

## Effect of carbon, nitrogen source and other conditions for enhancing phycocyanin production

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### ABSTRACT

Cyanobacteria (BGA) are prokaryotic photoautotrophs capable of doing photosynthesis and nitrogen fixation simultaneously. They have photosynthetic pigments like R-Phycocyanin, B-Phycocyanin,  $\alpha$ -Phycocyanin, C-Phycocyanin, Allophycocyanin, Phycocyanin-566, and Phycocyanin. Recent approaches are aimed to harvest these pigments for use as bio pigments in food and dye industries. Phycocyanin is a natural pigment gaining importance over synthetic colors, as they are non-toxic and non-carcinogenic. Among the different cyanobacterial genera screened for the maximum phycocyanin pigment production the genus *Westiellopsis* was found to be superior in phycocyanin production. The phycocyanin production was significantly enhanced by the parameters sodium carbonate as carbon source and potassium nitrate as nitrogen source. Among the cyanobacterial cultures studied, *Westiellopsis*-ARM 48 produced maximum phycocyanin content.

**Key words :** Cyanobacteria, Biopigment, Carbon, Nitrogen, *Westiellopsis*

### INTRODUCTION

Cyanobacteria are recognized as a rich but not yet extensively studied as a source of pharmacological as well as structurally interesting secondary metabolites (Belay *et al.*, 1993). Earlier the microalgal system alone is considered in the production of biopigments. It is important to exploit microalgae as well as cyanobacteria in these aspects. The most striking feature of cyanobacteria is the presence of brilliantly colored accessory pigments, the phycobiliproteins (Glazer and Fang, 1973), which accounts for 40 per cent of the total protein and 1-10 per cent (Fay, 1969) of the cell dry weight in *Anabaena cylindrica*. Cyanobacteria are considered to be a potential source of biocolors for food industries due to their versatile growth and abundant pigment production. The pigment content and composition of cyanobacteria is influenced by the environmental conditions. It is well known that cyanobacterial pigment concentration is influenced by environmental and nutritional factors (Rodriguez *et al.*, 1989). The present study was undertaken to examine the effectiveness carbon and nitrogen sources and other conditions as a potential factor to enhance the phycocyanin content in the cyanobacterial cultures.

### MATERIALS AND METHODS

#### *Optimization of carbon and nitrogen sources :*

The effect of different carbon and nitrogen sources on phycocyanin production was assayed by substituting the original carbon and nitrogen sources in the BG-11 medium with various carbon sources like ammonium

carbonate, glucose, fructose, sodium carbonate, sucrose and nitrogen sources such as ammonium sulphate, potassium nitrate, sodium nitrate and urea at one per cent level. The flasks containing the contents were incubated in used manner and the phycocyanin content was expressed as  $\mu\text{g ml}^{-1}$  of the cyanobacterial culture.

#### *Effect of different wavelength and intensity of light on phycocyanin production :*

The flasks were inoculated with selected cyanobacterial cultures and incubated under different wavelength of light such as red (640nm – 740nm), green (520nm – 570nm), blue (450nm – 520nm), yellow (570nm – 600nm) and unscreened white light (350nm – 740nm) by wrapping the flasks using different color papers. The desired intensity of light (1000 – 6000 lux) for the experimental cultures was obtained by wrapping the day light fluorescent tubes with layers of black nylon cloth. Light intensity was measured with a lux meter (Lutron electronics, USA) in the plane of culture vessel and oriented at right angles to the light source.

### RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been presented under following heads:

#### *Effect of carbon and nitrogen sources :*

The data revealed that sodium carbonate is the best preferred carbon source followed by fructose, while ammonium carbonate is the least preferred carbon source

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**Table 1 : Effect of carbon and nitrogen sources on phycocyanin pigment production by *Westiellopsis* sps**

