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SCREENING, EXTRACTION AND ESTIMATION OF PHYCOBILIPROTEINS FROM CYANOBACTERIA

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Abstract:-Cyanobacteria are known for the production of specialized and photosynthetic pigments Phycobiliproteins. The phycobiliproteins constitutes of three different pigments, which are c-phycocyanin, allophycocyanin and phycoerythrin. All the three pigments have their pharmaceutical value. This present study deals with the analysis of the quantitative production of phycobiliproteins from the cyanobacterium. About eight Cyanobacterial strains were chosen for this study and their growth curve and phycobiliprotein production were evaluated. The Cyanobacterial strains, *Synechococcus elongatus*, *Chroococcus minor* and Anabaena strains were found to grow well when compared to the other Cyanobacterial strains in this present study. The mass cultivation of different Cyanobacterial strains also correlated with the growth curve the same such Cyanobacterial strains found to yield more amounts of wet and wet-dried biomasses. The phycobiliprotein extraction using 90 % acetone has yielded more phycobiliproteins which was high in the Cyanobacterial strains *Synechococcus elongatus*, *Chroococcus minor, Anabaena* strains and *Nostoc* sp. The Nostoc sp. yielded relatively more phycobiliproteins but the production of biomasses was poor. This current research work concludes that the Cyanobacterial strains Synechococcus elongatus and Chroococcus minor are marine forms along with fresh water filamentous forms Anabaena strains and Nostoc sp. are found to yield high phycobiliprotein and can be an alternative source for the biological production of phycocyanin pigment.

Keywords: Cyanobacteria, Screening, Extraction, Phycocyanin and Estimation.

1.INTRODUCTION

The Cyanobacteria are the widespread group of photosynthetic prokaryotic microorganisms which are cosmopolitan to the global environment and one of the primary producers of energy (Chisholm *et al.*, 1988; Waterbury *et al*., 1979). The Cyanobacteria are otherwise known as Blue-green algae due to the production of specialized photosynthetic blue-green pigments called Phycocyanin (Whitton and Potts 2000). The presence of microcystin can be predicted due to production of extracellular fluorescent pigment phycocyanin on the water surface (Gerhardt and Bodermer 2000; Bastein *et al.*, 2011).

Phycocyanins are phycobiliproteins consists of three different sub-classes c-phycocyanin, Allophycocyanin and Phycoerythrin. Phycocyanin pigment has pharmaceutically high commercial value, because phycocyanin protects the photosystems from free radicals and can be able to prevent oxidative damage caused by free radicals. Phycocyanin is a blue-green light-harvesting pigment specifically found in Cyanobacteria exceptional to some algal genera, belongs to Rhodophyta and Cryptophyta. As a phycobiliprotein, phycocyanin consists of α and ß subunits and can be found as trimers or as hexamers. The phycocyanin has a range of molecular weight between 140-210 kDa. The phycobiliproteins are water soluble, strongly fluorescent and has potent antioxidant properties. Therefore, the phycobiliproteins are employed in biotechnological applications as in food, cosmetics, diagnostics and pharmaceutical industries. The production and applications of phycobiliproteins alone yield 236 patents among

them biological production of phycobiliproteins engulfs 55 patents and pharmaceutical applications engulfs 30 patents and rest of them on applications on the fluorescence properties of phycobiliproteins (Sekar and Chandramohan, 2008).

All the available phycobiliproteins are multi-chain holo-proteins with an apo-protein along with covalently bound phycobilins. Among the other phycobiliproteins, recognition and functional resemblance of phycocyanorubin and bilirubin has evolved c-phycocyanin as a major pharmaceutical agent other than nutrient foods (Eriksen, 2008). Thus, the phycocyanin extracted from Porphyra yezoensis can be a potent candidate to treat some kinds of cancers (Zhang *et al*., 2011). The phycobiliproteins are the specialized proteins which protects the photosystems from the oxidative damage during photosynthesis rather than the other protective agents such as superoxide dismutase, catalase, and glutathione peroxidase. The ß-carotene also used as an anti-oxidative agent during high light intensity (Rakhimberdieva *et al.*, 2004). The

The major objectives in this study were screening, growth curve analysis, biomass cultivation, extraction and estimation of phycobiliproteins from the Eight (8) different Cyanobacteria.

2.MATERIALS AND METHODS

The Cyanobacteria taken for this study including *Anabaena sp.*, *Anabaena variabilis, Anabaena spiroides, Nostoc sp., Chrospococcus minor, Lyngbya sp., Phormidium sp.* and *Synechococcus slongatus*. These Cyanobacterial cultures were obtained from the algal culture laboratory, Department of Plant Biology and Plant Biotechnology, Presidency College (Autonomous), Chennai, Tamil Nadu, India. The aforementioned Cyanobacteria were analyzed for growth curve, mass cultivated, pigments were extracted and phycobiliproteins were estimated from the crude extract.

