Photosynthetic and biochemical characterization of pigments and UV-absorbing compounds in *Phormidium tenue* due to UV-B radiation

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Abstract We report the effect of UV-B radiation (0.8 ± 0.1 mW cm^{-2}) and UV-B radiation supplemented with low-intensity PAR (~80 μmol photons m^{-2} s^{-1}) on the photosynthesis, photosynthetic pigments, phosphoglycolipids, oxidative damage, enzymatic antioxidants, and UV-absorbing compounds in *Phormidium tenue*, a marine cyanobacterium. UV-B radiation resulted in a decline in photosynthesis and photosynthetic pigments leading to lower biomass. *P. tenue* synthesized UV-absorbing compounds like mycosporine-like amino acids (MAAs) and scytonemin in response to UV-B radiation. Quantity of MAAs and scytonemin was higher when UV-B was supplemented with low-level PAR. UV-B treatment also resulted in quantitative changes in phosphoglycolipids of the membrane. The UV-B treatment resulted in a slight increase in the level of peroxidation of cell membrane and very little increase in the activity of superoxide dismutase (SOD). Results indicate that UV-B affected photosynthesis and that the main protective system was the synthesis of MAAs and scytonemin-like compounds rather than antioxidant enzymes such as SOD.

Keywords Chlorophyll fluorescence · Cyanobacteria · Lipids · MDA formation · Mycosporine-like amino acids · Pigments · Phycobilisomes · Scytonemin · Superoxide dismutase · UV-B

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Introduction

The springtime stratospheric ozone (O$_3$) layer over the Antarctica is thinning by as much as 50% (Yip 2000), resulting in increased mid-ultraviolet (UV-B 280–320 nm) radiation reaching the surface of the earth and ocean. UV radiation can cause a broad spectrum of genetic and cytotoxic effects in aquatic organisms. However, these responses are partly offset by various protection strategies such as morphological adaptations (Ma and Gao 2009), avoidance, screening, photochemical quenching, and repair (Roy 2000). One of the important concerns with ozone depletion is the potential contribution to global greenhouse gas warming by reducing the sink capacity for atmospheric carbon dioxide through UV inhibition of marine primary production (Takahashi et al. 1997). Multiple harmful effects of UV-B on marine primary producers have been reported and include the direct influences on molecular targets such as nucleic acids, proteins, and pigments (Franklin and Forster 1997) and depression of photosynthesis that ultimately results in cell death (Bhandari and Sharma 2007).

High energetic UV-B radiation has greater potential for cell damage caused by both direct effects on DNA and proteins and indirect effects via the production of reactive oxygen species (ROS; Vincent and Neale 2000). There are several targets for the potentially toxic ROS including pigments, lipids, DNA, and proteins; moreover, damage to photosynthetic apparatus is also partially mediated by ROS (He and Häder 2002). Morphology, cell differentiation, survival, growth, pigmentation, motility and orientation, nitrogen metabolism, phycobiliprotein composition, protein profile, DNA, and CO$_2$ uptake have been reported to be affected by UVR (Ma and Gao 2009; Lesser 2008).

An important physiochemical adaptational mechanism against biologically harmful UV radiation involves the biosynthesis and accumulation of photoprotective UV screening compounds like mycosporine-like amino acids (MAAs) and scytonemin. Widespread occurrence of MAAs in cyanobacterial strains isolated from various habitats exposed to strong radiation has been reported supposedly to protect the cell by absorbing incoming UV radiation (Garcia-Pichel et al. 1993). MAAs are small, water-soluble molecules of imino carbonyl derivatives of cyclo-hexanone with absorption maxima between 280 and 360 nm. Scytonemin, a water-insoluble molecule, occurs in the extracellular mucilaginous sheath surrounding cyanobacterial cells and also considered to be a photoprotective compound (Garcia-Pichel and Castenholz 1991).

In this study, we investigated the effect of UV-B and UV-B radiation supplemented with low-intensity PAR (80 µmol photons m^{-2} s^{-1}) on photosynthesis, photosynthetic pigments, phosphoglycolipids, lipid peroxidation, antioxidant enzymes such as superoxide dismutase, and UV-absorbing compounds like MAAs and scytonemin in the marine cyanobacterium, *Phormidium tenue*.

Materials and methods

Phormidium tenue (Meneghini) Gomont (strain GU 3) was isolated from marine habitats of Goa, India. The species was isolated and identified at Biological Oceanography Division, National Institute of Oceanography, Dona Paula, Goa. *Phormidium tenue* is filamentous, with filaments unbranched, parallel, and sheathed. Trichomes are isopolar, more or less straight, uniserial, composed of cylindrical up to slightly barrel-shaped cells, constricted or unconstricted at the cross walls. Cell content is usually blue-green, thylakoids situated perpendicularly to the cell wall. Heterocytes and akinetes are absent, reproduce by means of fragmentation.

The cultures were routinely grown in autoclaved ASN III medium according to Rippka et al. (1979). Culture was maintained in 100-mL conical flasks filled to 40% of their volume and kept on a shaker set to a temperature of 30±2°C under cool white fluorescent tubes providing approximately 80 µmol photon m^{-2} s^{-1} PAR at the culture level with a 12-h photoperiod. The alga was allowed to grow for 30 days to obtain the balanced growth phase based on biomass (logarithmic phase based on growth, Vonshak 1985). The balanced growth phase (31–33 days) was determined on the basis of F_v/F_m ratio, chlorophyll content, fresh weight, and dry weight measurements (Fig. 1).

