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COMPARATIVE GROWTH POTENTIAL AND PHOTOSYNTHETIC PIGMENT ACCUMULATION IN SELECTED RIVULARIAN MEMBERS OF BLUE GREEN ALGAE

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SUMMARY

The growth as dry matter accumulation, generation time and pigment profiles (chlorophyll *a*, carotenoids and phycobiliproteins) were studied in ten filamentous diazotrophic, heteropolar rivularian members of blue green algae under controlled laboratory conditions. Out of the total isolates examined *Gloeotrichia* sp. exhibited a maximum dry weight accumulation while *Calothrix membranacea* accumulated lowest dry weight. The specific growth rate varied amongst various genera examined and the highest specific growth rate was shown by *Gloeotrichia* sp.. Generation time was maximum for *Calothrix javanica* and minimum for *Gloeotrichia* sp.. The highest chlorophyll concentration was observed in *Calothrix marchica* var. *crassa* and *Calothrix membranacea* and lowest in *Gloeotrichia raciborskii.* The carotenoid production was highest in *Calothrix fusca* var. *crassa* and lowest in *Calothrix elenkinii. Gloeotrichia raciborskii* produced highest phycobiliproteins (89.5% of total pigments) and the lowest production was by *Calothrix fusca* var. *crassa* (63.09 % of total pigments). Interestingly, all the rivularian isolates examined produced substantial amount of phycobiliproteins, a characteristic which is typical of the members of family Rivulariaceae.

Key words: **Blue green algae, growth, pigments, Rivulariaceae.**

INTRODUCTION

The blue green algae (BGA) occupy a unique position, as they possess an autotrophic mode of growth like eukaryotic plant cells and a metabolic system as that of bacteria. These organisms offer the growth potential of the microbial cells along with the light harvesting properties of plant cells making them ideal candidates for solar energy conversion. Algologists are, therefore, beginning to tap the vast reservoir of genetic resources and harness their potential to human needs. Blue green algae have a wide range of physiological and biochemical attributes and their potential for the production of biochemicals *vis-a-vis* pigments, hydrocarbons, vitamins, antibiotics, polysaccharides, essential fatty acids, bioflocculants, biosurfactants, enzymes and plant growth regulators has been recently recognized.

There is a shift in the need for production of natural pigments for use as food colours because of the proven carcinogenicity of the use of coltar base dye colours. This had led to commercial interest for selection of algal forms, which are technologically amenable for production of pigments. Blue green algae contain chlorophyll *a*, carotenoids and phycobiliproteins and these pigments under specialized physiological conditions may

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accumulate in larger amounts. Chlorophyll is a prime photosynthetic pigment and only chlorophyll *a* is present in blue green algae. Carotenoids have a wide commercial application and are used for natural food colouring (Emodi 1978), as feed adhesives, to enhance flesh colour of fish and egg yolk colour (Foss *et al.* 1984, Schiedt *et al.* 1985). They are also known to improve the health and fertility of low fed cattle (Jakson *et al.* 1981). These also function as an important photoprotective agents (Krinsky 1976) and as accessory pigments in photosynthesis. They play an important role in the physiology of vision and reproduction (Sporn *et al.* 1984 a,b). Carotenoid derivatives such as abscisic acid and vitamin A ratinol act as growth promoters and aid in cancer prevention (Peto *et al.* 1981). Biliprotein is another group of potentially important pigments. These are chromoproteins in which the prosthetic group is a bile pigment tightly bound by a covalent linkage to its apoprotein. Phycobiliproteins are expensive fine chemicals and are used as natural colours and as fluorescent dye (phycoflour probe) in immuno assay and are important components of diagnostic kits.

The first blue green alga *Calothrix indica* was isolated from Assam, India and is a member of family Rivulariaceae (Montagne 1949). In addition to *Calothrix, Gloeotrichia* and *Dichothrix* have also been studied extensively with mainly taxonomic consideration in mind. However, the data on their growth potentials and the pigment accumulation is sparse in the literature. Therefore, it was considered desirable to evaluate and study the comparative growth potential in terms of dry matter accumulation, specific growth rate, generation time and relative pigment composition (chlorophyll *a*, carotenoids and phycobiliproteins) in ten morphologically distinct selected blue green algal strains of family Rivulariaceae, order Nostocales obtained from different districts of Uttar Pradesh, India.

