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Qualification and quantification of cyanobacterial biochromes from the Gulf of Mannar in Tamil Nadu, India

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Abstract

Marine cyanobacteria are a class of extinct photosynthetic bacteria that go back 3.5 million years. The most precious marine microorganisms are produced for various pigment extraction. Their production of beneficial secondary metabolites is abundant. Pigments are bright substances that are used to give other materials color. True pigments make up the majority of these coloring agents, which are also referred to as biological pigments or biochromes. These biological pigments are typically applied as a mixture of solid and liquid, as they are insoluble in water. The pigments are produced by living things, and particular light absorption methods give them their color. Natural selection has refined their metabolites over millions of years to have effects on a range of biological targets. The food, feed, cosmetic, pharmaceutical, nutraceutical, and aquaculture sectors all make extensive use of marine pigments. The objective of this study was to evaluate the many marine cyanobacterial species *Microcystis sp*., *Lyngbya limnetica, Oscillatoria roai, Oscillatoria acuminata,* and *Oscillatoria princes,* which have garnered some interest. These species were chosen for this examination of their biochromes, such as chlorophyll pigments, phycocyanin, beta-carotene, and phycoerythrin. Preliminary research had been done on all biochromes estimations, including those for beta-carotene, carotenoid content, phycocyanin, phycoerythrin, and chlorophyll pigments. At 5.9%, *Oscillatoria princes* had the greatest quantities of chlorophyll-a. The phycocyanin contents of *Oscillatoria acuminata* and *Oscillatoria princes* are higher, 0.78% and 0.85%, respectively. The amounts of carotenoid were 1.7% and 1.8% for *Microcystis sp*. and *Lyngbya limnetica*, respectively. *Microcystis sp*. exhibited up to 1.5% beta-carotene levels.

Keywords

biochrome; cyanobacteria; marine source; microorganisms; photosynthetic pigments

Introduction

In the past several decades, photosynthetic microorganisms such as microalgae and cyanobacteria have attracted attention due to their ability to fix carbon dioxide (CO_2) owing to their photosynthetic activity and their high adaptability. Microalgae and cyanobacteria exhibit different shapes and sizes, from one to several hundred micrometers (1, 2). Photosynthesis is the source of energy for cyanobacteria, sometimes referred to as Cyanophyta, a class of microorganisms. Blue-colored microorganisms are referred to as

"cyanobacteria". Because cyanobacteria are prokaryotic plants that produce oxygen gas as a byproduct of photosynthesis, it is believed that they are responsible for converting Earth's early reducing atmosphere to an oxidizing one. One unusual phytonutrient, phycocyanin, is a blue protein pigment in naturally occurring cyanobacteria. The production of white blood cells is controlled by phytocyanin (3). The majority of microalgae and cyanobacteria are photoautotrophic and thus consume CO_2 by using energy from light. Some of them are able to grow in the absence of light, using organic carbon (4). In addition, some microalgae and cyanobacteria are able to grow using both autotrophic and heterotrophic metabolisms, and they are hence called mixotrophic microorganisms (5). In this case, there is a synergistic effect between photosynthesis and aerobic respiration from both metabolisms, with the O_2 and CO_2 produced in one process being used in the other, thereby leading to better growth rates and enhancement of biomass productivity (6). Cyanobacteria, red algae, and cryptophytes include phycobiliproteins, which function as biochromes that absorb light during photosynthetic processes. Thus, the primary component accountable for the antioxidant action is phycobiliprotein.

The chromophores of phycobiliproteins are called phycobilins, which are open-chain tetrapyrroles made from protoheme (7). The initial studies on the anti-inflammatory and antioxidant properties of phycocyanin were released by Romay et al. (8). An increase in phycocyanin concentration was linked to an increase in antioxidant activity in different fractions, according to Pinero et al. (9). More than 100 animal studies have demonstrated that beta-carotene and vitamin A can stop the formation of various tumors and cancers. Several human epidemiological investigations have found a protective effect of high vitamin A intake against cancer (10). Shekelle et al. (11) found that preformed Vitamin A from animal sources was not linked to a lower risk of cancer, but -carotene was associated with decreased cancer incidence.

