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COMPARATIVE GROWTH ANALYSIS OF CYANOBACTERIA FROM DIVERSE HABITATS WITH REFERENCE TO PIGMENTS

Rupali Kaur [1], Akhlaqur Rahman [2], Manjita Mishra [1], Shailendra Kumar Singh [1], Shanthy Sundaram [*1] and Shashi Alok [3]

Centre of Biotechnology [1], Nehru Science Centre, Centre of Biotechnology, University of Allahabad, Prayagraj - 211002, Uttar Pradesh, India.
Department of Biotechnology [2], S.S. Khanna Girls Degree College, Attarsuiya, Prayagraj - 211003, Uttar Pradesh, India.
Institute of Pharmacy [3], Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India.

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Correspondence to Author:
Prof. Shanthy Sundaram

Centre of Biotechnology, Nehru Science Centre, Centre of Biotechnology, University of Allahabad, Prayagraj - 211002, Uttar Pradesh, India.

E-mail: shanthy.cbt@gmail.com

ABSTRACT: Cyanobacteria are a gram-negative group of microorganisms with unique features and found in diverse conditions of the environment. These tiny bio-factories are rich in pigment molecules like chlorophylls, carotenoids and phycobiliproteins. Studies on physiological and biochemical characteristics of different cyanobacterial strains will not only help to determine their biotechnological potential but also determine and interpret the appropriate growth conditions of these tiny microorganisms in their natural habitat. This paper focuses on the growth, chlorophyll, carotenoid and phycobilin production in two freshwaters (*Anabaena cylindrica* and *Anabaena* PCC7120), one halotolerant (*Anabaena* KP737864) and two marine cyanobacterial strains (*Synechococcus* BDUSM-13 and Nostoc BDUMM-70). Significant differences were observed among the strains with regard to their pigments. Maximum chlorophyll and phycobiliprotein content were observed on the 14th day of cultivation in all strains. The filamentous cyanobacteria *Anabaena cylindrica* recorded maximum production of phycocyanin, marine *Synechococcus* BDUSM-13 produced maximum phycoerythrin (17.33 µg/ml). *Anabaena* KP737864 was reported to acquire more amount of allophycocyanin (12.66 µg/ml) of phycobiliproteins.

INTRODUCTION: Cyanobacteria are the ancient lineage of photo-oxygenic bacteria on the Earth more likely to be present since 2600 million years ago [1]. Due to their competence in performing oxygenic photosynthesis, cyanobacteria are linked with the changes of ancient reductive to the lively oxidative state of atmosphere [2].

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Earlier they have been grouped with eukaryotic algae, but due to their specific physiological and morphological characteristics of gram-negative bacterial cell structure, they are currently considered as prokaryotes [3].

Cyanobacteria possess a range of characteristic shapes, sizes, smells, textures, second light-harvesting antenna complex, heterocyst for nitrogen fixation, *etc.*, which allow them to adapt to the harsh environmental changes from terrestrial to aquatic habitats [4, 5, 6]. They have been described as excellent sources of chlorophyll, carotenoids, phycobiliproteins, exo-polysaccharides, proteins and other biologically active metabolites [7].

Cyanobacteria contain only one form of chlorophyll that is chlorophyll A which is green pigment present at the photosynthetic reaction center. In addition, they also contain various yellowish carotenoids, the blue pigment phycobilin, and in some species, the red pigment phycoerythrin [8]. Phycobilins are water-soluble pigments and are therefore found in the cytoplasm, or in the stroma of the chloroplast. They occur only in Cyano-bacteria and Rhodophyta. These bioactive metabolites of cyanobacteria are of special interest in the development of new products for medical, pharmaceutical, cosmetic and food industries.

This biotechnological potential seems to increase in cyanobacterial strains that can tolerate the extreme conditions to partially avoid competition from other, less tolerant organisms. Thus, halotolerant organisms are very attractive candidates for their mass culture in hypersaline or arid environments with great solar radiation; otherwise, it is not useful in normal conditions. The growth of cyanobacteria in aquatic environments could be controlled by a variety of environmental factors like appropriate nutrients, temperature, pH and light conditions that are necessary for their culture. Knowledge of their physiological and biochemical characteristics in laboratory cultures, in a wide range of parameters, not only helps to determine their biotechnological potential but also to determine and interpret in a more efficient way to observe the results of the growth of these microorganisms in their natural environment. Thus, this work will evaluate the pigment production potential of two freshwater, one halotolerant and two marine cyanobacterial strains for commercial exploitation.

