Photoadaptation, photoinhibition and productivity in the blue-green alga, *Spirulina platensis* grown outdoors

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**ABSTRACT**

Two *Spirulina platensis* strains, SP-G and SP-RB, resistant and sensitive to photoinhibition of photosynthesis, respectively, were grown outdoors in dense cultures and under different photon fluxes provided by shading. Cultures of both strains grown under full sunlight were more resistant to photoinhibition than those grown under nets with 15–50% decreases in the incident photon flux. Cultures grown outdoors were more resistant to photoinhibition than the laboratory ones. At noon, the photosynthetic activity, as expressed by O₂ evolution, was higher for cultures grown under 50% shade, as compared with unshaded cultures. Productivity of the shaded cultures, in terms of biomass produced per day, was always higher when the cultures were protected from photoinhibition.

Key-words: *Spirulina*; blue-green algae; cyanobacteria; photoadaptation; photoinhibition; photosynthesis; productivity; biomass.

**INTRODUCTION**

Photoadaptation and photoinhibition have been studied intensively in microalgae and higher plants, but mainly under laboratory conditions (Powles 1984; Kyle & Ohad 1986; Oquist 1987). Photoadaptation has also been studied in algal populations mainly in relation to its ecological impact, i.e. the appearance and dominance of specific algal strains in a given aquatic habitat (Neale 1987). Laboratory algal cultures exposed to different photon fluxes and kept at constant cell concentrations may adapt to the specific light level to which they are exposed (Gendel, Ohad & Bogorad 1979; Levi & Gantt 1988). In outdoor cultures, the cells are exposed to two different rhythms of light/dark regime. The first, a relatively fast one, is induced by the turbulent flow in the culture, the rate of which is dictating the frequency of the light/dark cycle (Laws et al. 1983). Thus algal cells shift between near full sunlight, when located in the upper layer of the culture, and virtual darkness (below 10 μmol m⁻² s⁻¹) at the bottom of the culture, usually at a depth of 12–15 cm. The second regime, a relatively slower one, is the daily change in solar radiation from sunrise to sunset.

The potentially deleterious effect of photoinhibition on the productivity of outdoor algal cultures has been discussed in many studies, but is based on circumstantial rather than factual evidence (Ben-Amotz & Avron 1981; Richmond 1987). To the best of our knowledge, no work has actually demonstrated that photoinhibition does exist in dense outdoor cultures or the degree of loss in productivity that can be attributed to this phenomenon.

Previously (Vonshak et al. 1988), we described two *Spirulina* strains, designated SP-G and SP-RB, differing in their resistance to high photon flux density (HPFD). We suggested that the higher resistance of SP-G as compared with SP-RB was due to the lower rate of degradation of the PSII components at HPFD rather than to a higher rate of recovery. In the present work, we examine the extent to which these strains can adapt to different light regimes. We also evaluate the performance of the two strains under outdoor conditions, commonly employed in mass cultivation of *Spirulina*.

**MATERIALS AND METHODS**

**Strains and culture conditions**

Two *Spirulina* strains, designated SP-G and SP-RB (Vonshak et al. 1988), were used in this study. Laboratory cultures were grown as previously described (Vonshak et al. 1988). Outdoor cultures were grown in a modified Zarouk’s medium (Vonshak 1986) in 2-5 m² ponds. Culture depth was 10 cm, stirring was provided by a paddle-wheel at 15 rpm (Vonshak et al. 1982), and biomass concentration was kept almost constant at 0.5–0.6 OD₅₆₀nm by daily harvesting of the biomass on a 350-mesh screen. All nutritional requirements were supplied in excess in order to prevent nutrient deficiency. Shade was provided by standard shade nets used in greenhouses (Ben-Tzur & Drouianoff, Herzliyya, Israel). Nets were used to decrease incident photon flux by 15, 25–30 and 50%. Photon flux was measured with a Li-Cor 185 photometer and a quantum sensor. After two weeks of growth under present conditions, samples were taken from each treatment. Cells were harvested,
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Figure 1. Decrease in photosynthetic activity in Spirulina platensis SP-RB (a) and SP-G (b) grown under different light conditions and exposed to high photon flux density. Outdoor cultures grown under no shade (○), 15 (△), 25–30 (●) or 50% (■) shading nets. Laboratory cultures were grown at 150 μmol m⁻² s⁻¹ (Vonshak et al. 1988). One hundred per cent activity for all the cultures was in the range of 450–500 μmol O₂ h⁻¹ mg ch⁻¹. The results are average of three different measurement with a standard deviation of no more than 8%.

resuspended in fresh medium to a constant concentration of chlorophyll [2.5 g (chl) m⁻³], and photoinhibition in each strain grown under the different light regimes was monitored.

