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NATURAL PIGMENTS PRODUCTION BY TWO STRAINS OF *TOLYPOTHRIX* KUTZING, SCYTONEMATACEAE, BLUE-GREEN ALGAE

RAMA KANT

Department of Botany, Ramkrishna Mahavidyalaya, Kailashahar, North Tripura

ABSTRACT : The synthesis of different natural pigments viz. chlorophyll-a, carotenoids and phycobiliproteins (C-phycocyanin, allophycocyanin and C-phycoerythrin) were studied in two strains of genus *Tolypothrix* Kutzing (*Tolypothrix*-19 and *Tolypothrix*-24) of Scytonemataceae, isolated from the tree bark of angiosperms. *Tolypothrix*-19 and *Tolypothrix*-24 were isolated from tree bark of *Coccus nucifera*, Palmaceae and *Albizia lebbeck*, Leguminosae respectively. The concentrations of chlorophyll-a and C-Phycoerythrin were observed highest 30.25% and 50.91% respectively in *Tolypothrix*-19 and lowest 19.57% and 43.57% in *Tolypothrix*-24. The production of carotenoids, C-Phycocyanin and Allophycocyanin was highest 3.82%, 24.15% and 8.89% respectively in *Tolypothrix*-24 and lowest 2.10%, 12.47% and 4.29% respectively in *Tolypothrix*-19.

Key words : *Epiphytic, Natural pigments, Chlorophyll-a, Carotenoids, Phycobiliproteins.*

INTRODUCTION

Blue green algae/Cyanobacteria are a group of extra ordinary diverse Gram-negative prokaryotes that originated 3.5 billion years ago and their diversity ranges from unicellular to multicellular, coccoid to branched filaments. Blue green algae are photoautotrophic microorganism and capable to grow in almost all types of known habitats. They grow on moist soil, tree barks, rocks, walls, roofs and in fresh and marine water bodies and many specialized habitats including thermal springs and polar regions. They synthesize their food with the help of different light harvesting pigments. These pigments are called natural pigments and they include chlorophyll-a, carotenoids, C-Phycoerythrin, C-Phycocyanin and Allophycocyanin. The chlorophyll-a, is considered as the main photosynthetic pigment. However carotenoids, C-Phycoerythrin, C-Phycocyanin and C-Allophycocyanin are considered as the accessory pigments. Last three pigments are also known as phycobilinpigments and Phycobiliproteins. Although prime photosynthetic pigment is chlorophyll-a, but accessory pigments like phycoerythrin, phycocyanin, carotenoides are also play an important role in cyanobacteria and combination ratio of all these pigment results variation in colour of thallus.

The chlorophyll-a, is found in thylakoid membrane, which is not soluble in water. Carotenoids are accessory pigment and not soluble in water. These are found in the peripheral region of protoplast in cyanobacteria (Fogg,1950). Carotenoids have a wide commercial application and are used for natural coloring (Emodi,1978), they are also known to improve the health and fertility of low fed cattle (Jackson *et al.*, 1981). Carotenoids derivatives such as abscisic acid and vitamin A retinol act as growth promoters and aid in cancer prevention (Peto *et al.*,1981). The phycobilin pigments are water soluble pigments and are found attached to the outer surface of thylakoid (Gantt,1975). Phycobiliproteins are also accessory pigment, which absorbs light and transfer energy to chlorophyll (Chapman,1973, O'Carra and Oh Eocha,1976). phycobiliproteins are expensive fine chemicals, used as natural colours and fluorescent dye (phycoflour probe) in immuno assay and are important component of diagnostic kits. In present study efforts have been made to find out the good natural pigments producing strains of the genus *Tolypothrix*, Scytonemataceae, Nostocales of blue-green algae to utilize these strains as the natural pigments rich in future for commercial production.

MATERIAL AND METHODS

i) Samples Collection site : Samples of epiphytic blue-green algae were collected from tree barks of plants of Tripura growing near the boundary line of Indo-Bangladesh, Kailashahar, North Tripura district of Tripura; Belonia and Sipahijala wild life sanctuary, West Tripura district of Tripura.

ii) Isolation, purification and identification of Epiphytic Blue-green algal strains : The samples were collected from tree barks of plants of Tripura and transferred into culture medium and enrichment cultures were raised in BG-11 medium (Stanier *et al.*,1971) in absence and presence of nitrogen (1.5g $NaNO_3$/L). Isolation and purification of epiphytic blue-green algal strains were carried out with the help of methods described by Kant *et al.* (2004a) and finally four blue-green algae strains of genus *Tolypothrix* (*Tolypothrix*-15, *Tolypothrix*-19, *Tolypothrix*-20 and *Tolypothrix*-24) of family Scytonemataceae, order Nostocales were developed as unialgal strains. *Tolypothrix*-15 and *Tolypothrix*-19 strains were isolated from the tree bark of *Coccus nucifera* Linn. of family Arecaceae and *Tolypothrix*-20 and *Tolypothrix*-24 were isolated from the tree bark samples of *Albizia lebbeck* Benth of Leguminosae (Fabaceae). Isolated strains were identified with the help of available monographs (Geitler,1932 and Desikachary,1959).