MATERIALS AND METHODS:

Collection of algal strain: Five cyanobacterial strains were selected. Freshwater strains, Anabaena cylindrica, and Anabaena PCC7120 were obtained from the algal collection center of the University of Allahabad, India. However, a halotolerant species Anabaena KP737864 was procured from Algal Culture Collection Centre, Indian Agriculture Research Institute, New Delhi, India. Marine strains Synechococcus BDUSM-13 and Nostoc BDUMM-70 were received from National Facility for Marine Cyanobacteria (NFMC) Tiruchirappalli, India. All cultures were maintained in their appropriate medium under aseptic conditions.

Growth and Culture Maintenance Conditions of Strains: The freshwater cyanobacterial strains *Anabaena cylindrica* and *Anabaena* PCC 7120 were cultivated in BG 11-ve (without NaNO3) growth medium [9]. However, halotolerant Anabaena KP 737864 was grown in TI medium, *i.e.*, Turk's Island Salt solution [10, 11]. The marine cyanobacterial species *Synechococcus* BDUSM-13 and *Nostoc* BDUMM-70 were grown in ASN + and ASN – media (Artificial sea medium), respectively [12].

The medium constituents were sterilized separately by autoclaving at 121 °C for 15 min and were mixed thoroughly before inoculation. The chemicals used in this study were of reagent grade. All cultures were maintained for 20 days in autoclaved 250 ml conical flasks containing 150 ml of medium, pH 7.5, at 20º ± 2 ºC temperature, and 60 lE m2s1 light intensity with a dark: light cycle of 16:8 h. After 20 days, biomass was harvested by centrifugation at 3000 ×g for 5 min, filtered, dried at 40 ºC for 48 h and analyzed for pigment analysis.

Extraction of Photosynthetic Pigments: For extraction of phycocyanin, one gram of freeze-dried cell biomass was suspended in 100 ml of sodium phosphate buffer (0.1 M, pH 7.0, containing 1 mM sodium azide). Suspended cell biomass was disrupted by sonication for 60 s and repeated freezing at -20 ºC and thawing at room temperature, followed by the further extraction of phycobiliproteins. The mixture was subsequently centrifuged at 10,000g for 30 min at 4 ºC, and a phycobiliprotein containing blue color clear supernatant was collected and used for further analysis according to protocol.

Estimation of Chlorophyll: Growth was measured by extracting total chlorophyll A of the culture. The methanol extracted supernatant was estimated for cellular chlorophyll a by employing the standard extinction coefficient (13.42) for a solution of 1 mg/ml of chlorophyll [13]. 2 ml of homogenized algal suspension was taken in a centrifuge tube and done centrifugation at 7000 rpm for 10 min and then discarded the supernatant and transferred the algal pellet to a test tube and added 2 ml of 90% methanol. The contents were shaken and the tubes were placed covered with aluminum foil in a water bath at 60 ºC for 30 min. The absorbance of the

supernatant was measured at 665 nm against methanol blank (UV-visible spectrophotometer).

Estimation of Carotenoids: For estimation of carotenoids, 96% acetone was used as a solvent [14]. The absorbance of acetone extract was taken using 96% acetone as blank at 460 nm by UV Spectrophotometer. The total amount of carotenoids in µg/ml was calculated by using a UV-visible spectrophotometer.

Estimation of Phycobiliproteins: The absorbance of phycobiliprotein containing supernatant was measured in a UV–Vis spectrophotometer at wavelengths 620, 652 and 562 nm for calculating the concentrations of C-PC (C-phycocyanin), APC (Allophycocyanin) and PE (Phycoerythrin) respectively using the following equations.

$$CPC\ (mg/ml) = [A_{620} - 0.474\ A_{652}]/5.34$$

$$APC\ (mg/ml) = [A_{652} - 0.208\ A_{620}]/5.09$$

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

Separation of Phycocyanin by Ammonium Sulphate Precipitation: Ammonium sulfate was gradually added to 100 ml crude extracts to achieve 25% and 50% saturation with continuous stirring. The resulting solution was kept for 2 h and centrifuged at 12,000 g for 30 min. The obtained blue precipitate was dissolved in 0.005 M Na-phosphate buffer (pH- 7.0) [15]. At each extraction step, the C-PC concentration was calculated by the method of Boussiba and Richmond [16] and the purity was calculated by the method of Bennett and Bogorad [17]. This pigment was used for further studies.

