EFFECTS OF SALT AND IRRADIANCE STRESS ON PHOTOSYNTHETIC PIGMENTS AND PROTEINS IN *DUNALIELLA SALINA* TEODORESCO

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Abstract

The aim of this study was to examine the effects of salinity and light intensity on the chlorophylls, ß-carotene and protein contents in *Dunaliella salina* Teod. The algae were grown in inorganic medium containing 0, 0.9, 1.8 (control), 2.6 and 3.5 M NaCl under three illumination regimens [4500 (control), 9000 and 11000 Lux]. The results showed that most electrophoresis protein bands were separated in media with 1.8 and 2.6 M NaCl under continuous illumination with white fluorescent lamps at 4500 Lux and the most pigment contents were extracted in culture with concentration of 2.6 M NaCl under the same light intensity.

Introduction

Urmia Lake and saline evaporative little pools around it are natural habitats of *Dunaliella salina* in Iran. *D. salina* is a halotolerant, unicellular, motile, green alga with exceptional morphological and physiological properties [14]. This algae accumulates massive amounts of ß-carotene when exposed to high light intensities, nutrient depriviation and other stress conditions [2].

When *Dunaliella salina* is grown at a high irradiance a number of changes occur including a decrease in Chl/Cell and an increase in the Chl/Cell ratio [16]. Salt related difference in the abundance of a protein of approximately 150 KD is barely detectable in cells grown in 3.0 M salt and above [14]. A further consequence of salt stress in this organism includes the accumulation of ß-carotene [2,7]. These physiological responses appear to function as mechanisms designed to protect the organism against stress, in particular high-intensity irradiational [3] and either hypo- or hypersalinity [10].

The present work is based on the isolation of pigments (chlorophylls, ß-carotene) and proteins from *Dunaliella salina*. The results revealed that pigment and protein contents are varied in different treatment and related to growth conditions.

Keywords: ß-carotene; Chlorophylls, *Dunaliella salina* Teodoresco

Materials and Methods

Growth Conditions

Dunaliella salina was isolated from evaporative, hypersaline and shallow pools around Urmia Lake and pured by single cell isolation technique in ESM enrich-ment medium ($NaNO_3$ 120 $mg.L^{-1}$, KH_2PO_4 5 $mg.L^{-1}$, EDTA-Fe 0.26 $mg.L^{-1}$, EDTA-Mn 0.33 $mg.L^{-1}$, vitamin B1-HCl 0.1 $mg.L^{-1}$, vitamin B12 10 $\mu g.L^{-1}$, biotin 1 $\mu g.L^{-1}$, tris-buffer 1 $g.L^{-1}$) [12]. The clones were

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characterized by growth in modified Johnson's medium [7] at five different salinities (0, 0.9, 1.8 (control), 2.6 and 3.5 M NaCl).

The cultures were grown in 250 ml flasks with 250 ml medium and shaken gently. Three different light intensity regimes were used to test pigment contents:

(I) A growth chamber illuminated with halogen lamps providing light at 9000 Lux with 16 h light (HL).

(II) A growth chamber under continuous illumination with cool white fluorescent lamps providing light at 4500 Lux.

(III) A growth chamber under continuous illumination with cool white fluorescent lamps providing light at 11000 Lux (HL).

Two different facilities were used to extract proteins. The growth chamber II with 16 h light, the growth chamber III with the same photoperiod. The cultures were maintained at a constant growth temperature of 27°C±2, and the cells were harvested in their exponential growth phase.

Pigment Determination and Cell Counts

Chlorophylls and ß-carotene were extracted from an algal pellet with 100% acetone, diluted to 80% acetone with water (v/v) and assayed according to Arnon [5].

Isolation of ß-carotene globules was performed according to Ben-Amotz *et al.* [1]. Corrected spectra were recorded on a UV-02 spectrophotometer. Samples for growth measurements were taken 7 times during the 40-d incubation period, preserved with 4% formalin and counted using a haemocytometer.

