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Extraction and Native PAGE Separation of Phycobiliproteins from Some Cyanobacteria Collected from Their Natural Habitats

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ABSTRACT: Phycobiliproteins are a group of coloured proteins present in cyanobacteria and algae. They can be divided into three types based on their absorption spectra. These are phycocyanin, phycoerythrin and allophycocyanin. These pigment proteins are used as potential natural colorant in chewing gums, candies, soft drinks, dairy products and cosmetics like lipstick and eyeliners. They are also extensively commercialized for fluorescent applications in clinical immunological analysis. The phycobiliproteins from cyanobacteria have also been reported to have ant-cancerous, anti-inflammatory and antioxidant properties. In view of the increasing demand of these fluorescent pigments, it becomes important to find new species of cyanobacteria and exploit them for their phycobiliprotein content. In present work some commonly available cyanobacteria were collected from their natural habitats and analysed for their phycobiliprotein content. The extraction of phycobiliprotein was done in phosphate buffer and quantitative analysis of the pigment components was done. The study showed that all the cyanobacteria are the potential source of phycocyanin whereas phycoerythrin is significantly present in species of *Lyngbya* and *Oscillatoria*. The phycobiliprotein components were separated on Native PAGE which can be partially purified by electroelution. The percentage loss of phycobiliprotein content in stored cyanobacterial biomass for two months showed that phycoerythrin was more stable in *Lyngbya* and *Oscillatoria* as compared to the phycocyanin. The species of *Scytonema* showed good amount of phycocyanin content whereas allophycocyanin was significantly present and was stable in *Aulosira sp*.

KEYWORDS: Applications, Native PAGE, Cyanobacteria, extraction, fluorescent, phycobiliproteins.

INTRODUCTION

Phycobiliproteins are brilliantly coloured, highly fluorescent, water soluble accessory pigments found in red algae, cyanobacteria, cryptomonads and cyanelles. The phycobiliproteins are classified into three major groups depending on their absorption spectra and absorption maxima: allophycocyanins (APCs), phycocyanins (PCs) and phycoerythrins (PEs). These biliproteins are composed of two subunits (α and β), whereas phycoerythrin contain a third subunit (γ) as linker peptide. Some cyanobacteria have a fourth type of biliprotein in the place of phycoerythrins, the phycoerythrocyanin. [1,2] α and β in the form of a trimer $(\alpha\beta)_3$ constitute a building block for the individual PBPs, and are coupled to form hexameric disks $(\alpha\beta)_6$ as in PE (λmax 540-570 nm), whereas phycocyanin (λmax 610-620 nm) and allophycocyanin (λmax 650-655 nm) have the structure (αβ)3.[3] The hexameric disks are stabilized by linker polypeptides which also ensure the correct structural assembly of different phycobiliprotein within the rods.[4] The phycobiliproteins of both cyanobactertia and rhodophyceae form supramolecular extra-thylakoidal complexes called phycobilisomes (PBSs).

The phycobiliproteins have gained tremendous importance in recent years as natural colour and in food, chewing gums, dairy products, jellies, etc.[5,6,7]as a marker in gel electrophoresis and isoelectrofocusing.[8] and cosmetics such as lipstick and eyeliners in Japan, Thailand and China. [9, 10] Delange and Glazer (1989) [11] have suggested about application of phycobiliproteins in measurement of peroxy radical damage. Phycocyanin is used mainly as a colourant whereas phycoerythrin is used in fluorescent applications. [12] The recent studies on the bioactivity of phycobiliproteins have suggested their role as an anti-cancerous, anti-inflammatory antioxidative agent. [13] The study is mainly focused on the phycocyanin from cyanobacteria and is effective in different types of cancer cells like liver, leukemia, melanoma, breast etc. [14] The anti-inflammatory activity of phycobiliproteins is related mainly to the expression of enzymes inhibiting pro-inflammatory mediators. [15] The potential of phycocyanobilins as anti-diabetic agent has also been demonstrated in mice. [16]

