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Optimization of cultural conditions for enhancing biopigment - phycocyanin production by *Westiellopsis* species

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ABSTRACT

To increase the phycocyanin production and to use them as natural colorants, the following different approaches *viz.*, screening of the cyanobacterial cultures and standardization of culture conditions for maximum phycocyanin production was studied. 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 *viz.*, 35^{0}C temperature, alkaline pH (9.0), red color light, 3000 lux light intensity, 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, Phycocyanin, pH, Temperature

INTRODUCTION

The potential of cyanobacteria as biofertilizers is well known, the attention has been recently focused on the biotechnological potentials of cyanobacteria for obtaining industrially valuable compounds like pigments, amino acids, fatty acids, restriction enzymes and antibiotic compounds. 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). So far only limited genera of microalgae *viz., Spirulina, Dunaliella, Chlamydomonas* and *Haemotococcus* were exploited for their biopigments. It is important to exploit other 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. 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 of cultural condition variables as a potential factor to enhance the phycocyanin content in the cyanobacterial cultures.

MATERIALS AND METHODS

Screening of fresh water cyanobacterial cultures for high phycocyanin production :

Eighteen cyanobacterial cultures available in the culture collection centre of Algal Biotechnology Laboratory, Tamil Nadu Agricultural University, India were screened for their phycocyanin pigment content and the potential cultures were selected based on their pigment production for further optimization studies. In brief, one ml of selected cyanobacterial cultures was well homogenized and transferred aseptically to 100 ml of sterilized nitrogen free BG – 11 medium in 250 ml conical flasks. The cultures were grown photoautotrophically at 28 ± 1^0 C with 3000 lux light intensity with periodical shaking for 3 weeks in BG-11 medium as described previously (Rippka, 1988). The purity of the cyanobacterial cultures was checked periodically by microscopic observation.

Extraction of Phycocyanin from cyanobacterial biomass :

The phycobiliprotein phycocyanin extraction and estimation was done as described by Bennett and Bogorad (1971). Ten ml of cyanobacterial culture was homogenized and centrifuged at 5000 rpm for 5 min. The pellet was washed and suspended in 2.0 ml of 0.05M phosphate buffer (pH 6.8). The aqueous phase was subjected to freezing and thawing. The content was centrifuged at 5000 rpm for 5 min and then the supernatant was collected and stored at 4°C. The pellet was subjected to freezing and thawing until a colorless supernatant was obtained. The supernatant containing pigment was pooled and the final volume was recorded. The pigment absorption was measured at 615 and 652 nm in a Beckman DU-64 spectrophotometer against 0.05M phosphate buffer as blank. The concentration of phycocyanin was calculated using the formula:

$$\text{Phycocyanin (PC)} = \frac{E_{615} - 0.474\,(E_{652})\,*}{5.34}$$

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$*E_{615}$ $*E_{652}$ are the absorbances at 615 and 652 nm, respectively.

Effect of pH and temperature on phycocyanin production :

We have used the conventional method for the cultural condition optimization (variation of one factor at a time) throughout the study. The influence of cultural conditions parameters *viz.*, pH (3, 5, 7, 9, 11 and 13), temperature (25^0C, 35^0C, 45^0C), on the pigment production was investigated by inoculating the selected cyanobacterial cultures at the rate of 1ml in the 250ml conical flasks containing 100ml sterile BG–11 medium. The flasks were kept over a orbital incubator shaker (CIS 24 model) under the light intensity of 3000 lux at 28 ± 1^0 C for 28 days incubation period and the phycocyanin content was determined as described earlier and expressed as µg ml^{-1} of the cyanobacterial culture.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Screening of cyanobacterial cultures :

The Eighteen cyanobacterial cultures of *Anabaena, Fishcerella, Nostoc, Oscillatoria* and *Tolypothrix* genera were screened for higher phycocyanin pigment production and the results are given in Fig 1. Among the cultures, *Westiellopsis* sp produced more phycocyanin pigment than *Anabaena, Fishcerella, Nostoc, Oscillatoria* and *Tolypothrix* sp. Among the 11 *Westiellopsis* cyanobacterial cultures, *Westiellopsis*-ARM 48 (34.39 µg ml^{-1}), *Westiellopsis*-HT-SGK-1(28.90µg ml^{-1}), *Westiellopsis* -$4A_2$ (28.20µg ml^{-1}), *Westiellopsis* –PSG (32.18 µg ml^{-1}) and *Westiellopsis* – ST (29.01 µg ml^{-1}) were selected as the best ones, based on phycocyanin pigment production and selected for the further optimization studies.

