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COLLECTION, ISOLATION AND IDENTIFICATION OF HAEMATOCOCCUS PLUVIALIS FLOTOW FROM HIGH ALTITUDE REGION OF PITHORAGARH DISTRICT, UTTARKHAND, INDIA

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Abstract: Isolation and Identification of a new strain Haematococcus pluvialis Flotow from water samples collected from high altitude regions of Uttarkhand Himalayas in India was the major objective of this research study. The water samples were collected from Pithoragarh region of Pithoragarh district of Uttarkhand state in India has an efficient astaxanthin producing Green microalga Haematococcus pluvialis Flotow (Chlorophyceae) species in it. Different vegetative and encyst (aplanospore) stages of Haematococcus pluvialis Flotow were recorded and standardized the BBM media for microalga cultivation.

Keywords: Pithoragarh, Haematococcus pluvialis flotow, modified BBM media, Astaxanthin

INTRODUCTION

There are about more than 7000 species of Green alga (Chlorophyceae) are reported around the world in varying habitats. Most of them are involved in various applications such as Phycoremediation of industrial effluents, renewable biofuels, Aquaculture feed and isolation and extraction of various bioactive compounds for pharmaceutical purposes etc. Haematococcus pluvialis is an eukaryotic, unicellular, biflagellate, green, fresh water microalga capable under photoautotrophic and heterotrophic condition (Kang et al., 2005 and Sarada et al., 2002). It exists as biflagellate under favourable growth conditions and becomes green vegetative cells to red immotile aplanospores during unfavourable conditions (Harker et al., 1996). Haematococcus pluvialis was first described by Flotow in 1844, and by Braun in 1851 who added details about the morphology of it. The alternation of generation in life cycle between motile cells and resting cells were studied by Herrick in 1899. The first detailed description of the life history of Haematococcus was given by Hazen in 1899 in a report named Torrey Botanical club. The well explained life history with hand drawn images of different stages of Haematococcus was given by Peebles in 1901. Cellular morphology to the life history of the microalga and four types of cells were distinguished, microzoids, large flagellated macrozoids, non-motile palmella forms and haematocysts which is large red cells with resistant cell wall (Elliot, 1934). Pocock in 1961 and Almgren in 1966 described the ecology and distribution of Haematococcus strains in Africa and Sweden respectively. Haematococcus typically inhabited in rock pools, and suitably considerably suited under extreme conditions of fluctuations in light, temperature and salt concentration than most of the microalgae, due to its ability to encyst (Proctor, 1957). In encysted phase (aplanospore) the cells are accumulated with huge amount of carotenoid substances in which 'Astaxanthin' content will be higher. The major objective of this research study is to collect Haematococcus pluvialis Flotow at high altitude regions near Himalayas and modified the BBM media was standardized for microalgae cultivation.

MATERIALS AND METHODS

Study area

The geographical area of the Pithoragarh district is 7,110 km2, found in eastern Himalayan range of the state of Uttarkhand and rich in Floral and Faunal ecological diversity. Being average altitude region of 1635 m ASL (above sea level)

and variations in altitude, it has extreme variations in climatic temperature also and has an average temperature of 5.5-8.0ºC. In low altitude regions near river the temperature may raise up to 40ºC. But in high altitude regions of 3500 m ASL, the snowfall may reduce the atmospheric temperature. The seasonal cycle starts from winter (cold weather) during December to March, summer (hot weather) during March to June, general rainy season during mid-June to mid-September especially from North-West monsoon and retreating monsoon during September to November. The average annual rainfall is 36.7 cm. The soil is rich in minerals such as Magnesium ore, Copper ore, Limestone and Slate.

