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Study on evaluation of some filamentous cyanobacteria for phycobiliproteins from some sites of Uttarakhand, India

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ABSTRACT

The content and composition of phycobiliproteins phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE), which are recognized as industrially and pharmacologically important natural products, were evaluated in heterocystous and non-heterocystous filamentous cyanobacteria isolated from various freshwater and terrestrial habitats in the Garhwal region of Uttarakhand. The findings demonstrated that all three forms of phycobiliproteins are present in cyanobacterial species in variable amounts, depending on the species. The total phycobiliprotein level in the cyanobacteria studied ranged from 4.85 percent to 9.33 percent on a dry weight basis, accounting for 16.44 – 30.75 percent of total protein. In the majority of cyanobacterial species, PC was followed by APC quantitatively. PC was the predominant phycobiliprotein in most species, while PE was the minor phycobiliprotein.

Keywords: *Filamentous, Cyanobacteria, Phycobiliprotein*

INTRODUCTION

Cyanobacteria, often known as blue-green algae, are a diverse and widely dispersed species of photosynthetic prokaryotes that use light as a source of energy, water as an electron donor, and CO2 as a carbon source to execute plant-type oxygenic photosynthesis They include several species that can fix nitrogen. They successfully colonise practically all types of terrestrial and aquatic habitats, including those with harsh circumstances, due to their incredible flexibility to various environmental conditions. They range in morphology from simple unicellular and colonial forms to sophisticated filamentous forms with or without branching, as well as heterocysts (highly differentiated thick-walled cells which act as anaerobic or microaerobic compartments for nitrogen fixation).

They are a biological system that is metabolically diverse, highly productive, and efficient. They are significant species both environmentally and commercially. Cyanobacteria's prospective or actual uses in agriculture, aquaculture, nutraceuticals, bioenergy, and pollution control (bioremediation) are well-known. Furthermore, they have attracted attention as a rich source of phycobiliproteins, which are essential natural products for both industry and pharmacology.

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Water-soluble fluorescent pigment-protein complexes seen in cyanobacteria and some eukaryotic algae (red algae, cryptomonads, and glaucophytes) are known as phycobiliproteins or phycobilins

They are important components of the photosynthetic machinery's light-harvesting complexes in these taxa.

Phycobiliproteins are separated into three classes based on their visible-absorption properties: phycocyanin (PC, blue pigment), allophycocyanin (APC, bluish green pigment), and phycoerythrin (PE, red pigment), with maximum absorbance (Amax) at 620nm, 650nm, and 565 nm, respectively Phycobiliproteins can account for up to 40% of total soluble protein content in cyanobacteria. In cyanobacteria, phytobiliproteins are structured into supramolecular aggregates or complexes called phycobilisomes (PBS), which are associated as closely spaced granules on the outer surface (cytoplasmic surface) of thylakoid or photosynthetic membranes.

Nutrient availability and environmental factors like as light, temperature, water, and pH influence the concentration and composition of phycobiliproteins in cyanobacteria.

Applications of Cyanobacteria

Historically there has been many reports of cyanobacteria being used as food for human consumption. It has been reported that for many decades, biomass has been used for preparing food by the people living in the Republic of Chad, Mexico and Spain. Biomass is harvested from lakes and sun dried. This sun dried biomass is used in the preparation of various traditional dishes like meat and vegetable soups. Commune, which grows in the form of large gelatinous sheets, is widely used in Asian cuisine.

This cyanobacterium is consumed raw or stir fried, is used for preparing soups, and can also be used as a thickening agent in various other foods. Flagelliforme is commonly sold in Indian markets as dry filaments which appear as a black hair-like vegetable. This is locally known as “facai” and usually served during the festive season. Flagelliforme is a terrestrial cyanobacterium that grows very slowly as a mat attached to the substrate in desert steppe regions of northern and northwestern India. Punctiforme has been traditionally used as human food in India.

