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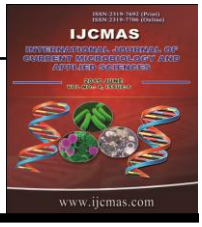

Original Research Article

Enumeration, pigment analysis and nitrogenase activity of cyanobacteria isolated from unexplored rice fields of Manipur, India falling under Indo-Burma biodiversity hotspots

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Biochemical, Cyanobacteria, Nitrogenase activity, Phycobili proteins, Repository

A B S T R A C T

The present study focussed on the enumeration of cyanobacteria from unexplored rice fields of Manipur, India falling under Indo-Burma biodiversity hotspots and estimation of natural pigment production, and nitrogenase activity. Total 112 unialgal diverse cyanobacterial strains were enumerated, successfully cultured, deposited to the national cyanobacterial repository with accession number. Out of 112 strains, 22 fast growing and potent strains were selected for detailed characterization particularly for estimation of nitrogenase activity and natural pigment production in controlled culture conditions. *Anabaena spiroides* BTA084; *Calothrix javanica* BTA024; *Phormidium arthurensis* BTA042; *Nostoc muscorum* BTA087 were characterized in details as these strains were considered as the potent candidates for the production of chlorophyll-a, phycoerythrin, phycocyanin, allo-phycocyanin, total carotenoids respectively. *Nostoc hatei* BTA037 yielded highest nitrogenase activity in culture conditions to be utilized for biofertilizer purpose particularly for terraced hill rice culture conditions.

Introduction

Cyanobacteria are a group of gram negative, morphologically diverse, aerobic phototrophs whose distribution is ubiquitous in nature and are found everywhere. Their morphology varies from unicellular to multicellular. Some species have unique cells called heterocysts, which are capable of fixing atmospheric nitrogen. Cyanobacteria are known to be one of the promising supplements to nitrogenous fertilizer, but the process biological nitrogen fixation, mediated through the enzyme nitrogenase may be inhibited in presence of readily available nitrogen sources.

Chlorophyll provides a chelating agent activity which can be used in ointment, treatment for pharmaceutical benefits especially liver recovery and ulcer treatment. It also repair cells, increases haemoglobin in blood and faster the cell growth (Puotinen, 2009). Chlorophyll-a is another component of biomass, which can be estimated as a measure of growth (Kobayasi, 1961). Carotenoids are widely used as natural colourant for food, drug and cosmetic products. They served as a second line density protein. Phycobilins are high concentration of pigment occurring under some conditions which leads to the bluish colour of the organisms and are known as blue-green algae. Cyanobacteria possess all the known phycobiliproteins (phycocyanin, phycoerythrin, phycoerythrocyanin and allophycocyanin). Among them, phycocyanin and phycoerthrin are commercially valuable. Phycocyanin is used in water-insoluble, dairy products and soft drinks (Cohen, 1986).

The use of microalgae in industry encourage the development of better cultivation system in order to optimize the production of algae rich in active substances such as vitamins, proteins, amino acids, fatty acids and trace elements. The merits of an organism for commercial exploitation are maximum yield and utility of cellular constituents (Borowitzka and Borowitzka, 1988). Higher growth rate and nutrient profile of cyanobacteria make them a potentially valuable source of nutrients (Cannell, 1989). Growth of a living organism is defined as an increase in mass or size accompanied by synthesis of macromolecules, leading to the production of newly organized structure. Microbiologists use a variety of techniques to quantify microbial growth other than determining direct cell count. These include the measurement of macromolecules in the cell (Healy and Henzdel, 1976), the cell quota of specific elements (Rhee and Gotham, 1980) or the kinetic parameters for nutrient uptake (Zevenboom et al., 1982).Venkataraman and Mahadevaswamy (1992) pointed out that good culture management with suitable strain is one of the basic needs to get promising yields with quality material on commercial scale. Therefore, cultivation techniques are to be improved with the main objective of obtaining higher algal biomass that exhibits specific qualities (Lobban and Herrison, 1994). Mass cultivation of cyanobacteria is essentially a complex process involving a large number of variables for successful growth of essential requirements of the organism as possible. The environmental factors may be either physiological such as acidity and pH or chemical which provide all the raw materials used for structural cells and protoplasmic synthesis of cyanobacterial cells (Becker, 1994). Physical and chemical factors such as temperature, acidity and light (Lobban and Herrison, 1994), aeration (Chen and Johns, 1991) or nutrient concentration (Bjornsater and Wheeler, 1990) influence the biochemical composition, physiological status and ultrastructure of the cyanobacteria. Culture medium has been found to play a significant role in the growth kinetic of algae, since it has to stimulate the natural conditions as closely as possible.

Materials and Methods

Preparation of the medium, processing of the collected samples and microscopic observation: From the stock solutions of BG-11 medium (Stanier et al., 1971), took 1ml for each stock solutions in 1000 ml distilled water with or without sodium nitrate for isolation of non heterocystous and heterocystous cyanobacteria respectively with addition of 15 g bacteriological agar in 1000 ml liquid medium for solid petriplates and slants. For isolation and culturing of cyanobacteria the

samples were inoculated in the enrichment BG-11 medium. The samples were incubated at 28±2ºC for 10 to 15 days or till the appearance of algal patches in growth media. A portion of unialgal cyanobacteria was taken from periphery and mount on a cleaned slide with glycerol covered by a cleaned cover slip and observed under phase contrast microscope (NIKON-80*i*) at different interval of their growth phase. Identification of cyanobacterial isolates were carried out using morphological and reproductive structure compared with Desikachary (1959) and Komarek and Anagnostidis (2005). Photo documentation was performed under different objective magnifications by using Carl Zeiss Microscope (Carl Zeiss-A1) (Table 1).

Preparation of inoculums: A loopful cyanobacterial biomass were inoculated in 250 ml conical flasks and incubated in culture room at 28±2ºC of 4KLux light intensity provided by white fluorescent tubes 14/10 light dark phase. 15 days old biomass was homogenized uniformly. 5 ml well homogenized cyanobacterial inoculums of each strain were inoculated into 250 ml conical flasks with 100 ml culture medium for investigation in exponential phase. Every day agitation was done to avoid clumping of the cells and for faster growth.

Estimation of phycobiliproteins: Estimation of phycobiliproteins was determined by the method described by Bennett and Bogorad (1973). 10 ml algal suspension was centrifuged at 7000 rpm for 10 min (refrigerated centrifuged eppendorf 5430 R). The pellets were suspended in 5 ml phosphate buffer. The contents were repeatedly freezed in 4ºC and thawed at room temperature. The supernatants were pooled and the absorbance was measured at 562 nm, 615 nm and 652 nm for phycocyanin, allophycocyanin and phycoerythrin respectively using UV-visible spectrophotometer (Shimadzu 1800).

Estimation of total carotenoids: Estimation of total carotenoids was determined by the method described by Jensen (1978). 10 ml homogenized algal suspension was taken and centrifuged at 6500 rpm for 10 min (refrigerated centrifuged eppendorf 5430 R). Discarded the supernatant and added 3 ml 85% acetone and subjected to repeat freezing and thawing until the pellet becomes colorless. Measured the volume of the extract and make up the final volume upto 10 ml with 85% acetone and read the O.D. at 450 nm using 85% acetone as blank and calculated the total amount of carotenoids in µg/ml using UV-visible spectrophotometer (Shimadzu 1800).

