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Pigment Analysis of Some Blue Green Algae

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ABSTRACT

The present study deals with the physiological characterization are 24 different strains of blue green algae. It includes the pigment profiles which deals the estimation of chlorophyll, carotenoids and phycobilins (phycocyanin, allophycocyanin and phycoerythrin) in different strains. The pigment analysis showed that the maximum chlorophyll-a was estimated in Microchaete uberrima. The ratio of pigment in all the strains indicates the occurrence of maximum amount of phycobilins and then carotenoids. All these pigments are not present always in same relative proportion.

Key words: Chlorophyll, Carotenoids, Phycobilins, Pigment, Blue green algae

Blue-green Algae having Prokaryotic cell organization on one hand and oxygen producing photosynthetic apparatus on the other hand fall in twilight zone of Prokaryote and eukaryote. The thallus organization and occurrence in natural habitats of Blue-green algae are like that of the other algae but their certain biochemical attributes are like those of Bacteria. Blue-green algae are the only organism known to have simultaneously both the photosystems (Photosystem-I and Photosystem-II) and nitrogen fixing capability in the same organism. Photosynthetic and diazotrophic nature of Blue-green algae makes them perfect autotrophic and versatile organisms. Blue-green algae contain the pigments C-phycocyanin and C-phycoerythrin along with chlorophyll-A and carotenoids. These accessories pigment impart bluish colour in addition to green colour and perform photosynthesis. Numerous works are involved in revealing the qualities and capabilities of these organism (De 1939, Singh 1939, 1961, Mitra 1951, Pandey 1965, Padmaja 1968, Goyal 1971, 1984, Venkataraman 1972, 1981, Singh 1968, Singh 1973, 1979, 1989) and many others. The importance of the blue-green algae to enriching the tropical soils, particularly rice fields have been emphasized by De (1939), Stewart *et al*. (1969), Tiwari (1972, 1975, 1979), Venkataraman (1979). Blue-green algae are also known to improve the physic-chemical properties of the soils. Improvement in soil aggregation due to algal growth was reported by Sankaram (1971), Kaushik and Subhashini (1985).

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MATERIALS AND METHODS

Blue- green algae are manageable systems and can produce in bulk with ease (Watanabe and Yamamota 1970, Fao 1977, Venkataraman 1981). Cultural studies of selected strains have done at standard laboratory conditions in sterilized glassware and media. Estimation of algal strains at controlled conditions has given all the information about their physiological characteristics.

Chlorophyll-a, carotenoids and phycobilins were extracted by using methanol (95%), acetone (85%) and buffer (phosphate buffer) respectively. According to method given by Mackney (1941) the centrifuged algae pellets were taken in tube and poured 10ml 95% methanol in it. After 15-20 mts. Chlorophyll dissolved in it and reading for optical density were taken at 650nm and 665nm at spectrophotometer. Similarly, in centrifuged algal samples 85% acetone was added and freeze it overnight. Next day at room temperature reading were taken for optical density at 450nm. For phycobilins, chilled phosphate buffer was prepared and added in each tube containing centrifuged algal pellet. Kept in freezer and continuously freezing and thawing was done till 72 hours. Purple or blue coloured phycobilins were dissolved in buffer and readings were taken at 560nm, 615 and 652 nm respectively.

Observation: The colour of different strains depends on certain pigments, which varies in their proportions and combinations in different groups. The pigment can be distinguished into three types:

I. Chlorophyll
II. Carotenoids
III. Phycobilins.

Again, there are three categories of phycobilins:

Phycocyanin (PC)
Allophycocyanin (APC)
Phycoerythrin (PE)

Phycocyanin is the characteristic pigment of Cyanobacteria (BGA) which imparts blue colour to the thallus and chlorophyll and imparts green colour, so mostly Blue- green algae are blue green in appearance. But due to presence of relative proportion of more red-phycoerythrin, few members of BGA gives reddish brown colour to the thallus. All these pigments are not present always nor are in same relative proportion.

