Indian Hydrobiology, 13(2) : 138 - 155, 2011

Changes in membrane lipids in response to high light in freshwater and marine cyanobacteria

Rupali Bhandari[1], *C.G Naik*[2] *and Prabhat Kumar Sharma*[1]*

*[*1]Department of Botany, Goa University, Goa - 403 206, India*

[2]Department of Bioorganics Chemistry

National Institute of Oceanography, Goa - 403 004, India

ABSTRACT

Effect of high light exposure (500 mmol $m^{-2} s^{-1}$ of photosynthetic active radiation: PAR) on *Nostoc spongiaeforme* Agardh, a fresh water cyanobacterium and *Phormidium corium* Agardh (Gomont), a marine cyanobacterium was studied with respect to photosynthesis, lipid peroxidation, antioxidant activity, membrane lipids and its fatty acid composition. The F_v/F_m ratio, which is indicative of photosynthetic efficiency, decreased due to the treatment. The damage to photosynthetic efficiency occurred at the level of antenna system, as Fo was affected and at the PS II level, as Fm was also affected. Peroxidation of membrane lipids was observed, indicating oxidative damage to lipids. Activity of superoxide dismutase (SOD) increased as a result of the treatments to mitigate oxidative stress. FTIR profile of the membrane lipids showed presence of both saturated as well as unsaturated fatty acids in both species in the ratio of 13:9. NMR profile of the membrane lipids showed peaks, representing major lipids. Quantitative changes in phospho and glycolipids were also observed. Unsaturation level of fatty acids of total lipids remains unchanged while saturation level decreased, suggesting changes in the membrane fluidity. The changes in membrane lipids and fatty acids under our experimental conditions may primarily suggest an adaptation strategy by the organism against the high light conditions, then the oxidative damage, as unsaturated fatty acids remained unaffected by the treatment.

Abbreviations: DGDG - digalactosyldiacylglycerol; HL - high light; MGDG - monogalactosyldiacylglycerol; PAR - photosynthetic active radiation; PG - phosphatidylglycerol; PS II - photosystem II; ROS - reactive oxygen species; SQDG-sulfoquinovosyldiacylglycerol.

Introduction

Photosynthetic organisms are dependent on solar energy for synthesizing their food and survival. In aquatic ecosystem, solar radiation affects physical, chemical and biological processes such as thermal stratification and the vertical distribution of nutrients, photoautotrophs, herbivore and consumers. Many photosynthetic organisms are capable of active movements and daily vertical migrations of up to 15 m to regulate their light requirement. Photosynthetic active zone has been reported as deep as 40 m in deep ocean (Menon *et al.*, 2005). Exposure of algae or plants to higher level of PAR can lead to inhibition of photosynthesis, a process known as photoinhibition (Han *et al.*, 2001; Krause 1994). Photoinhibition of photosynthesis affects photochemical reaction, resulting in generation of reactive oxygen species (ROS) which can oxidize membrane lipids, proteins and pigments resulting in membrane unstability as well as photobleaching of the photosynthetic pigments, affecting growth and survivability

of organism (Sharma and Hall 1996; Sharma and Singhal 1992).

Formation of ROS by stress factors, such as high light and temperature, is a common process in living organisms, including algae. However, excessive production of ROS can cause damage to DNA, proteins and lipids (Alscher *et al.,* 1997). Lipids are one of the most sensitive targets for oxidation by elevated ROS, particularly at sites where poly-unsaturated fatty acids occur in high concentrations (Dotan *et al.,* 2004), thus affecting the fluidity of membrane and, in turn, various other biochemical processes, including photosynthesis. An increase in unsaturation and decrease in the chain length of esterified fatty acids can lower the phase transition temperature of membrane lipids (Hazel 1995; Murata and Los 1997) and desaturation of lipids can provide protection against oxidative stress (Sakamoto and Bryant 2003).

Membrane lipid plays both structural and regulatory roles in organism's adaptation and survival when subjected to stress conditions. Lipids, though catalytically inactive, are reported to exhibit diverse biological functions mainly in providing hydrophobic environment for catalyst and structural proteins to function properly and adaptation against changing environment. Survival of algae under changed irradiance conditions can be attributed to the functioning of the membranes, because the degree of fatty acid changes resulting in the extent of unsaturation has been considered as one of the most important factors controlling membrane fluidity and functionality (Mikami and Murata 2003). Changes in fatty acid composition due to stresses can be regarded as an adaptive response to the change in surrounding environment (Wada and Murata 1998). There is currently considerable interest in lipid and fatty acid profile of organism with reference to changing climatic conditions. In this study, we present comparative account of changes taking place in membrane lipids and fatty acids profile and photosynthetic response with reference to high light in *Nostoc spongiaeforme* Agardh GUCC 1, a freshwater cyanobacteria present in rice fields and *Phormidium corium* Agardh (Gomont) GUCC 2, a cyanobacterium isolated from coral reefs off Lakshadweep Island, India.

Materials and methods

Culture conditions

The cultures were routinely grown in autoclaved liquid culture medium, BG 11 (for fresh water cyanobacterium) and ASN III (for marine cyanobacterium) according to Rippka *et al.* (1979). Cultures were maintained in 100 ml conical flasks filled to 40 % of their volume and kept on a shaker set to a temperature of 30°C ± 2°C under cool white fluorescent tubes providing approximately 80 mmol m^{-2} s^{-1} PAR at the culture level with a 12 h of photoperiod. *Nostoc spongiaeforme* and *Phormidium corium* were allowed to grow for 14 days and 30 days respectively to obtain their respective logarithmic phase of growth (balance growth phase).

Exposure to high light

The cyanobacterial cultures were exposed to irradiance of approximately 500 mmol m^{-2} s^{-1} PAR (Li-cor, Model Li-189), which are experienced under their natural growth environment, at the culture level at 30 °C up to 6 h in a double walled cuvette (2 cm diameter) circulated with temperature controlled water using two slide projectors (halogen lamp 25V, 150W) from opposite sides. Culture was kept constantly stirred at a slow speed to avoid shading effect during the treatment without causing mechanical damage to the cells (Bhandari and Sharma 2006a).

