



Research Article

CUMULATIVE EFFECTS OF ULTRAVIOLET RADIATION AND  
PHOTOSYNTHEICALLY ACTIVE RADIATION ON PHYCOBILIPROTEINS OF A  
HOT-SPRING CYANOBACTRIUM *NOSTOC* SP. STRAIN HKAR-2

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**Abstract**

Cyanobacteria are cosmopolitan in distribution and have adapted to diverse habitats. Adaptation of cyanobacteria is one of the key factors to withstand harsh environmental conditions. We have investigated the effects of photosynthetically active radiation (PAR; 400–700 nm), ultraviolet-B (UV-B; 280–315 nm) radiation and PAR+UV-B radiations on phycobiliproteins (PBPs) of a hot-spring cyanobacterium *Nostoc* sp. HKAR-2. There was a continuous induction of both phycoerythrin (PE) and phycocyanin (PC) after exposure of PAR up to 300 min. However, there was an induction in the synthesis of both PE and PC up to 240 min exposure of UV-B and PAR+UV-B radiations. Further exposure showed decline in the synthesis due to rapid uncoupling, bleaching and degradation of PBPs. Similarly, emission fluorescence also showed an induction with a shift towards longer wavelengths after 240 min of UV-B and PAR+UV-B exposure. These results indicate that short duration of UV radiation may promote the synthesis of PBPs that can be utilized in various biotechnological and biomedical applications.

**Keywords:** Cyanobacteria; Fluorescence; Phycocyanin; Phycoerythrin; UV radiation.

**Introduction**

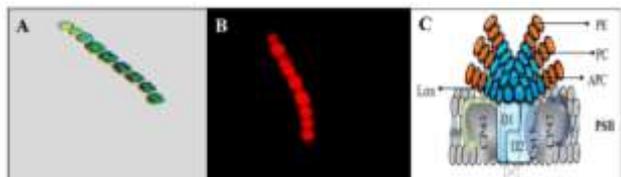
Cyanobacteria are the most ancient and dominant group of Gram-negative photosynthetic prokaryotes having cosmopolitan distribution from moderate to extreme habitats (Fischer, 2008; Ballaré *et al.*, 2011). Cyanobacteria are the most abundant nitrogen-fixing microorganisms in the rice paddy fields. They play a significant role in improving soil fertility, maintaining the nitrogen cycle and energy dynamics in ecosystem (Roger and Kulasoorya, 1980; Sinha and Häder, 1996; Sinha *et al.*, 2001; Stock *et al.*, 2014). Moreover, cyanobacteria are also considered as model organisms in biotechnological and space research due to their survivability in extreme abiotic and biotic stressed habitats (Olsson-Francis *et al.*, 2013; Rastogi *et al.*, 2014). Cyanobacteria are well characterized for their various applications in several fields of biotechnology and biomedical sciences (Richa *et al.*, 2011; Kannaujiya and Sinha, 2016a).

Solar spectrum is the primary source of photonic energy for storing carbon products in photosynthetic organisms on the Earth's surface. Solar spectrum is physically divided into

three major groups such as infrared (IR; 780 nm), photosynthetically active radiation (PAR; 400–700 nm), and ultraviolet radiation (UVR; 100–400 nm) that reaches on the Earth's surface. UVR can be divided into three spectral regions (based on wavelengths and energy) such as UV-A (315 - 400 nm), UV-B (280 - 315 nm), and UV-C (100 - 280 nm). The lower wavelength and highly energetic UV-C radiation is extremely harmful, fortunately it never reaches to the Earth's surface due to complete absorption by ozone (Madronich *et al.*, 1998, Kannaujiya *et al.*, 2014; Kannaujiya and Sinha, 2015). The fraction of solar radiation consists of reduced energy UV-A (95 %) that reaches to the Earth's surface (Santos *et al.*, 2013). About 5% UV-B radiation which is not completely absorbed by stratospheric ozone layer reaches to the Earth's surface and is harmful to all living organisms (Polo *et al.*, 2014).

