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HETEROCYSTOUS CYANOBACTERIA: PHOTOCHROMATIC ADAPTATION STUDIES

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ABSTRACT

The present algal investigation involves heterocystous cyanobacterial strains belongs to two genera namely *Nostoc* and *Scytonema*. In the present study we have taken four different species of *Nostoc* and a single species of *Scytonema*. These strains were isolated from rice fields of Allahabad District Uttar Pradesh, India. These strains were examined for its growth attributes and pigment composition (phycobilin). These strains are having special concern in microbiology as they are widely used as biofertilizer. Nostoc punctiforme Nostoc ellipsosporum Nostoc muscorum and Nostoc paludosum are the four different species of Nostoc selected for the present study while we have taken single species of Scytonema i. e., Scytonema hofmanni.

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

INTRODUCTION

Blue-green algae are prokaryots and are having oxygen evolving photosynthetic system. Inspite of that most of them are capable of fixing of atmospheric nitrogen. These blue green algae are also natural source of accessory pigment like chlorophyll, carotenoid and phycobiliproteins, They appear blue-green due to the presence of a pigments and this character makes them unique.

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 centers of photosynthesis.

MATERIAL AND METHODS

The effect of light quality was studied by pigment analysis of 05 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

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

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

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

Preparation of Phosphate buffer

Reagent KH_2PO_4 and K_2HPO_4. This solution was prepared bymixing equal 1 M of KH_2PO_4 and 0.1 M of K_2HPO_4.

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.

Table 1a: Observation of *Nostoc punctiforme.*

Light	PHYCOBILINS µg/ml					
	Phycoerythrin		Phycocyanin		Allophycocyanin	
Green	0.379	0.645	0.302	0.541	0.147	0.274
Red	0.495	0.176	0.478	0.170	0.245	0.069
White	0.306	0.228	0.292	0.221	0.113	0.084

Anova Table 1a: of *Nostoc punctiforme.*

Source of variations	Degrees Of Freedom	SS	MSS	F cal	F tab
Colour	2	0.160018	0.080009	3.75598	4.26
Pbs	2	0.092953	0.046476	2.181808	4.26
Pbs*colour	4	0.011193	0.002798	.131361	3.63
Error	9	0.191716	0.021302		
Total	17	0.45588			

Table 1b: Observation of *Nostoc ellipsosporum*.

Light	PHYCOBILINS µg/ml					
	Phycoerythrin		Phycocyanin		Allophycocyanin	
Green	0.167	0.108	0.384	0.256	0.144	0.085
Red	0.143	0.128	0.331	0.332	0.071	0.066
White	0.095	0.092	0.208	0.203	0.208	0.116

Anova Table 1B: of *Nostoc ellipsosporum.*

Source of variations	Degrees of Freedom	SS	MSS	F cal	F tab
Colour	2	0.004267	0.002134	1.196504	4.26
Pbs	2	0.11821	0.055911	31.3557	4.26
Pbs*colour	4	0.026357	0.006589	3.495395	0.63
Error	9	0.016048	0.001783		
Total	17	0.164882			

FIG. 12A: PHOTOCHROMATIC ADAPTATION IN *Nostoc punctiforme*

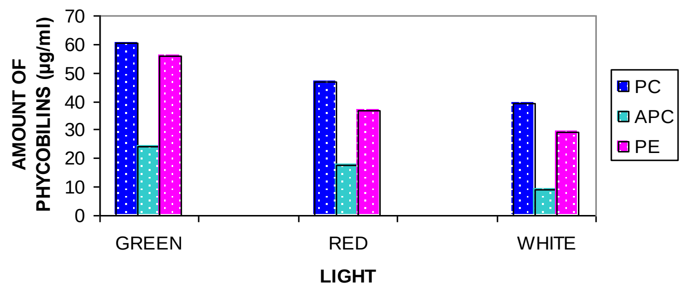
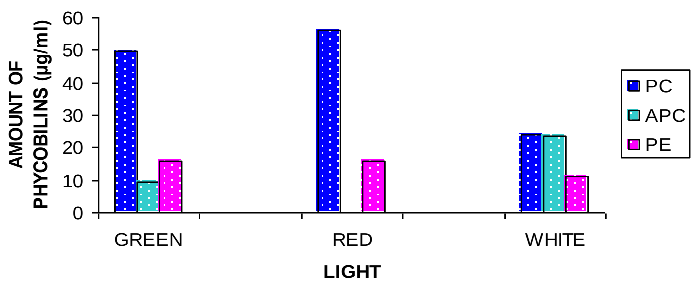


