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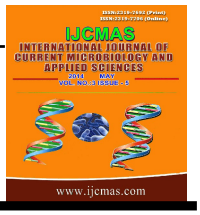

Original Research Article

Factors regulating phycobiliprotein production in cyanobacteria

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A B S T R A C T

Cyanobacteria (Blue- green algae) are a morphologically diverse and widely distributed group of prokaryotic organisms which show plant-type oxygenic photosynthesis. Cyanobacterial phycobiliproteins are water-soluble florescent accessory photosynthetic pigments which include phycocyanin, allophycocyanin and phycoerythrin. The commercial or biotechnological applications of phycobiliproteins in food and cosmetic industries as well as in nutraceuticals and pharmaceuticals are known. Different factors regulating phycobiliprotein production in coccoid (*Synechocystis* sp. and *Gloeocapsa* sp.) and filamentous (*Anabaena* sp. and *Lyngbya* sp.) cyanobacteria, isolated from high altitude freshwater and terrestrial habitats, were investigated. The study revealed that the content of phycobiliprotein varied with factors like pH, temperature, light intensity and light-dark period in the cyanobacteria investigated. Maximum level of phycobiliprotein was recorded at pH 8, temperature 35 ^{o}C, light intensity 2000 lux and light-dark period 16:08 h. The results indicate that the production of phycobiliproteins in cyanobacteria can be optimized by regulating these factors.

K e y w o r d s

Cyanobacteria, Phyco-biliproteins, Phycocyanin, Allo-phycocyanin, Phycoerythrin

Introduction

Cyanobacteria (Blue-green algae), are an ancient, diverse and highly adaptable group of photosynthetic prokaryotes, exhibiting oxygenic photosynthesis (Stanier and Cohen-Bazire, 1977). They inhabit a wide range of terrestrial and aquatic habitats, including those with extreme conditions (Tandeau de Marsac and Houmard 1993; Ward and Castemholz, 2000; Oren, 2000). Their morphology vary from simple unicellular and colonial to complex filamentous forms (branched or unbranched) with or without heterocysts, the thick-walled differentiated cells carrying nitrogen fixation. In addition to the applications of cyanobacteria in agriculture, nutraceuticals, bioenergy and bioremediation (Abed *et al*., 2009; Patterson, 1996), they have received considerable attention as a rich source of phycobiliproteins. Phycobiliproteins are water-soluble accessory photosynthetic pigments found in cyanobacteria, red algae and cryptomonads (Rowan, 1989). They

include blue colored phycocyanin, bluish-green colored allophycocyanin and red colored phycoerythrin, showing maximum absorbance at wavelengths 620nm, 650nm and 565 nm, respectively (Grossman *et al*., 1993). They assemble to form supramolecular complexes, called phycobilisomes (PBS), located on outer surface of thylakoid membranes (Cohen-Bazire and Bryant, 1982). Phycobiliproteins may comprise up to 40% of total soluble protein content in cyanobacteria.

Phycobiliproteins are regarded as non-toxic and non-carcinogenic natural food colorants alternative to the widely used synthetic food colorants/additives having potential toxicity and carcinogenicity (Cohen, 1986; Mille-Claire *et al*., 1993; Chaneva *et al*., 2007). They are known to possess certain pharmacologically important activities, such as antioxidative, neuroprotective, anticancerous, anti-inflammatory and hepatoprotective (Rimbau *et al*., 1999; Liu *et al*., 2000; Romay *et al*., 2003). Moreover, they have applications in cosmetics, biomedical research and clinical diagnostics (Dainippon Ink and Chemicals, 1985; Kronick, 1986; Araoz *et al*., 1998). The potential use of various cyanobacterial species for commercial production of phycobiliproteins have been reported by many workers (Takano et *al*., 1995; Chen *et al*., 1996; Chaneva *et al*., 2007). Factors like light, temperature, pH and nutrient availability are known to influence the amount of various phycobiliproteins in cyanobacteria (Takano *et al*., 1995; Chaneva *et al*., 2007; Grossman *et al*., 1994; Simeunovic *et al*., 2013; Hemlata and Fatma, 2009). The necessity of detailed investigation of the effects of environmental factors or growth conditions, such as temperature, light intensity, photoperiod and pH on phycobiliprotein content in cyanobacteria has been provoked by the requirement of optimization of culture or growth conditions for yield maximization of phycobiliproteins. Knowledge of individual factor would be helpful in producing both cyanobacterial biomass and phycobiliproteins in desired quantity. The present study was undertaken to investigate the factors regulating phycobiliprotein production in selected cyanobacteria isolated from high altitude freshwater and terrestrial habitats.

