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Effects of Ultraviolet and Photosynthetically Active Radiation on Phycocyanin of Habitat Specific Cyanobacteria

Deepak Kumar¹, Vinod K. Kannaujiya², Jyoti Jaiswal¹ and Rajeshwar P. Sinha^{1*}

¹Laboratory of Photobiology and Molecular Microbiology, Center of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi. rpsinhabhu@bhu.ac.in

²Department of Botany, MMV, Banaras Hindu University, Varanasi

Abstract: Cvanobacteria are widely distributed and evolutionary ancient Gram-negative prokaryotes on Earth. They are highly susceptible to incoming ultraviolet radiation (UV) in the form of solar spectrum. The accessory light harvesting proteins are the first site of action of UV which affects the process of photosynthesis. We have studied the response of photosynthetically active radiation (PAR) and combination of PAR + Ultraviolet-A (UV-A) + Ultraviolet-B (UV-B) (PAB) irradiations on phycocyanin (PC), carotenoids and chlorophyll a (Chl a) of five heterocystous and nonheterocystous cyanobacterial species. We have found that Chl a had continuously declined and carotenoids was increased significantly after 24 h of exposure of PAB radiation in both heterocystous and non-heterocystous cyanobacteria. We have also recorded more decline in amount and intensity of emission fluorescence of phycocyanin (PC) in non-heterocystous as compared to heterocystous cyanobacteria in the same period of time. Similarly, fluorescence wavelength was also shifted towards shorter wavelengths. These results indicate that photosynthetic pigments of heterocystous cyanobacteria were more stable than nonheterocystous cyanobacteria. These cyanobacteria may be used as stable source for the production of value added pigments and applications in diverse field of biomedical, pharmaceutical and biotechnological sciences.

Index Terms: Carotenoids, Cyanobacteria, Fluorescence, Phycocyanin, Ulltraviolet.

I. INTRODUCTION

Cyanobacteria are key source of biomass producers in both aquatic and terrestrial ecosystems. They belong to primitive group of Gram-negative, oxygenic photoautotrophic prokaryotes with cosmopolitan distribution in diverse habitats (Norval et al., 2015; Kannaujiya and Sinha, 2015). Apart from cosmopolitan distribution, cyanobacteria play indispensable role in fixation of atmospheric nitrogen as well as improving soil fertility and regulating energy dynamics in ecosystem (Stock et al., 2014; Häder et al., 2015). Cyanobacteria are also considered as model organisms for genetic engineering and space research.

Although, recent progress in industrializations has resulted in an increase in amount of halogen compounds, chlorocarbons, organobromides and other halogen based compounds in atmosphere which considerable deplete the lifesaving stratospheric ozone layer (Kramlich & Linak, 1994; Häder et al., 2015). The prominent depletion of ozone layer has resulted in an increase in harmful ultraviolet radiations on Earth surface. Broadly, ultraviolet radiation can be categorized into three major spectral regions such as UV-A (315 - 400 nm), UV-B (280-315 nm), and UV-C (100-280 nm) respectively (Kannaujiya et al. 2017a). The harmful ultraviolet radiations cause drastic inhibition in growth rate, photosynthesis, nitrogen fixation, lipid, CO2 uptake, nutrient uptake, DNA damage and destruction of protein biomolecules in several organisms (Schmidt et al., 2010; Richa et al., 2016). The internal factors for UV-induced damage are accelerated by generation of reactive oxygen species, superoxide anion radicals, hydroxyl radicals, hydrogen peroxide, hydroperoxyl radicals and hypochlorous acid (Schmitt et al., 2014).