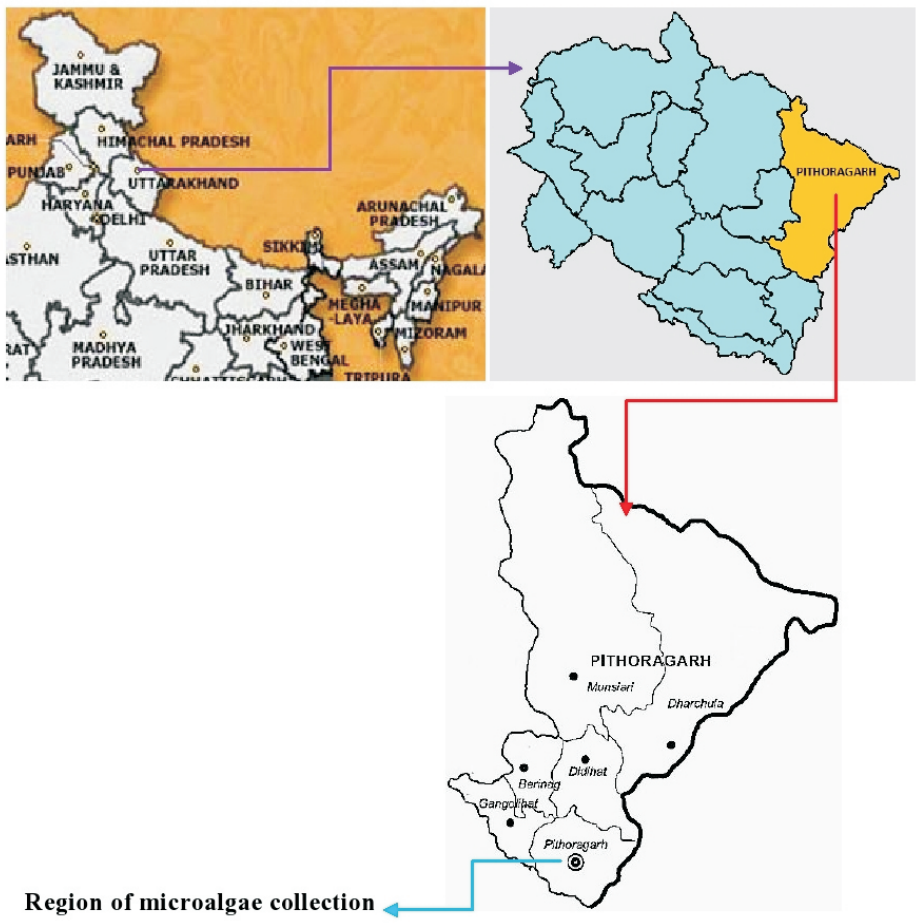

Fig 1. Study Area in Map View

Sample collection

Various Microalgae were collected from natural water bodies of Pithoragarh region of Pithoragarh district of the state of Uttarkhand. Collections of microalgae were done by random sampling method from fresh water bodies of Pithoragarh district during the month of November 2012. The Petri plates with BBM and BG11 media were used to streak instantly at the time of water sample collection. Along with Petri-plates, water samples of 50 ml in plastic vials with green mass of microalgae were also collected for isolation of microalgae under laboratory condition.

Isolation and Identification

Streaked Petri-plates were analyzed and checked every day under compound microscope (OLYMPUS CH20i) for microalgae growth, photographed and maintained under light 12:12 hrs Light:Dark photo period in laboratory condition at 24ºC. The colonized microalgae grown in solid media from the water samples were isolated separately and pure culture was maintained. Each and every isolated microalga were subjected to grow in liquid media for 15-20 days and checked microscopically for contamination. Some of the microalgae contaminated were purified by repeated plating and isolation methods. All the Isolated pure microalgae culture was morphologically identified by Dr. V. Krishnamurthy, Krishnamurthy

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Media standardization

After successive identification of Haematococcus pluvialis Flotow, the modified BBM medium was standardized for the newly collected Haematococcus pluvialis Flotow in our study.

