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Light acclimation and photoinhibition in three *Spirulina platensis* (cyanobacteria) isolates

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Abstract

Three isolates of *Spirulina platensis* (Norst) Geitler marked BP, P4P and Z19/2 were compared with respect to their response and acclimation capability to high photon flux densities (HPFD). Cultures exposed to HPFD (1500–3500 μmol photon m^{-2} s^{-1}) exhibited a marked decrease in light-dependent O_2 evolution rate. P4P was more sensitive to HPFD than the two other isolates. All three isolates recovered from photoinhibition when placed under low PFD. The BP isolate was able to recover also in the dark but to a lower extent and at a lower rate, while no recovery was observed in the other two isolates under dark conditions. No recovery was observed when protein synthesis was inhibited using chloramphenicol. Cultures grown at 200 μmol photon m^{-2} s^{-1} differed from cultures grown at 120 μmol photon m 2 s-1 by their lower maximal photosynthetic rate (P_{max}) and higher light saturation (I_k) value, while being more resistant to HPFD stress. The ability of *Spirulina* isolates to acclimate and withstand HPFD may provide useful information for the selection of strains useful for outdoor mass cultivation.

Introduction

It is generally accepted that photoinhibition occurs when the level of light absorbed by the photosynthetic apparatus exceeds the rate by which it is consumed in photosynthetic reactions (Richter et al, 1990; Falk & Samuelsson, 1992). Photoinhibition occurs in higher plants and in algae (Critchley, 1981; Powles 1984; Greer et al., 1986; Krupa et al., 1990). It is believed that the primary site of the photoinhibitory response (damage) is located in the PSII and is reflected as a reduction in light limited oxygen evolution or CO_2 uptake rates (quantum efficiency). Recovery from photoinhibition may be observed in algal cells when placed under favorable conditions (Ohad et al., 1984; Samuelsson et al., 1987; Vonshak et al., 1988). This recovery is a result of a repair mechanism that goes on continuously even when the cells are exposed to high photon flux densities (HPFD). This repair mechanism may be crucial in preventing irreversible photooxidation under prolonged photoinhibition.

The cyanobacterium *Spirulina platensis* is used as a potential source of protein and valuable chemicals (Richmond, 1987). *Spirulina* is currently cultivated commercially in several countries (USA, Thailand, Taiwan, Mexico). However, because of the high cost, several plants have discontinued their production. One of the main reasons for the high cost is the marked decrease in productivity upon scaling-up of production. A possible explanation is a decrease in photosynthetic activity due to photoinhibition (Vonshak & Guy, 1992).

In this work, we studied three isolates of *Spirulina* for their sensitivity to high light and their ability to adapt to such conditions. Such comparisons may help in selecting strains which exhibit greater resistant to high PFD and a higher productivity under outdoor conditions.

Materials and methods

Strains and growth conditions

Spirulina platensis, BP, P4P and Z19/2 isolates, were grown in batch cultures in Zarouk's medium (Vonshak et al., 1982). BP, a gas-vacuolated isolate, was isolated from a waste water treatment pond of a tapioca factory in Thailand. P4P, a non gasvacuolated isolate, originated from a cell culture acclimatized to 4% NaCl for many generations in our laboratory (KMITT Thailand). Z19/2, a non gas-vacuolated isolate, is a Sandoz 9785 [BASF 13-338 4-chloro-5(dimthylamino)-2-phenyl-3(2H) pyridazinone] tolerant strain. The alga was grown at 35 °C and kept in suspension by bubbling CO_2-enriched (1%) air. Illumination was provided by cool white fluorescent lamps with a photon flux density of 120 μmol photon m^{-2} s^{-1}.

Measurement of photosynthesis

Cultures were diluted to a final concentration of 2.5 μg mL^{-1} chl with fresh Zarouk's medium (Zarouk, 1966). Their photosynthetic activity was assayed by measuring the rate of O_2 evolution using a Clark-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 photon m^{-2} s^{-1}.