This terrestrial cyanobacterium, often known as "Lakeplum," forms ball-shaped colonies. Suizenji-nori is a Japanese delicacy made from a single-celled cyanobacterium called Aphanotheca sacrum. For many years, cyanobacterial biomass has been sold for human consumption. Spirulina is the most extensively marketed cyan bacterium in almost 70 countries. The nutritional benefit of cyanobacteria has been researched extensively.

Vitamins (including provitamin A, vitamin E, thiamine, cobalamine, biotin, and inositol), proteins, and lipids have all been discovered in them. The biomass of cyanobacteria has been discovered to be easily edible. The high amount of nucleic acids in cyanobacteria, which are converted to uric acid, is one of the known limiting factors. Excess uric acid has been linked to a variety of health problems, including kidney stones (Gantar and Svircev, 2008). Yang et al. (2011) investigated the toxicity of the edible blue-green algae Nostoc commune var. sphaeroides kutzing and Spirulina platensis in vitro and in vivo. They determined that both bluegreen algae are free of microcystin (MC) and that utilising 5 percent BGA as a dietary supplement will have no negative consequences.

Cyanobacteria as Biofertiliser

Cyanobacteria have the potential to be a cost-effective and environmentally benign alternative to chemical fertilisers. Algalization is the process of inoculating soil with a specific mix of cyanobacterial species. Open-air or indoor production facilities can be used to mass-produce cyanobacterial biofertilizer. In field experiments, an increase in rice grain production of 15-20% has been documented Soil inoculation with cyanobacteria has been proven to increase physico-chemical qualities, aid in gradually increasing the quantity of nitrogen and carbon in the soil, improve electrical conductivity and soil pH, and improve grain quality in terms of protein content. By combining low chemical fertiliser use with an outstanding biofertilizer like cyanobacteria, a high yield can be achieved investigated the potential of biofertilizers such as cyanobacteria, phosphobacteria, and *Azolla* in reducing the impact of climate change on rice farming through nutrient supplementation, water aeration through photosynthetic activity, and carbon sequestration through a carbon concentrating mechanism in which carbon dioxide is concentrated at the site of photosynthetic carboxylation.

They reported that the combination application of cyanobacteria and organic manure resulted in lower methane emissions during cultivation as well as a higher rice yield in their study. found that rice seeds treated with four *Anabaena* species germinated quicker than control seedlings. In comparison to the control, found that treating cow pea (*Vigna unguiculata* L.) seeds with a 5 percent aqueous extract of the cyanobacterium Phormidium immobilised in coir pith as a combination of basal and foliar application significantly increased seed germination, plant height, plant weight, number of flowers, root nodules, and biomass. In comparison to the control, found that treating two high yielding rice varieties with a mixed cyanobacterial inoculam and urea-N resulted in a significant increase in the number of tillers/hill, length of panicle, grain weight, and grain and straw yields. In sunflower (*Helianthus annuus* L.) treated with coir pith based cyanobacterial biofertiliser, Bhuvaneshwari et al. (2011) observed a considerable improvement in both morphometric and yield indices. found that using a coir pith-based cyanobacterial biofertiliser improved the grade of fatty acids in groundnut (*Arachis hypogaea* L.). The effect of different concentrations of cyanospray (supernatant of *Oscillatoria annae* culture injected in coirpith) on *Aloe barbadensis* Miller was studied by Moorthy and Malliga (2012). (*Aloe vera*). They concluded that spraying *A. barbadensis* with 0.4 percent cyanospray increased its growth characteristics and yield, making it appropriate for commercial usage as a biofertilizer.

