Research Paper

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Growth rate Measurement and Morphometry of isolated Microalgae from north India region

Life Science

KEYWORDS : Microalgae habitat, algal classification, Growth rate, Morphometry.

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ABSTRACT

Microalgae are the much more diversified group of microorganisms. These are found in a wide range of habitat. In this work we report the isolation of various microalgal strains from terrestrial and aquatic sites. A total of 60 samples of terrestrial and aquatic bodies were collected, out of them 8 algal strains were successfully isolated and purely cultured in lab and maintained. Genus level identification was done with the help of light microscope. Morphometry of algal strains was done using Imagepro+ software (version 4.5). Growth rate was measured by hemocytometer. Growth pattern was observed irregular but a constant growth was observed. Microalgal strains namely Chlorella, Scenedesmus and Chlorococcum showed a highest growth among all 8 isolated strains.

Introduction

Microalgae are the group of tiny microscopic microorganisms. They are photosynthetic cellular organization capable of using sunlight and CO_2 of environment, in return which produce O_2 and other bio actives. There is no easy definition of an alga. These are generally microscopic organisms and usually thought of as simple aquatic plants which do not have roots, stems or leaves and shows primitive methods of reproduction. They are carbon fixing and oxygenating organisms. However some algae display primitive animal features such as motility. While blue-green algae differ markedly from plants and all other algae. They have a cellular structure and function that is more common to bacteria than to the plant kingdom. Algae live in a wide range of aquatic environments and are a natural component of most aquatic ecosystems. Additionally, a great number of algae are terrestrial, living in soil, snow, or in association with other organisms, especially fungi (lichens), and animals. Aquatic algae are found in both fresh and marine water. They range in size from microscopic to large kelp (in metres). Some algae have an economic importance because they are a source of carotene, pigments, glycerol and alginates and can be converted into a food source for aquaculture.

Algae vary considerably in size, shape, and growth form. These can be either Single celled or multiple celled, either colonially or as filaments of cells or elaborate plant bodies with differentiated cell types Free floating in the water column (planktonic). Microalgae are small organisms, which can be divided into 4 size categories as the microplankton (20 to 1000 µm), the nanoplankton (2 to 100 µm), the ultraplankton (0.5 to 15 µm) and the picoplancton (0.2 to 2 µm) (Callieri &Stockner, 2002; Gopinathan, 2004). These comprise the microscopic unicellular algae and colonial and filamentous algae, known as "phytoplankton."Growing as a film on rocks on the bottom (benthic) or on plants growing in the water (epiphytic). These may be single celled or small colonial and filamentous species. Growing out into the water column but attached to a substrate at one point. These comprise the larger filamentous algae, and macro algae (e.g. seaweeds).

Blue-green algae or Cyanobacteria are microscopic cells that grow naturally in Australian fresh and salt waters. They are a type of bacteria, but in some ways act like plants by using sunlight to manufacture carbohydrates from carbon dioxide and water, a process known as photosynthesis. In doing so, they release oxygen. They grow in dams, rivers, creeks, reservoirs, lakes and even hot springs. Green algae range in size from microscopic to large plants, and can be single celled, colonial, or filamentous. Some of the single celled and colonial green algae have small tails or "flagella" attached to each cell, which they use to swim. However many green algae are non-motile. Green algae may be either planktonic or attached. They show the greatest diversity of shapes, sizes and species of any group of freshwater algae. Green chloroplasts are frequently observable within the cells of green algae when looked at under a microscope.

Material and Methods

Sampling

Soil and water samples were collected from various sites of North India. Aquatic and terrestrial samples were collected in four stages from different sites and stored in polybags and sample containers at low temperature. A sum of total 60 samples was collected. Moist soil samples 16, fresh water sample 2, sewage water sample 15, pond water sample 5, cropland soil sample 14, lake water sample 4, running water sample 2, shallow water sample 2 were collected

Table 1: List of sampling sites (District wise)

Sampling Site	Shamli	Baghpat	Garhmukhteshwar	Meerut	Nanital/ Ranikhat	Total
Terrestrial	2	6	4	16	2	30
Aquatic	2	6	4	16	2	30

Isolation Methods

Enrichment culture techniques were used to obtain unialgal colonies of microalgae using standard microbiological methods using Bold Basal medium (Stein, 1963) in culture room at 3.5klux light intensity with 16:8 light and dark period and 28±2^{0}C temperature. The pH of the medium was maintained at 8.0 for optimal growth of cultures.

