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Effects of light on pigments and photosynthetic activity in a phycoerythrin-rich strain of *Spirulina subsalsa*

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ABSTRACT: Data on acclimation to 2 photon flux densities (15 and 100 µmol photons m^{-2} s^{-1}) in *Spirulina subsalsa* strain 3F, a highly fluorescent phycoerythrin-rich cyanobacterium isolated from the brackish Lake Faro (Messina, Italy), indicated plasticity of the photosynthetic apparatus of this organism. High-irradiance grown cells showed the greatest photosynthetic capacity even though they had a lower chlorophyll and phycobiliprotein content. Carotenoids decreased to a lesser extent but their composition changed. β-carotene decreased, while the amount of myxoxanthophyll more than doubled. The stability of both C-phycoerythrin and C-phycocyanin ratios in cells grown under different light quality (green and red) demonstrated the lack of complementary chromatic adaptation in *S. subsalsa*. This factor, combined with the efficient utilization of low-wavelength light, indicates the strong adaptation of this strain to its habitat.

KEY WORDS: Pigments · Oxygen evolution · Photoacclimation · *Spirulina subsalsa* · Cyanobacteria

INTRODUCTION

Cyanobacteria of the genus *Spirulina* Turpin are frequently found in thermal springs and in brackish or marine waters, mostly eutrophic, where they can form dense populations, and make major contributions to primary productivity (Anagnostidis & Golubic 1966, Tomaselli Feroci & Balloni 1976, Castenholz 1977, Bazzichelli et al. 1978). The common species *S. labyrinthiformis* Gomont, *S. major* Kuetzing and *S. subsalsa* Oersted are characterized by a blue-green pigmentation due to the presence of C-phycocyanin, the main light-harvesting pigment of most cyanobacteria. The present study concerns a strain of *Spirulina subsalsa*, isolated from the brackish meromictic Lake Faro near Messina (Italy), which shows an unusual red pigmentation, due to phycoerythrin-rich phycobilisomes (Tomaselli et al. 1990). The phycoerythrin in this strain possesses a high degree of autofluorescence which increases with exposure to increases in photosynthetic active radiation (PAR) (Tomaselli et al. 1993).

We have examined the effects of distinct photon flux density (PFD) and spectral quality on growth, pigment composition and photosynthetic activity of this particular strain. Our results, besides contributing to the understanding of photoadaption in *S. subsalsa*, could provide useful information for the possible exploitation of this strain as a source of natural fluorescent dye in immunofluorescent assays (Strier et al. 1985).

MATERIALS AND METHODS

Organism and growth conditions. *Spirulina subsalsa* strain 3F was isolated from a water sample collected at a depth of 10 m in Lake Faro. Stock cultures were maintained under 25 µmol photons m^{-2} s^{-1} of continuous PAR, at 26°C on MN medium (Rippka et al. 1979). For the experiments on photoacclimation, cells were cultured in a Gallenkamp orbital incubator at 26°C, in an atmosphere of CO_2-enriched air (5%). Continuous irradiation of 15 µmol photons m^{-2} s^{-1} (LI) and 100 µmol photons m^{-2} s^{-1} (HI) was provided by cool-white fluorescent lamps. Cultures were allowed to acclimate to irradiance levels by diluting them every week with fresh medium. In this way, cells had been acclimated for at least 6 generations before biochemical and physiological determinations were made. Sim-

ilarly, to determine the effects of spectral quality on cell pigment content, *S. subsalsa* cultures were incubated for a month in white (25 µmol photons $m^{-2} s^{-1}$), red (30 µmol photons $m^{-2} s^{-1}$) and green light (28 µmol photons $m^{-2} s^{-1}$). Chromatic illumination was obtained by interposition of appropriate colored plastic filters between the cultures and the light source (400 W incandescent lamp).

Biomass and pigment analyses. Growth was determined by cell dry weight; trichomes were collected by filtration through a membrane filter of 3 µm pore size, washed and dried at 70°C to constant weight. Protein, carbohydrate and lipid cell contents were determined as previously reported (Tredici et al. 1988). Chlorophyll *a* (chl *a*) and phycobiliprotein (PBP) contents were determined spectrophotometrically using the methods of Vonshak et al. (1985), and of Bennet & Bogorad (1973), respectively. Total carotenoid content was estimated spectrophotometrically as reported by Paoletti (1969). Specific carotenoids were isolated by thin layer chromatography (Kieselgel 60, Merck) and identified spectrophotometrically in different solvents, following Davies (1965).