Cyanobacterial culture (1 mL) of the respective growth phase was taken out from well-stirred subcultured conical flask and centrifuged for 10 min at 3,000×g. The excess
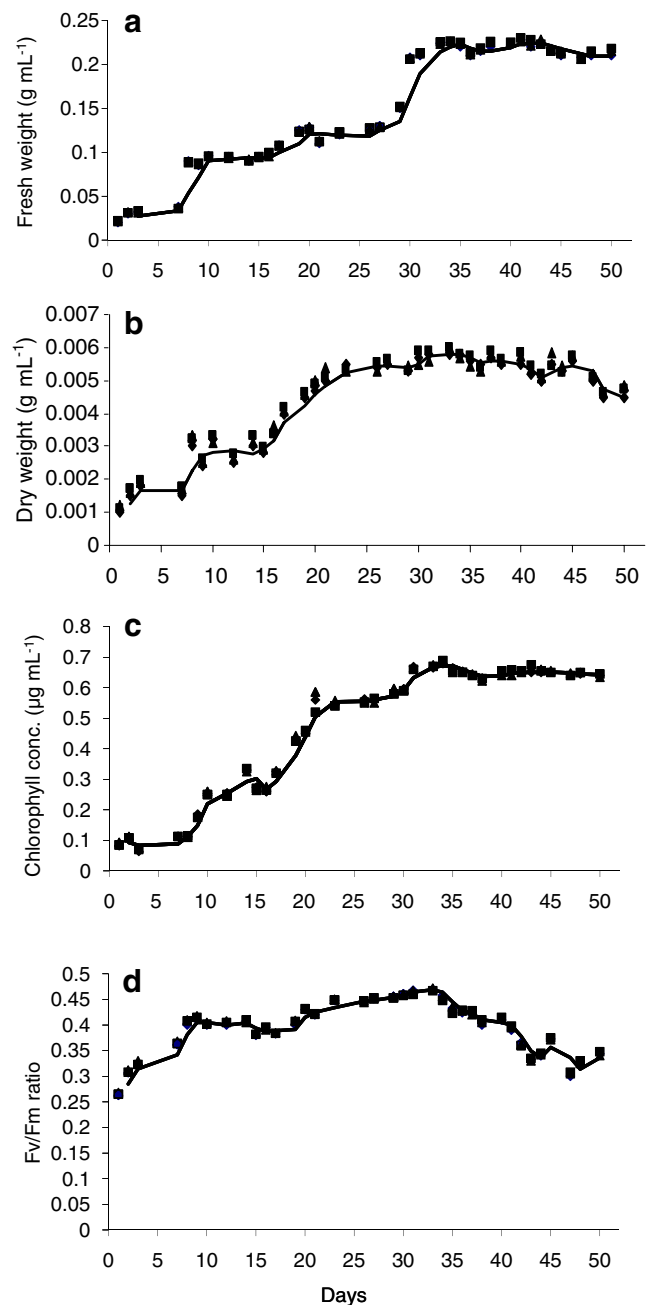
Fig. 1 Determination of balance growth phase based on fresh weight (**a**), dry weight (**b**), chlorophyll measurement (**c**), and chlorophyll fluorescence measurement (F_v/F_m ratio) (**d**) in *P. tenue*

water in the pellet was drained by gently petting it with blotting paper and fresh weight was taken on a digital balance. The pellet of cyanobacterial cells used for fresh weight measurement was dried in an oven at 70°C for 36 h and dry weight was measured.

The UV-B source (Vilbour-Lourmat, France T-6M source with a λ_{max} at 312 nm) was fitted in a BOD chamber. Cyanobacterial culture in an open Petri plate (without lid) was directly exposed to UV-B radiation (0.8±

0.1 mW cm^{-2}) from the top in the BOD chamber at 30°C up to 6 h while keeping the algal culture continuously stirred using a small magnetic flea to avoid the shading of the culture. UV-B radiation was measured using a UV-B radiometer procured from the same supplier (VLX-312 with a λ_{max} at 312 nm). The level of UV-B radiation that the cells received was 0.8±0.1 mW cm^2. UV-B radiometer sensor was placed exactly at the same position where the Petri plate with the culture was placed during the experiments. The distance between the UV-B source and culture was maintained to provide a specific dose (0.8 mW cm^{-2}) of the UV-B radiation. For certain experiments, white light of 80 μmol photons m^{-2} s^{-1} PAR was supplemented using light source with fiber optics at a 60° angle to the culture during the UV-B treatment.

The F_v/F_m ratio, an indicator of photosynthetic efficiency, was measured according to Sharma et al. (1998) using chlorophyll fluorometer PAM 101 with actinic light PAM 102 (Walz, Germany). Culture was dark adapted for 10 min prior to measurement at room temperature. The dark-adapted culture was exposed to a modulated light with an intensity of 3–4 μmol photons m^{-2} s^{-1} to measure initial fluorescence (F_0). This was followed by an exposure to a saturating pulse of white light of 4,000 μmol photons m^{-2} s^{-1} to provide the maximum fluorescence (F_m). Variable fluorescence (F_v) was determined by $F_v=F_m-F_0$ and the F_v/F_m ratio was calculated.