Sterilized Bold Basal medium was poured in 250 ml conical flasks and autoclaved. Each 1 ml of collected water samples were inoculated in each flask and kept in culture room for a period of 10-15 days, which is maintained with optimal light intensity and temperature. After incubation period green coloured growth of algal cells was observed and this was picked with the help of Pasteur pippets in order to obtain unialgal cells. Unialgal cell was re-cultured in another 100ml conical flask containing 100ml sterilized Bold Basal medium, incubated again for 10-15 days.

Solid agar based medium was prepared by dissolving 15-18 gm of purified agar in 1 liter of medium and autoclaved before use for maintenance of the cultures. Plates were incubated in cul-

ture room for a period of 10-15 days. The isolated colonies were picked up repeatedly and microalgae strains were purified by repeated sub culturing, plating and streaking on the appropriate medium. Isolated microalgae strains from selected habitat were grown and maintained in Bold Basal medium under similar conditions. Cultures were regularly streaked on plate having appropriate solid media to maintain purity of cultures which was examined several times by microscopic observation at regular intervals. Discrete colonies were inoculated in fresh medium and used for the study at exponential phase.

2.2.1. Serial dilution method

Enrichment sample containing a large number of microalgal cells were isolated by serial dilution method. A dilution from 10^{-1}- 10^{-10} was made in sterilized test tubes containing 9 ml BBM. One ml of enrichment sample was transferred into each test tube labeled dilution factor from 10^{-1}- 10^{-10}. Dilution factor 10^{-6}- 10^{-10} was used for spreading onto the freshly prepared agar plates. Petri dishes were placed onto rack maintained with light intensity and temperature. After incubation period of 15-20 days a number of colonies of green color was observed.

2.2.2. Streak plating method

A loopful enrichment sample of aquatic and soil sample (1 gm soil in 10 ml distilled water) containing a number of microalgal cells were taken to streak on petri dish containing agar BBM medium. Petri dishes were placed onto rack maintained with light intensity and temperature. After incubation period of 15-20 days a number of colonies of green color was observed.

2.2.3. Axenic culture maintenance

A 25 µl volume of triple antibiotic solution was added in 5 ml nutrient broth. Nutrient broth was inoculated with 0.5 ml of homogeneous microalgal suspension. Culture tubes were incubated for 5 hrs at 25±1ºC temperature in an incubator. After incubation nutrient broth having microalgal culture, was transferred in sterilized centrifuged tube under laminar flow bench and suspension was centrifuged at 3500 rpm. The supernatant was discarded in a flask containing dilute phenol solution. 5 ml sterilized distilled water was added to pellet in centrifuge tube, vortexed it and centrifuged again at 3500 rpm. Washing was repeated three times with sterilized distilled water. After washing, the pellet was inoculated in 5 ml culture tubes of BG-11 medium. The culture tubes were incubated in growth room under a light intensity of 3.5 - 4 klux at 28±2ºC. After 14 days of growth, two loops of microalgal cultures were inoculated in 5 ml of nutrient broth. This was incubated along with one control tube overnight at 28±1ºC in an incubator. Culture tubes were examined for bacterial contamination in comparison to control. Axenization process was repeated in few cases, where purity of culture was not achieved (Kaushik, 1987).

