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RESEARCH ARTICLE

STUDIES OF CYANOBACTERIUM *APHANOTHECE STAGNINA* (SPRENG.) A. BR. BTA9019 ITS MORPHOLOGICAL AND BIOCHEMICAL ANALYSIS

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ABSTRACT

The present investigation deals with the cultural studies, pigment analysis, lipid profiling, total sugar content, extracellular ammonium excretion and molecular characterization of cyanobacterium *Aphanothece* sp. BTA 9019. The study of the bioactive compounds showed a high and comparable amount of sugar, pigments, chlorophyll, carotenoids, phycocyanin, phycoerythin, allophycocyanin including high protein and ammonia excretion in culture conditions. Total carbohydrates were carried out and this study strain produced high content of carbohydrates. The study also revealed that investigated organism produced good amount of secondary metabolites in culture conditions during decline phase of growth; saturated fatty acid and unsaturated fatty acid i.e. palmitic acid (C16:0) and palmitoleic acid (C16:1) were found high. Furthermore, this alga could be considered as a source of supplement of food, therapeutic agent or for use as an ingredient in cosmetics in the future.

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INTRODUCTION

The unicellular cyanobacterium *Aphanothece* sp. are distinguished from other prokaryotes by their generally low rates of endogenous respiration and by limited ability to utilized organic substances as a source of carbon and energy in the dark. Microalgae are eukaryotic, as the green algae (Chlorophyta), or prokaryotic photosynthetic microorganisms, as the cyanobacteria (Cyanophyceae) (Mata *et al*, 2010). Cyanobacteria are photosynthetic organisms with high protein content on biomass, called of single-cell protein (SCP) and capable of simple organic molecules consumption on heterotrophic in the dark (Fay, 1983). The traditional cyanobacterial form-genus *Aphanothece* was established according to morphological criteria in the middle of the 19th century by Nageli. It comprises unicellular morphotypes with oval or cylindrical cells arranged irregularly in amorphous mucilaginous colonies. Up to now, more than 80 morphospecies have been described, mostly from various freshwater aquatic habitats. The genus *Aphanothece* is heterogeneous in the traditional morphological concept as well as according to modern molecular criteria. According to Komarek (1998) *Aphanothece* are unicellular-colonial, colonies many-celled, microscopic and mucilaginous, irregularly spherical with cells irregularly, densely arranged through the whole colony, greenish coloured; colonial slime diffluent usually without structure, surface yellowish. The ecophysiological characteristics of *Aphanothece* constitute important traits in polyphasic studies. The aim of this study was a thorough characterization of a strain *Aphanothece* through analysis of their morphology and biochemistry.

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MATERIALS AND METHODS

Growth condition

The studied strain was obtained from Department of Botany, University of Allahabad, Allahabad, U.P, India. Unialgal biomass was inoculated in Erlenmeyer flask containing BG-11 (-N) broth medium (Stanier *et al*., 1971). The flasks were kept in culture room under light: dark cycles of 14:10h conditions maintained at 28±2ºC under illumination provided by cool white fluorescent tubes of 54-67µmol photons $m^{-2}s^{-1}$.

Morphological study

The trinocular Carl Zeiss microscope with Axio Vision Viewer 4.8 software was used for image analysis referred the key of Desikachary, (1959).

Extracellular ammonium excretion

Ammonia excretion was determined by measuring absorbance at 640 using UV-Vis spectrophotometer model 1800 Shimadzu with extinction coefficient described by Solorzano, (1969).

Total sugar

Total sugar was measured at 620 nm against blank and total sugar content was calculated from the standard graph following the method described by Spiro, (1966).

Phycobiliproteins (PBS)

Phycobiliproteins was recorded by measuring OD at 615, 652 and 562 as described by Bennett and Bogorad, (1973).

Total carotenoids

Estimation of total carotenoids was determined by the method described by Jensen, (1978) O.D was measured at 450 nm using 85% acetone as blank.

