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Pigments analysis of cyanobacterial strains

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Abstract
The pigment variation is specific features among cyanobacteria. The main objective here is the importance of the blue green algae especially because of the pigments present in this class of algae. It is characterized by their specific phycobilin pigments, also other pigments such as chlorophylls and carotenoides are found. Phycobilins are an important group of pigments that through complementary chromatic adaptation optimize the light-harvesting process in phytoplankton cells, exhibiting great potential as cyanobacteria species biomarkers. Cellular chlorophyll concentration is one of the central physiological parameters, routinely followed in many research areas ranging from stress physiology to biotechnology. Carotenoids concentration is often related to cellular stress level; combined pigments assessment provides useful insight into cellular physiological state. So, for analysis of chlorophyll, carotenoids, phycobilins (phycocyanin, allophycocyanin and phycoerythrin) five cyanobacterial strains *viz*. *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertilissima*, *Westelliopsis prolifica*, and *Tolypothrix tenuis* were examined. These strains were grown in BG-11medium and their pigments content were analysed at an interval of 7, 14, 21 and 28 days of incubation. Significant differences were observed among the strains with regard to these pigments. Maximum chlorophyll content was observed at 7th day of incubation whereas the other parameters were maximum at 14th day of incubation in all the cases, followed by a gradual decline. *Anabaena variabilis* showed maximum amount of chlorophyll and phycocyanin whereas *Tolypothrix tenuis* showed maximum amount of carotenoids, allophycocyanin and phycoerythrin.

Keywords: Cyanobacteria, chlorophyll, carotenoids, phycobilins, phycocyanin, allophycocyanin, phycoerythrin

Introduction
Pigments are chemical compounds that absorb light in the wavelength range of the visible. Cyanobacteria are the photosynthetic organisms which possess the ability to synthesize chlorophyll and a number of valuable compounds such as carotenoids (used as antioxidants) as accessory pigments to absorb light energy. Accessory pigments confer extended ability to harvest light for photosynthesis and, in some cases, protection from UV and other light-induced cell damage. Chlorophylls are associated with membranous thylakoids similar to those of plants and other algae. The association- of carotenoids with chlorophyll prevents the formation of highly reactive singlet oxygen radicals that would otherwise cause irreparable damage to lipids, proteins and other molecules (Bartley and Scolnick, 1995) [1]. In addition to carotenoids and chlorophyll, cyanobacteria possess another group of pigments not expressed in plants, the phycobiliproteins, water-soluble pigments that transfer captured energy to chlorophyll a. Three types of phycobiliproteins are produced *viz.*, phycocyanin (Blue), allophycocyanin (Blue grey) and phycoerythrin (Red). However, not all taxa that contain phycobiliproteins produce all the three types. Additionally, these pigments represent the major constituents of red algae and cyanobacteria and may represent up to 60% of the total protein content of the cell (Viskari and Colyer, 2003) [27]. The evaluation of the pigment contents in the blue green algae is essential to indirectly check the measure of culture growth and also as a parameter to check the trophic levels of waters. Additional economic uses of natural pigments are, such as, use of phycobiliprotein as an additive in foods, cosmetic products and medical diagnostic reagents. Cyanobacteria, especially the *Anabaena*, *Nostoc* and *Spirulina* genera, have been used for centuries as food. Little attention has been given to the general features of physiology of blue green algae with the specific attention given to nitrogen fixation and some aspects of photosynthesis (Fogg, 1947; Brown and Webster, 1953) [10, 5]. A number of studies

have used blue green algae as reliable tools for studies involving growth and other related parameters (Kratz and Myers, 1955) [14]. Currently cyanobacteria are appealing because of the increasing demand for natural pigments and antioxidants over the synthetic ones which may trigger allergic and carcinogenic processes (Botterweck *et al.*, 2000) [4]. Now days they are produced in closed photo-bioreactors and in open tanks to be used as food and for the isolation of many products, such as drugs, fluorescent markers, biological pigments, enzymes, antioxidants and exopolysaccharides for use as gelling agents, emulsifiers, flocculants and moisturizers (Otero and Vincenzini, 2003; Tokusoglu and Unal, 2003; Cepoi *et al.*, 2009) [23, 26, 7]. Currently, the therapeutic effects of phycobiliproteins are well explored, including their antiplatelet, immunomodulatory (Jensen *et al.*, 2001) [13], antidiabetogenic (Soni *et al.*, 2009) [25], anti-hepatotoxic (Gallardo-Casas *et al.*, 2010; Chiu *et al.*, 2006) [11, 8], anti-inflammatory and anti-carcinogenic effects, their use in the treatment of Parkinson's disease and Alzheimer's disease (Rimbau *et al.*, 2001) [21] and their effect in reversing the multiple drug resistance phenotype of several types of tumor cells (Morlière *et al.*, 1998; Rodríguez *et al.*, 2006) [19, 22]. Carotenoids, on the other hand, have physiological functions in addition to their use as dyes.