Preparation of Samples for Extraction of Proteins and Protein Electrophoresis

Culture samples containing 2.5×10^6 cells were centrifuged at 2000 g for 10 min, and the pellet was immediately resuspended in 0.1 ml of H_2O, followed by the addition of 50 µl of 3×loading buffer [11] and incubation at 90°C for 2 min. Protein extracts were precipitated overnight by 80% acetone at -20°C. The pellet was resuspended in loading buffer and incubated for 2 min at 90°C [9]. Protein fraction was analyzed by Gelpolyacrylamid Electrophoresis with SDS-PAGE [13].

Statistical Analysis

Statistical analysis of the pigment and protein contents were carried out using the Bishop method [6].

Results and Discussion

Chlorophylls and Carotenes Content under Salt and Irradiance Stress Conditions

To test whether salinity and light intensity effect on chlorophylls and ß-carotene contents in *D. salina*, we used the different treatments according to "Materials and Methods" and the results are summarized in Figures 1 and 2. Our results showed that total chlorophyll and ß-carotene contents were varied in different salinities and light intensities, as well as we concluded that although chlorophyll content mostly decreased in each algal cell under stress conditions but in the same volume of the cell suspension, the growth rate, carotenogenesis and the other factors effect on chlorophyll content. To study the relationships between the growth rate and the photosynthetic pigments production in *D. salina*, we counted the cells each five days and growth curves for all cultures are shown in Figure 3, with actual growth rates.

Figure 1 showed that in the chamber I, chlorophyll content in *D. salina* were lower than other chambers.

Our results suggested the reduced chlorophyll content depended to high light intensity and photoperiodism. In this chamber, photoperiodism relatively reduced carotenogenesis (Fig. 2) and consequently chlorophyll molecule was not sufficiently protected against high light intensity.

Figure 2 showed that the maximum ß-carotene content varied in each chambers.

In the chamber I, ß-carotene and chlorophyll content in the culture with 1.8 and 3.5 M NaCl were higher than the other salinities (Figs. 1 and 2).

Figure 3a showed that in the Culture with 1.8 M NaCl, the growth rate was higher than the other cultures. Therefore, inhanced ß-carotene and chlorophyll contents related to the increase of growth rate.

On the other hand, in the culture with 3.5 M NaCl inhanced ß-carotene content was due to hyperosmotic shock (Fig. 2).

Recent Laboratory data [15] show that the carotenogenesis of *Dunaliella* and cell growth are two separate biological processes of different controls, and that carotenogenesis can be induced physiologically at any stage of the cell cycle. Based on this laboratory study [15], a large scale, two-phase *Dunaliella* cultivation system for production of ß-carotene was developed by Ben-Amotz [4]. Although this technique is useful but need to a long period and expensive. Therefore, we attempted to find an intermediate salinity and light intensity that optimized carotenogenesis and growth rate.

Thus our results can be explained in terms of a balance between the photosynthetic pigments production and growth rate. Figures 1 and 2 showed that in a constant light intensity, there were the different

