Available online at www.journalijmrr.com

INTERNATIONAL JOURNAL OF MODERN RESEARCH AND REVIEWS

Int. J. Modn. Res. Revs.

Volume 10, Issue 1, pp 001-005, January, 2022

ISSN: 2347-8314

ORIGINAL ARTICLE

STUDIES ON PIGMENTAL ANALYSIS OF *ANABAENA* AND *OSCILLATORIA* (CYANOBACTERIA)

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Article History: *Received 3rd January,2021 Accepted 30th January,2022, Published 31st h January,2022*

ABSTRACT

Blue green algae lived in the principal gram negative photosynthetic bacteria that were believed to have evolved on earth approximately 3.8 vast periods previously. The present study is aimed to analyse pigmental analysis such as chlorophyll 'a' phycocyanin, phycoerythin and allphycocyanin were observed in blue green algae were isolated from the paddy field of the Nalanthethu and Srineduncherry Cuddalore District and the isolated blue green algae of *Anabaena* and *Oscillatoria* were maintained in BG11 medium for 10 days. The present study shows the the level of pycocyanin was more in Anabaena and Oscillatoria than chlorophyll 'a', Phycoerythrin and allophycocyanin

Keywords: : Pigment analysis, Anabaena sp, Oscillatoriasp..
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1.INTRODUCTION

Cyanobacteria are an antique collection of unique prokaryotic organisms with the capability to perform mutually compatible finctions like niterogen obsession and photo synthesis. Evidence that the collection of blue-greens is indispensable to comprehensive the algae dynamics and interface with other microorgnisms. Current Research on cyanobacteria miscellany has improved amusing importance especially after the acceptance about their part trendy the common environment and its competence towards provide another source of dynamism. Moreover, they form simple models for understanding various basic singularities for example cellular metabolism, synthesis of micromolecular compounds, cell differentiation and directive of genetic expression. The quantity about numerous cyanobacteria to dose the atmospheric nitrogen is a substantial natural procedure about economic significance (Venkataraman, 1981, Santra, 1993).

Paddy fields are an appropriate milieu for the growing of nitrogen fixation, oxygen cyanobacteria, by furthermore appropriate nutrient, temperature nutrient besides aquatic ameniteies. In reappearance, cyanobacteria transport a substantial quantity of nitrogen and phosphours, which are situated the greatest required nutrients on the period of rice cultivation. Apart from nitrogen and phosphorus fixation, they also excrete several biological acids that increase and maintain territory richness, nutrient availability and water plot capability (Roger and Reynaued, 1982; Saadatia and Riahi, 2009; Wilson *et al*., 2006)

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2.MATERIALS AND METHODS

2.1 PHYSIOGRAPHY OF THE RESEARCH REGION

The study zone (Station I: Nalanthethu 11.47N 79.63 E) which is selected for the current research investigation were situated trendy the Bhuvanagiri Taluk and Station II Srineduncherry: 11 45 '0' Latitude N and 79 45'0' Longitude) which is located in Taluk of Srimushnam, District of Cuddalore, Tamilnadu, India.Station-I (Nalanthathu) is a small Village/hamlet amid Bhuvangiri Taluk among the Cuddalore Region about Tamil Nadu State in India. The situation comes under Azhichikudi Panchayath. It's situated 45 KM from state capital from District headquarters Cuddalore. Four kilometers from Melbhuvangiri, 227 kilometers from state capital Chennai. Station-II

(Srinedunchery) as a village trendy Srimushnam Taluk in Cuddalore and Chidambaram is located slightly arresting Srinedunchery remains a township in Srimushnam Taluk in District of Cuddalore, TamilNadu State, India. It is situated 60 Km towards South as of District headquarters Cuddalore, 7 Km from Srimushnam and 238 KM from state capital Chennai. Srinedunchery remains nearby Kammapuram Brick to South, Andimadam Block towards North, Kattumannarkoil Brick just before West and Melbhuvangiri Block towards East. Neyveli, Virudhachalam, Sethiyathope, Andimadam exist the neighboring Towns towards Srinedunchery. Here area is trendy the border about the Cuddalore and Ariyalur District.