Statistical Analysis: The experimental data are presented as mean ± SD of three replicates. All analyses were conducted using Graph pad Prism version-5.0 (Graph Pad Software, San Diego, CA, USA).

RESULTS AND DISCUSSIONS:

Growth Analysis of Different Cyanobacterial Species: Growth analysis was done by taking 2 ml of the culture of different cyanobacteria in eppendorf tubes and their O.D were taken at 700 nm in UV Visible spectrophotometer. All cyanobacterial species showed growth and grew luxuriantly in the flasks in laboratory conditions. The marine species also showed good growth but at a slower rate. These marine species were grown in low light environments. The growth conditions were good due to the proper temperature and light availability. The green color of the cultures represented the growth of cultures where the number of cells increased. The mean values of growth O.D at 16th day were maximum in *Anabaena cylindrica*, which was 0.361, whereas the slow-growing marine *Synechococcus* BDUSM - 13 mean growth O.D was 0.166 showed in **Fig. 1**.

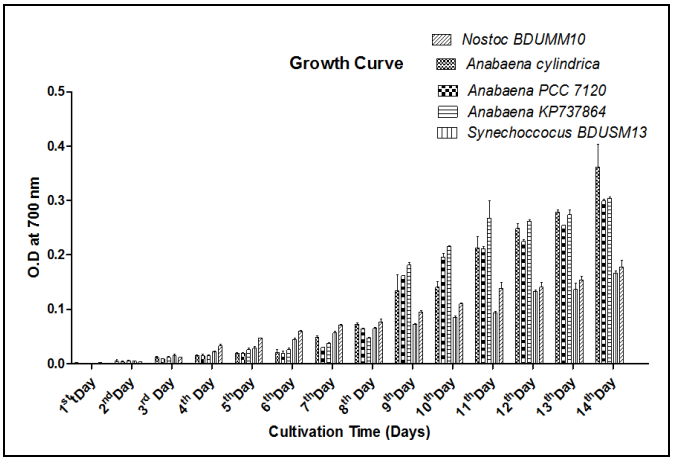

FIG. 1: SHOWING THE GROWTH ANALYSIS CURVE OF FIVE DIFFERENT CYANOBACTERIA

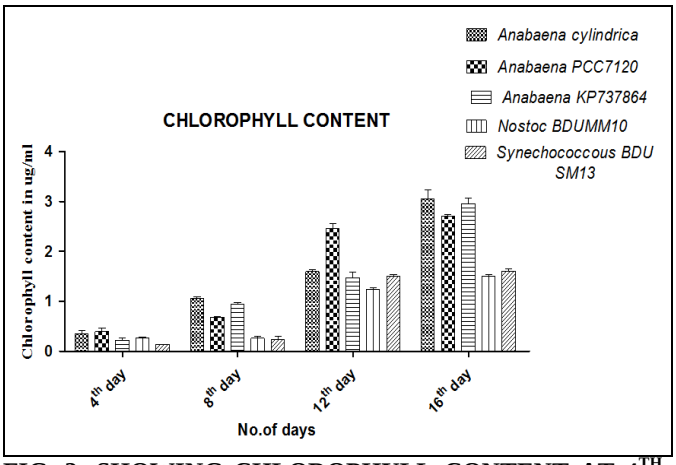

FIG. 2: SHOWING CHLOROPHYLL CONTENT AT 4TH, 8TH, 12TH AND 16TH DAY. Mean values are found from three independent determinations and are shown with ± standard deviation

Chlorophyll Analysis: Chlorophyll content was noted in each cyanobacterial strains. *Anabaena cylindrica* (3.06 µg/ml) showed the highest chlorophyll content at 16th day while *Synechococcus* BDUMM-13 showed minimum value at 1.60 ug/ml. On 4th day *Anabaena PCC7120* showed maximum value at 0.395 µg/ml whereas a minimum of *Synechococcus BDUMM-*

13, which was 0.134 ug/ml, respectively, shown in **Fig. 2**.