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Many red algae and some cyanobacteria have been exploited for their phycobiliproteins content. Cyanobacteria are prokaryotes inhabiting all the possible natural habitats. Some cyanobacterial species are also inhabitants of extreme environments. [17] These can vary in their structure from single celled, coenobial to filamentous. All cyanobacteria contain phycobiliproteins as accessory pigments in addition to chlorophyll a. The phycobiliproteins in cyanobacteria constitute almost 50% of total protein. [18] The type and amount of phycobiliproteins vary amongst species depending upon the habitats and availability of different abiotic factors. Some cyanobacteria have been studied for their phycobiliproteins mainly phycocyanin but extraction and purification of phycobiliproteins have been reported in very few species. The most studied cyanobacteria is *Spirullina platensis* and many studied have been focussed on the phycocyanin (C-PC) content and its purification.[19,20] Other cyanobacteria studied for their phycocyanin content are *Nostoc*, *Anabaena* and *Synechococcus*. The phycoerythrins have been extracted and purified mainly from the members of rhodophyceae. R-PE has been purified from the species of *Polysiphonia urecolata*. [21] and B-PE from the *Porphyridium cruentum*. [22] The extraction and purification of phycoerythrin has been reported in *Lyngbya arboricola* inhabiting Mango tree bark. [23] Looking into the importance and increasing demand of phycobiliproteins that may be because of its spectral characteristics or bioactivity, it becomes important to find new cyanobacterial strains which could be a potential source of these phycobiliproteins. The present study has been carried out to explore new species of cyanobacteria having significant amount of phycobiliproteins which can further be purified and commercialized. Study is also taken up to see the stability of these phycobiliproteins in desiccated cyanobacterial biomass. The separation of phycobiliprotein components on Native PAGE is seen which will facilitate further purification of these pigments to be used in future and its commercialization.

MATERIALS AND METHODS

Some cyanobacteria were collected from their natural habitats from different places in Sasaram. All collected cyanobacteria were identified to generic level with the help of microscope using the conventional method. [24] The species of *Oscillatoria*, *Lyngbya* and *Microcystis* were collected from the stagnant and polluted water body. The *Scytonema sp.* was collected from the roof top of Sri Shankar College, Sasaram. The species of *Nostoc*, *Aulosira* and *Gloeotrichia* were collected from the rice fields of Sasaram.

Dry weight: The algal samples which were collected in the form of mats and flakes such as *Lyngbya*, *Oscillatoria*, *Scytonema* and *Aulosira* were washed thoroughly with distilled water and other algal forms like *Nostoc*, *Anabaena*, *Gloeotrichia* and *Microcystis* were as such kept in a desiccator over silica gel in dark at 25ºC±1ºC for 12h. The dry weight was taken separately.

Preparation of cell free extract and extraction of phycobiliproteins: The preparation of cell free extract was not the same for all the genus collected. The mucilaginous forms like *Nostoc* and *Gloeotrichia* were first crushed by adding washed and dried sand. The cells of *Microcystis sp*. were disrupted by repeated freeze-thaw method. All the cyanobacterial samples were then homogenized separately in extraction buffer (10% w/v) containing 0.05M K-phosphate buffer (pH 7.5), 10 mM $MgCl_2$, 1 mM Na-EDTA, 1mM β-mercaptoethanol, 0.3M sucrose and 1 mM NaN_3 at 4ºC and then sonicated (130 W, 20 kHz, Sonic & Materials USA) for 2 min at 40% amplitudes.The homogenate was centrifuged at 20,000 × g for 30 min at 4ºC. The supernatants were collected. The pellets were re-extracted by repeating this procedure. The supernatants were pooled and treated as crude extract of phycobiliproteins.

Spectroscopic analysis: The absorbance was recorded at 565, 620 and 650nm on a Varian (Cary100-Bio, USA) UV-Visible spectrophotometer with a 1 cm light path and the different phycobiliprotein content i.e. phycoerythrin, phycocyanin and allophycocyanin was calculated using the formula of Tandeu de Marsec. [25]

Native PAGE: Native polyacrylamide gel electrophoreses (PAGE) was carried on 8% resolving gel with 4% stacking gel at 4ºC. Native PAGE was performed by adopting the methods described by Sambrook et al. (1989) [26] by using the chemical composition mentioned by them. There was not any application of a denaturant like SDS (sodium dodecyl sulphate) in the gel. The electrophoresis was carried out till the separation of phycobiliproteins (~5min) at the rate of 1-2 mA per well constant current.