Photoinhibition and recovery treatments

Cultures at the log phase of growth were harvested by centrifugation and suspended in fresh medium to give a chlorophyll concentration of 25 μg mL^{-1}. The cultures were placed in a thermoregulated double-jacket cylindrical glass vessel and then illuminated at HPFD (as indicated in the results) using a high intensity halogen lamp (OSRAM 220–230 V, 1000 W). The PFD between 400 and 700 nm was measured with a LI-180 (LiCor) photometer and a quantum sensor. At time intervals samples were drawn out and tested for their photosynthetic activity. The photosynthetic activity at zero time was used as 100% for the calculation of the degree of photoinhibition.

For recovery photoinhibited cells were diluted to a concentration of 2.5 μg Chl mL^{-1} with fresh medium and incubated either under dim light (70 μmol photon m^{-2} s^{-1}) or in complete darkness. At time intervals their photosynthetic activity was measured.

Table 1. Effect of exposure to HPFD (for 30 min.) on the degree of photoinhibition in three *Spirulina* isolates. Values are mean ± S. E. n=3,4, *(μmol photon m^{-2} s^{-1}

PFD*	% Photoinhibition		
	BP	P4P	Z19/2
1500	21.3 ± 1.2	20.0 ± 0.8	21.0 ± 0.5
2500	38.9 ± 2.5	43.5 ± 2.1	39.0 ± 1.6
3500	50.2 ± 2.1	63.0 ± 2.9	54.0 ± 2.2

Chlorophyll was determined by the method of Bennet and Bogorad (1973). The results are averages of three different experiments ± SE Statistical analysis of the data was performed using Super Anova soft-ware.

Results

When cultures of the three *Spirulina platensis* isolates were exposed to HPFD (1500, 2500 and 3500 μmol photon m^{-2} s^{-1}) the light-dependent O_2 evolution rate markedly decreased. The rate and extent of the decline increased with the increase in the irradiance or the exposure time (Figure 1). The light intensities used are similar to the one used in photoinhibition studies and do not imply that algal cultures are indeed grown in such high intensities continuously. In order to compare among isolates, the degree of inhibition after 30 min of exposure to the different irradiance is summarized in Table 1, indicating that the degree of photoinhibition was directly related to the PFD which the cells were exposed to. Of the three isolates P4P cells appeared to be the most sensitive to HPFD, especially when exposed to 3500 μmol photon m^{-2} s^{-1}. At the relatively low light stress, (1500 μmol photon $m^{-2}s^{-1}$), no significant difference among the trains was observed. Increasing the stress level to 3500 μmol photon m^{-2} s^{-1} resulted in a 3 fold increase in the inhibition level of the P4P cells (from 20 to 63%) as compared to a 2.5 fold in the other two isolates. The degree of inhibition at 3500 μmol photon m^{-2} s^{-1} was the highest in the P4P strain 63% as compared to 50 and 54% in the two others.

In order to evaluate the capability of the isolates to recover from the photoinhibitory stress, cells partially photoinhibited (by ca. 50%) were transferred to dim light (70 μmol photon m^{-2} s^{-1}) or darkness. Under dim light BP cells recovered faster and to a greater extent (86 ± 1%) as compared to P4P and Z19/2 cells

Table 2. Effect of light intensity during growth on photosynthetic characteristics of *Spirulina* isolates

Spirulina strain	BP		P4P		Z19/2	
	growth light intensity (μmol photon m^{-2} s^{-1}					
Parameters	120	200	120	200	120	200
μ	0.048	0.047	0.043	0.044	0.044	0.045
I_k	145 ± 15	258 ± 20	115 ± 13	185 ± 15	165 ± 15	325 ± 40
P_{max}	625 ± 8	536 ± 4	614 ± 5	585 ± 10	645 ± 13	585 ± 12
α	4.8 +0.4	2.3 ± 0.3	6.3 ± 1.0	3.85 ± 0.9	3.85 ± 0.7	2.1 ± 0.5

