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Influence of Various Culture Media on Growth and Carbohydrates Content in a Cyanobacterium *Nostoc muscorum*

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Nostoc muscorum, Carbohydrates, BG-11, Fogg's medium, Allen and Arnon medium, Zarrouk's medium and CFTRI medium.

ABSTRACT: *Nostoc muscorum* was isolated from the collected soil samples from different locations. Identification was carried out using morphological variation and taxonomical approaches according to Desikachary (1959). The axenic culture of *Nostoc muscorum* was obtained in the laboratory. For the biomass production, different culture media were used namely BG-11, Fogg's medium, Allen and Arnon medium, Zarrouk's medium and CFTRI medium. The biomass was harvested by filtration through double layered muslin cloth and dried using air blower. After harvesting, the biomass obtained was subjected to the growth analysis. The total carbohydrates were estimated by following Anthrone method (Hedge and Hofreiter, 1962). Out of the different culture media used, BG-11 medium supported the growth of *Nostoc muscorum* properly as compared to other media used. The total carbohydrates content was more in *Nostoc muscorum* grown in CFTRI medium followed by the Allen and Arnon medium.

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INTRODUCTION

Cyanobacteria (blue–green algae, BGA) are morphologically diverse group of phototrophic prokaryotes, which occur in almost every habitat on earth and useful to mankind in various ways (Thajuddin and Subramanian, 2005). They constitute a vast potential resource in varied applications such as food, feed, fuel, fertilizer, medicine, industry and in combating pollution (Thajuddin and Subramanian, 2005). Until past few decades of research, cyanobacteria were of academic interests and were mostly ignored as nuisance but, now are proved as potential organisms for much biotechnological utilization (Richmond, 1990; Sundararaman and Sekar, 2001; Thajuddin and Subramanian, 2005). The interest in these organisms as generators of pharmacologically active and industrially important compounds has been stimulated by recent results (Singh *et al*.,2002). The carbohydrates produced by cyanobacteria have important commercial uses. Since carbohydrates are non-toxic, they are desirable and used in the food industry (Bauernfeind, 1981). Carbohydrates are frequently used in dietary additives for poultry and aquaculture farming (Hirschberg and Chamoritz, 1994).

MATERIALS & METHODS

Method of collection: The soil samples from 5-10 cm deep soil layers were collected using the scalpels. Soil samples were collected in polythene bags of size 6 x 4 inches.

Nutrient media: The different culture media namely BG-11 (Rippka*et. al*. 1979); Fogg's medium 1949; Jacobson, 1951); Allen and Arnon's medium (Allen and Arnon, 1955); CFTRI medium (Venkataraman and Becker, 1984) and Zarrouk's medium (Zarrouk, 1966) were used for the rich growth of *Nostoc muscorum*. These media were separately used in different sets.

Isolation of cyanobacterial species: The dry soil samples were spread in petri dishes and moistened with sterilized distilled water and cultures were incubated in light. When the visible growth of cyanobacteria begins to appear in the cultures, these cultures were used for the isolation of unialgal cultures of *Nostoc muscorum*.

Identification of the algal samples: Morpohometric studies were carried out by using ocular and stage micrometer. The identification of *Nostoc muscorum* was carried out using monograph and keys of Desikachary (1959).

Biomass production: For production of biomass, glass bottles (300 mL capacity) were used. The bottles were filled with 100 mL medium and autoclaved. The inoculum was ground in the sterile mortar and pestle in laminar air flow. Then the bottles were inoculated with 5 mL of unialgal suspension of *Nostoc muscorum* and labeled properly. All the cultures were maintained in the culture room at temperature 28±2°C under 8-h light/16-h dark photoperiod with a photosynthetic photon flux density of 40 μmoles^{-2}S^{-1}provided by cool white fluorescent tube lights .After harvesting, the biomass obtained was subjected to the growth analysis

Estimation of carbohydrates: The carbohydrates were estimated by following Anthrone method (Hedge and Hofreiter, 1962). For the estimation of total carbohydrates, 1 mL of cell suspension was mixed with 4 mL of 2M H_2SO_4, and placed in boiling water bath for 3 hours after which, the solution was cooled to room temperature and centrifuged. Total carbohydrates were estimated from the supernatant liquid. D-glucose was used as a standard. The amount of carbohydrates is expressed as % of total carbohydrates on dry weight basis.

