Physiological Attributes of Selected Cyanobacterial Strains Isolated from Paddy Fields of Western U.P.

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ABSTRACT

Aim of the present work was to study the physiological attributes of six cyanobacterial isolates (*Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., *Calothrix* sp., *Cylindrospurmum* sp. and *Phormidium* sp.) from paddy fields of western Uttar Pradesh (Situated between 23°52'N and 31°28'N latitudes and 77°3' and 84°39'E longitudes). The major constituents like total chlorophyll, carotenoids, total proteins, total carbohydrates and phycobiliproteins content(s) of the cyanobacterial isolates were analysed and compared. Maximum content of chlorophyll-a was observed in *Anabaena*, carotenoids in *Oscillatoria*, protein in *Nostoc*, carbohydrate in *Phormidium*, Phycocyanin in *Anabaena*, and Allophycocyanin in *Phormidium*. The results reveal the potential of these cyanobacterial isolates in food, pharmaceutical, cosmetics industries and biotechnological applications.

Key words: Cyanobacterial strains, Nutraceuticals, Physiological parameters, carotenoids, phycobiliproteins and Phycocyanin

INTRODUCTION

Cyanobacteria comprise a large group of structurally complex and ecologically significant gram-negative prokaryotes, which exhibit a wide range of nutritional capabilities ranging from obligate phototrophy to heterotrophy (Rippka, 1972; Vasudevan *et al*., 2006; Prasanna *et al*., 2009), although the majority of forms examined so far exhibit phototrophy. Cyanobacteria occupy almost every niche of the earth, including fresh and salt waters, paddy (*Oryza sativa*) fields, hot springs, arid deserts, and polar regions (Thajuddin and Subramanian, 2005; Wilkie *et al*., 2011).Their diversity ranges from acidophilic to alkalophilic, autotrophic to heterotrophic, coccoid to branched filaments, nearly colourless to intensely pigmented, planktonic to barophilic, psychrophilic to thermophilic, unicellular to multicellular, fresh water to marine including hypersaline (salt pans). Cyanobacteria are an ancient group of prokaryotes with the ability to perform functions like biological N fixation and photosynthesis. The capacity of several cyanobacteria to fix atmospheric N is a significant biological process of economic importance (Santra, 1993).

Cyanobacteria are a remarkable group of prokaryotes, which are known to exist independently and in symbiotic/facultative associations with a diverse range of members of the plant kingdom, including Gymnosperms, Pteridophytes and Bryophytes (Rai and Bergman, 2002). However, their associations with crop plants are less explored (Nilsson *et al*., 2005). They are well adapted to a wide range of environmental conditions and have been widely employed as bio-inoculants for enhancing soil fertility and improving soil structure, besides enhancing crop yields, especially in rice (Venkataraman, 1972; Kaushik, 2004; Nayak *et al*., 2004; Dhar *et al*., 2007).

The paddy-field ecosystem represents a unique aquatic-terrestrial habitat, which provides a favourable environment for growth of and N fixation by cyanobacteria, meeting their requirements for water, elevated temperature, light and nutrient availability. This, in turn, is considered to be one of the major reasons for the relatively stable yield of rice under flooded conditions and maintenance of the productivity of rice fields (Roger *et al*., 1993). Cyanobacteria also add organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter content of the soil, provide O_2 to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants (Mandal *et al.*, 1998; Kaushik, 2004)

Cyanobacteria are recognized to be prolific producers of bioactive compounds drawing interests as a source of various nutraceuticals, biomass and pigments (Ciferri *et al*., 1985; Pulz *et al*., 2004; Cardozo *et al*., 2007; Tan, 2007). The present investigations aimed to obtain cyanobacterial isolates from paddy fields of western Uttar Pradesh (Situated between 23°52'N and 31°28'N latitudes

and 77°3' and 84°39'E longitudes), which have the ability to produce the metabolites such as proteins, exopolysaccharides, pigments and carbohydrates which are valuable substances with potential applications in the food, pharmaceutical and cosmetics industries.

MATERIALS AND METHODS

Isolation of cyanobacterial strains from soil samples

The soil samples were collected from rice fields of Muzaffarnagar (29.4727° N, 77.7085° E) and Meerut (28.9845° N, 77.7064° E) region of western Uttar Pradesh (India). Ten gram of the soil sample was transferred to 250 ml Erlenmeyer flask containing 90 ml sterile distilled water and homogenized at 120 rpm for 30 min. Standard serial dilution method was followed. Serial dilution (upto 10^{-4}) was made and 1ml aliquots were spread and grown in chemically defined BG-11 (-N and/or +N) medium (Stanier *et al*., 1971) with pH 7.2±0.2 at 28±2 ^{o}C under a light intensity of 52-55 µmol photon m^{-2} s^{-1} and L: D cycles of 16:8 h. The obtained cyanobacterial isolates were maintained on BG-11 media at 28±2 °C under laboratory conditions and repeated sub-culturing was performed to obtain respective pure culture (Rippka *et al*., 1979).

