ELSEVIER

Comparative Biochemistry and Physiology Part A 120 (1998) 113–118

CBP

Review

# Adaptation of *Spirulina platensis* to salinity-stress

Ming-Tao Zeng [a,*], Avigad Vonshak [b]

[a] *Department of Molecular and Cell Biology, University of California at Berkeley, 401 Barker Hall, Berkeley CA 94720-3202, USA*
[b] *Micro-algal Biotechnology Laboratory, The Jacob Blaustein Institute For Desert Research, Ben-Gurion University of the Negev, Sede Boqer 84990, Israel*

Received 24 March 1997; received in revised form 2 August 1997; accepted 6 August 1997

**Abstract**

*Spirulina platensis* cells, growing photoautotrophically in optimal media under 100 or 200 $\mu$mol $m^{-2}$ $s^{-1}$ photon flux densities (PFD), were exposed to different concentrations of sodium chloride, up to 0.75 M. After an initial acclimation phase, in which growth rate, photosynthetic activity and endogenous respiration were inhibited, a new steady state was established and a recovery in the photosynthetic activity was observed. Furthermore an increase in the respiration rate took place, exceeding the initial rate of the non-stressed cells. Photosynthetic light-response curves (*P-I*) of stressed cells showed that the light compensation points were increased and light saturation values were decreased under the different salinity-stress conditions. Photoinhibition of photosynthesis was significantly enhanced under salinity-stress. Photosystem II activities of cells substantially decreased after a salt-shock. The results show that, cells grown in higher PFD are less tolerant to salinity-stress than those grown in lower PFD.


*Keywords:* Growth; Photoinhibition; Photosynthesis; Photosystem II activity; Respiration; Salinity-stress; *Spirulina*

## 1. Introduction

When tested, many cyanobacteria demonstrate a considerable tolerance to salinity-stress. The tolerance ability depends on several physiological mechanisms including the accumulation of inorganic or organic osmoregulators [8,13,14,23] and active extrusion of sodium from the cell interior [5,12]. The cyanobacterium *Spirulina platensis*, is commercially produced as a nutrient source in health food, feed and pharmaceutical industries especially in developing countries [15,24]. Salinity-stress is encountered as a common problem during its continued biomass production. Eventually, when the natural sunlight is high in outdoor *Spirulina* production, salinity-stress is usually accompanied by photoinhibition of photosynthesis [19]. It was indicated that *S. platensis* adapted to salinity-stress by increasing carbohydrates metabolism in cells [22,20,9]. Much evidence has verified that the reaction center cores both in photosystem II (PSII) and photosystem I (PSI) suffered structural damage including protein degradation during photoinhibition of photosynthesis [17,1,2,6]. It has been suggested that salinity-stress enhances photoinhibition of photosynthesis in green alga *Chlamydomonas reinbardtii* [11].

In this work, we comparatively studied the adaptation process of *S. platensis* to salinity-stress under different cultivation light conditions, by measuring the changes in growth, photosynthesis, respiration, light response curve of photosynthesis, photoinhibition of photosynthesis, and photosystem II activity.

* Corresponding author. Tel.: +1 510 6425915; fax: +1 510 6435035; e-mail: mtch@uclink4.berkeley.edu

1095-6433/98/$19.00 
*PII* S1095-6433(98)10018-1

## 2. Materials and methods

### 2.1. Growth

*Spirulina platensis*, was grown in Zarouk's medium in glass column (550 ml) bubbling with air-$CO_2$ mixture(99:1,v/v), and illuminated under 100 or 200 $\mu$mol $m^{-2}$ $s^{-1}$ PFD, respectively. Cultivation temperature was kept at 35°C constantly [18]. Growth was measured by following the increase in chlorophyll (chl) content or change in absorbance at 560 nm of the culture. Chl was assayed as described previously [3].

### 2.2. Salinity-stress

When cells were grown to the exponential phase after inoculation, different amounts of NaCl were added into the cultures. The controls were grown in Zarouk's media without additional salt supplement. The initial chl concentration of the culture was 1.5 $\mu$g $ml^{-1}$ before salt-shock.