Photosynthesis measurements

Photosynthesis measurement was taken using chlorophyll fluorometer (PAM 101-102, Walz, Germany) according to Sharma *et al.* (1998). Culture was dark adapted for 10 minutes prior to measurements at room temperature. The dark adapted cultures were exposed to a modulated light with an intensity of 4 mmol $m^{-2}s^{-1}$ to measure initial fluorescence (F_o). This was followed by an exposure to a saturating pulse of white light of 4000 imol $m^{-2}s^{-1}$ to obtain the maximum fluorescence (F_m). Variable fluorescence (F_v) was determined by deducting the F_o from F_m ($F_v=F_m-F_o$) and the F_v/F_m ratio was calculated.

Peroxidation of cell membrane lipids

Lipid peroxidation was determined by the production of TBA-MDA adduct formation according to method described by Sharma *et al.* (1998). Cyanobacterial culture was harvested by centrifuging at 8000 g for 15 min and pellet was homogenized in 0.5 % TCA. The homogenate was made up to 5 ml and centrifuged at 8,000 *g* for 15 minutes. The supernatant was collected and used for measuring the peroxidation of membrane lipids. 1 ml of the supernatant was added to the test tube containing 2.5 ml of 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}.

Assay of superoxide dismutase

Superoxide dismutase was assayed according to Boveris (1984). Wet pellet of cyanobacteria (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 6000 g. Supernatant was used for SOD assay. 100 ml of tissue extract was added to 2.6 ml of assay buffer containing 6 mM EDTA in 10 mM sodium carbonate buffer (pH 10.2) and 300 ml of 4.5 mM epinephrine. Absorbance was recorded at 480 nm using UV-Visual Spectrophotometer. 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 of total lipids

Total lipids were extracted according to Turnham and Northcote (1984). Briefly freshly harvested cyanobacterial pellet was boiled in 5 ml of isopropanol for 2 min to inhibit the lipase activity and then dried under nitrogen gas. The dried pellet was homogenized in chloroform: methanol (1:2 v/v) to make the final volume 15 ml with 0.01% BHT added as an antioxidant in the lipid extraction solvent system. Lipid extract was centrifuged for 5 min at 2000 *g* to remove cell debris and to the supernatant, 0.8 ml of distilled water was added followed by 5 ml of chloroform and 5 ml of 0.88% potassium chloride 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 and concentrated under nitrogen gas. The dried lipid extract was redissolved in 5 ml of chloroform and was used for quantitative determination of different classes of lipids.

Qualitative determination of lipids

Separation of phosphoglycolipids and neutral lipids into individual lipid classes was carried out in a thin layer chromatography (TLC) on silica gel H according to Liljenberg and Von Arnold (1987). The eluting solvents

were the following: chloroform: methanol: glacial acetic acid:water (85:15:10:3.5) for phospho glycolipids and diethyl ether: water (90:1) for neutral lipids. The spots were visualized in an iodine chamber and identified against standards and R_f values.

Quantitative estimation of glycolipids

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 reagent blank. For calibration, standard glucose of different concentration was used. The amount of sugars in the unknown sample was read from calibration curve, prepared at the same time by performing the reaction on known amount of standard glucose solution.

Quantitative estimation of phospholipids

Phospholipid was estimated by determining the amount of phosphorus in total lipid extract according to Bartlett method (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 calibration curve, prepared at the same time using sodium bisphosphate.

Esterification of fatty acids

The fatty acid methyl esters were prepared for GC analysis according to Christie (1982). The internal standard (1mM heptadeconoic acid) was added to lipid sample and was subjected to methanolysis in the presence of methanolic-HCl at 68-70^{o}C for 2h. The methyl esters were extracted with three successive portions of hexane and was treated with 5 ml of saturated solution of sodium bicarbonate and washed with 5 ml of distilled water and upper solution was evaporated to dryness in a water bath at 35-40^{o}C with the help of nitrogen gas (Bhandari and Sharma 2006b). Briefly the methyl esters were taken in small volume of fresh hexane and 2 µl of sample was injected to the injector port of gas chromatography. Methyl esters of fatty acids were run on a Nucon gas chromatograph equipped with flame ionization detector and chromatopack data processor. The column (6 mm x 2 mm i.d, stainless steel) was packed with DEGS 10% on 80-100 mesh chromosorb W-HP (Chemlabs, Bangalore). Column temperature was 180°C and injector temperature was 220°C and nitrogen was used as carrier gas (flow rate 30 ml/min). Fatty acid methyl esters peaks were identified by comparing their retention times with methyl esters of pure fatty acid standards and were quantified by using the peak areas of individual fatty acids calculated using the program given by the manufacturers of the instrument. The instrument was programmed to give the mole % of different fatty acid directly.

Analysis of lipids by FTIR spectrometry

Extract of total lipids for FTIR analysis was prepared as described above. Lipid sample in chloroform was evaporated to dryness using nitrogen gas and immediately dissolved in 1 ml carbon disulphide (CS_2). A drop of the sample is placed on a sodium chloride (NaCl) window and fixed to sample holder of the FTIR spectrophotometer (Schimadzu FTIR 8201PC). The spectrum was recorded in percent transmittance mode with 4.0 cm^{-1} resolution and absorption bands were identified by Maquelin et al. (2002).