Determination of acetylene reduction activity: Acetylene reduction activity was determined by the method described by Hardy et al., 1973. A known volume of algal biomass was taken into 13 ml capacity test tubes. Stopper the tubes and remove the gas phase equivalent to 10% of the remaining volume of the tubes and injected equivalent volume of acetylene (C_2H_2).Vials were incubated for 120 min under light conditions (4 Klux) at 28±2°C interval shake was done. A gas sample of known volume (0.1 to 1.0 ml) was withdrawn with gas tight syringe and injected into injection port of the gas chromatograph (Thermo Chemito Ceres 800 plus).

Estimation of chlorophyll-a: Estimation of chlorophyll-a was determined by adapting the method described by Mckinney (1941). 10 ml of homogenized algal suspension was taken in centrifuge tube and done centrifugation at 7000 rpm for 10 min and then discarded the supernatant and transferred the algal pellet to a test tube and added 10 ml of 90% methanol. Shaked the

contents and placed the tubes covered with aluminium foil in a water bath at 60˚C for 30 min. The absorbance from supernatant was measured at 665 nm against methanol blank (UV-visible spectrophotometer (Shimadzu 1800).

Result and Discussion

One hundred twelve (112) cyanobacterial strains belong to 16 genera including non heterocystous (07 genera 40 strains) and heterocystous (09 genera 72 strains) namely; *Myxosarcina* (01), *Limnothrix* (03), *Phormidium* (16), *Oscillatoria* (01), *Spirulina* (01), *Lyngbya* (08) *Plectonema* (10) *Cylindrospermum* (04), *Anabaena* (32), *Nostoc* (13), *Aulosira* (02), *Scytonema* (03), *Calothrix* (09), *Microchaete* (06), *Dichothrix* (02) and *Westiellopsis* (01) were isolated from the rice fields of Manipur, India. These unialgal cyanobacterial strains were investigated for yielding of chlorophyll-a, phycobiliproteins, total carotenoids and acetylene reduction activity. Out of 112 screened twenty two (22) strains were selected for detailed characterization on the basis of fast growth rate in culture conditions. *Calothrix javanica* BTA024 (80.9 µg/ml), *Anabaena spiroides* BTA84 (73.9 µg/ml), *Phormidium arthurensis* BTA42 (69.2 µg/ml), *Microchaete grisea* BTA07 (60.7µg/ml) and *Anabaena oryzae* BTA50 (53.4 µg/ml) were released good amount of phycoerythrin (Table 2; photoplate 1). *Phormidium arthurensis* BTA042 (286.1 µg/ml), *Nostoc muscorum* BTA87 (273.1 µg/ml), *Spirulina platensis* BTA174 (196.1 µg/ml), *Nostoc muscorum* BTA27 (148.3 µg/ml) and *Nostoc piscinale* BTA947 (128.7 µg/ml) were released relatively high amount of phycocyanin (Table 2; photoplate 2). *Nostoc muscorum* BTA87 (159.7 µg/ml), *Anabaena fertilissima* BTA35 (130.7 µg/ml), *Nostoc piscinale* BTA947 (72.9 µg/ml), *Cylindrospermum indicum* BTA960 (79.2µg/ml) and *Oscillatoria agardhii* BTA170 (72.4 µg/ml) were produced justifiable amount of allo-phycocyanin (Table 2; photo plate-3).

Nostoc muscorum BTA87 (80.3 µg/ml), *Phormidium puspurescens* BTA82 (66.5µg/ml), *Calothrix marchica* BTA195 (66.4 µg/ml), *Phormidium tenue* BTA222 (66.1µg/ml) and *Nostoc carneum* BTA38 (58.9 µg/ml) produced comparatively high amount of total carotenoids (Table 2; photoplate 4). *Nostoc hatei* BTA037 (90.9 nmole C_2H_4/µg of Chl-a/hr), *Dichothrix baueriana* BTA1059 (89.5 nmole C_2H_4/µg of Chl-a/hr), *Anabaena anomala* BTA927 (13.7 nmole C_2H_4/µg of Chl-a/hr), *Anabaena oryzae* BTA919 (13.4 nmole C_2H_4/µg of Chl-a/hr) and *Cylindrospermum muscicola* BTA904 (12.1 µg/ml) were showed sufficient and comparatively high acetylene reduction activity expressed in nmole C_2H_4/µg of Chl-a/hr respectively (Table 2; photoplate 4). *Anabaena spiroides* BTA84 (31.1µg/ml), *Nostoc piscinale* BTA947 (25.2 µg/ml), *Nostoc carneum* BTA38 (19.1 µg/ml), *Nostoc muscorum* BTA87 (17.2 µg/ml) and *Calothrix marchica* BTA 26 (16.6 µg/ml) were observed as the high chlorophyll-a producing cyanobacteria (Table 2; photoplate 6). The present investigation revealed that *Calothrix javanica* BTA024 was produced highest amount of phycoerythrin; *Phormidium arthurensis* BTA042 produced highest amount of phycocyanin; and *Nostoc muscorum* BTA087 produced highest amount of allophycocyanin; *Anabaena spiroides* BTA084 produced highest amount of chlorophyll-a which were comparable with already commercialized strains for said purpose (Cohen 1986; Dainippon Patent 1980; Dainippon Patent 1981; Borowitzka 1994).