Materials and Methods

Sampling and identification

Cyanobacteria, both coccoid (*Synechocystis* sp. and *Gloeocapsa* sp.) and filamentous (*Anabaena* sp. and *Lyngbya* sp.), employed in the present study were isolated from high altitude freshwater and terrestrial habitats located in Uttarakhand, India according to the guidelines of Rippka (1988). Clonal and axenic cultures of cyanobacteria were established by repeated sub-culturing and antibiotic (cyclohexamide, streptomycin sulphate) treatment at standardized dose following standard methods (Rippka, 1988). Cyanobacteria were identified on the basis of morphological characteristics (nature, shape and dimensions of cells, colonies and filaments; presence/absence and position of heterocysts and akinetes; shape of intercalary and end cells; presence/absence and pattern of sheath) using standard literature (Desikachary, 1959; Rippka, 1979).

Growth and culture conditions

Cyanobacteria were grown in sterilized BG-11 culture medium (Rippka *et al*.,1979) in cotton-stoppered 250-mL

Erlenmeyer flasks at 26±2°C and under continuous illumination (light intensity, 1.5 Klux PAR) provided by cool-white fluorescent tubes. The source of combined nitrogen ($NaNO_3$) was omitted from the medium for the growth and maintenance of heterocystous cyanobacteria. Cultures were shaken twice a day for 15 min on rotary shaker. Cyanobacteria growth was monitored spectrophotometrically by recording the absorbance of homogenous liquid culture at 650 nm with UV-Vis spectrophotometer at regular intervals (Sorokin, 1973). Biomass was harvested from cultures (15-days old) by centrifugation (5,000x*g*, 10 min).

Estimation of phycobiliproteins

Phycobiliproteins were extracted from harvested biomass in 0.01 M phosphate buffer (pH 7.0), employing repeated freezing (-20 °C) and thawing (5 °C) and supernatants were obtained by centrifugation (10,000x*g*, 10 min). The absorbance of phycobiliprotein containing cell-free supernatants was measured at 562 nm, 615 nm and 652 nm. These wavelengths correspond to the absorption maxima of phycoerythrin, phycocyanin and allophycocyanin, respectively. The concentration of phycobiliproteins-phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) was determined spectrophotometrically using the equation given by Bennett and Bogorad (1973):

$$PC = (A_{615} - 0.474 \times A_{652})/5.34$$

$$APC = (A_{652} - 0.208 \times A_{615})/5.09$$

$$PE = [A_{562} - (2.41 \times PC) - (0.849 \times APC)]/9.62$$

$$\text{Total phycobiliprotein} = PC + APC + PE$$

The values were expressed as µg/ml of culture and presented as means of triplicate measurements.

Results and Discussion

Effect of pH

Figure 1 shows the effects different factors (pH, temperature, light intensity and light-dark period) on the production of phycobiliprotein in coccoid (*Synechocystis* sp. and *Gloeocapsa* sp.) and filamentous (*Anabaena* sp. and *Lyngbya* sp.) cyanobacteria. In order to assess the influence of pH on the levels of phycobiliprotein in cyanobacteria, the pH of the culture medium was adjusted at values 6, 7, 8, 9 and 10 using 1N HCl and 1N NaOH, the other parameters (e.g. temperature, light intensity and light-dark period) being optimally constant. As shown in the figure, the pH value of 8 of the culture medium was found to be optimum, yielding the maximum total phycobiliprotein in the cyanobacteria examined with the values 57.8 µg/ml in *Synechocystis* sp., 65.2 µg/ml in *Gloeocapsa* sp., 86.5 µg/ml in *Anabaena* sp. and 104.1 µg/ml in *Lyngbya* sp. This finding suggests that the optimum pH (7.6–8) required for the growth of cyanobacteria can be correlated with the optimum pH for phycobiliprotein production. The observed pH optimum value for the maximum production of phycobiliprotein is consistent with the values reported for the cyanobacteria *Synechocystis* (Hong and Lee, 2008) and *Anabaena* NCCU-9 (Hemlata and Fatma, 2009). The increase in pH, from 7 to 9, of the culture medium has been reported to increase the total phycobiliprotein content in *Nostoc* sp. UAM 206 (Poza-Carrion *et al*., 2001). pH is an important factor which not only determines diversity, distribution, abundance and growth of

cyanobacteria in various freshwater and terrestrial ecosystems, but also influence their metabolic or biochemical activities considerably in laboratory cultures (Whitton, 2000; Sardeshpande and Goyal 1981; Richmond, 1986; Rafiqul et al., 2005). Cyanobacteria generally prefer neutral to slightly alkaline pH for optimum growth (Singh 1961; Kaushik, 1994).