Analysis of lipids by NMR spectrometry

Extraction of total lipids for NMR analysis was prepared as described above. Sample in chloroform was evaporated to dryness using dry nitrogen gas and dissolved in deuterated chloroform ($CDCl_3$). Sample was analyzed using 300 MHz, NMR Spectrometer (Bruker, Avance 300) according to method

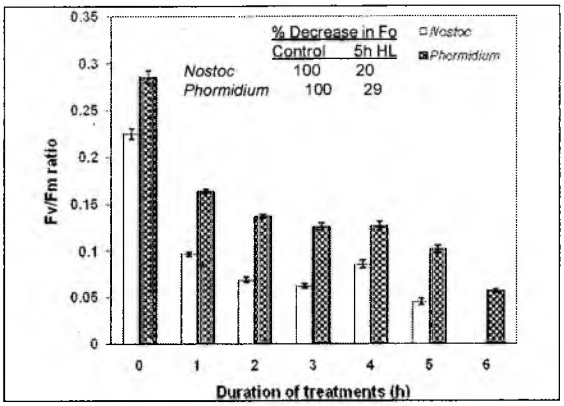

Fig. 1. Effect of high light treatment up to 6 h on Fv/Fm ratio in *N. spongiaeforme* and *P. corium* [Each bar represents the mean ± S.D, n = 5]

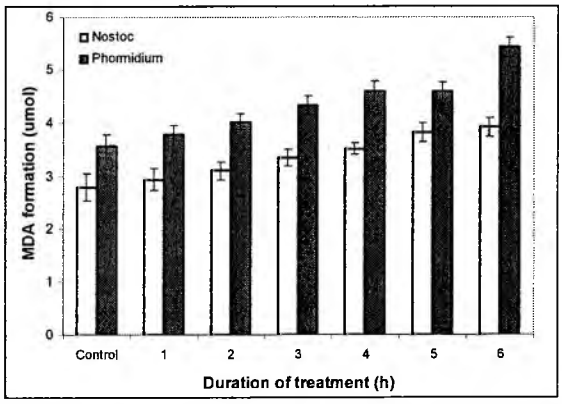

Fig. 2.Effect of high light treatment up to 6 h on lipid peroxidation in *N. spongiaeforme* and *P. corium*. Each bar represents the mean ± S.D. n=5

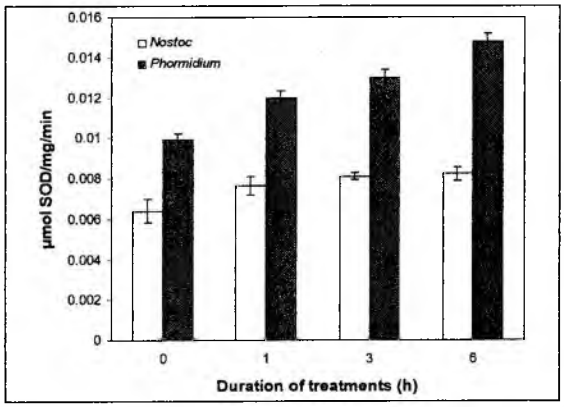

Fig. 3. Effect of high light treatment up to 6 h on superoxide dismutase (SOD) activity in *N. spongiaeforme* and *P. corium.* [Each bar represents the mean ± S.D, n = 4]

described by Tilvi *et al.* (2005). Chemical shift for $CDCl_3$ is observed at 7.26 ppm.

Results

Effect on chlorophyll fluorescence

Figure 1 shows the effect of high light on chlorophyll fluorescence (Fv/Fm ratio) in *Nostoc spongiaeforme* and *P.hormidium corium*. There was a decrease in the Fv/Fm ratio in response to increasing duration of the high light treatment in both *N. spongiaeforme* as well as *P. corium*. High light treatment for 6 hours resulted in complete inhibition of Fv/Fm ratio in *N. spongiaeforme* while 84% decrease was observed in *P. corium*. Fo level was also decreased due to the treatment in both the species studied (Fig. 1, inset).

Effect on peroxidation of lipids

Peroxidation of membrane lipids (measured as MDA formation) was observed in both *P. corium* as well as in *N. spongiaeforme* as a result of the high light treatment (Fig. 2). The high light treatment for 6h resulted in 40% increase in peroxidation level of the membrane in *N. spongiaeforme* and 52% increase in *P. corium* as compared to their respective controls.

Effect on superoxide dismutase

An increase in the activity of superoxide dismutase (Fig. 3) was observed in both *N. spongiaeforme* and *P. corium* as a result of high light treatment. Six hours of high light treatment resulted in an increase in the activity of superoxide dismutase by 28% in *N. spongiaeforme* and 48% in *P. corium* as compared to their respective controls (Fig. 3).

Qualitative changes in phospho-glycolipids and neutral lipids

Figure 4 shows TLC profile of phospho-glycolipids in *N. spongiaeforme* (a) and *P. corium* (b). Quantitative changes in phosphoglycolipids were observed due to high light treatment. The TLC bands were identified based on their R_f values with reference to their standards in identical stationary and mobile phase. The phosphoglycolipids observed on TLC profile were digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), phosphatidylglycerol (PG), sulfoquin ovosyldiacylglycerol (SQDG) and sulpholipid.

Figure 5 shows neutral lipids in *N. spongiaeforme* (a) and *P. corium* (b). Neutral lipids present were triglyceride, diglyceride and monoglyceride. Only slight change in neutral lipids due the high light was seen in both the cyanobacterial species (Fig. 5a & b).

Quantitative estimation of glycolipids and phospholipids

P. corium showed greater decrease in glycolipids and phospholipid content than observed in *N. spongiaeforme* (Fig. 6). High light treatment for 6 h resulted in 55% decrease in the glycolipids content in *P. corium* while the decrease in *N. spongiaeforme* was 35% for the same duration of treatment. Similarly phospholipid content resulted in only 17% decrease in *N. spongiaeforme* (Fig. 6a) as compared to 46% decrease observed in *P. corium* (Fig. 6b).