RESULTS

In the present study different genus of cyanobacteria were collected from their natural habitats and cell free extracts were analysed for their phycobiliprotein content (% dry wt.). All the three major components of phycobiliproteins (PE, PC and APC) were present in the studied cyanobacteria in more or less quantity. Among these, the species of *Lyngbya* and *Oscillatoria* shows a good amount

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of both phycoerythrin and phycocyanin. The species of *Scytonema*, *Nostoc* and *Microcystis* showed high content of PC (>3% dry weight) whereas APC is the predominant pigment in the species of *Aulosira* (Fig. 1).

The extract of phycobiliprotein when subjected to gel electrophoresis showed the separation of phycoerythrin, phycocyanin and allophycocyanin components of phycobiliprotein on Native PAGE. The phycobiliproteins are light and heat sensitive pigments so gel electrophoresis has to be stopped just after the separation of the pigments otherwise the phycobilin part of the pigment protein will disappear. The separation of PE, PC and APC showed that these pigments can be partially purified when required in low concentration through the process of electroelution at 4ºC (Fig. 2).

The phycobiliprotein content of desiccated cyanobacterial biomass kept in dark for two months shows different patterns for different phycobiliproteins and it also varies with the species of cyanobacteria. The phycoerythrin of *Lyngbya* and *Oscillatoria* are found to be more stable than phycocyanin. However the phycocyanin was present in considerable amount in *Oscillatoria* and *Scytonema*. The allophycocyanin was found to be stable in *Aulosira* only (Fig.3). The percentage loss of phycoerythrin and phycocyanin was compared between species of *Lyngbya* and *Oscillatoria* in mats stored for two months in dark (Fig.4). The loss percentage was more in case of phycocyanin in both species whereas it was less for phycoerythrin (<6% dry weight).

