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# PHOTOCHROMATIC ADAPTATION OF CERTAIN HETEROCYSTOUS CYANOBACTERIA OF RICE FIELDS OF ALLAHABAD U.P.

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**ABSTRACT** The present investigation deals with five heterocystous cyanobacterial strains belonging to two genera namely Anabaena(2) and Calothrix( 3) were isolated from rice fields of Allahabad District Uttar Pradesh.These strains were examined for the growth attributes and pigment composition(phycobilin).The selected strains may prove useful in biofertilizer technology and other applied aspects.

**KEYWORDS** : Blue green algae, rice fields, pigment composition, phycobilins.

## Introduction

Blue-green algae, also known as cyanobacteria are a major group of photosynthetic algae mainly refer to as 'pond scum'. Blue-green algae are most often blue green in colour, but can also be blue-green algae generally grow in lakes, ponds and slow-moving stream. The appearance of the colour of thallus of blue-green algae is due to the presence of pigments. Thus, pigments play an important role in characterization of blue-green algae. Phycobiliproteins which are water soluble pigments are the major accessory light harvesting pigment in blue-green algae, red algae and cryptomonad. These phycobiliproteins are broadly classified into three groups based on the spectroscopic properties viz. Phycoerythrin (red pigment, λ max 562 nm), Phycocyanin (blue pigment λ max 615 nm), Allophycocyanin (bluish green pigment λ max 652 nm). Each phycobiliproteins are comprised of two sub units α and β to which linear tetrapyrroles are covantely attached by a cystienthio ether bond. The chromophoric protein along with some colourless linker polypeptide form organized structure called phycobilisomes. The phycobilisomes absorb light energy from different light qualities and transfer it finally to chlorophyll a of PS II. Phycoerythrin (PE) absorb green light and fluoresces orange. The transfer of excitation energy from PE to chlorophyll a is facilitated by phycocyanin (PC) and allophycocyanin (APC). The former absorbs in the orange and fluoresces in the red. The fluorescence band of the latter overlaps the absorption band of APC, so that the excitation energy can be funneled into chlorophyll a. This light harvesting system is highly efficient and allows blue-green to absorb and transfer green light to chlorophyll. Thus, blue-green algae could change their pigmentation (particularly phycobiliproteins in response to wave length of light incident upon these organisms). This control of pigmentation by light was termed as complementary chromatic adaptation.

According to their response to spectral composition of light blue-green algae can be divided into three groups (Carr&Whitton, 1973):

**Group-1** Organism synthesizes constant level of PE and PC irrespective of light. Blue-green algae can alter phycobilins size and number in response to light used but do not markedly alter the absorbance characteristic of their phycobilisomes.

**Group-2** Phycoerythrin is regulated by light quality in the sense that green light produces higher levels whereas red light lower levels of PE. There are no such changes in PC. Blue-green algae can alter the levels of PE in the phycobilisomes.

**Group-3** They show elevated level of PC and reduced levels of PE in response to red light but just reverse in green light. Blue-green algae can modulate both PE and PC levels of Phycobilisomes via a process of complementary chromatic adaptation.
The excitation energy absorbed by PE is transferred sequentially to PC, APC and then to the chlorophyll molecules associated with the reaction centres of photosynthesis.

Material and Methods
The effect of light quality was studied by pigment analysis of 10 strains of Blue-green algae. Cellular pigment compositions of phycobiliproteins were determined after photoautotrophic growth under green, red and white light after 30 days. Phycobilins were estimated by the method as described by Bennet and Bogorad (1971).

**Reagent** – Potassium phosphate buffer.

## PROCEDURE

The blue-green algal suspension was centrifuged at 3000 – 5000 rpm for 20 – 30 minutes, chilled phosphate buffer (pH = 7.5) was prepared and added in each tube containing algal pellet. Tubes were kept in freezer and continuously freezing and thawing was done till 72 hours. When pigments were extracted, it was left at room temperature and optical density was taken by spectrophotometer at 562 nm, 615 nm and 652 nm wavelengths. Phycobilins were calculated by using following formula :-

$$\text{Phycocyanin } (\mu g / ml) = \frac{OD_{615} - 0.474\,(OD_{652})}{5.34}$$

$$\text{Phycoerythrin } (\mu g / ml) = \frac{OD_{562} \times 2.41\,(PC) - 0.849\,(APC)}{9.62}$$

$$\text{Allophycocyanin } (\mu g / ml) = \frac{OD_{652} - 0.208\,(OD_{615})}{5.09}$$

**Preparation of Phosphate buffer:** Reagent KH2PO4 and K2HPO4. This solution was prepared by mixing equal 1 M of KH2PO4 and 0.1 M of K2HPO4.