GC analysis of membrane lipids

In *N. spongiaeforme* and *P. corium*, the fatty acid groups present were saturated fatty acids such as Lauric acid C_{12}, Myristic acid C_{14}, Palmitic acid C_{16}, Stearic acid C_{18}, and unsaturated fatty acids such as Oleic acid $C_{18:1}$, Linoleic acid $C_{18:2}$ and Linolenic acid $C_{18:3}$. In *N. spongiaeforme*, high light resulted in slight decrease in saturated fatty acids more so in *N. spongiaeforme* than in *P. corium*, while unsaturated fatty acids showed no appreciable change. In *P. corium*, both saturated and unsaturated fatty acid remained virtually unchanged (Table 1).

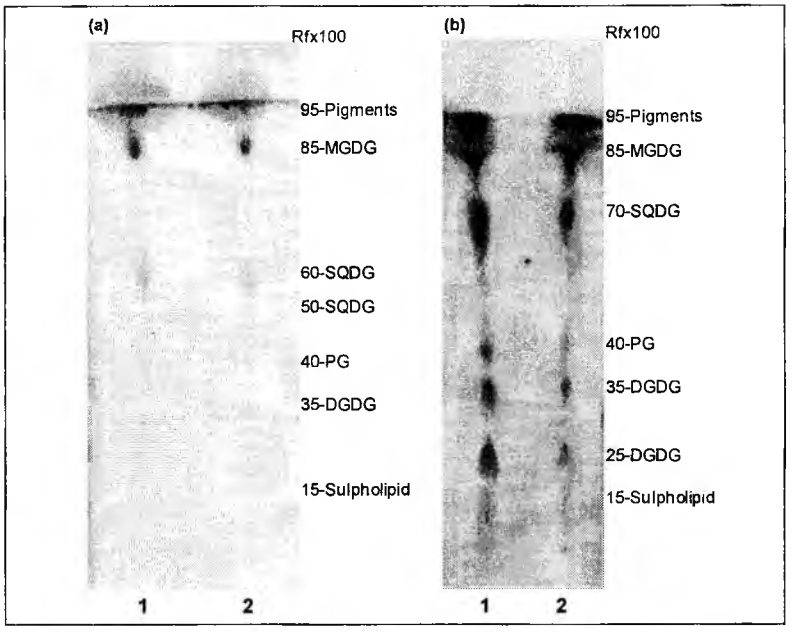

Fig. 4. TLC profile of phosphoglycolipids in (a) *N. spongiaeforme* and (b) *P. corium* due to high light treatment for 6h. Lane1- control, Lane 2- exposure to high light.

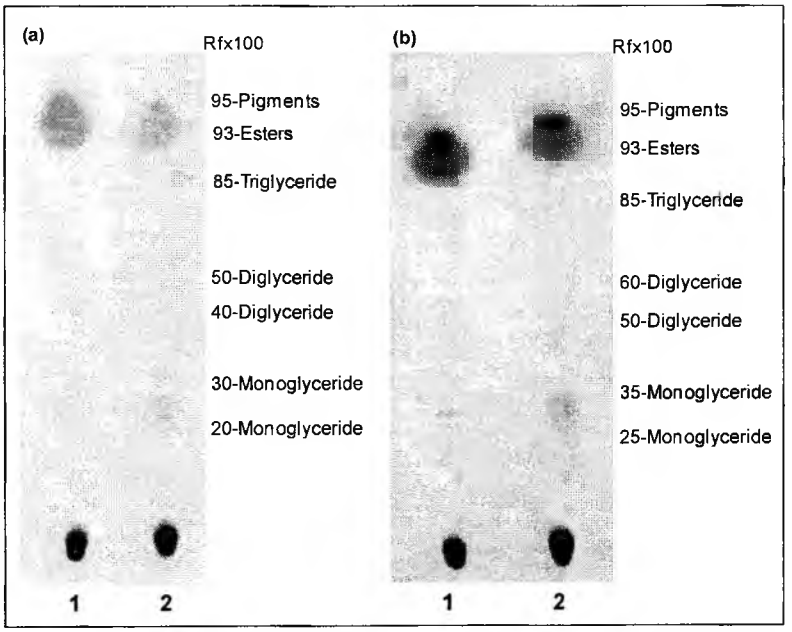

Fig. 5. TLC profile of neutral lipids in (a) *N. spongiaeforme* and (b) *P. corium* due to high light treatment for 6h. Lane1- control, Lane 2- exposure to high light

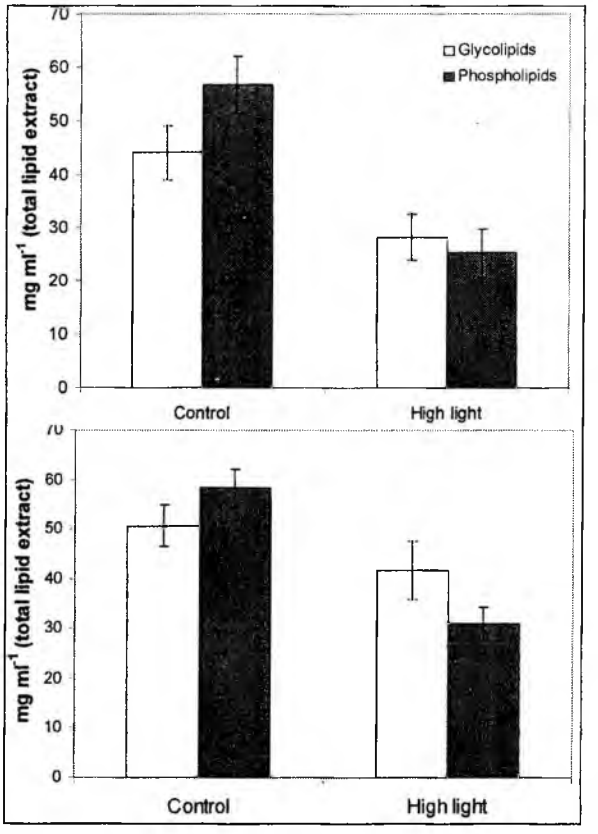

Fig. 6. Effect of high light treatment up to 6 h on glycolipids and phospholipid content in *N. spongiaeforme* (a) and *P. corium* (b). Each bar represents the mean ± S.D. n=3

