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ENHANCING THE PHYCOBILIN PIGMENT SYNTHESIS IN *CALOTHRIX ELENKINII* THROUGH OPTIMIZATION OF LIGHT CONDITIONS

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ABSTRACT
An extensive range of pigments including phycobiliproteins are present in algae. Cyanobacterial phycobiliproteins are water-soluble fluorescent photosynthetic accessory pigments which include phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE). The commercial or biotechnological applications of phycobiliproteins in food and cosmetic industries as well as in nutraceuticals and pharmaceuticals benefits are known. Due to increasing awareness of society towards the usage of natural products, the present study was designed to find the effect of light intensity and light quality on phycobilinprotein synthesis in cyanobacterium *Calothrix elenkinii.* The maximum phycobiliprotein synthesis was recorded at 32 W/m^2 and red light condition especially phycocyanin. Highest amount of phycoerythrin was found at green and blue light conditions. Hence the results showed that the production of phycobiliproteins in this cyanobacterium can be optimized by regulating light conditions for commercial aspect.

KEY WORDS: Phycobilin Pigment, Phycocyanin, Allophycocyanin, Phycoerythrin, *Calothrix elenkinii*.

INTRODUCTION

Cyanobacteria are organisms capable to fix atmospheric nitrogen and enriching the soil, as natural biofertilizers and biofuels (Abed *et al*., 2009). Besides chlorophyll, phycobiliproteins such as phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) are the major photosynthetic accessory pigments in cyanobacteria, rhodophytes and cryptomonads. These phycobilin pigments have been used successfully in various biological and pharmacological properties (Miranda *et al*., 1998, Romay *et al*., 2003). Moreover these molecules are used in food industries due to its antioxidant activity (Mille-Clarie *et al*., 1993). The fluorescence properties of phycobilins are so distinctive and reliable, may be used as chemical "tags" (Kronick, 1986). For all photosynthetic organisms, including cyanobacteria light energy (Takano *et al*., 1995) is essential factor plays a critical role in the growth and physiology of cyanobacteria. The detailed investigation of environmental factor like light intensities and qualities on cyanobacterial growth is necessary (Richardson *et al*., 1983) to provoke the requirement of optimization of the culture growth conditions for its phycobiliproteins yield maximization. While knowledge of individual factor would be useful in producing both cyanobacterial biomass and phycobiliproteins is essential. The present study was undertaken to investigate the light intensities and wavelength regulating phycobiliproteins production in *C. elenkinii* quantitatively for commercialization.

MATERIALS & METHODS

Cells of *C. elenkinii* were grown in 250 ml Erlenmeyer flasks in BG_{11} liquid medium (Ripka *et al*., 1969) with 16:8 light dark conditions at 26±1˚C.The source of combined nitrogen ($NaNO_3$) was omitted from the medium for the growth and maintenance of this heterocystous cyanobacterium. For different light intensities *i.e.,* high light (HL)- 48 Wm^{-2} , medium light (ML)-32 $Wm^{-2}$and low light (LL)-16 $Wm^{-2}$and wavelength study *i.e*., red light (RL), blue light (BL) and green light (GL); light sources and culture set up were described as in Vijaya and Anand (2009), the transmission spectra of the filters were shown in Figure 1. The culture flasks were shaken daily for 30 min on shaker. The biomass was determined in 10 ml of 15 days old culture medium by centrifugation for 10 min under 5,000 rpm. The supernatant of medium were removed and kept open in room temperature to remove the excess water and wet biomass was analyzed. For phycobilin pigment extraction to the 10 ml of pelleted cell, 10 ml of 80% cold acetone was added and stored overnight in dark at 4ºC. Subsequently, the sample was centrifuged at 5,000 rpm for 10min and the supernant was removed. To the pellet, 10 ml of 0.1 Molar cold potassium phosphate buffer (pH 6.7) was added, consequently the cells were broken in cell mill and centrifuged at 15,000 rpm for 20 min at 4ºC. The absorbance of the supernatant was measured at 615 (PC), 652(APC) and 562(PE) nm (Bennet and Bogorad, 1973). The mean value of triplicates was taken.