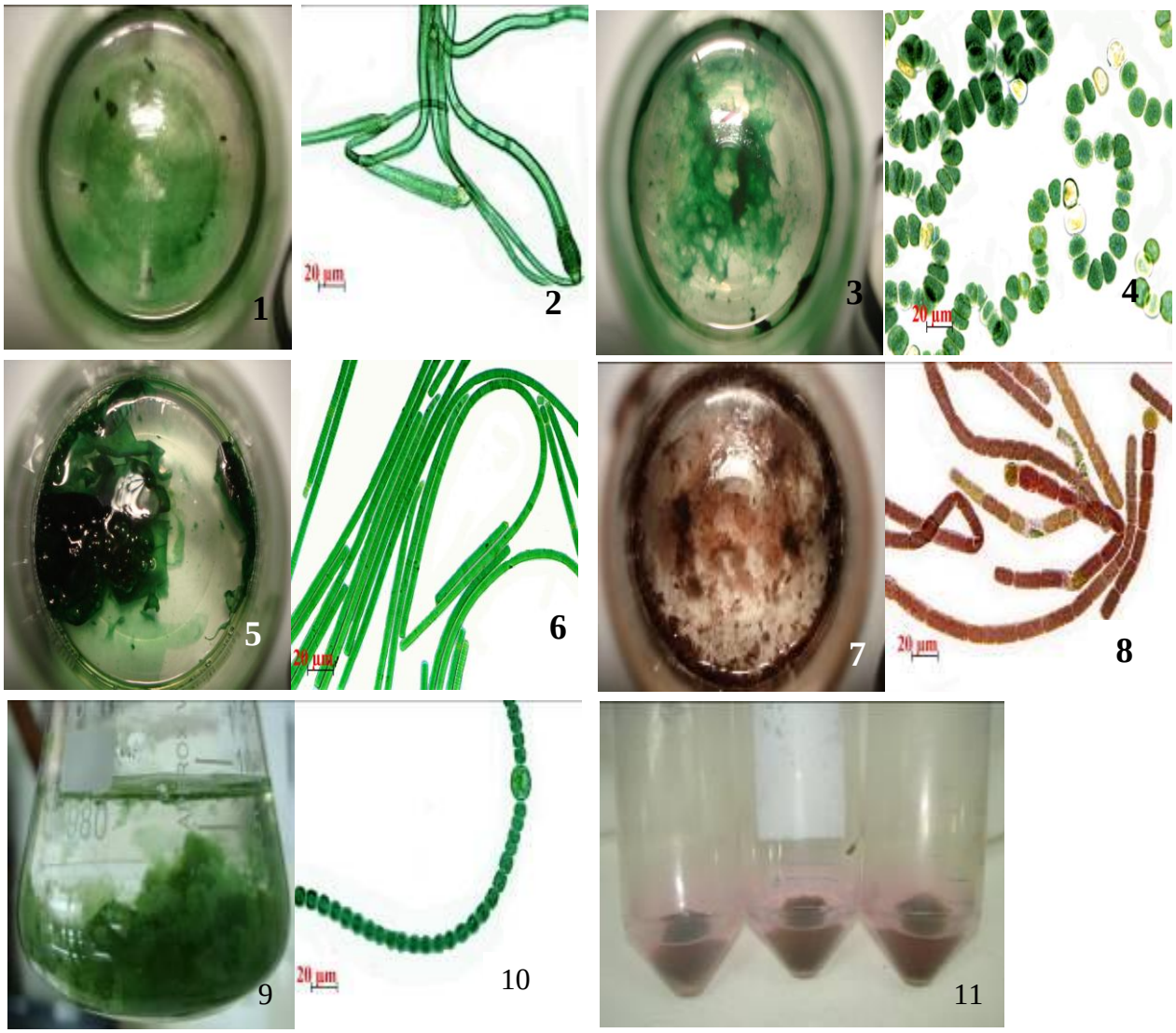
Table.1 History, morpho/taxomical characterization of cyanobacterial strains of rice fields of Manipur, India

Cyanobacterial strains	Habitat of strains	GPS location & date of collection	Taxonomical enumeration of strains		
			Heterocyst/ Akinetes	Cell colour / Filaments	Trichome ends
Microchaete grisea Thuret ex Born. et BTA007	Rice fields, Imphal East, Manipur, India	Alt: 775 m N24°49'26.4" E093°57'52.0" DOC: 06-04-2004	Basal	Brown	Broad ends
Calothrix javanica de Wilde BTA024	Rice fields, Imphal West, Manipur, India	Alt: 780 m N24°49'36.0" E093°53'25.5" DOC:21-04-2004	Basal	Light green	Pointed
Calothrix marchica Lemmermann BTA026	Rice fields, Imphal West, Manipur, India	Alt: 792 m N24°50'33.6" E093°56'23.4" DOC: 21-04-2004	Basal and spherical	Olive green aggregated	Conical
Nostoc muscorum Ag. ex Born. et Flah. BTA027	Rice fields, Imphal West, Manipur, India	Alt: 786 m N24°50'31.0" E093°66'23.0" DOC:21-04-2004	Spherical	Light green	Broad rounded
Anabaena fertilissima Rao, C. B. BTA035	Rice fields, Bishnupur, Manipur, India	Alt: 776 m N24°43'15.5" E093°50'27.5" DOC: 21-04-2004	Spherical	Green single	Rounded
Nostoc hatei Dixit BTA037	Rice fields, Moirang, Manipur, India	Alt: 761 m N24°30'12.3"E093°46'46.4" DOC:12-05-2004	Spherical	Yellowish green coil	Flattened board
Nostoc carneum Ag. ex Born. et Flah. BTA038	Rice fields, Moirang, Manipur, India	Alt: 767 m N24°30'12.3" E093°46'46.4" DOC: 12-05-2004	Oblong	Olive green loosely contorted	Rounded
Phormidium arthurensis Novis & Visnovsky BTA042	Rice field, Bishnupur, Manipur, India	Alt: 764 m N24°32'21.4" E093°45'27.6" DOC:12-05-2004	Absent	Pale blue green entangled	Rounded
Anabaena oryzae Fritsch BTA050	Rice field, Thoubal, Manipur, India	Alt: 769 m N24°29'25.1" E094°00'43.7" DOC:12-05-2004	Terminal intercalary	Deep green aggregated	Conical
Phormidium purpurascens (Ag) Gomont BTA081	Rice field, Thoubal, Manipur, India	Alt: 776 m N24°29'25.1" E094°00'23.7" DOC:16-06-2004	Absent	Yellowish blue green, entangled	Attenuated

Anabaena spiroides Klebahn BTA084	Rice field, Thoubal, Manipur, India	Alt: 769 m N24°29'25.1" E094°00'43.7" DOC:16-06-2004	Subspherical	Deep green slight spiral	Rounded
Nostoc muscorum Ag. ex Born. et Flah. BTA087	Rice field, Imphal West, Manipur, India	Alt: 782 m N24°48'14.3" E093°54'18.3" DOC:16-06-2004	Oblong	Olive green loosely contorted	Broad rounded
Oscillatoria agardhii Gomont BTA170	Rice field, Churachandpur, Manipur, India	Alt: 835 m N24°20'36.7" E093°41'50.3" DOC:10-12-2006	Absent	Brilliant blue green, straight	Gradually tapering
Spirulina platensis Nordst. Gomont BTA174	Rice field, Bishnupur, Manipur, India	Alt: 764 m N24°32'21.9" E093°45'27.6" DOC: 27-10-2006	Absent	Blue green spiral	Broadly rounded
Calothrix marchica Lemmermann BTA195	Rice field, Churachandpur, Manipur, India	Alt: 835 m N24°20'36.7" E093°41'50.3" DOC:10-12-2006	Basal	Yellowish blue green board	Rounded
Phormidium tenue Menegh Gomont BTA222	Rice field,Thoubal, Manipur, India	Alt: 795 m N24°45'01.3" E093°52'40.2" DOC:19-04-2007	Absent	Pale blue green. Slightly bent	Acute conical
Cylindrospermum muscicola Kutzing ex Born. et. Flah. BTA904	Rice field, Imphal East, Manipur, India DOC: 06-04-2010	Alt: 775 m N24°49'26.4" E093°57'52.0"	Oblong akinete present	Dark blue green cylindrical	Quadrate
Anabaena oryzae Fritsch BTA919	Rice field, Bishnupur, Manipur, India	Alt: 761 m N24°30'12.3" E093°46'46.4" DOC:15-03-2011	Terminal and intercalary	Light green curved	Conical
Anabaena anomala Fritsch BTA927	Rice field, Thoubal, Manipur, India	Alt: 782 m N24°39'18.5" E093°59'18.6" DOC:15-03-2011	Intercalary	Deep green irregular torn	Rounded
Nostoc piscinale Fremy BTA947	Rice field, Thoubal, Manipur, India	Alt: 805 m N24°29'28.7" E094°00'24.1" DOC:15-03-2011	Intercalary subspherical	Greenish flexuous	Cylindrical
Cylindrospermum indicum Rao, C. B, orth.mut. De Toni BTA960	Rice field, Thoubal, Manipur, India	Alt: 782 m N24°39'18.5" E093°59'18.6" DOC:15-03-2011	Oblong akinete	Light green cylindrical	Rounded
Dichothrix baueriana Grun. Born. et Flah. BTA1059	Rice field, Bishnupur, Manipur, India	Alt: 773 m N24°42'09.6" E093°48'22.3" DOC: 12-09-2011	Basal	Olive green ultimate branches	Rounded