FTIR analysis of membrane lipids

FTIR spectra of *N. spongiaeforme* and *P. corium* showed absorption bands at various wavelengths (Fig. 7a & b, Table 2). *N. spongiaeforme* showed qualitative changes as a result of high light treatment, which caused only slight change in the unsaturation level seen as spectral band at 1620 cm^{-1}. Spectral band at 1701 cm^{-1}, indicative of C=O group of carbonic acid disappeared after high light condition. While additional absorption peaks were observed at 1739 cm^{-1} indicative of C=O stretch of esters, 1697 cm^{-1} indicative of amide I group, 1161 cm^{-1} and 970 cm^{-1} indicative of deformation of carbohydrates. Slight changes in C-H stretch band at 2852 and 2923 cm^{-1} was also observed. Absorption band at 1683, 1651, 1541 and 1510 cm^{-1} indicative of amide groups disappeared due to the treatment. Also hydroxyl group observed at absorption band 3840 cm^{-1} was not seen after the treatment (Fig. 7a & b, Table 2). A strong signal is observed at 1697 cm^{-1} which is suggestive of complexation of protein with other molecules resulting in the absorption of amide band at slightly higher frequency. There were quantitative decreases in the amide groups indicating significant changes in membrane protein structure after exposure to high light treatment.

Table 1. Effect of high light (500 mmol m^{-2} s^{-1} PAR) for 6 h on fatty acid composition of total lipids in *Nostoc spongiaeforme* and *Phormidium corium* (mole percent). [Values represent the mean ± S.D, n = 3].

Fatty acids	*Nostoc spongiaeforme* Control	HL	*Phormidium corium* Control	HL
Lauric acid (C_{12})	11.4±0.5	10.3±0.4	11.3±0.4	11.3±0.6
Myristic acid (C_{14})	18.2±0.8	17.6±0.7	17.6±0.7	17.5±0.6
Palmatic acid (C_{16})	15.4±0.7	14.8±0.7	14.8±0.8	14.7±0.7
Stearic acid (C_{18})	9.5±0.4	9.10.6	9.1±0.4	9.0±0.5
Oleic acid ($C_{18:1}$)	3.4±0.3	3.40.3	3.2±0.1	3.2±0.2
Linoleic acid ($C_{18:2}$)	20.9±0.9	20.7±0.8	19.9±0.5	19.9±0.4
Linolenic acid ($C_{18:3}$)	3.5±0.2	3.5±0.3	3.3±0.2	3.3±0.4

Table 2. FTIR profile of lipids indicating qualitative changes of lipids in *Nostoc spongiaeforme* and *Phormidium corium* due to high light treatment. + indicates presence of peak and – indicates absence of peak. + and - do not indicate quantitative chnages.

Peaks	Components	*Nostoc spongiaeforme* Control	HL	*Phormidium corium* Control	HL
3840	O-H stretch of hydroxyl groups	+	-	-	-
3674	O-H stretch of hydroxyl groups	-	-	+	+
2923	C-H stretch of CH_2	+	+	+	+
2854	C-H stretch of CH_2 in fatty acids	-	-	+	-
2852	C-H stretch of CH_2 in fatty acids	+	+	-	+
1739	C=O stretch of esters	-	+	-	+
1735	C=O stretch of esters	+	-	+	-
1701	C=O stretch of carbonic acid	+	-	+	-
1697	Amide I	-	+	-	-
1683	Amide I	+	-	-	-
1651	Amide I	+	-	-	-
1541	Amide II	+	-	+	+
1510	Tyrosine band	+	-	-	+
1458	C-H deformation of CH_2	+	+	+	-
1269	Amide III	-	-	+	-
1166	C-O, C-C stretch, C-O-H, C-O-C def of carbohydrates	+		-	- +
1161	C-O, C-C stretch, C-O-H, C-O-C def of carbohydrates	-		+	- -
1122	C-O, C-C stretch, C-O-H, C-O-C def of carbohydrates	-		-	+ -
1066	C-O, C-C stretch, C-O-H, C-O-C def of carbohydrates	+		-	- +
1035	C-O, C-C stretch, C-O-H, C-O-C def of carbohydrates	+		+	+ -
970	C-O, C-C stretch, C-O-H, C-O-C def of carbohydrates	-		+	- -
734	Fingerprint region	-	+	-	-
671	Fingerprint region	-	+	-	+
551	Fingerprint region	-	-	+	-