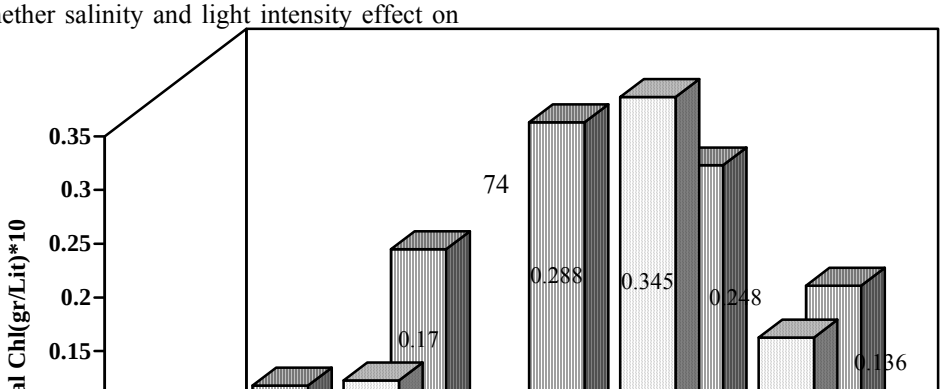

Figure 1. Changes of total Chl content in *D. salina* under different salinities (0, 0.9, 1.8, 2.6 and 3.5 M NaCl) and light intensities (S1, Chamber I; S2, Chamber II; S3, Chamber III).

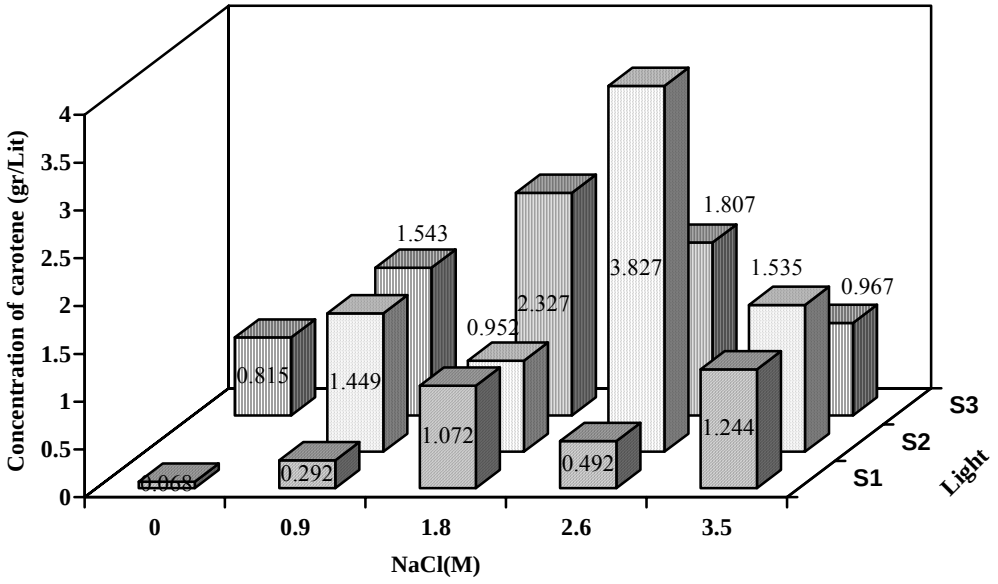

Figure 2. Changes of carotene content in *D. salina* under different salinities (0, 0.9, 1.8, 2.6 and 3.5 M NaCl) and light intensities (S1, Chamber I; S2, Chamber II; S3, Chamber III).

pigment contents in different salinities. As well as in a certain salinity, there were different productivities at different light intensities. The concentration of 2.6 M NaCl under 4500 Lux light intensity caused carotenogenesis but was not sufficiently adverse to prevent the cell growth and decrease chlorophyll content. Thus, carotenogenesis helps to survive chlorophyll molecule and elevate Chl contents in this condition. In this culture, there was a balance between the carotenogenesis and the growth rate and therefore the moat *ß*-carotene content extracted from this sample (Fig. 2).

