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Adaptation of cyanobacteria to the light regime within Antarctic microbial mats

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Introduction

Benthic cyanobacteria are a ubiquitous component of freshwater ecosystems. In Antarctic lakes, ponds and streams these organisms form epilithic mats and films that often dominate the total ecosystem biomass (Vincent 1988). These communities persist throughout winter darkness and then experience a continuous light regime throughout summer, with maximum irradiances around 1200 μmol photons $\cdot m^{-2} \cdot s^{-1}$.

Although there is now a large literature on the mechanisms of photoadaptation by cyanobacteria in culture, and an increasing level of information about the structure and physiology of microbial mats, there have been very few attempts to combine both laboratory and field approaches towards understanding the adaptative characteristics of cyanobacteria in the natural freshwater environment. Previous work in Antarctica has shown that the thick lake and stream mats are vertically differentiated, with a surface layer often rich in carotenoids and a bottom layer rich in phycobiliproteins (Howard-Williams & Vincent 1989). This pigmentation gives rise to a shift in the spectral quality as well as intensity of light passing down through the mat profile (W. F. Vincent & R. W. Castenholz unpubl. data).

In this paper we examine the photoadaptive responses of an Antarctic cyanobacterium to changes in light intensity and quality. Our aim has been to examine the implications of the in situ irradiance regime of Antarctic microbial mats for cellular growth and pigmentation. We selected a small pond on the McMurdo Ice Shelf ablation zone (78° S, 166° E) that, like other ponds throughout the region, contained a thick epilithic film of cyanobacteria. We made environmental and microbial community measurements in this pond, and then brought one of the cyanobacterial dominants into culture. We then measured the physiological responses of this isolate to a range of irradiances, including spectral conditions similar to those we had recorded in the field.

Methods

Sampling was conducted over two successive summers (1989/90; 1990/91) at Orange Pond, a small (90 m^2), shallow (maximum depth of 1.5 m) waterbody on the McMurdo Ice Shelf near Bratina Island. This ablation zone contains a 1200 km^2 region of freshwater, brackish or saline lakes, ponds and streams, with extensive sheets of benthic cyanobacterial mats (see Howard-Williams et al. 1989). Orange Pond had a conductivity of 3 mS $\cdot$ cm^{-1}, a pH of 9.5 to 10.1 and a summer temperature in the range 0 to 6 °C. The cyanobacterial mat formed a 2–3 mm thick layer with a 0.6–0.8 mm surface orange layer that overlaid a 1.5–2.0 mm thick blue-green coloured bottom layer. The mat was dominated by several oscillatorian species. One of these was brought into culture on BG-11 culture media during the 1990/91 season, and was identified as *Phormidium murrayi* (West & West), Anagnostidis & Komarek, previously called *Lyngbya murrayi* (West & West) (P. A. Broady pers. comm.).

The penetration of spectral irradiance through the cyanobacterial mat was measured with a Licor 1800 Scanning Spectrophotometer with sections of fresh mats (2 cm diameter) laid over the detector. For pigment analysis cores of 10 mm diameter were frozen at −20 °C. They were later ground in 5 ml of 90 % acetone with a Teflon grinder, diluted with 5 ml of 90 % acetone and sonicated for 5 min. Samples were left in the dark at 4 °C for 4 hours. Finally they were centrifuged and filtered through a Whatman (grade GF/F) glass fibre filter. The pigments were separated using reverse-phase HPLC with a binary solvent system. Solvent A was 85 % methanol–15 % 0.17 M ammonium acetate buffer, with a gradient of 90 % acetone – 10 % water (solvent B) at 10 % per minute over the first 10 min. Solvent B continued for 20 min. The peaks were detected and identified with a Shimadzu photodiode array.

The isolated *Phormidium* strain was grown in unialgal culture (non axenic but low bacterized) on BG-11 medium (Rippka et al. 1979). Cultures were incubated at 15 °C under a 24 h light cycle, with cool fluorescent light. Light measurements (photon flux density, PFD) were made with a Biospherical Instruments Inc. 4 π light meter.