Carotenoid Pigment: The mean values of carotenoids calculated were highest during peak growth stage (*i.e.* 16th day of incubation) followed by a decline thereafter. There was a marked difference amongst the cyanobacterial strains for this parameter. *Anabaena cylindrica* showed the highest carotenoid content at 16th day about 0.715 µg/ml and a minimum of 0.011 µg/ml, which was of *Synechococcus* BDUSM-13 respectively **Fig. 3**.

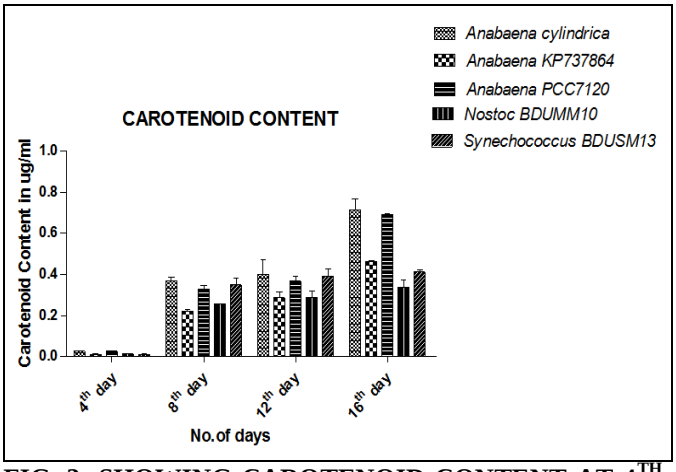

FIG. 3: SHOWING CAROTENOID CONTENT AT 4TH, 8TH, 12TH AND 16TH DAY. Mean values are found from three independent determinations and are shown with ± standard deviation

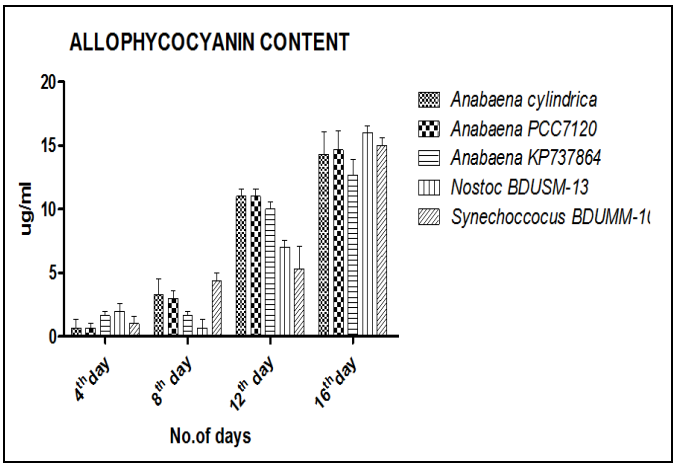

FIG. 4: SHOWING ALLOPHYCOCYANIN CONTENT AT 4TH, 8TH, 12TH AND 16TH DAY. Mean values are found from three independent determinations and are shown with ± standard deviation

Phycobiliproteins: Phycobilins comprising allophycocyanin (APC), phycoerythrin (PE) and phycocyanin (PC) were measured individually during the incubation time and showed in **Fig. 4**, **Fig. 5** and **Fig. 6,** respectively. The highest phycocyanin contents were observed at the 16th day of incubation in *Anabaena cylindrica* 24 µg/ml and a minimum 16 µg/ml phycocyanin of Nostoc BDUMM-10 on 16th day. Phycoerythrin content in *Synechococcus* BDUSM-13 was 17µg/ml, while in *Anabaena*, PCC7120 was 5 ug/ml on the 16th day. Allophycocyanin content on the 16th day in *Anabaena* KP 737864 was 12 µg/ml and that of 16 µg/ml in Nostoc BDUMM-10, respectively.

