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Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress

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Abstract

The response of *Spirulina platensis* cells to salinity stress was studied. Once adapted to the higher osmoticum, photosynthetic parameters such as the maximum rate of photosynthesis under saturating irradiance (P_{max}) and the initial slope of the P–I curve (α) are reduced by 15% and 25% in 0.5 M NaCl grown cells, respectively. Salt-adapted cells have a modified biochemical composition; reduced protein and chlorophyll content, and an increased level of carbohydrates. The reduction in the photosynthetic capacity of the salt-adapted *Spirulina* cells reflects a lower ability to utilize light energy and results in an increase in the susceptibility of the stressed cells to photoinhibition. This conclusion is supported by the finding that cultures exposed to salt stress show not only a decrease in growth rate (μ), but lose the ability to respond to increased irradiance with an increase in growth. The use of variable fluorescence as a fast and reliable measurement to follow the changes in PSII of salt-stresses *Spirulina* cells enables following the early events of salinity shock. It indicates that as soon as the cells are exposed to salt, a protection mechanism is induced. This mechanism does not require any protein synthesis and may take place even in the dark, though at somewhat reduced effectiveness. The significance of the result in providing a better understanding of the interaction between two environmental stresses – light and salinity – and their application in the outdoor mass cultivation of *Spirulina* are discussed.

Introduction

Photosynthetic micro-organisms are exposed in nature to a continuous fluctuation in growth conditions such as temperature, light intensity and an increase in the osmoticum due to evaporation. The effect of each of those parameters was studied separately under laboratory conditions (Lewin, 1962). In many cases interaction between these environmental parameters plays a major role in the ability of the cells to withstand and adapt to the environmental stress. Cyanobacteria were isolated from a wide range of aquatic environments ranging from freshwater to hypersaline habitats. Many species of this group easily adapt to great fluctuations in salt concentration (Borowitzka, 1986). Significant progress has been made in understanding the physiological strategies for salt adaptation, including avoidance of internal toxic levels of inorganic ions and the synthesis and accumulation of osmoprotective compounds (Blumwald et al., 1983; Reed & Stewart, 1985).

Spirulina platensis a filamentous cyanobacteria, has been isolated from a wide range of habitats which differ in their water quality from low to high ionic strength in salty and alkaline waters (Ciferri, 1983). It has been reported that in *S. platensis* α-glucoside accumulates as an osmoregulant in salt-stressed cells (Martel et al., 1992). In a previous work, Vonshak et al. (1988) demonstrated that *S. platensis* is capable of adapting to high NaCl concentrations. This adaptation is associated with an increase in respiratory

activity of the cells. Such an increase associated with salt tolerance has also observed in a marine *Spirulina* strain (Gabbay-Azaria et al., 1992). The effect of salinity stress in cyanobacteria was studied, using cells in the steady state adapted stage, by Hagemann et al. (1989a), as well as its effect on enzyme activity (Hagemann et al., 1989b) and protein synthesis (Hagemann et al., 1990). Less information is available on the initial response of the cells to salt stress and the effect of light on the initial steps of adaptation as related to the photosynthetic apparatus.

Photoinhibition is defined as a reduction in photosynthetic efficiency due to damage caused by photon flux densities above that required to saturate photosynthesis. The phenomenon of photoinhibition has been studied intensively, it is well documented in algae and higher plants (Kyle & Ohad, 1986; Öquist, 1987). Recent studies have suggested that this reduction in activity represents a regulatory response rather than real damage (Baker & Bowyer, 1994). As already pointed out, the level of photoinhibition correlates highly with other environmental stress such as salinity and temperature (Powles, 1984). Neale and Melis (1989) demonstrated changes in photosynthetic activity and the response to high photon flux density in an eukaryotic green alga *Chlamydomonas reinhardtii* subjected to salinity stress.

In recent years considerable interest has been expressed in outdoor cultivation of *Spirulina* for commercial biomass production (Vonshak, 1990). In cultures grown outdoors in open ponds under arid and semiarid conditions, daily evaporation of 1–2 cm occurs leading to a progressive increase in the salt concentration (Vonshak, 1987). Thus *Spirulina* cells cultivated outdoors are exposed to a continuous fluctuation in light availability and salinity. In a previous study (Vonshak et al., 1996), we have pointed out the importance of light acclimation and photoinhibition in mass culture of *Spirulina*. In the current study, we are trying to provide a better understanding of the interaction between light and salinity that may help to optimize the productivity of algal cultures outdoors.

