Bioscience Discovery, 5(1):60-63, Jan. 2014

ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Received: 02-11-2013, Revised: 06-12-2013, Accepted: 16-12-2013

Full Length Article

Influence of Various Culture Media on Growth and Production of Carotenoides in a Cyanobacterium *Lyngbya bipunctata* Lemm.

Nehul J N

Dada Patil Rajale College, Adinathnagar Tal-Pathardi, Dist-Ahmednagar- 414505(MS, India).
jnnehul@gmail.com

ABSTRACT

*Lyngbya bipunctata*was isolated from the collected soil samples from different locations of Pathardi Tehsil of Ahmednagar district (MS,India) in the month of July And August2010. Identification was carried out using morphological variation and taxonomical approaches according to Desikachary (1959). The axenic culture of *Lyngbya bipunctata* was obtained in the laboratory by streak plate method. 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. Carotenoids were estimated by spectrophotometer method according to Gowenlock (1988). Out of the different culture media used, BG-11 medium supported the growth of *Lyngbya bipunctata* properly as compared to other media used. The carotenoids content was more in *Lyngbyabi punctata*grown in BG-11 medium followed by the BG-11 medium.

Key words: *Lyngbyabipunctata*BG-11, Fogg's medium, Allen and Arnon medium, Zarrouk's medium and CFTRI medium.

INTRODUCTION

Lyngbya bipunctata is acyanobacterium and found in moist babitat like soil and water. 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). A Variety of carotenoids produced by cyanobacteria have important commercial uses. Since carotenoids are non-toxic, they are desirable and used as coloring agents in the food industry (Bauernfeind, 1981). Carotenoids are frequently used in dietary additives for poultry and aquaculture farming (Hirschberg and Chamoritz, 1994).

MATERIALS AND METHODS

Area of Collection-*Lyngbya bipunctata* was isolated from the collected soil samples from different locations of Pathardi Tehsil of Ahmednagar district (MS,India) in the month of July And August2010.

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 *Lyngbya bipunctata*. 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 began to appear in the cultures, these cultures were used for the isolation of unialgal cultures of *Lyngbya bipunctata*.

Identification of the algal samples

Morpohometric studies were carried out by using ocular and stage micrometer. The identification of *Lyngbya bipunctata* 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 *Lyngbya bipunctata* 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 carotenoids

Carotenoids were estimated by spectrophotometer method according to Gowenlock (1988). Absorbance of carotenoids solution in n- hexane was determined at 440nm and the amount was calculated by comparing with standard. The amount of carotenoids is expressed as % on dry weight basis.

RESULTS AND DISCUSSION

Out of the different culture media used, BG-11 medium supported the growth of *Lyngbya bipunctata* 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 *Lyngbya bipunctata* but the growth rate was very slow.

Table: Influence of different media on growth and carotenoides in *Lyngbya bipunctata*

Sr.no	Medium	Fresh Weight(g)	Dry Weight(g)	Carotenoids %
1	BG-11	1.80±0.09^{a}	0.17±0.00^{a}	1.89±0.02^{a}
2	Allen &Amon	1.64±0.02^{b}	0.15±0.03^{b}	1.16±0.03^{b}
3	Fogg's Medium	1.13±0.07^{d}	0.09±0.01^{c}	1.23±0.07^{b}
4	Zarrouk' Medium	1.13±0.00^{d}	0.10±0.00^{c}	1.34±0.01^{b}
5	CFTRI	1.37±0.01^{c}	0.10±0.00^{c}	0.75±0.00^{c}

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 *Lyngbya bipunctata* 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 carotenoids content was more in the *Lyngbya bipunctata* grown inBG-11 medium followed by the Fogg's Medium. CFTRI and Allen and Arnon medium showed poor response for the carotenoids 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 *Lyngbya bipunctata*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. *Lyngbya bipunctata*is from moist soil habitat, which may not require high concentration of Na^+ ions in the medium.

Production of pigments depends on composition of medium and its pH. In BG-11 medium composition and pH is moderate which resulted in higher accumulation of carotenoides in the biomass of *Lyngbya bipunctata.* Cifuentes and co-workers (1996 a,b) demonstrated that low nitrogen content results in higher accumulation of carotenoides in *Dunaliella* sp.This response can be explained by the well-known effect of limitation in this nutrient as an inductive factor of carotenogenesis in *Dunaliella* (Ben-Amotz*et al*., 1982). Fogg's medium does not contain nitrogen source, therefore the higher production of carotenoides may be due to low nitrogen content of the medium.

