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The response of phycobiliproteins to light qualities in *Anabaena circinalis*

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ABSTRACT

The effect of different light qualities (white, blue, green and red light) on the accumulation of phycobiliproteins was studied in *Anabaena circinalis*. The results also showed that responding to different light, this strain change the composition of their light-harvesting pigments, phycoerythrin (PE) and phycocyanin (PC). Photosynthetic pigment's content (PE, PC and APC) were high in white light, phycoerythrin (31.53 µgmg^{-1}), phycocyanin (135.01 µgmg^{-1}) and allophycocyanin (35.92 µgmg^{-1}) after 15 days of growth. In contrast, the lowest phycocyanin was found under blue light (20.01 µgmg^{-1}). Red light favoured the accumulation of phycoerythrin (30.29 µgmg^{-1}) than blue and green but lower than white light. Increasing awareness of harmful effects of synthetic compounds and towards the usage of natural products have led to the exploitation of microalgae as a source of natural pigments. The findings revealed that phycobiliproteins composition and colour of strain drastically changed in response to light quality through complementary chromatic adaptation. Phycocyanin content was significantly correlated with fluorescent white light indicating its important role in cyanobacteria.

1. INTRODUCTION

Phycobiliproteins are the major photosynthetic accessory pigments in cyanobacteria which are brilliantly coloured, water soluble proteins, bearing covalently attached open chain tetrapyrroles [1]. They are naturally occurring fluorescent pigments derived from cyanobacteria and some eukaryotic algae, serving as accessory or antenna pigments for the photosynthetic light-harvesting complex. These pigments include the blue pigment phycocyanin and the red pigment phycoerythrin. Additionally, these pigments represent the major constituents of red algae and cyanobacteria and may represent up to 60% of the total protein content of the cell [2]. The growing awareness on the importance of natural colours, especially in food and cosmetics, colourants has placed great demand on biological sources of natural colours. Global attention has been focused on cyanobacteria for their potential applications in mariculture, food, feed, fuel, fertilizer, biopolymers, natural colorants, vitamins, toxins, enzymes, pharmaceuticals, pharmacological fluorescent probes, and pollution abatement [3]. The prices of phycobiliproteins vary from US$ 3-25 mg^{-1} for food/cosmetic grade pigments but they can reach US$ 1500 mg^{-1} for highly purified molecular markers (with antibodies or other fluorescent molecules). In 1997, the value of these pigments in the commercial sector was estimated to be US$ 50 million worldwide [4]. Among various cyanobacterial and algal strains, only a few like *Spirulina*, *Porphyra*, *Porphyridium* spp. etc. were well-characterized and exploited commercially [5]; [6]. Phycocyanin and phycoerythrin are the two currently used natural pigmented and fluorescent proteins having versatile applications. They are stable at low temperature [7] with some preservative like citric acid [8]; [9] in acidic and basic solutions and therefore, they could be leveraged as food colorant (chewing gum, jellies), health drink and colouring agent in sweet confectionary and cosmetics [10]. The photosynthetic system of many cyanobacteria is highly adaptable to quality and quantity of light in different environments. These stress signals has significant role in influencing the synthesis of physiologically important phycobiliproteins [11]. Most cyanobacteria are shade-adapted organisms, possessing efficient mechanisms to counteract the harmful effects of solar radiation, especially freshwater forms exposed to high tropical irradiances. Light too plays a vital role for growth and pigment accumulation in cyanobacteria and the colourful process in which cyanobacteria dramatically alters its pigmentation in response to ambient light colour changes, i.e. change in the colour phenotype is known as complementary chromatic adaptation [12]; [13].

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The amount of light harvesting proteinic pigment influences the accumulation of target products in the microalgae cultivated under different light conditions [14]. The chromatic acclimation process allows the cells of cyanobacteria to alter its light absorption characteristics in order to regulate photosynthesis according to light availability in different environments. As a consequence of this phenomenon, the pigment which absorbs the incident wavelengths of light most strongly becomes predominant [15]; [16]. Variable spectral proportions of light like red:far red, blue:red, green:red and blue:green affect the relative pigment composition and possibly act as photo-morphogenic signals in algae [17]. However, composite effect of individual light quality remains scanty. Thus, to understand the factors affecting, different light penetrations to algal suspension must be investigated in terms of pigment production. Instead of chemical mutagenesis which has been employed as a tool to improve the pigment content of cyanobacterial strains [18], the identification of promising strains and cultural/ environmental conditions for maximizing production of specific pigment(s) would be a more viable option. In the present investigation, it was evaluated how the changes in light quality affect the production of phycobiliproteins pigment in *Anabaena circinalis*. Although, normally the content of pigments depends on the species of cyanobacteria and cultivation conditions, no such information exists with respect to *Anabaena circinalis*.