Materials and methods

Organism and growth conditions

Spirulina platensis strain M-2 of the Culture Collection of the Centro di Studio dei Microrganismi Autotrofi of Florence was used. It was grown in Zarouk's medium, containing 200 mM sodium bicarbonate (Vonshak, 1986). The algae were grown at 35°C and kept in suspension by bubbling through CO_2-enriched (1%) air. Illumination was provided by cool-white fluorescent lamps of 60 W, and light intensity was modified by changing the distance of the light source from the algal culture.

NaCl stress

Exponentially growing cells were harvested and resuspended in fresh medium containing 0.5 M or 0.75 M NaCl as indicated (exclusive of Na already present in the medium) to a final chlorophyll *a* concentration of 1 μg ml^{-1}. Those two concentrations were chosen after preliminary studies that demonstrated that cells can still recover and grow after a period of adaptation. Growth was measured by following the increase in chlorophyll *a* content or the change in absorbency at 560 nm of the culture.

Oxygen evolution

Samples were diluted with fresh Zarouk's medium to a final concentration of 2.5 μg chl ml^{-1}. Their photosynthetic activity was assayed by measuring the rate of O_2 evolution using a Clarke-type O_2 electrode in a double jacket thermoregulated glass vessel. The temperature (35°C) was kept constant, and illumination was provided by a side projector lamp at PFD of 160 μmol m^{-2} s^{-1}.

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 (Torzillo & Vonshak, 1995). Algal samples were incubated in the dark for 10 min to allow for full dark adaptation. Measurements were performed using the Plant Efficiency Analyzer (Hansatech UK).

Photoinhibition

Algal cells in the log phase of growth were harvested and resuspended in fresh medium to a final chlorophyll *a* concentration of 25 μg ml^{-1}. The cultures were placed in a thermoregulated, double-jacket, cylindrical glass vessel and then illuminated by a high intensity halogen lamp (OSRAM 220–230 V, 1000 W) at 3500 μmol m^{-2} s^{-1}. The PAR photon flux density was measured by Li-180 photometer and a quantum sensor. At time intervals, samples were drawn out, diluted with fresh medium to 2.5 μg chl ml^{-1}, and

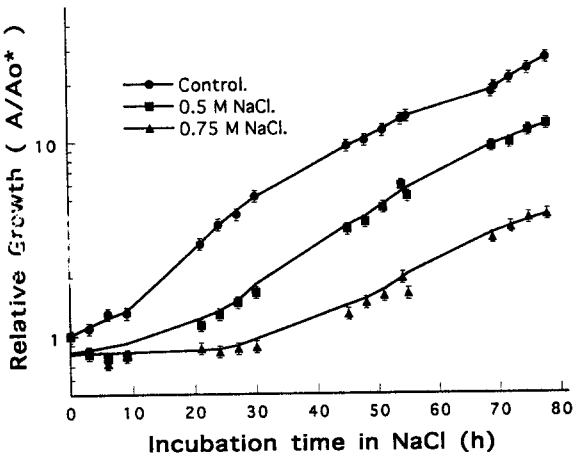

Figure 1. Growth of *Spirulina* exposed to different NaCl concentrations (-●- control, -■- 0.5 and -▲- 0.75 M NaCl.

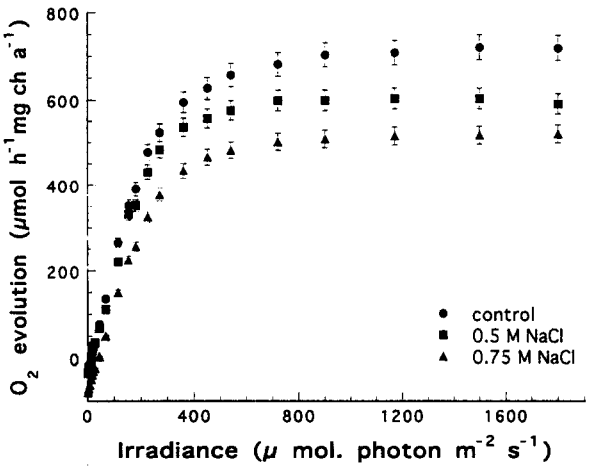

Figure 2. Light response curves of *Spirulina* cells grown in: -●- control, -■- 0.5 and -▲- 0.75 M NaCl containing medium.

their photosynthetic activity was measured under light limiting conditions.