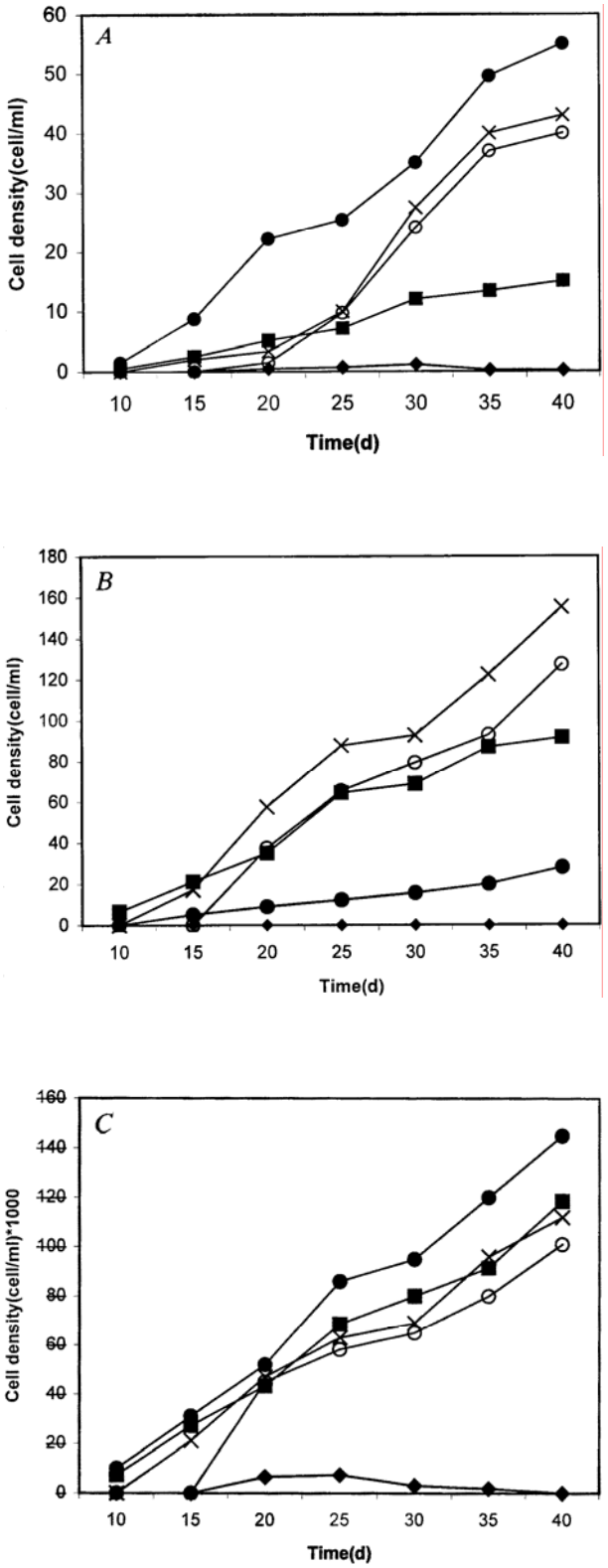

Figure 3. Growth curves of *D. salina* (A) Chamber I; (B) Chamber II; (C) Chamber III; in five salinities, 0(♦), 0.9(■), 1.8(●), 2.6(×), 3.5(○) M NaCl.

In the culture with 1.8 M NaCl at the same light intensity, the quantity of produced ß-carotene could not protect Chl molecule against continuous light intensity. Thus, the growth rate and consequently Chl content decrease in these cultures, whereas this salinity was optimum for producing the photosynthetic pigments in the chamber III (Figs. 1 and 2).

Grobbelaar [8] reported that there is no simple relation between nutrient stress, especially nitrogen, high irradiance and ß-carotene production.

Figure 3b showed that in the culture with 0 M NaCl under 4500 Lux light intensity, none of the cells grew. As well as, in the cultures with 0 M NaCl under 11000 and 9000 Lux light intensities, a long time lag in growth was evident at the start of the incubation period and growth ceased after 30 d (Figs. 3a, c). This case may be due to increase predators. In the other samples, growth continued until the end of the experimental period although eventually at a slower rate in cultures with 1.8 M NaCl under 4500 Lux light intensity (Fig. 3b). As before cited, in this culture Chl content were very lower than the other cultures. In summary, in this study we attempted to find the adverse salinity and light intensity to equilibrate the ß-carotene production and the growth rate. We believe that although *D. salina* has very tolerance to stress conditions but it should be considered that this alga need to optimum conditions for its growth and vital activities. Thus we applied the different salt and/or irradiance stress to find the most ß-carotene content and determined the growth rate and electrophoretic protein bands in this alga.

