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EFFECT OF LIGHT AND TEMPERATURE ON THE PHOTOSYNTHETIC ACTIVITY OF THE CYANOBACTERIUM *SPIRULINA PLATENSIS*

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Abstract—The response of the photosynthetic activity of *Spirulina platensis* M2 to temperature and light stress was studied. The optimal temperature for photosynthesis was 35 C, while dark respiration was highest at 45 C. At temperature extremes outside those optimal for growth, both respiratory and photosynthetic activity declined. However, the sensitivity of respiration to such extremes was significantly greater than the sensitivity of photosynthesis under the same conditions. Under conditions where respiration was completely inhibited, photosynthetic oxygen evolution was maintained at about 30% of the optimum value. Exposing *Spirulina* cells to high photon flux densities results in a significant reduction in all the photosynthetic parameters (i.e. initial slope, light-saturated rate and the convexity of the photosynthesis–light response curve). The photoinhibitory stress resulted in much larger decreases in the quantum yield than in the light-saturated rate of photosynthetic oxygen evolution. The extent of photoinhibition was much higher when applied at temperatures over or below the optimum for photosynthesis. The implications of these findings are discussed with respect to the outdoor cultivation of *Spirulina*.

INTRODUCTION

The cyanobacterium *Spirulina platensis* has been used for the last 10 years as a model organism in many studies on outdoor cultivation of algal biomass as a source of protein and chemicals.[1] Although much progress has been made in this field, resulting in the set-up of several commercial production sites all over the world (Japan, Thailand, Taiwan, U.S.A.), the original goal of producing a cheap alternative source of protein has not yet been achieved. The cost of production is still one order of magnitude higher than that of conventional sources of protein. One of the pre-requisites in further development of this biotechnology is the better understanding of the physiology of *Spirulina* and the response of its photosynthetic machinery to environmental stress.

In outdoor dense cultures, light is considered to be one of the most important limiting factors.[2] Nevertheless, the fact that outdoor algal cultures are continuously exposed to changes in environmental factors such as light and temperature, which are limiting for the growth of any photosynthetic organism, imposes a special physiological situation in which light limitation or inhibition is not only defined by the photon flux intensity but also by the given temperature associated with it. It is thus of interest and of practical importance to understand the effects of light and temperature on the photosynthetic response of *Spirulina*.

In this work we studied the effect of high photon flux densities (HPFD) and temperature on the photosynthetic activity of *Spirulina*, higher when the exposure to the light stress is carried out at temperatures above or below the optimal temperature. The implications of these findings are discussed with respect to the outdoor cultivation of *Spirulina*.

MATERIALS AND METHODS

Organism and culture conditions

Spirulina platensis strain M2 isolated from Mombolo Lake in Chad by Luisa Tomaselli from the Centro di Studio dei Microrganismi Autotrofi.—C.N.R.—of Florence (Italy) was used. It was grown in batch culture in Zarrouk's medium, containing 200 mM sodium bicarbonate.[3] The cells were grown in 500 ml glass tubes immersed in a water bath at 30 C and kept in

suspension by bubbling CO_2-enriched (1%) air. Illumination was provided by a set of cool fluorescent tubes to give 100–120 μmol $m^{-2} s^{-1}$ at the center of the tube.

Photon flux density was measured with a Li-Cor 185 photometer equipped with a quantum sensor, measuring in the 400–700 nm range. Chlorophyll content was determined according to Bennet and Bogorad.[3]

Photoinhibition experiments

Cells in the log phase of growth were harvested by filtration using a GFC filter and resuspended with fresh medium to 25 μg chl ml^{-1}. Cells were placed in a thermoregulated double-jacket cylindrical glass vessel (internal diameter 1.5 cm) and then exposed to inhibitory conditions by illumination at a PFD of 2500 μmol $m^{-2} s^{-1}$ at 25°, 35° and 40°C. Prior to photoinhibitory treatment, the cells were allowed to equilibrate to each temperature tested for 15 min. At indicated time intervals, samples were withdrawn and tested for their photosynthetic activity.