2. MATERIALS AND METHODS

2.1 Organism and growth conditions

Cyanobacterial strain used in this study was isolated from Loktak Lake, Manipur, India (a Ramsar site, located between longitudes 93º46' to 95º55' E and latitudes 24º25' to 24º42' N at an elevation of 768.5 m) and purified in our laboratory of Freshwater Cyanobacterial and Microalgal Repository (National facility created by the Department of Biotechnology, Government of India with reference No. BT/PR 11323/PBD/26/171/2008 dated 31-03-2009) at Institute of Bioresources and Sustainable Development (IBSD), Imphal, Manipur, India. The study of the strain was carried out using trinocular research microscope (NIKON Eclipse 80*i*) and Carl Zeiss fluorescence microscope, Axio Scope A1 coupled with Carl Zeiss Imaging Systems 32 software AxioVision 4.7.2 followed by taxonomical characterization referring to key [19]. A log phase culture was homogenized and 1 ml of the culture was inoculated into 250 ml cotton-plugged Erlenmeyer flask containing 100 ml of BG11 medium (nitrate free) [20] and kept in the culture room. The culture room rack was fitted with photoperiodic automatic model timer coupled with Biotech room temperature controller to provide alternative light and dark phases. White light (WL) grown cells were illuminated using cool white fluorescent tubes. Red (RL), green (GL) and blue light (BL) grown cells were exposed to a similar light filtered through layers of appropriate coloured cellophane papers wrapped around the culture flasks providing chromatic illumination. Cultures were allowed to grow in light intensity of 40 µmol photons m^{-2} s^{-1} provided by cool white fluorescent tubes following light/dark cycles of 14:10 h condition maintained at 28±2ºC for 15 days. The flasks were stirred twice daily to allow uniform light penetration and circulation of air and nutrients. The fresh cyanobacterial cells were harvested after 15 days of incubation.

2.2 Extraction and quantification of phycobiliproteins

Phycobiliproteins were extracted from harvested biomass by centrifugation at 5000x*g* for 10 min in 0.05 M phosphate buffer (pH 7.0), employing repeated freezing (-20ºC) and thawing (room temperature) method, till coloured supernatant was obtained from the pellet. The absorbance of phycobiliprotein containing cell-free supernatants obtained by the centrifugation was measured at 562 nm, 615 nm and 652 nm using phosphate buffer as a blank. These wavelengths correspond to the absorption maxima of phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) respectively and was determined spectrophotometrically in spectrophotometer (UV-1800, Shimadzu, Japan) using the formula [21].

2.3 Determination of absorption spectra

Absorption spectra were determined by scanning the supernatant in a range of 400-700 nm wavelengths by spectrophotometer at room temperature.

2.4 Statistical analysis

The data showing the effects of different light qualities on phycobiliproteins production were subjected to one-way analysis of variance. The data were analyzed using SPSS version 19.0 software (IBM, Chicago, USA). Data obtained was subjected to analysis of variance and least significant difference (LSD) at 5% were tested to separate the means. The criteria for statistical significance (LSD) was set at $p<0.05$. Furthermore, Pearson's correlation analysis was used to analyze the correlation among phycobiliproteins content and light qualities.

3. RESULTS AND DISCUSSION

Thallus was dark green, mucilaginous, submerged and floccose. Filament flexuous, hyaline and colourless sheath, cells cylindrical, rarely barrel shape, heterocyst intercalary and spherical. A difference in the production of phycobiliproteins was observed when the strain was cultured in different light qualities. PC concentrations in fluorescent WL was significantly higher than other three light during the study period (LSD test, $p<0.05$). Therefore, we hypothesized that PC concentrations can be enhanced in other light qualities also. The flasks showing the growth pattern of the studied strain before and after chromatic illumination were shown (Fig 1a and 1b). Our experiments proved that among different light qualities, phycocyanin production was found to be obviously elevated in WL. It was found that PC and APC underwent partial modulations under WL while APC remained almost constant under all other three light qualities used. The order of suitable chromatic regime for the phycocyanin

production was found to be white> red > green> blue. PC concentration was significantly higher (LSD test, $p<0.05$) in WL compared with the other three i.e. RL, GL and BL. The correlation between PE, PC, APC and different light qualities was presented in Table 1 and was found to be significant ($p<0.01$).