Chlorophyll *a* was determined by the method of Bennet and Bogorad (1973). Protein was determined following the procedure of Karkwell et al. (1981) and carbohydrates were determined using the Antharon reagent method (Hassid & Abraham, 1957). The results are averages of three different experiments ± S.E. Statistical analysis of the data was performed using 'Super Anova' software.

Results and discussion

Upon exposure of *Spirulina* cells to a salinity stress of 0.5 or 0.75 M NaCl, an immediate cessation of growth is observed. After a lag period, which is directly correlated to the extent of the stress, growth resumes as measured by the increase in chlorophyll concentration in the culture (Figure 1). When *Spirulina* cultures are grown at optimal temperature and nutrient conditions, light is considered to be the only limiting growth factor. Previous studies (unpublished data) have shown that growth is saturated at photon flux densities in the range of 160–200 μmol m^{-2} s^{-1}. Indeed, when comparing the growth rate of *Spirulina* cultures grown under control conditions at 100 or 200 μmol m^{-2} s^{-1}, an increase in the specific growth rate is observed with increasing light intensities (Table 1). However, increasing light intensity from 100 to 200 μmol m^{-2} s^{-1} decreases the growth rate of the 0.5 and 0.75 M NaCl grown cultures by 35% and 50% respectively (Table 1).

The change in the growth response of salt-stressed cultures to light may reflect a change in their photosynthetic capacity. When the response of the photosynthetic activity to different light levels (P–I curve) of control cultures was compared to that of salinity grown cultures (0.5 M and 0.75 M NaCl), it was evident that a marked change was taking place (Figure 2). A reduction in the light-saturated maximal photosynthetic activity (P_{max}) is observed as well as a reduction in the initial rate (α) which is an indicator of the efficiency of photosynthesis (Henley, 1993). Dark respiration activity and the compensation point of the 0.75 M NaCl grown culture are both also significantly increased when compared to the control or the 0.5 M NaCl grown cultures (Table 2). These changes in the photosynthetic parameters indicate that *Spirulina* cells grown under elevated concentration of NaCl show a reduced ability to utilize light energy absorbed by their photosynthetic pigments. This reduced ability may thus entail an increased susceptibility to photoinhibition a tendency indeed reflected in the observed reduction in growth rate of salinity grown cultures at 200 μmol m^{-2} s^{-1} as compared to the growth rate of salt stressed cells grown at 100 μmol m^{-2} s^{-1} (Table 1).

In order to further evaluate the increased susceptibility of salt stressed cells to light, *Spirulina* cultures were grown in 0.5 or 0.75 M NaCl. After being acclimated to the salt stress, as indicated by establishing a constant logarithmic growth, the cells were subsequently exposed to high irradiance stress (photoinhibition). The elicited response was estimated by measuring the light-dependent O_2 evolution rates of

Table 1. Effects of salinity stress on the steady-state growth of *Spirulina platensis* under different light intensities. Values are mean ± S.E. ($n = 3$)

	μ-Specific Growth Rate (h^{-1})		Doubling Time (hrs)	
NaCl (M)	100 μE	200 μE	100 μE	200 μE
control	0.043 ± 0.002	0.060 ± 0.008	16.1 ± 0.8	11.6 ± 1.5
0.50	0.037 ± 0.002	0.025 ± 0.003	18.7 ± 1.0	27.7 ± 3.3
0.75	0.026 ± 0.001	0.013 ± 0.001	26.7 ± 1.1	53.3 ± 4.1

Table 2. Photosynthetic parameters of *Spirulina* cells grown under control and salinity conditions P_{max} – Maximal rate of light saturated photosynthesis (μmol O_2 h^{-1} mg chl^{-1}), α – Initial slope at the P–I curve (mmol O_2 h^{-1} mg chl^{-1}/(μmol m^{-2} s^{-1}), dark respiration-(μmol O_2 uptake. h^{-1} mg chl^{-1}), Compensation point- the light intensity in μmol · m^{-2} s^{-1} were no net oxygen uptake or evolution was observed. Values are mean ± S.E. ($n = 3$)