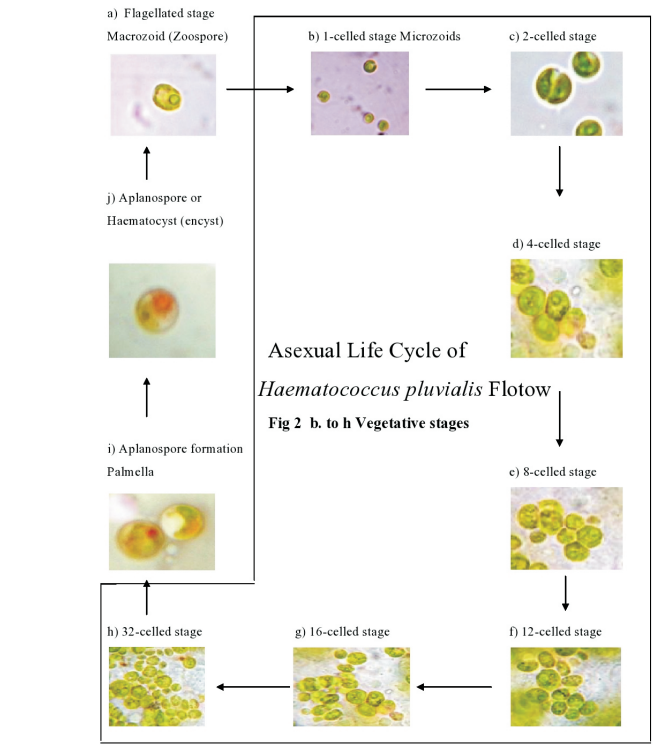
Results and discussion

The green microalga Haematococcus pluvialis Flotow were collected from the water samples of high altitude regions of Pithoragarh region, Pithoragarh district. The modified BBM medium (Table. 1) was standardized and different stages of microalga were recorded [Vegetative to cyst (aplanospore)] (Fig. 2). Isolation of a new strain of green microalga Haematococcus pluvialis Flotow paved the way for high production of carotenoid substances which consists of an anti-cancer compound Astaxanthin in it. The Asexual life cycle of Haematococcus pluvialis Flotow has been studied and recorded in our research study (Fig. 2).

The coccoid cells underwent cytokinesis and formed up to 32 daughter celled stage when transplanted in to fresh medium (Wayama et al., 2013). The same process took place in our work from single celled stage to 32 celled stages including Aplanospore stage (Fig. 2).

Table 1. Standardized media for Haematococcus pluvialis Flotow

S. No.	Ingredients	Amount used
1.	Sodium nitrate (NaNO3)	0.103 g
2.	Calcium chloride (CaCl2.2H2O)	0.038 g
3.	Sodium hydrogen carbonate (NaHCO3)	0.001 g
4.	Magnesium sulphate (MgSO4.7H2O)	0.075 g
5.	Dipotassium hydrogen phosphate(K2HPO4)	0.001 g
6.	Sodium EDTA (Na2 EDTA)	0.001 g
7.	Citric acid (C6H8O7)	0.006 g
8.	Calcium nitrate [Ca(NO3)2.6H2O]	0.00004 g
9.	Ammonium ferric citrate [Fe(III)NH3 citrate]	0.001 g
10.	A5 metals solution	1 ml
	A5 Metals solution	
1.	Boric acid (H3BO3)	2.860 g
2.	Manganese chloride (MnCl2.4H2O)	1.810. g
3.	Zinc sulphate (ZnSO4.7H2O)	0.222 g
4.	Sodium molybdate (Na2MoO4.2H2O)	0.017 g
5.	Copper sulphate (CuSO4.5H2O)	0.079 g
	Distilled H2O	1000 ml



In this study, the asexual life cycle of Haematococcus pluvialis Flotow showed four types of cell formation such as Macrozoids, Microzoids, Palmella and Aplanospore stages as reported (Elliot, 1934). Macrozoids are zoospores pear shaped and biflagellated (Fig. 2a), Microzoids are single celled stage formed during favourable conditions and are the early vegetative growing stage (Fig. 2b). Each and every microzoids are subjected to form 2-32 daughter cells at the time of vegetative growth (Fig. 2c to 2h). Palmella are non-motile and expanded in shape formed during unfavourable conditions, and it is described as early stage of encyst formation (Fig. 2i). Aplanospores are formed with rigid cell wall and reddish orange coloured during stressed condition and accumulates more amount of Astaxanthin in it (Fig. 2j). Macrozoids are 20-50 µm in size and exhibit motility after their release from gametocysts and the microzoids are smaller cells with less than 10µm in size (Triki et al., 1997). During the transformation of vegetative cells into red aplanospores, nearly 50 % of chlorophyll pigment is getting reduced (Boussiba et al. 1999, Han et al. 2012).

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