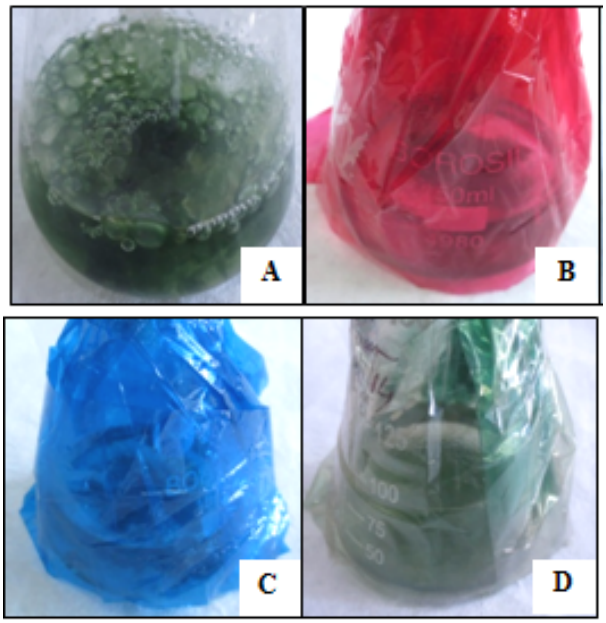

Fig 1a. Culture flasks of *Anabaena circinalis* wrapped with coloured cellophane papers providing chromatic illumination.

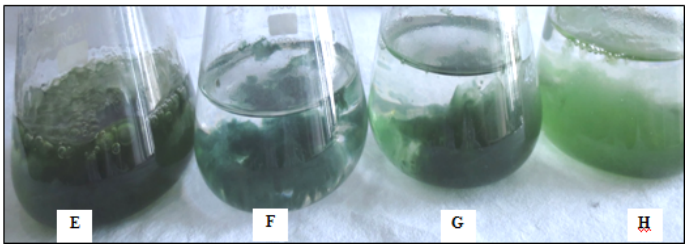

Fig. 1b: Growth pattern of *Anabaena circinalis* after chromatic illumination.

Table 1: Correlation between phycobiliproteins and different light qualities.

Different variables	Phycoerythrin (PE)	Phycocyanin (PC)	Allophycocyanin (APC)
White light	0.768**	0.981**	0.920**
Red light	0.596**	0.598**	0.418**
Green light	0.216*	0.223*	0.184*
Blue light	0.148*	0.154*	0.129*

* Correlation is significant at $p<0.05$; ** Correlation is significant at $p<0.01$.

However, there were no significant differences among the treatments. PE and PC content also increased in all treatments, except BL. The increase of PC was greater than that of PE. Furthermore, the content of PE was significantly ($p < 0.05$) higher in WL and RL than in BL and GL, but there were no significant differences among treatments in PC content. The absorption spectrum analysis of phycocyanin (PC) showed that WL was found to exhibit highest absorbance of 0.680 followed by RL of 0.313, GL of 0.264 and BL of 0.190 at 615 nm (Fig 3a-3d). The spectra showed a peak at 615 nm for all four lights for PC. The absorption spectra of PC from WL grown cells showed a sharp peak at 615 nm when compared to the other three light. Thus, these results suggest that the 615 nm absorbing component was under the light quality control and WL enhanced the levels of this component.

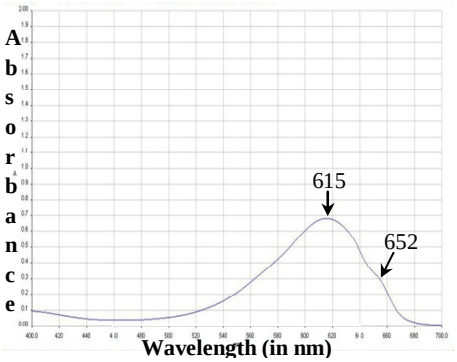

Fig. 3a: Fluorescent white light (WL).