For pigment analysis, cyanobacterial cells were collected after centrifugation of culture at 8,000×g for 15 min and 0.1 g of cells was extracted in 1 mL of 80% (v/v) methanol in a homogenizer at 4°C under dim light, followed by centrifugation at 6,000×g for 10 min at 4°C. The samples were filtered through a 0.2-μm filter prior to HPLC analysis.

The pigments were separated by HPLC according to Sharma and Hall (1996) using reverse phase column (Waters Spherisorb ODS 25 μm×4.6 mm×250 mm) and a PDA detector (Waters 2996). Twenty microliters of the filtered sample was injected into the HPLC. The gradient for separation was 0–100% ethyl acetate in acetonitrile/water (9:1) over 25 min with flow rate of 1.2 mL min^{-1}. The quantity of pigments was calculated from peak area value using β-carotene as external standard. Identification of pigments was carried out using retention time against standards and using the spectral profile of individual peaks using PDA detector in the range of 400–700 nm.

Cell samples were concentrated by centrifugation for 15 min at 6,000×g and 0.1 g of pellet was resuspended in 5 mL of 20 mM sodium acetate buffer, pH 5.5. Cells were broken using sonicator (Bandelin UW 2200, Germany) at 50% power with nine cycles for 1 min. Phycobilisomes were precipitated by incubating with 1% streptomycin sulfate (w/v) for 30 min at 4°C and collected by centrifugation at 8,000×g for 30 min at 4°C. The amount

of phycocyanin, allophycocyanin, and phycoerythrin were calculated according to Liotenberg et al. (1996).

Total lipids were extracted according to Turnham and Northcote (1984). Freshly harvested algal pellet (8 g) was boiled in 5 mL of isopropanol for 2 min to inhibit the lipase activity. This isopropanol solvent was dried using nitrogen gas and the algal pellet was homogenized in chloroform/methanol (1:2, v/v) containing 0.01% BHT as an antioxidant agent to make the final volume to 15 mL. Lipid extract was centrifuged for 5 min at 2,000×g to get rid of cell debris, and to the supernatant, 0.8 mL distilled water was added followed by 5 mL chloroform and 5 mL of 0.88% KCl in a separating funnel to make the ratio of chloroform/methanol/water 1:1:0.9. The mixture was shaken vigorously for 5 min and allowed to separate for 30 min. The solvent phase was collected in screw-capped vials and concentrated under nitrogen gas. The dried lipid extract was redissolved in 5 mL chloroform and was used for qualitative and quantitative determination of different class of lipids.

Total sugars in glycolipids were determined in total lipid extract by the phenol–sulfuric acid method according to Kushwaha and Kates (1981). The absorbance of the orange color was read at 490 nm against a reagent blank. For calibration, standard glucose was used.

Phospholipid was estimated by determining the amount of phosphorus in the total lipid extract according to the Bartlett method by Christie (1982). A blank and series of standard samples was analyzed simultaneously which was measured at 830 nm. The amount of phosphorus in the unknown sample was read from the curve, prepared at the same time.

Lipid peroxidation was determined by the production of TBA-MDA adduct formation according to method described by Sharma et al. (1998). Algal culture was harvested by centrifuging at 8,000×g for 15 min. The algal pellet was homogenized in a tissue homogenizer and redissolved in fresh culture medium with a ratio of 1:5 (w/v). Resuspended algal culture (5 mL) was again centrifuged and algal pellet was homogenized in 0.5% TCA. The homogenate was made up to 5 mL and centrifuged at 8,000×g for 15 min. The supernatant was collected and used for measuring the peroxidation of membrane lipids. One milliliter of the supernatant was added to the test tube containing 2.5 mL freshly prepared (0.5%) TBA in (20%) TCA and allowed to incubate for 30 min at 90°C in a water bath. After incubation, it was allowed to cool at room temperature and centrifuged for 2 min at 1,000×g to settle the debris and non-specific precipitation. The optical density was taken at 532 nm (Schimadzu, UV-250). Peroxidation of lipids was measured using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Superoxide dismutase was assayed according to Boveris (1984). Wet pellet of alga (0.5 g) was extracted

in 5 mL of 50 mM sodium dihydrogen phosphate buffer (pH 7.8) using tissue homogenizer. The extract was centrifuged for 10 min at 6,000×g and supernatant was used for the SOD assay. One hundred microliters of tissue extract was added to 2.6 mL of the assay buffer containing 6 mM EDTA in 10 mM sodium carbonate buffer (pH 10.2) and 300 μL of 4.5 mM epinephrine. Absorbance was recorded at 480 nm. A set of standard with epinephrine but without tissue extract was also assayed separately to calculate the activity. Protein concentration of the supernatant was determined according to Lowry et al. (1951).

Extraction and purification of MAAs was carried out according to the method of Sinha and Häder (2000). Cyanobacterial cells (0.1 g) were homogenized using 20% (*v/v*) aqueous methanol (HPLC grade) and incubated at 45°C for 2.5 h. The pellet was removed and supernatant was evaporated to dryness. The dried supernatant was dissolved in 0.2% acetic acid and analyzed using HPLC (Waters Spherisorb ODS 25 μm×4.6 mm×250 mm), detection program (Waters 2996 PDA detector) at 280 nm with a flow rate of 1.0 mL min^{-1} using isocratic mobile phase of 0.2% acetic acid. Spectra of MAAs were measured using a PDA detector (Waters, 2996) at the wavelength range of 200–700 nm.