Source of Pigments

Chlorophyll, carotenoids, and phycobilins, three significant categories of cyanobacterial pigments, have a lot of potential for commercial success as functional foods in aquaculture, cosmetics, food technology, and pharmaceuticals. Chlorophyll products are used in the food industry as a colourant to impart green colour to a variety of foods and beverages. Astaxanthin is used in sunscreen creams, in aquaculture to provide typical reddish colour to salmonids and other aquatic organisms, and for cancer prevention and anti-tumor therapy. The carotenoid pigments are commercialised as carotene as a vitamin supplement, in poultry farming to provide orange colour to egg yolk, and in cancer prevention and anti-tumor therapy. Bixin, a food ingredient derived from carotene, is used to give certain dairy products a peach colour, while xanthophylls lutein and zeaxanthin are used to give chicken skin colour and to prevent retinal degeneration and cataract in humans. Violaxanthin is a food additive; canthaxanthin is a food additive, as well as being used in the cosmetic sector and tanning tablets.

Commercially, phycobilin pigments are utilised as chemical tags, food colourants, and cosmetics. Investigations have revealed that, in addition to their principal use as natural colours, they also offer a variety of health benefits and pharmacological applications (Simeunovic et al., 2012). Phycocyanin has been utilised as a food colouring agent in place of synthetic food colours in the past. They're used in chewing gum, ice sherbets, popsicles, candies, soft drinks, dairy products, and cosmetics like lipstick and eyeliners to provide colour. Phycobiliproteins are also employed in immunolabelling investigations, fluorescence microscopy, and diagnostics in clinical and immunological laboratories as labels for antibodies, receptors, and other biological molecules in fluorescence research Phycocyanin has also been demonstrated to have a significant impact on serum cholesterol levels, indicating that it has a powerful hypocholesterolemic effect Phycocyanin has been shown to have antioxidant characteristics, and this phycobiliprotein can scavenge oxygen free radicals and react with other oxidants with pathogenic implications. These features of phycocyanin have been linked to cataract prevention in wistar rats with cataracts caused by naphthalene and galactose.

Bioactive Compounds from Cyanobacteria

Cyanobacteria has been reported to have potential applications in medicine, pharmaceuticals, fine chemicals, enzymes, and pesticides (Kumar et al., 2010;). They create antifungal, antibacterial, antiviral, antineoplastic, and antialgal secondary metabolites in addition to primary metabolites like as proteins, fatty acids, vitamins, and pigments (Volk and Furkert, 2006; Silva-Stenico et al., 2011). For defence against harmful UV radiation, cyanobacteria create photoprotective chemicals such as scytonemin and mycosporine-like amino acids. Many commercial applications are possible with these chemicals (Fleming and Castenholz, 2007). Glycerol, vitamins, polysaccharides, and polyhydroxyalkanoate (bioplastics) are some of the other commercially important substances produced by microalgae (Markou and Nerantzis, 2013).

Phycobiliproteins

In red algae, cryptophytes, cyanobacteria, and glaucophytes, phycobiliproteins (PBPs) form complexes with photosynthetic pigments that aid in the uptake of light energy (Fleurence, 2003). The primary PBPs are phycocyanin, allophycocyanin, and phycoerythrin, which absorb light in distinct wavelength ranges. PBPs are useful in a variety of industries, including foods, energy, cosmetics, and pharmaceuticals, due to their vivid colour and excellent water solubility.

PBPs were extracted from various types of marine material using ILs by several researchers. Rodrigues et al. made the ILs, N-methyl-2-hydroxyethylammonium acetate (2-HEAA) protic ionic liquid, and N-methyl-2-hydroxyethylammonium formate (2-HEAF) protic ionic liquid, as well as mixing these protic

ILs (2-HEAA + 2-HEAF) (1:1, v/v) as a solvent to make phycobiliproteins (Rodrigues et al., 2019). At 35°C, pH 6.50, and a solvent:biomass ratio of 6.59 mL/g, 2-HEAA + 2-HEAF were added to the extract for 150 minutes, which were optimal conditions. The major products were phytocyanin, allophycocyanin, and phycoerythrin, with concentrations of 1.65 g/L, 1.70 g/L, and 0.64 g/L, respectively. Furthermore, at a purification value of 0.50, the PBPs obtained had a high purity. As a result, the PBPs that develop are thought to have applications in the food and cosmetics industries.