Identification of Cyanobacterial species

Cyanobacterial species were identified microscopically based on morphological as well as taxonomical observations *e.g.* the length and the width of the vegetative cells, the width of the sheath, type of spores, presence/absence of hormogonia, presence/absence of spores and its position, number of heterocysts and its repetition, presence of akinetes and its type, the nature of cell wall, presence/absence of gas vacuoles and pigment colour (Desikachary, 1959; Santra, 1993). The photomicrographs were taken with a fluorescent microscope (Fig 1a-f).

Physiological parameters

Estimation of Chlorophyll (McKinney, 1941)

About 10 mL of homogenized cyanobacterial suspension was taken and subjected to centrifugation (4000g, 10 min). The chlorophyll was extracted from pellet with equal volume of methanol (95%; v/v) in a water bath (60 ^{o}C, 30 min). The suspension was centrifuged and the absorbance of the supernatant was measured at 650 and 665 nm against 95% methanol as blank.

Estimation of Carotenoids (Jensen, 1978)

About 10 mL of homogenized cyanobacterial suspension was centrifuged at 3000 rpm for 10 min. The pellet thus, obtained was washed with distilled water to remove traces of adhering salt. To that, 2-3 mL of acetone (85%; v/v) was added and subjected to repeated freezing and thawing. Extractions were performed till acetone became colourless. The acetone fractions, thus, obtained were pooled; and the final volume was recorded. The content of total carotenoids was estimated from the maximum absorbance measured at 450nm using 85% acetone as blank.

Estimation of Total soluble proteins (Lowry *et al*., 1951; Herbert *et al*.,1971)

(i) Reagents:

(a)1N sodium hydroxide solution

(b) (i) 5% sodium carbonate

(ii) 0.5% copper sulphate ($CuSO_4.5H_2O$) solution in 1% sodium potassium tartarate

2 mL of reagent B (ii) was mixed with 50 ml of freshly prepared reagent B (i)

(c) 1N Folin-Ciocalteau reagent

About 0.5 mL of homogenized cyanobacterial suspension was taken in test tubes. To this, 0.5 mL of reagent (a) was added. The tubes were then heated in a boiling water bath for 10 mins. and cooled undar running tap water. Subsequently, 2.5 mL of reagent (b) was added in each and the tubes were incubated at 23 ^{o}C for 10 mins. After this, 0.5 mL of reagent (c) was added and the tubes were kept at 23 ^{o}C for 15 min. The intensity of blue colour was read as absorbance at 650 nm. The protein content was estimated using a standard curve prepared using bovine serum albumin (BSA).

Estimation of Total Carbohydrates (Spiro, 1966)

(i) Reagent: 100 mg anthrone and 1 g thiourea was dissolved in 100 mL of 75% sulphuric acid. The mixture was kept in water bath at 85 ^{O}C to dissolve the ingredients completely.

(ii) Procedure: About 0.5 mL of homogenized suspension was taken in test tubes and volume was made up to one mL with distilled water. To that, 4 mL of anthrone reagent was added and tubes were transferred to boiling water bath. After 10 min., the tubes were cooled to room temperature and absorbance was read at 625 nm. The sugar content was calculated using the standard curve made with glucose.

Estimation of Phycobiliproteins (Bennett and Bogard, 1973)

(i) Reagent: 0.05 M phosphate buffer

(ii) Procedure: A known volume of homogenized algal suspension was centrifuged at 3000 rpm for 10 min and pellet was suspended in equal volume of 0.05 M phosphate buffer, pH 7.5 (obtained by mixing equal volume of 0.1M KH_2 PO_4 and 0.1 M K_2HPO_4). Repeated freezing and thawing was done till the pellet became colourless and the pigment was released in the supernatant. The absorbance was measured at 562, 615 and 652 nm against 0.05M phosphate buffer as blank.

RESULTS AND DISCUSSION

Six Cyanobacterial species namely *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., *Calothrix* sp., *Cylindrospurmum* sp. and *Phormidium* sp. were isolated from paddy fields. The biochemical analysis of Chlorophyll A, Carotenoids, Proteins, Carbohydrates, and phycobiliproteins content of all Cyanobacterial strains were analysed (Table 1). The Chlorophyll A content was found in significant amounts.