It has been reported that cyanobacterial light harvesting proteins absorb > 99 % total solar radiation (Lao and Glazer, 1996), but < 1 % UV-B radiation is enough to cause several deleterious effects on living organisms. Phycobilisomes (PBSs) constitute 50 % of the total soluble proteins in

cyanobacteria. It has been characterized into three groups such as phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) with absorbance ranging from 450 to 660 nm (Grossman *et al.*, 1993; Sinha and Häder, 2003). Structurally, PBSs are arranged in trimeric-hexameric macromolecular assembly (3-7M Da) with colorless linker polypeptides in six to eight rods and a core having two to five cylinders (Adir, 2008) (Fig. 1A, B, C).



**Fig. 1:** Microphotograph of *Nostoc* sp. strain HKAR-2 in light microscope (A) and fluorescence microscope (B). Structure of phycobilisome (C).

The monomer unit of each of PBS consists of  $\alpha$  and  $\beta$  subunits with molecular mass 15-20 kDa and 17-22 kDa respectively (Sinha *et al.*, 1995a; Kannaujiya *et al.*, 2016). In addition to PBS, 15 % non-pigmented linker polypeptides also play distinguished role in the stabilization of complex (Tandeau de Mersac and Cohen-Bazire, 1977). Some cyanobacterial strains have the capability to grow at high temperature in hot springs having highly stable PBS composition (Pumas *et al.*, 2011).

The aim of present investigation was to study the cumulative effects of UV-B and PAR irradiations on PBPs composition of a hot-spring cyanobacterium *Nostoc* sp. strain HKAR-2. Our results elucidate the spectroscopic and chromophore fluorescence stability of PC and PE. This investigation may provide an insight for the production and utilization of PBPs from cyanobacteria.

## Materials and Methods

### Organism and Growth Condition

The cyanobacterium *Nostoc* sp. strain HKAR-2 (Rastogi *et al.*, 2012) was isolated from the hot-springs of Bihar, India (25°04'5"N 85°25'7"E), at 74 m above sea level. Isolated culture was purified and routinely grown in BG-11<sub>0</sub> medium (Rippka *et al.*, 1979) at 20 ± 2 °C and illuminated with day light fluorescent tubes having photon flux density of 94  $\mu\text{m}^{-2}\text{s}^{-1}$  at the surface of the vessels with a 14/10 light/dark cycle. The cyanobacterium *Nostoc* sp. HKAR-2 (FJ939126) was identified by using the standard taxonomic keys as well as by 16S rRNA gene sequencing.

### Radiation Conditions

Cyanobacterial suspension culture was transferred into three sterile Petri dishes (150 x 25 mm). Petri dishes were wrapped with 295, 395 nm cut-off filter foils (Ultraplan, UV Opak Digefra, Munich, Germany) for the effects of PAR, UV-B and PAR+UV-B radiations. Petri dishes were kept on rotary shaker in a self-assembled opaque UV chamber equipped with fluorescent white light (OSRAM L

36 W: 32 Lumilux deluxe warm white and Radium NL 36 W: 26 Universal white, Germany) and UV-B tube (Philips Ultraviolet-B TL 40 W: 12, Holland) at constant temperature (20±5 °C). The exposure of cyanobacterium was fixed at ~0.7  $\text{Wm}^{-2}$  and 55.08± 9.18  $\mu\text{m}^{-2}\text{s}^{-1}$  under UV-B irradiation and PAR respectively. The cultures were exposed to UV-B and PAR irradiation for 300 min and aliquots were withdrawn after 30, 60, 120, 180, 240, 300 min of radiation.

### Extraction and Purification of Phycobiliproteins

The extraction and purification of PBPs were done by adopting the method of Sinha *et al.* (1995a). Suspended aliquots of cyanobacterial culture were washed with 0.75 M potassium phosphate buffer (pH 7.5) and resuspended in reaction mixture containing 1 mM phenylmethanesulfonyl fluoride (PMSF), 10 % (w/v) EDTA, and 5 % (w/v) sucrose. The separation and purification of PBPs were done by the method as described earlier (Kannaujiya and Sinha, 2016b).