FIG. 12B: PHOTOCHROMATIC ADAPTATION IN *Nostoc ellipsosporum*



RESULT

From the ANOVA Table A of *Nostoc punctiforme*, it can be seen that Fcal<Ftab for all the three i.e. for colour, PBS and the interaction of PBS*COLOUR. Hence, it was decided that the variations in all the three are insignificant at 5%level of significance. Thus, it was concluded that it belongs to group 1.

However, from the ANOVA Table B of *N. ellipsosporum*, it can be noted that Fcal>Ftab in phycobilisomes which are significant at 5% level of significance while in colour and interaction effect of PBS*colour Fcal<Ftab that means colour and interaction effect both are insignificant at 5% level of significance. Hence, this was classified in to group 1.

The strain Nostoc punctiforme & Nostoc ellipsosporum belongs to group I$^{st.}$ After examining this group it was found that neither phycoerythrin synthesis nor phycocyanin production is affected by the spectral composition of light (Fig. 12 A & B).

According to N. Tandeau De Marsac, 1976 not all phycoerythrin containing cyanobacteria can adopt chromatically. No adaptation was observed in 12 of the 44 strains examined. In such cyanobacteria of group Ist the relative rate at which 3 phycobiliproteins are synthesize seen to be constant and characteristic for each strain either grown in white light, in chromatic light or in the dark.

Group 2

Phycoerythrin is always present and its synthesis is increased in green light Phycocyanin level remains unchanged.

None of the species belonging to this group

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: Observation of *Nostoc muscorum.*

Light	PHYCOBILINS µG/ML					
	Phycoerythrin		Phycocyanin		Allophycocyanin	
Green	.202	.193	.101	.104	.032	.025
Red	.093	.101	.111	.114	.034	.035
White	.096	.085	.113	.088	.021	.02

Anova Table 2b: of *Nostoc muscorum*.

Source of variations	Degrees Of Freedom	SS	MSS	F cal	F tab
Colour	2	3.37	1.68	0.315563	4.26
Pbs	2	0.033785	0.016892	316.7316	4.26
Pbs*colour	4	0.014173	0.003543	66.43753	3.63
Error	9	0.00048	5.33		
Total	17	3.418438			

Table 2c: Observation of *Nostoc paludosum.*

Light	PHYCOBILINS µG/ML					
	Phycoerythrin		Phycocyanin		Allophycocyanin	
Green	0.536	0.520	0.201	0.188	0.103	0.093
Red	0.231	0.359	0.464	0.788	0.229	0.410
White	0.431	0.334	0.237	0.166	0.178	0.176

Anova Table 2d: of *Nostoc paludosum.*

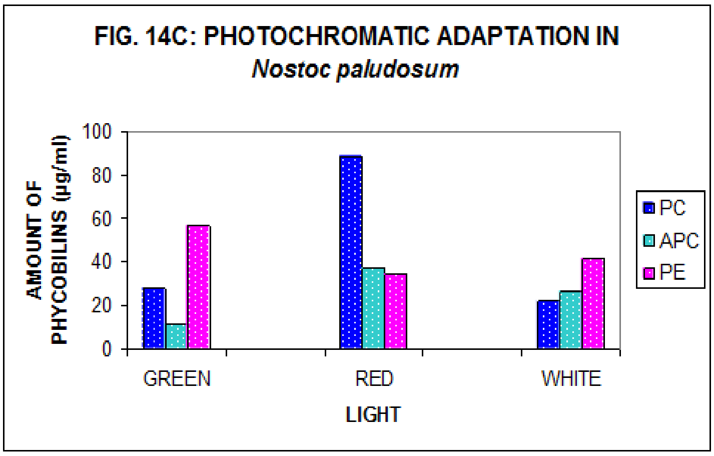
Source of variations	Degrees of freedom	SS	MSS	F cal	F tab
Colour	2	0.130594	0.065297	6.952077	4.26
Pbs	2	0.09092	0.04546	4.840078	4.26
Pbs*colour	4	0.25879	0.064698	6.888249	3.63
Error	9	0.084532	0.009392		
Total	17	0.564836			