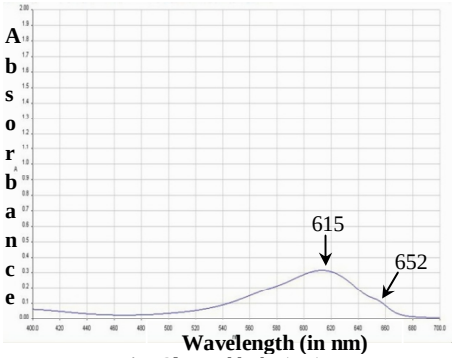

Fig. 3b: Red light (RL)

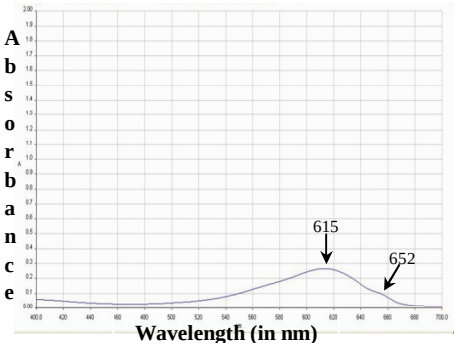

Fig. 3c: Green light (GL)

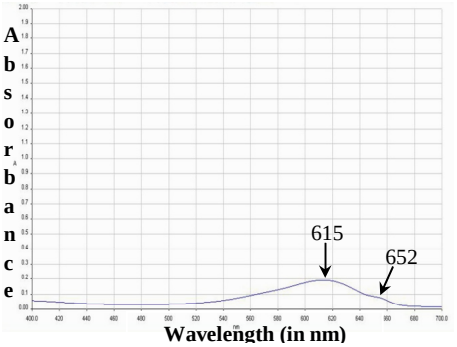

Fig. 3d: Blue light (BL).

Figs 3a-3d. Absorption spectrum of crude phycocyanin extract in different qualities of light on *Anabaena circinalis*.

As can be seen from the concentration of phycocyanin, there is a direct relationship between WL and phycocyanin. Under WL, C-phycocyanin production was highest and C-allophycocyanin production got drastically decreased. PE concentrations in cultures grown under WL and RL were significantly higher than those in cultures grown under all other light colour treatments. PE concentrations under WL and RL were around twofold those under BL. PC concentrations under WL were significantly greater than those for all other light quality conditions (Fig 2); there was no significant difference between the BL and GL treatments. APC concentrations under WL were significantly higher than those under RL, BL and GL. Our results were consistent with the studies of [22].

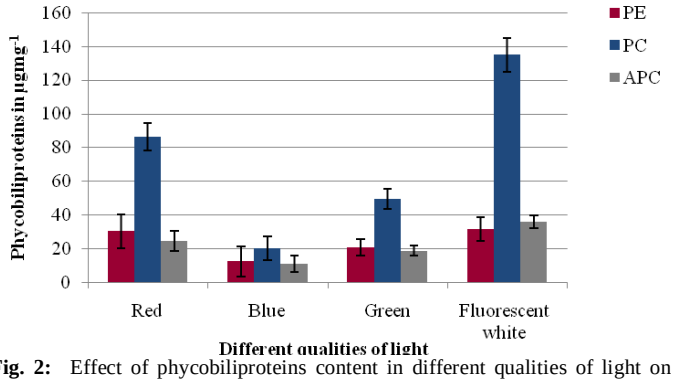

Fig. 2: Effect of phycobiliproteins content in different qualities of light on *Anabaena circinalis*. Values are means ± SD (n = 3).