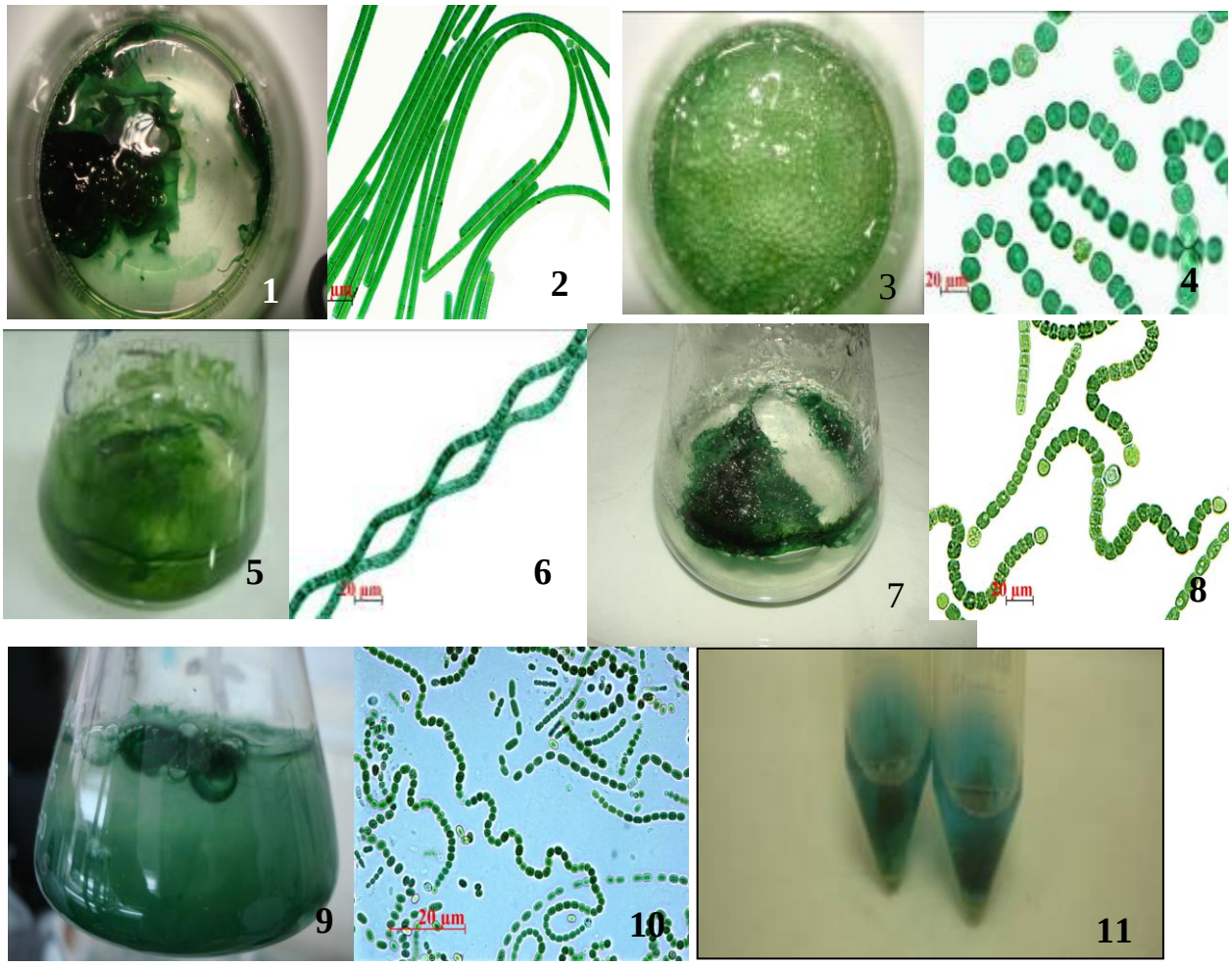
Table.2 Pigment analysis and ARA activity of fast growing cyanobacteria of rice fields of Manipur, India

Strains name and repository accession no.	Chlorophyll-a (µg/ml)	ARA (nmole C_2H_4 /µg of Chl-a/hr)	Carotenoids (µg/ml)	Phycobiliproteins (µg/ml)		
				PE	PC	APC
Microchaete grisea BTA007	10.8±2.04	0.15±0.04	42.1±1.75	60.7±1.78	70.1±1.76	47.3±1.89
Calothrix javanica BTA024	14.4±1.17	0.94±0.10	26.3±1.82	80.9±0.37	27.9±3.38	20.0±2.05
Calothrix marchica BTA026	16.0±1.09	0.72±0.04	13.1±0.68	25.3±1.54	49.1±1.98	28.2±1.97
Nostoc muscorum BTA027	9.00±1.09	0.21±0.04	19.4±2.25	4.85±0.93	148.3±1.24	32.7±1.54
Anabaena fertilissima BTA035	4.48±0.48	1.43±0.03	31.7±1.00	21.1±5.40	150.4±1.12	130.7±1.46
Nostoc hatei BTA037	5.51±0.60	90.9±0.09	21.5±2.50	4.34±2.10	16.7±5.38	10.3±5.47
Nostoc carneum BTA038	19.1±0.29	8.68±0.10	58.9±1.80	11.2±1.85	98.8±8.44	39.9±6.32
Phormidium arthurensis BTA042	12.5±0.69	ND	30.4±6.50	69.2±7.40	286.1±1.00	46.8±4.37
Anabaena oryzae BTA050	12.4±1.30	5.31±0.06	32.3±1.30	53.4±1.50	72.7±1.25	39.6±3.64
Phormidium purpurascens BTA081	5.71±0.13	ND	66.5±1.56	11.2±1.08	16.2±1.11	25.6±0.01
Anabaena spiroides BTA084	31.1±3.55	0.25±0.05	40.3±2.30	73.9±2.70	61.3±2.11	1.38±2.14
Nostoc muscorum BTA087	17.2±0.00	9.00±0.06	80.3±1.40	43.9±1.30	273.1±5.68	159.7±3.29
Oscillatoria agardhii BTA170	7.12±0.12	ND	46.5±4.67	28.7±3.10	48.7±1.27	72.4±1.63
Spirulina platensis BTA174	10.0±0.12	ND	55.1±0.00	8.72±0.00	196.1±0.01	56.8±0.02
Calothrix marchica BTA195	6.25±0.82	7.01±0.00	66.4±1.36	25.6±1.36	22.3±1.20	21.0±0.90
Phormidium tenue BTA222	10.5±0.01	ND	66.1±1.99	1.10±1.46	1.96±1.01	1.87±0.76
Cylindrospermum muscicola BTA904	0.89±0.05	12.1±0.05	2.66±0.19	7.51±0.20	16.5±3.34	21.2±6.30
Anabaena oryzae BTA919	1.72±0.40	65.6±6.65	2.66±0.19	12.2±1.60	20.7±1.27	7.75±3.46
Anabaena anomala BTA927	0.69±0.50	13.7±0.06	11.4±1.57	28.3±5.70	52.9±5.90	22.1±3.20
Nostoc piscinale BTA947	25.2±0.05	4.61±0.13	32.6±1.30	15.6±2.40	28.7±1.89	72.9±2.49
Cylindrospermum indicum BTA960	4.21±0.01	3.97±0.02	5.67±1.00	10.3±0.50	67.3±0.04	79.2±1.49
Dichothrix baueriana BTA1059	6.68±1.05	19.5±0.16	21.3±0.74	9.52±1.71	10.3±0.98	8.01±3.71