Cyanobacteria have accessory light harvesting protein complexes or phycobiliproteins (PBPs) which absorb > 99 % of total solar radiation (Lao & Glazer 1996), however, < 1 % of UV-B radiation invariably cause deleterious effects on biochemical and physiological processes in aquatic as well as terrestrial organisms (Ballaré et al., 2011; Häder et al., 2015). PBPs have been characterized into three groups such as phycocyanin (PC, 610-620 nm), phycoerythrin (PE, 540-570 nm) and allophycocyanin (APC, 650- 655 nm) (Kannaujiya et al., 2016a). They are water soluble, brilliantly coloured and multi-chain proteins assembled into supramolecular lightharvesting complexes on the thylakoid membranes of chloroplast. The effects of UV-B radiation primarily occurred on absorbance and fluorescence of PBPs which affects the photosystem II complex of cyanobacteria (Chis et al., 2016; Kannaujiya & Sinha 2017b).

In the present investigation, we have shown the impacts of ultraviolet radiation and photosynthetically active radiation on physiological and biochemical responses of PC as well as carotenoids and chlorophyll a (Chl a) in different heterocystous and non-heterocystous cyanobacteria.

II. MATERIALS AND METHODS

A. Organisms and growth conditions

We have used various heterocystous and non-heterocystous cyanobacterial species isolated by using standard microbiological technique (Sinha et al., 1995a) (Table I). All organisms were routinely grown in axenic BG-11 medium (Rippka et al., 1979) whereas *Spirulina platensis* was grown in Zarrouk's medium. Culture was illuminated under white fluorescent tubes with an intensity of 12 ± 2 Wm⁻² in 14/10 L/D cycle. All experiments were performed in log phase or exponential growth phase.

Table	I:	Different	strains	of	heterocystous	and	non-heterocystous		
cyanobacteria of different habitats, temperature and pH.									

Species	H	abitats	Temperature	pH
Heterocystous	Source	Place	(°C)	-
Anabaena	Fresh	Uttar	20-30	7-8
cylindrica	water	Pradesh,		
		India		
Fischerella sp.	Rocks	Rajasthan,	30-40	7.5-8
HKAR-5		India		
Nostoc sp. HKAR-	Hot-	Bihar, India	35-40	6-7
2	spring			
Nostoc sp. HKAR-	Rice-	Uttar	20-30	7-8
11	field	Pradesh,		
		India		
<i>Rivularia</i> sp	Hot-	Bihar, India	35-40	6-7
HKAR-4	spring			
Non-heterocystous				
Arthrospira	Soda	Rajasthan,	35-40	8-9
Platnesis	lake	India		
Cyanothece sp.	Hot-	Bihar, India	35-40	6-7
HKAR-1	spring			
Gloeocapsa sp.	Rice-	Uttar	20-30	7-8
	field	Pradesh,		
		India		
Phormidium sp.	Fresh	Uttar	30-35	7-7.5
	water	Pradesh		
		India		
Synechococcus sp.	Fresh	Uttar	20-30	7-8
HKAR-10	water	Pradesh,		
		India		

B. Experimental setup and source of radiation

Exponentially growing cyanobacterial cultures (under photon flux density of 12 \pm 2 Wm⁻²) were transferred into sterile transparent Petri dishes and placed on a rotary shaker for uniform exposure of ~1 Wm⁻² UV-B (Philips Ultraviolet-B TL 40 W:12, Holland) and PAR (55.08±9.18 µmol m⁻² s⁻¹) (OSRAM L 36 W: 32 Lumilux deluxe warm white and Radium NL 36 W: 26 Universal white, Germany) and UV-A (Philips Ultraviolet-A TL 40 W:12, Holland) glass tubes fitted in the same chamber. The Petri dishes were covered and irradiation was restricted with 295 and 395 nm cut off filters (Ultraphan,

UV OpakDigefra, Munich, Germany) to get desired irradiation regimes of PAR (400-700 nm), UV-A (315-400 nm) and UV-B (280-315 nm) (PAB). Cultures were withdrawn at regular intervals during 24 h of exposure. During the entire period of experiment, temperature was maintained at 20 ± 2 °C.