### 2.3. Photosynthesis and respiration

Photosynthetic activity and endogenous respiration were measured by following the rate of $O_2$ evolution and consumption, using a Clark-type electrode (Yellow Springs, OH). Samples were harvested and re-suspended to final chl concentration of 2.1–2.2 $\mu$g $ml^{-1}$ with fresh media. Temperature was kept constant at 35°C during the measurement. Illumination was supplied by a slide projector lamp at 160 $\mu$mol $m^{-2}$ $s^{-1}$ PFD [20]. Samples were placed in dark for the endogenous respiration measurement.

### 2.4. Light-response curve of photosynthesis

Cells, grown in exponential phase at different salt concentration, were harvested and re-suspended in fresh media to a final chl concentration of 5 $\mu$g $ml^{-1}$. Photosynthetic rates were measured from dark to 5000 $\mu$mol $m^{-2}$ $s^{-1}$ PFD.

### 2.5. Photoinhibition of photosynthesis

Cells, grown in exponential phase at different salt concentration, were exposed to 3500 $\mu$mol $m^{-2}$ $s^{-1}$ PFD at 35°C for different lengths of time at chl concentration of 25 $\mu$g $ml^{-1}$. Then samples were diluted with fresh media to a chl concentration of 2.2 $\mu$g chl $ml^{-1}$. The rate of $O_2$ evolution was measured at 160 $\mu$mol $m^{-2}$ $s^{-1}$ PFD.

### 2.6. Photosystem II activity

Phosphate buffer (pH 7.0) containing 0.5 M mannitol and the same salt concentration as in stressed culture was prepared freshly. Cells were harvested, washed with the buffer and re-suspended in 0.1% *p*-benzoquinone for 3 min in dark. $O_2$ evolution was measured in 0.01% *p*-Benzoquinone solution under chl concentration of 4 $\mu$g $ml^{-1}$ at 350 $\mu$mol $m^{-2}$ $s^{-1}$ PFD.

### 2.7. Statistical analysis

Data are presented as means ± S.E.M. Statistical significance was determined using repeated measures analysis of variance (ANOVA). Significant differences were recognized for $P < 0.05$.

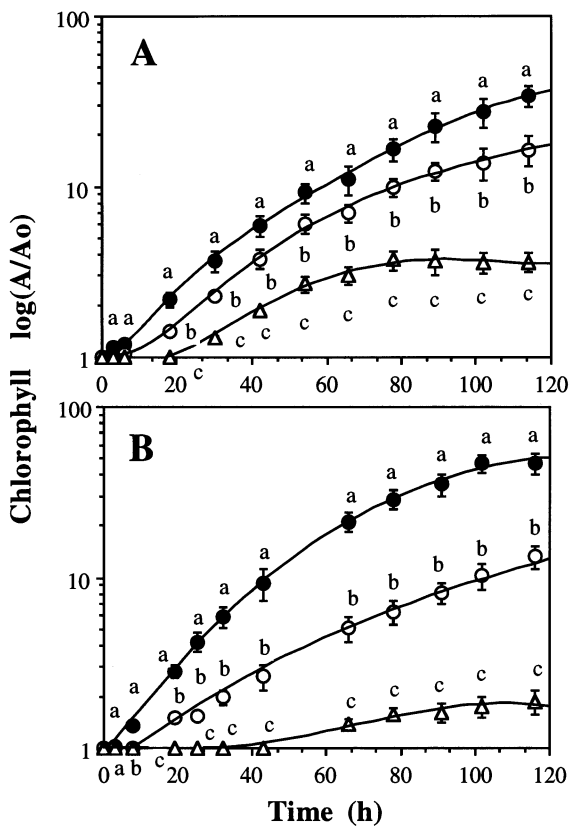


Fig. 1. Growth of *Spirulina platensis* after a salt-shock. Cells were grown under different photon flux densities: (A) 100 $\mu$mol $m^{-2}$ $s^{-1}$ (B) 200 $\mu$mol $m^{-2}$ $s^{-1}$. In exponential phase, cells were exposed to zero (control, ●), 0.5 M (○) and 0.75 M (△) NaCl. Growth was measured by following the changes in absorbance at 560 nm. $A_0$, absorbance at time zero; $A$, absorbance at any given time. Data are presented as means ± S.E.M. ($n = 5$). Significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$).