### Spectroscopic Measurements

Absorption spectra of PC (615 nm) and PE (563 nm) were measured by using UV-Vis double beam spectrophotometer (U-2910, 2J1-0012, Hitachi, Tokyo, Japan). Excitation fluorescence spectra of PC and PE were measured by spectrofluorometer (F-2500, Hitachi, Tokyo, Japan) at 615 nm and 563 nm respectively. The peak analyses of emission wavelength of PC (642 nm) and PE (575 nm) were performed by using the software FL Solution provided by Hitachi corporation. The immobilization of cyanobacterial filaments was done by using 4 % gelling agarose (Reize and Melkonian, 1989) by using the method as described earlier (Sinha *et al.*, 2002). Fluorescence images were taken by using a fluorescence microscope (Nikon Eclipse-E800) after excitation at a wavelength of 550 nm up to 600 ms.

### Statistical Analyses

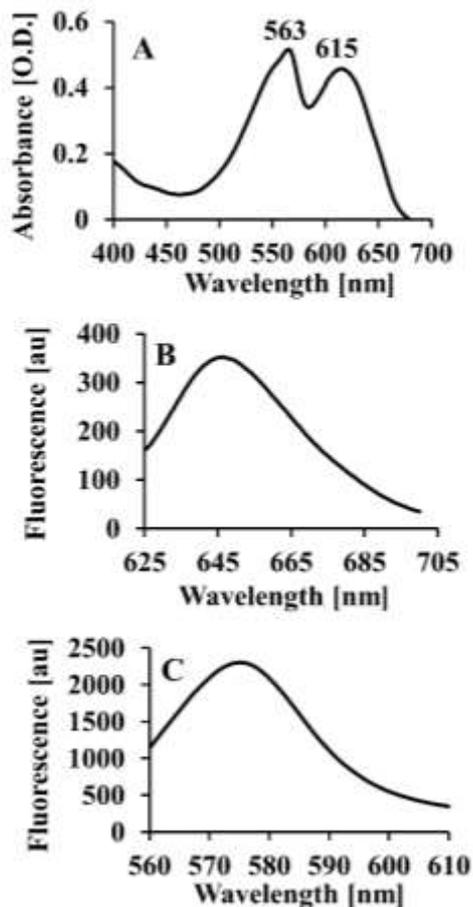
All the experiments were repeated thrice with at least three replicates. One-way analyses of variance with multiple comparison modes were applied to evaluate the significance of data ( $P \leq 0.05$ ). SPSS-20 and Sigma Plot 11 softwares were used for statistical analyses.

## Results and Discussion

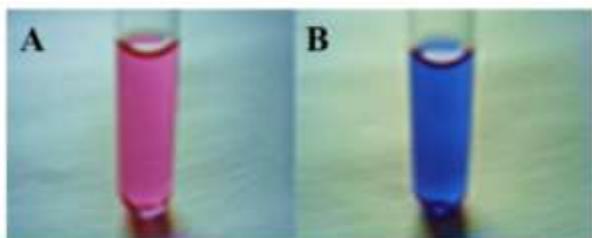
### Extraction and Purification of PBPs

Absorption spectrum of the crude extracts (Fig. 2A) showed that the cyanobacterium *Nostoc* sp. strain HKAR-2 contains mainly PE ( $\text{Ex}$ :563 nm) and PC ( $\text{Ex}$ :615 nm) components of the PBPs. The fluorescence emission of purified PC and PE was recorded after excitation at  $\text{Ex}$ :615 nm and  $\text{Ex}$ :563 nm respectively. A sharp peak at the emission wavelengths for individual PC (Fig. 2B) and PE (Fig. 2C) was recorded at the wavelength of ~574 and ~646 nm, respectively. After completion of purification processes the color of PE appeared as pink (Fig. 3A) and that of PC blue (Fig. 3B). Intense color of PBPs is dependent on the high purity ratio.

Highly purified PBPs are used for making high quality products and therefore there is a tremendous increasing demand for pure PBPs (Kannaujiya and Sinha, 2016b). Purity ratio of around 0.7, 3.9 and >4.0 has been considered for making food grade, reactive grade and analytical grade bioproducts respectively (Chaiklahan *et al.*, 2012).