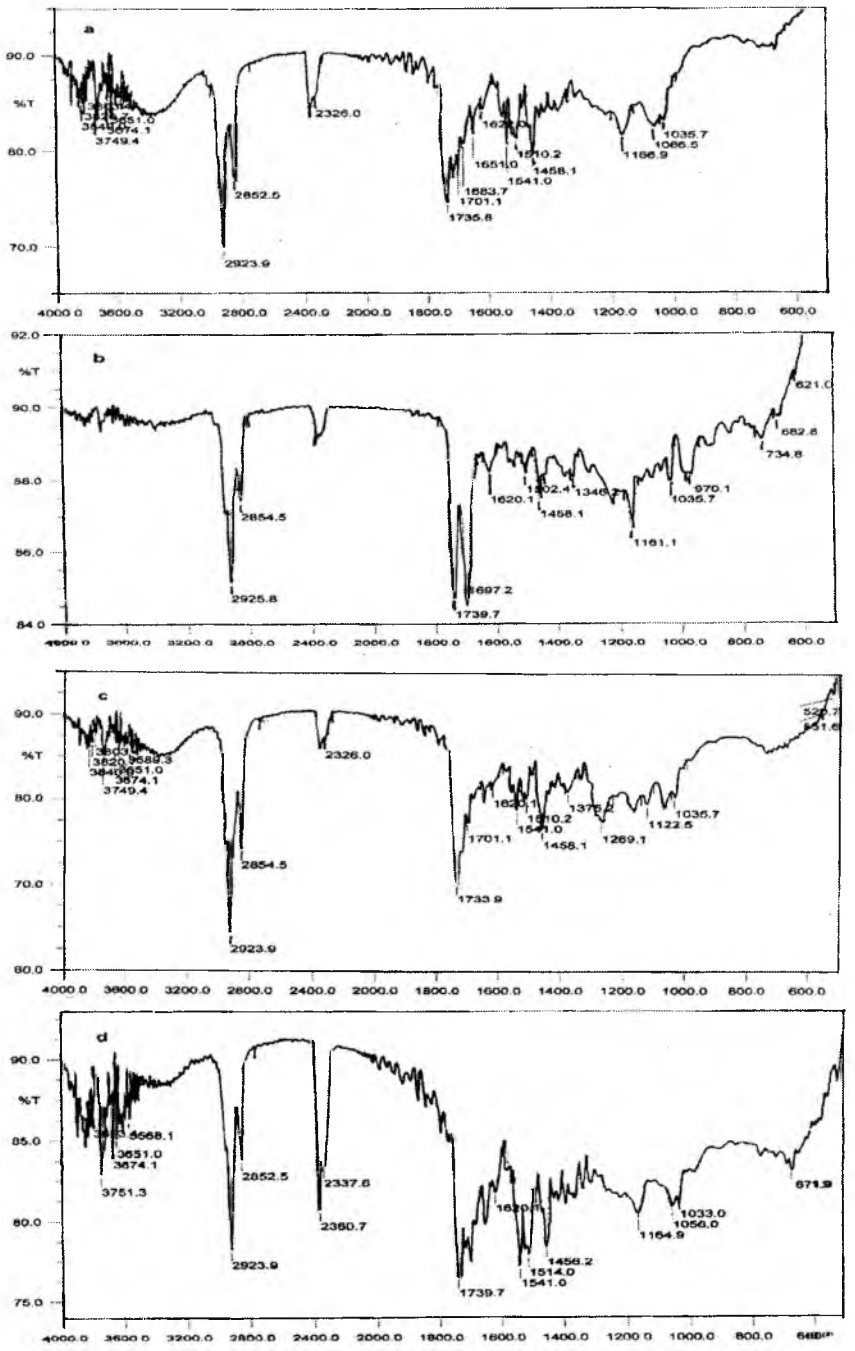

Fig. 7. FTIR profile of lipids in *N. spongiaeforme* (Fig 6a: control, Fig 6b: exposure to high light) and in *P. corium* (Fig 6c: control, Fig 6d: exposure to high light)

In comparison to *N. spongiaeforme*, FTIR spectra of *P. corium* showed slightly different spectral bands (Fig. 7c & d, Table 2). Spectral band at 2854 cm^{-1} indicative of C-H stretch of fatty acids and 1701 cm^{-1} indicative of C=O stretch of carbonic acid decreased slightly as a result of the treatment compared to control while spectral band at 2923 cm^{-1} indicative of C-H stretch and hydroxyl group detected at 3674 cm^{-1} did not change (Fig. 7c & d). Additional spectral bands seen at 2852 cm^{-1} indicative of C-H stretch, at 1739 cm^{-1} indicative of C=O stretch of esters, at 1514 cm^{-1} indicative of amide II, at 1456 cm^{-1}, indicative of C-H deformation, and at 1164, 1056 and 1033 cm^{-1} indicative of deformations of carbohydrate were observed as a result of the high treatment but not seen in control. The changes in C=O and C-H stretch was observed in *Nostoc* as a result of the treatments but was not seen with *Phormidium*.

NMR analysis of membrane lipids

NMR spectra of *N. spongiaeforme* and *P. corium* showed peaks at d0.85, d1.25, d1.43, which are indicative of aliphatic fatty acid chain and d5.3 which are indicative of unsaturation level (Fig. 8a, b, c & d, Table 3). In control *N. spongiaeforme,* NMR spectra showed peak at d2.17, but was absent during high light treatment. Additional peaks at d1.59 and d2.035 were observed during high light treatment in *Nostoc*.

The peak at d1.31 and d4.2 indicative of aliphatic fatty acid chain was seen in case of *P. corium,* which was not seen in *N. spongiaeforme*. Also appearance of peaks at d7.0 and d8.03, indicating the presence of peptide moieties, was also observed in *Phormidium* as a result of high light treatment but not seen in *Nostoc*.

Discussion

High light treatment resulted in a decrease in photosynthetic efficiency which was due to decrease in the F_o as well as F_m (Fig. 1). Decrease in the Fo is an indicator of decrease in the excitation energy reaching the photosynthetic reaction centre II probably due to loss of pigments (chlorophyll and phycobiliproteins) in the light harvesting complex II (Papageorgiou 1996). While decrease in the Fm is an indicator of damage

Table 3. NMR profile of lipids indicating qualitative changes of lipids in *Nostoc spongiaeforme* and *Phormidium corium* due to high light treatment. + indicates presence of peak and – indicates absence of peak. + and - do not indicate quantitative chnages.