2.7 CULTURE ABOUT CYANOBACTERIAL SPECIES

The *Anabeana fertilissima* and *Oscillatoria princeps* inaccessible as per of the rice fields of Cuddalore District. The filamentous *cyanobacterium Anabeana fertilissima and Oscillatoria princeps* were established in culture conical flask at 30°C ± 2°C trendy the nitrogen-free form of GG-11 liquid and nitrogen average respectively (Rippika *et al*., 1979) and pH existed attuned to 7.0 *Anabeana sp.*was established trendy BG-11 middling deprived of nitrogen source (Stanier *et al.*, 1971). Their cultures were maintained in laboratory situations in sunshine fluorescent ducts for 16 h a day for 10 days. The cultured cyanobacterial species were used for the estimation of pigment analysis.

Estimation of Cholrophyll 'a'

The estimation about Chlorophyll **'a'** was done by the process of McKinney's (1941).The principles existed taken and centrifuged at 7,000× g for 10 minutes. The pill was lapped with distilled water, adjourned trendy 4 mL of 80% methanol and vortexed thoroughly. Then the conduits exist covered by aluminium thwart towards prevent solvent evaporation then cultivated amid a water bath set at 60°C for 1 h in dark with occasional shaking. After 1 h the contents were taken, cooled and centrifuged at 7000× g for 5 minutes. The supernatant was saved and the exceeding process repeated once again to ensure complete quarrying of the pigments. The pooled supernatant live rouged towards recognized measurements with 80% methanol. The absorbance lives slow at 663 nm using a spectronic 20 against methanol as blank.

Chlorophyll 'a' =

$$\frac{A663 \times 12.63 \times \text{vol. of sample}}{\text{Vol. of sample}} \text{«g/L}$$

Where,663= Absorbance at 663 nm

12.63 = Correction factor

Estimation of phycobiliproteins

The estimatiopn of phycobiliproteins was done the technique of Moreno *et al*., (1995).The values existed centrifuged at 7000×g for 10 min. The pellets stayed wash away with distilled water, suspended in 3 mL of phosphate buffer (0.05m) and homogenized. The contents were freze thawed, repeated and cebtrifuged at 7000×g for 5 min.The supernatant was stored in refrigerator. The overhead procedure existed recurrent to ensure complete extraction. The absorbance about the pooled supernatant existed measured at 565 nm, 615 nm and 652 nm to quantify phycoerythrin, phycocyanin and allophycocanin respectively against phosphate buffer as blank.

C-Phycocyanin (PC) =

$$\frac{A615 \times 0.474\,(A652)}{5.34} \text{«g/mL}$$

Allophycocyanin (APC) = $\frac{A652 \times 0.208\,(A615)}{5.098}$ «g/mL

C.Phycoerthrin (PE) = $\frac{A565 \times 2.41(PC) - 0.849}{9.62}$ «g/mL

3.RESULTS

PIGMENTS STUDIES IN CYANOBACTERIA

The level of chrophyll 'a'

Table.1. Appear the quantity of chlorophyll 'a' witnessed in *Anabeana fertilissima* (1.96 ± 0.37mg/mL) besides *Oscillatoria princeps* (1.72± 0.26 mg/mL) at 10th day. The extent about chorophyll 'a' existedmore in *Anabeana fertilissima* and *Oscillatoria princeps* (Table 1).

Table.1. The level of chlorophyll 'a', phycocyanin, Phycoerythrin and allophycocyanin in *Anabeana fertilissima and Oscillatoria princeps*

Parameters	*Anabaena fertilissima*	*Oscillatoria princeps*
Chlorophyll 'a' (mg/mL)	**1.96±0.37**	**1.72±0.26**
Phycocyanin (mg/mL)	**5.20±0.78**	**5.16±0.11**
Phycoerythrin (mg/mL)	**2.69±0.19**	**1.12±0.32**
Allophycocyanin (mg/mL)	**2.47±0.79**	**1.19±0.62**

Values expressed as mean ± SE of three individual observations

The level of phycocyanin, phycoerythrin besides allophycocyanin

Table. 1. Shows the extent of phycophiliprotein (phycocyanin, phycoerythrin, allophycocyanin) *Anabeana fertilissima and Oscillatoria princeps* at 10th day. Among the pigments, phycocyanin, phycoerythrin besides allophycocyanin existed additional trendy *Anabaena*

fertilissima (5.20 ± 0.78 mg/mL,2.69±0.19 mg/mL and 2.47±0.79 mg/mL) than *Oscillatoria princeps* (5.16 ± 0.11 mg/mL, 1.12±0.32 mg/mL and 1.19± 0.62 mg/mL) correspondingly once likened towards phycoerythrinin and allophycocyanin (Table 1).