Experiments were run in liquid media in Petri dishes, incubated under different coloured light filters: red (peak transmittance (80 %) at 700 nm, falling to less than 20 % at 600 nm), green (transmittance peak at 515 nm (Tmax = 80 %) with a bandwith at 0.5 Tmax of 110 nm

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and 0.2 Tmax of 140 nm) and orange (65% transmittance from 600 to 700 nm, Fig. 1 B), all with the same quantum irradiance of 33 μmol · m^{-2} · s^{-1}. The cultures were harvested after 5–10 days of growth and then homogenized with a Teflon grinder in 10 ml vials. The cell turbidity (optical density O.D.) was immediately measured at 750 nm and then in vivo absorbance spectra were run with a Spectronic 1001 spectrophotometer, operating under the program SPEC.SCAN by Milton-Roy. In vivo spectra were run from 200 to 750 nm at 500 nm · min^{-1} in order to minimize cell sinking effects. The Phycobiliprotein (PB) quantitation followed a modification of Wyman & Fay (1986). Cells were centrifuged then resuspended in glycerol. A portion of Whatman (GF/C grade) glass fibre filter (a quarter of a 45 mm diameter filter) was added in small pieces, followed by homogenization with a Teflon grinder until an homogeneous paste was obtained. This was kept in the dark for 30 min at 4 °C, and then nine volumes of distilled water were rapidly added. The extract was homogenized again and centrifuged, the disruption was repeated one more time. Supernatants were filtered through 0.45 μm Millipore filters to eliminate big pieces of thylakoids. Concentrations were determined using the equations of Bennet & Bogorad (1973).

For chlorophyll *a* (chl *a*) and carotenoids another cell aliquot was resuspended in 90% acetone and homogenized with the Teflon grinder, and then kept in the dark at 4 °C for 30 min. The extract was centrifuged, the pellet then re-extracted and the extracts combined. The concentrations were determined using equations from Marker et al. (1980) for chl *a* and from Britton (1985) for carotenoids. Pigment concentrations were expressed per unit cell concentration as measured by culture turbidity. The latter is considered linearly related to number of cells (Wyman & Fay 1986) within the range of these experiments. Furthermore there was always a strong correlation (r = +0.98) between turbidity and nucleic acid content.

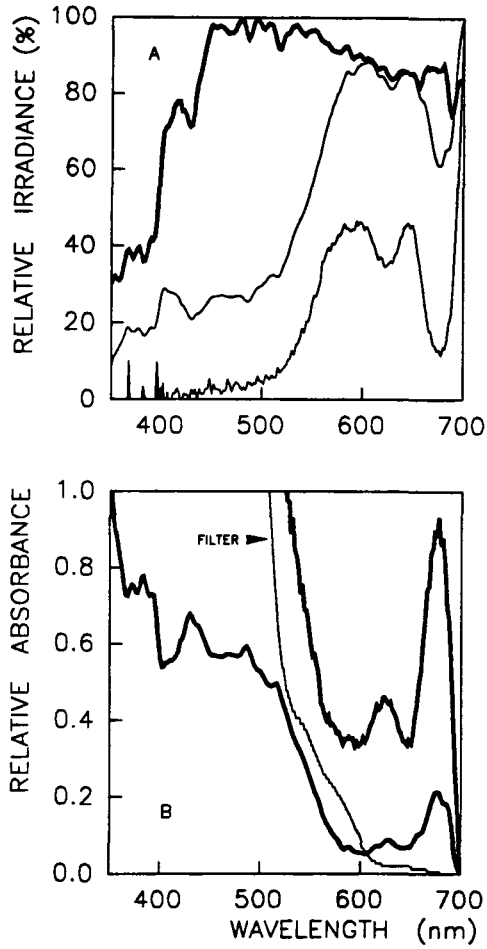

Fig. 1. Light inside the cyanobacterial mat in Orange Pond, Antarctica. A) Relative irradiance (% relative to maximum), above the mat (upper curve), 0.3 mm depth (middle curve) and at 2 mm depth (lower curve). B) Relative absorbance by the mat from 0 to 0.3 mm (upper curve) and 0 to 2 mm (lower curve). The thin line shows the absorbance spectrum for the orange filter used in the subsequent colour experiments.