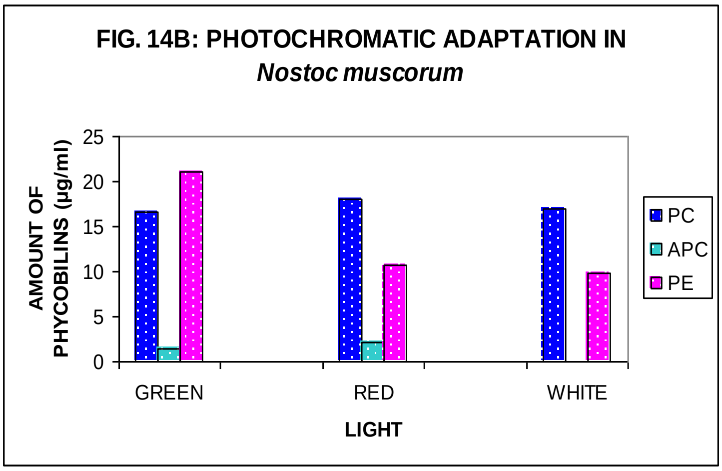
Table 2e: Observation of *Scytonema hofmanni.*

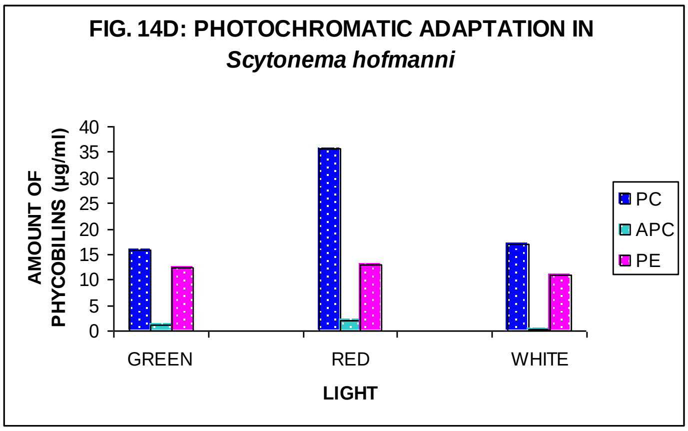
Light	PHYCOBILINS µg/ml					
	Phycoerythrin		Phycocyanin		Allophycocyanin	
Green	0.113	0.114	.097	.095	021	0.030
Red	0.115	0.114	0.205	0.207	0.025	0.04
White	0.087	0.112	0.102	0.103	0.022	0.025

Anova Table 2e: Of *Scytonema Hofmanni.*

Source of variations	Degrees of freedom	SS	MSS	F cal	F tab
Colour	2	0.006727	0.003363	63.72757	4.26
Pbs	2	0.03795	0.018975	359.5288	4.26
Pbs*colour	4	0.00888	0.00222	42.06473	3.63
Error	9	0.000475	5.28		
Total	17	0.054032			







DISCUSSION

From ANOVA Table F of *Nostoc muscorum*, it can be noted that the interaction effect PBS*colour is significant which means that the variation in colour and that of PBS making a significant effect when combined together. Hence, this was classified into group 3.

From the ANOVA Table G of *Nostoc paludosum*, 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 H of *Scytonema hofmanni*, 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 *Nostoc muscorum, N. Paludosum, Scytonema hofmanni* 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.

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 there was no colour changes in *Nostoc punctiforme* and *Nostoc ellipsosporum* due to effect of incident light (green, red and white),because synthesis of phycoerythrin and phycocyanin was not affected that is why both strains belong to group Ist.

However, in *N. muscorum*, *N. paludosum*, and *S. hofmanni* 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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