Proteins

A number of results obtained from electrophoresis of proteins revealed that in different treatments used in experiment the number of protein bands and their density were varied (Fig. 4).

The protein band 150 KD in 2.6 M NaCl under 4500 Lux light intensity was observed. This protein that induced under salinity stress is a glicoprotein in surface of membrane that is soluted in detergents and has a role in osmose adaptation of this alga under high rate of salinity in medium [14].

Probably this protein which was formed in 2.6 and 3.5 M NaCl under 11000 Lux light intensity, but in gel, it was not observed due to lower protein content.

A 75 KD protein was observed in all samples. It is possible that this protein was a constant protein occurring in all conditions. As well as a 174 KD protein, a heavy protein band, was observed in beginning of gel in all cultures. A 106 KD protein was observed in 1.8, 2.6 and 3.5 M NaCl under 11000 Lux light intensity and in 1.8 and 2.6 M NaCl at 4500 Lux light intensity. This band probably is not formed in hypoosmotic medium and can play a key role in stress tolerance.

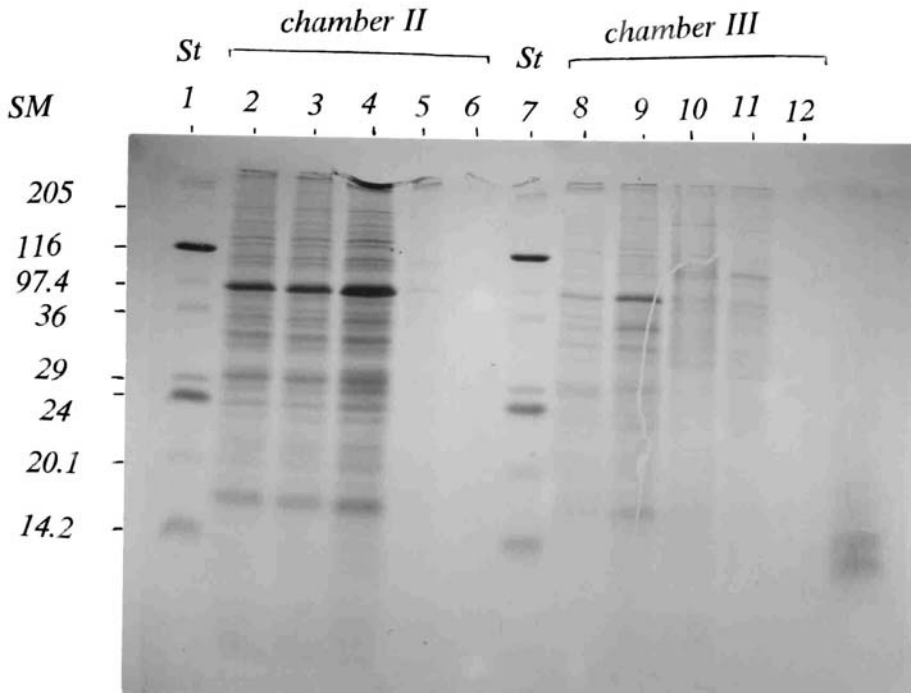

Figure 4. SDS-PAGE, analysis of proteins in *D. salina* cells grown under different treatments. Lanes 1 and 7 represent standard proteins (Albumin serum). Lanes 2 to 6 are about protein samples (2.6, 2.6 (repeat), 1.8, 0.9 & 0 M NaCl, respectively, 3.5 M NaCl not detectable). Lines 8 to 12, protein samples (3.5, 2.6, 1.8, 0.9 & 0 M NaCl, respectively). SM, position of molecular weight standards (KD); arrow, position of P150.

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