μ - Specific growth rate (h-1)
I_k - Irradiance at the onset of light saturation (μmol photon m^{-2} s^{-1})
P_{max} - Maximal rate of light saturated photosynthesis
α - Initial slope at the P-I curve (μmol O_2 h^{-1} mg Chl^{-1})/(μmol photon m^{-2} s^{-1}) The values are mean ± S.E. n=3

Table 3. Effect of light intensity during growth on the degree of photoinhibition (%) in response to HPFD (for 30 min.) in three *Spirulina* isolates.

	Light intensity during growth					
Spirulina strain	BP		P4P		Z19/2	
HPFD*	120	200	120	200	120	200
1500	21.3 ± 1.2	19.0 ± 2.2	20.0 ± 0.8	19.7 ± 3.1	21.0 ± 0.5	12.0 ± 0.5
2500	38.9 ± 3.5	32.8 ± 2.0	43.5 ± 2.7	39.5 ± 2.0	39.0 ± 1.6	33.9 ± 1.4
3500	50.2 ± 2.1	48.8 ± 2.5	63.0 ± 2.9	53.0 ± 2.2	54.0 ± 2.2	47.2 ± 1.7

The values are mean ± S.E. n=3,4
HPFD The irradiance used for the photoinhibitory treatment
μmol photon m^{-2} s^{-1}

(80 ± 1.5% and 77 ± 1.5% respectively ($P<0.05$) (Figure 2). No lag phase was observed in the recovery process when HPFD-stressed cells were transferred to dim light. This may indicate, as already found in other algae, that the repair process is going on continuously. In the dark, only BP cultures had the capability to partially recover (Figure 2), but at a lower rate than in dim light. Chloramphenicol, a protein synthesis inhibitor, at a concentration of 100 μg ml^{-1} prevented recovery of photoinhibited cells under dim light even after 60 min of incubation (Figure 2).

The rate of recovery seems to be inversely related to the degree of sensitivity to photoinhibition. The BP strain recovered faster and to a greater degree than P4P and Z19/2 (Figure 2), and it was also more resistant to HPFD than the others (Table 1). However, this relationship was not observed when comparing P4P and Z19/2: both recovered at almost the same rate and almost to the same degree, but Z19/2 seemed more resistant to photoinhibition than P4P at least at the 2500 and 3500 μmol photon m^{-2} s^{-1} treatment, indicating that differences in the total response of the strains to the photoinhibitory stress may involve different protection mechanisms and not only the capability of recovery from the HPFD stress.

Falk and Samuelsson (1992) suggested that some of the recovery process may take place in the presence of chloramphenicol. This difference may be a result of the fact that we used much higher irradiances as well as to the fact that their measurements were done on a eukaryotic algae as compared to *Spirulina* which is a prokaryote. The partial recovery of BP cells in the dark suggests that residual protein synthesis activity may be taking place in the dark using the existing energy pool in the cells, which is presumably greater than those of P4P and Z19/2 cells.

It has been demonstrated that photoinhibition and its recovery in unicellular cyanobacteria is directly correlated to the growth irradiance (Samuelsson et al., 1985; 1987). Using *Spirulina* strains grown under 120 or 200 μmol photon m^{-2} s^{-1}, a range of irradiance at which growth was saturated we have tried to distinguish between the effect of light on growth rate and its effect on adaptation to light stress. The effect of irradiance on growth rate, some photosynthetic parameters, and the response to photoinhibition of the three strains