Photoinhibition

Cells were illuminated at high PFD of 3500 μmol m⁻² s⁻¹ at 30°C for varying lengths of time. At time intervals, samples were withdrawn, diluted with fresh medium to a final concentration of 2.5 g (chl) m⁻³ and their O₂ evolution activity was measured. In outdoor cultures, O₂ evolution activity was measured in samples preincubated under dim light for 2 h to allow full recovery from photoinhibition, if this had indeed occurred (Vonshak et al. 1988). O₂ evolution rate was measured after this incubation, and the difference between the two activities, if any, may be accounted for by photoinhibition. All samples were harvested and resuspended in fresh medium to the same chlorophyll concentration.

Oxygen evolution

Under laboratory conditions

Photosynthetic activity was measured with a Clark-type electrode. Samples were harvested and resuspended to a final concentration of 2.5 g (chl) m⁻³ with fresh Zarouk’s medium. The temperature (30°C) was kept constant and illumination was provided by a slide projector lamp at an intensity of 170 μmol m⁻² s⁻¹ (light limited conditions) as described by Vonshak et al. (1988).

Under outdoor conditions

Photosynthesis was measured by following the increase in O₂ concentration in the pond after being reduced to about 100–110% of saturation by bubbling N₂ through the cultures for 5–10 min (Guterman, Vonshak & Ben-Yaakov 1989).

Productivity

Biomass concentration was measured daily by determining the dry weight (Vonshak 1986) of samples drawn out of the culture before and after harvest. Chlorophyll content was determined according to Bennet & Bogorad (1973).

RESULTS AND DISCUSSION

Two Spirulina strains, SP-G and SP-RB, were grown outdoors under different shade conditions and constant cell concentrations. As shown previously (Vonshak 1986) under laboratory conditions, the SP-G strain was found less sensitive to the HPFD treatment than the SP-RB strain: after 30 min of exposure to HPFD, the SP-G strain lost about 60% of its photosynthetic activity (Fig. 1b), while SP-RB lost almost 80% of its activity (Fig. 1a). In this study, more striking differences were observed in the same strain for the different shade conditions. It is clear that in both strains, cells grown under full sunlight were much more resistant to HPFD stress than the laboratory-grown cells (Fig. 1). Likewise, the smaller the light level in growth, the higher the resistance to the light stress.

The nature of this photoadaptation is still not clear. Preliminary chemical analysis of the cells suggests that the photoadaptation is associated with an increase of the carotenoid to chlorophyll ratio (data not shown) and with changes in the chlorophyll to phycocyanin ratio. This kind of modification has been well documented in other algae studied under laboratory conditions (Gendel et al. 1979; Cunningham et al. 1989). Whether they are the cause or the effect of photoadaptation cannot be answered from the current study.

The questions of whether the two strains grown outdoors in dense well-mixed cultures are photoinhibited, at what time of the day, and to what extent were explored by measuring the photosynthetic activity in the...
Figure 2. Photosynthetic activity of *Spirulina platensis* SP-RB (a) and SP-G (b) grown outdoors under different shade conditions. The results are average of four different measurements with a standard deviation of no more than 8% (morning 0800-0900 h; noon 1200-1300 h).