## OBSERVATION

For statistical analysis we are using two-way analysis of variance (ANOVA) for which we propose the following NULL HYPOTHESIS regarding the effect of the colours on phycobilisomes. The proposed hypothesis is as follows:

Ho1: there are no variations in colours.
Ho2: there are no variations in phycobilins (PBS)
Ho3: there are no variations in interaction among phycobilins and incident light (PBS*COLOUR).

**Group 1:** Neither phycoerytrin synthesis nor phycocyanin production affected by the spectral composition of light.

**NONE OF THE SPECIES COMES UNDER THIS CATEGORY**
**Group 2** Phycoerythrin is always present and its synthesis is

increased in green light. Phycocyanin level remains unchanged.

**TABLE 1A:** Observations of Anabaena doliolum

| LIGHT | PHYCOBILINS µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | PHYCOERYTHRIN | PHYCOCYANIN | ALLOPHYCOCYANIN | | | |
| GREEN | 0.317 | 0.216 | 0.288 | 0.233 | 0.11 | 0.136 |
| RED | 0.192 | 0.284 | 0.190 | 0.120 | 0.047 | 0.087 |
| WHITE | 0.182 | 0.192 | 0.133 | 0.136 | 0.05 | 0.064 |

**ANOVA TABLE1 A** of Anabaena doliolum

| SOURCE OF VARIATIONS | DEGREES OF FREEDOM | SS | MSS | F cal | F tab |
|---|---|---|---|---|---|
| COLOUR | 2 | 0.032164 | 0.016082 | 13.16283 | 4.26 |
| PBS | 2 | 0.070955 | 0.035478 | 29.03777 | 4.26 |
| PBS*COLOUR | 4 | 0.008996 | 0.002249 | 1.840658 | 3.63 |
| ERROR | 9 | 0.010996 | 0.001222 | | |
| TOTAL | 17 | 0.123091 | | | |

**TABLE 1B:** Observation of Calothrix fusca

| LIGHT | PHYCOBILINS µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | PHYCOERYTHRIN | PHYCOCYANIN | ALLOPHYCOCYANIN | | | |
| GREEN | 0.860 | 0.806 | 0.282 | 0.265 | 0.260 | 0.240 |
| RED | 0.627 | 0.369 | 0.370 | 0.249 | 0.359 | 0.346 |
| WHITE | 0.770 | 0.744 | 0.338 | 0.330 | 0.278 | 0.323 |

**ANOVA TABLE 1B** of Calothrix fusca

| SOURCE OF VARIATIONS | DEGREES OF FREEDOM | SS | MSS | F cal | F tab |
|---|---|---|---|---|---|
| COLOUR | 2 | 0.036999 | 0.0185 | 14.12176 | 4.26 |
| PBS | 2 | 0.780932 | 0.390466 | 298.0656 | 4.26 |
| PBS*COLOUR | 4 | 0.012788 | 0.003197 | 2 .440458 | 3.63 |
| ERROR | 9 | 0.01179 | 0.00131 | | |
| TOTAL | 17 | 0.842509 | | | |

**FIG. 13A: PHOTOCHROMATIC ADAPTATION IN *Anabaena doliolum***

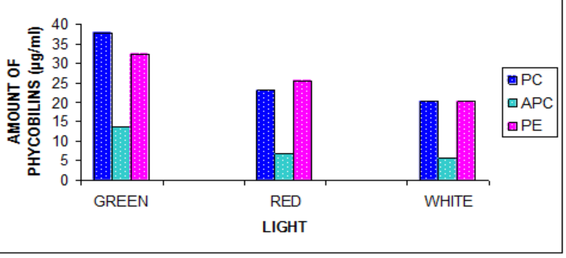
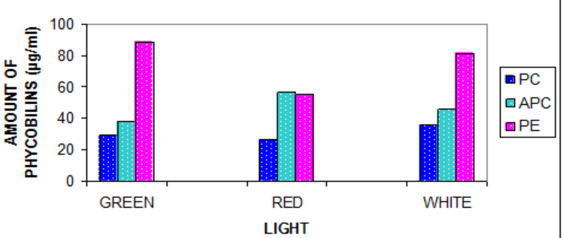



**FIG. 13B: PHOTOCHROMATIC ADAPTATION IN *Calothrix fusca***




## RESULT & DISCUSSION

From the ANOVA Table C of Anabaena doliolum, it can be seen that Fcal>Ftab for only the colour and PBS while it was found that Fcal<Ftab for the interaction effect. Hence, it was decided that the variation in colour and PBS are significant at 5% level of significance while the interaction effect may not be significant. Thus, this was classified in to group 2.