Effect of temperature

In order to investigate the influence of temperature on phycobiliprotein production, the cultures of cyanobacteria were incubated at different temperature (20 ^{0}C, 25^{0}C, 30^{0}C, 35^{0}C, 40^{0}C, 45^{0}C) for a specific duration in a fluorescent tubes/lamps equipped BOD incubator, keeping other culture conditions (e.g. light intensity, light-dark and pH) optimally constant. In the present study, the optimum temperature for maximum phycobiliprotein production was found to be 35^{o}C for all the cyanobacteria investigated with the values 57.8 µg/ml in *Synechocystis* sp., 64.9 µg/ml in *Gloeocapsa* sp., 88.1 µg/ml in *Anabaena* sp. and 100.9 µg/ml in *Lyngbya* sp. Other researchers have reported 37^{o}C as optimum temperature for the production of phycobiliprotein in *Arthronema africanum* (Chaneva *et al*., 2007), 36 ^{o}C in *Synechococcus* (Sakamoto and Bryant, 1998) and 30^{o}C in *Anabaena* NCCU-9 (Hemlata and Fatma, 2009). Temperature is an important physical factor which strongly influences growth as well as various physiological and biochemical processes in cyanobacteria (Inoue *et al*., 2001; Murata, 1989; Vonshak, 2003; Shukla and Kashyap, 1999).

Effect of light intensities

The influence of light intensity was investigated by incubating the cultures at different light intensities (500, 1000, 1500, 2000, 2500, 3000 lux) for specific duration. Light intensities were adjusted to desired levels by changing the distance/orientation/angle of culture flasks from the light source and/or by changing the number/power of fluorescent tubes/bulbs growth chamber. The other culture conditions (e.g. temperature, light-dark period and pH) were kept optimally constant. Maximum phycobiliprotein production was observed at 2000 lux, yielding phycobiliprotein concentration 57.7 µg/ml in *Synechocystis* sp., 63.9 µg/ml in *Gloeocapsa* sp., 86.9 µg/ml in *Anabaena* sp. and 100.6 µg/ml in *Lyngbya* sp. Light intensity of 25 µmol photons $m^{-2}s^{-1}$ (=1850 lux) has been reported to be optimal for phycobiliprotein production in cyanobacteria *Synechococcus* NKBG 042902 (Takano *et al*., 1995), *Spirulina subsalsa* and *S. maxima* (Tomasseli *et al*., 1995, 1997), *Synechocystis* (Hong and Lee, 2008) and *Anabaena* NCCU-9 (Hemlata and Fatma, 2009).

Cyanobacteria are known to prefer low light intensities and stimulate phycobiliprotein synthesis because of their low specific maintenance energy and pigment composition (Grossman *et al*., 1993; Mur and Elema, 1983). For all photosynthetic organisms, including cyanobacteria, light (primary energy source) is an essential factor or requirement for growth and development, and the intensity, quality and duration of light plays a critical role in the growth and physiology of cyanobacteria, enabling them to carry out all the necessary metabolic processes. In addition to quantitative changes in pigments, the qualitative composition of pigments may change with the variation in light intensity (Richardson *et al*., 1983).