Photoplate-1: High quantity phycoerythrin producing strains.

1&2: *Calothrix javanica* BTA24 (thallus behaviour & microphotograph respectively)
3&4: *Anabaena spiroides* BTA084 (thallus behaviour & microphotograph respectively)
5&6: *Phormidium arthurensis* BTA42 (thallus behaviour & microphotograph respectively)
7&8=*Microchaete grisea* BTA07 (thallus behaviour & microphotograph respectively)
9&10= *Anabaena oryzae* BTA50 (thallus behaviour & microphotograph respectively)
11: Extraction of phycoerythrin in phosphate buffer



Photoplate-2: High quantity phycocyanin producing strains.

1&2: *Phormidium arthurensis* BTA42 (thallus behaviour and microphotograph respectively)

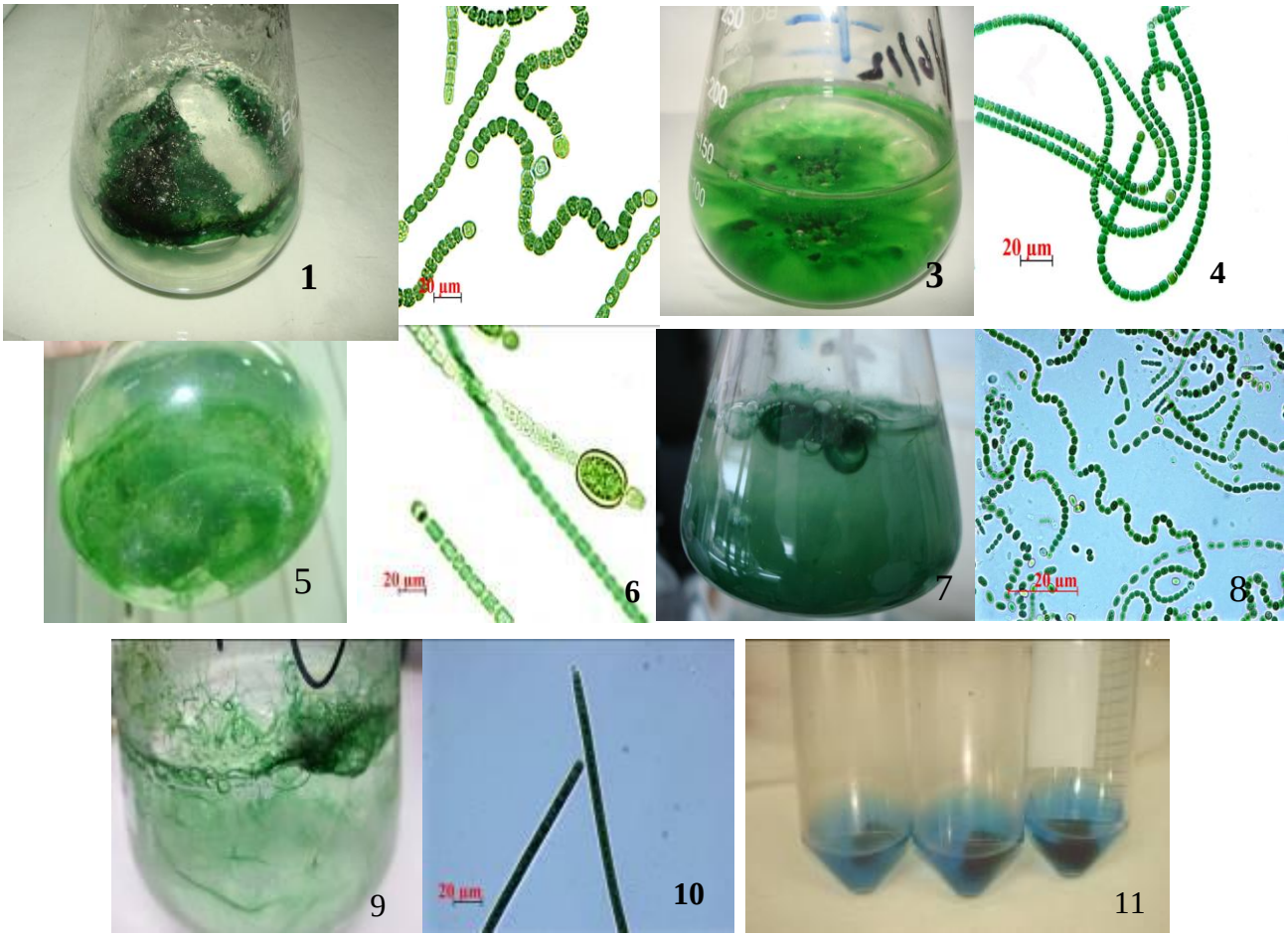
3&4: *Nostoc muscorum* BTA087 (thallus behaviour and microphotograph respectively)

5&6: *Spirulina platensis* BTA174 (thallus behaviour and microphotograph respectively)

7&8: *Nostoc muscorum* BTA27 (thallus behaviour and microphotograph respectively)

9&10: *Nostoc piscinale* BTA947 (thallus behaviour and microphotograph respectively)

11: Extraction of phycocyanin in phosphate buffer



Photoplate -3: High quantity Allo phycocyanin producing strains.