O_2 evolution of photoinhibited cultures was measured at 35°C on a ten-fold diluted sample with fresh Zarrouk's medium to give a final concentration of 2.5 μg chl ml^{-1}. O_2 evolution was recorded for 5–10 min to obtain a constant rate, using a Clark type electrode. A PFD of 160 μmol $m^{-2} s^{-1}$ was provided using a slide projector lamp (Gabin 150 M Industrial Co., Ltd). The extent of photoinhibition was expressed as a percentage of the photosynthetic activity of cells before exposure to the HPFD zero time.

The photosynthesis–light response curves of control and photoinhibited cultures were obtained by exposing culture samples (4 μg ml^{-1} chl) to different light intensities from 0 to 1900 μmol $m^{-2} s^{-1}$. Photoinhibited cultures at about 50% of the initial photosynthetic activity were obtained by exposing the cells to 2500 μmol $m^{-2} s^{-1}$ at 35°C for 45 min. In order to prevent recovery from photoinhibition during the measurements, chloramphenicol was added to the photoinhibited and control samples to a final concentration of 0.05 mM.

Light-saturated photosynthesis of control and photoinhibited cultures was calculated as the mean of the last two values in the asymptotic region of the P–I curve. Light-limited slopes of the P–I curves (α) were calculated by regression using the rates at the lowest 6–10 PFDs. The strictly linear region of the curves was judged on the basis of the maximum r^2. I_k corresponded to the intersection of the extrapolated linear part of the curve with the horizontal line at P_m. Respiration rates are those measured as O_2 uptake in the dark.

Short-term responses of photosynthesis and respiration to temperature of *Spirulina* cells were measured in the 10–50°C range. O_2 evolution was measured at a PFD of 160 μmol $m^{-2} s^{-1}$. Cells were allowed to equilibrate for 15 min at each temperature tested prior to the measurement. Gross photosynthesis was calculated as the sum of net O_2 evolution and O_2 uptake rates at each temperature tested. All data presented are an average of three different experiments.

RESULTS AND DISCUSSION

Effect of temperature

Net biomass productivity of an algal culture is considered to be directly correlated to the net rate of CO_2 fixation and the rate of respiration. Those two metabolic activities are highly dependent on temperature, while only the CO_2 fixation or O_2 evolution are also light dependent. The O_2 evolution rate of *Spirulina* cells measured at different temperatures is shown in Fig. 1. The optimal temperature for photosynthesis was 35°C; however, activities of 28% and 23% of the optimum could be measured at the minimum and maximum temperatures tested (10° and 50°C, respectively).

A temperature-dependent exponential relationship was observed, with the dark respiration rate increasing as temperature increased up to

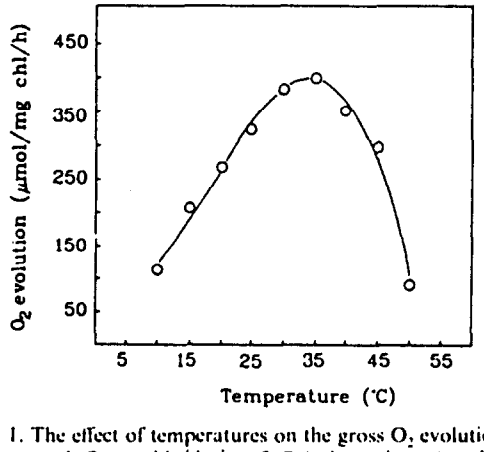

Fig. 1. The effect of temperatures on the gross O_2 evolution rate (μmol O_2 mg $chl^{-1} h^{-1}$) of *Spirulina platensis* cells. Cells were allowed to equilibrate at each temperature for 15 min before the measurement.