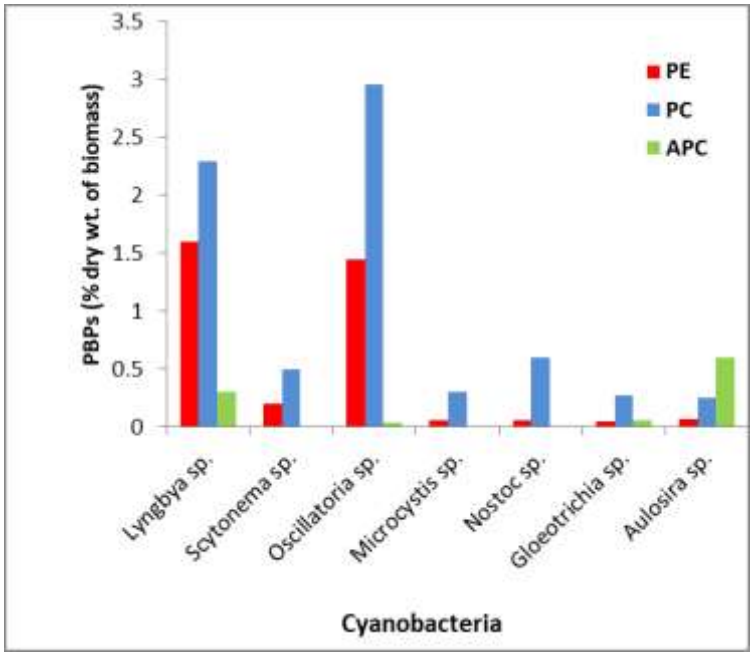

Fig.1. Phycobiliproteins in freshly collected cyanobacteria

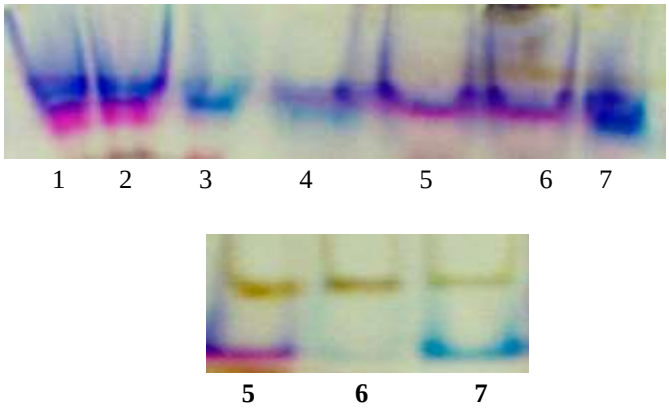

Fig. 2. Native PAGE separation of phycobiliproteins from cyanobacteria

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Lyngbya sp. (1 & 2), *Scytonema sp*. (3 & 4). *Oscillatoria sp*. (5 & 6), Microcystis sp. (7), *Nostoc sp*. (8), *Gloeotrichia sp*. (9), *Aulosira sp.* (10)