From the ANOVA Table D of Calothrix fusca it can be seen that Fcal>Ftab for only the colour and PBS while Fcal<Ftab for the interaction effect. Hence, it was decided that the variation in colour and PBS are significant at 5% level of significance while the interaction effect may not be significant. Thus, this was classified in to group 2.

In the strain Anabaena doliolum and Calothrix fusca belonging to group IInd. It was found that phycoerythrin is always present and its synthesis is increased in green light, phycocyanin level remains unchanged (13 A& B).

N. Tandeau De Marsac, 1976; among cyanobacteria that do adopt chromatically, two physiological species can be distinguished. In 7 strains of group IInd only the synthesis of phycoerythrin is significantly affected, the rate of its formation being much higher in green than in red light. However, phycoerythrin synthesis never ceases completely in red light.

**Group 3** Phycoerythrin synthesis induced in green light and suppressed jn red light, phycocyanin is always present and its synthesis is enhanced in red light.

**TABLE 2A:** Observations of phycobiliproteins in Anabaena variabilis

| LIGHT | PHYCOBILINS µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | PHYCOERYTHRIN | PHYCOCYANIN | ALLOPHYCOCYANIN | | | |
| GREEN | 0.385 | 0.393 | 0.105 | 0.134 | 0.093 | 0.107 |
| RED | 0.262 | 0.240 | 0.909 | 0.857 | 0.336 | 0.332 |
| WHITE | 0.206 | 0.228 | 0.558 | 0.596 | 0.233 | 0.216 |

**ANOVA TABLE 2A** of Anabaena variabilis

| SOURCE OF VARIATIONS | DEGREES OF FREEDOM | SS | MSS | F cal | F tab |
|---|---|---|---|---|---|
| COLOURS | 2 | 0.24642 | 0.12321 | 340.0461 | 4.26 |
| PBS | 2 | 0.666971 | 0.333486 | 920.3837 | 4.26 |
| PBS*COLOUR | 4 | 0.075725 | 0.018931 | 52.24785 | 3.63 |
| ERROR | 9 | 0.003261 | 0.000362 | | |
| TOTAL | 17 | 0.992377 | | | |

**TABLE 2B:** Observation of Calothrix braunii

| LIGHT | PHYCOBILINS µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | PHYCOERYTHRIN | PHYCOCYANIN | ALLOPHYCOCYANIN | | | |
| GREEN | 0.155 | 0.192 | 0.068 | 0.085 | 0.074 | 0.077 |
| RED | 0.110 | 0.116 | 0.178 | 0.229 | 0.087 | 0.108 |
| WHITE | 0.260 | 0.303 | 0.140 | 0.161 | 0.086 | 0.093 |

**ANOVA TABLE 2B** of Calothrix braunii

| SOURCE OF VARIATIONS | DEGREES OF FREEDOM | SS | MSS | F cal | F tab |
|---|---|---|---|---|---|
| COLOUR | 2 | 0.011041 | 0.005521 | 14.21181 | 4.26 |
| PBS | 2 | 0.029796 | 0.014898 | 38.35297 | 4.26 |

| | | | | | |
|---|---|---|---|---|---|
| PBS*COLOUR | 4 | 0.036677 | 0.009169 | 23.60505 | 3.63 |
| ERROR | 9 | 0.003496 | 0.000388 | | |
| TOTAL | 17 | 0.08101 | | | |

**TABLE 2C:** Observation of Calothrix javanica

| LIGHT | PHYCOBILINS µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | PHYCOERYTHRIN | PHYCOCYANIN | ALLOPHYCOCYANIN | | | |
| GREEN | 0.207 | 0.190 | 0.086 | 0.075 | 0.08 | 0.074 |
| RED | 0.237 | 0.220 | 0.532 | 0.524 | 0.228 | 0.222 |
| WHITE | 0.01 | 0.011 | 0.032 | 0.034 | 0.006 | 0.004 |