Chlorophyll-a

Chlorophyll-a was determined by measuring O.D. at 665nm as described by McKinney, (1941).

Total soluble proteins

Estimation of total soluble proteins was determined by measuring OD at 650 nm as described by Herbert *et al*., (1971).

Lipid profiling

The total lipid and fatty acid composition were extracted described by Bligh & Dyer (1959).

RESULTS AND DISCUSSION

Aphanothece sp. under 40X and 63X microscope visualized spherical pale blue-green with cell oblong colonies multicellular, with cells arranged irregularly, loosely in common mucilage, gelatinous. The color of the algae in nature as well as in the lab condition showed dirty brown due to phycoerythin pigment. By all these features this species name as *Aphanothece stagnina* (Spreng.) A. Br (Fig. 1). In this study it was observed that *Aphanothece stagnina* produced 23.0 µg ml^{-1} in 15^{th} day growth and 57.3 µg ml^{-1} in 30^{th} day growth of total carbohydrates. Chlorophyll content showed high at 30^{th} day of more biomass (4.23 µg ml^{-1}). The amount of extracellular ammonium excretion showed 12.5 µg ml^{-1} and 23.3 µg ml^{-1} in 15^{th} and 30^{th} day. Phycoerythin content 17.1 µg ml^{-1} in 15^{th} day and 28.2 µg ml^{-1} in 30^{th} day also for phycocyanin 17.6 µg ml^{-1} and 44.1 µg ml^{-1} and allophycocyanin 3.74 µg ml^{-1} and 13.2 µg ml^{-1} for 15^{th} and 30^{th} day growth. Total soluble proteins content soared high at 30^{th} day (66.6 µg ml^{-1}) than 15^{th} day growth. Dietary intake of carotenoids is positively correlated with chemoprevention of cancer and other degenerative diseases.

Table 1. Biochemical characterization of *Aphanothece stagnina* BTA9019

Name of the strain and code	Biochemical/ physiological characterization (µg ml^{-1})			
			15^{th} day	30^{th} day
Aphanothece stagnina BTA9019	Total carbohydrates		23.00±2.61	57.33±1.62
	Total soluble proteins		50.00±0.00	66.60±4.16
	Chlorophyll-a		01.41±0.05	04.23±1.19
	Ammonia excretion		12.50±4.17	23.30±8.68
	Total carotenoids		01.49±0.80	06.94±3.80
	Phycobiliproteins	PE	17.09±6.40	28.19±5.40
		PC	17.57±6.60	44.08±5.60
		APC	03.74±2.40	13.02±3.60

Table 2. Lipid profiling and fatty acid composition of *Aphanothece stagnina* BTA 9019