Results

The spectral distribution of light above and through the mat is given in Fig. 1 A. At the surface the irradiance distribution was practically homogeneous between 450 and 700 nm. The second curve represents the relative irradiance spectrum found at 0.3 mm depth, showing a similar spectral irradiance between 550 and 650 nm, but with troughs at 680 and 625 nm. The bottom curve is for 2 mm depth and shows deeper troughs at 625 and 680 nm. The same data expressed as relative absorbances at two different depths (Fig. 1 B) show the typical cyanobacterial in vivo absorbance spectrum with two peaks, 680 and 625 nm related to chl *a* and c-phycocyanin, plus a broad band of carotenoid absorbance. The thin line in the graph shows the relative absorbance spectrum of the orange filter subsequently used in the orange light experiments, indicating its similarity to the in situ spectral distribution of light.

Studies on the distribution of pigments in the field material showed a deep chl *a* maximum within the mat between 1 and 2 mm depth (Fig. 2 A). The carotenoid content per unit chl *a* (Fig. 2 B)

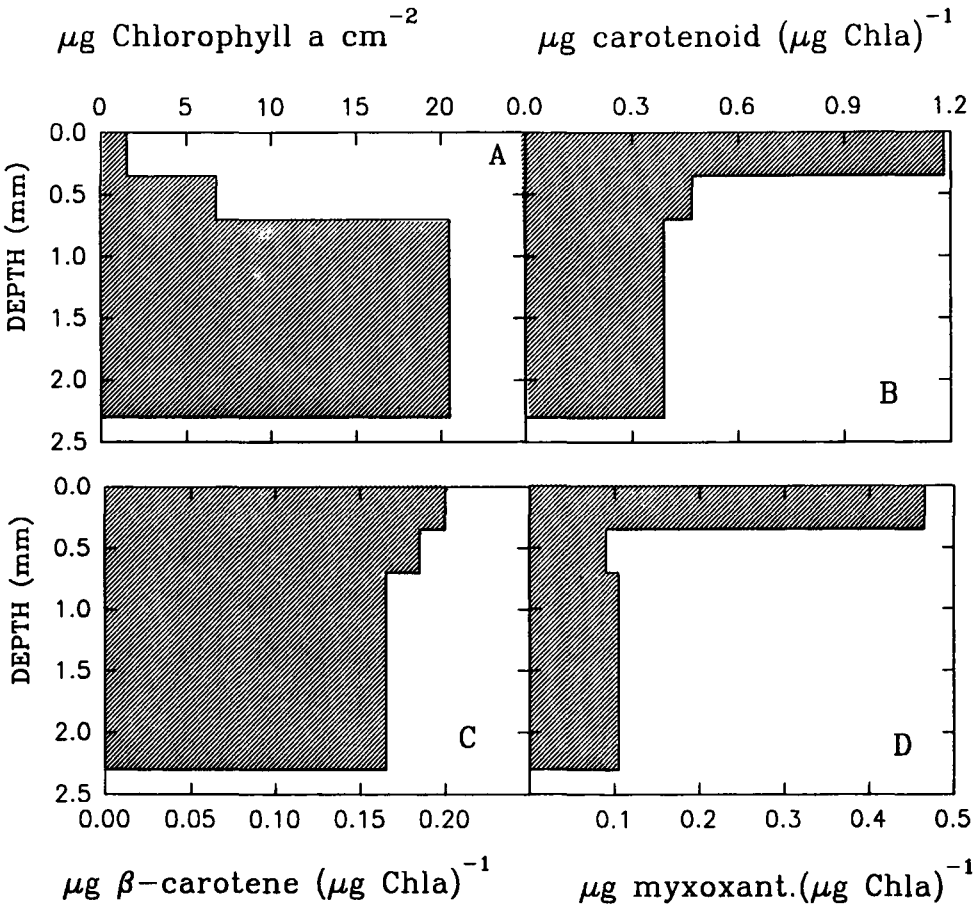

Fig. 2. Pigment profiles through the Orange Pond mat. A) Chl *a* per unit area. B) Carotenoid content, per unit chl *a*. C) β-carotene per unit chl *a*. D) Myxoxanthophyll per unit chl *a*.

showed a gradient in the opposite direction; the maximum was at the very top of the mat with a 50% reduction between 0.5 and 1 mm depth. Values were stable around 0.34 μg carotenoid (μg Chl *a*)$^{-1}$ from the second millimetre to the bottom of the mat. These values are very similar to those found in laboratory experiments in low and medium light intensities (Table 1).