Material and Methods

Estimation of pigments

Chlorophyll: For estimation of chlorophyll, a known *volume* of homogenized suspension was centrifuged for 5 min (3000 x *g).* Chlorophyll from the pellet was extracted with 95% *(v/v)* methanol at 60 °C for 30 minutes. After centrifugation, the final *volume* was made up and the chlorophyll was quantified by measuring optical density at 650 and 665 nm. Total chlorophyll was calculated according to the formula *given* by Mackinney (1941) [17].

Carotenoids: A known volume of homogenized algal suspension was centrifuged at 3000 x *g* for 10 minutes. The pellet thus obtained was washed with distilled water to remove traces of adhering salts. To that, 2-3 mL of 85% acetone was added and subjected to repeated freezing and thawing. Extractions were performed till acetone became colourless. The acetone fractions thus obtained was pooled; and the final total volume was recorded. The content of total carotenoids was estimated from the maximum absorbance measured at 450 nm using 85% acetone as blank (Jensen, 1978) [12].

Phycobilins: A known volume of homogenized algal suspension was centrifuged at 5000 x *g* for 10 minutes and pellet was suspended in equal volume of 0.05 M phosphate buffer, pH 7.5 (obtained by mixing equal volume of 0.1 M KH_2P0_4 and 0.1 M KH_2P0_4). Repeated freezing and thawing was done till· the pellet became colourless and the pigments oozed out in the supernatant. The absorbance was measured at 562, 615 and 652 nm against 0.05 M phosphate buffer as blank. The amounts of phycobilin pigments (Phycocyanin, PC; Allophycocyanin, APC; and Phycoerythrin, PE) were calculated by using the following equations (Bennett and Bogorad, 1973) [2].

Results

Chlorophyll: Chlorophyll content exhibited peak value at 7th day of growth followed by a gradual decline. There was a significant difference among the strains for this parameter and the chlorophyll content ranged between 9.12 to 21.95 μg mg^{-1} dry weight. *Anabaena variabilis* showed highest chlorophyll content followed by *Westelliopsis prolifica. Nostoc muscorum*, *Aulosira fertilissima* and *Tolypothrix tenuis* ranked 3rd, 4th and 5th respectively in terms of chlorophyll content (Fig. 1).

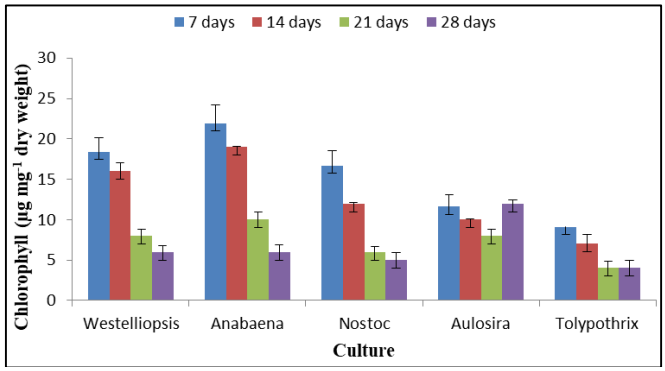

Fig 1: Chlorophyll content (µg mg^{-1} dry weight) in blue green algal biofertilizer strains at different days of incubation

Carotenoids

Mean values of carotenoids calculated were highest during peak growth stage (*i.e.*, 14th day of incubation) followed by a decline thereafter. There was a marked difference amongst the cyanobacterial strains for this parameter. *Tolypothrix tenuis* showed highest carotenoid content (0.79μg mg^{-1} dry weight) and *Nostoc muscorum* showed lowest carotenoid content (0.22μg mg^{-1} dry weight). Carotenoid content at 14th day by *Anabaena variabilis* assumed 2nd position. *Westelliopsis prolifica* and *Aulosira fertilissima* showed almost similar carotenoid content and ranked 3rd and 4th respectively (Fig. 2).