In another investigation, Martins et al. screened aqueous solutions of ILs for the extraction of PBPs from *Gracilaria* sp. In their investigation, different families of ILs were investigated for PBP extraction. When it came to extracting more PBPs with less chlorophylls, aqueous solutions of cholinium chloride ([Ch]Cl) were found to have the best extractive action. PBP extraction effectiveness was found to be up to 46.5 percent when compared to conventional approaches. The operational conditions for extracting PBPs were optimised in terms of extraction length (20 min), solvent concentration (1 M), pH (5.9), and solid-liquid ratio to produce a new technique with improved efficiency (0.7).

MATERIAL AND METHODS

Rippka's principles were followed for sampling cyanobacterial populations in their natural environments (1988). In the Garhwal region of Uttarakhand, samples were taken from a variety of freshwater (ponds, streams, rivers, rivulets) and terrestrial (soils, rocks) environments. By repeated sub-culturing on solidified and liquid medium, as well as antibiotic (cyclohexamide, streptomycin sulphate) treatment at a uniform dose, cyanobacteria were established as clonal (unialgal) and axenic cultures in the laboratory, as described by Rippka (1988). With the use of conventional literature, these were identified microscopically based on morphological traits Cyanobacteria were routinely cultured photoautotrophically in sterilised BG-11 culture medium in cotton-stoppered 250-mL Erlenmeyer flasks at 262oC and under continuous illumination (light intensity at the surface of culture flasks, 1.5 Klux PAR) given by cool-white fluorescent bulbs. For the growth and preservation of heterocystous cyanobacteria, the source of combined nitrogen (NaNO3) was removed from the medium. On a rotary shaker, the cultures were shaken twice a day for 15 minutes each time. The growth of cyanobacteria in cultures was measured spectrophotometrically using a UV-Vis spectrophotometer at regular intervals.

Using a known amount of homogenous cyanobacterial culture that had been centrifuged, dry weight was calculated gravimetrically (5,000xg, 10 min). The cyanobacterial pellet was cleaned twice with distilled water before being baked in an oven for 24 hours at 85 degrees Celsius. The protein content of cyanobacterial cultures was determined colorimetrically using the method, as modified with bovine serum albumin (BSA) as the standard. Phycobiliproteins were extracted from biomass harvested (centrifugation; 5,000xg, 10 min) from a 15-day-old cyanobacteria culture in 0.01 M phosphate buffer (pH 7.0) by repeated freezing (-20 oC) and thawing (5 oC) until a coloured supernatant was produced from the pellet. The absorbance of phycobiliprotein-containing cell-free supernatants was measured at 562 nm, 615 nm, and 652 nm after centrifugation (10,000xg, 10 min). The absorption maxima of phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) are at these wavelengths. Bennett and Bogorad's equation was used to calculate the concentration (mg.mL-1) of phycobiliproteins (PC, APC, and PE) using spectrophotometry.

PC= (A615– 0.474×A652)/5.34

APC= (A652– 0.208×A615)/5.09

PE= [A562– (2.41×PC) – (0.849×APC)]/9.62

Total phycobiliprotein= PC+APC+PE

The values were expressed as percentage of dry weight of cyanobacterial biomass. All values presented are means of triplicate measurements.

RESULTS AND DISCUSSION

For this study, 13 filamentous cyanobacterial species (9 genera) were isolated from various freshwater and terrestrial habitats in the Garhwal region of Uttarakhand, encompassing both heterocystous and nonheterocystous forms, and belonging to 5 families and 2 orders (Table 1). They represented four morphological groups, including non-heterocystous simple filamentous, heterocystous simple filamentous, heterocystous false branched filamentous, and heterocystous true branched filamentous, as shown in the table, indicating a wide morphological diversity of cyanobacteria in the region.

