PHYSIOLOGICAL ATTRIBUTES OF SELECTED CYANOBACTERIAL STRAINS ISOLATED FROM PADDY FIELDS OF WESTERN U.P.

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ABSTRACT

The aim of the present work was to study the biochemical constituents of six strains of Cyanobacteria isolated from paddy fields of western Uttar Pradesh. The biochemical constituents were analysed in terms of total chlorophyll, carotenoids, total proteins, total carbohydrates and phycobiliproteins content of all Cyanobacterial strains Anabaena sps., Nostoc sps., Oscillatoria sps., Calothrix sps., Cylindrus purpurescens, and Phormidium sps. were analysed. The analysis showed that maximum chlorophyll-a content in Anabaena, Carotenoids in Oscillatoria. Protein in Nostoc, Carbohydrate in Phormidium, Phycocyanin in Anabaena, Allophycocyanin in Phormidium, Phycocyanin in Anabaena respectively. The results of this experiment revealed that in food, pharmaceutical, cosmetics industries and biotechnological applications.

Key words: Cyanobacterial strains, Nutraceuticals and Physiological parameters.

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. 2008), although the majority of forms examined so far exhibit phototrophy. Cyanobacteria occupy almost every niche of the earth, including fresh and salt waters, paddy fields, hot springs, arid deserts, and polar regions (Thajuddin and Subramanian, 2005, Wilkie et al., 2011). Their diversity ranges from unicellular to multicellular, coccolid to branched filaments, nearly colourless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkaliophiles, planktonic to barophilic, fresh water to marine including hypersaline (salt pans). Cyanobacteria are an ancient group of prokaryotic with the ability to perform functions like nitrogen fixation and photosynthesis. The capacity of several Cyanobacteria to fix atmospheric nitrogen 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 & 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 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 favorable environment for growth of and nitrogen fixation by cyanobacteria, meeting their requirements for light, water, elevated temperature 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 oxygen 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 (Cifferi et al., 1985; Pulz et al., 2004; Tan, 2007; Cardozo et al., 2007). This work aimed to obtain cyanobacterial isolates from paddy fields, 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 strains from soil samples

The soil samples were collected from selected rice fields of Muzaffarnagar, Meerut region of western U.P. Ten gram of the soil sample was transferred to a 250ml flask containing 90ml sterile distilled water and shaken (120 rpm) for 30 mins. 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 ,at 28 ± 2°C under a light intensity of 52-55 \(\mu\)mol photon m\(^{-2}\) s\(^{-1}\) and L: D cycles of 16:8 hours. The resulting cyanobacterial isolates were maintained in their isolation media at 28±2°C in laboratory conditions and repeated subculturing was performed a number of times until pure axenic culture were obtained.(Rippka et al., 1979). Protocol was optimized for better results (Kumar et al).

**Identification of Cyanobacterial species:**

Identification of cyanobacterial species were done microscopically based on morphological as well as taxonomical observation, the length and the width of the vegetative cells ,also the width of the sheath, type of spores, presence or absence of hormogonia, presence or absence of spores and its position, number of heterocysts and its repetition, presence of akinetes and its type, the nature of cell wall, presence or absence of gas vacuoles, as well as pigment color was taken in consideration according to standard monographs (Desikachary, 1959; Santra, 1993). The photomicrographs were taken using with a fluorescent microscope (Fig.1).

**Physiological parameters**

**Estimation of Chlorophyll (McKinney, 1941)**

A known volume (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%) in a water bath (60°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)**

A known volume 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 85% acetone 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) 5% sodium carbonate

(ii) 0.5% copper sulphate (CuSO\(_4\).5H\(_2\)O) 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

A known volume (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 in running tap water. Subsequently, 2.5 mL of reagent (b) was added in each and the tubes were incubated at room temperature for 10 mins. After this, 0.5 mL of reagent (c) was added and the tubes were kept at room temperature for 15 mins. The intensity of blue colour was read as absorbance at 650 nm against appropriate blank. The protein content was estimated using a standard calibration curve prepared from bovine serum albumin and expressed in terms of mg/mL.

**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°C to dissolve the ingredients completely.

(ii) Procedure: A known volume (0.25-0.5 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 mins., the tubes were brought to room temperature and absorbance was read at 625 nm. The sugar content was calibrated against the standard curve made with glucose and expressed in terms of mg/mL.

**Estimation of Phycobiliproteins** (Bennet 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\(_2\)HPO\(_4\)). 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 652nm against 0.05M phosphate buffer as blank.