1&2: *Nostoc muscorum* BTA87 (thallus behaviour and microphotograph respectively)

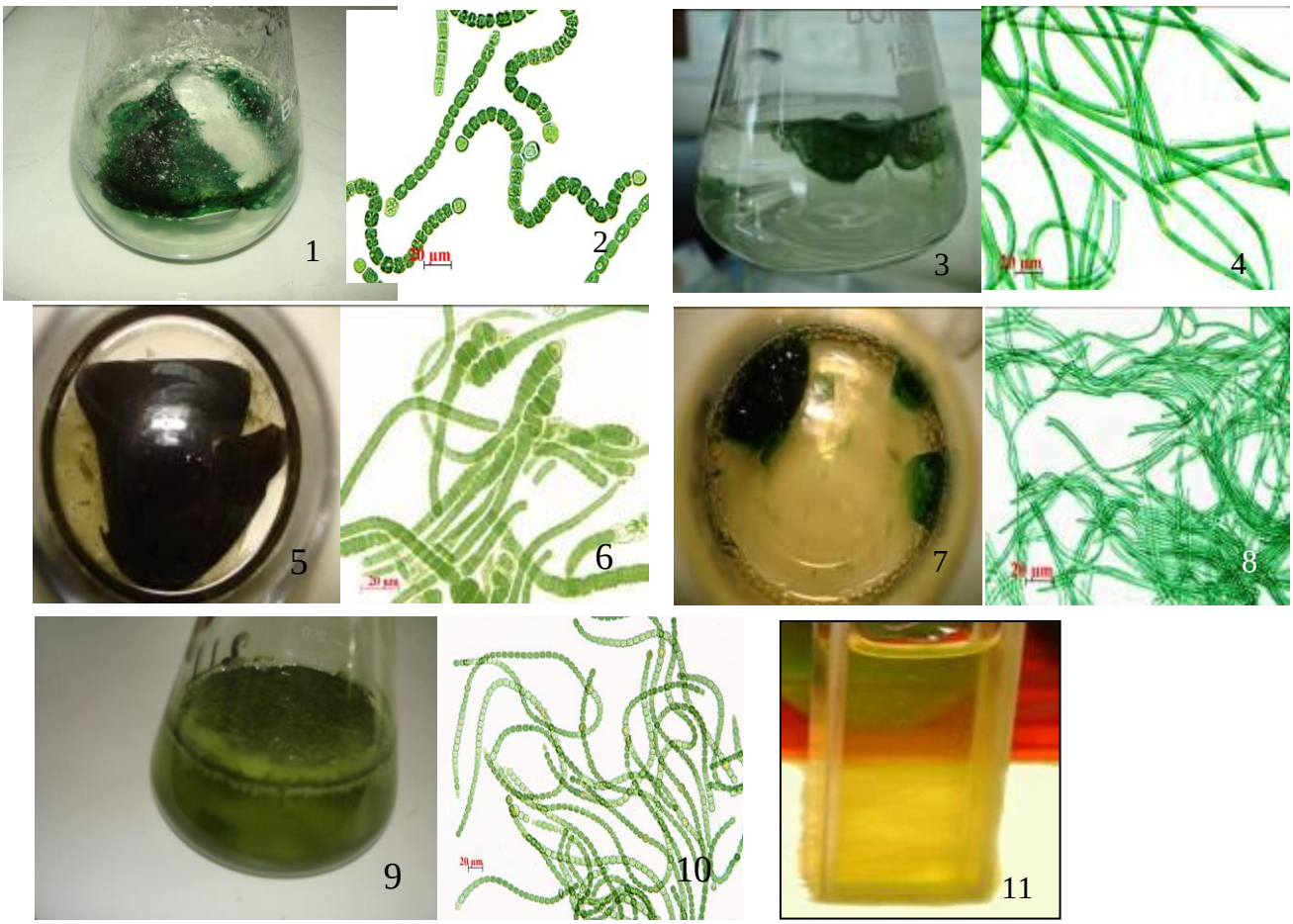
3&4: *Anabaena fertilissima*BTA35 (thallus behaviour and microphotograph respectively)

5&6: *Cylindrospermum indicum* BTA960 (thallus behavior and microphotograph respectively)

7& 8: *Nostoc piscinale* BTA947 (thallus behaviour and microphotograph respectively)

9&10: *Oscillatoria agardhii* BTA170 (thallus behavior and microphotograph respectively)

11: Extraction of Allophycocyanin in phosphate buffer



Photoplate-4: High quantity Carotenoids producing strains

1&2: *Nostoc muscorum* BTA87 (thallus behaviour and microphotograph respectively)

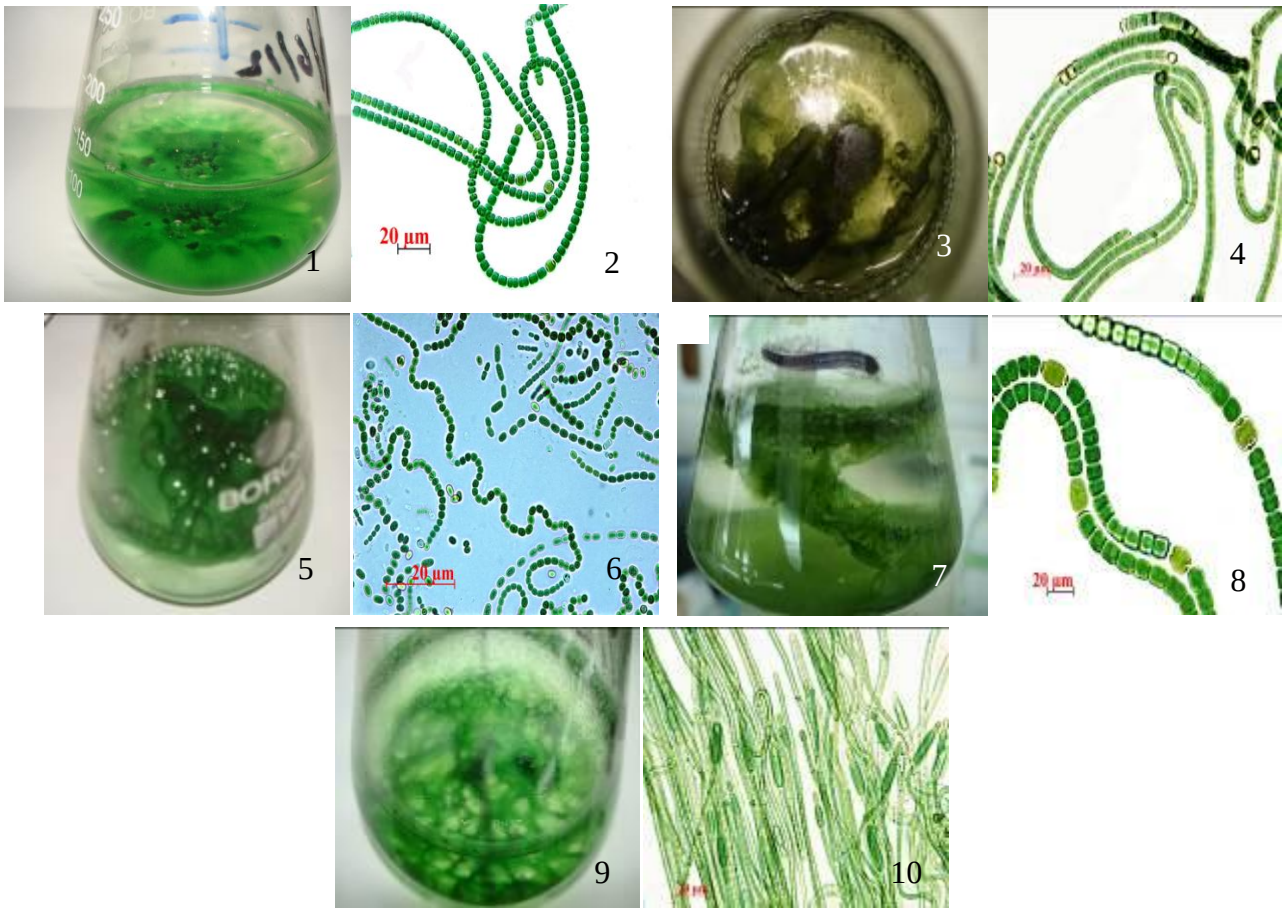
3&4: *Phormidium purpurescens* BTA81 (thallus behavior and microphotograph respectively)