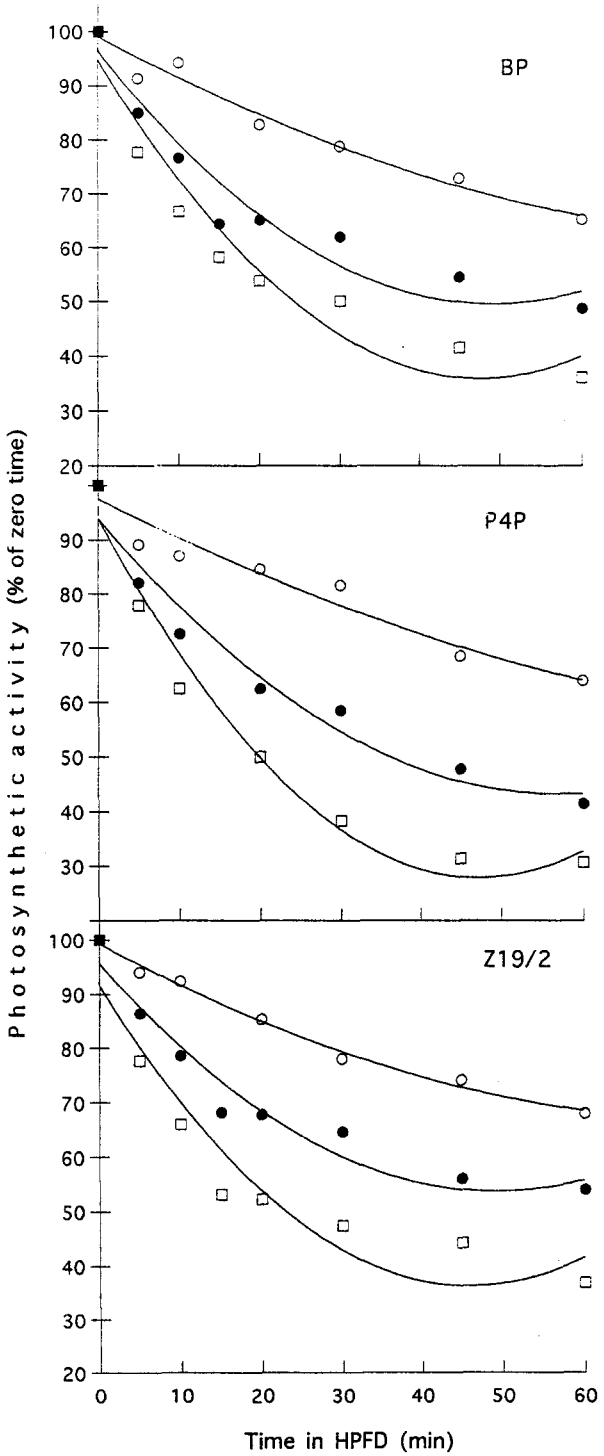

Figure 1. Kinetics of decline in oxygen evolution by three *Spirulina* isolates exposed to a photoinhibitory stress of high photon flux density (1500, 2500, 3500 μmol photon m^{-2} s^{-1}).

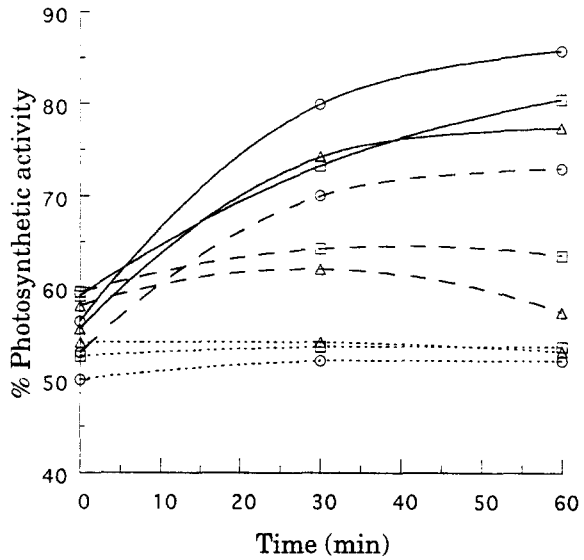

Figure 2. Effect of light (——), dark (– – –) and chloramphenicol (· · · · · ·) on the recovery of photosynthetic activity of photoinhibited *Spirulina* isolates. BP , P4P and Z19/2 Δ. (results are % of non-photoinhibited control cells).