Malignant tumors in hamsters' mouths that received a beta-carotene solution either completely vanished or reduced in size (12). Twenty hamsters were administered a beta-carotene extract; none of them developed mouth cancer, although they were predisposed to it. Tissue samples included an immune-stimulating agent believed to have killed cancer cells before they could spread (12). About fifteen research between 1975 and 1986 found a correlation between a lower incidence of lung cancer with beta-carotene and vitamin A. One study found that as serum beta-carotene levels decreased, the risk of lung cancer increased (13). Nine research between 1974 and 1986 found a correlation between reduced risk of digestive tract cancer (oral, stomach, colon, and gastrointestinal) and beta-carotene and vitamin A. A connection between lower breast and cervical cancer was found in two studies, including female participants. Daily doses of vitamin E, selenium, and beta-carotene were reported to reduce cancer mortality incidence by 13% in a five-year study with 29,000 participants completed in 1993 in China (14). Carotenoids are important for human health because of their provitamin action, anti-cancer activity, immune system stimulation, and other biological functions. The human body cannot create carotenoids on its own; instead, it must consume them.

Nevertheless, the carotenoid content of many plants is very low, and the chemical synthesis of several carotenoid types can be difficult. Thanks to advances in the understanding of the carotenoid biosynthesis pathway and the cloning of genes encoding similar enzymes from microbes and higher plants, the production of carotenoid compounds can now be controlled by genetic engineering (15). A Japanese patent (16) states that a daily little dose of phycocyanin retains or accelerates normal regulatory cell processes, which either prevents cancer from developing or limits its growth or recurrence. The presence of "biochromes" in cyanobacteria is measured and classified using present studies.

Materials and Methods

Source of Organisms

Marine cyanobacterial species from several marine bodies in Tamil Nadu were gathered for this investigation. *Microcystis* sp., *Lyngbya limnetica, Oscillatoria roai, Oscillatoria acuminata*, and *Oscillatoria princes* are the five cyanobacterial species that were collected in the period of (2014-2017) in the Gulf of Mannar (Koswari, Puvarsanpatti, Krusadai, Talairi).

Incubation conditions

The marine cyanobacterial strains were maintained at room temperature 27°C under cool white fluorescent illumination (approximately 1500 lux). ASN III culture medium was prepared and used. Agar was used as a solidifying agent at a concentration of 1.5% with the liquid media (ASN III).

Cultures conditions

Cultures were kept in 250 mL Erlenmeyer flasks with 100 mL of sterile ASN III media and cultured at room temperature of 27°C with a 14:10 hr L/D cycle while exposed to white cool fluorescent light 13.7 Einsteins $m^{-2}s^{-1}$.

Dry weight determination (g)

Dry weight measurements were taken every specified number of days, such as 5, 10, 15, 20, and 25, to measure the growth of cyanobacteria. The superspin R-V/FM, plastic crafts centrifuge, was used to centrifuge the cyanobacterial suspensions for 15 minutes at 10,000 rpm. Using filter paper, the pellets were taken out of the extra water. The electronic balance was then used to determine the fresh weight. The pellet was placed in reweighed aluminium foil and dried in a hot air oven for two hours at 100°C. Weight was applied to the dried pellet. The weight was given in milligrams per gram of fresh weight (17, 18).

Estimation of organic constituents by colorimetric method

The colorimetric approach can be used to quantitatively determine the majority of organic elements. The Beer's law, or state, is the foundation of colorimetric analysis. The concentration of the coloring agent determines how

much light is absorbed in colored solutions. Spectrophotometers or photoelectric colorimeters can be used to measure the absorbance. In colorimetric analysis, a color is first created through a series of chemical processes that are traits of the constituent being studied. Then, using several standard solutions with known concentrations, a standard curve is created. The standard curve is used to determine the concentration of the unknown based on its absorbance value (17, 18).

Bio-chromes

Estimation of Chlorophyll – a (mg / gm of dry weight)

The colorimetric approach can be used to quantitatively determine most organic elements. The Beer's law, or state, is the foundation of colorimetric analysis. The concentration of the component that gives colours their colour influences how much light is absorbed in coloured solutions. Spectrophotometers or photoelectric colorimeters can be used to measure the absorbance. In colorimetric analysis, a colour is first created through a series of chemical processes that are traits of the component being studied. Then, using many standard solutions with known concentrations, a standard curve is created. The standard curve is used to determine the concentration of the unknown based on its absorbance value (19, 20, 21). Centrifuging the cyanobacterial suspensions at 10,000 rpm for 15 min. The filter paper was used to remove extra water from the pellets. The material was then divided into stoppered test tubes with known weights, and 5 ml of 80% methanol was added to each tube. For one hour, the tubes were maintained in a water bath at 60 °C. Avoid using bright lighting. After cooling the tubes, the material was centrifuged at 5,000 rpm for 10 min. The procedure was carried out three times, and the collected supernatant was combined and diluted with methanol to make 10 ml. At 663 nm, the absorbance was measured against a methanol blank. The following formula was used to compute the chlorophyll- a.