Table 1
The effect of salinity-stress to the growth of *Spirulina platensis* under different photon flux densities (PFD)

| NaCl (M) | Lag time (h) | | Specific growth rate ($\mu$) | |
|---|---|---|---|---|
| | PFD = 100 | PFD = 200 | PFD = 100 | PFD = 200 |
| 0 | $0 \pm 0.00^{a}$ | $0 \pm 0.00^{a}$ | $0.043 \pm 0.003^{a}$ | $0.060^{+}0.002^{a}$ |
| 0.5 | $6 \pm 0.41^{b}$ | $9 \pm 0.62^{b}$ | $0.037 \pm 0.005^{b}$ | $0.025^{+}0.002^{b}$ |
| 0.75 | $18 \pm 0.73^{c}$ | $33 \pm 1.20^{c}$ | $0.026 \pm 0.002^{c}$ | $0.013^{+}0.001^{c}$ |

The unit of PFD is $\mu$mol $m^{-2}$ $s^{-1}$.
Data are presented as means $\pm$ S.E.M. ($n = 5$).
[a,b,c] Means with different letters in a column differ significantly at least at $P < 0.05$.

## 3. Results

The growth rate of *S. platensis* was significantly inhibited immediately after cells were exposed to a salt-shock. A lag phase was observed before a new steady state of growth was established (Fig. 1). The specific growth rate $\mu$ at all elevated NaCl concentrations was lower than the control (Table 1). Cells grown in lower PFD (100 $\mu$mol $m^{-2}$ $s^{-1}$) can adapt to salinity-stress faster than cells grown in higher PFD (200 $\mu$mol $m^{-2}$ $s^{-1}$). The algae failed to adapt to higher salinity environment as more than 1.0 M NaCl either under lower PFD (100 $\mu$mol $m^{-2}$ $s^{-1}$) or under higher PFD (200 $\mu$mol $m^{-2}$ $s^{-1}$) (not shown).

Photosynthesis of *S. platensis* was inhibited in the initial lag phase after a salt-shock (Fig. 2). With the establishment of a new steady state, photosynthetic activity recovered to 95% of the initial activity under relatively lower PFD (100 $\mu$mol $m^{-2}$ $s^{-1}$) and lower salinity-stress (0.5 M NaCl) conditions, while photosynthetic activity could recovered partially (about 50% of the initial activity) under relative lower PFD (100 $\mu$mol $m^{-2}$ $s^{-1}$) but higher salinity-stress (0.75 M NaCl) conditions (Fig. 2A); Photosynthetic activity had less recovery under relatively higher PFD (200 $\mu$mol $m^{-2}$ $s^{-1}$) at both levels of salinity-stress (0.5 M or 0.75 M NaCl) (Fig. 2B).

In contrast to the photosynthetic activity, endogenous respiration of *S. platensis*, after a salt-shock, was inhibited in the early lag phase. Then a significant increase, above the control level in the respiration rate, took place (Fig. 3). This observation is in agreement with our previous studies [21,20].

The relationship between light irradiance and photosynthetic rate can be described as $P$ versus $I$ curve (light-response curve of photosynthesis) [7]. $P$ is defined as photosynthetic rate and $I$ is defined as light irradiance (or photon flux density). Fig. 4 showed that, with the increase of salinity-stress, light compensation point and light saturation point ($P_{max}$) of the cells declined while the curvature of the light response curve abased. The light response curves were bent gradually while the salt concentration increased. The effect of salinity-stress was stronger when cells were grown under relatively higher PFD (200 $\mu$mol $m^{-2}$ $s^{-1}$) than under lower PFD(100 $\mu$mol $m^{-2}$ $s^{-1}$) (Table 2).