studied are shown in Table 2. Increasing the growth PFD from 120 to 200 μmol photon m^{-2} s^{-1} did not change the growth rate significantly. When the light response curves (PI) of the cultures grown at 120 and 200 μmol photon m^{-2} s^{-1} were compared (Figure 3). The irradiance required for the onset of saturation of photosynthetic activity (I_k) increased significantly in all three strains (Table 2). Furthermore, P_{max} and the initial slope (α) in all cells grown at 200 μmol photon m^{-2} s^{-1} were lower than in cells grown at 120 μmol photon m^{-2} s^{-1}. All these changes are considered to be indicators of a photoinhibitory stress. These results suggest that continuous exposure to 200 μmol photon m^{-2} s^{-1} is already somewhat photoinhibitory. At this relatively low light the adaptive nature of 'photoinhibition' is more pronounced rather then the damage process i.e. a big change in I_k and α as compared to the reduction in P_{max} (Falk & Samuelsson, 1992).

When the cultures grown at the two irradiances were exposed to HPFD (1500, 2500 and 3500 μmol photon m^{-2} s^{-1}) a lower degree of photoinhibition was observed in cells grown at 200 μmol photon m^{-2} s^{-1} than in those grown under 120 μmol photon m^{-2} s^{-1} (Table 3). This result clearly indicates the adaptability of the cultures to high PFD. Even P4P the most sensitive strain, acquired significant resistance to photoinhibition when grown at 200 μmol photon m^{-2} s^{-1}.