5&6: *Calothrix marchica* BTA195 (thallus behaviour and microphotograph respectively)

7&8: *Phormidium tenue* BTA222 (thallus behaviour and microphotograph respectively)

9&10: *Nostoc caeneum* BTA38 (thallus behavior and microphotograph respectively)

11: Extraction of total Carotenoids in 80% Acetone



Photoplate -5: High determination of nitrogenase activity producing strains

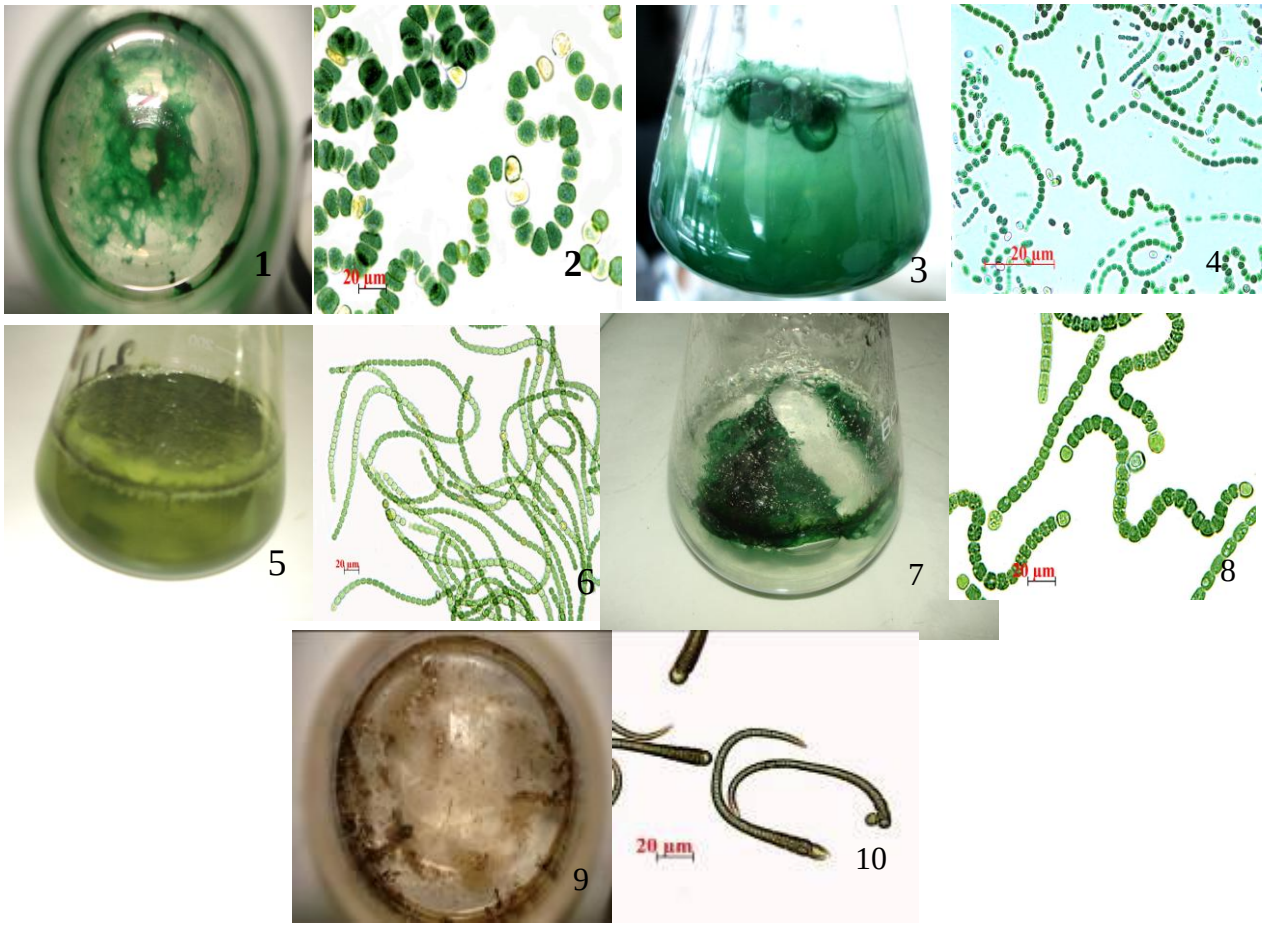
1&2: *Nostoc hatei* BTA37 (thallus behaviour and microphotograph respectively)

3&4: *Dichothrix baueriana* BTA1059 (thallus behaviour and microphotograph respectively)

5&6: *Anabaena anomala* BTA927 (thallus behaviour and microphotograph respectively)

7&8: *Anabaena oryzae* BTA919 (thallus behavior and microphotograph respectively)

9&10:*Cylindrospermum muscicola* BTA904(thallus behavior and microphotograph respectively)



Photoplate-6: High quantity of chlorophyll-a producing strains

1&2: *Anabaena spiroides* BTA84 (thallus behaviour and microphotograph respectively)
3&4: *Nostoc piscinale* BTA947 (thallus behaviour and microphotograph respectively)
5&6: *Nostoc carneum* BTA38 (thallus behavior and microphotograph respectively)
7&8: Nostoc *muscorum* BTA87 (thallus behaviour and microphotograph respectively)
9&10: *Calothrix marchica* BTA26 (thallus behaviour and microphotograph respectively)

Phycocyanin is the major and important phycobiliproteins which exhibited anti-cancer activity, stimulation of immune system and ability to treat ulcers and haemmorrhoidal bleeding. Since the culture conditions are known to be changed the biochemical composition of the algae the constituents can be improved upon further by manipulating culture conditions (Ciferri, 1983). It was observed that *Nostoc muscorum* BTA087 was recorded highest carotenoids under culture conditions which was comparable to previous workers reported the carotenoids in *Phormidium laminosum* (Fresnedo et al., 1991); *Synechococcus* sp. (Gombos and Vigh 1986) and *Nostoc commune* (Olie and Potts 1986; McPherson. 1986; Clement et al., 1967; Becker, 1986). The rate of acetylene reduction activity by cyanobacteria generally ranges from 1-10 nmol C_2H_4/µg chl-a/hr (Fogg et al., 1973). In our study it was recorded that *Nostoc hatei* BTA037 may be the potent diazotrophic

cyanobacteria as expressed very high ARA activity in culture conditions. Vargas et al., 1998 reported that when nitrogen fixing cyanobacteria were grown under diazotrophic conditions, protein, carbohydrates and lipid content varied organism to organism. The present findings given indication that the *Calothrix javanica* BTA024, *Phormidium arthurensis* BTA042, *Nostoc muscorum* BTA087, *Nostoc hatei* BTA037 and *Anabaena spiroides* BTA084 may be the potential strains for commercial exploitation for one or other reason but detailed and thorough investigation to be done systematically before final conclusion. *Nostoc muscorum* BTA87 may be promising strains for exploiting as natural pigment producing organism since it showed the good content of allophycocyanin and carotenoid pigments.