The pigments allophycocyanin, phycocyanin and phycoerythrin were high in RL when *Westiellopsis iyengarii* was subjected to different light qualities as reported by [23]. In their study, all the three phycobilin pigments were absent in GL condition. However, [24] observed that C-phycoerythrin production was more in GL and BL as contrast to our findings. In their studies, phycocyanin production during RL treatment was highest (14.9 mgl^{-1}) which gradually decreased in GL (3.7 mgl^{-1}), BL (3.5 mgl^{-1}) and WL (2.1 mgl^{-1}) treatment whereas our findings showed more phycocyanin in the WL followed by RL and GL. The research findings of [25] revealed that BL enhanced the pigment synthesis in cyanobacterium *Anabaena ambigua*. These above discrepancies could be due to various reasons e.g. differences in species, physiological age and light quality and regulation to chromatic adaptation. Effect of chromatic light on phycobiliproteins induction was also reported as strain specific [26]; [27] indicated that WL for *Anabaena* sp. and GL for *Nostoc* sp. as most promising for the phycobiliproteins synthesis. However, for enhancement in the yield of phycocyanin, it was necessary to optimize the colour of light in the present investigation. In this particular strain, chromatic adaptation has shown an important role in enhancing the phycocyanin pigment content by 1.6-fold increase which is beneficial during the purification process, where suppression of other phycobiliproteins occur and simultaneously induced C-PC content through this phenomenon. This process is economical, scalable and fast to achieve high production of phycocyanin. *Anabaena circinalis* cells adjust the phycobiliprotein composition in response to light quality, in order to make use of the light source to conduct photosynthesis and other life relating activities. Under white (WL) and red light (RL), the relative content of phycocyanin and phycoerythrin increased and their contents reached the highest which intensified the absorption of the white and red light. It can be seen that phycoerythrin and phycocyanin with the main absorbances in the white and red light regions were the main components in cells, which implies that this strain tends to absorb the white (WL) and red light (RL) for photosynthesis and other activities. However, for allophycocyanin, it was considered that its maximal absorbance is near 645 nm (RL region) [28], but its content reached the highest in the WL. However, it was reported that RL stimulate phycobiliprotein production in *Nostoc* UAM206 [29] and *N. muscorum* [30], while BL positively affect phycobiliprotein production in red algae *Chondrus crispus* [31]; *Halymenia floresii* [32]; *Porphyra lecosticta* [33]. In the present communication, assuming presence of a PXB type of chromophore in the cyanobacterium and this chromophore undergoes light quality dependent modulations. Phycobiliprotein levels in the extracts of cyanobacterium exhibited partial modulations in the levels of PC but not APC under the light qualities mentioned. It may be hypothesized that in white light (WL) some kind of structural changes occur in some of the native apoproteins of PC which in turn are exposed to isomerases responsible for the transformation of PCB chromophores into PXB chromophores. These kinds of changes were suggested earlier by [34]; [35]. PC synthesis in *Nostoc* sp. was highest in WL as reported by [36] which supported our findings. Recently, information about how light quality regulates vegetative cell shape and integrity of cyanobacterial filaments has begun to emerge [37]. When incubated under RL, GL and BL, cultures changed pigmentation towards blue, green and red colour. In this study, in GL and BL the culture appeared to remain the same. On the other hand, the present study showed that in RL the culture appeared bluish-green that was consistent with the study of [38]; [39] and [40] and which showed that phycoerythrin is replaced by phycocyanin in RL to optimize light harvesting, and cells appear blue-green in colour.

Similarly, light quality has a strong influence in light harvesting system of the cyanobacterium [41]. In general, during chromatic adaptations, only the inducible PC gene set modulates its gene expression depending on the light quality [42], while the constitutive PC gene set expresses itself under green and red light. The synthesis of PC in non-chromatic adapting cyanobacteria is photo-reversible and it has also been postulated that the reversible nature of these biliproteins was under the control of photoreversible photoreceptors [43]; [44].

But neither photo-reversible photoreceptors mentioned above nor inducible PC gene sets are present in the cyanobacterium like *Anabaena* sp. Although the results will be useful in optimising the pigments yield from this organism for commercial purpose. In North-east India, research in the field of cyanobacterial products such as phycobiliproteins and their commercial exploitation is very nascent and need adequate

attention. This study also provides valuable information for further extensive research on the exploitable potential of the species for the desired pigments in an efficient manner.

4. CONCLUSIONS

The present study was focused on the influence of light quality on the phycobiliprotein production in *Anabaena circinalis*. On the basis of our findings, it is concluded that this strain chromatically adapted to different light quality producing different quantities of phycobiliproteins which could be a good basis for the exploitation as a source of biopigments. To understand the photochemical process in cyanobacteria, more knowledge of the photochromatic properties of biliprotein subunits and linker assembly is needed at the molecular levels. This would allow us to determine better the contribution of different light and pigment production in cyanobacteria. However, efforts have to be made in order to achieve economical overproduction of phycobiliproteins by recombinant DNA technology.

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6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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