Peaks	Components	*Nostoc spongiaeforme*		*Phormidium corium*	
		Control	HL	Control	HL
0.85	Fatty acid chain	+	+	+	+
1.25	Fatty acid chain	+	+	+	+
1.31	Fatty acid chain	-	-	+	-
1.43	Fatty acid chain	+	+	+	+
1.59	Fatty acid chain	-	+	-	+
2.035	Fatty acid chain	-	+	-	-
2.17	Fatty acid chain	+	-	-	-
4.2	Fatty acid chain	-	-	+	-
5.3	Unsaturation	+	+	+	+
7.26	Unsaturation	+	+	+	+
7.52	Micropeptides	-	-	-	+
7.69	Micropeptides	-	-	+	-

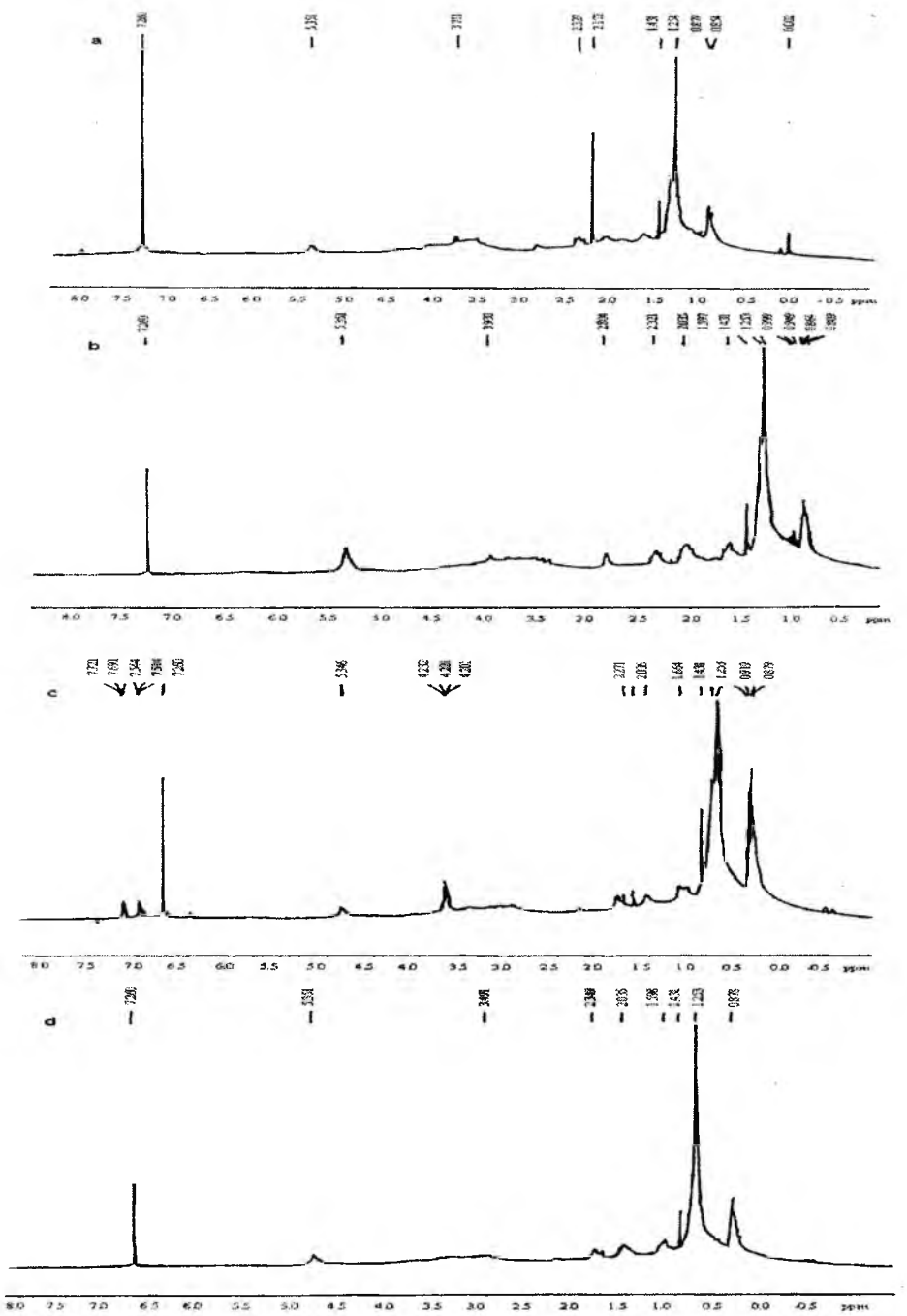

Fig. 8. NMR profile of lipids in *N. spongiaeforme* (Fig 7a: control, Fig 7b: exposure to high light) and in *P. corium* (Fig 7c: control, Fig 7d: exposure to high light).

to the PS II reaction centres itself. The results obtained may represent direct damage to key components within the photosystem such as D1 protein of PS II (Papageorgiou 1996) as well as loss of photosynthetic pigments and xanthophyll cycle due to over energization of photosynthetic apparatus (Tyystjarvi *et al.* 2001). High light is known to affect quantum yield of photosynthesis and the PS II reaction centre (Bischof *et al.* 2002; Bhandari and Sharma 2006a). Decrease in F_v/F_m ratio and changes in the pigments due to high light in green alga *Spongiochloris*, *Chlorella* and *Scenedesmus* is also reported by Vass (1997), Xenopoulos *et al.* (2002) and Koblizek *et al.* (1999). Observed decrease in Fo may be due to movement of phycobilisome from PS II to PS I (Masojidek *et al.* 1999). Miskiewicz *et al.* (2000) observed that cyanobacterium, *Plectonema boryanum* UTEX 485 responds to either varying growth temperature or varying growth irradiance by adjusting the ability to absorb light through decreasing the cellular contents of chlorophyll *a* and light-harvesting pigments and screening of excessive light by myxoxanthophyll predominantly localized in the cell wall/cell membrane to protect PS II from over-excitation.

The increase in MDA formation due to high light treatment, observed in this study is an indicator of increased oxidative damage caused to the cell membrane (Fig. 2). Also more MDA formation was seen in *P. corium* as compared to *N. spongiaeforme*. UV-B radiation is known to cause oxidation of membrane lipids (Malanga *et al.* 1997) which may affect biochemical processes including photosynthesis as membrane provide the surrounding atmosphere for enzyme to function.