Photoinhibition is considered to be a decline in the photosynthetic activity as a result of an exposure to high PFD [6,11]. The degree of inhibition is highly dependent on the growth conditions of the organism. Fig. 5 showed that salinity-stress enhanced photoinhibition in *S. platensis*. With the increase of salinity-stress, stressed cells had lower photosynthetic activity than the controls after an exposure to extremely high PFD (3500 $\mu$mol $m^{-2}$ $s^{-1}$). It also demonstrated that, stressed cells, grown under higher PFD (200 $\mu$mol $m^{-2}$ $s^{-1}$), showed lower photosynthetic activity after photoinhibition than that grown in lower PFD (100 $\mu$mol $m^{-2}$ per s).

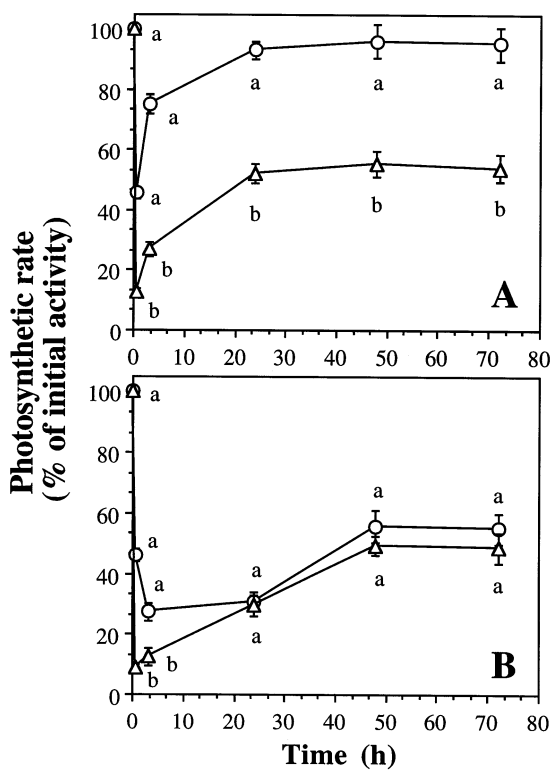


Fig. 2. Recovery of photosynthesis in *Spirulina platensis* after a salt-shock. Cells were grown under different photon flux densities: (A) 100 $\mu$mol $m^{-2}$ $s^{-1}$ (B) 200 $\mu$mol $m^{-2}$ $s^{-1}$. In exponential phase, cells were exposed to 0.5 M (○) and 0.75 M (△) NaCl. The initial photosynthetic activities were set as 100% activities before salt-shock. Data are presented as means $\pm$ S.E.M. ($n = 3$). Significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$).

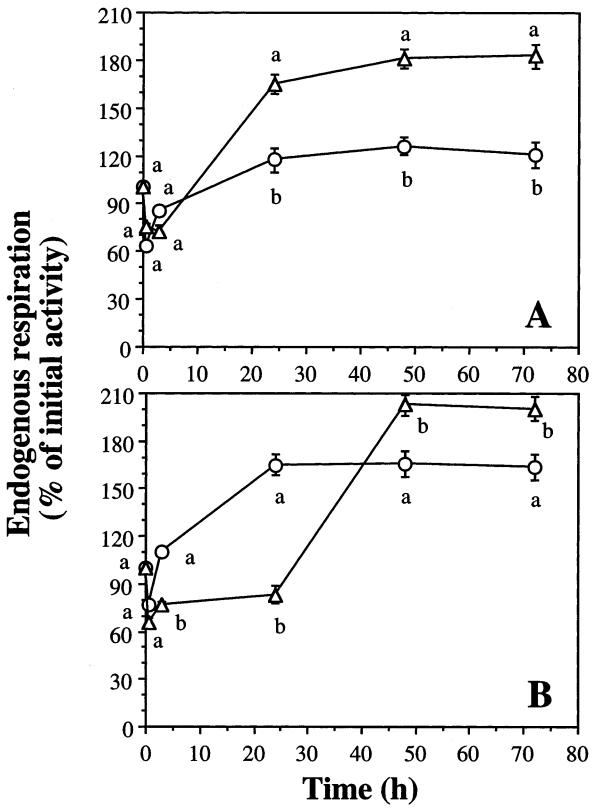


Fig. 3. Endogenous respiration of *Spirulina platensis* after a salt-shock. Cells were grown under different photon flux densities: (A) 100 $\mu$mol $m^{-2}$ $s^{-1}$ (B) 200 $\mu$mol $m^{-2}$ $s^{-1}$. In exponential phase, cells were exposed to 0.5 M (○) and 0.75 M (▲) NaCl. The initial endogenous respiration was set as 100% before salt-shock. Data are presented as means ± S.E.M. ($n = 3$). Significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$).