The maximum Chlorophyll A was reported in case of *Anabaena* (2.54±0.06), followed by *Nostoc* (2.08±0.34), *Ocillatoria* (1.87±0.21), *Calothrix* (1.13±0.43, *Phormidium* (0.45±0.01) and minimum in *Cylindrospermum* (0.09±0.08).

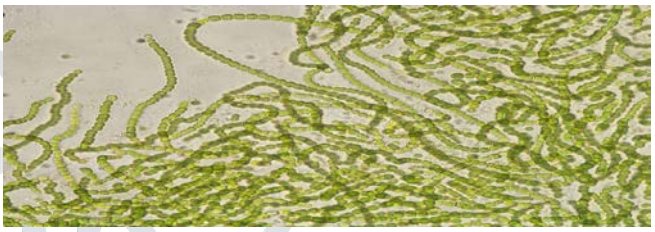
The Carotenoid was found to be highest in *Oscillatoria* (1.55±0.01), followed by *Nostoc* (1.23±0.25), *Anabaena* (0.93±0.14) *Cylindrospermum* (0.68±0.07), *Phormidium* (0.66±0.07) and lowest in *Calothrix* (0.56±0.02). The maximum protein content was observed in *Nostoc* (2.84±0.09) and minimum in *Cylindrospurmum* (1.13±0.07). The carbohydrate content was highest in case of *Phormidium* (3.97±0.79) followed by *Anabaena* (3.23±0.25), *Nostoc* (3.06±0.23), *Oscillatoria* (2.30±0.02), *Calothrix* (1.98±0.07) and lowest in *Cylindrospurmum* (1.11±0.06). The phycocyanin content was also significant *i.e. Anabaena* (4.99±0.06), *Oscillatoria* (3.13±0.12), *Nostoc* (3.3±0.02), *Cylindrospurmum* (2.1±0.01), *Phormidium* (2.0±0.06) and *Calothrix* (0.97±0.02), respectively. The allophycocyanin content in *Phormidium* (3.8±0.07) followed by *Calothrix* (2.64±0.51), *Anabaena* (2.57±0.72), *Nostoc* (2.4±0.18), *Oscillatoria* (1.03±0.01) was reported. The lowest content of allophycocyanin was observed in *Cylindrospurmum* (0.6±0.02). The Phycoerythrin content was found maximum in *Anabaena* (2.21±0.03), followed by (1.44±0.12 & 1.29±0.06) in *Calothrix* and *Phormidium* ; in *Ocillatoria* and *Nostoc* (1.22±0.05 & 0.87±0.05), respectively. The lowest content was found in *Cylindrospurmum* (0.21±0.04).

In the present study the highest Chlorophyll A was reported in case of *Anabaena*. Results indicate that chlorophyll A content increased with increasing incubation period. Similarly Sheekh *et al*. (2015) recorded significant Chlorophyll A in *N. calcicola*, *A. variabilis* and *N. Linkia*. Amalina and Jayashree (2017) reported significant amount of Chlorophyll-a in the *L. holdenil*. The highest Carotenoid content was observed in *Oscillatoria*. The results are in accordance with Narayanan *et al*. (2006); Bakiyaraj *et al*. (2014) who reported the good amount of carotenoids content in *O. pseudogeminata* and *Nostoc*.

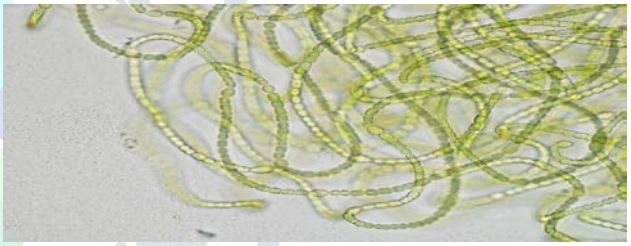
During our study the amounts of protein and carbohydrate were analysed in *Nostoc* and *Phormidium*. From the literature, maximum protein content has been reported in *N. punctiforme*. Similarly,Thamizh and Sivakumar (2012) has reported increased total soluble proteins and total carbohydrate contents. The protein content was more than the carbohydrates in *A. aconstricta*. But in *O. curviceps*, the level of total carbohydrates was higher. Gribovskaya *et al*. (2009) reported that the significant protein and carbohydrates were found in the *Oscillatoria* sp. Mishra *et al*. (2004) analysed the protein and carbohydrates in *Anabaena* sp. and *Calothrix* sp. in the soil cyanobacteria. The present study shows that the level of phycocyanin was more than allophycocyanain and phycoerythrinin in the isolated cyanobacterial strains. The highest Phycocyanin pigment was observed in *Anabaena*. Badrish *et al*. (2006) reported similar type of results regarding phycocyanin in *Oscillatoria* sp. The highest allophycocyanin content was recorded in *Phormidium*. Narayan *et al*. (2006) reported that the highest content of allophycocyanin was present in *Anabaena*, whereas the maximum content of Phycoerythrin in *N. Punctiforme*. In case of *Nostoc* and *Calothrix*, Phycoerythrin was highest and *N. calcicola* showed highest significant amount of phycoerythrin (Sheikh *et al*., 2015).

