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Biochemical and spectroscopic changes in phycobiliproteins of the protein-rich Cyanobacterium, *Spirulina fusiformis* induced by UV-B radiation

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Abstract: The present study indicated that the Increase in UV-B exposure (312.67 nm, 2.5 Wm^{-2}) to *Spirulina fusiformis*, a highly protein-rich (60-71%) helically coiled, unicellular, polymorphous cyanobacterium not only bleached phycocyanin (PC) but also decreased its content Absorption spectra of UV-B treated culture showed significant decline in the absorbance at the peak 620nm indicating the presence of PC. The absorbance decreased by increasing UV-B exposure. One hour simultaneous UV-B+PAR (Photosynthetic Active Radiation) exposure protected 33 percent damage of phycocyanin.

Keywords : UV-B, PAR., Phycobiliprotein, *Spirulina*

INTRODUCTION

The increasing size of the ozone hole over Antarctica, Australia and New zealand, due to stratospheric ozone depletion and consequent enhancement in UV-B intensity reaching to the earth's surface , has become a major golobal problem in recent years (Rowland, 2006). The size of the ozone hole has increased from 5 million square km (1994) to 28 million square km (2000), 18 Million square km (2003) and 25-30 million square km (2004) and it will not recover until 2065 (Hanson, 2003; and Rowland , 2006). Deleterious UV-B rays damage protein, enzymes, uptake mechanisms, photosynthetic pigments and ultimately photosynthesis by dimer formation in genes (Häder *et al*., 2003 and Prasad *et al*., 2006).

Cyanobacteria are the oldest, phylogenetically primitive group of photosynthetic prokaryotes of pre cambrian period (2.8-3.5x10^8 yr) (Sinha and Hader, 1996a) and rich in photosynthetic pigments especially phycobilisomes (Cai *et al*, 2001). *Spirulina fusiformis* is an aseptate, filamentous, polymorphic, protein-rich microalgae (60-70%).It is rich in various useful biologically active compounds like phycobiliproteins (PBPs).

PBPs are major light harvesting pigment and classified into phycoerythrin (PE, red, max 540-570nm) phycocyanin (PC, blue, max 610-620nm and allophycocyanin (APC blue-green, max 650-655nm).It transfers the captured radiant energy to chlorophyll in the order PE®PCAPC®PRC (Photosynthetic reaction centre). Destruction of PBPs by UV-B was found reported in various cyanobacteria like Anabaena sp., Nostoc carmium, Aulosira fertilissima, Spirulina platensis, Calothrix sp. and few marine red algae (Sinha *et al*., 1995; and Banerjee *et al*., 1998., Rajagopal *et al*., 1999., Sinha and Häder, 2003 and Sinha et al 2005).

MATERIALS AND METHODS

Test organism and growth condition: Alkaline and Saline Sambhar Salt Lake strain *Spirulina fusiformis* is a filamentous, nonheterocystous, aseptic, helically coiled polymorphous, cyanobacterium in three distinct forms, S- type. C-type and H- type (Jeeji Bai and Seshadri, 1980). The polymorphs readily transformed from one type to another by change in environmental and nutritional factors. But, the freshly transferred liquid culture contained s-type filaments. It was grown in Zarrouk's medium at 9.5 pH (Zarrouk, 1966) in 250ml Erlenmeyer flask containing 100ml medium at 25±1^{0}c in culture room illuminated with 14.4 Wm^2 fluorescent light for 14 h d^{-1}. Growth experiments were performed in culture tubes each containing 10 ml basal medium.

Source and mode of UV-B treatment: The source of UV-B irradiation was a UV-B lamp (Cat No. 3-4408, Fotodyne, Inc, USA) with peak emission at 312 nm. Culture suspension of 0.2 optical density was taken in 75 mm petri dish with lid removed and irradiated under UV-B lamp. The desired intensity of UV-B (2.5 Wm^{-2}) was obtained by adjusting the distance between UV-B source and the sample. The UV-B intensity was measured by a Black –Ray J-221 long wave UV-B intensity meter (UVP Inc, San Gabriel, California). The suspension was stirred magnetically during UV-B exposure.