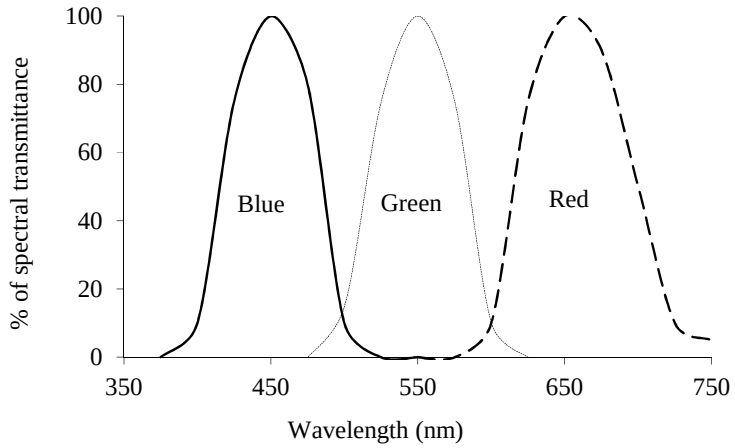

FIGURE 1: Transmittance spectra of the different-colored filters used for *C. elenkinii* cell cultivation [Blue (——); Red (-------); Green (- - - -)].

RESULTS & DISCUSSION

Effect of light intensities and wavelength in biomass

The biomass of *C. ellenkinii* grown under different light intensities and wavelength study shows dissimilarity (Fig. 2). Among different light intensities, the highest fresh weight biomass was recorded in ML (78.20 g/l) followed by LL (65.46 g/l) and least weight was observed in HL (51.15/l). The different wavelength grown cells showed highest biomass in RL (142.29 g/l) followed by BL (103.97 g/l). The lowest biomass was observed in GL (83.20 g/l). Based on the result it can be considered that RL is the most favorable light followed by BL, GL and ML sources for the considerable growth of *C. elenkinii*. Further LL (16 Wm^{-2}) and HL (48 Wm^{-2}) conditions can be considered as insufficient for better growth of this organism.

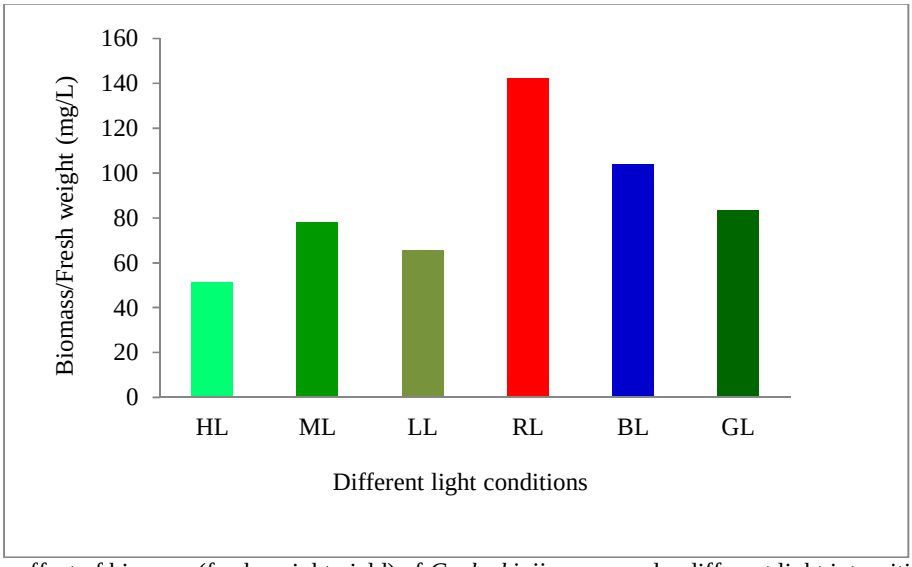

FIGURE 2: The effect of biomass (fresh weight yield) of *C. elenkinii* grown under different light intensities. Values were average of triplicates (HL- High Light; ML- Medium Light: LL-Lowlight; RL- Red Light; BL- Blue Light; GL- Green Light)

Effect of light intensities in phycobilin pigment synthesis

In *C. elenkinii* PC synthesis was observed maximum at all light intensities (HL- 48 Wm^{-2}, ML-32 Wm^{-2}, LL-16 Wm^{-2}) (Fig. 3) while compare to APC and PE. Among the three different light intensities study, the maximum production of PC (17.2 mg/l) was observed in ML condition (32 Wm^{-2}) and minimum of 6.46 mg/L in HL (48 Wm^{-2}) condition. The influence of different intensities showed variation in the yield of phycobiliprotein (PE, PC, and APC) at all light conditions (Chaneva *et al*., 2007). Based on the inference *C. elenkinii* prefers moderate light intensity (32 W^{-2}) for its growth and phycobilin synthesis (Hemlata and Fatma, 2009; Maurya *et al.*, 2014).