45°C. From Fig. 2 the following equation can be derived to describe this relation.

$$R = 0.771\, e^{(0.0636T)},$$

where R is the respiration rate (μmol O_2 mg^{-1} chl h^{-1}) and T is the temperature (°C). Dark respiration rates dropped almost to zero when *Spirulina* cells were exposed to 50° or 15°C (data not shown). An Arrhenius plot (Fig. 3) for respiration showed an activation energy for *Spirulina* of 48.8 kJ mol^{-1}. The temperature coefficient (Q_{10}) of the organism at the temperature range was calculated by the following equation being deducted from the Arrhenius equation:[4]

$$\log Q_c = \frac{Ea}{2.303R} \times \frac{10}{(T+10)T},$$

where E_a is the activation energy (kJ mol^{-1}) and R is the universal gas constant (8.31 J K^{-1} mol^{-1}). Between 20° and 45°C, a Q_{10} of 1.85 was calculated.

The respiration-to-photosynthesis ratio in *Spirulina* was 1% at 15°C and reached 4.6% at 45°C. These rather low values confirm the general assumption that cyanobacteria have low respiration rates.[5] However it should be noted that those rates were measured in laboratory cultures grown under relatively low light. As reported previously, dark respiration rate is affected by the light intensity at which the cells were grown. The higher the light intensity at which cells were grown, the higher their dark respiration rates.[6,7] Indeed, measurements of the dark respiration losses in outdoor cultures of *Spirulina* have demonstrated that up to 34% of the biomass produced during the daylight period may be lost through respiration at night.[7,8] This will be useful in mass culture operation where cells are exposed to high light intensity to keep the night temperature of the culture as low as possible in order to reduce the extent of night biomass loss throughout respiration, as demonstrated previously by Vonshak *et al.*[2]

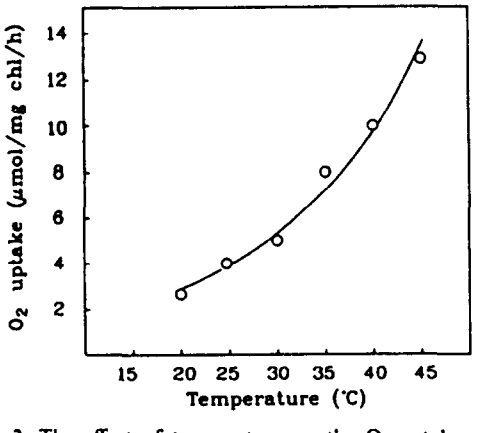

Fig. 2. The effect of temperature on the O_2 uptake rate (μmol O_2 mg chl^{-1} h^{-1}) of *Spirulina platensis* cells. Cells were allowed to equilibrate at each temperature for 15 min before the measurement.

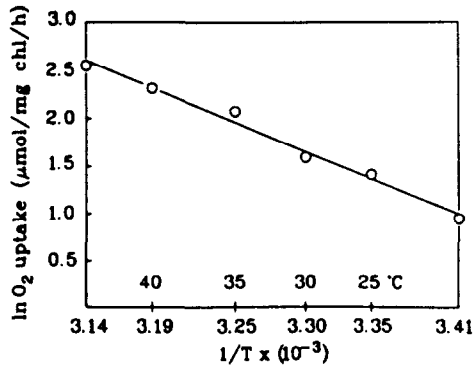

Fig. 3. Arrhenius plot of the effect of temperature on respiration rate of *Spirulina platensis*.

It may be concluded that, in our *Spirulina* strain, the respiratory activity has a much higher optimum temperature than the photosynthetic activity. Nevertheless, the photosynthetic activity of the cells was more resistant than dark respiration at the extreme temperatures tested. It should be noted that the 15 min incubation time used in the experimental procedure does not allow a real adaptation process to take place. Nevertheless, in outdoor cultures, cells are continuously exposed to a gradual change in temperature, which is also too fast to allow a real adaptation. We are aware that these results should be used as a general indication of the potential of the particular algal strains tested.

