



BLUE LIGHT ENHANCE THE PIGMENT SYNTHESIS IN CYANOBACTERIUM *Anabaena ambigua* Rao (NOSTACALES)

Velu Vijaya and Narayanaswamy Anand

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India

E-Mail: veluvijaya@gmail.com

ABSTRACT

Light intensity and quality are the most significant environmental factors influencing the photosynthetic pigments in Cyanobacteria. Cyanobacterium *Anabaena ambigua*, was subjected to different light intensities (highlight-48 W m⁻², medium light-32 W m⁻² and low light 16 W m⁻²) and different light qualities (blue, red and green light). The content of Chl *a* decreased with the increase in light intensity and reached the highest amount of 21% at low light 16 W m⁻², whereas other biliprotein and total protein were relatively less. It was reversible in highlight 48 W m⁻², except Chl 11%; the total protein 90%; phycoerythrin 4%, phycocyanin 25% and allophycocyanin 19% content were found to be relatively high. At ML and LL, PC content remained constant (21%). Since light quality seem to play an important role in regulation of pigment synthesis, Comparatively in RL and GL, 29% and 23% of PC content were found and other pigments were less than BL. Significantly all the photosynthetic pigment's content was high in blue light cells, as high as Chl *a* 22%, total protein 95%; phycoerythrin 11%, phycocyanin 45% and allophycocyanin 17%. Besides other light conditions, blue light triggered high photosynthetic pigments synthesis, especially high PC synthesis of this cyanobacterium.

Keywords: light, intensity, quality, cyanobacteria, phycobiliproteins, phycocyanin.

INTRODUCTION

Algal cultures are influenced by a variety of environmental factors and they play a significant role in the production and composition of the photosynthetic pigments. 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. Hence cyanobacteria will optimize the harvesting of light for available irradiance and spectral composition by modulating their antenna pigment composition (Bennet and Bogorad, 1973; Grossman *et al.*, 2001, Tandeau De Marsac, 2003).

Especially phycobilisomes represent a major biosynthetic component of the cyanobacterial cell, as they can comprise 50% (Morena *et al.*, 1995). Factors like low light intensities stimulate the synthesis of PBS and increase the rod's length (Öquist, 1974; Lundell and Glazer, 1981). Laczko' and Kaiseva, 1987) have studied that light energy absorbed by PBS is transferred to photosystem II antenna chlorophylls with higher efficiency in the highlight (100 W m⁻²) than low light (15 W m⁻²) adapted *Anabaena cylindrica*. The effect of irradiance in *Nostoc* sp. showed that total PBS was enhanced from 18%-28% when photon flux density was decreased from 1400-100 E m⁻² s⁻¹ (Rodriguez *et al.*, 1991). Along with light intensity, temperature also influences the growth, phycobiliprotein content in *Arthonema africanum* (Chaneva *et al.*, 2007).

Similarly light quality has a strong influence in light harvesting system of the cyanobacterium (Bennet and Bogorad, 1973; Korbee *et al.*, 2005). Nevertheless 'chromatic adaptation' to light of different spectral qualities has been confirmed in diatoms *Haslea ostrearia* (Mouget *et al.*, 2004). Lönneborg *et al.*, 1985) have

studied that light shift from white light to red light increases the PC and chlorophyll synthesis of *Anacystis nidulans*. Red light or blue light was essential for phycocyanin production of *Synechococcus* sp. NKBG 042902 (Takano *et al.*, 1995). Metabolically, protein synthesis or enzyme activation in unicellular green algae and higher plants is triggered by blue light (Senge and Senger, 1991). Contrarily, a low photosynthetic efficiency as well as the growth rate was found under blue light than red and white light in *Synechococcus* strains (Hauschild *et al.*, 1991) and *Porphyra leucosticta* (Korbee *et al.*, 2005). In 1994, Leukart and Luning, demonstrated that green light, at very low intensity (0.5 μmol m⁻²S⁻¹) was more effective than red or blue light germinating growth in several red algae. Interestingly in red algae *Phorphyra umblicalis* phycocyanin accumulation was stimulated after a short period of illumination with red light, while an increase in PC occurs after pulses of green light treatment (Rudiger and Figueroa, 1992). However, allophycocyanin synthesis was independent to spectral light (Marsac and Houmard, 1993).

Although cyanobacteria have been exploited as bio-fertilizers and their biochemical potential, especially phycobiliproteins in terms of production as valuable compounds (Ascencio *et al.*, 2004). Our aim is to analyze the synthesis of this commercially important phycobilin pigments. Since light plays an important roll in the pigment synthesis, in our study we correlated the impact of different light intensities and spectral qualities on photosynthetic pigment production of *Anabaena ambigua*.