The cyanobacteria were homogenized in acetone (100%) and kept for extraction overnight in a refrigerator at 4°C. The resulting suspension was centrifuged and the supernatant was filtered through a 0.2-μm pore filter. Further analysis of the samples was done by HPLC (Waters) according to Sinha et al. (1999). Wavelength range for detection was 240–500 nm with a flow rate of 1.5 mL min^{-1}, and a mobile phase of solvent A (double distilled water) and solvent B (acetonitrile/methanol/tetrahydrofuran, 75:15:10, *v/v/v*) was used with a 0- to 3-min linear increase from 30% solvent B to 3–23 min at 100% solvent B.

Results

Figure 2a shows the effect of UV-B radiation and UV-B radiation supplemented with low-intensity PAR on morphological changes in *P. tenue*. Decrease in fluorescence intensity, indicative of chlorophyll degradation and breakage of filaments, was observed due to the treatments (Fig. 2a). The decrease in chlorophyll fluorescence and breakage of filaments resulted in a linear decrease in F_v/F_m (Fig. 2b). The decrease in F_v/F_m is due to the decrease in F_0 as well as in the F_m (Fig. 2b, inset). Six hours of UV-B treatment as well as UV-B radiation supplemented with low-intensity PAR resulted in a decrease of ~65% in F_v/F_m compared to the respective control (Fig. 2b).
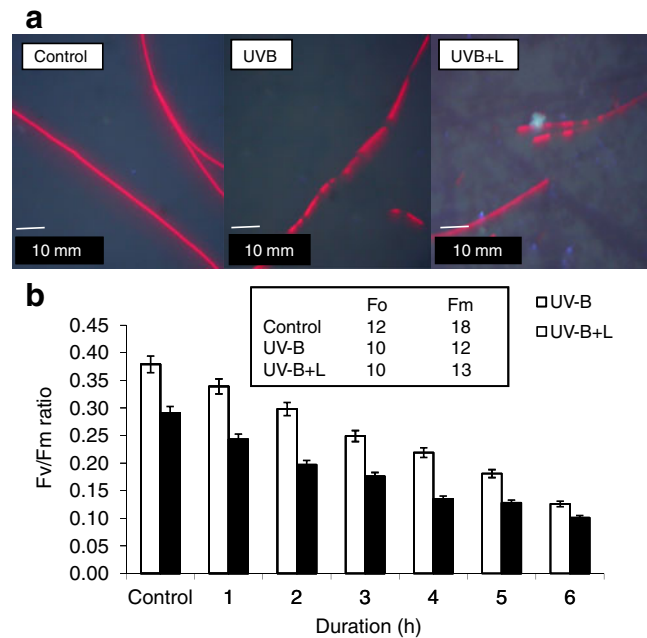
Fig. 2 Effect of UV-B radiation and UV-B radiation supplemented with low-intensity PAR treatment up to 6 h on morphological changes (a) and F_v/F_m ratio (b) in *P. tenue*. *Bar* represents the mean ± SD, *n*=6

Photosynthetic pigments observed in *P. tenue* were chlorophyll a, β-carotene, alloxanthin, and myxoxanthophylls (Table 1). As a result of UV-B alone as well as UV-B supplemented with low-intensity PAR treatments, an overall decrease in pigment content was observed. After 6 h of UV-B treatment, chlorophyll a decreased by 74%, myxoxanthophylls decreased by 81%, and β-carotene decreased by 86% as compared to control (Fig. 3). UV-B radiation supplemented with low-intensity PAR resulted in a greater decline in all the pigments as compared to UV-B radiation alone (Table 1).

UV-B treatment resulted in a 13% decrease in phycocyanin within 1 h, followed by 30% and 55% decrease after 3 and 6 h, respectively, compared to control. Allophycocyanin and phycoerythrin content also showed a linear decrease due to the UV-B treatment (Fig. 4a). UV-B radiation supplemented with low-intensity PAR treatment also gave more or less the same results (Fig. 4b). It was observed that phycocyanin content was much higher than the content of allophycocyanin (59%) and phycoerythrin (16%).

UV-B treatment decreased glycolipids to 55% and phospholipids to 64% compared to the control. However, UV-B radiation supplemented with low-intensity PAR for the same duration did not cause much of a decrease in glycolipids (8%) compared to the control. Percent decrease in phospholipids due to UV-B radiation supplemented with low-intensity PAR was more or less the same as observed for UV-B treatment (Fig. 5a).