Photosynthetic activity determination. Rates of photosynthetic oxygen evolution and respiratory oxygen uptake were measured on *Spirulina subsalsa* cultures, diluted to a final concentration of about 2.5 mg chl *a* l^{-1} with fresh medium containing 1 g l^{-1} $NaHCO_3$, using a Clark-type O_2 electrode, at 26°C under PFDs ranging from 25 to 760 µmol photons $m^{-2} s^{-1}$ (PAR). Red and green light was obtained as described above.

Statistical analyses. Effects of PFD and spectral quality on pigment content and photosynthetic activity were compared by an analysis of variance (ANOVA). The significance between pairs of variable means was analyzed by a least significant difference (LSD) analysis.

RESULTS

Laboratory cultures of *Spirulina subsalsa* strain 3F showed the prevalence of the C-phycoerythrin (CPE) over the other photosynthetic pigments. In cultures growing at low irradiance (below 40 µmol photons $m^{-2} s^{-1}$) CPE cell content, which represented about 20% of total cell protein, was more than 10 times greater than that of chl *a*. It was also about 4 times greater than the sum of the contents of C-phycocyanin (CPC) plus allophycocyanin (APC). Eight distint carotenoids were revealed by thin layer chromatography, 4 of which were present in significant amounts: β-carotene, myxoxanthophyll, zeaxanthin and a carotenoid having spectral characteristics and Rf values similar to β-cryptoxanthin.

Effect of PFDs and spectral quality on pigment content

Spirulina subsalsa cultures exposed to increased PFD showed a general reduction in pigment cell content. PBPs content decreased more markedly than chl *a* content (about 70% and 57%, respectively) (Table 1). Therefore, with increasing PFD, there was a reduction in the CPE:chl *a* and CPC:chl *a* ratios. Total carotenoid content was reduced with increasing PFD, but to a lesser extent than chl *a* content, resulting in an increase of the total carotenoids:chl *a* ratio. Decreases in the relative abundance of β-carotene in the carotenoid pigments coincided with increasing PFD, while myxoxanthophyll increased noticeably. Due to the reduction of β-carotene, zeaxanthin was the prevalent carotenoid at higher irradiance (Fig. 1).

Cell pigment composition of *Spirulina subsalsa* was altered little by spectral quality (Table 1). The ratio of phycoerythrin contents after growth in red and green light [CPE (red):CPE (green)] and the one of phycocyanin contents under the same conditions [CPC (red):CPC (green)] did not vary by more than 15%, indicating a lack of chromatic adaptation in this strain.

Photosynthetic activity of LI and HI cultures

The curves of photosynthetic activity to increasing PFD of *Spirulina subsalsa* 3F cultures grown at LI or HI showed that the strain underwent some degree of

Table 1 Effects of photon flux density (PFD) and spectral quality on pigment composition (mg g^{-1} dry weight) of *Spirulina subsalsa* strain 3F. Data are means ± standard deviation (n = 4). For each experiment, variable means with the same letter are not significantly different ($p > 0.05$)

Light conditions	Chl *a*	CPE	CPC	APC	Carotenoids
White light (100 µmol photons $m^{-2} s^{-1}$)	4.02 ± 0.18 a	29.32 ± 1.94 a	6.09 ± 0.68 a	0.88 ± 0.15 a	0.97 ± 0.10 a
White light (25 µmol photons $m^{-2} s^{-1}$)	9.42 ± 0.32 b	96.50 ± 2.49 b	22.90 ± 1.35 b	1.58 ± 0.12 b	1.39 ± 0.15 b
Red light (30 µmol photons $m^{-2} s^{-1}$)	8.87 ± 0.26 c	88.55 ± 2.84 c	22.42 ± 0.72 bc	1.32 ± 0.11 c	–
Green light (28 µmol photons $m^{-2} s^{-1}$)	9.73 ± 0.51 b	100.62 ± 2.63 d	21.40 ± 0.71 c	1.46 ± 0.15 bc	–