2.1. Growth curve analysis of different Cyanobacteria

All the Cyanobacteria were allowed to grow in 400 ml of BG11 medium in 1l flasks with standard pH-7.0 at room temperature (29^{o}C) under white fluorescent light for 12:12 hours of light and dark period respectively. Then the Cyanobacteria cultures were analyzed under UV-Vis spectrophotometer (Hitachi U-2900) at 690 nm for every day up to 15 days. Growth curve for all the Cyanobacteria were analyzed and interpreted.

2.2. Mass cultivation of Cyanobacteria

Every Cyanobacterial cultures were then subjected to mass cultivation in 2.5 l of BG11 medium in 5 l flasks with standard pH of 7.0 at a room temperature (29OC) under white fluorescent light for 12:12 hours of light and dark period respectively. After successive incubation for 15 days of mass cultivation, all the Cyanobacteria were harvested by centrifugation at 8000 rpm for 5 min. the wet biomass and wet-dried biomass were estimated gravimetrically.

2.3. Extraction of phycobiliproteins

The dried biomasses from the different Cyanobacteria are the sources for the extraction of pigments. The wet dried biomasses were homogenized with Mortar and Pestle for 15 min. All the fine powdered biomasses were separately subjected to extraction with 90% acetone and for complete extraction kept undisturbed overnight. After successive extraction, all the extractions were centrifuged at 10,000 rpm for 5 min. at room temperature. The cell debris was settled down in the pellet and supernatant consists of the crude extract, which was stored in a separate container.

2.4. Estimation of phycobiliproteins

Three different phycobiliproteins such as c-phycocyanin, Allophycocyanin and Phycoerythrin were measured photometrically at 620, 652 and 562 nm respectively using UV-Vis. spectrophotometer based on the Siegelman and Kycia method (Siegelmen and Kycia, 1978).

3.RESULTS

3.1. Growth curve analysis of different Cyanobacteria

Cyanobacterial strains such as *Synechococcus elongatus* and *Chroococcus minor* are the single cellular cyanoprokaryotes whereas the other Cyanobacterial strains are filamentous forms. The growth curve analysis of eight different Cyanobacteria have shown that the Cyanobacterial strains such as *Synechococcus elongatus, Chroococcus minor, Anabaena sp., Anabaena variabilis, Anabaena spiroides* were found to obtain a good growth

curve when compared to other Cyanobacterial strains including *Nostoc* sp., *Lyngbya* sp. and *Phormidium* sp. (Fig. 1).