$$\text{Chlorophyll a (g/100g dry wt.)} = \frac{A663 \times 12.63 \times \text{Vol.of methanol} \times 100}{\text{Dry weight of the sample}}$$

Estimation of Phycobilliproteins (mg/gm of dry weight)

Each Cyanobacterial sample was pulverized in a pestle and mortar after being suspended in 3 ml phosphate buffer (0.05 M, pH 6.8). The suspensions were centrifuged at 5,000 rpm for 15 minutes after being periodically freeze-thawed (at least three times). To ensure thorough extraction, the operation was repeated with the supernatant conserved. In a spectrophotometer, the pooled supernatant (5 ml) was measured at 565, 615, and 652 nm in comparison to the buffer blank. The following formula was used to compute the C- phycocyanin (PC), allo-phycocyanin (aPC), and C- phycoerythrin (PE) (21).

$$\text{1. C-Phycocyanin (PC)} = \frac{A615 - 0.474\,(A652)}{5.34}$$

$$\text{2. allo-Phycocyanin (aPC)} = \frac{A(652) - 0.208\,(A\,615)}{5.09}$$

$$\text{3. C-Phycoerythrin (PE)} = \frac{A562 - 241\,(PC) - 0.849\,(aPC)}{9.62}$$

Estimation of Carotenoids (mg/gm of dry weight)

Using 95% ethanol, a cyanobacterial sample was polarised. In a bath of hot water, this suspension was refluxed for approximately 30 minutes. Filtered and diluted with 25 mL of 85% ethanol was the clear supernatant. Petroleum ether was used to replicate the extracted solution. Cool the extract before adding ether to make up to 100 mL. Five milliliters of 85% acetone were added to a tube containing the cyanobacterial suspension. After 60 minutes at room temperature, the solutions were centrifuged to separate the supernatant. To get rid of all the biochromes, the extraction technique was repeated. With 85% acetone, the pooled extracts were diluted to a final volume of 10 mL, and the absorbency was measured at 450 nm in a spectrophotometer against an acetone blank.

$$\text{Carotenoids (mg/ml)} = D \times V \times f \times 10/2500.$$

Where, D = absorbency at 450 nm, V = volume of the sample, f = dilution factor, 2500 = extinction coefficient. The total carotenoids were calculated from the above formula, and the results were expressed in mg/100 g dry weight of the sample (22).

Estimation of β - Carotene (mg/gm of dry weight)

1 gram of cyanobacterial sample and an equivalent volume of ethanolic KOH (1N KOH in 90% ethanol) were taken and thoroughly mixed in a glass stoppered tube. The tubes were cooled at room temperature after being incubated in a water bath at 60° C for 30 minutes. Ten milliliters of mild petroleum ether were used to extract the unsaponifiable material three times. To get rid of the alkali that sodium sulphate passed through, the petroleum ether extracts were rinsed with water. Immediately after being dried, the residues were dissolved in 3 mL of cyclohexane, and a spectrophotometer was used to determine the absorbance at 460 nm. Purified carrot-carotene in concentrations ranging from 1 to 10 g/mL was used to produce the standard graph, and the results were presented in mg/100 gm (22).

Results and Discussion

Five species *Microcystis* sp., *Lyngbya limnetica, Oscillatoria roai, Oscillatoria acuminata,* and *Oscillatoria* princes were used in the current investigation for quantifying their biochromes. The cyanobacterial strains were used to estimate the biochromes. Results were given as mg for every gram of dry weight. The qualitative and quantitative analyzed of the cyanobacterial biochromes produced by *Microcystis* sp., *Lyngbya limnetica, Oscillatoria roai, Oscillatoria acuminata*, and *Oscillatoria princes.*