**Table 1.** Photoinhibition in outdoor *Spirulina* cultures*

<table>
<thead>
<tr>
<th>Shade (%)</th>
<th>Time of sampling</th>
<th>Per cent of photoinhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain SP-G</td>
<td>SP-RB</td>
</tr>
<tr>
<td>None</td>
<td>Morning**</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td></td>
<td>Noon***</td>
<td>13±0.8</td>
</tr>
<tr>
<td>25–30</td>
<td>Morning</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Noon</td>
<td>4±0.3</td>
</tr>
</tbody>
</table>

* Per cent of photoinhibition was calculated from the increase in the photosynthetic activity of samples from outdoor cultures incubated in dim light for 2 h to allow recovery from photoinhibition (see ‘Materials and methods’). The results are averages of at least three different measurements.

** Between 0800 and 0900 h.
*** Between 1200 and 1300 h.

As mentioned previously, the outdoor *Spirulina* cultures were grown in a semi-continuous mode maintaining an almost constant cell concentration by daily harvesting. Measuring dry weight concentration before and after the harvest allows estimation of the productivity of the culture. The productivity thus assessed (Table 2) showed decreases which corresponded with the degree of photoinhibition. When the resistant strain of *Spirulina* SP-G was provided with some protection from photoinhibition, i.e. 15–20% shade, a slight increase in productivity was observed. A further increase in the degree of shading seems to lower the productivity of the cultures.

**Table 2.** Productivity of *Spirulina* cultures grown under different shade conditions. Results are average of 30 d of operation

<table>
<thead>
<tr>
<th>Shade (%)</th>
<th>Productivity (g m⁻² dw⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain SP-G</td>
</tr>
<tr>
<td>None</td>
<td>18.3±1.2</td>
</tr>
<tr>
<td>15</td>
<td>20.8±1.5</td>
</tr>
<tr>
<td>25–30</td>
<td>15.0±1.1</td>
</tr>
<tr>
<td>50</td>
<td>—</td>
</tr>
</tbody>
</table>

* *Spirulina* cultures had a lower photosynthetic activity than their morning counterparts the activity increased with the degree of shading. SP-G and SP-RB cultures under 25–30% shade had 33 and 50% higher activity, respectively, at noon than in the morning (Fig. 2a,b). These results strongly suggest that even dense algal cultures grown outdoors may be photoinhibited to some degree at noon, and that shading of the cultures may help overcome this inhibition.

An attempt was made to estimate the degree of photoinhibition imposed on each of the strains tested under full sunlight or partial shade. By measuring the photosynthetic activity of the cultures before and after a 2 h period of incubation under dim light for recovery (see ‘Materials and methods’), the extent of photoinhibition at sampling time may be estimated. The results presented in Table 1 indicate that cultures of both strains of *Spirulina* grown outdoors, shaded or unshaded (25–30%), were not photoinhibited in the morning at a photon flux of 1100 μmol m⁻² s⁻¹ and at temperature significantly below the optimum for these strains. At noon, when photon flux was higher than 2000 μmol m⁻² s⁻¹, both strains were photoinhibited when exposed to full sunlight, the sensitive strain SP-RB being much more inhibited than the resistant strain SP-G. Shading which decreased the incident sunlight by 25–30% prevented the reduction in O₂ evolution, i.e. prevented photoinhibition.
overall productivity, most likely due to light limitation. Shading had a much more pronounced effect on the SP-RB strain. For all the shade conditions, there was an increase in productivity versus the unshaded culture, the protection of the photosynthetic apparatus from photoinhibition of the total solar radiation (Table 2).

The divergence between the figures of the degree of protection of the photosynthetic apparatus from photoinhibition by different shade conditions (Table 1) and the productivity figures (Table 2) is most likely due to the different nature of the measurements. While the first measures photosynthetic activity over a few minutes, the second is an average value, integrating the total activity throughout the day. Furthermore, shade was provided throughout the day, not only when light was above a certain threshold. In all treatments, both strains would be light limited for some time during the morning and late afternoon. Hence, if shading could have been provided only when needed, i.e. when photon flux is excessive, the productivity of the shaded cultures could have been even higher.

Mass production of microalgae is a developing aspect of biotechnology used in the production of high-value products (Richmond 1986; Vonshak & Richmond 1988). The future development of this biotechnology is highly dependent on the optimization of the biological system to increase productivity per unit area. These results indicate that photoinhibition is a significant factor reducing productivity. This has to be taken into consideration when new strains of algae or a new production system are chosen or developed.

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REFERENCES


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