MATERIALS AND METHODS

Axenic culture of *Anabaena ambigua* Rao was obtained from culture collection CAS in Botany. A log phase (15 days old) cultures were homogenized and



inoculated into 250 ml glass vials containing 100 ml BG₁₁ medium (Rippka *et al.*, 1979) at 26±1°C, light dark regime (16:8) as triplicates. To study the effect of different light intensities cultures were grown under the highlight (HL) (48 W m⁻²) medium light (ML) (32 W m⁻²) and low light (LL) (16 W m⁻²), provided by cool white fluorescent tubes (Philips). For different spectral studies, culture flasks were

covered with blue (BL), red (RL) and green (GL) cellophane paper (Figure-1) and were exposed to HL intensity of 48 W m⁻². The flasks were stirred daily to allow uniform light penetration and circulation of air and nutrients. Samples were harvested after a period of 2 weeks for analysis.

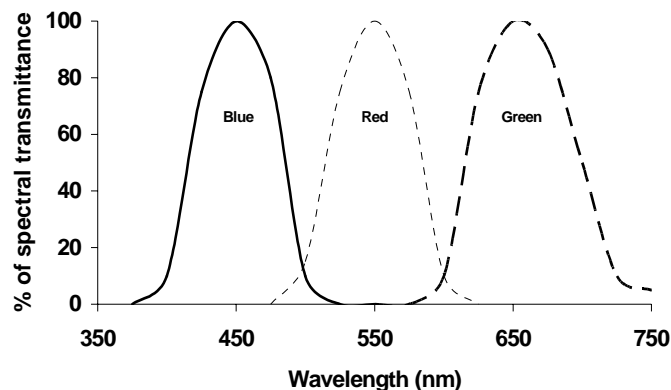


Figure 1. Spectral distribution of colour filters.

Chlorophyll *a* (*Chl*) was determined according to Mackinney, (1941) and total protein by the method of Bradford, (1976) with Bovine serum albumin as the standard. After the removal of *Chl* by cold acetone (80%) extraction, the cell pellet was washed twice in extraction buffer of 0.1M-phosphate buffer, pH 6.7 at 4°C and disrupted until all the cells were completely broken using Labsonic 2000 sonicator. The disrupted cells were incubated prior to the centrifugation in the presence of 2% Triton X-100 for 30 min in dark to separate the PBS (phycobilisomes) from PBS-membrane complex. The homogenized suspension was centrifuged at 12096-x g for 15 min Tandeu De Marsac, (1977). The amount of PE (phycoerythrin), PC (phycocyanin) and APC (allophycocyanin) was quantified and calculated according to Bennett and Bogorad, (1973), and average of the data was taken from the triplicates.

RESULTS AND DISCUSSIONS

Studies of the environmental factors that affect the growth and metabolism of microalgae are necessary because they contribute to control the cellular metabolism and optimization of certain biosynthetic products. *Anabaena* is an environmentally significant cyanobacterium inhabiting diverse ecological niches, moreover commonly observed in rice fields. For all photosynthetic microorganisms light plays a critical role in growth, multiplication of cells and physiology of the cyanobacteria.

In the present study, cells of *A. ambigua* grown under different light intensities exhibited their own specific levels of *Chl*, total protein, PE, PC, and APC. This

rapid acclimation process was seen when cells were shifted to different light intensities and qualities. The ratio of PBS to *Chl* variation in algae shows difference in culture colour depending on the quality and intensity of the light they grow (Bogorad, 1975; Olaizola and Duerr, 1990). Under LL intensity (16 Wm⁻²), cells were found to be dark green in colour with a high *Chl* content of 21%. (Figure-2a) relatively, cells under HL were found to be apple green in colour with a very less *Chl* content (11%). Blue light adapted cells were bluish green in colour that could be ascribed to spectral quality and accumulation of low irradiance (Figuroa *et al.*, 1995). Even though the result obtained from LL and GL (*Chl*) mimics as BL, the bilinpigments and protein synthesis are drastically retarded (Figures 2a and 2b). As described by Armstrong and Apel, (1998) low accumulation of *Chl* in HL, ML and RL can be related to the reduced activity of plastid localized NADPH-photochlorophyllide oxido-reductase, which mediates chlorophyll biosynthesis and is extremely light dependent.