**Fig. 2:** Absorbance and fluorescence spectra of PC (615 nm) and PE (563 nm) of *Nostoc* sp. strain HKAR-2.

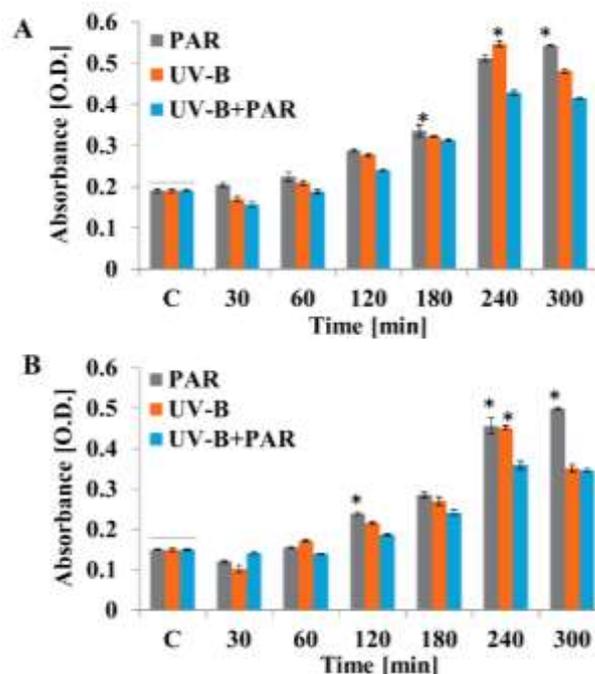


**Fig. 3:** Purified PE (A) and PC (B).

### Spectrophotometric Analyses of PBPs

Current depletion of ozone layer is the primary cause of elevated UV-B radiation that is potentially harmful to all the living organisms including cyanobacteria (Sinha *et al.* 1996; Häder and Sinha, 2005; Sinha *et al.*, 2008). It is a well-established fact that longer duration of exposure is more deleterious (Rastogi *et al.*, 2011; Kannaujiya *et al.*, 2014; Häder *et al.*, 2015; Kannaujiya and Sinha 2015,

2016c). To determine the short duration impacts of PAR and UV-B radiations on PBPs, exponentially growing cyanobacterium was exposed to PAR, UV-B and PAR+UV-B irradiations up to 300 min. Absorption of PE was continuously increased up to 2.8 fold as compared to control after 300 min exposure of PAR with distinct significant difference ( $P < 0.05$ ). The same absorption was increased up to 2.9 fold ( $P < 0.05$ ) after 240 min of UV-B radiation. Further exposure with UV-B radiation reduced the absorption of PC. The combination of UV-B and PAR irradiation reduces the amount up to 2.1 fold as compared to control with no significant differences ( $P > 0.05$ ) (Fig. 4A). Absorption of PE was also continuously increased up to 3.1 fold as compared to control after 300 min exposure of PAR. In contrast to PAR, absorption of PC was increased up to 3 fold with significant differences ( $P < 0.05$ ) after 240 min exposure of UV-B radiation and declined after further exposure. The combined effects of UV-B and PAR irradiation was the same as UV-B radiation as compared to control with no significant differences ( $P > 0.05$ ) (Fig. 4B). Similar time-dependent photobleaching and destruction of PBPs have been reported in *Anabaena* sp. and *Nostoc carmium* (Sinha *et al.*, 1995a), *Nostoc* sp. (Sinha *et al.*, 1995b) and *Aulosira fertilissima* (Banerjee *et al.*, 1998). However, short duration and weak exposure may promote induction of PBPs in certain cyanobacteria (Kannaujiya and Sinha, 2016c).