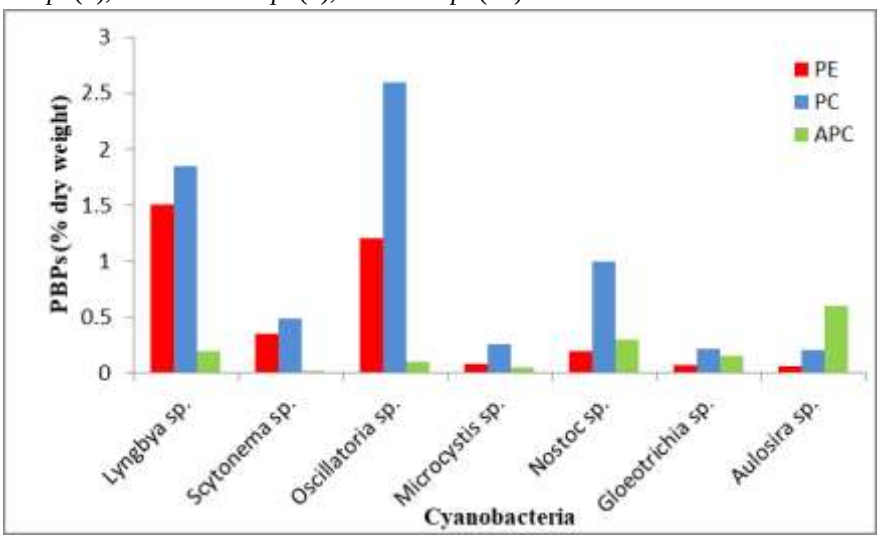

Fig.3. Phycobiliproteins in two months stored desiccated cyanobacteria

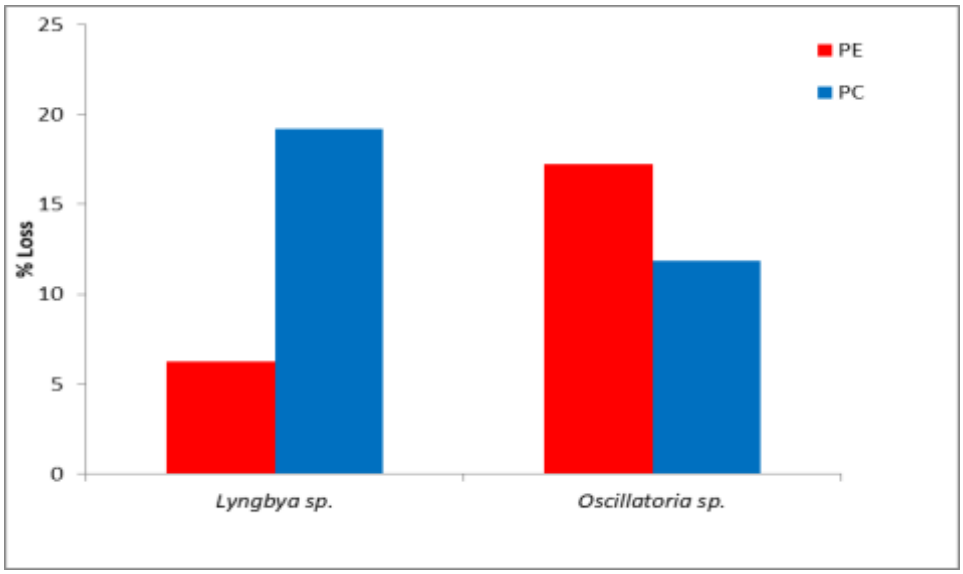

Fig.4. Loss in phycoerythrin (PE) and phycocyanin (PC) in desiccated cyanobacteria after two months

DISCUSSION

Cyanobacteria are the potential source of different phycobiliproteins. They are known to contain C-PE, C-PC and APC. Cyanobacteria are the rich source of phycocyanin followed by phycoerythrin and allophycocyanin. [27] But till date only few of them have been exploited for the purification of these pigments. Under this category a single genus *Spirulina* has been exploited commercially mainly for the production of purified C-PC. Different methodologies have been reported for the efficient extraction of PC from this alga. [28] But the extraction and purification of phycoerythrin are mainly confined to red algae. However, Rodriguez et al., (1989) [29] have shown exceptionally very high amount of C-PE (8.3% of dry weight) in *Anabaena sp*.

India being a tropical country is very rich in cyanobacterial diversity so more species of cyanobacteria need to be exploited for their phycobiliprotein content. The study here reflected that all the cyanobacteria possess two of these pigments (PC and APC) while the species of non heterocystous cyanobacteria *Lyngbya* and *Oscillatoria* contain a significant amount of PE in addition to PC and APC.

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The species of *Scytonema*, *Nostoc*, *Aulosira* can be a good source of either PC or APC or both. The yield of phycobiliproteins (as % dry weight) obtained in present study can be increased by adopting different extraction methodologies like using liquid nitrogen. The Native PAGE was performed after extraction of these pigments. The native gel shows a clear separation of these pigments which indicates that these can be separated on the basis of their molecular weight on a gel filtration chromatography and can be purified further. Though some initial purification steps will be required like $(NH_4)_2SO_4$ precipitation or acetone precipitation to remove some UV absorbing pigments like MAA (Mycosporin like amino acid) and Scytonemin found naturally in cyanobacteria. The phycobiliprotein content in desiccated cyanobacterial mats stored for two months in dark at 25ºC ±1ºC shows the stability of phycoerythrin in *Lyngbya* and *Oscillatoria* which suggests that these pigments may have antioxidative properties owing to which they survive in desiccated states. These prokaryotes are well known to survive desiccation for many years. [30] The phycocyanin content was much lowered in case of *Nostoc* and *Microcystis* (<0.1% dry weight) whereas it shows some stability in *Oscillatoria* and *Scytonema*. The allophycocyanin was almost lost in all the cyanobacteria except *Aulosira*. This suggests that allophycocyanin could be extracted efficiently and purified from the species of *Aulosira*. The phycobiliproteins when purified above the purity index >3 becomes very sensitive to light and temperature. Many chemicals are required to store the purified pigments and hence it becomes expensive to be used in biomedical or other research work. The study here shows that the cyanobacterial biomass can be stored as a source of phycobiliproteins and pigments can be purified when required for diagnostic or research purposes. The loss percentage of phycoerythrin in *Lyngbya sp*. was less than 6% which shows that it is very stable in dried mats which indicates its role in desiccation tolerance. To use these phycobiliproteins from cyanobacteria some easy and efficient purification procedure are required so that these pigments can be purified when required.

CONCLUSION

Cyanobacteria are the potential source of phycobiliproteins but very few species of cyanobacteria have been exploited for their fluorescent pigment. The study taken up here shows that many species of cyanobacteria can also be a good source of phycoerythrin and allophycocyanin in addition to the phycocyanin which is the main pigment studied and purified from these prokaryotes so far.

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