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Unifying features of a high lipid accumulating marine microalga (*Chlorella* sp.) collected from south coast of Odisha and the effects of nitrate on its growth and lipid profile

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ABSTRACT

The study was undertaken to isolate and screen microalgae from South coast of Odisha and further see the effects of nitrate on growth and lipid accumulating ability of a selected green alga. In all ten isolates were pure cultured, which include both cyanobacteria and a green alga. An identified *Chlorella* sp. was found to accumulate high amount of neutral lipids in its stationary phase of growth and subsequently the effects of nitrate on its growth and lipid profile was carried out. Growth was retarded in cultures without nitrate followed by reduced biomass productivity and specific growth rate. Photosynthetic pigments like chlorophylls and carotenoids were less synthesized in nitrate deprived growth medium. Complete lipid profile analysis under normal growth conditions showed that the alga contained maximum PUFA (49.1 %) followed by saturated fatty acids (32 %) and MUFA (18.9 %). Under deprived N condition the occurrence of PUFA (43.6 %) was reduced while saturated fatty acids (36.1 %) and MUFA (20.3 %) increased to 12.8 % and 7.4 %, respectively. It is necessary to grow the organism both in nutrient optimum or deprived condition so that the alga can be used as a source of high nutraceutical compounds or biofuel.

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1. Introduction

Microalgae include the prokaryotic or eukaryotic group of photosynthetic organisms which like land plants capture the solar radiation and could fix CO_2 to biomass. They are present over a wide range of temperature, pH and salinity and found to be present in all type of ecosystems (Falkowski and Raven, 1997). They can modify their physiological and biochemical response according to the changes in the environmental parameters which enable them to inhabit in almost all climatic conditions on earth. They are mostly found as a member in fresh water, marine or brackish water environments (Richmond, 2004).

Their growth requirement is very simple which includes a light source e.g. solar energy or artificial illumination system, water and some mineral nutrients. Nutrients, may be in the form of inorganic or organic compounds, are used for growth apart from CO_2 and water required for cellular functions (Neenan *et al*., 1986). These include macro as well as micro elements and the requirement is different from species to species in regard of one or a few of them. Nitrogen is the most vital element after carbon as a constituent of cellular molecules like protein, nucleic acids and amino acids. Algae take it either in the form of NO_3^-, NH_4 or $(NH_2)_2CO$ (urea). The most used N source for the culture of microalgae is nitrate (NO_3^-).

During favourable conditions of growth the microalgae grow rapidly and accumulate large biomass. They contain very high amount of nutrients, especially protein, carbohydrates and lipids. Under optimal growth, the relative content of the various nutrients is fairly similar among species (Hu, 2004). When the nutrient, salinity, temperature and light quality and quantity are not supporting the growth they can modify their metabolism to strive through the harsh conditions. Many algae follow different adaptation strategies

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to survive adverse environmental conditions in different habitats (Seckbach, 2007; Barsanti *et al*., 2008).

Mainly microalgae change the lipid metabolism to unusual pattern under environmental variations and hence able to survive and proliferate under stress (Hu *et al*., 2008). Under unfavourable growth they modify the lipid biosynthetic pathway towards neutral lipids (20-50% of DCW) mostly triacylglycerol (TAG) in nature. In comparision to the algae, cyanobacterial lipids mostly contain diacylglycerol (DAG).

In response to nitrogen limitation, accumulation of lipids, particularly TAG has been observed as a regular strategy in numerous species of various algae (Thompson, 1996; Merzlyak *et al*., 2007). Cyanobacteria are also topic of research and hence subjected to screening for lipid production (Basova, 2005). In contrast to eukaryotic algae, considerable amounts of total lipids have not been found in cyanophycean organisms examined in the laboratory. The accumulation of neutral lipids as triacylglycerols has not been observed in naturally occurring cyanobacteria.