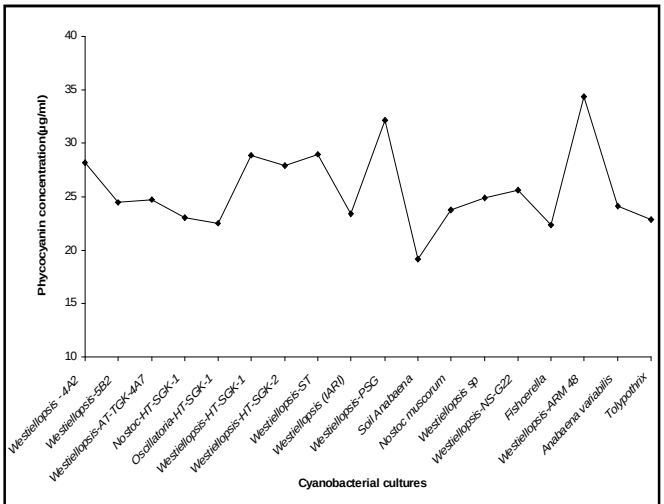

Fig. 1 : Screening of the cyanobacteral cultures for higher phycocyanin pigment production

The selection of algal strains is an important criteria for the production of high value chemicals in industrial level (Vonshak *et al*., 1996). Malathy (2000) reported that *Westiellopsis* cultures could tolerate stresses like acidity and salinity without an appreciable loss of biochemical constituents of cell. The versatile adaptation nature of the *Westiellopsis* species to nutrient and environmental factors may be the reason for their higher phycocyanin production in the present study. Growth conditions are known to affect the pigment composition, protein and lipid composition in cyanobacteria (Fatma *et al*., 1994). Although, the content of phycobiliproteins depends on the species of cyanobacteria and cultivation conditions, no such information exists with respect to *Westiellopsis* species.

Effect of pH :

The phycocyanin pigment production was affected in acidic pH (Table1). Except the acid tolerant cyanobacterial *Westiellopsis* $4A_2$ (15.10 µg ml^{-1}), none of the other cyanobacterial cultures were able to produce phycocyanin at pH 5.0. However, pigment production was linearly increased with pH 5.0 – 9.0 of the growth medium and started to decrease in the pH range of 9.0 – 13.0. Among the cyanobacterial cultures; *Westiellopsis*-ARM 48 (53.28 µg ml^{-1}) and *Westiellopsis* –PSG (45.18 µg ml^{-1}) produced significantly higher phycocyanin at pH 9.0 compared to the stress tolerant cultures.

In cyanobacteria the decreasing tendency of pigment chlorophyll-a content with increasing salinity was observed (Anantani and Vaidya, 1983). The hydrogen ion concentration affects the growth as well as the biochemical constituents of cells of cyanobacteria (Singh, 1974). In the present study, maximum phycocyanin production was observed by the cyanobacterial cultures at pH 9.0. The present results were at par with Silva *et al.* (1989) suggesting that the acid tolerant cyanobacterial culture synthesize chlorophyll pigment normally even in the pH of 4 and 5 and the pigment synthesis was higher in alkaline pH in comparison with the acidic pH in normal cyanobacterial cultures. Temperature of incubation has profound influence on pigment production and cyanobacterial biomass yield.

Effect of temperature on C-Phycocyanin production:

The maximum phycocyanin production was recorded

Table 1 : Effect of pH and temperature on phycocyanin pigment production by *Westiellopsis* sps#

Cyanobacterial cultures	Phycocyanin content (µg ml^{-1})								
	pH*						Temperature*		
	pH 3	pH 5	pH 7	pH 9	pH 11	pH 13	25^{0}C	35^{0}C	45^{0}C
Westiellopsis-4A$_2$	-	15.1 ± 0.4	28.36 ± 0.6	42.78 ± 1.1	39.24 ± 0.2	35.65 ± 0.3	28.6 ± 0.2	41.95 ± 0.5	9.57 ± 0.9
Westiellopsis-ARM 48	-	-	35.29 ± 0.7	53.28 ± 0.2	45.42 ± 0.2	40.30 ± 0.4	38.44 ± 0.2	46.72 ± 0.3	10.46 ± 0.3
Westiellopsis- HT-SGK-1	-	-	24.32 ± 0.7	41.23 ± 0.3	36.54 ± 05	32.24 ± 0.3	25.97 ± 0.4	33.57 ± 1.1	8.79 ± 1.6
Westiellopsis-ST	-	-	29.58 ± 0.3	45.18 ± 0.5	41.70 ± 0.3	37.89 ± 0.5	27.47 ± 1.3	41.68 ± 0.2	11.23 ± 1.3
Westiellopsis-PSG	-	-	32.40 ± 0.2	49.67 ± 0.6	43.65 ± 1.2	39.20 ± 0.2	35.94 ± 1.4	45.61 ± 0.3	9.31 ± 0.4

*Reported as the mean ± S.E.M for three independent replicates
#The results are those for day 28 of growth

at 35º C and drastically reduced at 45º C (Table 1). Among, all the five cyanobacterial cultures tested Westiellopsis-ARM 48 registered the maximum phycocyanin production (46.72 µg ml^{-1}) followed by Westiellopsis –PSG (45.61µg ml^{-1}) at 35º C. Stress tolerant cyanobacterial cultures; Westiellopsis 4A$_2$ and Westiellopsis –ST were at par in the phycocyanin production, while Westiellopsis - HT-SGK-1 recorded minimum phycocyanin content (33.57µg ml^{-1} among the five cyanobacterial cultures.

The metabolism and the cellular processes of cyanobacteria are temperature dependent with their rates changing exponentially with temperatures increase (Reynolds, 1984). Rodriguez *et al*. (1989) observed the fluctuation in the phycobiliproteins is in the temperature range between 25^0 C to 35^0 C. The extreme high and low temperature affects the chloroplast and pigments of the cyanobacteria. In the present study it has been observed that the phycocyanin pigment production by the cyanobacterial cultures was maximum at 35^0 C. The temperature ranging below and above 35^0 C affected the pigment content of the cell.

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