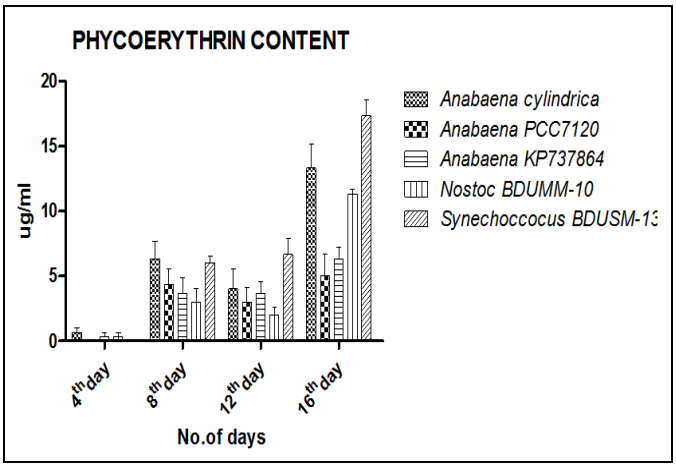

FIG. 5: SHOWING PHYCOERYTHRIN CONTENT AT 4TH, 8TH, 12TH AND 16TH DAY. Mean values are found from three independent determinations and are shown with ± standard deviation

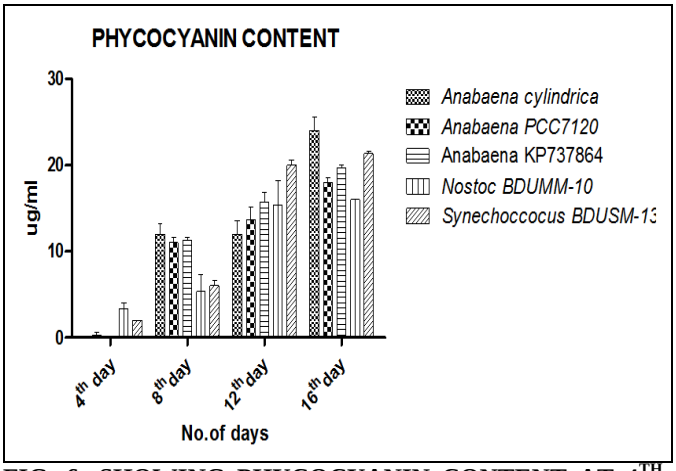

FIG. 6: SHOWING PHYCOCYANIN CONTENT AT 4TH, 8TH, 12TH AND 16TH DAY. Mean values are found from three independent determinations and are shown with ± standard deviation

DISCUSSION: In the present time, microalgae are the richest sources of biologically active pigments with the high medicinal property. Amongst these microalgae, gram-negative microorganisms, cyano-bacteria, are unique producer of diverse pigments. These cyanobacteria are also reported to produce the general photosynthetic pigments like chlorophyll-A and β-carotene as in other

microalgae, but they are different from microalgae by synthesizing phycobiliproteins which may be one of the biomarkers for cyanobacteria. From this present study, five different cyanobacterial strains were analyzed for their biomass and phycobiliprotein production. All the *Anabaena* strains (Anabaena PCC7120, *Anabaena cylindrica* and *Anabaena* KP787364 found to grow well in media, which was being analyzed by their growth curve and pigment production while *Synechoccoccus* BDUSM-13 showed slow growth.

Anabaena cylindrica shows maximum growth with absorbance mean value of 0.361 at 700 nm, and Nostoc BDUMM-10 shows an absorbance value of 0.166 at 700 nm. Carotenoid content at 16th day was 0.715 ug/ml was in *Anabaena cylindrica* and 0.011 µg/ml in *Anabaena* KP737864. It was interesting to observe that strain Anabaena cylindrica showed the highest phycocyanin (PC) content. Prasanna [18] also observed a wide range (0.11–3.40 µg ml^{-1}) with respect to chlorophyll accumulation in different Anabaena strains. Here the chlorophyll content was highest at about 3.06 µg/ml in *Anabaena cylindrica*. Prasanna [18] observed differences in the protein and phycobiliprotein content of *Anabaena* strains isolated from different geographical locations. The chlorophyll and carotenoids in the organisms were related to the physiological capability of the cells in the case of cyanobacteria isolated from hyper-saline environments.