Fig. 6 showed the changes in photosystem II (PSII) activity of cells under salinity-stress. It indicated that PSII activity of the cells decreased in the lag phase after a salt-shock. When salt concentration in the media was increased, PSII activity of the cells declined. PSII activity of stressed cells, grown under higher PFD (200 $\mu$mol $m^{-2}$ $s^{-1}$), decreased more than that grown under lower PFD (100 $\mu$mol $m^{-2}$ $s^{-1}$). When salt concentration in the media was lower (0.5 M), a partial recovery of PSII activity was observed after a new steady state was established.

## 4. Discussion

In a previous study we demonstrated that the response of *Spirulina platensis* to salinity-stress consisted of a relatively short shock stage, followed by an adaptation process [20]. The data presented here show that the light at which algae are grown affects the ability of cells to response and adapt to salinity-stress. In general, our results illustrated that, under higher cultivation PFD, the shock stage is prolonged and the adaptation ability of cells to salinity-stress is declined (Fig. 1).

Enhanced respiration, following salinity-stress, was also reported for various cyanobacteria [4,10,5]. The results showed that the process of the adaptation to salinity-stress was an energy consuming process. Molitor et al. [10] found that the increase in respiration in *Anacystis nidulans* grown in NaCl-enriched medium was due to the enhanced activity of the plasma membrane cytochrome oxidase. It was suggested that the entry of $Na^+$ and $Cl^-$ triggered enhanced or activated respiration electron transport, involving constitutive respiratory system in cells [5].

Sammuelsson et al. [16] showed that higher light acclimated cyanobacterium *A. nidulans* had a higher rate of recovery after photoinhibition than that lower light acclimated *A. nidulans*. Our results presented here further support this observation (see controls in Fig. 5). But the susceptibility of photosynthesis to photoinhibition was changed to the opposite way under salinity-stress condition. Higher light grown cells show lower

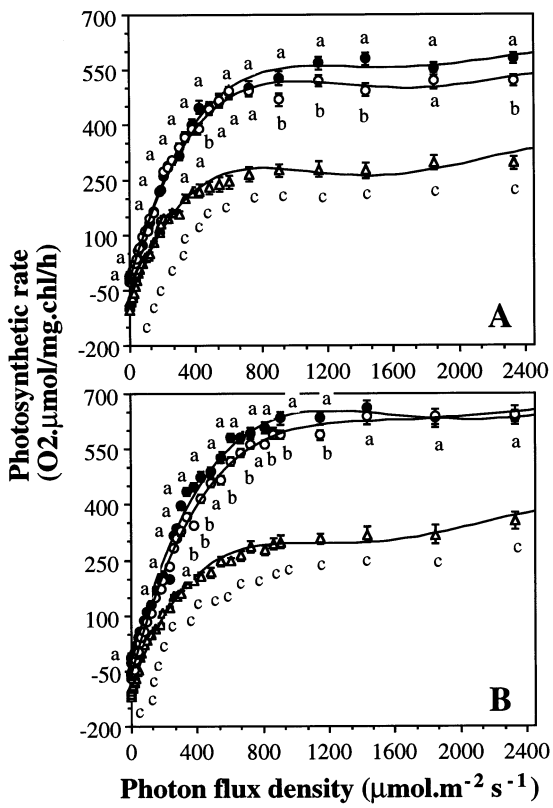


Fig. 4. Light response curve of *Spirulina platensis* under salinity-stress. Cells were grown in the media, containing zero (control, ●), 0.5 M (○) and 0.75 M (△) NaCl. The growth photon flux densities were: (A) 100 $\mu$mol $m^{-2}$ $s^{-1}$ (B) 200 $\mu$mol $m^{-2}$ $s^{-1}$. In exponential phase, photosynthetic rates were measured from dark to 5000 $\mu$mol $m^{-2}$ $s^{-1}$ pFD. Data are presented as mean ± S.E.M. ($n = 3$). Significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$).