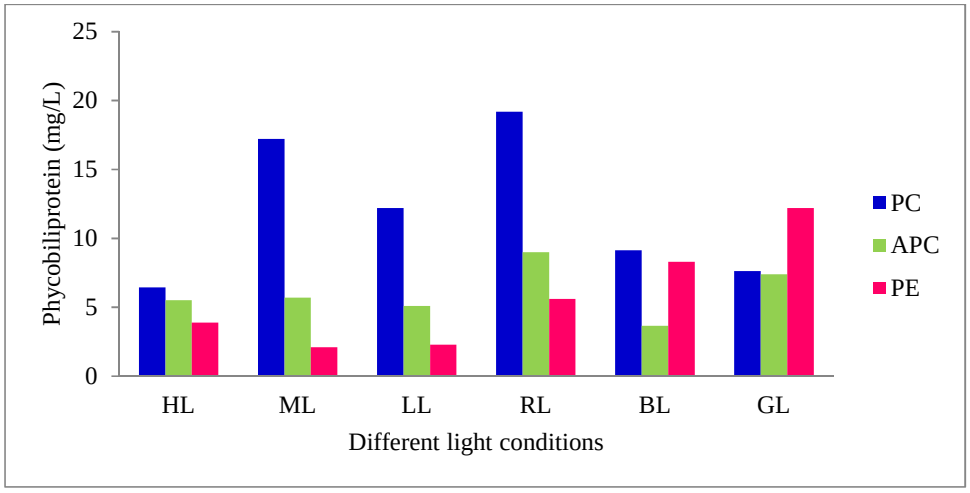

FIGURE 3: The effect of phycobiliproteinquantity (PC-Phycocyanin; APC-allophycocyanin; PE-Phycoerythrin) of *C. elenkinii* grown under different light intensities and different wavelength. Values are average of triplicate. (HL- High Light; ML- Medium Light: LL-Lowlight; RL- Red Light; BL- Blue Light; GL- Green Light)

Effect of light wavelengths in phycobilin pigment synthesis

The effect of different wavelengths on the influence of the phycobilin pigment contents (PC, APC and PE) is showed in Figure 4. Among different wavelength of lights RL cells reached highest amount of PC pigment (19.19 mg/l) and least amount of PE (5.6 mg/l). Inversely in GL cells the content of PE pigment reached highest (12.2 mg/l) with minimum amount of PC (7.64 mg/l). It was seen that in BL the contents of PC and PE was almost similar (PC- 9.15 mg/l; PE-8.3 mg/l) in its pigment proportion. The result shows that not only RL and GL, *C. elenkinii* can also use BL for the accumulation of PE and PC pigments and improves the light efficiency for the growth and phycobilin pigment accumulation in the cells. Under RL, the relative proportion/ratio of PC and APC was increased which intensified the absorbance of red light by this organism for its growth. Cells grown in GL showed enhanced absorbance of this GL by increasing its PE synthesis/ratio. This phenomenon showed the ability of complementary chromatic adaptation of *C. elenkinii* which was studied in other cyanobacteria and most phycobiliprotein containing organisms, by adjusting the composition of pigments to make use of the light sources for its photosynthesis (Campbell 1996, Bezy*et al*., 2011).