In terms of biochromes like phycocyanin, phycoerythrin, and carotenoids, *Microcystis* sp., *Lyngbya limnetica*, and *Oscillatoria roai* of these Marine forms of cyanobacteria had more chlorophyll - a than the other species, but *Microcystis* sp. had more carotene than chlorophyll - a. Despite being based on dubious research, the current great demand for chlorophyll as a deodorant may provide a steady market for chlorophyll at high prices. The economics of mass culture would allow for the growth of cyanobacteria largely as a source of chlorophyll, with the leftovers being used as a byproduct for feed. Chlorophyll was first isolated from Chlorella, a eukaryotic green algae; however, cyanobacteria are currently the source of interest. Large-scale cultivation of cyanobacteria is necessary for various reasons; the production of chlorophyll is unquestionably enticing as a byproduct. The results of the estimation of the chlorophyll in the chosen cyanobacteria revealed that *Oscillatoria princes* had a very high level of chlorophyll at 5.9%, making it a viable source of chlorophyll in the future. Comparatively, less than 0.2 percent of dehydrated lucerne, a commercially available chlorophyll-containing extraction, was recorded (8).

The Marine form of cyanobacterium *Oscillatoria raoi* had 3.41% chlorophyll - a with 0.44 % phycocyanin pigment, 1.01% carotenoid, and 1.22% b -carotene. *Lyngbya limnetica* had nearly 1.1% b-carotene, 1.8% carotenoids, and 2.65% chlorophyll-a (Table 1; Fig. 1-4). *Oscillatoria acuminata* and *Oscillatoria princes* were analyzed in different biochromes such as Chlorophyll–a, Phycocyanin, Carotenoids, and b -carotene which was compared, they were more in an amount in all the biochromes and *Microcystis sp.* was 2.08%, 0.56%, 1.77% and 1.55 % of Chlorophyll–a, Phycocyanin, Carotenoids and b -carotene respectively. Biochromes are commercially highly useful for industrial purposes. Phycocyanin was reported to be present in many of the cyanobacteria. Phycocyanin is found in cyanobacteria that contain open-chain tetrapyrroles with possible scavenging properties, and c-phycocyanin has antioxidant and anti-inflammatory properties (8). From this study, the phycocyanin result showed 0.78 % and 0.85% in *Oscillatoria acuminata and Oscillatoria princes* respectively.

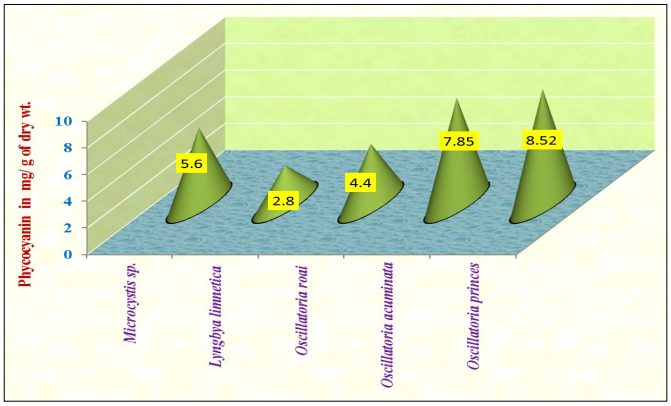

Fig. 2. Phycocyanin content of five marine cyanobacterial species.

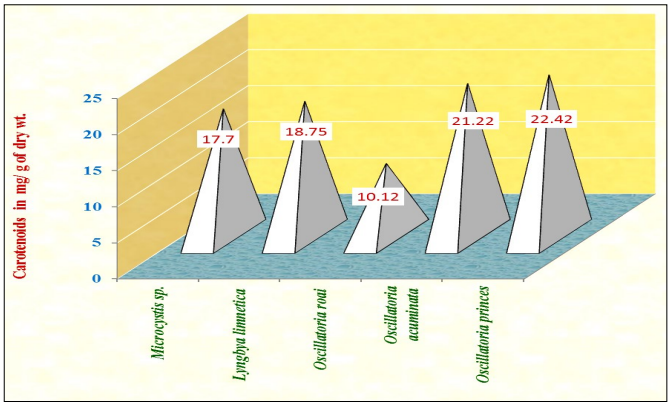

Fig. 3. Carotenoids content of five marine cyanobacterial species.

Table 1. Quantification of biochromes in marine cyanobacterial species.

S. No.	Cyanobacteria	Chlorophyll – a mg / gm of dry weight	Phycocyanin mg / gm of dry weight	Pycoerythrin mg / gm of dry weight	Carotenoids mg / gm of dry weight	ß-Carotene mg / gm of dry weight
1.	*Microcystis* sp.	20.81	5.6	-	17.7	15.5
2.	*Lyngbya limnetica*	26.5	2.8	-	18.75	11
3	*Oscillatoria roai*	34.1	4.4	-	10.12	12.2
4	*Oscillatoria acuminata*	41.25	7.85	-	21.22	17.6
5	*Oscillatoria princes*	59.22	8.52	-	22.42	18.22

Note: (-) is not detected.