Survival and phycocyanin estimation: For determining the percent survival 1 ml irradiated samples were withdrawn at known time intervals and diluted 10^4 times. Form that diluted samples 0.1 ml were plated on agar plates and after 41h (generation time) of dark pre incubation exposed to fluorescent light. Colony counts were performed after 15 days of growth and plotted

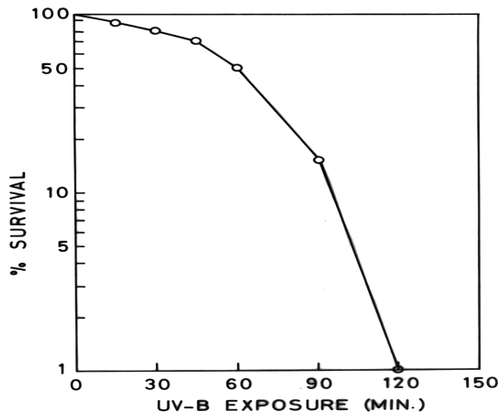

Fig. 1. *Effect of UV-B on survival of S. fusiformis.*

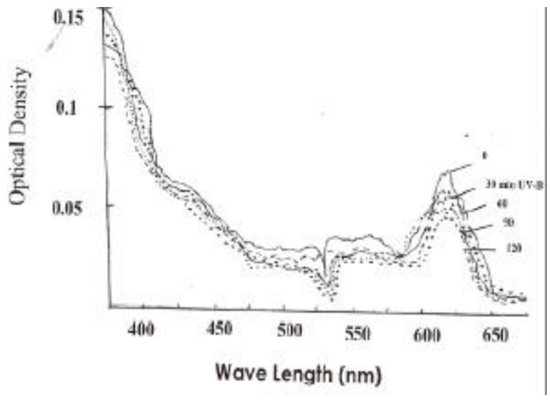

Fig. 3. *Effect of UV-B on absorption spectrum of phycocyanin pigment in S. fusiformis.*

semilogarithmically to determine percent survival (Prasad *et al.*, 2006).

Similarly, samples were withdrawn at desired intervals after UV-B exposure in two sets of tubes one for immediate observation and other for 41h dark incubation. After dark incubation, 1 ml sample was diluted and from that diluted sample 0.1ml were poured in culture tube having 10 ml culture. Such 15 sets of culture tubes were exposed to fluorescent light for phycocyanin estimation. Phycobiliprotein was extracted by the method of Boussiba and Richmond (1979) and estimated according to Brody and Brody (1961).

Phycobiliprotein isolation: Pellet left during chlorophyll estimation by 50% acetone method was mixed with 5 ml of 0.1ml Na phosphate buffer (pH 7.0) containing 100ug ml^{-1} lysozyme and 100 mM Na EDTA, sonicated for 2-3 min in a sonicator (Heat system ultrasonic Inc.) The solution was stored at room temperature for 10h in dim light and then incubated overnight at 4°C. The slurry was then centrifuged at 10,000xg for 20 min to remove the debries and to yield blue colour supernant. Optical density was observed at 620 and 655 nm for phycocyanin (PC) and allophycocyanin (APC) in the spectrocolorimeter.

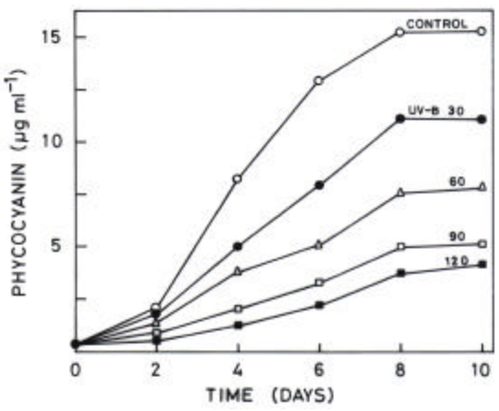

Fig. 2. *Effect of fluorescent light on UV-B induced phycocyanin of S. fusiforms.*

Protection : Phycocyanin estimation of Spirulina culture, treated with photosynthetic active radiation (PAR) and UV-B at a time and UV-B alone were performed. The source of PAR was fluorescent light of 400-700nm emitting 14.4Wm^{-2} fixed at 18 cm from the sample. the intensity was measured with the Luxmeter.