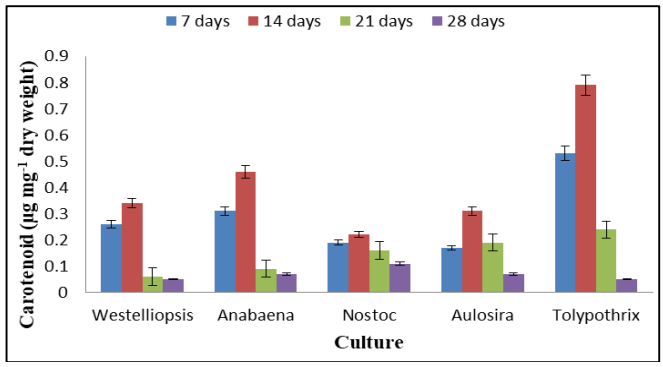

Fig 2: Carotenoid content (µg mg^{-1} dry weight) in blue green algal biofertilizer strains at different days of incubation.

Phycobilins

Phycobilins comprising phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APe) were measured individually in BGA strains grown in nitrogen free BG-11 medium during the incubation time. Highest phycocyanin, allophycocyanin and phycoerythrin contents were observed at 14thday of incubation. *Anabaena variabilis* showed highest phycocyanin content (41.07μg mg^{-1} dry weight) followed by *Tolypothrix tenuis* (39.01μg mg^{-1} dry weight) whereas *Aulosira fertilissima* showed lowest phycocyanin content (8.61μg mg^{-1} dry weight). *Nostoc muscorum* ranked 3rd whereas *Westelliopsis prolifica* and *Aulosira fertilissima* ranked 4th and 5th in terms of phycocyanin content (Fig. 3). *Tolypothrix tenuis* showed highest allophycocyanin content (20.04μg mg^{-1} dry weight) followed by *Anabaena variabilis* (15.01 μg mg^{-1} dry weight) (Fig. 4). *Nostoc muscorum* ranked 3rd whereas *Westelliopsis prolifica* and *Aulosira fertilissima* showed

almost similar value. *Tolypothrix tenuis* also showed highest phycoerythrin content (42.04*µg* mg^{-1} dry weight) whereas *Aulosira fertilissima* showed lowest (7.37 *µg* mg^{-1} dry weight). *Nostoc muscorum* ranked 2nd whereas *Anabaena variabilis* and *Westelliopsis prolifica* ranked 3rd and 4th respectively in terms of phycoerythrin content (Fig. 5).

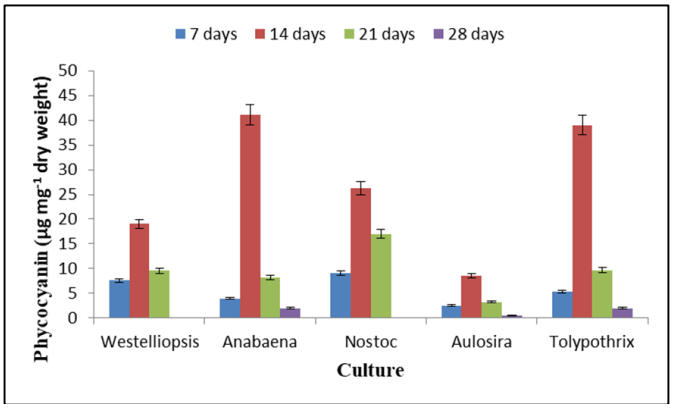

Fig 3: Phycocyanin content (µg mg^{-1} dry weight) in blue green algal biofertilizer strains at different days of incubation.

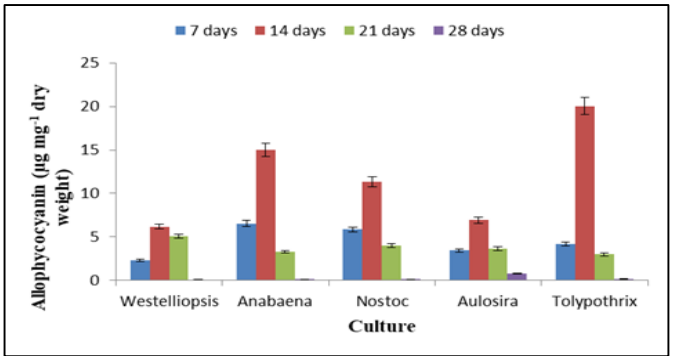

Fig 4: Allophycocyanin content (µg mg^{-1} dry weight) in blue green algal biofertilizer strains at different days of incubation.