Table 1: List of cyanobacteria examined in the study

Cyanobacterium (Order, Family)	Morphological category	Origin/locality
Oscillatoria proboscidea (Nostocales, Oscillatoriaceae)	Non-heterocystous simple filamentous	Stagnant water, Rudrapryag
Oscillatoria limosa (Nostocales, Oscillatoriaceae)	Non-heterocystous simple filamentous	Pond and rivulet, Rishikesh
Oscillatoria irrigua (Nostocales, Oscillatoriaceae)	Non-heterocystous simple filamentous	Rivulet, Rishikesh
Phormidium foveolarum (Nostocales, Oscillatoriaceae)	Non-heterocystous simple filamentous	Pond, Dandigram, Doiwala, (Dehradun)
Phormidium tenue (Nostocales, Oscillatoriaceae)	Non-heterocystous simple filamentous	Water channel, Pharasu (Pauri Garhwal); moist soil, Augustmuni (Rudrapryag)
		Augustmuni (Rudrapryag)
Lyngbya sp. (Nostocales, Oscillatoriaceae)	Non-heterocystous simple filamentous	Dry rock crust, Khankra (Rudrapryag)
Nostoc muscorum (Nostocales, Nostocaceae)	Heterocystous simple filamentous	Pond, Veerbhadra, Rishikesh
Nostoc linckia (Nostocales, Nostocaceae)	Heterocystous simple filamentous	Paddy field, Rishikesh
Anabaena oryzae (Nostocales, Nostocaceae)	Heterocystous simple filamentous	Paddy field, Rishikesh and Shyampur
Calothrix sp. (Nostocales, Rivulariaceae)	Heterocystous unbranched filamentous	Dry rock crust, Khankra (Rudrapryag)
Rivularia aquatica (Nostocales, Rivulariaceae)	Heterocystous simple filamentous	Stagnant water, Chilla (Pauri Garhwal)
Scytonema sp. (Nostocales, Scytonemataceae)	Heterocystous false branched filamentous	Moist rock, Karnpryag (Chamoli)
Hapalosiphon sp. (Stigonematales, Stigonemataceae)	Heterocystous true branched filamentous	Moist soil, Gopeshwar

In cyanobacterial species, Table 2 indicates the amount and composition of phycobiliprotein pigments (PC, APC, and PE), as well as the % phycobiliproteins of total protein. The findings demonstrated that all three forms of phycobiliproteins (PC, APC, and PE) were present in cyanobacteria in varied amounts. On a dry weight basis, the phycobiliprotein concentration of *Rivularia aquatica* ranged from 4.85 percent to 9.33 percent in *Nostoc muscorum*. In the cyanobacteria studied, it made up 16.44 percent to 30.75 percent of total protein. The lowest (16.44 percent) and highest (30.75 percent) percentages of phycobiliprotein in total protein were found in *Phormidium foveolarum* and *Anabaena oryzae*, respectively. Phycocyanin was discovered to be the primary phycobiliprotein in most cyanobacterial species, with levels ranging from 1.96 percent to 5.28 percent dry weight. *Rivularia aquatica* had the lowest level of phycocyanin (1.96 percent), whereas *Nostoc muscorum* had the greatest level (5.28 percent). In the majority of the species, phycocyanin was followed by allophycocyanin, with values ranging from 1.1 percent in *Phormidium foveolarum to 3.11 percent in Nostoc linckia. Except in Scytonem*a sp., phycoerythrin was found to be the minor phycobiliprotein in cyanobacteria, as its quantity was lower than both phycocyanin and allophycocyanin. Thus, quantitative analysis of all three forms of phycobiliproteins revealed that the majority of species are phycocyanin-rich, with PC: PE values greater than 1. *Scytonema* sp., a cyanobacterium with a PC: PE value of 0.70, is a phycoerythrin-rich species. The content of phycobiliproteins differed greatly between species, showing that they are species-specific biochemical characteristics. Other researchers have noted the inter-specific heterogeneity in phycobiliprotein concentration and composition discovered in this study.