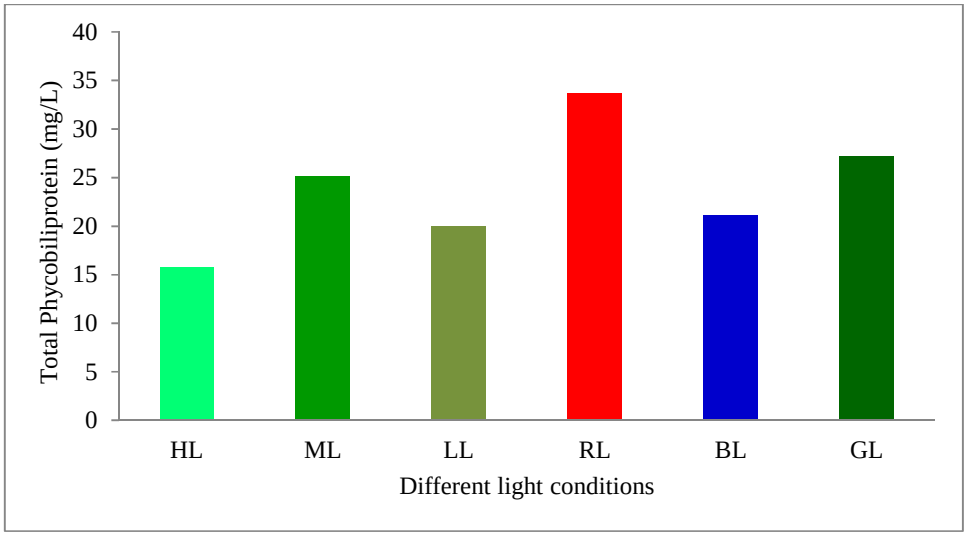

FIGURE 4: The effect of total phycobiliproteins of *C. elenkinii* grown under different Light intensities and different wavelengths. Values are average of triplicate.

CONCLUSION

Based on our result we inferred that the complementary chromatic behavior is more advantage criteria of *C. elenkinii*. Cells had maximum amount of PC production at RL and ML conditions and maximum PE content under GL. The yield of these pigments (PC/PE) could be increased by further adapting the cells for successive generation in

respective light condition for several generations. The changes in the particular phycobilin pigment synthesis under different light helps to optimize the culture condition for large scale production.

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REFERENCES

Abed, R.M.M., Dobretsov, S. and Sudesh, K. (2009) Applications of cyanobacteria in biotechnology. J. Appl. Microbiol. 106 1-12.

Bennett, A. & Bogorad, L. (1973) Complementary chromatic adaptation in a filamentous blue green alga. J. Cell Biol. 58: 419-435.

Bezy, R. P., Wiltbanka, L. and Kehoea, D. M. (2011) Lightdependent attenuation of phycoerythrin gene expression reveals convergent evolution of green light sensing in cyanobacteria. Proc. Natl. Acad. Sci. USA 108: 18542-18547.

Campbell, D. (1996) Complementary chromatic adaptation alters photosynthetic strategies in the cyanobacterium *Calothrix.* J. Microbiol. 142: 1255-1263.

Chaneva, G., Furnadzhieva, S., Minkova, K. and Lukavsky, J. (2007) Effect of light and temperature on the cyanobacterium *Arthronemaafricanum*- a prospective phycobiliprotein-producing strain. J. Appl. Phycol. 19: 537-544.

Hemlata & Fatma, T. (2009) Screening of cyanobacteria for phycobiliproteins and effect of different environmental stress on its yield. Bull. Environ. Contam. Toxicol. 83:509-515.

Kronick, M.N. (1986) The use of phycobiliproteins as fluorescent labels in immunoassay. J. Immunological Methods 92: 1-13.

Maurya, S.S., Maurya, J.N. & Pandey, V.D. (2014) Factors regulating phycobiliprotein production in cyanobacteria. Ind. J. Cur. Sci. Appl. Sci. 3: 764-771.

Miranda, M.S., Cintra, R.G., Barros, S.B., Mancini, F.J. (1998) Antioxidant activity of the micro alga *Spirulina maxima.* Braz. J. Med. Biol. Res. 31:1075–1079

Richardson, K., Beardall, J. and Raven, J.A. (1983) Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol. 93: 157-191.

Rippka, R., Deruelles, J., Waterbury, J., Herdman, M. and Stanier, R.Y. (1979) Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria Microbiology. 111:1 1-61.

Romay, C., González, R., Ledon, N., Remirez, D. and Rimbau, V. (2003) C-Phycocyanin: A biliprotein with antioxidant, anti-inflammatory and neuro protective effects. Cur. Protein and Peptide Sci. 4: 207-216.

Takano, H., Arai, T., Hirano, M. and Matsunaga, T. (1995) Effect of intensity and quality of light on phycocyanin production by a marine cyanobacterium *Synechococcus* sp.NKBG042902. Appl. Microbiol. Biotechnol. 43: 1014-1018.

Vijaya, V. & Anand, N. (2009) Blue light enhances the pigment synthesis in cyanobacterium *anabaena ambigua rao* (nostacales), ARPN. Jr. of Agri. and Biol. Sci. 4: 1-8.