South coast of Odisha harbours many forms of microalgae due to the presence of varied environments such as Chilka Lake, Huma salt pan, Bay of Bengal etc. Chilka is the largest brackish water lagoon of Asia situated at the East coast of India (19°28'2" and 19°54'2" N latitude and 85°06'2" and 85°35'2" E longitude).On one side it is connected with Bay of Bengal and on the other side many rivers like Daya, Bharghavi and Luna fall into it. So it is a habitat for both fresh and brackish water microalgae, due to mixing of sea water with fresh water at many places along the coast. Seasonal fluctuations in the physico-chemical parameters of water along the South coast promote the growth of many species of algae in different seasons.

A random collection of samples from all these environments of South coast of Odisha tempted us to focus on a few of which one was found having high lipid accumulating capacity vis-à-vis variable nitrogen concentrations. The complete lipid profile due to unavailability of nitrate in the culture medium in comparison with the normal growth condition was worked out simultaneously.

2. Materials and methods

2.1 Selection of study sites

South coast of Odisha was chosen as the study site and algal samples were collected during three season viz. winter, summer and rainy for one year from October 2013 to October 2014 taking dry and wet soil, water samples and sediments from Kaluparaghat, Balugaon, Huma salt pan and sea shore of Gopalpur while sampling both planktonic and benthic algal organisms were screened out from the aquatic environments using mess size and macro-forms were rejected at the site. The salinity and temperature of the water was measured using a salinity meter and a thermometer, respectively. The samples were kept in sterile zipper bags and taken to the laboratory. Water (50lit each) from each site was collected for cultivation of the organisms.

2.2 Pre-treatment of the samples

The soiled samples were washed in tap water thoroughly overnight to remove the impurities and again washed along with other samples repeatedly in distilled water. All the samples were serially diluted and used for isolation directly.

2.3 Preparation of culture medium and pure culture

To isolate the organisms BG-11 and nitrogen free BG-11 medium as per composition provided by Rippka *et al.* (1979) along with f/2 medium as per Guillard (1973) was used. The medium was prepared in water collected from the sampling site (autoclaved and kept standing in dark for 24hrs). After sterilisation, the liquid mediums in 250 ml Erlenmeyer flasks were kept for cooling. Solid agar plates were prepared using 1.5% algae culture agar (Hi-Media) in the BG-11, N_2 free BG-11 and f/2 liquid medium.

The water samples were transferred to agar plates using spread plate technique. The soil and sediment samples were transferred to the BG-11and N_2 free BG-11 agar plates. All the plates were then incubated at 28 ± 2^0 C and 250 µmol m^{-2} s^{-1} light intensity using white fluorescent tubes in a culture room. After 7 days all plates and flasks were checked for the growth and appearance of any organisms. The difference in any colour, texture in the coloured patches was marked and again subcultured in fresh plates. After sufficient growth, it was again sub cultured in fresh plates. The pure cultured organisms were observed under an inverted microscope and colonies were subsequently transferred to fresh, sterile liquid medium and incubated under above-mentioned conditions. With every subculture, the organisms were checked under a microscope for purity and transferred to fresh medium.

2.4 Maintenance of pure cultures

All the pure isolates were maintained in the laboratory at 28 ± 2^0 C and 250 µmol $m^{-2}s^{-1}$ light intensity using white fluorescent tubes with continuous illumination. All the flasks were manually shaken twice daily for aeration and to avoid settling down of the organisms at the bottom.

2.5 Selection of the experimental isolate

2.5.1 Morphological identification

The morphology of all the isolates was examined under normal phase contrast microscope using 40X objective. Micrographs were taken using Leica software. The fluorescence property of chlorophyll molecule was used to get a clear picture of the isolates. All the pure isolates were observed under 20X objective in TCS SP5 confocal microscope exciting with a blue light.

2.5.2 Spectral characteristics

Absorption spectra (360-800 nm) of the whole suspension were recorded using carry 300 Bio spectrophotometer at room temperature. The isolates were found to give different wavelength dependent electronic transitions referring to varied pigment types. The absorbance peak at specific wavelengths varied for cyanobacteria and algae, which was used to single out the cyanobacterial or green algal species.