**ANOVA TABLE 2C** of Calothrix javanica

| SOURCE OF VARIATIONS | DEGREES OF FREEDOM | SS | MSS | F cal | F tab |
|---|---|---|---|---|---|
| COLOUR | 2 | 0.301399 | 0.1507 | 714.2156 | 4.26 |
| PBS | 2 | 0.088558 | 0.044279 | 209.8531 | 4.26 |
| PBS*COLOUR | 4 | 0.052468 | 0.026234 | 124.3318 | 3.63 |
| ERROR | 9 | 0.000422 | 0.000211 | | |
| TOTAL | 17 | 0 .442847 | | | |

**FIG. 14A: PHOTOCHROMATIC ADAPTATION IN *Anabaena variabilis***

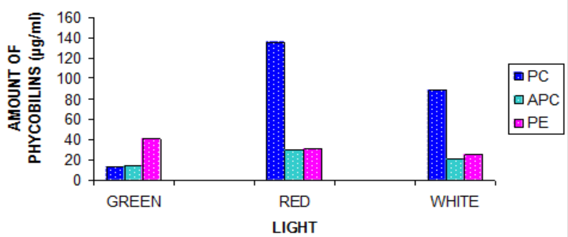



**FIG. 14E: PHOTOCHROMATIC ADAPTATION IN *Calothrix braunii***

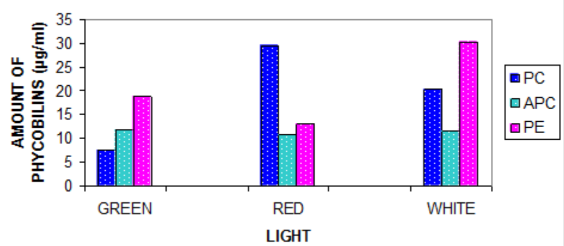
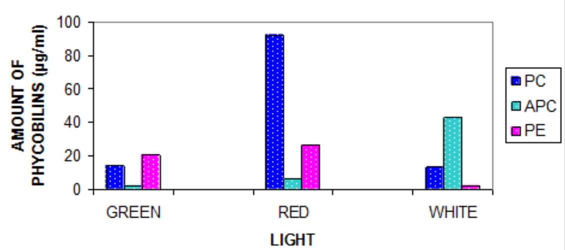



**FIG. 14F: PHOTOCHROMATIC ADAPTATION IN *Calothrix javanica***




## RESULT & DISCUSSION

From the ANOVA Table E of Anabaena variabilis, it can be seen that Fcal>Ftab for all the three i.e. for colour, PBS and the interaction PBS*colour. Hence, it was decided that the variations in all the three are significantly present at 5% level of significance. Thus, this was classified into group 3.

From the ANOVA Table I of Calothrix braunii, it can be seen that Fcal>Ftab for all the three i.e. for colour, PBS and the interaction PBS*colour. Hence, it was decided that the variations in all the three are significantly present at 5% level of significance. Thus, this was classified into group 3.

From the ANOVA Table J of Calothrix javanica, it can be seen that Fcal>Ftab for all the three i.e. for colour, PBS and the interaction PBS*colour. Hence, it was decided that the variations in all the three are significantly present at 5% level of significance. Hence, this was classified into group 3.

The Anabaena variabilis, Calothrix braunii, Calothrix javanica strains which belongs to group IIIrd were examined for photochromatic adaptation and found that phycoerythrin synthesis is induced in green light and suppressed in red light, phycocyanin is always present and its synthesis is enhanced in red light (14 A-F).

N. Tandeau De Marsac, 1976; observed that in group IIIrd light quality affects both phycoerythrin and phycocyanin synthesis. In green light the differential rate of phycoerythrin synthesis is much higher, than in red light, whereas that of phycocyanin is much lower.

## CONCLUSION

Photochromatic adaptation of 05 strains was studied. The result indicated that None of the species belongs to this group Ist In Anabaena doliolum and Calothrix fusca more red colour was produced in green light which indicated that phycoerythrin synthesis was higher in green light and their was no change in phycocyanin and so it was concluded that both strains belong to group Iind.

However, in Anabaena variabilis, C. braunii and C. javanica more red colour was produced in green light and more blue colour was produced in red light that revealed that all the five strain showed photochromatic adaptation in green and red light and all five strains belong to group IIIrd.

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Abberiviation:
PBS : Phycobilins
APC: allophycocyanine
PC: phycocyanine
PE: Phycoerythrine

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