Effect of light

Outdoor algal cultures are exposed to high light intensities much above those used in the cultivation of laboratory cultures. As previously reported[9] *Spirulina* cultures exposed to high photon flux densities (HPFD) are photoinhibited. By comparing the photosynthetic light response of *Spirulina platensis* cells (Fig. 4) before and after 45 min photoinhibitory treatment, it can be observed that photoinhibition influenced (1) the initial slope (quantum yield); (2) the asymptote (light saturated rate); (3) the value of the irradiance at the onset of light saturation; and (4) the convexity (rate of bending) of the photosynthesis–light response curve.[10] The extent of photoinhibition

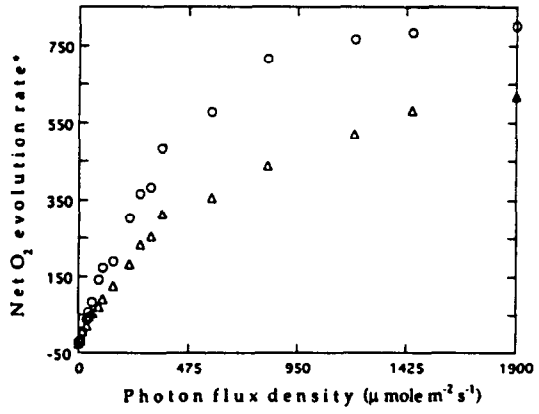

Fig. 4. The photosynthetic light response curve of *Spirulina platensis* cells (control—○ and photoinhibited—△). Rates are in μmol O_2 mg chl^{-1} h^{-1}.

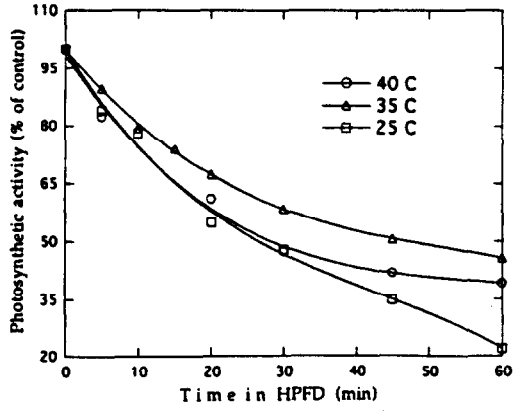

Fig. 5. The effect of temperature on photoinhibition. Cells grown at 30 C were incubated at 25 □, 35 △, and 40 C ○ for 15 min in dim light and then exposed to a HPFD of 2500 μmol m^{-2} s^{-1}. At time intervals samples were withdrawn and the O_2 evolution activity was measured at 35 C and 160 μmol m^{-2} s^{-1}.

quantified from the quantum yield and estimated by linear regression of the low-light data, was 48% as compared with the control. The reduction in P_{max} occurred at a lower extent, at about 25% of the control. This finding confirmed previous reports which demonstrated that photoinhibition results in a much larger decrease in the quantum yield than in the light-saturated rate of photosynthesis, even in intact leaves.[11,12] Similar behavior was also found in photoinhibited cells of *Chlamydomonas reinhardtii*.[10] These observations also indicate that the response to photoinhibition in green algae, higher plants and cyanobacteria is most likely similar in nature. Convexity of the light response curve, i.e. the transition region from light limitation to light saturation, became less abrupt in the photoinhibited culture. This is in agreement with the result of Leverenz *et al.*[10] who demonstrated that the convexity of the P–I curve depended on the degree of photoinhibition in *Chlamydomonas reinhardtii*. Finally, the dark respiration rate was about 29% higher in the photoinhibited culture. The changes in the photosynthetic parameters between a control and a photoinhibited culture are summarized in Table 1.

Table 1. Photosynthesis-irradiance (P-I) parameters estimated from *Spirulina platensis* M2 culture before and after 45 min photoinhibition at a PFD of 2500 μmol m^{-2} s^{-1} at 35 C. P_{max}—saturated photosynthetic rate; I_k—irradiance at the onset of light saturation; α—initial slope of the P-I curve; R—dark respiration

Parameter	Control	Photoinhibited
P_{max}*	800	600
I_k†	430	610
α‡	1.915	1.0
R*	−20.5	−26.4

*μmol mg^{-1} chl h^{-1}.
†μmol photons m^{-2} s^{-1}.
‡μmol O_2 [mg chl μmol photon m^{-2} s^{-1}]$^{-1}$ h^{-1}.