Parameter	α	P_{max}	Dark respiration	Compensation point
control	2.13 ± 0.06	716 ± 33	27 ± 3	10 ± 2
0.5 M NaCl	1.99 ± 0.05	598 ± 26	35 ± 5	15 ± 2
0.75 M NaCl	1.70 ± 0.09	517 ± 16	78 ± 7	49 ± 5

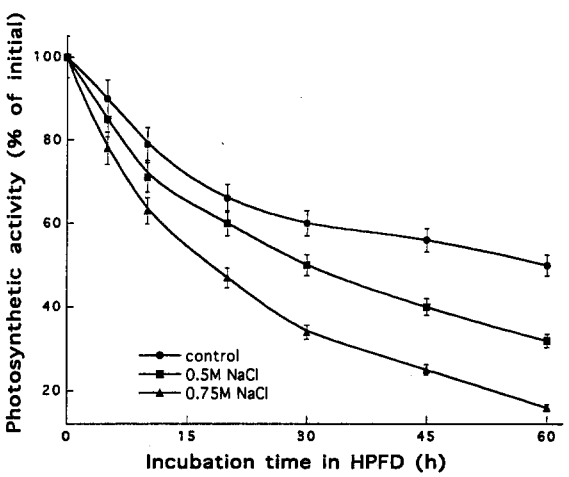

Figure 3. Kinetics of decline in oxygen evolution by *Spirulina* cultures grown in -●- control, -■- 0.5 and -▲- 0.75 M NaCl, and exposed to a photoinhibitory stress of 3500 μmol photon m^{-2} s^{-1} HPFD.

the cultures. As demonstrated in Figure 3, exposing the cultures to high photon flux density (HPFD) of 3500 μmol m^{-2} s^{-1} resulted in a decline in their photosynthetic activity. This reduction proceeded faster and to a lower level of activity in the salt stress cultures then in the control cultures. It is thus very clear that *Spirulina* cultures grown at high salinity are more sensitive to photoinhibition due to their reduced ability to utilize the light energy absorbed. The phenomenon of photoinhibition has been intensively studied in the last ten years (Kyle et al., 1987; Baker & Bowyer, 1994). It is generally accepted that the initial site of damage caused by photoinhibition is located in PSII. It has also been demonstrated that the reduction in PSII activity, be it a result of a damage or a process of down regulation is associated with the turnover of the D1 protein. Recovery from photoinhibition is at least partially attributable to the rate of protein synthesis required for the replacement of D1 damaged molecules. The higher sensitivity of salt-grown *Spirulina* cells to photoinhibitory stress may be partially due to their lower protein synthesis capacity (Hagemann et al., 1990).

As evident from Figure 1, *Spirulina* cells exposed to high salinity after an initial shock are capable of adapting and establishing a new steady state of growth. This new steady state is associated not only with modified photosynthetic activity (Figure 2 and Table 2) but also with a change in cell composition, mainly reflected in a reduction in protein content and a significant increase in carbohydrates (Table 3). The increase in the carbohydrate level reflects the need to increase the intracellular osmoticum in order to balance the higher osmoticum of the medium. Most of those osmoregulants are identified as amino acids or carbohydrates (Borowitzka, 1986).

The changes described so far are a result of an acclimation process that may take from five to ten generations before the establishment of a new balanced

Table 3. Chemical composition of *Spirulina* grown under salinity stress for at least 5 generations. Values are mean ± S.E. ($n = 3$)

Treatment	Chlorophyll (% dry weight)	Protein (% dry weight)	Carbohydrates (% dry weight)
Control	2.65 ± 0.15	56.1 ± 3.1	35.5 ± 2.7
+0.50 M NaCl	1.91 ± 0.12	44.1 ± 3.1	46.7 ± 3.2
+0.75 M NaCl	1.33 ± 0.10	36.1 ± 2.9	55.8 ± 4.9