2.5.3 Effects of nitrate on the experimental algae

Out of all only the selected alga proved to be a *Chlorella* sp. JD-2016 as released by NCBI (KU497645) was grown in 250ml Erlenmeyer flask using complete f/2 medium and nitrate deprived f/2 medium under laboratory conditions with 16:8 L: D photoperiod, 100 µmol/m^{2}S light intensity (white fluorescent bars) and at 24±2 °C. The organism cultured previously by Dash *et al*. (2016) exhibited growth curve in which the exponential growth phase was appeared between 6 to 12 days of culture. Accordingly the dry weight was estimated on 5, 8 and 13 days which fall in the linear region of the growth curve. The photosynthetic pigments concentrations were determined at different phases of the growth curve.

2.5.4 Growth and photosynthetic pigments under altered nitrogen doses

For dry weight determination of the algal biomass, 10 ml algal culture were harvested, washed and filtered through pre-weighed Whatman GF/C filters (0.45 µm) and dried at 80^{0}C till constant weight was obtained. Specific growth rate and biomass productivity was calculated from the data according to the equation; $K' = Ln (N_2/N_1)/(t_2-t_1)$ where N_1 and N_2 are biomass at time (t_1) and (t_2), respectively (Levasseur, 1993).

The pigment concentration (Chl-a, Chl–b and carotenoids) of the alga in the methanolic extract was determined following Porra *et al*. (1989) and Lichtenthaler (1987). The methanolic extract of the pigments was achieved by percolation method. In order to percolate the pigments, 1ml algal culture was centrifuged at 8000 g for 10 min. The pellet was washed with distilled water and incubated with 2 ml of absolute methanol keeping at - 4° C for 48-h in dark, by which time the extraction of the pigment was completed leaving behind a colourless pellet. The respective absorption peak values for carotenoids (470nm), Chl-b (652 nm) and Chl-a (665nm) in the methanolic extract were corrected for turbidity, if any, by subtracting the values obtained at 750nm.

2.5.5 Extraction of lipids and analysis of Fatty Acid Methyl Esters (FAME) profile

The *Chlorella* sp. grown in culture medium with optimum nitrate (0.082 mM) and without nitrate (0 mM) were further investigated to compare the effects of nitrate on the lipid profiles of the alga. Algal cells were harvested till early stationary phase of growth. The cells on the 14 days of culture were taken, centrifuged and lyophilized. Dry algal biomass (0.2 g) treated with 2% H_2SO_4 in methanol (5 ml) at reflux temperature (65^0 C) for 4 h was used for the conversion of lipid component present in the biomass to its fatty acid methyl esters. The reaction mixture was extracted with ethyl acetate (3 x 15 ml) and the extract was washed with water. The organic layer was dried over anhydrous sodium sulphate and concentrated to get the fatty acid methyl ester (FAME) mixture.

The FAME mixture was dissolved in minimum amount of chloroform and analysed by GC for fatty acid composition. GC was carried out with Agilent 6890 N-Series gas chromatograph equipped with a FID detector. The GC was performed using DB-225 capillary column (30 m × 0.25 mm × 0.25 µm) and the oven temperature was programmed for 2 min at 160 °C, raised to 230 °C at 5 °C/min and finally maintained at 230° C for 20 min. The carrier gas, N_2 flew at 1.0 ml / min maintaining the split ratio at 50:1.

3. Results

3.1 Isolation and maintenance of pure culture

The water temperature was almost equal everywhere with little variation (22 ± 5° C) in the predetermined sites. However, the salinity differed to a large extent. Gopalpur estuary had recorded maximum salinity of (35 ppt) as compared to water of Kaluparaghat, Balugaon, and Huma, which had salinity range 20-25 ppt.

After repeated culture and subculture, total 10 numbers of pure cultures were isolated. Isolates were named as S-1 to S-10 (S being abbreviated for sample).