Interaction of light and temperature

It is generally accepted that photoinhibition of photosynthesis is related to absorption of light energy by the pigment antennae in excess of what can be dissipated in an orderly fashion by photosynthesis.[13] It is thus expected that cells will be better able to handle excess energy when grown at the optimum temperature for photosynthesis. Indeed, as demonstrated in Fig. 5, cultures exposed to HPFD at temperatures above (40°C) or below (25°C) the optimal temperature are much more sensitive to the HPFD stress. The difference is more pronounced with prolonged exposure time to high irradiance (especially at 25°C) and fits well with the overall concept of photoinhibition. After 1 h of exposure of *Spirulina* cells to HPFD, the highest rate of inhibition (78%) occurred at 25 C, while at 35° and 40°C the rates of photoinhibition were 54.4% and 61%, respectively. The interaction of low temperature and HPFD is a common event in outdoor cultures of *Spirulina* in open ponds. Indeed, in such culture systems, even during summer, the morning temperature of the culture (almost 10°C below the optimum) prevents full exploitation of the photosynthetic capacity of the culture for a few hours and eventually makes the culture prone to photoinhibition. On the other hand, when the outdoor culture of *Spirulina* is carried out in closed systems, the combination of high temperature and full sunlight is very frequent in summer.[14]

In this case it is expected that a temperature of several degrees above the optimum will exacerbate photoinhibition which may lead to a reduction of the biomass yield.

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REFERENCES

1. A. Richmond, Microalgae of economic potential, in *Handbook of Microalgal Mass Culture* (edited by A. Richmond). CRC Press, Boca-Raton, FL (1986).
2. A. Vonshak, A. Abeliovich, S. Boussiba and A. Richmond, Production of *Spirulina* biomass: effects of environmental factors and population density. *Biomass* **2**, 175–185 (1982).
3. J. Bennet and L. Bogorad, Complementary chromatic adaptation in filamentous blue-green alga. *J. Cell Biol.* **58**, 419–435 (1973).
4. S. J. Pirt, *Principles of Microbe and Cell Cultivation*, Blackwell, Oxford (1975).
5. L. van Liere and L. Mur, Growth kinetics of *Oscillatoria agardhii* Gomont in continuous culture limited in its growth by the light energy supply. *J. Gen. Microbiol.* **115**, 153–160 (1979).
6. J. U. Grobbelaar and C. J. Soeder, Respiration losses in planktonic green algae cultivated in raceway ponds. *J. Plankton Res.* **7**, 497–506 (1985).
7. G. Torzillo, A. Sacchi, R. Materassi and A. Richmond, Effect of temperature on yield and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. *J. Appl. Phycol.* **3**, 103–109 (1991).
8. H. Guterman, A. Vonshak and S. Ben-Yaakov, Automatic on-line growth estimation method for outdoor algal biomass production. *Biotechnol. Bioengng* **34**, 143–152 (1989).
9. A. Vonshak, R. Guy, R. Poplawsky and I. Ohad, Photoinhibition and its recovery in two strains of the cyanobacterium *Spirulina platensis*. *Plant Cell Physiol.* **29**, 721–726 (1988).
10. J. W. Leverenz, S. Falk, M. Pilström and G. Samuelsson, The effect of photoinhibition on the photosynthetic light-response curve of green plant cells (*Chlamydomonas reinhardtii*). *Planta* **182**, 161–168 (1990).
11. O. Björkman, B. Demming and T. J. Andrews, Mangrove photosynthesis: response to high-irradiance stress. *Aust. J. Plant Physiol.* **15**, 43–61 (1988).
12. G. Oquist and G. Malmberg, Light and temperature dependent inhibition of photosynthesis in frost-hardened and un-hardened seedling of pine. *Photosynth. Res.* **20**, 261–277 (1989).
13. C. B. Osmond, Photorespiration and photoinhibition. Some implications for the energetic of photosynthesis. *Biochim. Biophys. Acta.* **639**, 77–98 (1981).
14. G. Torzillo, B. Pushparaj, F. Bocci, W. Balloni, R. Materassi and G. Florenzano, Production of *Spirulina* biomass in closed photobioreactors. *Biomass* **11**, 61–64 (1986).