In (1991), Babu *et al.*, have studied that APC synthesis was independent of light quality and remains constant under white, red and green light. Contrarily, in our studies, APC content increases with increasing light intensity, and maximum content was noticed in HL 19% and BL 17%, but significantly very less in RL and GL (Figure 3a and 3b). Though PE synthesis was more drastically reduced as compared to PC and APC, maximum PE value of 11% could be noted only in BL grown cultures. PE is known to be the most flexible phycobiliprotein, which facilitates adaptation to environmental changes (Maccoll and Guard-friar, 1987).



While, preferential enrichment of cells with PE at low irradiance was obtained in marine *Synechococcus* sp. (Alberte *et al.*, 1984).

The results infer that *A. ambigua* synthesized PC as their major biliprotein constituent. Among different light intensities, high PC (25%) content and total protein (90%) accumulation in HL (Figure-3a and b), suggests the diverse physiological adaptation of the organism that leads to increased protein synthesis (Tandeau De Marsac, 1977). And their total PBS content (PC+APC) and (PC+PE) was found to be 44% and 30% respectively. Almost similar total biliprotein content was found between ML and LL (PE+ PC) 24% and 23% and (PC+APC) 34% and 33% (Figure-3a).

Among different light qualities, PC production was found to be obviously elevated in blue light (Figure-4a) (21) and the total PBS was high as (PC+APC) 62%, (PE+PC) 56%, respectively. A high total protein content of about 95% was obtained in BL, whereas, in RL and ML it was constant (75%) and in GL and LL, it was 70% and 60% (Figure 3b and 4b).

While comparing the PC synthesis under different light intensity and light qualities, BL (45%)

seems to be the most suitable condition. Red (29%) and HL (25%) intensity shows second and third place in pigment synthesis. our result shows that major effects were observed under different light quality in the PBS synthesis (Damerval *et al.*, 1991; Figueroa *et al.*, 1995). Which shows that the spectral light triggered the synthesis of PC of the phycobilisome rods, but neither the length nor the total number of phycobilisomes per unit photosynthetic membrane remains uncertain. In general, during complementary chromatic adaptations, only the inducible PC gene set modulates its gene expression depending on the light quality (see review, Grossman, 2003), while the constitutive PC gene set expresses itself under GL and RL. The synthesis of PC and PE in nonchromatic 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 (Bogorad, 1975; Vogelmann and Scheibe, 1978). But neither photo reversible photoreceptors mentioned above nor inducible PC gene sets are present in the cyanobacterium like *A. ambigua*. Although the results will be useful in optimising the pigments yield from this organism for commercial purpose.



Figure-2a and b

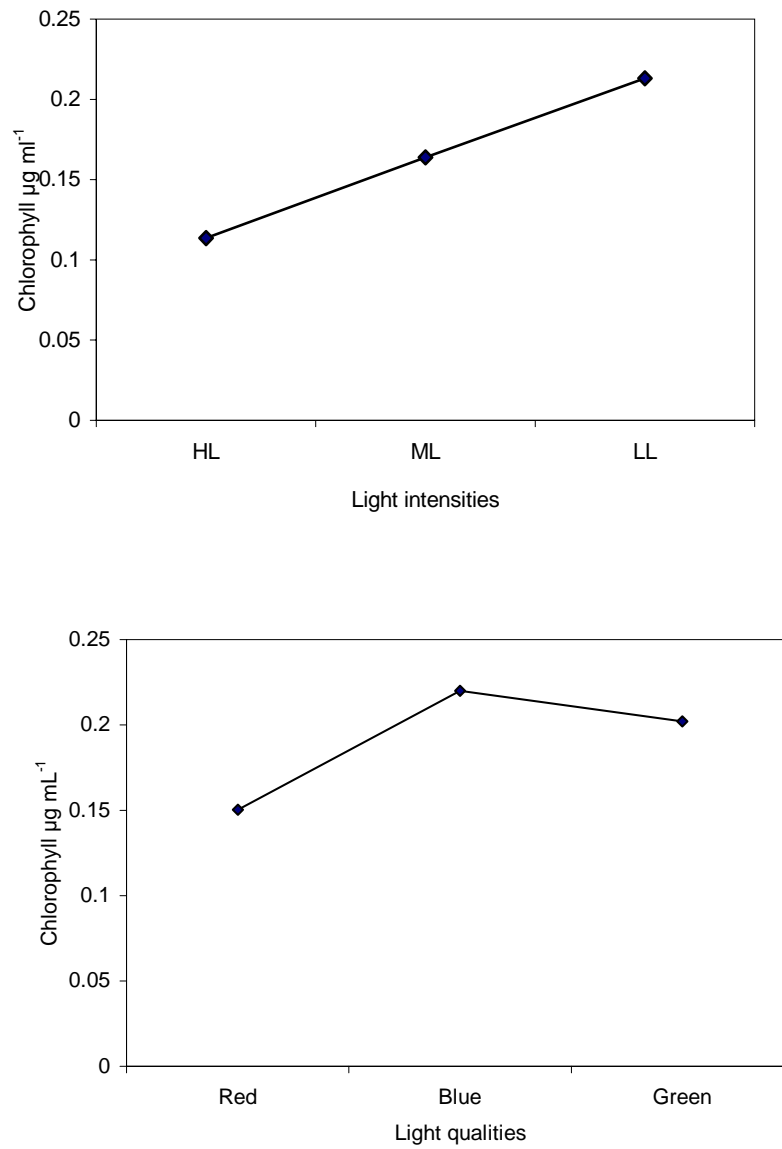
**Figure-2.** Effect of a) different light intensities and b) light qualities on chlorophyll synthesis of *A. ambigua*.