Estimation of chlorophyll-a

Chlorophyll-a was estimated after 15 days of growth in Bg-11 medium. From observations it is clear that after growth of 15 days maximum biomass in terms of chlorophyll-a was obtained in Microchaete-11 (13.27%) and Calothrix44 (10.86%), Aulosira-52 (7.42%) also responded very well, while minimum in Gloeotrichia-47 (3.11%), Nostoc.35 (3.17%), Tolypothrix-46 (3.83%) and Scytonema-12 (3.87%). The result indicates that all the stains showed chlorophyll-a production between 3.11% to 13.27%.

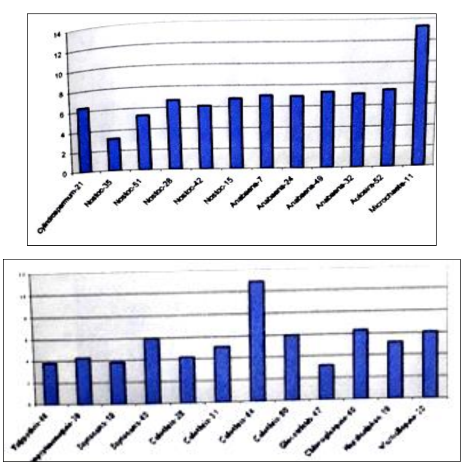

Fig 1 Percent of chlorophyll in different strains (In -N medium)

Following trend was observed

Michrochaete-11 (13.27%)> Calothrix-44 (10.86%)> Aulosira-52 (7.42%)> Anabaena-49 (7.31%)> Anabaena-24(6.95%)> Nostoc-15 (6.77%)> Nostoc-28 (6.67%) Cylindrospermum-21(6.39%)>Chlorogloeopsis-40 (6.34%)> Nostoc-42 (6.12%)> Westiellopsis-33 (6.06%)> Calothrix-50 (5.97%)> Scytonema-43(5.96)> Nostoc-51 (5.32%)> Haplosiphon-19 (5.17%)> Calothrix-31 (5.12%)> Camptylonemopsis-39 (4.25%)> Calothrix-25 (4.21%)>Scytonema-12 (3.87%)> Tolypothrix-46 (3.83%)> Nostoc-35 (3.17%)> Gloeotrichia-47 (3.11%) (Table 1, Fig 1).

Estimation of carotenoids

Carotenoid was determined after 15 days of growth in BG-11 medium. After growth of 15 days the result indicates that all the strains have carotenoids between 8.05% to 33.53%. Maximum carotenoid was observed in Nostoc-28 (33.53%) while minimum in Calothrix-31 (8.05%).

Following trend was observed

Nostoc-28 (33.53%) > Anabaena-49 (32.53%) > Nostoc-42 (31.52%) > Camptylonemopsis-39 (30.57%) > Calothrix-44 (29.19%) > Nostoc-15 (28.36%) > Westiellopsis-33 (27.04%) > Nostoc-51 (26.56%) > Scytonema-12 (26.32%) > Tolypothrix-46 (26.21%) > Aulosira-52 (25.65%) Haplosiphon-19 (25.62%) > Chlorogloeopsis-40 (24.53%) > Anabaena-32 (23.59%) > Anabaena-7 (23.36%) Cylindrospermum-21 (22.60%) > Calothrix-50 (21.45%) > Scytonema-43 (20.65%) > Gloeotrichia-47 (18.37%) > Nostoc-35 (17.46%) > Anabaena-24 (16.13%) > Calothrix-25 (13.70%) > Michrochaete-11 (9.42%) > Calothrix-31 (8.05%).