C. Isolation and purification of PC

Cultures were harvested by centrifugation (5,000g for 15 min) at 4 ± 2 °C. Pellets were washed with 50 mM phosphate buffer (PB) and inoculated in the same buffer with addition of reaction mixture containing 1 % (w/v)EDTA. 1 mM phenylmethylsulfonyl fluoride (PMSF), and 5 % (w/v) sucrose. Simultaneously, cells were sonicated (130 W, 20 kHz, 50 % amplitude for 3-5 min) and suspended aliquots were subjected to repeated freeze (-20 °C) and thaw (4 °C) cycles for desired extraction of PBPs (Kannaujiya & Sinha 2016ab). The crude extract of PC was precipitated by 20-60% ammonium sulfate and dialyzed through a fine dialysis tubing (Sigma, 12 kDa). PC was loaded on a sucrose density gradient (5-15%) for ultracentrifugation (Beckman coulter optima XPN-100, USA) and centrifuged at 35,000g for 60 min (Sinha et al., 1995 ab). The purity of PC was calculated by using the equation described by Kannaujiya and Sinha (2016b).

D. Spectrophotometric and fluorescence analysis

Absorbance of PC (λ^{615}) was recorded by a UV-Vis spectrophotometer (U-2910, Hitachi, Tokyo, Japan). Emission fluorescence of PC (λ^{642}) was measured by a fluorescence spectrophotometer (G9800AA, Agilent Technologies, Cary Eclipse, USA) and calculated wavelength shift of emission intensity.

E. Estimation of chlorophyll a and carotenoids

The cyanobacterial cell cultures were sonicated (20 kHz, 130 W, Sonic and Materials, USA) in 80 % acetone and kept for 15 min at 80 °C. The samples were centrifuged at 5000g for 10 min and the supernatant was mixed properly with an equal ratio of solvent (water and petroleum ether) and kept at 4 °C. The green layer of supernatant was collected and spectrum was taken in a UV-Vis spectrophotometer. Estimation of Chl *a* and carotenoids was done by the methods as described by Mackinney (1941) and Dere et al. (1998) respectively.

F. Statistical analysis

At least three replicates (mean \pm SD) were taken in our experimental results. All data were analyzed by one-way analysis of variance to confirm the significance of data (P \leq 0.05). Microsoft Excel (2010), Sigma Plot 11 and SPSS-16 software were used for the statistical analysis.

III. RESULTS AND DISCUSSION

A. Analyses of pigments and proteins

Total carotene, chlorophyll content and protein in cyanobacterial cells of heterocystous and non-heterocystous cyanobacteria were changed during light irradiation of PAB exposure as compared to control (Table 2). Chlorophyll content was declined about 27 % and 33 % in *Nostoc* sp. HKAR-11 and *Rivularia* sp. HKAR-4 respectively. However, other cyanobacteria such as *Nostoc* sp. HKAR-2, *Anabaena cylindrica* and *Fischerella* sp. HKAR-5 were exhibited insignificant

decline in same duration. It was already established that intensive irradiation of PAB accelerated rapid bleaching and inhibition of photosynthesis in cyanobacteria (Bhandari & Sharma 2006).

Table II: Concentration of pigments and proteins of heterocystous and non-heterocystous cyanobacteria under control (PAR) and after 24 h exposure of PAB irradiation.