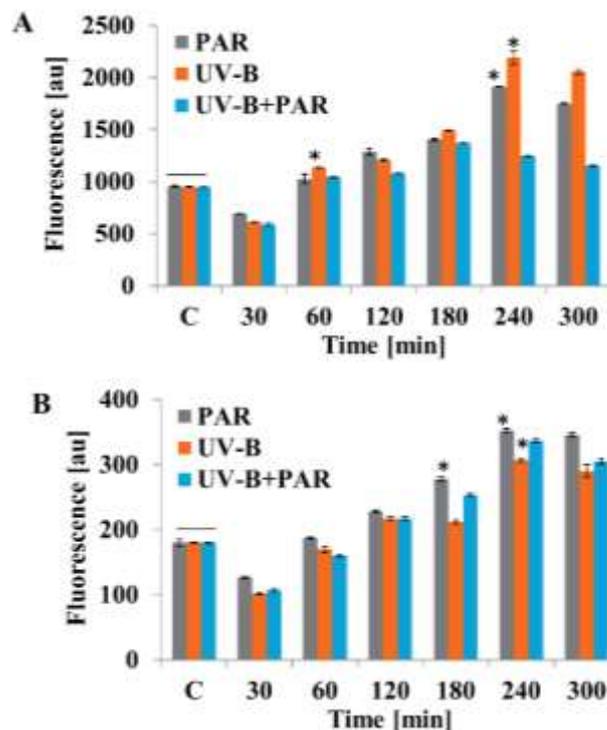
**Fig. 4:** Absorbance values of PE (A) and PC (B) after exposure of PAR, UV-B and PAR+UV-B radiations. Horizontal line over bars indicate no significant differences ( $P > 0.05$ ). Asterisk marks indicate significant differences ( $P < 0.05$ ) with the control (0 h). The error bar denotes standard deviations of means ( $n = 3$ ).

**Fluorometric Analysis of PBPs**

The fluorescence emission of PE was continuously increased up to 2 fold with no significant differences after 240 min of exposure to PAR. A slight decline in fluorescence was observed after 300 min. The reduction in fluorescence intensity of PBPs was most probably due to the phenomena of chromophore photobleaching and detachment from rods and core subunits. In contrast to PAR, UV-B radiation induced (2.2 fold) the intensity of PBPs after 240 min of exposure. Further exposure resulted in decline in fluorescence. However, combination of PAR+UV-B had resulted in slight increase in fluorescence intensity after 300 min of exposure in comparison to control (Fig. 5A). Similar effects have been observed with emission fluorescence of PC after exposure of PAR and UV-B radiation. Combination of PAR+UV-B resulted in a slight increase in PC after 300 min of exposure (Fig. 5B).

**Fluorescence Wavelength Shifting**

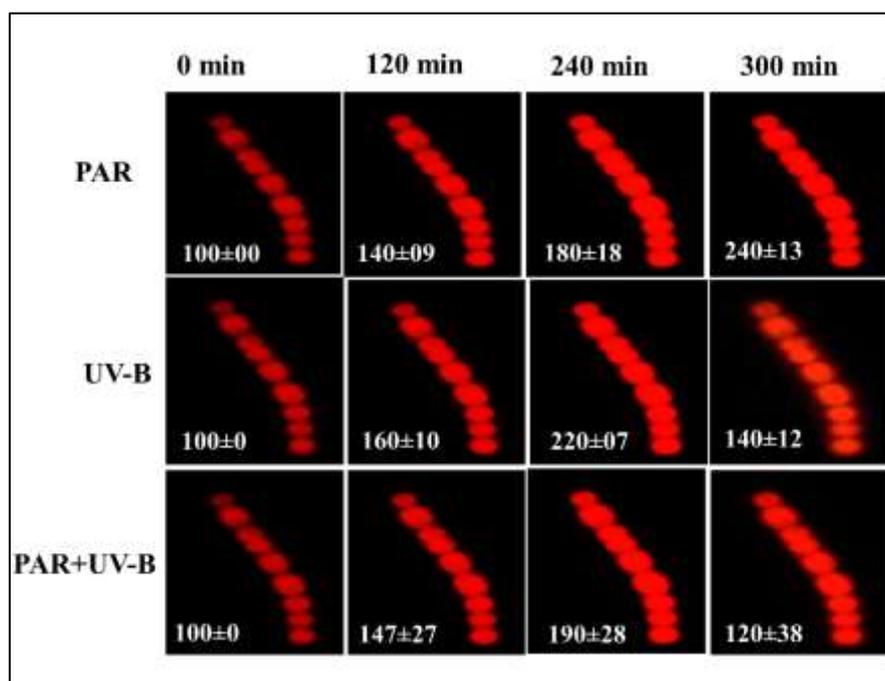
Both emission fluorescence of PE (574 nm) and PC (646 nm) were slightly shifted towards shorter or longer wavelengths as compared to control after exposure of PAR. In contrast to PAR, emission fluorescence of PE had shifted to longer wavelengths from 574–576 after 240 min of UV-B exposure. Similarly, a slight change in PE fluorescence was observed after 240 min of PAR+UVB radiation. The emission fluorescence of PC was shifted towards shorter wavelengths after UV-B and PAR+UV-B radiations after 300 min of exposure (Table 1)