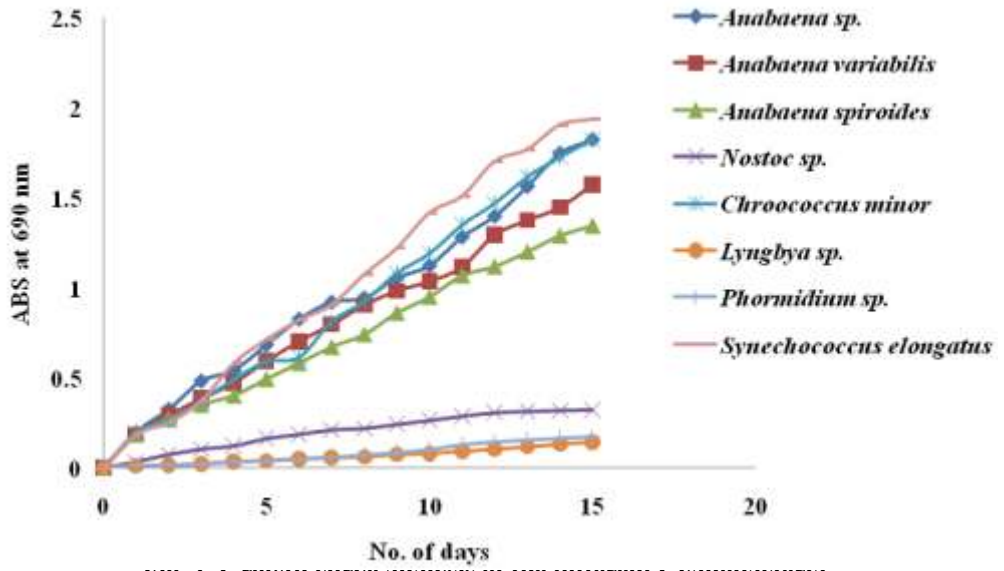

Fig. 1. Growth curve analysis of the different Cyanobacteria

3.2. Mass cultivation of Cyanobacteria

The biomasses harvested after mass cultivation were dried, before that the wet biomasses were measured gravimetrically. The wet and wet-dried biomasses of all the Cyanobacterial strains were estimated gravimetrically and interpreted. Among the Cyanobacterial strains mass cultivated, *Synechococcus elongatus* (81.094 g of wet weight and 2.149 g of wet-dried weight), *Chroococcus minor* (81.660 g of wet weight and 2.164 g of wet-dried weight), *Anabaena* sp. (84.377 g of wet weight and 2.236 g of wet-dried weight), *Anabaena variabilis* (80.867 g of wet weight and 2.143 g of wet-dried weight) and *Anabaena spiroides* (78.415 g of wet weight and 2.078 g of wet-dried weight) were yielded quantitatively high amount of wet as well as wet-dried biomass from 2.5 l of culture when compared to Nostoc sp. (65.773 g of wet weight and 1.743 g of wet-dried weight), *Lyngbya* sp. (58.716 g of wet weight and 1.556 g of wet-dried weight) and *Phormodium* sp. (57.886 g of wet weight and 1.534 g of wet-dried weight) which were yielded comparatively low biomass (Table 1).

Table 1. Wet and wet-dried biomasses of different Cyanobacterial strains

Different Cyanobacterial strains	Wet weight (g) in 2.5 *l* of mass cultivation	Wet-dried weight (g) in 2.5 *l* of mass cultivation
***Anabaena* sp.**	**84.377**	**2.236**
Anabaena variabilis	**80.867**	**2.143**
Anabaena spiroides	**78.415**	**2.078**
Nostoc sp.	65.773	1.743
Chroococcus minor	**81.660**	**2.164**
Lyngbya sp.	58.716	1.556
Phormidium sp.	57.886	1.534
Synechococcus elongatus	**81.094**	**2.149**

3.3. Extraction and Estimation of phycobiliproteins

Three different phycobiliproteins such as c-phycocyanin, allophycocyanin and phycoerythrin were measured photometrically. From which, *Synechococcus elongatus* (3.594 mg/ml of c-phycocyanin, 3.372 mg/ml of allophycocyanin and 2.993 mg/ml of phycoerythrin), *Chroococcus minor* (3.328 mg/ml of c-phycocyanin, 2.945 mg/ml of allophycocyanin and 2.626 mg/ml of phycoerythrin), *Anabaena* sp. (2.963 mg/ml of c-phycocyanin, 2.621

mg/ml of allophycocyanin and 2.991 mg/ml of phycoerythrin), Anabaena variabilis (2.853 mg/ml of c-phycocyanin, 2.137 mg/ml of allophycocyanin and 2.762 mg/ml of phycoerythrin) *Anabaena spiroides* (2.724 mg/ml of c-phycocyanin, 2.216 mg/ml of allophycocyanin and 2.573 mg/ml of phycoerythrin) and Nostoc sp. (2.273 mg/ml of c-phycocyanin, 2.183 mg/ml of allophycocyanin and 2.372 mg/ml of phycoerythrin)have found to produce huge amount of phycobiliprotein pigments when compared to other Cyanobacterial strains like Lyngbya sp. (0.542 mg/ml of c-phycocyanin, 0.345 mg/ml of allophycocyanin and 0.472 mg/ml of phycoerythrin) and Phormidium sp. (0.624 mg/ml of c-phycocyanin, 0.512 mg/ml of allophycocyanin and 0.619 mg/ml of phycoerythrin) with relatively poor amount of phycobiliprotein pigments (Fig. 2).