Table 1 Analysis of chlorophyll-a, protein and nitrogen BG-11 medium on 15 day of growth

Strains	Chlorophyll-a (ug/ml)
Cylindrospermum-21	0.656
Nostoc-35	0.435
Nostoc-51	0.425
Nostoc-28	0.515
Nostoc-42	0. 412
Nostoc-15	0.395
Anabaena-7	0.526
Anabaena-24	0.645
Anabaena-49	0.622
Anabaena-32	0.616
Aulosira-52	0.524
Microchaete-11	0.868
Tolypothrix-46	0.564
Camptylonemopsis-39	0.617
Scytonema-12	0.25988
Scytonema-43	0.293
Calothrix-25	0.342
Calothrix-31	0.397
Calothrix-44	0.452
Calothrix-50	0.376
Gleotrichia-47	0.292
Chlorogloeopsis-40	0.248
Westiellopsis-33	0.376
Hapalosiphon-19	0.289

Determination of phycobilins

There are three major categories of phycobilins %

Phycocyanin (PC)
Allophycocyanin (APC)
Phycoerythrin (PE)

Phycobilins were determined after 15 days of growth in BG-11 medium. From observation it is clear that maximum phycobilins was obtained in Calothrix-31 (86.83%) while minimum in Calothrix-44 (59.95%). Results indicate that all the strains showed phycobilins production between 59.80% to 86.83%.

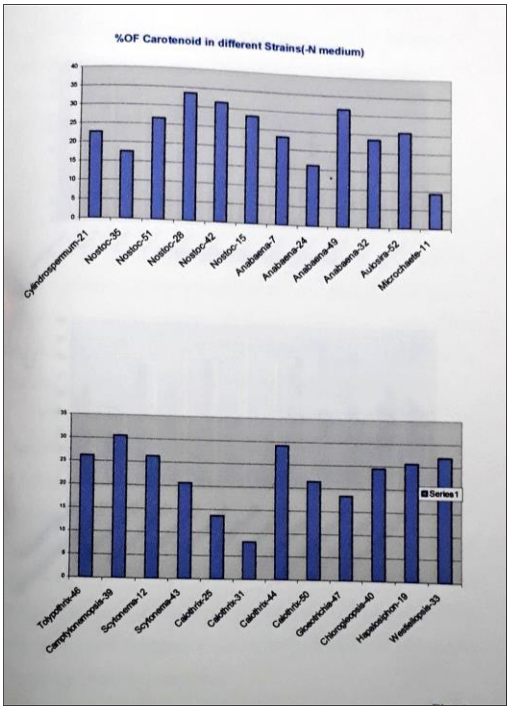

Fig 1 Percent of carotenoid in different strains (-N medium)

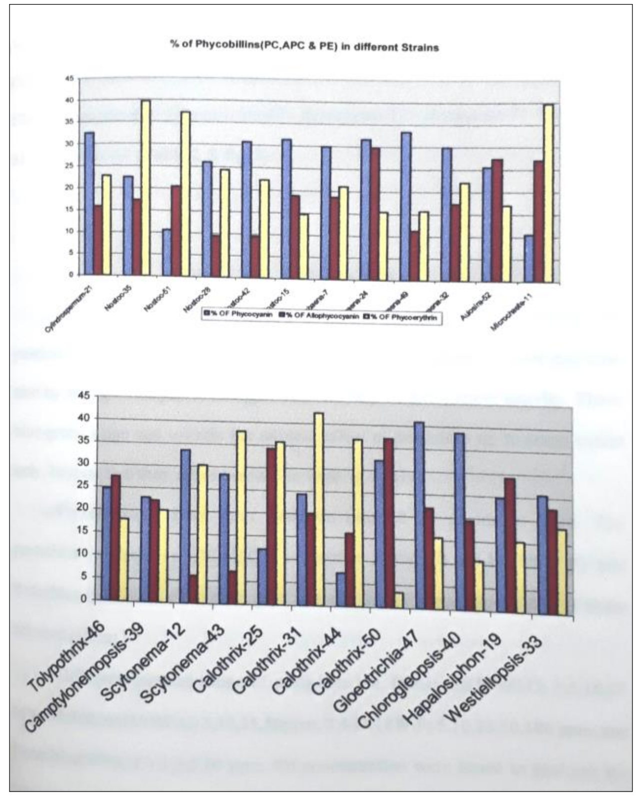

Fig 3 Percent of phycobolins (PC, APC and PE) in different strains

Table 2 Pigment analysis: Percentage of chlorophyll, carotenoids and phycobilins (PC, APC and PE) in strains