Results and Discussion

A total 8 microalgae were finally isolated and carried for growth rate measurement and characteristic morphometric analysis was done using Imagepro+ software (version 4.5). Isolation of microalgal isolates was carried by using Bold Basal medium (BBM), developed by stein (Stein, 1963). Samples of aquatic and terrestrial sites were collected randomly. Serial dilution method and streak plate method was used to isolate microalgal cells from enriched samples of aquatic and terrestrial sites. Culture room was maintained at 3.5klux light intensity with 16:8 light and dark period and 28±2ºC temperature. The pH of the medium was maintained at 8.0 for optimal growth of cultures. Morphometry of all eight isolates were carried out on the basis of color of thallus, trichomes and heterocyst. Isolates were identified at genus level. Measurement of growth rate was done on 14th day, 21st day and 28th day. A regular growth rate was observed in each stage of measurement. Highest growth rate was observed in isolate 10 while lowest growth rate was observed in isolate 1 with standard deviation 0.04 and 0.02 respectively (On 14th day). While highest growth rate was observed in isolate 1 and lowest growth rate was shown by isolate 15 with standard deviation 0.05 and 0.03 respectively (on 21st day). Again it was observed that highest growth rate was observed by isolate 02 while lowest growth rate was shown by isolate 09 with standard deviation 0.4 and 0.2 respectively (on 28th day). Although it may occur due to environmental stress like pH, salinity, carbon source, growth under nutrient deficient conditions (Taguchi & Smith, 1993).

Morphology and Morphometry

All isolated microalgal Strains were identified according to keys given by Desikachary (Desikachary, 1959) and Geitler (Geitler, 1932). Morphological studies were carried out on the specimens of exponential growth phase. All the measurements on fresh material were performed on each morphological variable by using a light microscope (Olympus, model: CX40RF200). Micrographs were taken by Olympus (CAMEDIA C-5060 WIDE ZOOM) digital compact camera**.** The pictures were processed with Imagepro+ software (version 4.5). Isolates were identified at genus level on the basis of color of thallus, trichomes and heterocyst etc.

Table 2: List of isolated Microalgal strains

S.No	Isolate code	Isolate No.	Sampling site	Generic Name
1.	VGPW 04	Isolate 09	Pond water sample	Ossilatoria sp.
2.	UFMS 50	Isolate 15	Moist soil sample	Aulosira sp.
3.	BFWS 12	Isolate 02	Fresh water sample	Chlorella sp.
4	KFWS 28	Isolate 03	Fresh water sample	Scenedesmus sp.
5	MUCF 31	Isolate 10	Crop land soil sample	Nostoc calcasie sp.
6.	ACSW 57	Isolate 06	Sewage water sample	Phormidium sp.
7.	UABS 46	Isolate 01	Moist soil sample	Chlorococcum sp.
8.	VDSW 68	Isolate 04	Sewage water sample	Chlamydomonas sp.

Table 3: Morphometery of isolated strains

S.No	Isolate code	Isolate No.	Shape (Vegetative Cells)	Length (in µm)	Breadth (in µm)
1.	VGPW 04	09	Cylindrical	3.56-6.56	3.34-6.67
2.	UFMS 50	15	Barrel shaped	2.35-4.13	1.8-2.48
3.	BFWS 12	02	Oblong	2.88-7.88	2.11-5.75
4.	KFWS 28	03	Elongated & almost rectangular	4.12-8.74	3.32-6.59
5.	MUCF 31	10	Cylindrical	3.12-5.69	2.59-5.55
6.	UABS 46	06	Elongated & almost rectangular	2.96-6.98	2.23-6.01
7.	ACSW 57	01	Barrel shaped	2.89-5.54	2.10-4.23
8.	VDSW 68	04	Cylindrical	3.10-5.45	2.78-3.61

Growth rate Measurement

Measurement of growth of all 8 isolated strains was carried with the help of haemocytometer (Schoen & Lobban, 1988). Growth was measured at three stages of time interval viz. 14th day, 21st day, 28th day. Statistical analysis of all three growth intervals was carried out.