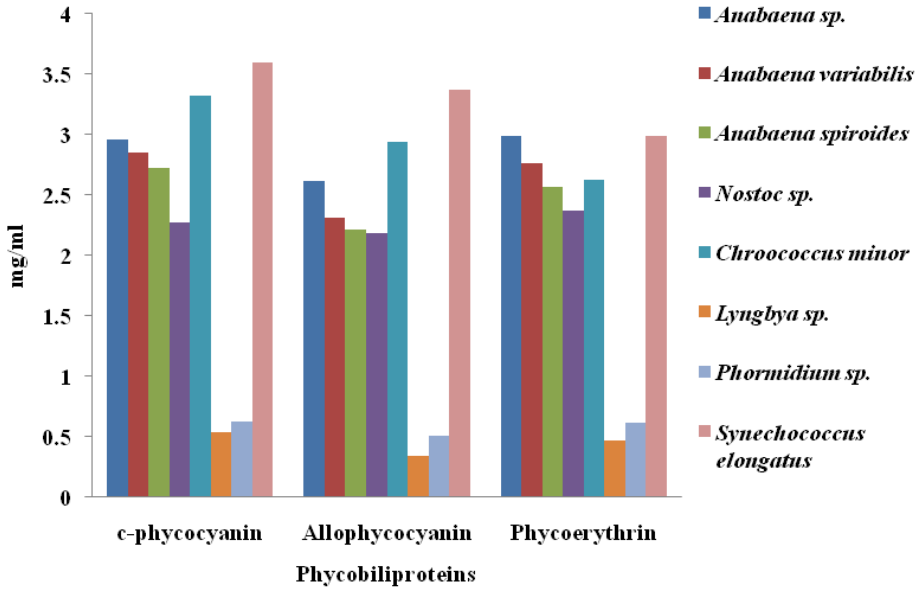

Fig. 2: Estimation of Phycobiliproteins from different Cyanobacteria

4.DISCUSSION

Nowadays, the microalgae are the rich sources of biologically active pigments with high medicinal property. Among the micro algae, Cyanobacteria are unique by producing different pigments like phycocyanin. The Cyanobacteria are also reported to obtain the general photosynthetic pigments like chlorophyll-a and β-carotene as in other micro algae, they are different from them by synthesizing phycobiliproteins which may be one of the biomarker for Cyanobacteria. Phycobiliproteins are coloured proteins involved as a light harvesting complex and transfer it to the reaction center of PSII (Glazer, 1984). Among all the proteins present in the Cyanobacteria phycobiliproteins alone constitute about 60 % of proteins (Bogard, 1975). From this present study, eight different Cyanobacterial strains were analyzed for its huge biomass and phycoiliprotein production. Among them, *Synechococcus elongatus*, *Chroococcus minor* and all the Anabaena strains found to grow well in the BG11 medium by analyzing their growth curve.

The c-phycocyanin has a free radical sites with it and it was the first report from Electron spin resonance spectra (ESR). Therefore, c-phycocyanin will be a potent candidate against free radicals. In this present study, *Synechococcus elongatus, Chroococcus minor, Nostoc* sp. and all the Anabaena strains found to produce more amount of c-phycocyanin when compared to other Cyanobacterial strains. The c-phycocyanin extracted from Lyngbya sp. has shown high peroxy radical scavenging potential when compared to the extract from Spirulina sp. and Phormidium sp. with an IC50 value of 6.63 µM (Patel *et al*., 2006). But the results from this current study have shown that Lyngbya sp. did not produce much amount of phycobiliproteins which is comparatively low. The c-pycocyanin was reported to show very food anti-cancer activity in the humans and in animals (Pardhasaradhi *et al.*, 2003). During cultivation, followed by incubation in high temperature, led the Cyanobacterial strain *Synechococcus* sp. WH8102 to produce huge amount of phycobiliproteins (Mackey et al., 2013).

The phycocyanin content from the dried biomasses of six Cyanobacterial strains from different regions were *Anabaena* sp. (Antartic) with 144.2 µg mg^{-1}, *Anabaena* sp. (Tropical) with 82.8 µg mg^{-1}, *Nostoc* sp. (Antartic) with 136.4 µg mg^{-1}, *Nostoc* sp. (Tropical) with 88.6 µg mg^{-1}, Phormidium sp. (Antartic) with 162.8 µg mg^{-1} and Phormidium sp. (Tropical) 95.5 µg mg^{-1} (Shukia *et al*., 2008). The Cyanobacterial strains used in this study were collected and isolated from tropical regions, but can produce feasible amount of phycobiliproteins. Pilot scale

cultivation using raceway ponds of Cyanobacterial strains such as Anabaena sp. and Arthospira platensis have yielded 14 to 23.5 and 0.82 to 1.32 m^{-2} day^{-1} respectively (Jiménez et al., 2003). At last from this current study, the Cyanobacterial strains including *Synechococcus elongatus*, Anabaena strains and *Chroococcus minor* were found to synthesize huge amount of phycocyanin pigments and can be an efficient alternate sources rather than the other sources employed for the commercial production of phycocyanin.

5.CONCLUSION

About eight Cyanobacterial strains were used in this study to quantitatively analyze the production of phycobiliproteins. The Cyanobacterial strains such as *Synechococcus elongatus, Anabaena* strains and *Chroococcus minor* found to acquire good growth curve and synthesized quantitatively high yield of phycobiliproteins when compared to other Cyanobacterial strains. The phycobiliproteins were extracted using solvent extraction methods, which can also reported to extract as protein extraction method using ammonium sulfate fractionation. About 90 % acetone extraction was also efficient in this study to extract the phycobiliproteins. The production and progression of wet and wet-dried biomasses also correlated with the quantitative production of phycobiliprotiens. The major conclusion of this study is that the marine Cyanobacterial forms including *Synechococcus elongatus* and *Chroococcus minor* has shown to produce quantitatively high amount of phycobiliproteins. In addition to this, some of the filamentous Cyanobacterial forms like *Anabaena* sp., *Anabaena* variabilis and *Anabaena spiroides* are also reported to acquire more amounts of phycobiliproteins.

6.ACKNOWLEDGEMENT

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