Upon microscopic observation it was found that except two microalgal isolates (S-8 and S-9) all others were filamentous algae of varied lengths. All were blue green or green in colour while isolates S-1, S-3, S-4, S-6, S-7 and S-10 were found to belong to different species of *Oscillatoria*,

S-2 and S-5 to be *Phormidium* sp. and S-8 was a unicellular microalga. On the otherhand, S-9 with very small filaments, under the confocal microscope appeared in diad and tetrad (Fig. 1a and Fig. 1b).

3.2 Screening of the isolates

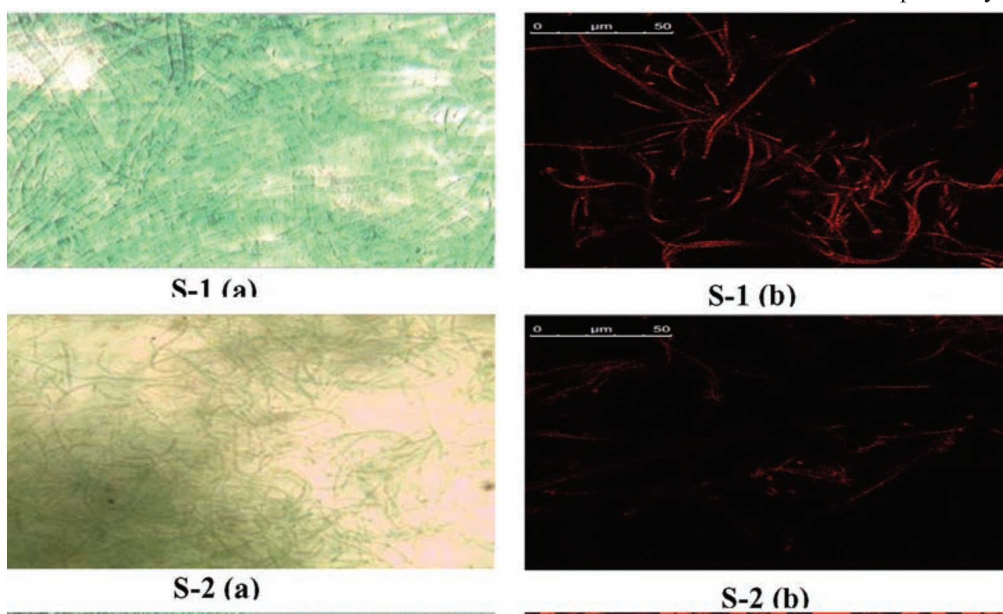
Taking the whole cell absorbance by scanning the cell suspensions of all the isolates were studied in a UV-VIS spectrophotometer from wavelength 360-800 nm. The absorbance peak at specific wavelength varies for cyanobacteria and algae, which was used to single out the cyanobacterial or green algal species. The cyanobacteria and algae, as expected, gave different wavelength dependent electronic transitions referring to the presence of various pigment types. All the isolates, except one, showed characteristic signature of electronic transition for cyanobacteria having a prominent transition peak at 620 nm due to phycocyanin pigments. Isolate S-9 which was apparently viewed as rods in diad and tetrad form under a confocal microscope, was confirmed to be cyanobacteria from its spectral characteristics. Only a single isolate (S-8) exhibited the presence of electronic transition for Chl-b at 652nm (Fig. 2 A) referring to a green alga.

3.3 Effect of nitrate on growth, chlorophyll and lipid profile of the Chlorella sp.

The biomass productivity per day of the alga declined in the cultures with low nitrate. The cell dry weight was lower in the nitrate deprived medium (301 mg/l) as compared to the optimum (610 mg/l) medium. So it is evident that growth in the limited supply of nitrate is reduced as compared to the optimum nitrate. Similarly, the cell division per day has decreased and doubling time has increased suggesting reduced metabolism. The specific growth rate of the alga in optimum nitrate culture medium was higher in comparison to the zero nitrate condition. Similar effects were observed on the synthesis of chlorophyll due to nitrogen deprivation in the culture medium. The synthesis of the chlorophyll per ml of the culture was affected by the absence of nitrate in the medium (Table 2). The chlorophyll synthesis was always more in higher nitrate concentrations. At a particular day of growth the C_a/C_b and total chlorophyll to carotenoids ratio were almost constant. But when the cultures were compared in the stationary phase (24 day), it is noticed that the chl / carotenoids ratio decreased as compared to exponential (12 day) and early stationary phase (18 day). This suggests that the low nitrate in the medium is not only the stressor but the cultures with ageing also exhibit stress which is indicated by the low ratio of total chlorophylls to carotenoids