Cyanobacterial cultures	Phycocyanin ( $\mu\text{g/ml}$ )								
	Carbon sources*				Nitrogen sources*				
	ammonium carbonate	fructose	glucose	sucrose	sodium carbonate	ammonium sulphate	potassium nitrate	sodium nitrate	urea
<i>Westiellopsis</i> -4A <sub>2</sub>	20.48 $\pm$ 0.5	28.92 $\pm$ 0.4	23.74 $\pm$ 0.6	24.25 $\pm$ 1.1	38.71 $\pm$ 0.2	36.36 $\pm$ 0.3	43.29 $\pm$ 0.2	37.57 $\pm$ 0.5	39.61 $\pm$ 0.9
<i>Westiellopsis</i> -ARM 48	34.60 $\pm$ 0.4	40.24 $\pm$ 0.2	36.20 $\pm$ 0.7	37.20 $\pm$ 0.2	55.53 $\pm$ 0.2	35.21 $\pm$ 0.4	55.91 $\pm$ 0.2	48.61 $\pm$ 0.3	51.21 $\pm$ 0.3
<i>Westiellopsis</i> - HT-SGK-1	22.60 $\pm$ 0.1	30.25 $\pm$ 0.2	24.81 $\pm$ 0.7	25.42 $\pm$ 0.3	40.40 $\pm$ 0.5	26.34 $\pm$ 0.3	40.25 $\pm$ 0.4	35.29 $\pm$ 1.1	37.34 $\pm$ 0.6
<i>Westiellopsis</i> -ST	27.81 $\pm$ 0.5	32.62 $\pm$ 0.5	28.84 $\pm$ 0.3	30.42 $\pm$ 0.5	45.24 $\pm$ 0.3	27.54 $\pm$ 0.5	46.28 $\pm$ 1.3	41.65 $\pm$ 0.2	43.38 $\pm$ 1.3
<i>Westiellopsis</i> -PSG	28.41 $\pm$ 0.2	35.65 $\pm$ 0.2	30.91 $\pm$ 0.2	32.83 $\pm$ 0.6	50.42 $\pm$ 1.2	29.68 $\pm$ 0.2	52.54 $\pm$ 1.4	44.37 $\pm$ 0.3	48.35 $\pm$ 0.4

\*Reported as the mean  $\pm$  S.E.M for three independent replicates

for the phycocyanin pigment production (Table 1). Among the cyanobacterial cultures, *Westiellopsis*-ARM 48 produces maximum phycocyanin (55.53  $\mu\text{g ml}^{-1}$ ) pigment production followed by *Westiellopsis*-PSG (50.42  $\mu\text{g ml}^{-1}$ ) with one per cent sodium carbonate as carbon source. Among the nitrogen sources potassium nitrate followed by urea increased the pigment production significantly, while the least pigment production was noticed with ammonium sulphate. Among the cultures *Westiellopsis*-ARM48 produced significantly higher phycocyanin (55.91  $\mu\text{g ml}^{-1}$ ) with potassium nitrate.

The highest content of C-Phycocyanin was obtained with CO<sub>2</sub> as a carbon source and nitrate as nitrogen source with a mean value of 20.1 per cent of the biomass dry weight in comparison with 11.5 per cent when both sources were added as mineral salts to the culture medium. The carbon concentration and nitrogen starvation have major influence on the C-phycocyanin content of cyanobacteria (Tandeau de Marsac, 1977 and Oquist, 1974). The C-Phycocyanin also acts as a nitrogen storage compound in cyanobacteria and this will be utilized in the nitrogen starvation conditions (Boussiba and Richmond, 1980). The normal growth of the cyanobacterial systems don't require any inorganic sources of carbon, because it can assimilate the required carbon from the atmosphere. But when we aim to increase the cell constituents like pigments, protein and biomass the exogenous application of carbon source becomes necessary. Becker and Venkataraman (1983) reported that, the addition of 3 to 4 g of sodium bicarbonate per liter of the medium was found to increase the biomass as well pigments in algal systems. In the present study, sodium carbonate at one per cent concentration in the BG-11 medium produces the maximum production of phycocyanin followed by the fructose. The sodium bicarbonate not only serves as a carbon source and also as a buffer to stabilize the pH which increases due to the ammonia excretion by the cyanobacterial cultures and helps for good growth.

Boussiba and Richmond (1980) reported that nitrogen concentration in the medium vary the phycocyanin content in cyanobacterial cultures. Liotenberg *et al.* (1996) showed that, when compared to nitrate, growth in the presence of ammonium resulted in intracellular steady-state levels 35% lower for phycoerythrin and 46% higher for phycocyanin. Nitrate nitrogen sources, ammonium and urea nitrogen sources increased pigment production only in lower concentrations and become toxic at higher concentrations. The nitrogen devoid conditions lead to the reduction in the phycocyanin content. The nitrate nitrogen sources at one per cent concentration are more favored for the phycocyanin production than the other nitrogen sources. The results are in agreement with Silva *et al.* (1989), suggesting that KNO<sub>3</sub>, Urea and NH<sub>4</sub>Cl as nitrogen source in the medium influence the phycocyanin pigment content in cyanobacteria.

