the salt stress may enhance PS Ⅱ tolerance to high photon flux density (Fig. 3).

Similar to high light intensity, removal of salt stress causes the decline of photosynthetic activity as well as PS Ⅱ efficiency of the salt pretreated cells under chilling temperature stress to gradually (within 48 h) return to the level of control cells (Fig. 4).

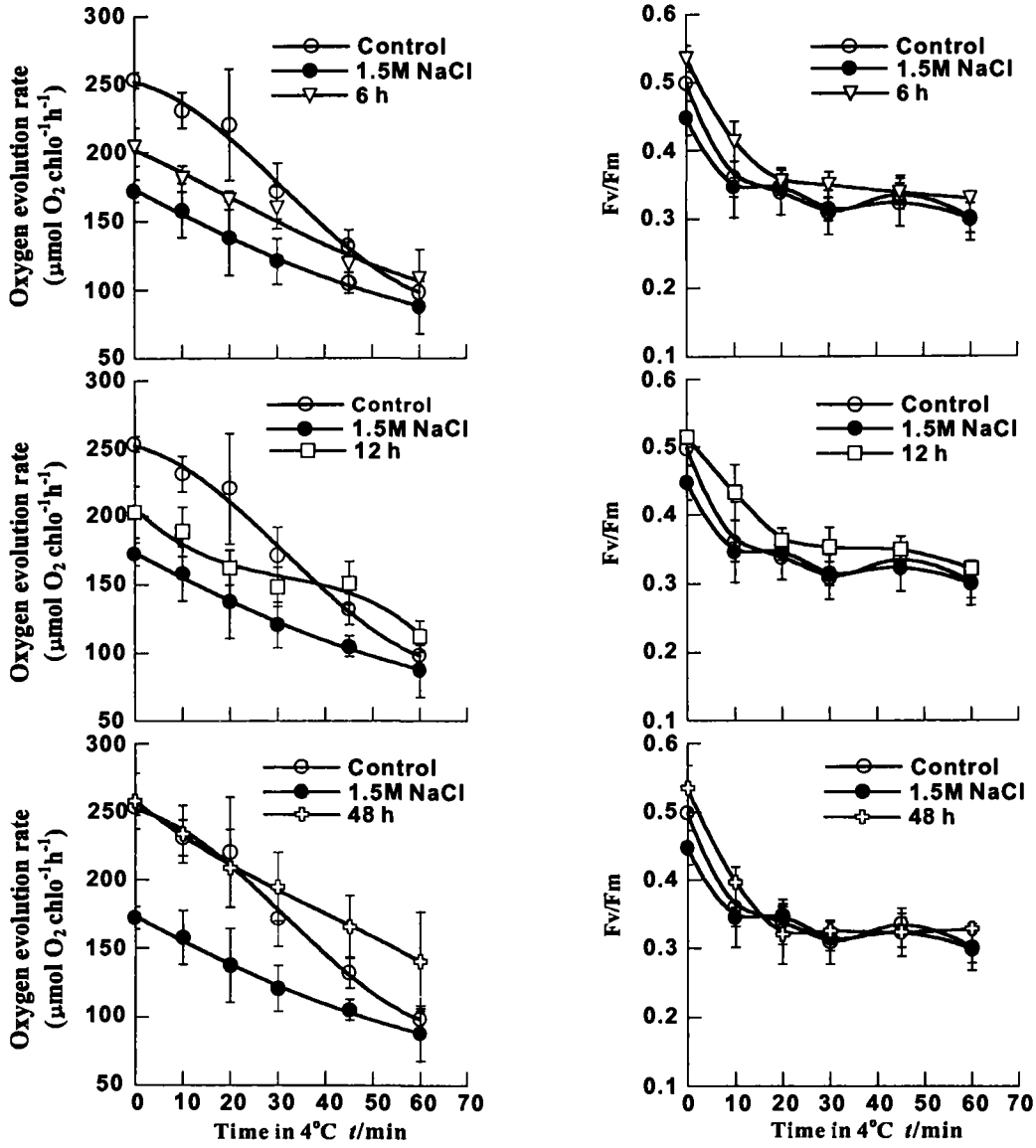

Fig. 4　Effect of 4 ℃ on the maximal PS Ⅱ photochemical efficiency (Fv/Fm) of *Porphyridium* sp. cultures grown with or without (as control) 1.5 M NaCl recovered for 6, 12, 48 h after salt was removed

4　Discussion

It was found in our experiment that saline stress induced accumulation of bound polysaccharide in *Porphyridium* sp. cells as occurred under nitrate and sulfate deficiencies(Arad, 1992). When saline stress was removed, the bound polysaccharide content started to decrease. Meanwhile, photosynthetic activity also gradually recovered. Our

results showed further that the elevated polysaccharide content induced by the salt stress seemed to enhance PSⅡ tolerance to high photon flux density. This indicated that the elevated polysaccharide contents observed in *Porphyridium* sp. stress cells modified responses of these cells to the stress. The polysaccharides exist in a typical gel form, which is a stable net structure formed by a solution of polymers. They may create a buffer layer around the cells to protect them from extreme environmental condition(Arad, 2000).

However, addition of 0.05% polysaccharide to cultures had no significant effect to prevent the decrease of photosynthetic rate and the maximal photochemical efficiency of PSⅡ (Fv/Fm) under HPFD (2 500 $\mu molm^{-2} s^{-1}$) or chilling temperature (4 ℃). Although in this experiment the preparation of polysaccharides added to the cultures was derived from *Porphyridium* sp. cells themselves, we still can be certain that the external polysaccharides evenly dissolved in the cultures could not function in the same way as do the salt-induced bound polysaccharides encapsulating the cells. Moreover, addition of external polysaccharide increases the viscosity of the medium, which might cause a decrease in the diffusion coefficients of nutrients.

Rotem *et al.* (1992) further proved that the polysaccharide inhibits the rate of carbon uptake by the cells and as a result photosynthesis of cells is also inhibited.

In conclusion, adaptation to increased concentration of NaCl modifies the response of *Porphyridium* sp. to HPFD and chilling temperature stresses. However, the relatively high level of bound polysaccharide of *Porphyridium* sp. cells induced by salt stress can somehow enhance PSⅡ tolerance to high photon flux density. We propose that the bound polysaccharide of *Porphyridium* sp. cells plays a role in the modification of cells in stress.

Further work may be required in order to better understand the correlation of the bound polysaccharide of *Porphyridium* sp. cells to a modification in PSⅡ.

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(上接第 154 页 Continue from page 154)

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