Strains	% of chlorophyll	% of carotenoids	% of phycocyanin	% of allophycocyanin	% of phycoerythrin	% of total phycobilins
Cylindrospermum-21	6.39	22.60	32.43	15.79	22.79	71.01
Nostoc-35	3.17	17.46	22.45	17.32	39.60	79.37
Nostoc-51	5.32	26.56	10.55	20.45	37.12	68.12
Nostoc-28	6.67	33.53	26.2	9.47	24.31	59.80
Nostoc-42	6.12	31.52	30.67	9.51	22.18	62.36
Nostoc-15	6.77	28.36	31.35	18.83	14.69	64.87
Anabaena-7	7.03	23.36	29.91	18.66	21.04	69.61
Anabaena-24	6.95	16.13	31.66	29.85	15.41	76.92
Anabaena-49	7.31	31.53	33.35	11.18	15.63	60.16
Anabaena-32	7.11	23.59	29.94	17.35	22.03	69.32
Aulosira-52	7.42	25.65	25.58	27.41	16.94	66.93
Microchaete-11	13.27	9.42	10.51	27.09	39.71	77.31
Tolypothrix-46	3.83	26.21	24.65	27.38	17.93	69.96
Camptylonemopsis-39	4.25	30.57	22.80	22.27	20.11	65.18
Scytonema-12	3.87	26.32	33.51	6.07	30.23	69.81
Scytonema-43	5.96	20.65	28.25	7.15	37.99	73.39
Calothrix-25	4.21	13.70	12.06	34.23	35.80	82.09
Calothrix-31	5.12	8.05	24.35	20.10	42.38	86.83
Calothrix-44	10.86	29.19	7.30	15.92	36.73	59.95
Calothrix-50	5.97	21.45	32.11	37.03	3.44	75.58
Gleotrichia-47	3.11	18.37	40.91	22.00	15.61	78.52
Chlorogloeopsis-40	6.34	24.53	38.71	19.53	10.89	69.13
Westiellopsis-33	6.06	27.04	25.55	22.58	18.49	66.62
Hapalosiphon-19	5.17	25.62	24.77	29.13	15.31	69.21

Following trend was observed

Calothrix-31 (86.83%) > Calothrix-25 (82.09%) > Nostoc-35 (79.37%) > Gloeotrichia-47 (78.52%) > Michrochaete-11 (77.31%) > Anabaena-24 (76.92%) > Scytonema-43 (73.39%) > Calothrix-50 (72.58%) > Cylindrospermum-21 (71.01%) > Tolypothrix-46 (69.96%) > Scytonema-12 (69.81%) Anabaena-7 (69.61%) > Anabaena-32 (69.32%) > Haplosiphon-19 (69.21%) > Chlorogloeopsis-40 (69.13%) > Nostoc-51 (68.12%) > Aulosira-52 (66.93%) > Westiellopsis-33 (66.62%) > Camptylonemopsis-39 (65.18%) > Nostoc-15 (64.87%) > Nostoc-42 (62.36%) > Anabaena-49 (60.16%) > Calothrix-44 (59.95%) > Nostoc-28 (59.80%) (Table 1, Fig 2)

RESULTS AND DISCUSSION

The pigment analysis showed that the maximum chlorophyll-a was estimated in Microchaete uberrima (Table 2, Fig 3). However, the pigment profile of this alga is being reported here for the first time. The result of (Table 2) show that generally increase of chlorophyll are also observed by other workers. The result of (Table 2) showed the percentage of various pigment (chlorophyll-a, carotenoids, phycocyanin, allophycocyanin and phycoerythrin) estimated in all the twenty-four strains (Fig 4). The ratio of pigment in all the strains indicated occurrence of maximum amount of phycobilins and then carotenoids. However, occurrence of chlorophyll-a is minimum.

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