The increase in the SOD activity observed in response to the high light treatment in this study (Fig. 3) indicates both that the treatment resulted in oxidative damage, which was also confirmed by MDA formation, and in response to oxidative damage the antioxidant enzymatic systems also responded to overcome the oxidative damage to the tissue due to the treatment, as SOD removes superoxide anion radical generated due to the over reduction of the photosynthetic electron transport. Higher activity of SOD is seen in response to higher oxidative damage (MDA formation) seen in *P. corium* than in *N. spongiaeforme*. Increased SOD activity has been shown to confer increased protection from oxidative damage. Lohrmann *et al.* (2004) observed that antioxidant enzymes play an important role in detoxifying ROS and regenerating the reduced forms of the antioxidant. Barros *et al.* (2003) observed higher tolerance against high light stress in plants by over producing SOD. He and Häder (2002) observed induction of ROS under *in vivo* condition due to UV-B stress in cyanobacterium, *Anabaena* sp., which was further enhanced when photochemical reactions of PS II were impaired. It has been reported that even a moderate increase in superoxide dismutase activity may lead to improved tolerance to oxidative stress.

Quantitative changes observed in phospho and glycolipids could be because of oxidative damage affecting the integrity of the membrane (Murphy 1983). In our study phospholipids seem to affect more as compared to glycolipids and that may have more physiological effect on energy transducing membrane of photosynthesis. The effect could also affect the mitochondrial membrane, however, it was not studied here. Funteu *et al.* (1997) have reported significant decrease in the level of sulfoquinosyl diacylglycerol and phosphatidylglycerol in the cyanobacterium, *Spirulina platensis* where as Tedesco and Duerr (1989) shown that high light slightly increased total lipids and percent

composition of polyunsaturated fatty acid (g–linolenic acid) in *Spirulina platensis*. Nishida and Murata (1996) suggested that part of the damage to phospho and glycolipids could be due to impairment of enzymes involved in lipid synthesis during stress conditions, however, we have not studied the enzymes of lipid biosynthesis. They attributed the survival of algae under higher irradiance conditions to membrane fluidity as a result of higher level of fatty acid unsaturation. However, Norman and Thompson (1985) have reported changes in fatty acid unsaturation level under high light treatment.

Though in the present study, we observed significant quantitative changes in phospho and glycolipids of cell membrane, only a slight change in the fatty acid composition was observed (Table 1). A 10% decrease in the saturated lipid content and no decrease in the unsaturated lipid content due to high light in this study (Table 1) may indicate a slight increase in the fluidity of the membrane, resulting in an adaptation of membrane under high light treatment. Some earlier studies have observed changes in fatty acid composition during light acclimation of algae (Sewon et al. 1997; Klyachko-Gurvich et al. 1999). Gombos et al. (1997) had reported variation in fatty acid composition of total lipids, which is advantageous adaptation against abiotic stresses. Walsh et al. (1997) reported that the changes in the fatty acid composition due to high level of light intensity might occur as a cellular response to reduce the susceptibility of the cyanobacterial membranes to photooxidation. Dawes *et al.* (1993) observed that light intensity influenced primarily the content of saturated and tetraunsaturated fatty acids in *Ulva pertusa* but no differences in the fatty acid composition was found in *Gracilaria* species. Khotimchenko and Yakovleva (2004) reported increase in the amount of saturated fatty acids of total lipids in lipid composition of the marine green alga *Ulva fenestrata* grown under irradiance conditions and suggested that changes in lipid composition can be considered as a mechanism of adaptation and survival of thalli subjected to variations in solar irradiance. Gupta *et al.* (2008) also showed that the changes in membrane in cyanobacterium, *Spirulina platensis* under UV-B stress is due to increased unsaturation of fatty acids for the tolerance of cyanobcterium to UV radiation, which keeps cells alive under stress.

Our results with FTIR analysis showed presence of both saturated as well as unsaturated fatty acids (Fig. 7). There were significant changes in the amide groups (NH_2) indicating significant changes in protein moieties within the cell membrane due to high light treatment. Also OH group were affected due to the treatment which may affect hydrophilic and hydrophobic interaction through OH and NH_2 groups, thus affecting various membrane associated physiological and biochemical processes. Klyachko-Gurvich *et al.* (1999) reported light dependent alterations in the content of polar lipids and proportion of fatty acids in them, may influence lipid interplay with proteins of the membranes. Changes in amide group and hydroxyl group may influence lipid interplay with proteins of the membranes.

NMR profile showed presence of aliphatic chain of fatty acids in both the species studied (Fig. 8). Al-Fadhli *et al.* (2006) isolated and identified three major galactoglycerolipids in the native form from the red alga, *Chondria armata* using NMR complemented with mass spectrometry. Shao *et al.* (2002) reported the presence of a new sulfonoglycolipid, crassicaulisine, with palmitoyl and myrsitoyl as acyl groups, from the red alga, *Chondrus crassicaulis*.

The results indicated oxidative

nature of damage to cyanobacteria under high light conditions which leads to increase in the SOD activity as a protective response. Decrease in the photosynthetic efficiency seems to be partly due to rearrangement of pigments in the LHC antenna system and partly due to effect on PS II reaction centre itself. Though there were quantitative changes in the phospho and glycolipids, no qualitative changes in the lipid composition was observed. A slight change in the saturation to unsaturation level of fatty acids was seen indicating adaptation to high light. Significant changes in amide groups (NH_2) indicate changes in lipid and protein moieties and their interplay within the membrane under high light conditions which may be related to adaptation and survival of the organism by maintaining membrane integrity and physiological functions, rather than consequences of direct damaging effect of high light.

Acknowledgement

Dr. C. Raghukumar, NIO, Goa, for providing pure culture of *Phormidium*, Prof. Sivkumar Swamy, Department of Botany, Karnatak University, Dharwad for helping with fatty acid analysis. Grants from CSIR, New Delhi (grant No. 38(1030)/02/EMR-II) and DST, New Delhi (grant No. SF/FT/LS-015/2007 to RB) are acknowledged.

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