Figure-3a and b

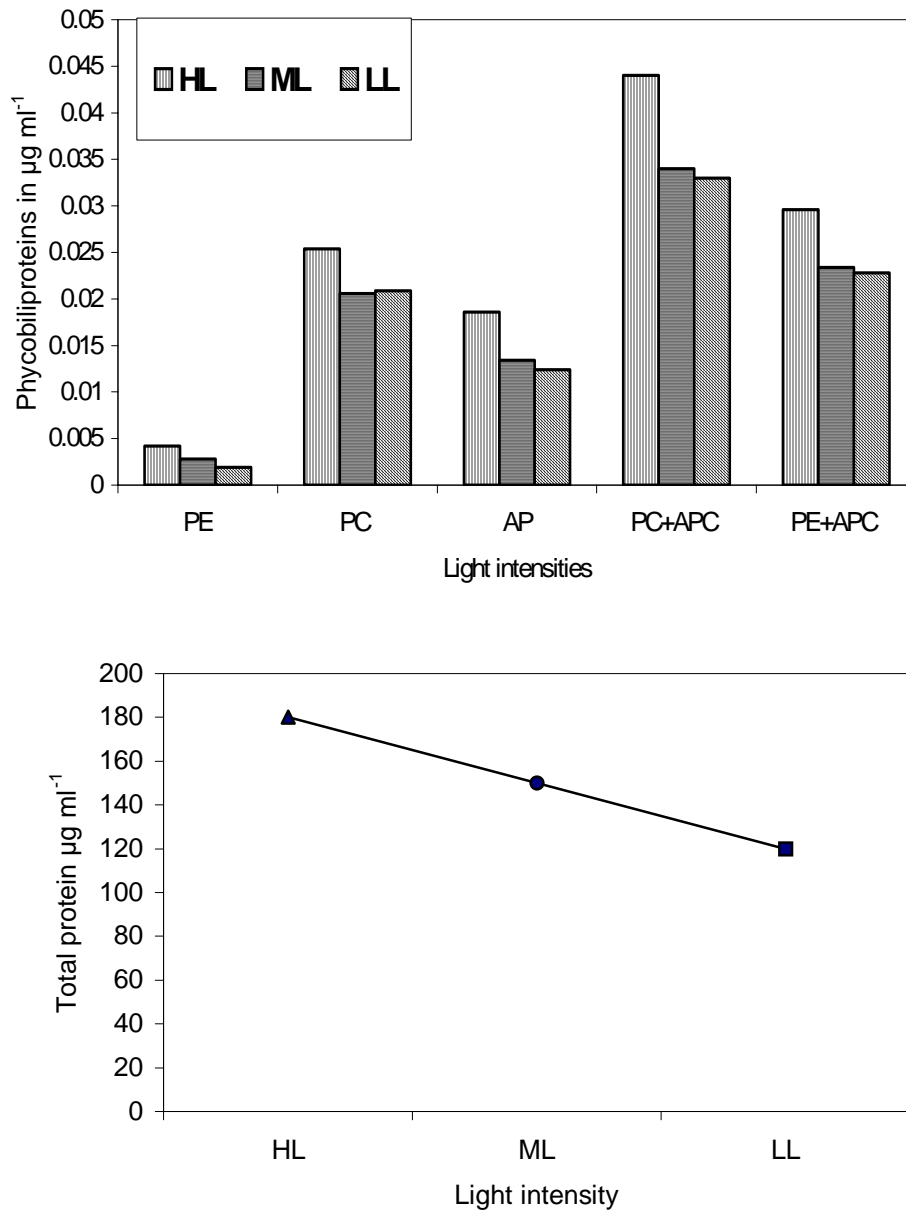


Figure-3. Effect of different light intensities on synthesis of a) phycobiliproteins, total phycobilin and b) total protein content of *A. ambigua*.