(iii) Estimation of natural pigments : For the assessment of natural pigments *viz.* chlorophyll a, carotenoides, C-phycocyanin, C-allophycocyanin and C-phycoerythrin, both strains of *Tolypothrix* were grown in BG 11 medium and incu-

*Author for correspondence (email : rkojha_1@rediffmail.com) Received 22.06.2011 Accepted 07.07.2012

bated for 20 days under standard laboratory conditions. The estimation of Chlorophyll a was made with the help methods described Mckinney (1941); Carotenoides were estimated by the method described by Jensen (1978); estimation of C-Phycocyanin, C-Allophycocyanin and C-Phycoerythrin were made with the help of method described by Bennet and Bogorad (1973). The optical density of the homogenized suspension of grown cultures were measured by using Spectrophotometer, Electronic (model 305), results were recorded in µg/ml and were converted into the percentage of total pigments. All the experiments were performed in three replicates.

RESULTS AND DISCUSSION

Present comparative study on two strains of *Tolypothrix* revealed a wide variation in the synthesis of natural pigment by different light harvesting pigment system. Results indicated that chlorophyll-a and C-Phycoerythrin were produced in greater percentage by *Tolypothrix*-19 in comparison to *Tolypothrix*-24. However, carotenoids, C-Phycocyanin and C-Allophycocyanin were produced in greater percentage by *Tolypothrix*-24 in comparison to *Tolypothrix*-19. The percentage of chlorophyll-a and C-Phycoerythrin were synthesized maximum 30.25% and 50.91 % respectively by *Tolypothrix*-19. However, percentage of Carotenoids, C-Phycocyanin and C-Allophycocyanin were synthesized maximum 3.82%, 24.15% and 8.89% respectively by *Tolypothrix*-24.

The percentage of Chlorophyll-a and C-Phycoerythrin were synthesized minimum 19.57% and 43.57 % respectively by *Tolypothrix*-24. However, percentage of Carotenoids, C-Phycocyanin and C-Allophycocyanin were produced minimum 2.10%, 12.47% and 4.27% respectively by *Tolypothrix*-19. The detailed results are given in graph (Fig.1).

Singh *et al.* (2008) have studied comparative pigment profile in ten strains of *Nostoc* and reported a wide range of pigments production in *Nostoc*. They reported Chlorophyll-a from 5.21% to 27.35%, Carotenoides from 1.58% to 20.12%, C-Phycocyanin 0.99% to 47.44%; C-Allophycocyanin 10.03% to 68.06%, C-Phycoerythrin form 6.49% to 41.57%. Kant *et al.* (2004b) have studied a comparative studied of pigments production in six strains of unicellular and colonial cyanobacteria and reported Chlorophyll-a from 6.80% to 11.93%, Carotenoides from 9.68% to 17.73%, total phycobilin from 75.38% to 82.13%. Mishra *et al.* (2003) made a comparative study on pigments production by rivularian members of blue-green algae and reported Chlorophyll-a production from 2.80% to 11.85%, Carotenoides from 1.58% to 20.12%, 7.22% to 26.50% and total phycobilin production 63.09% to 89.51%.

On the basis of results it was concluded that *Tolypothrix*-19 was beneficial strain for production of natural pigments chlorophyll-a and C-Phycoerythrin. However, *Tolypothrix*-24 was useful for the production of Carotenoids, C- Phycocyanin and C-Allophycocyanin.

Among natural pigments, C-Phycocyanin and C-Phycoerythrin are commercially valuable and are used for colouring candy, ice-cream, dairy products, soft drinks and Carotenoides are used as natural food colourant. These natural pigments after specific treatments with organic solvent and precipitation can be utilized for their commercial exploitation. Phycocyanin from *Spirulina* has been commercialized by Dainippon Ink and Chemicals of Japan under the trade name Linablue which gives brilliant blue colour with faint reddish fluorescence (Switzer,1981 and Kant *et al.*,2006).

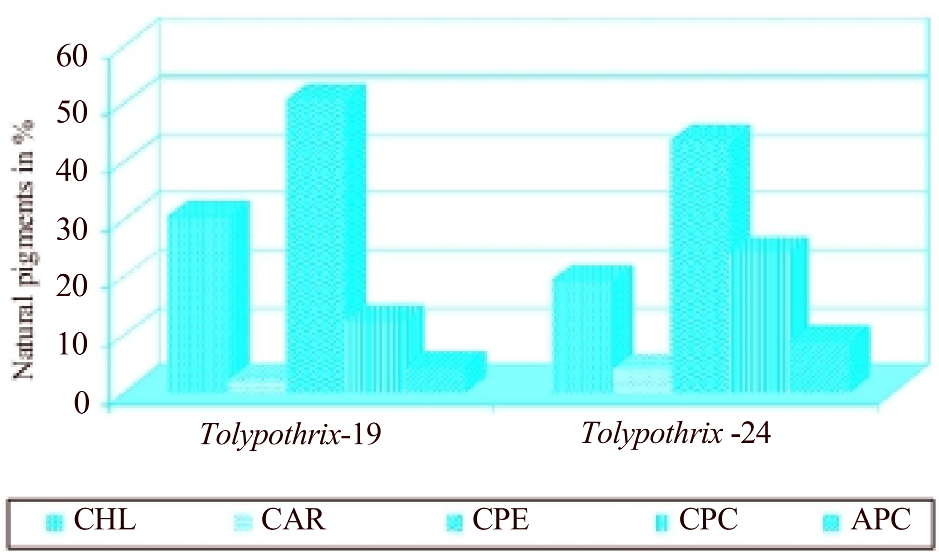

Fig. 1 Showing Chlorophyll-a (CHL), Carotenoids (CAR), C-Phycoerythrin (CPE), C-Phycocyanin (CPC) and C-Allophycocyanin (APC) production by *Tolypothrix* strains in percentage (%).

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