To further know the normal lipid profile of the alga and if any change upon omitting N from its culture medium is there, FAME were analyzed using gas chromatography with a flame ionizing detector (FID). The chromatogram is also provided that contains peaks for various fatty acids given by their area (Fig. 3 A, B; Fig. 4 A, B) The occurrence of saturated, monounsaturated and polyunsaturated fatty acids (%) upon deprivation and supplementation of nitrate in the culture mediums are tabulated (Table 3).

As it can be seen from the table that under normal growth conditions (optimum nitrate in culture), the algae contain maximum PUFA (49.1 %) followed by saturated fatty acids (32 %) and MUFA (18.9 %). When nitrate is not supplied in the culture medium i.e. under deprived N condition the occurrence of PUFA (43.6 %) has reduced while saturated fatty acids (36.1 %) and MUFA (20.3 %) increased to 12.8 % and 7.4 % respectively.



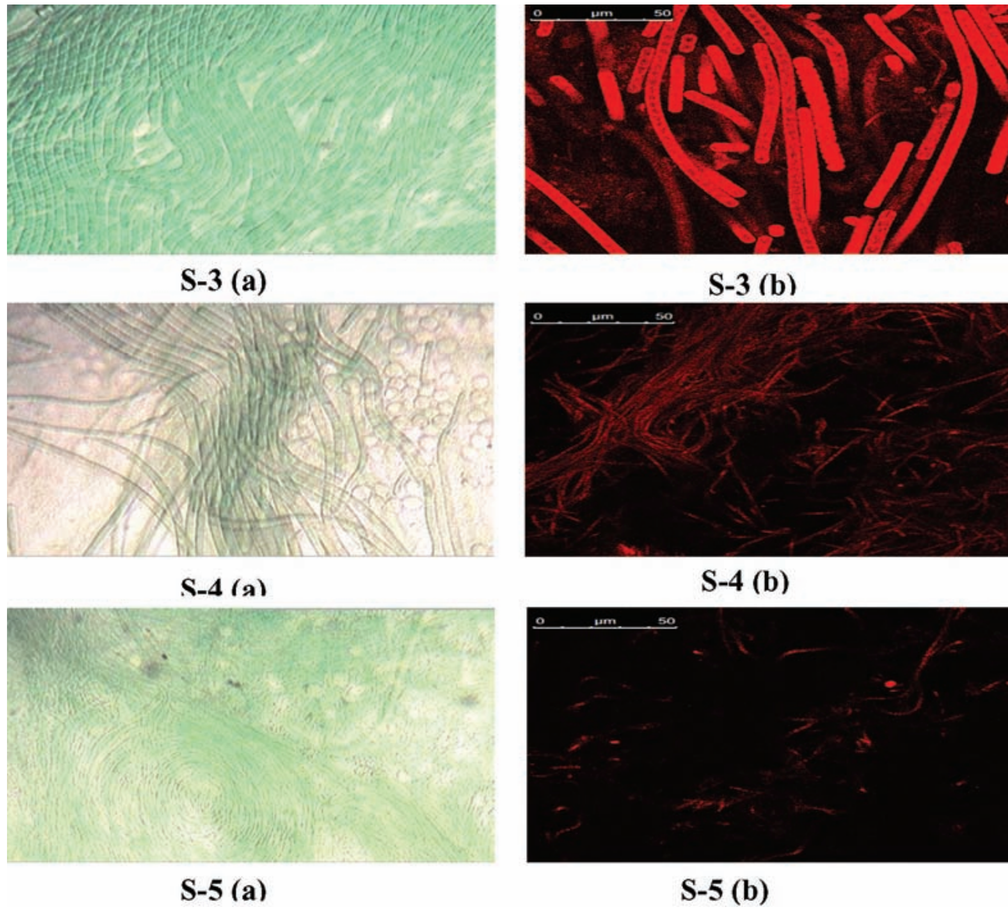
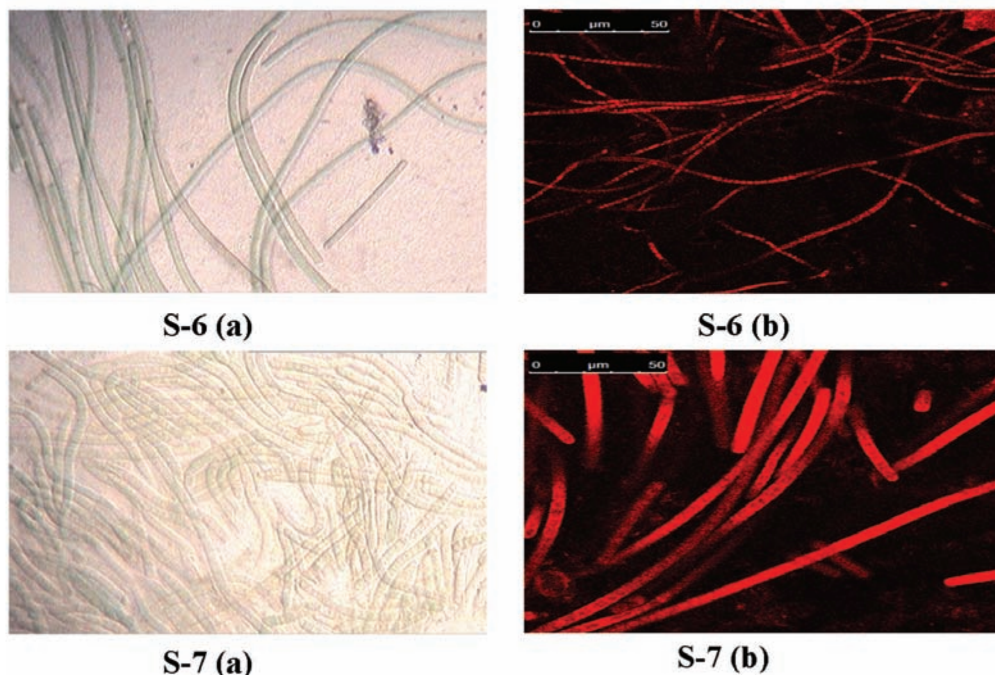