Table 1 Effect of UV-B and UV-B supplemented with low visual light for 6 h on photosynthetic pigments (μg g^{-1} fresh weight) in *P. tenue*

Sr. no.	Pigments	Retention time	Absorption spectra (nm)	Amount (in μg g^{-1} fresh weight)		
				Control	UV-B	UV-B+L
1.	Myxoxanthophyll	11.383	450, 475, 506	0.2681	0.0584	0.0494
2.	Alloxanthin	13.313	430, 454, 480	1.2912	0.2355	0.0849
3.	Chlorophyll a	15.030	430, 660	1.8196	0.4688	0.2093
4.	Phycobilins	19.1	410, 504, 534, 608, 666	0.0102	0.0152	0.0157
5.	β-carotene	19.82	448, 480, 600	0.5088	0.0688	0.0195

Fig. 3 HPLC profiles of photosynthetic pigments extracted from *P. tenue* at 445 nm after exposure to UV-B radiation and UV-B radiation supplemented with low-intensity PAR treatment for 6 h. Spectral profile of photosynthetic pigments extracted from *P. tenue* is shown in *inset* of the figure
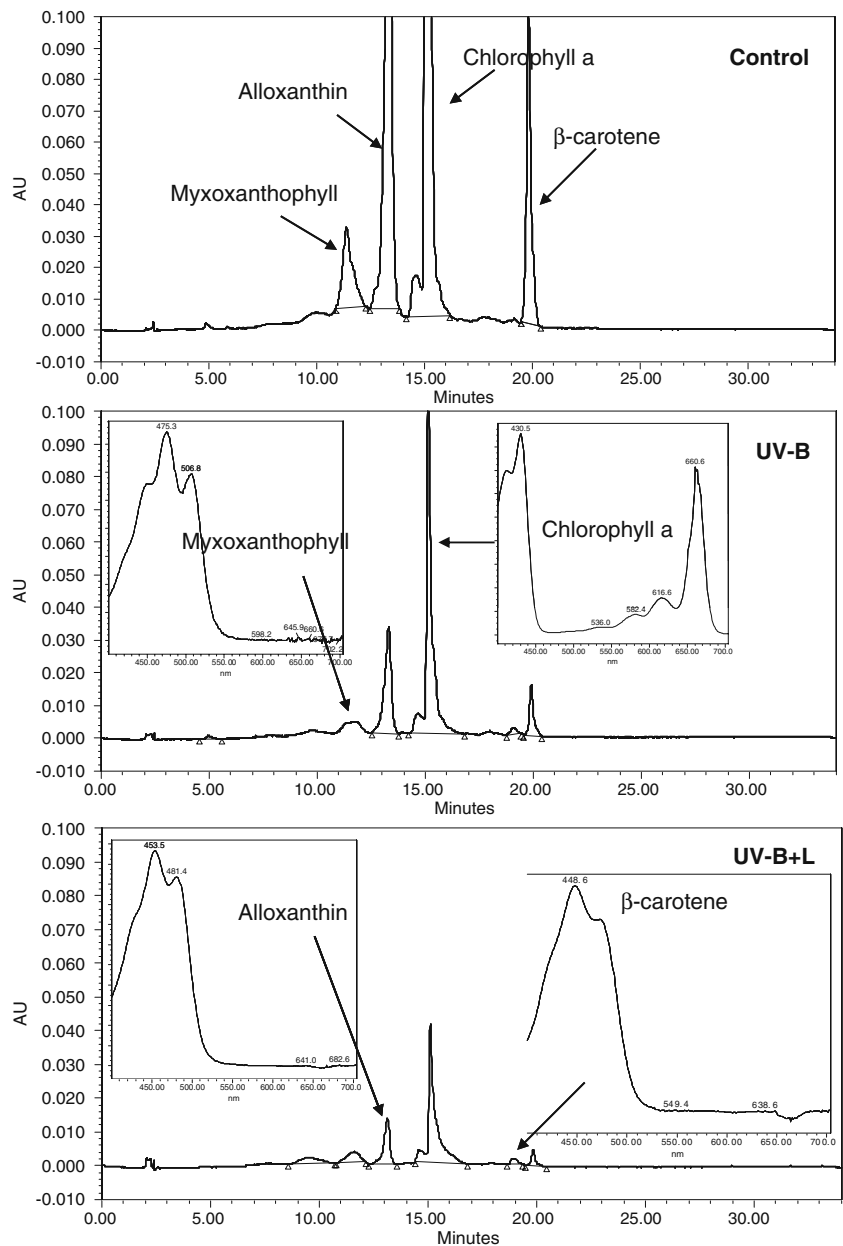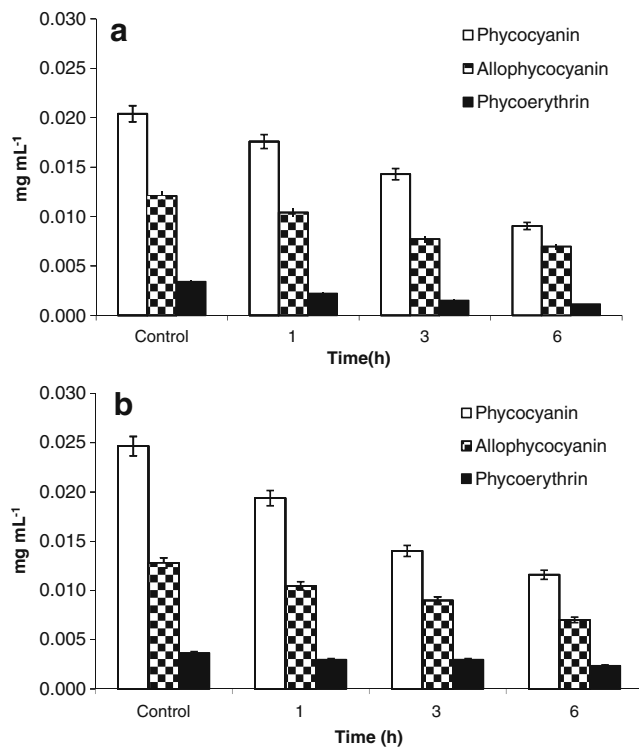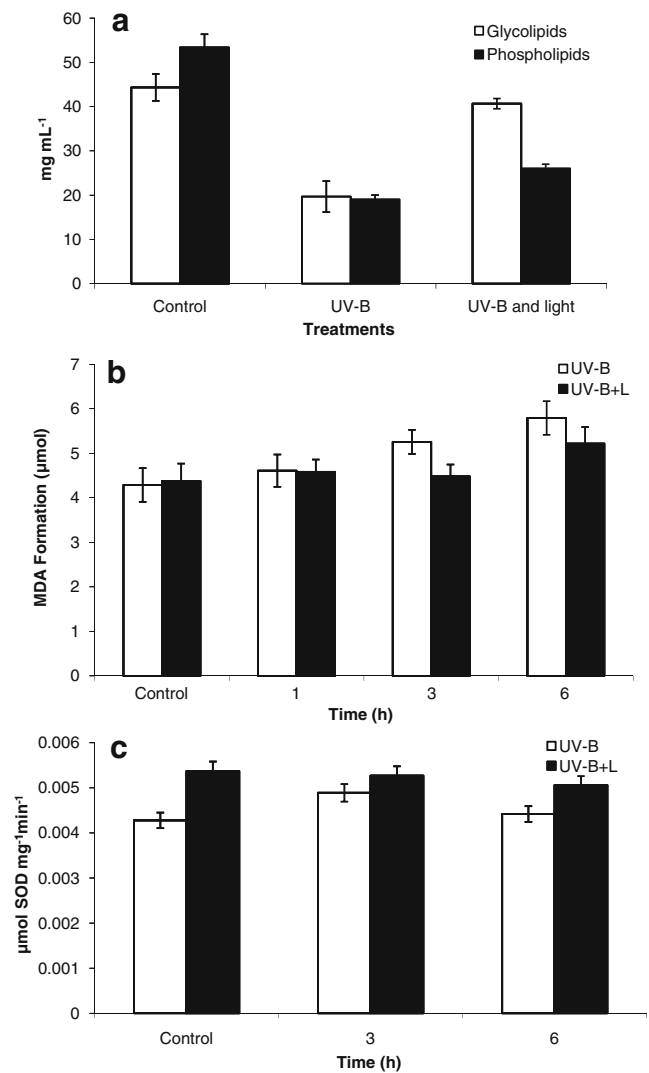
Fig. 4 Effect of UV-B radiation (**a**) and UV-B radiation (**b**) supplemented with low-intensity PAR treatment up to 6 h on phycocyanin, allophycocyanin, and phycoerythrin in *P. tenue*. Mean ± SD, *n*=3–6
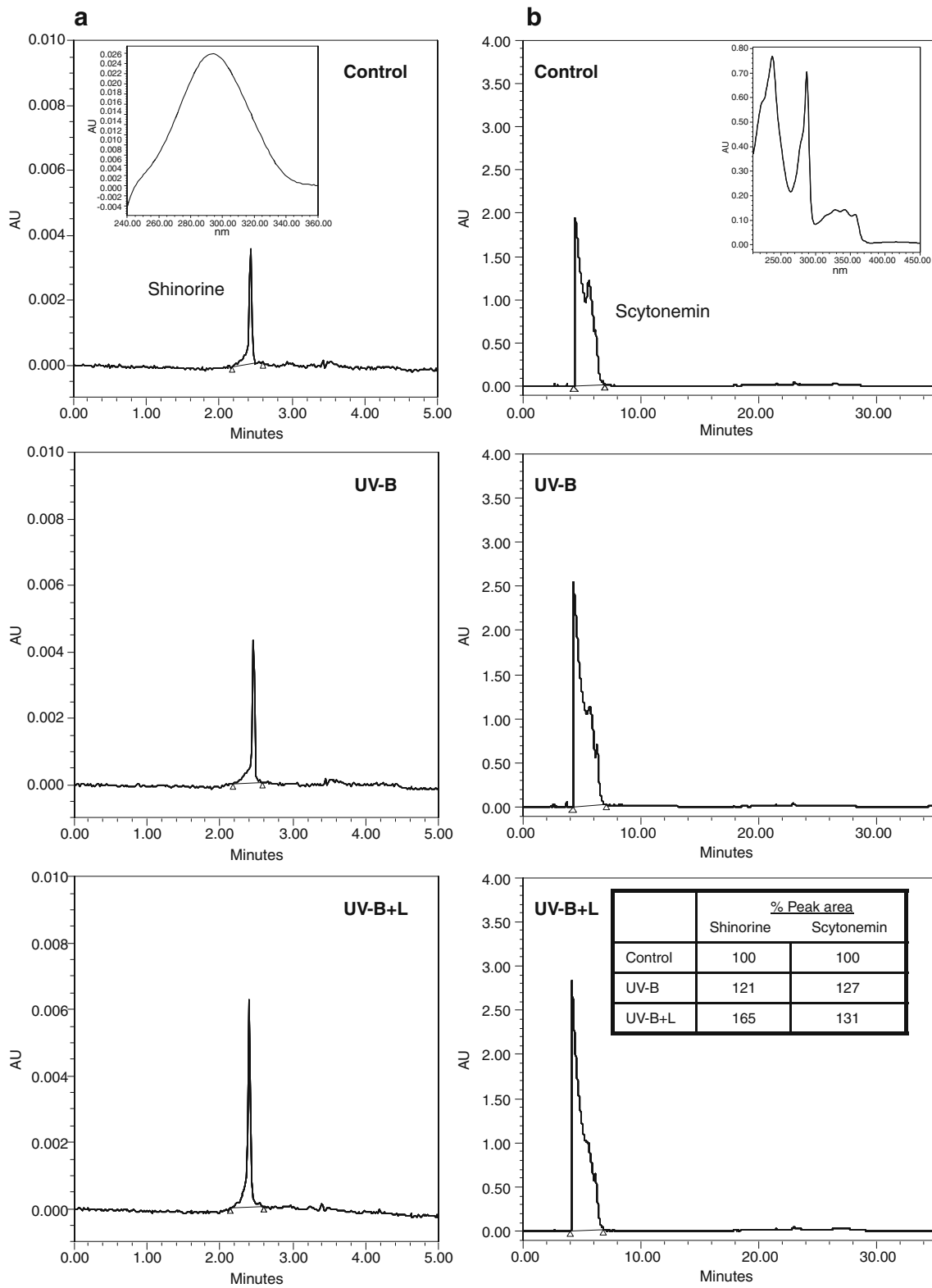