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References

Becker, E.D. (1986). "Nutritional properties of microalgae: Potential and constraints", in A. Richmond (ed.), *Handbook of microalgal mass culture*, *Boca Raton, Florida, USA, CRC Press*, 339-420.

Becker, E.W. (1994). *Microalgae*: *Biotechnology and microbiology*, Cambridge University Press.

Bennett, A., Bogorad, L. (1973). Complementary chromatic adaptation in filamentous blue-green algae, *J. Cell Biol*., 58: 419-433.

Bjornsater, B. R., Wheeler, P. A. (1990). Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis*, (Ulvales, Chlorophyta), *J. Phycol*., 26: 603-611.

Borowitzka, M. A. (1994). "Products from algae", In S.M. Phang, K. Lee, M.A. Borowitzka and B. Whitton (eds.), *Algal biotechnology in the Asia-Pacific region, Institute of Advanced Studies, University of Malaya, Kuala Lumpur*, 5-15.

Borowitzka, M.A., Borowitzka, L.J. (1988). Microalgal biotechnology, Cambridge University Press, Cambridge, 477.

Cannell, R. J. P. (1989)."Algal biotechnology", In H. Weetall (ed.), *Appl. Biochem.* and *Biotech*., *The Humana Press Inc*., 85-105.

Chen, F., Johns, M. R. (1991). Effect of C/N ratio and aeration on the fatty acid composition of heterotrophic *Chlorella sorokiniana*, *J. Appl. Phycol*., 3: 203-209.

Ciferri, O. (1983). *Spirulina*: The edible microorganism, *Microbiol. Rev*., 47(4): 551.

Clement, G., Rebeller, M., Zarrouk, C. (1967). Wound treating medicaments containing algae, *Fr. Med*., 5279.

Cohen, Z. (1986). "Products from microalgae", In A. Richmond (ed.), *Handbook of microalgae mass culture*, *CRC press, Florida, USA*, 421-454.

Dainippon Ink and Chemicals Inc.(1980). Production of highly purified alcoholophilic phycocyanin, Japanese Patent, 80: 77-890.

Dainippon Ink and Chemicals Inc. (1981). Cosmetics containing water soluble phycocyanin, Japanese Patent, 79: 138-755.

Desikachary, T. V. (1959). *Cyanophyta*, Indian Council of Agricultural Research, New Delhi, India.

Fogg, G.E., Stewart, W.D.P., Fay, P., Walsby, A. E. (1973). The blue green algae, Academic press, London and New York, 459.

Fresnedo, O., Gomez, R., Serra, J.L. (1991). Carotenoids composition in the cyanobacterium *Phormidium laminosum*: Effect of nitrogen starvation, *FEBS Lett*., 282(2): 300-304.

Gombos, Z., Vigh, L. (1986). Primary role of the cytoplasmic membrane in thermal acclimation evidenced in nitrate-starved cells of the blue-green alga *Anacystis nidulans*, *Plant Physiol*., 80: 415-419.

Hardy, R.W.F., Burus, R.C., Holsten, R.D. (1973). Application of the acetylene ethylene assay for measurements of nitrogen fixation, *Soil Biol. Biochem*., 5:47-81.

Healey, F.P., Henzdel, L.L. (1976). Physiological changes during the course of blooms of *Aphanizomenon flos-aquae*, *J. Fish. Res. Board Can*., 33: 36-41.

Jensen, A. (1978). "Chlorophylls and carotenoids", In J. A. Hellebust and I. S. Craige, (eds.), *Handbook of phycological methods*: *Physiological and biochemical methods*, *Cambridge University press*, 59-70.

Kobayasi, H. (1961). Chlorophyll content in sessile algal community of Japanese mountain river, *Bot. Mag. Tokyo* 74: 228-235.

Komarek, J., Anagnostidis, K. (1998). *Cyanoprokaryota 1. Chroococcales*, Gustav Fisher, Jena, Stuttgart Lubeck, Vlm.

Komarek, J., Anagnostidis, K. (2005). "Cyanoprokaryota 2. teil/ 2nd part: Oscillatoriales", In B. Budel, L. Krienitz, G. Gartner and M. Schagerl (eds.), *Susswasserflora von MitteleuropaI*, *19/2*, *Elsevier*/*Spektrum*, *Heidelberg*, 759.

Lobban, C.S., Harrison, P.J. (1994). *Seaweed ecology and physiology*, Cambridge University Press, NewYork.

Mckinney, G. (1941). Absorption of light by chlorophyll solution, *J. Biol. Chem*., 140: 315-322.

McPherson, K. R. (1986). "Dietary fiber a perspective", In G. Spiller (ed.), *Handbook of dietary Fiber in Human Nutrition*, *Boca Raton*, *FL. USA*, *CRC press*, 7-11.

Olie, J.J., Potts, M. (1986). Purification and biochemical analysis of the cytoplasmic membrane from the desiccation-tolerant cyanobacterium *Nostoc commune* UTEX 584, *Appl. Environ. Microbiol*., 52(4): 706-710.

Poutinen, C.J. (2009). Detoxification herbs natural body cleansing treatments, Suite 101.com, insightful writers. Informed readers.

Rhee, G. Y., Gotham, I. J. (1980). Optimum N:P ratios and co-existence of planktonic algae, *J. Phycol*., 16: 486-489.

Skulberg, O.M., Carmichael, W.W., Codd, G. A., Skulberg, R. (1993). "Taxonomy of the toxic cyanophyceae (cyanobacteria)", In: *Algal toxins in seafood and drinking water*, *Academic Press Ltd*., 144-164.

Stanier, R.Y., Kunisawa, M.M., Cohen-Bazire, G. (1971). Purification and properties of unicellular blue green algae (order Chroococcales), *Bact. Res*., 35: 171-201.

Vargas, M.A., Rodriguez, H., Moreno, J., Olivares, H., Del Campo, J.A., Rivas, J., Guerrero, M.G. (1998). Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. *J Phycol* 34:812–817.

Venkataraman, L.V., Mahadevaswamy, M. (1992). Pond management in *Spirulina platensis*, In Proceedings of ETTA. National Symposium, Madras, 113-118.

Zevenboom, W., De Vaate, A. B., Mur, L.R. (1982). Assessment of factors limiting growth rate of *Oscillatoria agardhii* in hypertrophic Lake Wollderwijd by use of physiological indicators, *Limnol. Oceanogr.*, 27: 39-52.