#### ***Effect of wavelength and intensity of light on phycocyanin production :***

Among the different light wavelength, red light (640nm – 740nm) enhanced the pigment production followed by unscreened white light (350nm – 740nm) in all the cyanobacterial cultures. The least pigment production was noticed in green light (520nm – 570nm). In general, the pigment production was higher in the case of non - stress induced cultures than stress tolerant cultures irrespective of wavelength used (Table 2). Between the two non - stress induced cultures the cyanobacterial culture *Westiellopsis*-ARM 48 produced significantly higher phycocyanin (58.40  $\mu\text{g ml}^{-1}$ ) than *Westiellopsis*-PSG (52.50  $\mu\text{g ml}^{-1}$ ) in the entire five wavelength tested. Among the cultures *Westiellopsis*- HT-SGK-1 (44.20  $\mu\text{g ml}^{-1}$ ) recorded least pigment production.

The effect on phycocyanin production to seven different light intensities such as 0 (dark), 1000, 2000, 3000, 4000, 5000 and 6000 lux was assessed and given in

Table 2 : Effect of wavelength and intensity of light source on phycocyanin pigment production by *Westiellopsis* sps

Cyanobacterial cultures	Wavelength of light source <sup>#</sup>					Intensity of light source (Lux) <sup>*</sup>						
	Phycocyanin (µg/ml)											
	Red	Green	Blue	Yellow	White	0(Dark)	1000	2000	3000	4000	5000	6000
<i>Westiellopsis</i> -4A <sub>2</sub>	46.8 ±0.2	18.6 ±0.1	32.3 ±0.1	22.9 ±0.2	40.2 ±0.4	17.5 ±1.2	19.6 ±0.1	21.4 ±0.6	44.2 ±0.3	40.8 ±1.4	24.6 ±0.4	15.4 ±0.2
<i>Westiellopsis</i> -ARM	58.4 ±0.4	25.3 ±0.1	42.1 ±0.4	32.2 ±0.4	49.1 ±0.9	18.2 ±0.6	21.4 ±0.1	23.4 ±0.6	56.7 ±0.2	55.8 ±0.6	32.6 ±0.4	15.4 ±0.5
48												
<i>Westiellopsis</i> -HT-	44.2 ±0.3	9.1 ±0.2	29.3 ±1.3	21.3 ±0.5	38.5 ±0.7	16.2 ±0.8	18.4 ±0.5	20.6 ±0.4	40.3 ±0.9	30.8 ±0.5	23.4 ±0.2	14.5 ±0.1
SGK-1												
<i>Westiellopsis</i> -ST	48.1 ±1.1	20.5 ±0.5	36.5 ±0.6	27.5 ±0.3	45.3 ±0.3	17.6 ±0.7	20.2 ±0.6	21.6 ±0.2	45.2 ±0.4	43.8 ±0.6	25.6 ±0.8	15.8 ±0.4
<i>Westiellopsis</i> -PSG	52.5 ±0.3	22.3 ±0.6	39.4 ±0.4	31.2 ±0.3	47.2 ±0.4	18.0 ±1.1	20.9 ±0.4	22.0 ±0.5	47.8 ±0.2	49.6 ±0.5	26.1 ±0.4	16.2 ±0.5

<sup>#</sup>Red: 640nm – 740nm, yellow: 570nm – 600nm, green: 520nm – 570nm, blue: 450nm – 520nm and unscreened white light: 350nm – 740nm.

<sup>\*</sup>Reported as the mean ± S.E.M for three independent replicates

Table 2. The results revealed that the phycocyanin pigment production was low, when the cultures were incubated in dark. However the pigment concentration increased with increased light intensity. Maximum phycocyanin production was observed between 3000 and 4000 lux and the amount decreased markedly above and below this range. In the present experiment, *Westiellopsis*-ARM 48 (56.70 µg ml<sup>-1</sup>) produced maximum phycocyanin followed by *Westiellopsis*-PSG (49.60 µg ml<sup>-1</sup>) at 3000 and 4000 lux light intensity, respectively.

The photoautotrophic metabolism of cyanobacteria makes light as a principal limiting factor in microalgal biotechnology. The fresh water cyanobacterial species *Microcystis aeruginosa* and *Aphanizomenon flosaquae* are extremely sensitive to high light intensities (Mur, 1983). More than one set of phycocyanin subunits was synthesized in light adapting cyanobacteria, which can be explained that multiple phycocyanin genes are differentially controlled under different light conditions. Results of the light intensity experiments indicated the cyanobacterial growth was very slow in the lowest light intensity at 1000 lux. Maximum phycobiliprotein production was observed between 3000 and 4000 lux and the quantity decreased markedly above and below this range and these results are agreeable with the results of Adhikary (1979). The cyanobacterial isolates become yellowish green with increasing light intensities as has been reported in other algae groups (Fogg *et al.*, 1973). The major groups of blue green algae assume yellow coloration at high light intensities as a result of a break down of nitrogen rich chlorophyll and phycobilin pigments and retention of non - nitrogen containing carotenoids.

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