Figs. 2 C and 2 D give the HPLC estimates of two important carotenoids found in this mat. The upper strata of the mat were highly enriched in myxoxanthophyll (per unit chl *a*) whereas the vertical gradient in β-carotene was much less pronounced.

Experiments undertaken to measure the effects of light intensity on this strain were carried out under an irradiance gradient from 20 to 140 μmol photon · m^{-2} · s^{-1}. Growth rates were similar at the two higher intensities but slightly reduced in the lowest light conditions (Table 1). Pigment spectra in Fig. 3 from two different light intensities show that in dim light (40 μmol photon · m^{-2} · s^{-1}) the chl *a* content was higher than in high light, but the carotenoid peak much reduced, indicating chromatic adaptation to different light intensities.

The chl *a* content per cell was almost double in the low light cultures by comparison with the higher light treatment while the carotenoid content per unit chl *a* almost halved across this comparison. The PB per unit chl *a* was 35% higher in the low light adapted cultures by comparison with high light.

Table 1. Growth and pigment composition of *Phormidium murrayii* grown under different light intensities. Growth rate in d^{-1}. O.D. = optical density at 750 nm. Chl *a* in $mg \cdot l^{-1}$, Pigment ratios are by weight. Irradiance conditions: low = 20, medium = 60, High = 140 μmol photons $\cdot m^{-2} \cdot s^{-1}$.

	Irradiance		
	Low	Medium	High
μ (O.D.)	0.13	0.17	0.17
Chl *a*/O.D.	8.10	4.19	3.91
Carot/O.D.	2.51	2.14	2.18
Pb/O.D.	78.48	31.84	27.80
Carot/Chl *a*	0.31	0.51	0.56
Pb/Chl *a*	9.69	7.60	7.11
Pb/Carot	31.26	14.90	12.69

Table 2. Growth and pigment composition of *Phormidium murrayii* grown under different spectral irradiance conditions (units as in Table 1).

	Spectral irradiance			
	White	Orange	Green	Red
μ (O.D.)	0.28	0.28	0.23	0.33
Chl *a*/O.D.	6.96	6.40	7.12	6.70
Carot/O.D.	2.50	2.11	2.20	2.54
Pb/O.D.	47.54	35.07	56.17	34.10
Carot/Chl *a*	0.36	0.33	0.31	0.38
Pb/Chl *a*	6.83	5.48	7.89	5.09
Pb/Carot	18.97	16.66	25.45	13.39

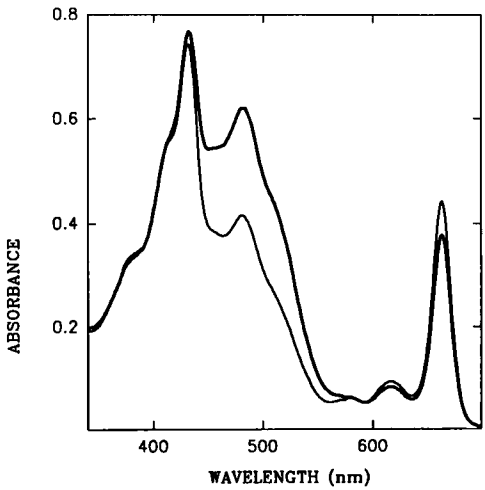

Fig. 3. Absorbance spectra of chl *a* and carotenoids extracts from cultures grown under two irradiance conditions. Thick line: high light (140 $\mu E \cdot m^{-2} \cdot s^{-1}$). Thin line: low light (40 $\mu E \cdot m^{-2} \cdot s^{-1}$).