MATERIALS AND METHODS

Ten heterocystous, heteropolar akinete forming blue green algal strains belonging to family Rivulariaceae, order Nostocales representing seven species of *Calothrix,* two of *Gloeotrichia* and one of *Dichothrix* were maintained in BG-11 medium (Stanier *et al.* 1971) under controlled illumination (4 K lux) and light and dark cycle (14:10 h) at 28±2°C. The blue green algal strains were examined for their growth potential in terms of dry matter accumulation, specific growth rate, generaion time and pigment content during exponential phase of growth (15th day incubation). The physiological parameters exhibit an enhancement during this phase, which is consistent with general studies on life phases of blue green algae and other prokaryotic systems (Bremner and Dennis 1987). The experiments were replicated three times and the data obtained were subjected to statistical analysis using DMRT (Duncan's Multiple Range Test).

Biomass in terms of dry weight was measured on fifteenth day using hot air oven maintained at 60°C until the samples achieved constant weight (Sorokin 1973). Generation time was calculated by incubation of unialgal isolates and measuring the optical density of homogenized suspension at 716 nm (Guillard 1973). Chlorophyll *a* was estimated by hot methanol extraction method (Mackinney 1941). Carotenoids were extracted in acetone and examined at 450 nm (Jensen 1978). Phycobiliproteins were estimated by the absorbances read at 615, 650 and 562 nm (Bennett and Bogorad 1973) in phosphate buffer.

RESULTS AND DISCUSSION

There was a wide variation observed among different isolates examined with respect to growth parameters and the differences observed for dry weight and generation time was statistically significant. The range of dry weight for *Calothrix* was 133.20 - 239.79 μg/ml, for *Gloeotrichia* 166.50 - 286.38 μg/ml and for *Dichothrix* 153.18 μg/ml (Table 1). Out of the total isolates examined, *Gloeotrichia* sp. exhibited a maximum dry weight (286.38 μg/ml) followed by *Calothrix braunii* and *Calothrix marchica* var. *crassa* showing similar ranking as per DMRT studies. *Calothrix membranacea* and *Calothrix* sp. produced lowest dry matter. Similarly specific growth rate also varied amongst various isolates studied and this could be mainly attributed to different habitats from where the strains have been isolated. This parameter was also obviously highest in *Gloeotrichia* sp.

The generation time also exhibited variation among various genera studied. In different species of *Calothrix,* generation time varied from 77-185 h, whereas in *Gloeotrichia* species it varied from 44-96 h. In single species of *Dichothrix* examined, the generation time was

Table 1. Dry weight, specific growth rate and generation time of selected blue green algal strains from the family Rivulariaceae, order Nostocales

Rivularian strains	**Dry weight (μg/ml)**	**Specific growth rate ($g\ g^{-1}\ day^{-1}$)**	**Generation time (h)**
Calothrix braunii	233.10^{b}	0.21	114.00^{c}
Calothrix marchica var. *crassa*	239.76^{b}	0.22	109.00^{cd}
Calothrix elenkinii	186.48^{d}	0.31	77.00^{f}
Calothrix membranacea	133.20^{f}	0.28	86.00^{ef}
Calothrix sp.	133.20^{F}	0.15	160.00^{b}
Calothrix javanica var. *fertilissima*	186.48^{d}	0.13	185.00^{a}
Calothrix fusca var. crassa	213.95^{c}	0.23	104.00^{cd}
Dichothix baueriana	153.18^{e}	0.15	160.00^{b}
Gloeotrichia sp.	286.38^{a}	0.55	44.00^{g}
Gloeotrichia raciborskii	166.50^{e}	0.25	96.00^{de}
CD at 5% p	5.22		5.11

160 h (Table 1). Generation time was maximum for *Calothrix javanica* var. *fertilissima* (185 h) indicating it to be a slow grower. A significantly shorter generation time was exhibited by *Gloeotrichia* sp. which interestingly also produced maximum dry matter. Studies conducted on the selected members of family Rivulariaceae have clearly indicated that *Gloeotrichia* sp. was a fastest grower as compared to other isolates examined under cultural conditions. Kratz and Myers (1955) have also reported a negative correlation between two parameters (dry matter accumulation and generation time) in their study on prokaryotic systems. The strains screened for different growth potential certainly merit attention for future in-depth studies for their kinetics and mode of growth and possible commercial exploitation.