**Results**

Six Cyanobacterial species were isolated from paddy fields which were Anabaena sps., Nostoc sps., Oscillatoriasps., Calothrix sps., Cylindrosparmumsps, and Phormidiurnspss. were analysed.. The biochemical analysis of Chlorophylla, Carotenoids, Protein,
Carbohydrates, and phycobiliproteins content of all Cyanobacterial strains were presented in (Table-1). The Chlorophyll-a had showed a very significant content. The maximum Chlorophyll-a present in Anabaena (2.54±0.06), followed by Nostoc (2.08±0.34), Oscillatoria (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 content in Oscillatoria (1.55±0.01) followed by Nostoc (1.23±0.25), Anabaena (0.93±0.14) Cylindrospermium (0.68±0.07), Phormidium (0.66±0.07) and lowest content was found in Calothrix (0.56±0.02). The maximum protein content was found in Nostoc (2.84±0.09) and minimum in Cylindrospermum (1.13±0.07), the protein content present in Anabaena (2.54±0.32), Oscillatoria (2.28±0.23), Calothrix (1.98±0.07) and Phormidium (1.5±0.01) respectively. The carbohydrate content was highest in 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 showed lowest content Cylindrospermum (1.11±0.06). The phycocyanin (PC) content had shown very significant higher content in Anabaena (4.99±0.06) followed by Oscillatoria (3.13±0.12), Nostoc (3.4±0.02), Cylindrospermum (2.12±0.01), Phormidium (2.0±0.06) and Calothrix (0.97±0.02) respectively. Likewise the allophycocyanin (APC), the highest content was recorded 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), however the lowest content of allophycocyanin was observed in Cylindrospermum (0.6±0.02). The Phycoerythrin (PE) content was maximum in Anabaena (2.21±0.03), followed by (1.44±0.12 & 1.29±0.06) in Calothrix and Phormidium In Oscillatoria and Nostoc was (1.22±0.05 & .87±0.05) respectively. Whereas the lowest content was observed in Cylindrospermum (0.21±0.04), under the study.

Discussion

The results of the biochemical analysis of Cyanobacteria isolated from paddy fields showed the high amount of biochemical contents. In the present study the highest Chlorophyll-a was present in the Anabaena. Result indicated that chlorophyll-a content increased with increasing incubation period. 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. holdenii. The highest Carotenoid content was recorded in Oscillatoria followed. The results are in agreement with Bakjyaraj et al., (2014), Narayanun et al., (2006) who stated that the maximum carotenoids content in O. pseudogeminata and Nostoc respectively. In the present study the level of protein and carbohydrate were observed in Nostoc and Phormidium. From the study, maximum protein content was observed in N. punctiforme. Similarly Thamizh and Sivakumar, (2012) suggests that the results on total soluble proteins and total carbohydrates increased. The protein content was more than the carbohydrates in A. aconstricta. But in O. curviceps, the level of total carbohydrates was high when compared with total protein. Gribovskaya et al., (2009) reported that the protein and carbohydrates were found in the Oscillatoria sp. Mishra et al., (2004) reported that the protein and carbohydrates were present in the Anabaena sp. and Calothrix sp. in the soil cyanobacteria. The present study shows the level of phycocyanin was more than allophycocyanin and phycoerythrin in the present investigation. The highest Phycocyanin pigment was observed in Anabaena. Badrish et al., (2006) reported similar type of result of phycocyanin in Oscillatoria sp. The highest allophycocyanin content was recorded in Phormidium. Similarly, Narayan et al., (2006) reported that the highest content of allophycocyanin presented in Anabaena, whereas 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 Sheik et al., 2015) respectively.

Conclusion

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

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References


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### Table 1: Comparative Chlorophyll, Carotenoids, Proteins and Phycobiliproteins (µg/ml⁻¹) in different cyanobacterial strains

<table>
<thead>
<tr>
<th>Cyanobacterial Species</th>
<th>Chlorophyll a (µg/ml⁻¹)</th>
<th>Carotenoids (µg/ml⁻¹)</th>
<th>Protein (µg/ml⁻¹)</th>
<th>Carbohydrate (µg/ml⁻¹)</th>
<th>Phycocyanin (µg/ml⁻¹)</th>
<th>Allo Phycocyanin (µg/ml⁻¹)</th>
<th>Phycoerythrin (µg/ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabaena</td>
<td>2.54±0.06</td>
<td>0.93±0.14</td>
<td>2.54±0.32</td>
<td>3.23±0.25</td>
<td>4.99±0.06</td>
<td>2.57±0.72</td>
<td>2.21±0.03</td>
</tr>
<tr>
<td>Nostoc</td>
<td>2.08±0.34</td>
<td>1.23±0.25</td>
<td>2.84±0.09</td>
<td>3.06±0.23</td>
<td>3.3±0.02</td>
<td>2.4±0.18</td>
<td>0.87±0.05</td>
</tr>
<tr>
<td>Oscillatoria</td>
<td>1.87±0.21</td>
<td>1.55±0.01</td>
<td>2.28±0.23</td>
<td>2.30±0.02</td>
<td>3.13±0.12</td>
<td>1.03±0.01</td>
<td>1.22±0.57</td>
</tr>
<tr>
<td>Calothrix</td>
<td>1.13±0.43</td>
<td>0.56±0.02</td>
<td>1.98±0.07</td>
<td>2.17±0.9</td>
<td>0.97±0.02</td>
<td>2.64±0.51</td>
<td>1.44±0.12</td>
</tr>
<tr>
<td>Cylindrospermum</td>
<td>0.09±0.08</td>
<td>0.68±0.07</td>
<td>1.13±0.07</td>
<td>1.11±0.06</td>
<td>2.1±0.06</td>
<td>0.6±0.02</td>
<td>0.21±0.04</td>
</tr>
<tr>
<td>Phormidium</td>
<td>0.45±0.01</td>
<td>0.66±0.07</td>
<td>1.50±0.01</td>
<td>3.97±0.079</td>
<td>2.0±0.06</td>
<td>3.8±0.07</td>
<td>1.29±0.06</td>
</tr>
</tbody>
</table>
Fig.1: Photo micrograph of Cyanobacterial strains

a) Nostoc
b) Anabaena
c) Calothrix
d) Cylindrospermum
e) Phormidium sp
f) Oscillatoria sp