Specific growth rate and statistical analysis: Specific growth rate (K) and generation time (G) were calculated as per method of Kratz and Myers (1955). Statistical analysis was made by student's test.

RESULTS AND DISCUSSION

Survival: 2.5 Wm^{-2} of UV-B after 60 min treatment was selected as mid inhibitory 50% survival dose in *S. fusiformis* and this dose was used for further experiments. 2.5 Wm^{-2} UV-B exposure for 60 min showed 50% survival and complete killing at 120 min in *S. fusiformis* (Fig.1). It is consistent with the sensitiveness of *Nostoc muscorum* (Tyagi *et al.*, 2003) and *N.sp*. (Sinha *et al.*, 1995) where 45 min and 30 min are the 50% survival dose at 6.7 mmol $m^{-2}s^{-1}$ and 5 Wm^{-2} UV-B exposures respectively.

Phycocyanin pigment: The inhibition of phycocyanin pigment in *S. fusiformis* with increasing UV-B exposure in pre and post dark incubation (Table 1) is consistent with the work of Sinha *et al*. (1995) and Quesada and

Table 1. Effect of UV-B Exposure on phycocyanin pigment in pre and post dark incubation in *S. fusiformis*.

UV-B (min)	Pre-dark incubation		Post-dark incubation	
	m g ml^{-1}	%	*m* g ml^{-1}	%
0	14.0	100	13.0	100
30	10.0	71.4	9.00	69.2
60	6.50	46.6	6.00	46.2
90	4.80	34.3	4.00	30.8
120	3.40	24.3	3.00	23.0

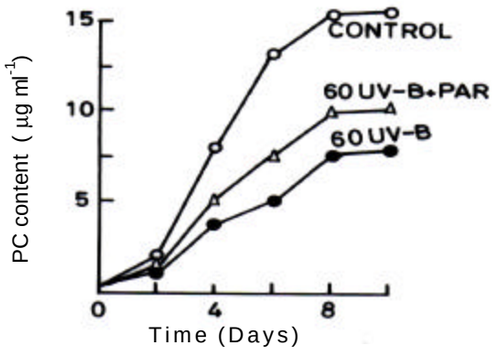

Fig. 4. *Effect of fluoresent light on PC content of S. fusiformis under simultaneous exposure of(UV-B + PAR) and UV-B for 60 min.*

Vincent (1997) in most of the cyanobacteria, which might be due to destruction of photosynthetic pigments during UV-B exposure. But, more inhibition of phycocyanin in dark incubated cultures after UV-B exposure might be due to failure of dark repairing (Prasad*et al.,* 2003). There were slower growth in PC content in cultures exposed to UV-B for higher durations (Fig.2).

Absorption spectrum: The drastic decline in absorption spectra of phycocyanin with increasing UV-B exposure in *S. fusiformis* is similar to *Calothrix sp*. (Sinha *et al.*, 1995). Similar time dependent destruction and bleaching of PBSs have been reported in *Aulosira fertilissima* (Banerjee et al, 1998) and*Spirulina platensis* (Rajagopal *et al*., 1999).

Protection by PAR: Although excessive PAR is highly photoxidative and cause severe PC bleaching in several cyanobacteria, but low PAR was found protective against photoxidation which was confirmed by increase in carotenoid contents at 230 mmol $m^{-2}s^{-1}$ (Cen and Bornman, 1990). Here, in *S. fusiformis* 60 min simultaneous PAR treatment with UV-B protected 33% of the PC content. It shows imparing of UV-B damage by fluorescent light. This slo0077 imparing of UV-B damaged PC by fluorescent light. It is consistent with work of Warner and Caldwell (1983). It might be due to photoreactivation by DNA photolyase and monomerization of dimmers (Karent et al. 1991) or the resynthesis of the UV damaged D_1-protein (Strid and Anderson, 1994). It is the first report in *S. fusiformis*.

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