Fig. 5 Effect of UV-B radiation and UV-B radiation supplemented with low-intensity PAR treatment up to 6 h on glycolipid and phospholipid content (**a**), lipid peroxidation (**b**), and superoxide dismutase activity (**c**) in *P. tenue*. Mean ± SD, *n*=4

UV-B treatment led to a linear increase in the peroxidation of cell membrane measured as MDA formation (Fig. 5b). MDA formation increased by 7% after 1 h of UV-B treatment, which was further increased to 22% after 3 h and 35% after 6 h of treatment as compared to control. UV-B radiation supplemented with low-intensity PAR upto 3 h did not show any significant increase in the MDA formation. A 6-h UV-B radiation supplemented with low-intensity PAR treatment, however, increased the MDA formation by only 19% compared to control (Fig. 5b), which was 16% less than that observed with UV-B alone treatment.

A mere 3% increase in the activity of superoxide dismutase was observed as a result of 6 h UV-B treatment as compared to control. UV-B radiation supplemented with low-intensity PAR treatment did not show any increase in the SOD activity (Fig. 5c).

HPLC analysis of *P. tenue* showed the presence of MAAs with an absorption maximum of 294 nm (shinorine; Fig. 6a, inset). A 6-h exposure of UV-B radiation resulted in a 21% increase in MAA as compared to the control. UV-B radiation supplemented with low-intensity PAR resulted in a further increase in the MAA content to 65% as compared to the control.

HPLC analysis also showed the presence of scytonemin with λ_{max} at 237, 273, and 334 nm (Fig. 6b, inset). Exposure of *P. tenue* to UV-B radiation for 6 h increased the scytonemin content by 27% as compared to control. UV-B radiation supplemented with low-intensity PAR further increased the scytonemin content to 31% as compared to control (Fig. 6b).

Discussion

Our results indicate that UV-B radiation affected photosynthesis and, thereby, growth and productivity of the cyanobacterium. The results obtained indicate direct damage to key components within the photosystem since F_m declined (Jansen et al. 1996) as well as loss of photosynthetic pigments since F_0 declined. Decrease in the F_0 is an indicator of a decrease in the excitation energy reaching the

Fig. 6 HPLC profile of (**a**) MAAs and (**b**) scytonemin extracted from *P. tenue* after exposure to UV-B radiation and UV-B radiation supplemented with low intensity PAR treatment for 6 h. Absorption spectra in inset shows spectra for MAAs at 330 nm and for scytonemin at 300 nm. Table in the inset shows the % change in MAA and scytonemin on peak area basis

photosynthetic reaction center II, largely due to the loss of pigments in the light-harvesting complex II, while a decrease in F_m is an indicator of damage to the PS II reaction center itself (Krause 1988). Both photoinhibition of photosynthesis and photobleaching of pigments were used as indicators of UV-B-induced photodamage to the cells. UV-B radiation is known to cause strong photoinhibition in photosynthetic microorganisms (Renger et al. 1989; Holm-Hansen 1996), and the main target has been identified as the PS II D-1 protein in several organisms. As a result of the impaired photochemical reactions of photosynthesis due to the UV-B treatment, ROS are generated, which may lead to bleaching of surrounding pigments, damage to D1 protein, and peroxidation of lipid membranes (Sharma 2002; Bischof et al. 2002; He and Häder 2002; Hideg et al. 1994). Decreases in F_v/F_m, photochemical quantum yield, pigments, and growth in various algal species under UV-B radiation have been observed by others (Fouqueray et al. 2007; Marcoval et al. 2007; Navarro et al. 2007; Gao et al. 2007; Wu et al. 2008). Morphological changes such as reduced trichome length and the differentiation of heterocyst have also been reported (Gao et al. 2007). We also observed breakage of filaments and loss of chlorophyll in *P. tenue* under UV-B treatment (Fig. 2a).