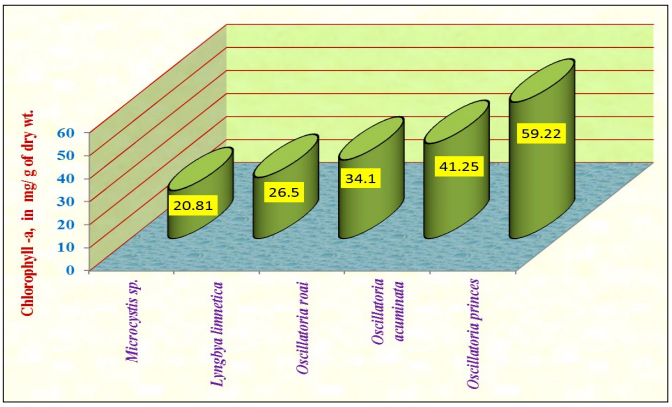

Fig. 1. Chlorophyll a content of five marine cyanobacterial species.

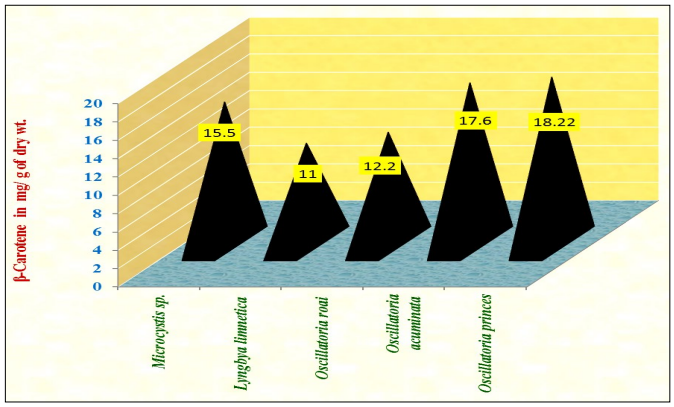

Fig. 4. ß – Carotene content of five marine cyanobacterial species.

In all the Marine forms, none of them had the pigment phycoerythrin (Table 1). The Marine form of cyanobacterium *Oscillatoria acuminata* had highly 4.1% chlorophyll-a, content with 2.1% in Carotenoids and 1.7% in b-carotene and *Oscillatoria princes* had maximal 5.9% chlorophyll - a content with 2.2 and 1.8 % in Carotenoids and b -carotene respectively (Table 1; Fig. 1-4; Plate -1). b- Carotene has been used primarily as a coloring agent for food to import, standardize, or enhance natural color (15).

When it was necessary to get appropriate yolk color and acceptable skin pigmentations in poultry fish, the feed industry employed carotenoids to standardize the natural pigment concentration in feeds of all the vitamin A produced; the majority is used to improve animal feeds. The fat-soluble yellow, orange, and red azo dyes, whose usage is prohibited by food law, have been introduced into food products via carotenoids. To color water-based foods, including orange-colored beverages, dairy products, cheese, butter, ice cream, cake mixes, puddings, dry and canned soups, etc., water-dispersible versions of beta-carotene have been produced. With their provitamin activity, anticancer activity, immune system stimulation, and other biological effects, carotenoids play a significant role in human health (15).

Conclusion

Microcystis sp, *Lyngbya limnetica, Oscillatoria roai, Oscillatoria acuminata,* and *Oscillatoria princes* were among the marine cyanobacterial species chosen for this investigation of their biochromes, and publications on their products have received a fair amount of interest. Preliminary research has been done on all pigment estimations, including those for chlorophyll, phycocyanin, phycoerythrin, carotenoids, and beta-carotene. *Oscillatoria princes* had the highest concentration of chlorophyll-a, which was 5.9%. *Oscillatoria acuminata* and *Oscillatoria princes* both have higher phycocyanin contents, with respective values of 0.78% and 0.85%. *Lyngbya limnetica* and *Microcystis sp.* both contained about 1.8% and 1.7% carotenoid, respectively. *Microcystis* sp. contained up to 1.5% -carotene.

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Authors' contributions

J S carried out field and research work; G S supervised the research work and checked manuscript; M R draft the manuscript and correction.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

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