Species (code)	Contr ol Chl a	24h PAB Chl a	Contr ol Carot	24h PAB Carote	Contro l Total Protei	24h PAB Total		
Heterocystous	(µg/gf w)	(µg/gf w)	en- oid (µg/gf w)	noid (µg/gf w)	n (µg/ml)	Protei n (µg/ml)		
Anabaena cylindrica (AC)	9.10±0 .50	7.0±0.1 0	3.50± 0.05	3.30±0. 50	209.0± 1.0	192.0± 1.50		
<i>Fischerella</i> sp. HKAR-5 (FH5)	33.50± 0.70	31.0±1. 250	6.70± 0.10	8.0±0.5 0	132.0± 0.50	104.0± 1.25		
Nostoc sp. HKAR-2 (NH2)	36.50± 0.50	26.0±0. 50	8.0±0. 10	8.70±0. 25	192.0± 0.50	186.50 ±1.0		
Nostoc sp. HKAR-11 (NH11)	25.75± 0.20	24.0±0. 10	7.0±0. 10	7.55±0. 10	197.0± 0.50	175.0± 1.0		
Rivularia sp. HKAR-4 (RH4)	4.50±0 .10	3.0±0.2 0	5.0±0. 01	5.25±0. 20	231.50 ±1.0	220.0± 1.40		
Non-heterocystous								
Spirulina platensis (SP)	11.0±0 .50	10.50± 0.10	1.50± 0.10	2.25±0. 15	463.0± 0.50	363.50 ±1.50		
Cyanothece sp. HKAR-1 (CH1)	35.0±0 .20	33.0±0. 10	12.25 ±0.50	14.0±0. 30	333.0± 0.50	256.75 ±1.50		
Gloeocapsa sp. (GS)	23.0±0 .10	19.50± 0.20	4.60± 0.10	4.15±0. 30	150.85 ±0.50	145.75 ±0.50		
Phormidium sp. (PS)	53.50± 0.40	44.50± 0.40	1.25± 0.05	4.0±0.0 5	37.75± 0.40	35.75± 0.50		
Synechococcus sp. HKAR-10 (SH10)	4.50±0 .10	4.20±0. 150	3.0±0. 05	4.0±0.0 5	218.0± 0.40	211.50 ±1.0		

It has been also established that UV-B induced more bleaching response and play significant role in molecular conversions of protochlorophyllide to chlorophyllide (Marwood and Greenberg 1996). In response of PAB radiation, total carotenoids in Fischerella sp. HKAR-5 was induced about 19 % whereas only 7-8 % synthesis of carotenoid was recorded in Nostoc sp. HKAR-2 and Nostoc sp. HKAR-11. Protein content was declined more in Fischerella sp. HKAR-5 (12 %) and least in Cyanothece sp. HKAR-4. In non-heterocystous cyanobacteria, about 17 % Chl a content was declined in Phormidium sp. whereas other cyanobacteria show insignificant decline in same content after exposure. The total carotene content was increased 3 times in *Phormidium* sp. whereas about 50 % increments were recorded in Spirulina platensis after exposure of PAB irradiation (Table II). About 22 % of protein content was declined in Spirulina platensis after exposure. Induction in carotenoids content is well established as photoprotective scavenging potential of reactive oxygen species (Pattanaik et al., 2008; Kannaujiya et al., 2014). In addition, it has been shown that significant bleaching effects occur not only in pigment composition but also in photosynthetic performance, yield, and

proteins profile in many cyanobacterial species (Fragoso et al., 2014). Thus, absorbance range of proteins makes them very susceptible to damage from UV radiation (Karsten et al., 2007; Hargreaves et al., 2007).

B. Spectrophotometric analyses of PC

The extracted PC from different cyanobacteria of heterocystous and non-heterocystous cyanobacteria had shown peaks of PC at 615 nm and characteristic fluorescence emission spectrum was observed at 642 nm. PC has been reported to be severely affected by intense solar radiation due to nature of light harvesting property (Sinha et al., 2003). Low light intensity and variable photoperiods play enhancing role in PC content in cyanobacteria such as *Nostoc calcicola* (Khajepour et al., 2015) and *Spirulina platensis* (Kumar et al., 2018). The effects of UV on heterocystous and non-heterocystous cyanobacteria showed decline in PC content after 24 h exposure due to bleaching, degradation and uncoupling effects as compared to control (PAR) with significance difference (P < 0.05) (Fig. 1).