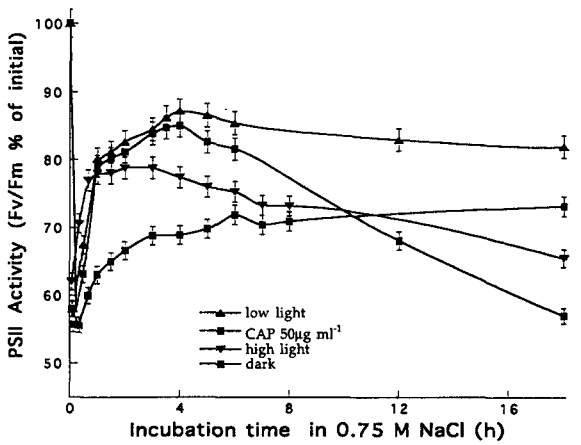

Figure 4. Changes in PS II activity of *Spirulina* cells exposed to 0.75 M NaCl and incubated in -▲- low light 80 μmol photon m^{-2} s^{-1}, -▼- high light 200 μmol m^{-2} s^{-1}, -■- in the dark, and -●- in low light with 50 μg ml^{-1} of chloramphenicol.

growth which reflects the new metabolic and energy requirements imposed on a photosynthetic organism in order to survive in its elevated new saline environment.

The question remains what happens to the photosynthetic machinery of a cell during its first few hours of exposure to high salinity before it is able to synthesize osmoregulants to control its intracellular water content? Is light important in this process? Is it necessary that an immediate protein synthesis take place? In order to track the initial response of the photosynthetic apparatus to salinity shock we have followed the changes in PSII activity of cells exposed to 0.75 M NaCl incubated under different conditions by monitoring the variable chlorophyll fluorescence of the cells (Fv/Fm ratio). As demonstrated in Figure 4, an immediate decrease in the Fv/Fm ratio occurs immediately after addition of the salt. Within the first hours after exposure, to the stress, recovery in the PS II activity is observed reaching a value of 80% of the activity prior to the exposure (except for the dark incubated cells). Recovery of activity in the dark incubated cells is much slower, reaching a value of 70% of their initial level after 8 hours, 75% after 24 hours of incubation.

Cells incubated at low light (80 μmol m^{-2} s^{-1}) with or without Chloramphenicol, an inhibitor of protein synthesis, recovered at the same rate and to the same extent after the first 4 hours to about 85% of the original activity. After the first 4 hours a difference in the response is observed; whereas PS II activity of the salt-stressed chloramphenicol treated cells started to decline the PS II activity of the cells which were not treated with chloramphenicol remained constant. It seems that response of the cells to the NaCl shock is instantaneous. Initial recovery does not require protein synthesis, and although it may not function as well as in the light, the basic recovery process may nevertheless take place in the dark.

It is worth noting that when the salt shocked cells are incubated at an irradiance of 200 μmol m^{-2} s^{-1} the initial response of the cells during the first hour is not modified. Thereafter, as in the chloramphenicol treated samples, a gradual decrease in PSII activity is observed. These results may suggest that the second decline in PSII activity observed in salt-treated cells incubated at high light or with chloramphenicol is actually the result of reduced or inhibited protein synthesis activity which is associated with the turnover of the D1 protein. The relatively rapid recovery response of the cells indicates the possible existence of an efficient Na^+ pump that is either activated or working continuously in this alkaliphilic alga. The fact that in the dark the rate and extent of recovery are lower may be attributed to lower energy supply in the dark incubated cells.

Results presented in this paper suggest that in the course of response of *Spirulina* to an increase in the NaCl concentration in its environment two processes may be distinguished. The first, a fairly rapid one, does not require the synthesis of new proteins; it is most likely associated with the activation of the energy depleting process of pumping sodium out of the cells. The second, which is slower and governs the adaptive process to the higher osmoticum, involves syn-

thesis of osmoregulants as well as modification of the photosynthetic activity of the cells. The lower photosynthetic capacity of the salt-adapted cells increases the susceptibility of *Spirulina* cells to photoinhibition. This susceptibility may be of critical importance in the mass production of *Spirulina* in outdoor open ponds, where high rates of evaporation and the use of saline or sea water lead to continuous increase in the salt concentration of the medium. In such cases, reduction in productivity may take place triggered by the salt stress and further reduced by photoinhibition.

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