Table 2
The effect of salinity-stress to the parameters of photosynthetic light-response curve of *Spirulina platensis* under different photon flux densities (PFD)

| Parameters | PFD = 100 $\mu$mol $m^{-2}$ $s^{-1}$ | | | PFD = 200 $\mu$mol $m^{-2}$ $s^{-1}$ | | |
|---|---|---|---|---|---|---|
| | Control | 0.5 M NaCl | 0.75 M NaCl | Control | 0.5 M NaCl | 0.75 M NaCl |
| $P_{max}$ | $570 \pm 23.1^a$ | $516 \pm 17.0^b$ | $294 \pm 19.7^c$ | $652 \pm 16.9^a$ | $540 \pm 24.0^b$ | $340 \pm 11.1^c$ |
| Respiration | $-22 \pm 1.9^a$ | $-26 \pm 1.6^a$ | $-82 \pm 2.1^c$ | $-24 \pm 2.0^a$ | $-60 \pm 3.2^b$ | $-102 \pm 9.1^d$ |
| Compensation | $16 \pm 0.6^a$ | $18 \pm 0.9^a$ | $59 \pm 3.0^c$ | $16 \pm 0.1^a$ | $37 \pm 2.0^b$ | $64 \pm 4.5^c$ |

The unit for $P_{max}$, respiration and compensation is $\mu$mol O2 $mg^{-1}$ chl $h^{-1}$.
Data are presented as means $\pm$ S.E.M. ($n = 5$).
[a,b,c,d] Means with different letters in a row differ significantly at least at $P < 0.05$.

capacity of recovery in the photosynthetic activity after photoinhibition than lower light grown cells. This may be a result of the fact that stressed cells have a lower protein synthesis capacity and thus a slower repair mechanism. Our studies also indicate that stressed cells show lower PSII activity than non stressed cells (Fig. 6). The reason for the declination of PSII activity of cells under salinity-stress remains open. We may think of a stress-induced damage or inactivation of PSII reaction center as it is in the case of photoinhibition of photosynthesis [2,6]. As refer to our observation, that higher light grown cells show lower photosynthetic activity after photoinhibition under salinity-stress than