The decrease in photosynthetic pigments due to UV-B treatment in *P. tenue* could be due to the generation of reactive oxygen species, which could also affect pigment content as pigments are highly sensitive to oxidation and peroxidation reactions (Sharma 2002). We observed increased oxidation of photosynthetic pigments due to the UV-B and UV-B radiation supplemented with low-intensity PAR treatment. Our results also showed a slight increase in lipid peroxidation of cell membrane after the UV-B treatment, again indicating oxidative damage to the organism under UV-B treatment in this study. The chromopheric compounds such as chlorophyll, phycobili-proteins, and quinones absorb UV-B radiation and photo-sensitize the generation of ROS, leading to the loss of pigmentation due to bleaching (Carreto et al. 2001; Xue et al. 2007). Lipids along with pigment–protein complexes are also some of the oxidative targets attacked by the elevated ROS, and lipid peroxidation occurs especially at sites where polyunsaturated fatty acids occur in high concentrations. Ultraviolet radiation has been shown to be very effective in inducing lipid oxidation of biological membranes (Kochevar 1990), polyunsaturated fatty acids (Yamashoji et al. 1979), and phospholipid liposomes (Pelle et al. 1990); however; the extent of lipid peroxidation in our study was considerably less. Decrease of photosynthetic pigments in various algal species under UV-B exposure has been also reported by others (De Lange et al. 2000; Cabrera et al. 1997; Germ et al. 2002). It is evident from this study that UV-B promoted the produc-

tion of ROS and induced oxidative stress, leading to the oxidation of pigment and lipid molecules.

Quantitative changes in phospho- and glycolipids observed with UV-B treatment in this study may also be due to oxidative damage caused to cell membrane or damage caused to enzymes involved in lipid synthesis, though not studied here. A significant decrease in the level of sulfoquinosyl diacylglycerol and phosphatidyl-glycerol in the cyanobacterium, *Spirulina platensis*, in response to environmental factors has also been reported earlier (Funteu et al. 1997).

UV-B treatment resulted in a slight increase in the activity of superoxide dismutase, which metabolizes super-oxide radicals. The increase in SOD provides protection against photodamage; however, the increase observed in this study is not significant. This may indicate that a slight increase in SOD activity may not play an important role in *P. tenue*'s defense mechanism as compared to the protection offered by the production of MAAs and scytonemin which results in screening of UV-B radiation. Accumulation of SOD had been reported in cyanobacterium in desiccated field to reverse the effect of oxidative stress imposed by UV-B irradiation (Canini et al. 2001; Xue et al. 2006, 2007). Increased activity of SOD had been shown by Araoz and Häder (1999) to confer increased protection from oxidative damage by detoxifying ROS and regenerating the reduced form of antioxidant. However, in this study, no significant increase in SOD and limited peroxidation of cell membrane (MDA formation) was observed.

Photosynthetic organisms possess features that limit UV-B exposure and reduce the amount of damage sustained. Carotenoids, MAAs, and scytonemin all absorb wave-lengths in the ultraviolet region and are suggested to act as natural sunscreens and/or antioxidant (Karentz et al. 1994). In this study, it was shown that *P. tenue* synthesizes a single MAA, i.e., shinorine, a bisubstituted MAA containing both a glycine and a serine group (Sinha et al. 2003) with absorption maximum at 294 nm, as a result of UV-B and UV-B radiation supplemented with low-intensity PAR treatment (Fig. 6a). The presence of shinorine has been reported in other cyanobacteria (Sinha et al. 2007; Singh et al. 2008) and has been suggested to provide protection against UV-B radiation (Cockell and Knowland 1999). Sinha et al. (1999) had shown that MAAs prevent three out of ten photons from hitting cytoplasmic targets and provide protection as a UV sunscreen pigment. The presence of high concentrations of MAAs in this study may indicate an important protective process by absorbing UV radiation.

Here, we also report the induction of scytonemin in *P. tenue* due to UV-B and UV-B radiation supplemented with low-intensity PAR treatment. There is strong evidence for the role of scytonemin as ultraviolet-shielding compound,

the accumulation of which has been reported in several cyanobacterial isolates mostly exposed to high irradiances (Sinha et al. 1999; Portwich and Garcia-Pichel 2000). In cyanobacterial cultures, as much as 5% of the cellular dry weight may be accumulated as scytonemin. The correlation between UV protection and scytonemin presence has been established under solar irradiance, and it is reported that as much as 90% of the incident UV-B radiation is screened due to the presence of scytonemin in the cyanobacterial sheaths (Brenowitz and Castenholz 1997). Once synthesized, it remains highly stable and carries out its screening activity without further metabolic investment from the cell.

The results indicate oxidative nature of damage to *P. tenue* under our experimental conditions, as indicated by damage to photosynthesis and peroxidation of lipids. We also showed that in response to UV-B radiation, there was a very little increase in the activity of SOD, but greater increase in UV-absorbing compounds like MAAs and scytonemin which probably provide primary protection against UV-B induced damage to the cell organelles and components from the full impact of deleterious effect of UV radiation, whereas SOD (antioxidant enzyme) may not be an efficient system in *P. tenue*, as reported in crop plants.

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