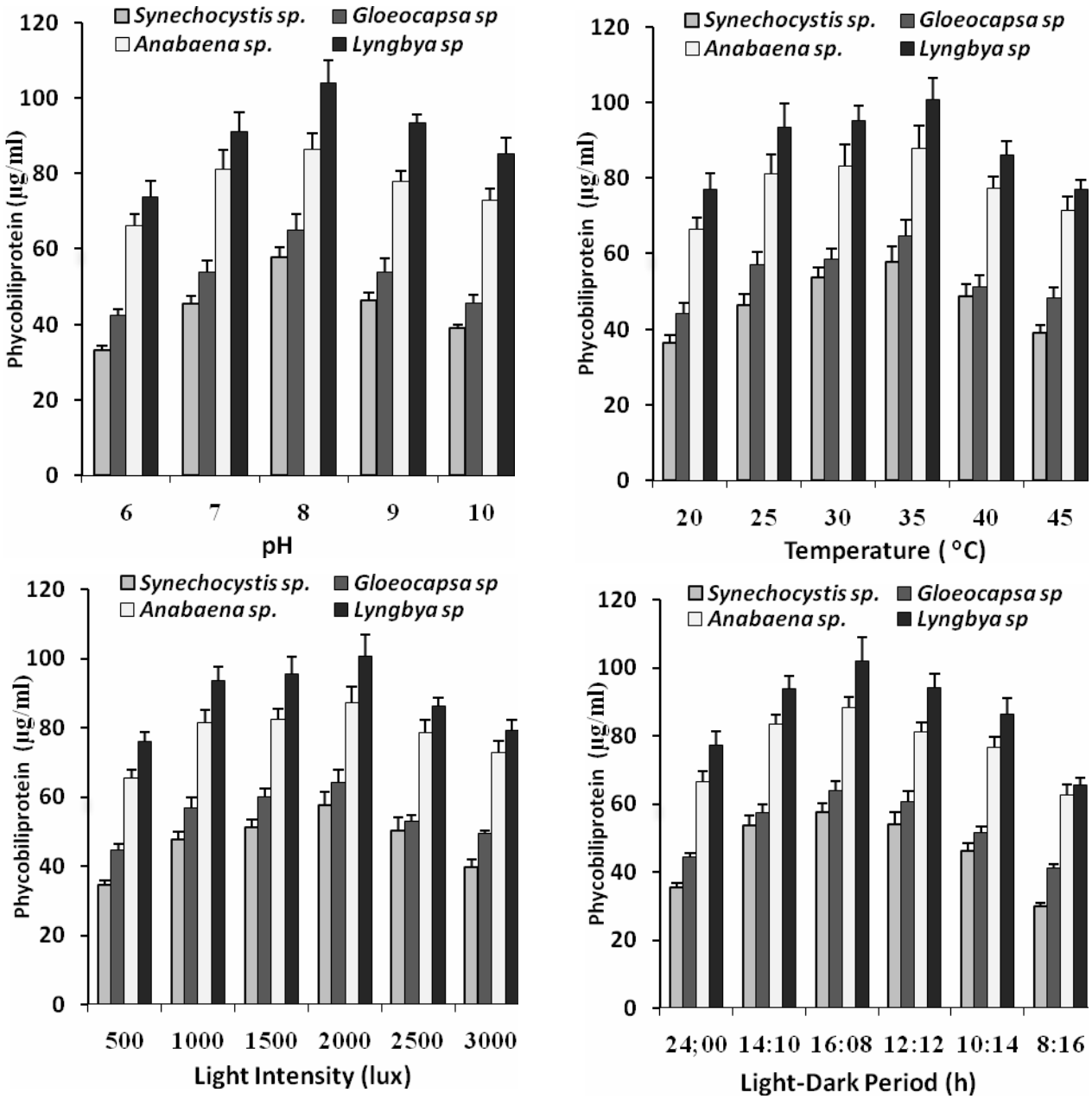

Figure 1. Effects of pH, temperature, light intensity and light-dark period on phycobiliprotein production in cyanobacteria. Values are means±SD (n=3)

Effect of light-dark period

Similarly, the influence of light-dark period (photoperiod) on phycobiliprotein production was investigated by adjusting different light: dark regime (24:00, 14:10, 16:08, 12:12, 10:14, 08:16 h), keeping other parameters (e.g. temperature, light intensity and pH) optimally constant. The results revealed that the photoperiod with light:dark regimes 16:08 h to be optimal for the production of phycobiliproteins in the cyanobacteria with the values 57.7 µg/ml in *Synechocystis* sp., 63.7 µg/ml in *Gloeocapsa* sp., 88.2 µg/ml in *Anabaena* sp. and 101.9 µg/ml in *Lyngbya* sp. A photoperiod with light:dark regimes 16:08 h has earlier been reported to be optimal for phycobiliprotein production in cyanobacteria *Calothrix elenkenii* (Prasanna *et al*., 2004) and *Anabaena* NCCU-9(Hemlata and Fatma, 2009).

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