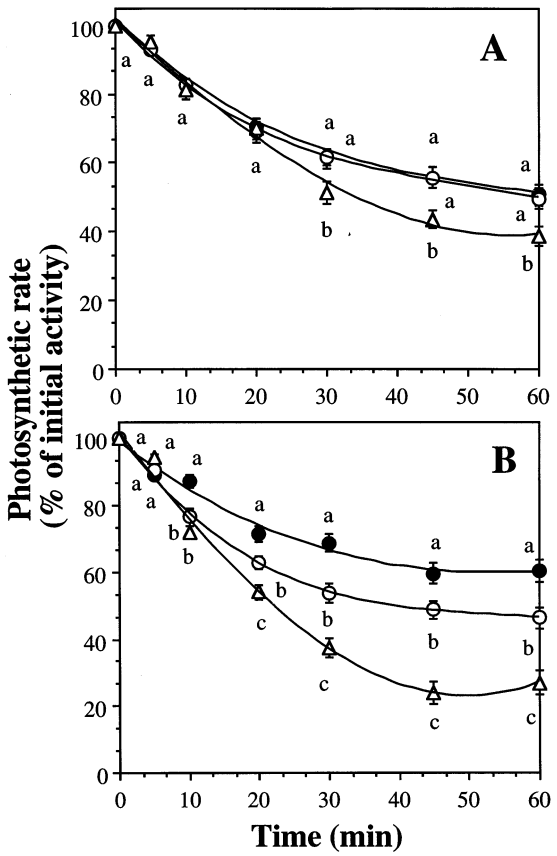


Fig. 5. Photoinhibition of *Spirulina platensis* under salinity-stress. Cells were grown in the media, containing zero (control, ●), 0.5 M (○) and 0.75 M (△) NaCl. The growth photon flux densities were: (A) 100 $\mu$mol $m^{-2}$ $s^{-1}$ (B) 200 $\mu$mol $m^{-2}$ $s^{-1}$. In exponential phase, photosynthetic rates were measured after cells were exposed to 3500 $\mu$mol $m^{-2}$ $s^{-1}$ PFD for different lengths of time. The initial photosynthetic activities were set as 100% activities before cells were exposed to 3500 $\mu$mol $m^{-2}$ $s^{-1}$ PFD. Data are presented as mean $\pm$ S.E.M. ($n = 3$). Significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$).

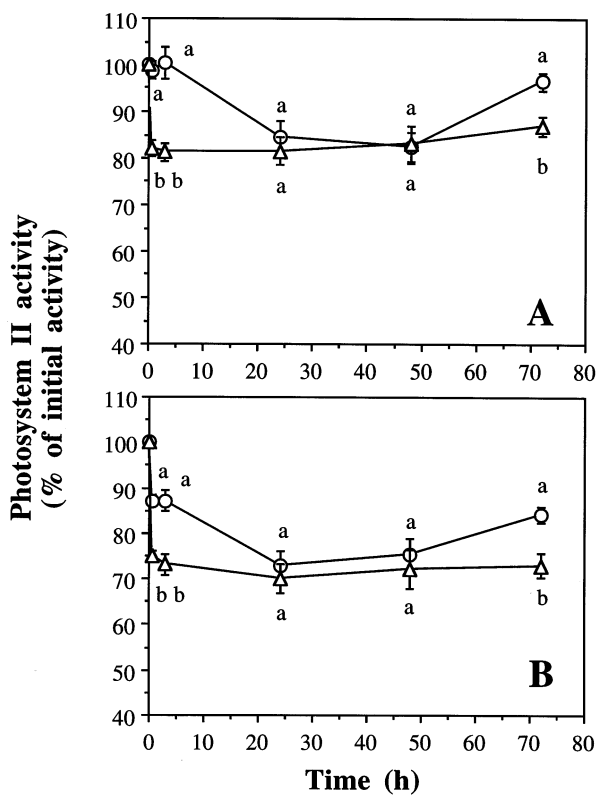


Fig. 6. Effect of salinity-stress to photosystem II activity of *Spirulina platensis*. Cells were grown under different photon flux densities: (A) 100 $\mu$mol $m^{-2}$ $s^{-1}$ (B) 200 $\mu$mol $m^{-2}$ $s^{-1}$. In exponential phase, cells were exposed to 0.5 M (○) and 0.75 M (△) NaCl. The initial photosystem II activities were set as 100% activities before salt-shock. Data are presented as mean $\pm$ S.E.M. ($n = 3$). Significant interaction at each time period is shown by letters next to symbols, and means with different letters are significantly different ($P < 0.05$).

lower light grown cells, we may suggest that salinity-stress enhances photoinhibition of photosynthesis through a direct effects on PSII reaction center.

## Acknowledgements

This work was partially supported by the Blaustein International Center for Desert Studies in the Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Israel.

## References

[1] Baba K, Itoh S, Hoshina S. Degradation of photosystem I reaction center proteins during photoinhibition in vitro. In: Mathis P, editor. Photosynthesis: from Light to Biosphere, vol. II. Dordrecht: Kluwer, 1995:179–82.

[2] Barber J. Molecular basis of photoinhibition. In: Mathis P, editor. Photosynthesis: from Light to Biosphere, vol. II. Dordrecht: Kluwer, 1995:159–64.

[3] Bennet A, Bogorad L. Complementary chromatic adaptation in a filamentous blue-green alga. J Cell Biol 1973;58:419–35.

[4] Fry IV, Huflejt M, Erber WWA, Peschek GA, Packer L. The role of respiration during adaptation of the freshwater cyanobacterium *Synechoccus 6310* to salinity. Arch Biochem Biophys 1986;244:686–91.