In comparison to the total pigments, the proportion of chlorophyll *a was* less (1-12% of total pigment) as compared to carotenoids and phycobiliproteins. This is a attribute in the members of family Rivulariaceae. Therefore, the strains may not have much commercial value for their chlorophyll content. The highest chlorophyll produced was by *Calothrix marchica* var. *crassa* and *Calothrix membranacea* while *Gloeotrichia raciborskii* produced lowest chlorphyll content (Table 2). *Calothrix braunii* and *Calothrix fusca* produced carotenoids above 25% of total pigments, whereas, *Calothrix elenkinii* produced 7.22% carotenoids out of the total pigment produced (Table 2). The strains, hence, can be tested for their commercial viability as these have immense biotechnological applications (Emodi 1978, Jakson *et al.* 1981).

Gloeotrichia raciborskii synthesized 89.51% and *Calothrix elenkinii* synthesized 88.12% phycobiliproteins out of the total pigments. *Calothrix marchica* var. *crassa* and *Calothrix fusca* var. *crassa* produced minimum amount of phycobiliproteins with the content as high as 63% (Table 2). The rivularian members are basically shade loving organisms and produce substantial quantities of these pigments particularly under conditions of specific light intensity, quality and nitrogen supply (Konopka and Schur 1981). These natural colours after specific treatments with organic solvents and precipitation can be utilized for their commercial exploitation. Phycocyanin from *Spirulina* has been commercialized by Dainippon Ink and Chemicals of Japan under the trade name Linablue which gives brilliant blue colour with faint reddish fluorescence (Switzer 1981).

Table 2. Chlorophyll, carotenoids and phycobilins accumulation (% of total pigments) of selected blue green algal strains from the family Rivulariaceae, order Nostocales.

Rivularian strains	**% Chlorophyll**	**% Carotenoids**	**% PC**	**% APC**	**% PE**	**% total Phycobilins (PC+APC+PE)**
Calothrix braunii	9.85^{a}	25.75^{ab}	13.50^{de}	40.00^{c}	10.90^{cd}	64.40^{f}
Calothrix marchica var. *crassa*	11.85^{a}	24.16^{b}	37.38^{a}	20.80^{g}	5.79^{e}	63.98^{fg}
Calothrix elenkinii	4.66^{cd}	7.22^{e}	22.85^{bcd}	25.51^{f}	39.77^{a}	88.12^{b}
Calothrix membranacea	11.12^{a}	24.32^{b}	9.73^{de}	16.94^{h}	37.88^{a}	64.55^{f}
Calothrix sp.	4.10^{d}	22.56^{c}	35.30^{ab}	34.71^{d}	3.32^{f}	73.33^{e}
Calothrix javanica var. *fertilissima*	4.79^{cd}	13.99^{d}	42.17^{a}	32.08^{e}	6.96^{e}	81.22^{c}
Calothrix fusca var. crassa	10.41^{a}	26.50^{a}	12.99^{de}	40.15^{c}	9.96^{d}	63.09^{g}
Dichothix baueriana	6.46^{bc}	14.32^{d}	18.20^{cde}	48.79^{b}	12.22^{c}	79.22^{d}
Gloeotrichia sp.	7.00^{b}	13.67^{d}	5.15^{e}	58.49^{a}	15.68^{b}	79.32^{d}
Gloeotrichia raciborskii	2.80^{d}	7.68^{e}	42.47^{abc}	31.53^{e}	15.51^{b}	89.51^{a}
CD at 5%	1.92	1.53	12.65	2.15	1.89	1.21

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

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