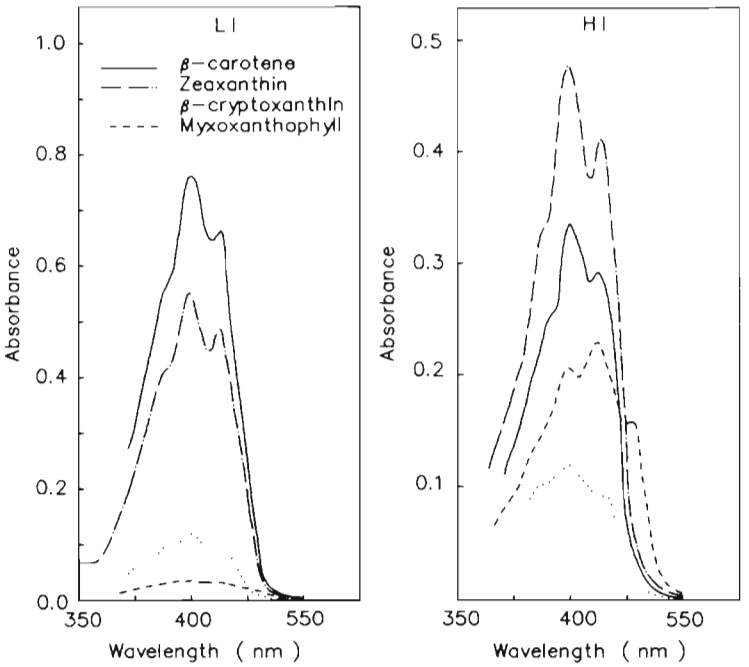

Fig. 1. Absorption spectra of the prevailing carotenoids from *Spirulina subsalsa* 3F cultures grown under 15 µmol photons m^{-2} s^{-1} (LI) and 100 µmol photons m^{-2} s^{-1} (HI) of continuous PAR. Note that the absorbance scales are different

acclimation (Fig. 2). In fact, at PFDs greater than 150 µmol photons m^{-2} s^{-1} the photosynthetic activity of the HI cultures was always higher than that of LI cultures. The maximum photosynthetic activity (P_{max}) in the former was more than 20% greater than in the latter. As expected, dark respiration was also greater in HI cultures (79 µmol O_2 mg^{-1} chl *a* h^{-1}) than in LI cultures (39 µmol O_2 mg^{-1} chl *a* h^{-1}). The greater photosynthetic activity observed at PFDs less than 150 µmol photons m^{-2} s^{-1} in cultures acclimated to LI (Fig. 2) is explained by the great capacity of this strain to alter light-harvesting through a considerable increase of pigment cell content, especially CPE (Table 1).

Effect of PFD on growth and cell composition

Growth rate increased with the increase of PFD levels and was enhanced up to 100 µmol photons m^{-2} s^{-1}. Cultures growing at high irradiance showed a mean generation time of 27 h, whereas it took 36 h in the cultures growing at 25 µmol photons m^{-2} s^{-1}. The biochemical composition of *Spirulina subsalsa*, expressed as % of dry weight, changed from low to high growth irradiance, shifting from a prevalence of proteins (47.7% compared to 33.2% of carbohydrates) to a prevalence of carbohydrates (43.6% compared to 36.8% of proteins). Lipids remained unchanged (7.1% and 6.8% under low and high irradiance, respectively).

Effect of spectral quality on photosynthetic activity

The photosynthetic activities of *Spirulina subsalsa* cultures exposed to white, red and green light at similar PFDs were compared (Table 2). Similar values for photosynthetic activity were observed under white and green light, whereas under red light the value was about 20% lower.

DISCUSSION AND CONCLUSIONS

Spirulina subsalsa strain 3F efficiently regulated pigment content in response to PFD changes. It followed classical photoacclimation mechanisms of cyanobacteria, which are decreases in cell content of both chl *a* and PBPs, following a shift from lower to higher growth irradiance (Post 1986, Wyman & Fay 1987, Millie et al. 1990, Falkowski & LaRoche 1991). The pigment required to sustain the energy needs of cells at their division rate decreases (Van Liere & Walsby 1982). The reduction in pigment content, accompanied by the decline of light-harvesting appa-

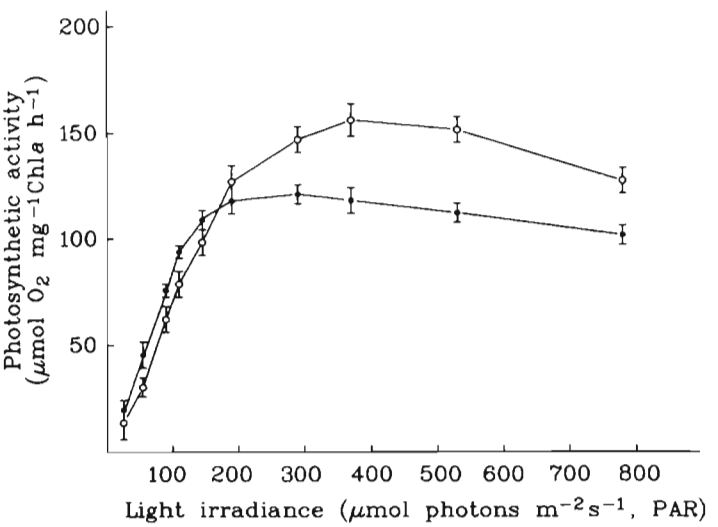

Fig. 2. Photosynthetic activity of *Spirulina subsalsa* 3F cultures acclimated at 15 µmol photons m^{-2} s^{-1} (•) and 100 µmol photons m^{-2} s^{-1} (○) of continuous PAR. Values are means and error bars are ± standard deviations (n = 4)