[5] Gabbay-Azaria R, Schofeld M, Tel-Or S, Messinger R, Tel-Or E. Respiratory activity in the marine cyanobacterium *Spirulina subsalsa* and its role in salt tolerance. Arch Microbiol 1992;157:183–90.

[6] Komenda J, Masojidek J. Functional and structural changes of the photosystem II complex induced by high irradiance in cyanobacterial cells. Eur J Biochem 1995;233:677–82.

[7] Lobban, CS, Harrison, PJ, Duncan, MJ. The Physiological Ecology of Seaweeds, Cambridge:Cambridge University Press; 1985:24–25.

[8] Mackay MA, Norton RS, Borowitzka LJ. Organic osmoregulatory solutes in cyanobacteria. J Gen Microbiol 1984;130:2177–91.

[9] Martel A, Yu S, Garcia-Reina G, Lindblad P, Pedersen M. Osmoic-adjustement in the cyanobacterium *Spirulina platensis*: presence of an a-glucosidase. Plant Physiol Biochem 1992;30(5):69–74.

[10] Molitor V, Erber WWA, Peschek GA. Increased levels of cytochrome oxidase and sodium-proton antiporter in the plasma membrane of *Anacystis nidulans* after growth in sodium enriched media. FEBS Lett 1986;204:251–6.

[11] Neale PJ, Melis A. Salinity-stress enhances photoinhibition of photosynthesis in *Chlamydomonas reinbardtii*. J Plant Physiol 1989;134:619–22.

[12] Peschek GA, Obinger C, Fromwald S, Bergman B. Correlation between immuno-gold lables and activities of cytochrome-c oxidase($aa_3$-type) in membranes of salt stressed cyanobacteria. FEMS Microbiol Lett 1994;124:431–8.

[13] Reed RH, Borowitzka LJ, Mackay MA, Chudek JA, Foster R, Warr SRC, Moore DJ, Stewart WDP. Organic solute accumulation in osmotically-stressed cyanobacteria. FEBS Microbiol Rev 1986;39:51–6.

[14] Reed RH, Richardson DL, Warr SRC, Stewart WDP. Carbohydrate accumulation and osmotic stress in cyanobacteria. J Gen Microbiol 1984;130:1–4.

[15] Richmond A. Spirulina. In: Borwitzka A, editor. Micro-algal Biotechnology. Cambridge: Cambridge University Press, 1988:85–121.

[16] Samuelsson G, Lonneborg A, Gustafsson P, Oquist G. The susceptibility of photosynthesis to photoinhibition and the capacity of recovery in high and low light grown cyanobacteria, *Anacystis nidulans*. Plant Physiol 1987;83:438–41.

[17] Shipton CA, Barber J. In vivo and in vitro photoinhibition reactions generate similar degradation fragments of D1 and D2 photosystem-II reaction center proteins. Eur J Biochem 1994;220(3):801–8.

[18] Vonshak A. Laboratory techniques for the culturing of microalgae. In: Richmond A, editor. Handbook for Microalgal Mass Culture. Boca Raton FL: CRC press, 1986:117–45.

[19] Vonshak A, Guy R. Photoadaptation, photoinhibition and productivity in the blue-green alga, *Spirulina platensis* grown outdoors. Plant Cell Environ 1992;15:613–6.

[20] Vonshak A, Guy R, Guy M. The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. Arch Microbiol 1988;150:417–20.

[21] Vonshak A, Richmond A. Photosynthetic and respiratory activity in *Anacystis nidulans* adapted to osmotic stress. Plant Physiol 1981;68:504–5.

[22] Warr SRC, Reed RH, Chudek JA, Foster R, Stewart WDP. Osmotic adjustment in *Spirulina platensis*. Planta 1985;163:424–9.

[23] Warr SRC, Reed RH, Stewart WDP. The compatibility of osmotica in cyanobacteria. Plant Cell Environ 1988;11:137–42.

[24] Zeng MT, Hu HJ. Studies on the cultivation of *Spirulina platensis* in alkaline water of Lake Chenghai. J Wuhan Bot Res 1992;10(1):73–82.