4.DISCUSSION

The extensively utilized pigments in bioindustry, are the phycobiliproteins (comprising phycocyanin, phycoerythrin and allophycocyanin), which account for about 20% of total dry weight (Prasanna and Nayak, 2007; Walsh, 1997) of many cyanobacteria. The phycobiliproteins represent the main photosynthetic auxiliary pigments in cyanobacteria; along with chlorophyll *a*. Phycobiliproteins are a family of exceedingly soluble and reasonably stable fluorescent proteins plagiaristic from cyanobacteria. There are three basic types of biliproteins allophycocyanin (APC), phycocyanin (PC) and phycoerythrin (PE).

Cyanobacteria have all three types of phycobilins: APC and PC are permanently present and PE is found in some organisms and not in others (Samsonoff and MacColl, 2001), but forms the most spectroscopically mutable class of phycobiliproteins. Light energy fascinated by PE migrates first to PC, then to APC and lastly to chlorophyll *a*. Phycocyanin is the chief constituent of the phycobilisomes, while APC functions as bridge pigment between phycobilisomes and photosynthetic lamellae (MacColl, 1998).

Cyanobacteria are photosynthetic micro-organisms, which lug out photosynthesis similar to higher plants. However, instead of chloroplasts they contain membranous structures called thylakoids, where phycobilisomes are found. These micro-organisms only contain chlorophyll a and almost 50%of the light obligatory is apprehended by the phycobiliproteins. Phycobiliproteins are antenna pigments, light harvesting, and consist of chromophores called bilins, which are attached to cysteine residues of the apoprotein (Samson off and MacColl, 2001).

In cyanobacteria, the primary photosynthetic pigment is chlorophyll 'a', which is a green coloured photo harvesting pigment. Phycoerythrin and phycocyanin (together known as phycobiliproteins) are the accessory pigments located in the phycobilisomes, which help in the photosynthetic activity by transferring light to reaction centre of photosystem I and II by overlapping respective absorption and fluorescence spectra between bilipigments and chlorophyll 'a' at the reaction centre (Rodrigo and Robaina, 1997*)*. Phycobiliproteins are a brilliantly colored family of water soluble proteins known as 'phycobilins'. On the basis of their visible absorption properties, the phycobilins have been assigned to four spectroscopic classes: Phycoerythrocyanine; phycoerythrins; phycocyanine; and Allophycocyanine; (Sidler, 1994).

In the present study, the Chlorophyll 'a', Phycocyanin, Phycoerythrin and Allophycocyanin were observed in *Anabaena and Oscillatoria.* The present study shows the level of phycocyanin was more than phycoerythrin, allophycocyanain at 10 day of culture of cyanobacteria. The present study shows the chlorophyll *'a'*, content is minor than phycocyanin, phycoerythrin, allophycocyanain which may be due to N fixing cyanobacteria. Kaushik, (2000) stated that among various cyanobacterial strains, heterocystous forms *Anabaena variabilis*, *Aulosira fertilissima*, *Hapalosiphon* sp. and *Tolypothrix tenuis* can be exploited for phycobilins; (Badrish *et al.,* 2006) observed the pycocyanin in Oscillatoria sp. (Mishra *et al.,* 2004) observed that highest chlorophyll gratified in *Anabaena sp.*in the soil. The present study concludes that pycocyanin is more than chlorophyll and among the biochemical substances the carbohydrate gratified is higher in oscilatoria which promotes the soil fertility in the paddy fields. (Kurger *et al.,* 1995)