RESULTS AND DISCUSSION

Out of the different culture media used, BG-11 medium supported the growth of *Nostoc muscorum* properly as compared to other media used. Allen and Arnon medium also supported growth but after 20 to 25 days, photo bleaching of biomass was observed. Other growth media, such as Fogg's medium and Zarrouk's medium supported the growth of *Nostoc muscorum* but the growth rate was very slow.

Table: Influence of different media on growth and carbohydrates in *Nostoc muscorum*

Sr.no	Medium	Fresh wt. (g)	Dry wt. (g)	Total Carbohydrates %
1	BG-11	2.79±0.12^{a}	0.21±0.05^{a}	15.23±1.24^{a}
2	Allen & Amon	2.50±0.10^{b}	0.19±0.04^{a}	13.19±0.95^{b}
3	Fogg's Medium	2.11±0.09^{c}	0.11±0.03^{b}	12.84±1.12^{b}
4	Zarrouk' Medium	2.28±0.15^{c}	0.13±0.05^{a}	13.43±1.23^{b}
5	CFTRI	2.42±0.22^{b}	0.70.07^{a}	12.88±1.80^{b}

Values are mean ± SE of three independent experiments:

Yield of biomass is one of the direct measures of quantity of biomass produced per unit area within a specific time. Higher yield indicates higher biomass produced per unit area. Comparison of *Nostoc muscorum* in different media showed that highest biomass per bottle in terms of dry weight was produced in BG-11 medium followed by Allen and Arnon medium. The carbohydrates content was more in the *Nostoc muscorum* grown in Fogg's medium followed by the CFTRI medium. Zarrouk's medium showed poor response for the carbohydrates content.

Cyanobacteria are photoautotrophic bacteria and require all the essential major and minor elements. The heterocystous cyanobacteria fix atmospheric nitrogen and they can use atmospheric nitrogen as a source of nitrogen. In bottles, the medium does not come in contact with atmospheric nitrogen and the source needs to be added in the culture medium. If the culture medium is devoid of nitrogen, it results in poor growth of cyanobacteria. Similar results were reported by Olatz (1991); medium lacking nitrogen source, results in yellowish green color of the cells which is a characteristic of nitrogen deficiency. In the culture methods like photo- bioreactors, pure nitrogen is continuously bubbled into culture medium, (Humberto *et al*., 1989; Vonshak, 1993; Roxana *et al*., 2000) so that cultures do not get affected due to nitrogen deficiency.

The growth of *Nostoc muscorum* was more in BG-11 medium than in other media. For optimum growth of cyanobacteria,

appropriate Ka^+: Na^+ ratio is required in the cytoplasm. High Na^+ is required by nitrogen fixing cyanobacteria for conversion of molecular nitrogen into ammonia (Becker, 1994). BG-11 medium consists moderate concentration of Na^+ and in Allen and Arnon medium, Zarrouk's medium and CFTRI medium there is high concentration of Na^+ while in Fogg's medium; there is no Na^+ source. *Nostoc muscorum* is from moist soil habitat, which may not require high concentration of Na^+ ions in the medium.

Production of carbohydrates depends on composition of medium and its pH. In Fogg's medium composition and pH is moderate which resulted in higher accumulation of carbohydrates in the biomass of *Nostoc muscorum.*Cifuentes and co-workers (1996 a,b) demonstrated that low nitrogen content results in higher accumulation of carbohydrates in *Dunaliella* sp. This response can be explained by the well-known effect of limitation in this nutrient as an inductive factor of carbohydrates accumulation in *Dunaliella* (Ben-Amotz*et al*., 1982). Fogg's medium does not contain nitrogen source, therefore the higher production of carbohydrates may be due to low nitrogen content of the medium.

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