CONCLUSION

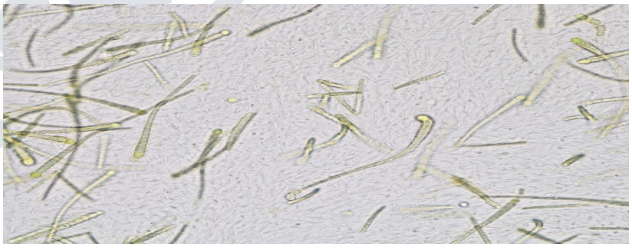
The present study reveals that the six Cyanobacterial strains isolated from paddy fields of Western U.P. region , seem to be a rich source of chlorophyll A, carotenoids, proteins, carbohydrate and phycobiliproteins. Due to rich biochemical contents these Cyanobacterial strains may be of use in the agricultural, food industry and biotechnological applications in near future.



a). *Nostoc* sp.

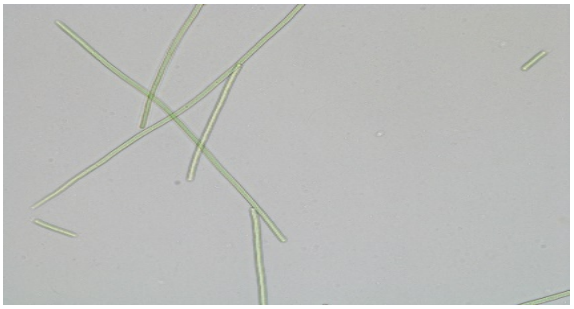


b). *Anabaena* sp.

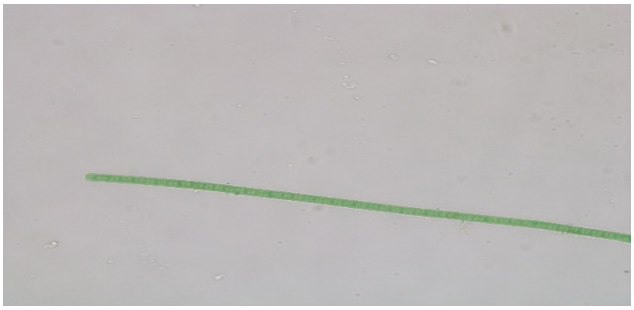


c). *Calothrix* sp.

d). *Cylindrospermum* sp.



e). *Phormidium* sp.



f). *Oscillatoria* sp.

Fig.1a-f: Photo micrograph of Cyanobacterial isolates

Table 1: Comparative chlorophyll, carotenoids, proteins, carbohydrate, phycocyanin, allophycocyanin and phycoerythrin content (µg ml^{-1}) of various cyanobacterial strains

Isolate(s)	Chl A	Carotenoid	Protein	Carbohydrate	Phycocyanin	Allophycocyanin	Phycoerythrin
Anabaena	2.54±0.06	0.93±0.14	2.54±0.32	3.23±0.25	4.99±0.06	2.57±0.72	2.21±0.03
Nostoc	2.08±0.34	1.23±0.25	2.84±0.09	3.06±0.23	3.3± 0.02	2.4± 0.18	0.87± 0.05
Oscillatoria	1.87±0.21	1.55±0.01	2.28±0.23	2.30±0.02	3.13±0.12	1.03±0.01	1.22±0.57
Calothrix	1.13±0.43	0.56±0.02	1.98±0.07	2.17±0.9	0.97±0.02	2.64±0.51	1.44±0.12
Cylindrospermum	0.09±0.08	0.68±0.07	1.13± 0.07	1.11±0.06	2.1± 0.06	0.6± 0.02	0.21± 0.04
Phormidium	0.45±0.01	0.66±0.07	1.50± 0.01	3.97±0.079	2.0± 0.06	3.8± 0.07	1.29± 0.06

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