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Table 4: Growth rate measurement of all 8 isolates

Isolate No.	14th day			21st day			28th day		
	Mean (±)	S.D	C.V	Mean (±)	S.D	C.V	Mean (±)	S.D	C.V
09	1.96±0.01	0.01	0.51	2.48±0.01	0.01	0.40	4.30±0.12	0.20	4.65
15	1.72±0.03	0.06	3.50	2.23±0.02	0.03	1.35	5.40±0.12	0.20	3.70
02	1.61±0.02	0.04	2.48	2.36±0.03	0.06	2.54	6.40±0.23	0.40	6.25
03	2.07±0.02	0.03	1.45	2.50±0.23	0.40	16.00	5.70±0.29	0.50	8.77
10	2.08±0.02	0.04	1.92	2.24±0.02	0.04	1.79	4.50±0.29	0.50	11.11
06	1.94±0.02	0.04	2.06	2.29±0.01	0.01	0.44	5.80±0.17	0.30	5.17
01	1.52±0.01	0.02	1.32	2.96±0.03	0.05	1.70	5.90±0.17	0.30	5.08
04	1.83±0.02	0.03	1.64	2.69±0.15	0.27	9.89	6.20±0.12	0.20	3.23

Conclusion

Microscopic examination of each algal strain confirmed the culture at generic level, which can enable for further use of particular species for biodiesel, biocosmetics, biofertilizers etc. Our study shows that there is totally random growth rate was seen in each isolate but regular growth rate was obviously seen. To our knowledge, the present study shows that growth rate may vary during the course of growth period of microalgal cells.

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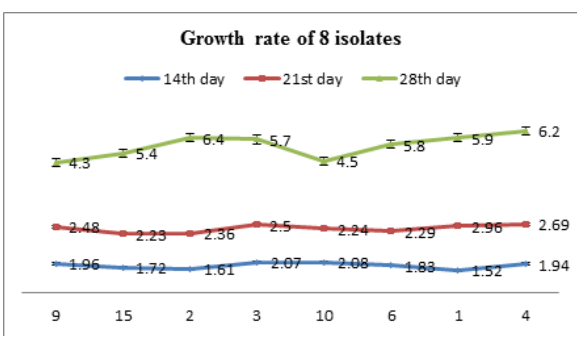


Figure 1: Graphical representation of Growth rate measurement of all 8 isolates.

Abbereviations: 9= Ossilatoria; 15= Aulosira; 2= Chlorella; 3= Scenedesmus; 10= Nostoc; 6= Phormidium; 1= Chlorococcum; 4= Chlamydomonas;

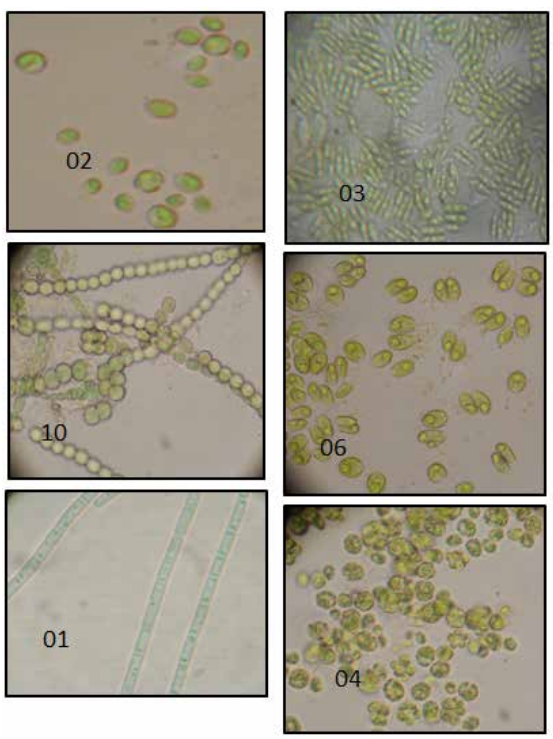
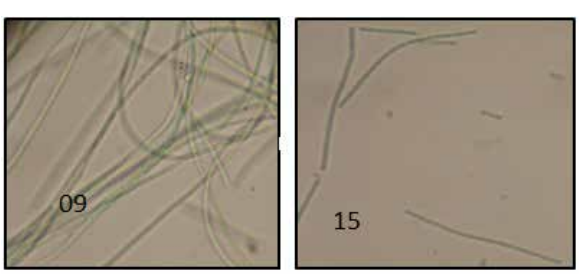


Figure 2: Micrographs of all 8 microalgae isolates.



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