Table 2. Effects of light spectral quality on photosynthetic activity of *Spirulina subsalsa* 3F cultures. Data are means ± standard deviation (n = 3). Variable means with the same letter are not significantly different ($p > 0.05$)

Light conditions	Photosynthetic activity (μmol O_2 mg^{-1} chl *a* h^{-1})
White light (250 μmol photons m^{-2} s^{-1})	115.00 ± 3.69 a
Red light (260 μmol photons m^{-2} s^{-1})	93.02 ± 3.17 b
Green light (240 μmol photons m^{-2} s^{-1})	117.45 ± 2.13 a

ratus, leads to considerable conservation of energy that may be directed toward the biosynthesis of other cell constituents (Wyman & Fay 1987). Indeed, in our experiments, when compared with cultures growing under low irradiance, those growing under high irradiance showed a reduction in cell contents of chl *a* and even more of PBPs, accompanied by a decreased mean generation time (27 instead of 36 h). The photosynthetic activities of HI grown cultures, measured at incident irradiances greater than 150 µmol photons m^{-2} s^{-1}, were also considerably higher than those of LI grown cultures. This may be a result of a lower incidence of the reciprocal shading of individual pigment molecules, which leads to a greater capacity of light-harvesting by each pigment molecule (Falkowski & LaRoche 1991).

During the acclimation to high irradiance, the observed decrease in the cell chl *a*:carotenoids ratio, specifically affecting the synthesis of chlorophyll, suggests a photoprotective role for carotenoid pigments in *Spirulina subsalsa* 3F, as has been well documented in other cyanobacteria (Kellar & Paerl 1980). Also, the increase in the relative abundance of myxoxanthophyll and zeaxanthin found in *S. subsalsa* 3F, similar to what Millie et al. (1990) observed in *Oscillatoria agardhii*, may be considered an adaptive response for protection against photooxidation. On the other hand, the greater carotenoid pigment content, observed in LI grown cultures of *S. subsalsa* 3F, seems to indicate also a considerable contribution of these lipophilic pigments to light-harvesting, as already proposed for bloom-forming cyanobacteria (Paerl et al. 1983, Paerl 1984). In particular, the β-carotene increase in LI grown cultures, quite comparable with that observed both in *O. agardhii* (Millie et al. 1990) and in *Spirulina platensis* (Olaizola & Duerr 1990), is in agreement with a light-harvesting or structural role of this pigment in the photosystem I (Olaizola & Duerr 1990). Therefore, our results indicate that in the strain 3F carotenoids may serve as an accessory pigment and for photoprotection.

The ability of *Spirulina subsalsa* to increase the β-carotene cell content may be of ecological relevance in the eutrophic, sulphide-rich and scarcely transparent waters of Lake Faro, where the most penetrating component of the downwelling radiation is the low-wavelength blue and green light absorbed specifically by β-carotene and phycoerythrin (Skulberg 1978). The efficient utilization of low-wavelength light shown by this strain also resulted from the greater photosynthetic activities measured under green light in respect to red light of similar quantum flux.

Therefore, *Spirulina subsalsa* strain 3F appears to be well adapted to the environment where it lives with its specific equipment of antenna pigments, the prevalence of CPE, and the capability of extensive accumulation of this pigment under low irradiance. Despite the fact that it grows in a relatively narrow range of low irradiance, this organism shows a certain degree of photoacclimation to high PFD. This is due not only to pigment regulation, but also to its capability to dissipate a greater fraction of the light energy absorbed by CPE as autofluorescence (Tomaselli et al. 1993). The latter phenomenon, previously observed in a marine phycoerythrin-rich *Synechococcus* strain, is considered to be of strategical importance in preventing photoinhibition (Wyman et al. 1985).

The behavior of *Spirulina subsalsa* 3F in the photoacclimation response to high growth irradiance, together with the lack of chromatic adaptation, could be a good basis for the exploitation of this organism as a source of phycofluors.

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