We have observed that PC declined in 47, 22, 24, 42 and 35 % in heterocystous cyanobacteria such as Anabaena cylindrica, Fischerella sp. HKAR-5, Nostoc sp. HKAR-2, Nostoc sp. HKAR-11 and Rivularia sp. HKAR-4 respectively after exposure. However, PC content was reduced about 32, 34, 58, 49 and 48 % in non-heterocystous cyanobacteria such as Spirulina platensis, Cyanothece sp. HKAR-1, Phormidium sp., Synechococcus sp. HKAR-10 and Gloeocapsa sp. respectively. The PAB exposure on cyanobacteria has accelerated production of free radicals which causes photobleaching and damage of PBPs (Kannaujiya & Sinha, 2015). It has been found that PC content was less affected in hot-spring cyanobacteria as compared to other cyanobacteria (Kannaujiya & Sinha 2017b). Apart from combination effects of PAR and UV-B radiation, it has been shown that UV-B radiation induces photodestruction in PC, PE and APC subunits of PBPs in Lyngbya sp. A09DM (Rastogi et al. 2015).

C. Fluorescence responses of PC and PE

It is well established that UV radiation induces disassembly and photobleaching in PBPs which affect the fluorescence property (Sinha et al., 2003). Similarly, a rapid photo-destruction in fluorescence band of PC was found in the cyanobacterium Nostoc sp. HKAR-2 (Kannaujiya & Sinha, 2017b). Biochemical investigation on fluorescence protein bands ($\alpha\beta$) of PC also provides major evidence for the site of action of UV radiation (Kannaujiya & Sinha, 2017a). Recently, it has been found that cysteine linked chromophores act as primary site for action of UV-B radiation and causes inhibition of fluorescence (Kannaujiya & Sinha, 2017b). Moreover, UV radiation may affect light and dark transition between PS-II and PBPs (Chukhutsina et al., 2015). Emission fluorescence of PC had declined in PAB as compared to control (PAR) with significance difference (P < 0.05) (Fig. 2 A, B). Similarly, the emission fluorescence intensity of PC was declined up to 25, 16, 21, 25 and 62 % in Spirulina platensis, Cyanothece sp. HKAR-1, Phormidium sp., Synechococcus sp. HKAR-10 and Gloeocapsa sp. respectively. However, heterocystous cyanobacteria such as Anabaena cylindrica, Fischerella sp. HKAR-5, Nostoc sp. HKAR-2 and Rivularia sp. HKAR-4 had shown lower reduction as compared to non-heterocystous cyanobacteria (Fig. 2 A, B).

PAB had shown maximum blue wavelength shift of PC ranging from 640-638 nm after 24 h of irradiation (Fig. 3A, B). The wavelength shifting was more pronounced in non-heterocystous cyanobacteria as compared to heterocystous counterpart. Therefore, fluorescence nature of chromophores in PC was slightly affected in PAB radiation in heterocystous cyanobacteria.



Fig. 1: PC content of heterocystous (A) and non-heterocystous (B) cyanobacteria under control (PAR) and 24 h exposure of PAB irradiation.

CONCLUSION

PAB irradiation influenced the physiology and biochemistry of heterocystous and non-heterocystous cyanobacteria in terms of chlorophyll, proteins and carotenoids content. The dynamic change in pigments and proteins content provides a significant insight for growth and development of cyanobacteria. Results indicate that exposure of PAB is more suitable for growth and stress adaptability in heterocystous cyanobacteria. The cyanobacterium of hot-spring habitats was more stable as compared to other cyanobacteria. Overall, heterocystous hotspring cyanobacteria are more stable as compared to nonheterocystous cyanobacteria. Thus, heterocystous hot-spring cyanobacterium could be used to for the production of stable photosynthetic and non-photosynthetic metabolites for biotechnological and biomedical applications.

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Fig. 2: Fluorescence emission intensity of PC of heterocystous (A) and non-heterocystous (B) cyanobacteria under control (PAR) and 24 h exposure of PAB irradiation.



Fig. 3: Fluorescence wavelength shift in PC of heterocystous (A) and non-heterocystous (B) cyanobacteria under control (PAR) and 24 h exposure of PAB irradiation.

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