**Fig. 5:** Fluorescence values of PE (A) and PC (B) after exposure of PAR, UV-B and PAR+UV-B radiations. Horizontal line over bars indicate no significant differences ( $P>0.05$ ). Asterisk marks indicate significant differences ( $P<0.05$ ) with the control (0 h). The error bar denotes standard deviations of means ( $n=3$ ).

**Table 1:** Fluorescence emission wavelength shifts in PE and PC of *Nostoc* sp. HKAR-2 after 300 min of exposure to PAR, UV-B and PAR+UV-B radiations. Em.: Emission;  $Em^0$ : Emission at 0 min of exposure;  $Em^n$ : Emission after n min of exposure.

Time (min)	PAR		UV-B		PAR+UV-B	
	PE- Em. ( $Em^0 \pm Em^n$ )	PC- Em. ( $Em^0 \pm Em^n$ )	PE- Em. ( $Em^0 \pm Em^n$ )	PC- Em. ( $Em^0 \pm Em^n$ )	PE- Em. ( $Em^0 \pm Em^n$ )	PC- Em. ( $Em^0 \pm Em^n$ )
Control	574 (0)	646 (0)	574 (0)	646 (0)	574 (0)	646 (0)
30	575 (+1)	643 (-3)	573 (-2)	642 (-4)	574 (0)	643 (-3)
60	574 (0)	643 (-3)	574 (0)	643 (-3)	574 (0)	643 (-3)
120	574 (0)	645 (-1)	575 (+1)	644 (-2)	575 (+1)	644 (-2)
180	574 (0)	645 (-1)	575 (+1)	644 (-2)	575 (+1)	645 (-1)
240	575 (+1)	646 (0)	576 (+2)	645 (-1)	574 (0)	646 (0)
300	574 (0)	646 (0)	568 (-6)	639 (-7)	570 (-3)	640 (-6)



**Fig. 6:** Fluorescence microscopic images of *Nostoc* sp. strain HKAR-2 after 0, 120, 240 and 300 min exposure of PAR, UV-B and PAR+UV-B radiations. Fluorescence percentage is indicated in brackets.

### *In-Vivo* Fluorescence of PBPs

*In-vivo* emission fluorescence of PBPs in cyanobacterial cells was visualized after 300 min of exposure to PAR, UV-B, and PAR+UV-B radiations. The internal fluorescence of PBPs had increased up to 2.4 fold after 300 min of exposure to PAR. However, internal fluorescence of PBPs had increased up to 2.2 and 1.9 fold after 240 min exposure of UV-B and PAR+UV-B radiations (Fig. 6). Further irradiation of UV-B and PAR+UV-B resulted in a decline in fluorescence after 300 min of exposure which indicates the photobleaching of cyanobacteria (Kannaujiya and Sinha, 2016c).

### Conclusion

The present work describes the short duration exposure effects of PAR and UV-B radiations on PBPs composition in a hot-spring cyanobacterium *Nostoc* sp. strain HKAR-2. The absorbance and emission fluorescence intensity of PC and PE indicated a rapid synthesis of PBPs up to 240 min of exposure to UV-B and PAR+UV-B radiations. This method can be used for the production of PC and PE from cyanobacterial sources for many biotechnological and biomedical applications.

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### Conflict of Interest

The authors have no conflicts of interest.

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