CONCLUSION: Cyanobacteria are the photosynthetic organisms that possess the ability to synthesize chlorophyll. About five cyanobacterial strains were used in this study to quantitatively analyze the production of phycobiliproteins. The cyanobacterial strains, such as Anabaena (both freshwater and halotolerant) are found to show good growth and synthesized high yield of phycobiliproteins in comparison to other marine cyanobacterial strains.

The filamentous cyanobacterial forms like *Anabaena cylindrica* was recorded as maximum phycocyanin, marine *Synechococcus* BDUSM-13 (17.33 µg/ml) was having highest phycoerythrin. Anabaena KP737864 was reported to acquire more amounts of allophycocyanin (12.66 µg/ml) of phycobiliproteins.

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CONFLICTS OF INTEREST: The authors have declared no conflict of interest.

REFERENCES:

1. Hedges S, Chen H, Kumar H, Wang DYC, Thompson AS and Watanabe H: A genomic timescale for the origin of eukaryotes. BMC Evol Biology 2001; 1: 4.
2. Singh S K, Bansal A, Jha MK and Dey A: An integrated approach to remove Cr (VI) using immobilized *Chlorella minutissima* grown in nutrient rich sewage wastewater. Bio resource Tech 2012; 104: 257-65.
3. Stanier RY, Kunisawa R, Mandel M and Cohen-Bazire G: Purification and properties of unicellular blue-green algae (order Chroococcales). N Bacteriological Reviews 1971; 35(2): 171-205.
4. Singh SK, Rahman A, Dixit K, Nath A and Sundaram S: Evaluation of promising algal strains for sustainable exploitation coupled with CO_2 fixation. Environmental Technology 2016; 37(5): 613-22.
5. Papke RT, Ramsing NB, Bateson MM and Ward DM: Geographical isolation in hot spring cyanobacteria. Environmental Microbiology 2003; 5(8): 650-59.
6. Comte K, Sabacka M, Carre-Miouka A, Elster J and Komárek J: Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium* -like strains evaluated by a polyphasic approach. FEMS Microbiol Ecology 2007; 59: 366-76.
7. Muramatsu M and Hihara Y: Acclimation to high-light conditions in cyanobacteria: From gene expression to physiological responses. Journal of Plant Research 2011; 125(1): 11-39
8. Glazer AN and Stryer L: Phycofluor probes. Trends Biochem Sci 1984; 9: 423-27.
9. Singh SK, Bansal A, Jha MK and Dey A: Comparative studies on uptake of wastewater nutrients by immobilized cells of *Chlorella minutissima* and dairy waste isolated algae. Indian Chemical Engineer 2011; 53(4): 211-19.
10. Garlick S, Oren A and Padan E: Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. Journal of Bacteriology 1977; 129: 623-29.
11. Codd GA, Cook CM and Stewart WDP: Purification and subunit structure of D-ribulose 1, 5-bisphosphate carboxylase from the cyanobacterium *Aphanothece halophytica*. FEMS Microbiol Lett 1979; 6: 81-86.
12. Rippka R, Deruelles J, Waterbury JB, Herdman M and Stanier RY: Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 1979; 111: 1-61.
13. Mackinney G: Absorption of light by chlorophyll solutions. Journal of Biological Chemistry 1941; 140: 315-22.
14. Hellebust JA and JS Craige: Handbook of Physiological and Biochemical Methods Cambridge University 1978; 59-70.
15. Silva LA, Kuhn KR, Moraes CC, Burkert CAV and Kalil SJ: Experimental design as a tool for optimization of C-

phycocyanin purification by precipitation from *Spirulina platensis*. J of the Brazilian Chem Soc 2009; 20(1): 5-12.
16. Boussiba S and Richmond A: Isolation and purification of phycocyanins from the blue-green alga *Spirulina platensis*. Arch. Microbiol 1979; 120: 155-59.
17. Bennett A and Bogorad L: Complementary chromatic adaptation in a filamentous blue green alga. Journal of Cell Biology 1973; 58: 419-35.
18. Prasanna R, Kumar R, Sood A, Prasanna BM and Singh PK: Morphological, physiochemical and molecular characterization of *Anabaena* strains. Microbiological research 2006; 161(3): 187-02.
19. Bryant DA: Phycoerythrocyanin and phycoerythrin: Properties and occurrence in cyanobacteria. Journal General Microbiology 1982; 128: 835-44.

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