Figure-4a and b

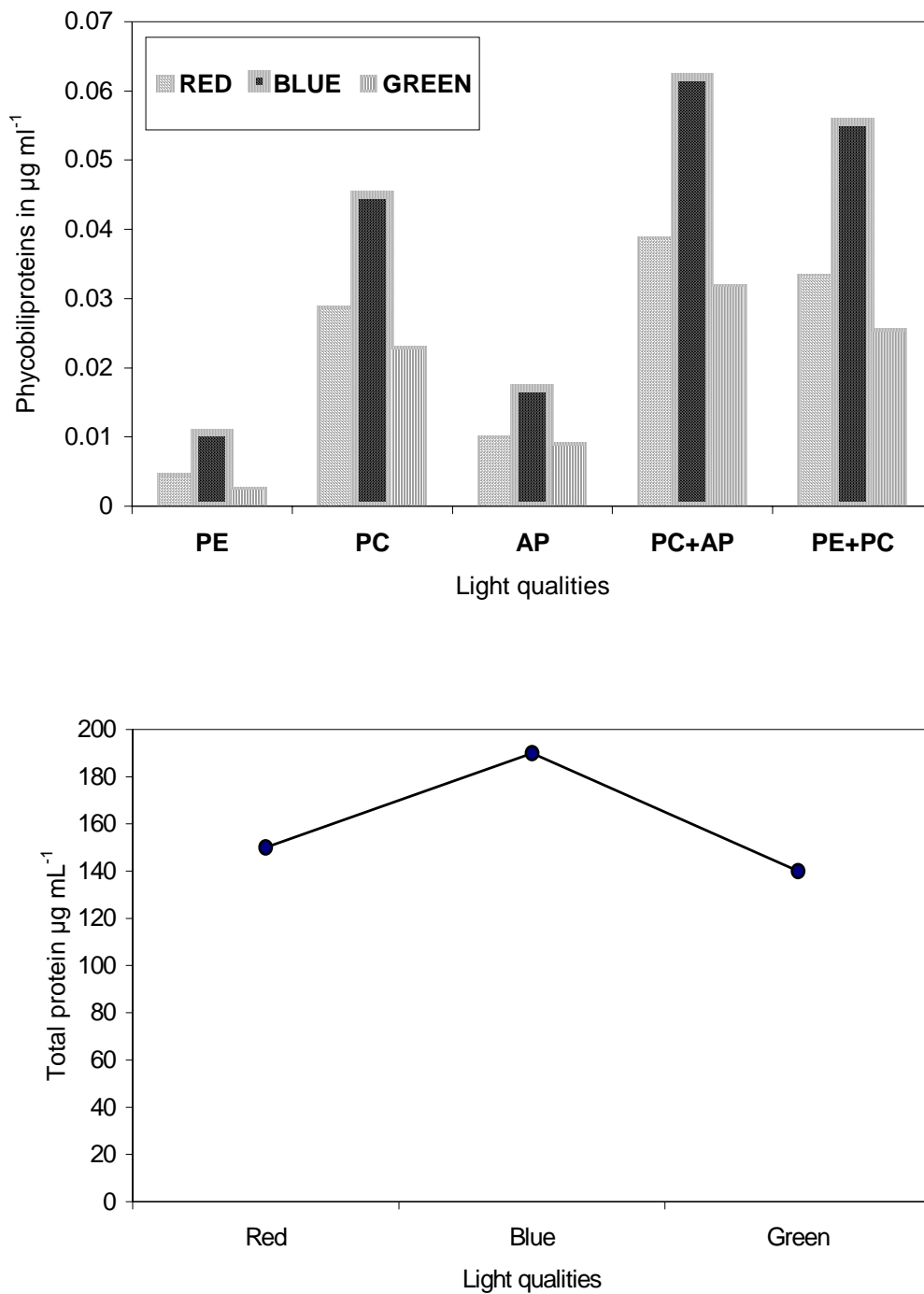


Figure-4. Effect of different light qualities on synthesis of **a)** phycobiliproteins, total phycobilin and **b)** total protein content of *A. ambigua*.

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