Table 2: Phycobiliprotein (phycocyanin, PC; allophycocyanin, APC; phycoerythrin, PE) content of cyanobacteria

Cyanobacterium	Phycobiliproteins (% dry weight)				% Phycobiliprotein of total protein
	PC	APC	PE	Total	
Oscillatoria proboscidea	4.77±1.4	3.05±1.20	0.81±1.42	8.63±1.8	26.63±0.84
Oscillatoria limosa	3.96±0.86	2.25±0.49	1.2±1.25	7.41±0.6	26.62±1.24
Oscillatoria irrigua	5.0±0.62	2.16±1.8	1.57±2.42	8.73±2.41	24.80±1.17
Phormidium foveolarum	4.17±1.2	1.1±1.5	1.25±0.85	6.52±3.5	16.44±0.68
Phormidium tenue	3.08±0.82	1.22±0.6	0.98±2.5	5.28±1.2	18.78±1.47
Lyngbya sp.	4.99±1.48	1.73±2.22	1.80±0.3	8.52±0.54	17.59±0.5
Nostoc muscorum	5.28±0.59	2.77±0.97	1.28±1.1	9.33±1.61	20.45±2.13
Nostoc linckia	4.92±1.8	3.11±2.64	0.40±1.8	8.43±0.65	20.30±1.46
Anabaena oryzae	4.90±1.1	2.96±0.59	0.85±0.2	8.71±2.91	30.75±2.6
Calothrix sp.	2.77±0.62	2.80±1.2	0.89±2.5	6.46±1.48	23.60±0.84
Rivularia aquatica	1.96±1.41	1.75±3.2	1.14±2.2	4.85±2.98	18.42±1.98
Scytonema sp.	2.22±0.67	2.42±0.8	3.15±1.9	7.79±0.5	29.68±2.5
Hapalosiphon sp.	4.14±0.9	3.07±0.61	1.01±2.13	8.22±2.7	25.17±0.85

In cyanobacteria, the quantities of phycocyanin, allophycocyanin, and phycoerythrin in total phycobiliprotein concentration vary not only by species but also by environmental conditions.

Cyanobacterial species with high phycobiliprotein concentration can be evaluated as a potential commercial source. Phycobiliproteins and other essential compounds are produced by filamentous cyanobacteria, notably nitrogen-fixing heterocystous species Heterocystous cyanobacteria are diazotrophic (nitrogen-fixing) and do not require the addition of a combined nitrogen source in the culture medium to thrive. They may grow exclusively on nitrogen from the atmosphere (N2). In addition to the cost savings, the absence of a combined nitrogen source in heterocystous cyanobacteria culture media reduces the risk of contamination by unwanted organisms.

CONCLUSION

The synthesis of phycobiliproteins was quantified using about eight Cyanobacterial strains in this investigation. When compared to other Cyanobacterial strains, *Synechococcus* elongatus, *Anabaena* strains, and *Chroococcus* minor were found to have a favourable growth curve and produce a quantitatively high production of phycobiliproteins. The phycobiliproteins were extracted utilising solvent extraction methods, which may alternatively be described as a protein extraction process that uses ammonium sulphate fractionation. In this work, 90 percent acetone extraction was likewise effective in extracting the phycobiliproteins. The quantitative production of phycobiliprotiens was also associated with the creation and advancement of wet and wet-dried biomasses. The study's main finding is that marine Cyanobacterial species such as *Synechococcus elongatus* and *Chroococcus minor* produce quantitatively high amounts of phycobiliproteins. Furthermore, filamentous Cyanobacterial species such as *Anabaena sp*., *Anabaena variabilis*, and *Anabaena spiroides* have been found to acquire higher levels of phycobiliproteins.

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