Our findings are comparable to the previous workers (Borowitzka, 1994). Similar observations were also reported by Koijam and Tiwari (2012) in *Anabaena fuellebornii*, *Nostoc spongiaeforme*. The results of the present study suggest that some *Nostoc* and *Anabaena* strains had much higher content of phycobiliproteins compared to values found by (Jelica *et al.,* 2012).Many studies displayed that the best methods for extracted of C-PC are freezing-thawing and lysozyme treatment (Soni *et al.,* 2006). The results of the biochemical scrutiny of cyanobacteria sequestered from paddy fields showed the high amount of biochemical contents. Similarly (El Sheekh *et al.,* 2015) recorded highest Chlorophyll-a in *N. calcicola*, *A. variabilis* and *N. linkia* and Amalina and Jayashree (2017) reported maximum Chlorophyll-a in the *L.holdenil*. The present study shows the level of phycocyanin was more than allophycocyanain and phycoerythrinin in the present investigation. The highest Phycocyanin colorant was observed in *O. princeps.* Badrish et al., (2006) reported analogous type of result of phycocyanin in *Oscillatoria* sp. The highest allophycocyanin gratified was recorded in *P. mucosum.* Similarly, Narayan *et al.,* (2006) stated that the uppermost gratified of allophycocyanin presented in *Anabaena*, while the maximum content of Phycoerythrin present in *N. punctiforme* while Narayan et al., (2006) reported in *Nostoc* and *Calothrix*, Phycoerythrin was highest observed and *N.calcicola* showed highest Phycoerythrin content (El Sheikh *et al.,*2015) respectively. Moreover, the optimum contents of Phycocyanin, phycoerythrin, and allophycocyanin reported by (Tyagi *et al.,* 1980).

The genus *Oscillatoria* is one of the dominant blue green algae (Cyanophyceae) which grows in various habitats *(*Castenholz *et al.,* 1989*)*. The Thallus is made up of single trichome (filament). Cells blue-green to violet-red in colour; wherein the green colour of Chlorophyll *a* is masked by carotenoids and accessory pigments Phycobilins ie. Phycocyanin and phycoerythrin (Douglas and Chloroplast, 1994). Some unique features that set it apart from other cyanobacteria are that it is motile and can demeanor anoxygenic photosynthesis. Within its long filamentous structure the cells are in the form of discoid and are surrounded by cell wall so it does not require heterocyst. In order to optimize their position in water many planktonic species possess gas vesicles. These are cytoplasmic inclusions that enable buoyancy to adjust their vertical position in water thus find a suitable niche for survival and growth.

Mostly this genus shows oscillating movement thus derives its name *Oscillatoria; O. princeps* is cosmopolitan in distribution and has wide range of variability in its

characteristic features (Geitler, 1932*)*. The occurrence is not only restricted to freshwater, but also in other habitats. It is very common and there are numerous gossips in most of the check lists and floras from India.(Nirmal Kumar and Cini Oomme, 2011) While studying the plankton community in Gujarat has observed *O. princeps* as one of the prevailing classes which occurs in most of the seasons.

The strain was originated to produce relatively morephycocyanin (PC) than phycoerythrin (PE) and allophycocyanin (APC). Similar consequences were achieved from the rice pitches of Dima Hasao district in Assam. North-East Indiasherein two strains of Nostoc commune were characterized on the basis of their pigements and biochemical analysis in addition to molecular stueies (Borah *et al.,* 2014).

The increase in carbohyfrate content of pea seedlings may be accredited to the increase in the rate of photosynthetic electron transport (Bograh *et al.,* 1997) and inspiration of colorant biosynthesis (Younis *et al.,* 1991). A steady increase in biochemical attributes, chlorophyll-a (Chl-a), total carotenoid contenets (TCC) and exoplysaccharides, (Choudhary and Bimal ., 2010) concluded that application of nitrogen-fixing cyanobacteria in the rice fields can be used for sustainable management of nitrogen fertilizer at dissimilar stages of paddy cultivation. Cyanobacteria are the group of photosynthetic organisms which can easily survive on bare minimum prerequisite of light, carbon dioxide (CO_2) and water (Woese et al,1987); (Castenholz *et al.,*2001). The present study concluded that the cyanobacteria were isolated from ground water samples with minimal survival factors. Cyanobacteria (Microcystis, Merismopedia amd Plectonema) was secluded from high TDS ground water samples and morphologically identified microscopically. Similar to other prokaryotic bacteria, the cyanobacteria are increasingly applied as bio-inoculants for improving soil fertility and environmental quality. They are also useful for waste water treatment, and have the ability to degrade the various toxic compounds including pesticides (Cohen, 2006). The present study shows the level of phycocyanin was more than allophycocyanain and phycoerythrinin in the present investigation. The highest Phycocyanin pigment was observed in *O. princeps.* (Badrish *et al.,* 2006) reported similar type of result of phycocyanin in *Oscillatoria* sp. The highest allophycocyanin gratified was recorded in *P. mucosum.*

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