LITERATURE CITED

Allen MB, Arnon DI, 1955. Studies on nitrogen fixing blue green algae. I. Growth and nitrogen fixation in *Anabaena cylindrica. Plant Physiol.* **30**:366-372.

Bauernfeind JC, 1981. *Carotenoids ascolorants andVitaminsAPrecursors*. London: Academic Press.

Becker EW, 1994. *In: Microalgae - Biotechnology and Microbiology.* Cambridge, Cambridge University Press, ISBN 0- 521 35020-4, pp 1-4.

Ben-Amotz A, Katz A, Avron M, 1982. Accumulation of b-carotene in halotolerant algae: purification and characterization of b-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). *J. Phycol.* **18**:529-537

Cifuentes A, González M, Parra O, 1996a. The effect of salinity on the growth and carotenogenesis in two Chilean strains of *Dunaliellasalina*Teodoresco. *Biol. Res.* **29**: 227-236.

Cifuentes A, González M, Parra O, Zúñiga M, 1996b. Cultivo de cepas de *Dunaliella salina* (Teodoresco 1905) en diferen tesmediosbajo condiciones de laboratorio. *Rev. Chilena Hist.* Nat. **69**: 105-112.

Desikachary TV, 1959. *A monograph on Cyanophyta*, ICAR Publication, New Delhi.1-686.

Fogg GE, 1949. Growth and heterocyst production in *Anabaena cylindrica*Lemm. II. In relation to carbon and nitrogen metabolism. *Ann. Bot.* **13**: 241-259.

Gowenlock AH, 1988. *Varley's Practical Clinical Biochemistry*. Sixth edition. London; Heineman Medical Books, 407-420.

Hirschberg J, Chamoritz D, 1994. In: *The Molecular Biology of Cyanobacteria, (Ed.).* Bryant, DADordrecht, The Netherlands: Kluwer Academic Publishers. pp. 559-579.

Humberto J, Silva IC, Rodolfo JE, 1989. Effect of nutritional factors on the culture of *Nostoc* sp.as a source of phycobiliprotiens. *Appl. Microbiol. Biotechnol.* **31**: 293-297.

Jacobson L. 1951. Maintenance of iron supply in nutrient solutions by a single addition of ferric potassium ethylene diamine tetra-acetate. *Plant Physiology,* **26**: 411-413.

Olatz F, Ramon G, Juan LS, 1991. Carotenoides composition in the Cyanobacterium *Phormidium laminosum*. Effect of nitrogen starvation. *FEBS Letters*, **282**: 300-304.

Richmond A (1990) In: *Algal Biotechnology*, (Eds.). Round FE, Chapman DJ, Bristol UK: Biopress Ltd. pp. 269-330.

Rippka R, Deruelles J, Waterbury JB, Herd man M, Stainer RY, 1979. Generic assignments. Strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**: 1-61

Roxana Olvera-Ramirez, Marisela Coria-Cedillo, Rosa Olivia Canizares-Villanuevea Fernando Martinez-Jeronimo, Teresa Ponce-Noyola, Elivira Rios-Leal, 2000. Growth evaluation and byproduct characterization of *Calothrix* sp. *Bioresource Technology*, **72**: 121-124.

Singh DP, Tyagi MB, Kumar A, Prasuna EG, 2002. Bioactive Secondary Metabolites from cyanobacteria. Pg.275-292. In Algological research in India. Edt. N. Anand, Pbl. Bsmps Dehradun. India

Sundararaman M, Sekar S, 2001. *Biotechnological potential of cyanobacteria. In: Algal biotechnology*, Trivedi PC (ed.), Pointer Publishers, Jaipur, India. pp 31–52.

Thajuddin N, Subramanian G, 2005. Cyanobacterial biodiversity and potential applications in biotechnology, *Curr. Sci.* **89**: 47-57.

Venkatarama, LV, Becker EW, 1984. Production and utilization of the blue-green alga *Spirulina*in India. *Biomass4*: 105-125.

Vonshak A, 1993. *Spirulina platensis*(Arthrospira): Physiology, Cell Biology and Biotechnology. Taylor &Francis, London. ISBN 0-7484-0674-3.

Zarrouk C, 1966. Contribution al'etuded' unecyanophycee. Influence de divers' facteurs physiques etchimiquessur la croissance et la photosyntheses de *Spirulinamaxima*.Ph.D.Thesis. University of Paris, France

How to Cite this Article:

Nehul JN, 2014. Influence of Various Culture Media on Growth and Production of Carotenoides in a Cyanobacterium *Lyngbya bipunctata* Lemm. *Biosci. Disc.,* **5**(1):60-63.