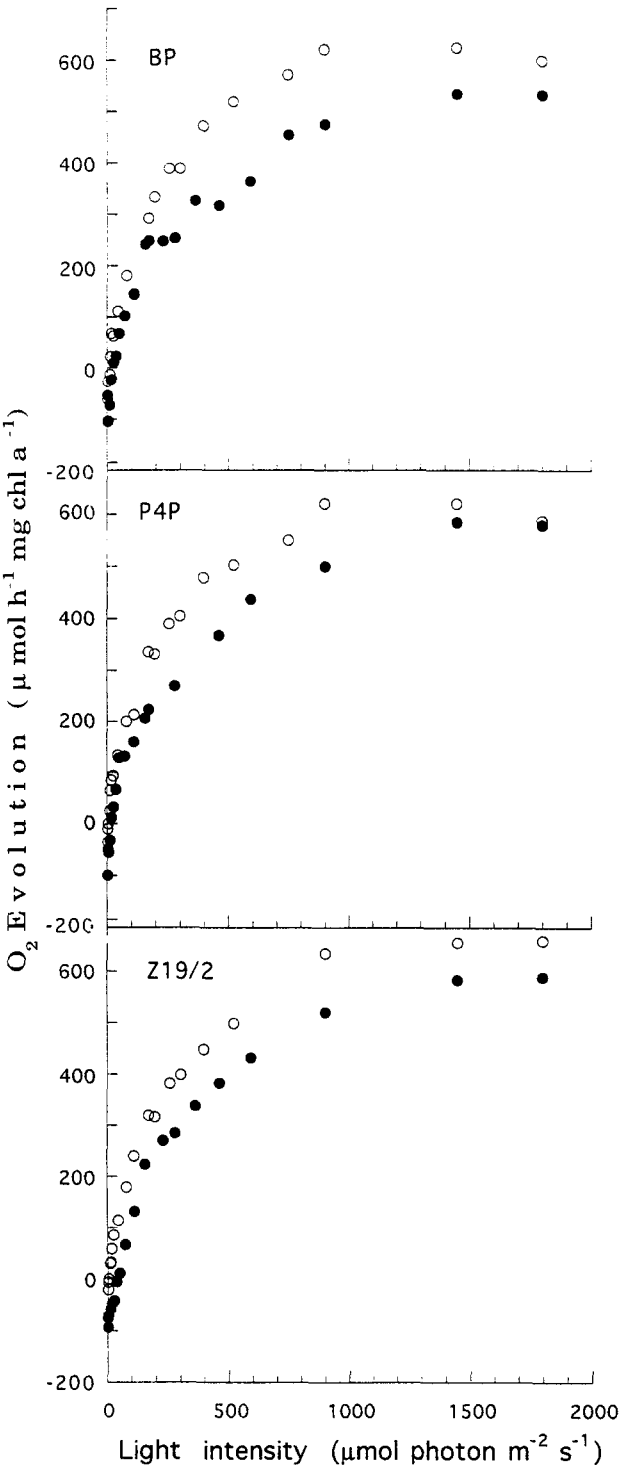

Figure 3. Light response curves of oxygen evolution rate in *Spirulina* isolates grown at two different light intensities: 120 or 200 μmol photon m^{-2} s^{-1}.

Discussion

Although photoinhibition has been studied intensively in the last ten years (Baker & Bowyer 1994, Kyle et al., 1987) very little has been done on the role of photoinhibition on productivity of algal production systems. The role of photoinhibition as an ecological factor in aquatic systems has been reviewed by Neals (1987) with the conclusion that the importance of photoinhibition in aquatic systems is emerging and that in order to be able and include it as a factor in estimating aquatic productivity much more work as to be carried out. Studies on photoinhibition in algae of commercial value are very limited. Vonshak et al. (1988) were the first to study photoinhibition in *Spirulina* under laboratory conditions followed by a later study to try and estimate the effect under out door conditions. In this later work (Vonshak & Guy, 1992) an attempt was made to quantify the effect of photoinhibition on productivity, estimating that at least 25% of the potential loss in outdoor production of *Spirulina* may be due to photoinhibition. Further understanding on the interaction between light and temperature stress in *Spirulina* was provided in the work of Jensen and Knutsen (1993) demonstrating that at temperatures below the optimal for growth *Spirulina* is much more sensitive to photoinhibition. Indeed Vonshak et al. (1994) demonstrated that in out door tubular reactors photoinhibitory damage may be prevented by increasing the temperature to the optimal during the day. Although much more work is needed in order to be able and estimate the exact effect of photoinhibition on productivity of dense out door algal cultures, it is of no doubt that at least in *Spirulina* photoinhibition may account for at least 25% of the total lose in potential productivity. In this work we have tried to point out that *Spirulina* strains do deafer in their response to a photoinhibitory stress. Even if one considers photoinhibition as a down regulation process that has a physiological importance, then by selecting of strains capable of recovering from such a decline a significant improvement in productivity may be expected.

The strains used in these study were chosen randomly and are used just to demonstrate the point that the response of algal isolated to a light stress may differ significantly even if grown under the same conditions. We have also demonstrated that the net outcome of the photoinhibitory stress as measured by the decline in O_2 evolution rate may be a result of many process. Either the rate of damage and destruction of a specific protein or the rate of recovery and replacement of damaged components in PS II. Furthermore cells grown at dif-

ferent light intensities can be acclimated to the high light stress and thus exhibit a lower damage level once exposed to HPFD.

Outdoor algal cultures are continuously exposed to changes in the environmental conditions. Laboratory studies of the photosynthetic activity of *Spirulina* isolates and their response to environmental stresses, such as light, temperature and salinity as well as their ability to adapt to the environmental stress may provide us with useful information for the selection of strains suitable for outdoor cultivation. Further studies on the response of these and other isolates to temperature and salinity stress may eventually lead to selection of improved strains for biomass production under outdoor conditions.

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