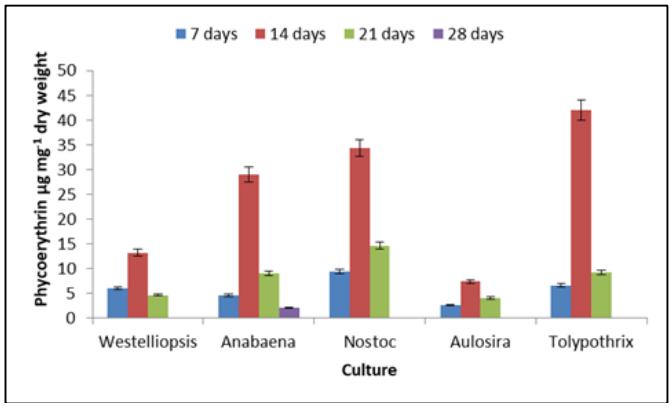

Fig 5: Phycoerythrin content (µg mg^{-1} dry weight) in blue green algal biofertilizer strains at different days of incubation.

Discussion

Cyanobacteria are the photosynthetic organisms which possess the ability to synthesize chlorophyll. In this study, a significant difference among the strains for this parameter have been reported and the chlorophyll content ranged between 9.12 to 21.95 *µg* mg^{-1} dry weight with *Anabaena variabilis* showed highest chlorophyll content. Maria *et al.* (2009) found 28771.5 and 35880 chlorophyll g^{-1} in *Anabaena* and *Nostoc* respectively. Prasanna *et al.* (2006) [24] also observed wide range (0.11–3.40 µg mL^{-1}) with respect to chlorophyll accumulation in different *Anabaena* strains. Besides chlorophyll, the filamentous cyanobacteria, particularly nitrogen-fixing heterocystous species, are regarded as attractive organisms for the production of phycobiliproteins, carotenoids and other important chemicals which serve as accessory pigments in photosynthesis. (Borowitzka, 1988; Moreno *et al.*, 1995) [3]. Carotenoids protect the cyanobacterial cell from photooxidative damage and are found associated with proteins. Prasanna *et al.* (2006) [24] reported carotenoid content in the selected *Anabaena* strains ranged from 0.4 to 21.63 µg mL^{-1}. In this study, *Tolypothrix tenuis* showed highest carotenoid content (0.79*µg* mg^{-1} dry weight) and *Anabaena variabilis* assumed 2nd position with 0.46*µg* mg^{-1} dry weight. The levels of phycocyanin, allophycocyanin and phycoerythrin in total phycobiliprotein content in cyanobacteria vary not only with species but are also influenced by environmental factors. Cyanobacterial species with high content of phycobiliproteins can be considered as their prospective source for commercial use. All naturally occurring cyanobacteria were found to produce PC (Bryant, 1982). Evans, (1988) also reported 0.044 and 0.030 µg mL^{-1}phycocyanin content in *Anabaena* and *Nostoc* respectively. It was interesting to observe that strain *Anabaena variabilis* showed highest phycocyanin (PC) content and the second highest rank for allophycocyanin (APC) content. Our observations were in consonance with those from an earlier work (Bryant, 1982) in that the PC-rich strains were more commonly found among cyanobacteria, especially Anabaena strains. *Tolypothrix tenuis* showed highest allophycocyanin content and also exhibited highest phycoerythrin content. It was observed that *Nostoc muscorum* ranked second in case of phycoerythrin content and ranked third both in terms of phycocyanin and allophycocyanin content. Similar observation were reported by Ankita and Suchit (2017) where they found phycoerythrin concentration 0.0047 µg mL^{-1} in *Anabaena* and 0.030 µg mL^{-1}in *Nostoc* strains. Kursar and Alberte (1983) [15] reported allophycocyanin content of 108.18 µg mL^{-1} 112.33 µg mL^{-1} in *Anabaena* and *Nostoc* strains respectively. The ranking of strains for PC, APC and PE content and also for their total phycobiliproteins can be very useful in selection of strains for their potential utilization as colouring agents, phycoflour probes or as additives in a range of cosmetic and pharmaceutical products (MacColl and Guard-Friar, 1987) [17].

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