SN	Fatty acid composition	Fatty acid content (%)
1	Butyric Acid Methyl Ester (C4:0)	0.02
2	Caproic Acid Methyl Ester (C6:0)	0.07
3	Caprylic Acid Methyl Ester (C8:0)	0.10
4	Capric Acid Methyl Ester (C10:0)	0.13
5	Undecanoic Acid Methyl Ester (C11:0)	0.33
6	Lauric Acid Methyl Ester (C12:0)	2.55
7	Tridecanoic Acid Methyl Ester (C13:0)	0.68
8	Myristic Acid Methyl Ester (C14:0)	1.72
9	Myristoleic Acid Methyl Ester (C14:1)	0.18
10	Pentadecanoic Acid Methyl Ester (C15:0)	0.03
11	*cis*-10-Pentadecenoic Acid Methyl Ester (C15:1)	0.51
12	Palmitic Acid Methyl Ester (C16:0)	45.8
13	Palmitoleic Acid Methyl Ester (C16:1)	33.6
14	*cis*-10-Heptadecenoic Acid Methyl Ester (C17:1)	0.13
15	Stearic Acid Methyl Ester (C18:0)	1.29
16	Elaidic Acid Methyl Ester (C18:1n9t)	0.47
17	Oleic Acid Methyl Ester (C18:1n9c)	1.49
18	Linolelaidic Acid Methyl Ester (C18:2n6t)	1.31
19	Linoleic Acid Methyl Ester (C18:2n6c)	0.13
20	Arachidic Acid Methyl Ester (C20:0)	1.63
21	*cis*-11-Eicosenoic Acid Methyl Ester (C20:1)	0.02
22	Linolenic Acid Methyl Ester (C18:3n3)	1.74
23	Heneicosanoic Acid Methyl Ester (C21:0)	0.52
24	*cis*-11,14-Eicosadienoic Acid Methyl Ester (C20:2)	0.19
25	Behenic Acid Methyl Ester (C22:0)	2.15
26	Erucic Acid Methyl Ester (C22:1n9)	0.29
27	*cis*-11,14,17-Eicosatrienoic Acid Methyl Ester (C20:3n3)	0.02
28	*cis*-13,16-Docosadienoic Acid Methyl Ester (C22:2)	0.16
29	Lignoceric Acid Methyl Ester (C24:0)	0.05
30	*cis*-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3)	0.28
31	Nervonic Acid Methyl Ester (C24:1)	2.09

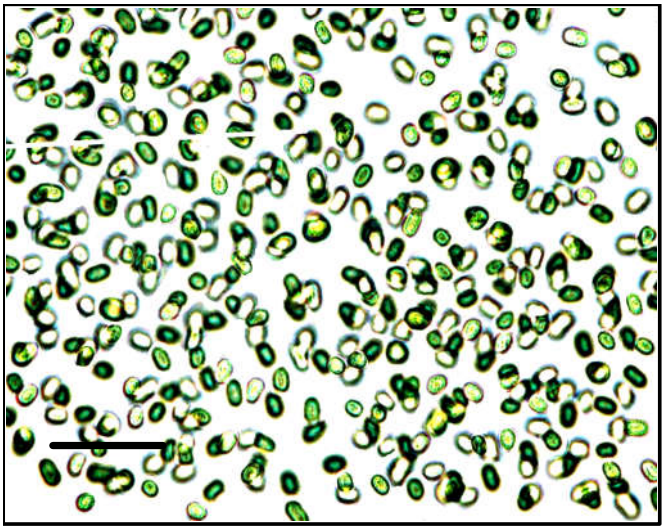

Fig.1. Photomicrograph of *Aphanothece stagnina* (Bar=10µm)

In this study it was observed that *Aphanothece stagnina* produced 1.49 µg $ml^{-1}$and 6.94 µg ml^{-1} in 15^{th} and 30^{th} day growth under culture conditions as shown in (Table 1). The content of lipid production show high in saturated fatty acid i.e. palmitic acid (45.8%) was found high and unsaturated fatty acid i.e. palmitoleic acid (33.8%) (Table 2). In the present study, age of culture play an important role in increasing carbohydrates content of *Aphanothece* biomass. Jones and Yopp (1979) also found that the extracellular carbohydrates increased with the age of cultures of *Aphanothece halophytica*. The carotenoids and phycobiliproteins, characteristic of cyanobacteria have high commercial value which possess in *Aphanothece stagnina* BTA9019. They may be used as natural food colourants in commercial aspects (Emodi, 1978).

Conclusion

The successful isolation and establishment of pure cultures of *Aphanothece stagnina* may contribute to studies of its culture conditions and may allow the establishment of effective culture methods for the cyanobacterium. Depending on the result of analysis of biochemical estimation *Aphanothece stagnina* may be suitable for producing useful materials for food, food additives or industrial use. Furthermore, as *Aphanothece* sp. has been consumed as a food since ancient times and as various kinds of cyanobacteria can easily be transformed it is expected that *Aphanothece* sp. may be used as a host for producing foods that contain useful physiologically active materials in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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