Table 2 summarizes the results obtained in the colour filter experiments. The cultures grew at the same rate under white and orange light, while the cells under green light had lower growth rates and those under red light had higher growth rates. The cellular content of chl *a*, carotenoids and PB differed between treatments, but the effects were small relative to those induced under the white light gradient. The greatest changes were in PB content, which was maximal under green light and c.a. 40% lower in the red and orange light treatments (Table 2).

Discussion

The results presented here confirm the high level of spectral partitioning down the mat profile in an Antarctic community of mat-forming cyanobacteria. The surface layer of the mat was rich in carotenoid pigments per unit chl *a* and effectively screened the high energy UV and blue wavelengths. Most of the microbial biomass was located well below the surface where the cells experienced a dim, orange-light, shade environment.

Laboratory assays indicated that many of the pigment characteristics observed in the field were a response to light intensity rather than to spectral quality. The white light gradient induced major changes in chl *a* and in the carotenoid: chl *a* ratio. These properties were relatively unresponsive to light quality. Cellular PB showed the strongest response to spectral conditions, but the changes were much less than those induced by the white light gradient. Furthermore, the PB content was lowest under orange light and highest under white light, completely contrary to the pattern observed in the field. These results imply that the pigment profiles in Antarctic cyanobacterial mats are primarily a reflection of changes in the absolute photon flux density down the profile and that changing spectral quality plays a much lesser role.

The influence of light intensity on chl *a* content conformed to the usual pattern observed in culture (e.g. Fontes et al. 1991) with an increased cellular concentration, and thus light absorption efficiency (e.g. Ganf et al. 1991), under reduced intensities. Contrary to expectation (e.g. Post 1987), however, the orange light treatment did not elict a similar response, implying that the PB pigmentation was able to harvest adequately the photons within this waveband. The efficiency of orange light capture by PB in this species is also suggested

by the comparison of PB/O.D.; this ratio was lowest under red light, but only marginally higher (< 5%) under orange light. The cellular PB content of *P. murrayii* was more responsive to changing intensities and spectral composition than chl *a*, and much more so than the total carotenoid content. This response flexibility by PB is well known in cyanobacteria (e.g. LORIMIER et al. 1992, BRYANT 1982, MILLIE et al. 1990), and probably reflects the high efficiency of light harvesting and energy transfer within the phycobilisome, the PB-containing pigment complex (e.g. MILLIE et al. 1990). The capacity for cellular PB adjustment, however, seems to vary enormously between genotypes. FONTES et al. (1991) found a constant ratio of PB: chl *a* under all PFD conditions, while WYMAN & FAY (1986) showed that the response of this ratio to light differed between 8 strains. Large differences in the PB: chl *a* ratio have been observed between different strains of the same genus isolated from Spanish ricefields (FERNANDEZ VALIENTE pers. comm.).

The total cellular content of carotenoids changed very little across the broad range of intensities and spectral conditions evaluated in our experiments. The maximum carotenoid: chl *a* ratios were observed under the highest PFDs, implying that the primary role of carotenoids was photoprotection (YOUNG 1991) rather than light-harvesting (cf. HAXO 1985). The differences in depth distribution of the carotenoids analysed by HPLC may reflect differences in their cellular location. β-carotene is found in close proximity to chl *a* on the thylakoid membrane where it may confer stability to the photosynthetic apparatus, especially photosystem II. Myxoxanthophyll, on the other hand, appears to be associated with cytoplasmatic structures (KANA et al. 1988) and may act as more general cellular protectant against photooxidation (MILLIE et al. 1990, YOUNG 1991).

In conclusion, this study shows that many of the physiological properties observed in an Antarctic cyanobacterial mat can be induced within a single species exposed to specific irradiance conditions. The changing PB, chl *a* and total carotenoid ratios appear to be primarily a response to changes in light quantity rather than quality down the mat profile. These changes strongly influence the in situ spectral irradiance profile through the mat, and result in a dim, orange-light environment at the depth of maximum microbial biomass. Such conditions are highly favourable for the growth of PB-rich cyanobacteria.

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