Fig.1a. Phase contrast (a) and confocal (b) micrograph of the isolates



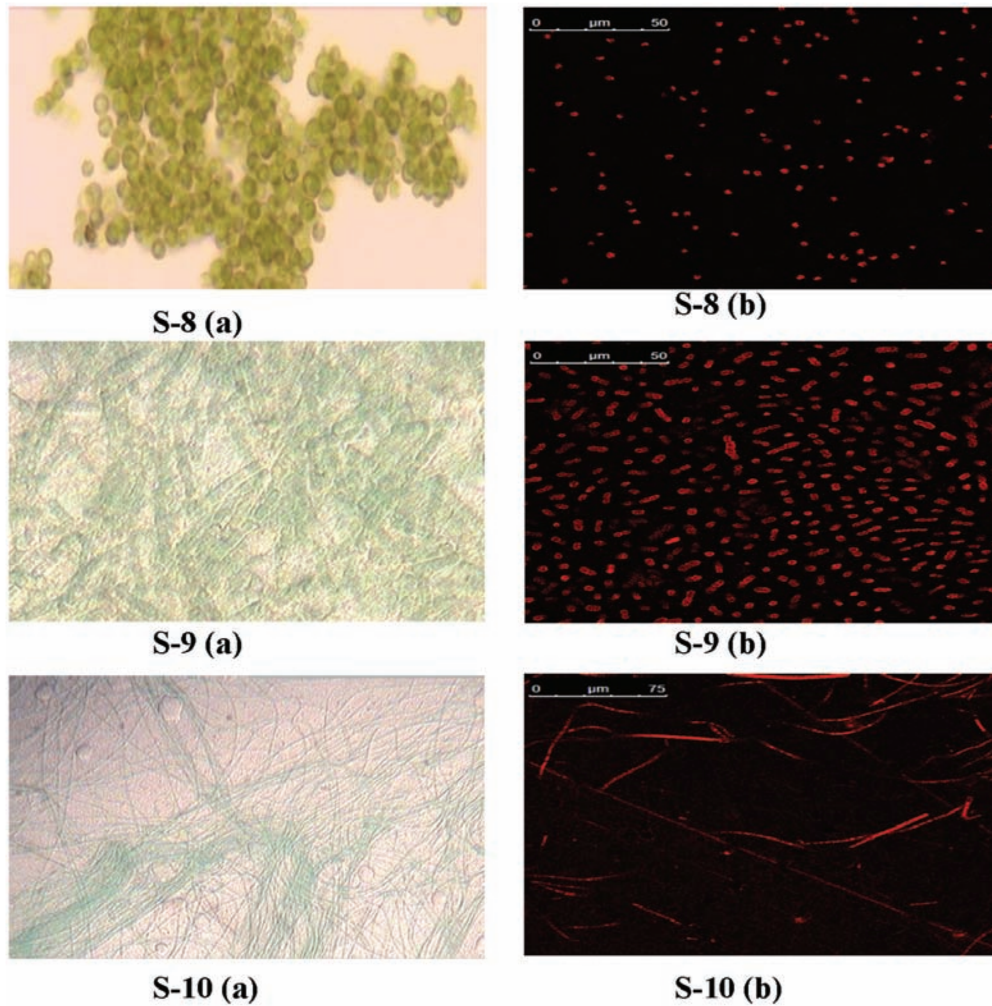


Fig.1b. Phase contrast (a) and confocal (b) micrograph of the isolates

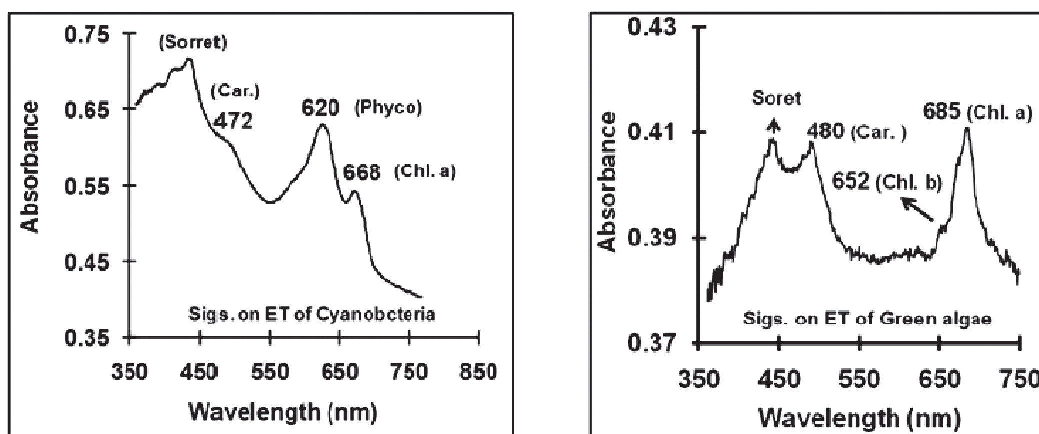


Fig. 2. Screening of green alga using spectral characteristics of the isolates

Table 1

The effects of nitrate on growth and biomass productivity of the *Chlorella* species

Concentration of Na NO_3	Dry weight (mg/l)	Biomass Productivity mg /l/day	Specific growth rate µ/day	Division/day	Generation/ doubling time
0	301±20	23	0.063	0.09	10.92
0.882	610±66	46.9	0.155	0.22	4.45

Values represent mean ± SE and results were significant at P< 0.01

Table 2

Change in pigment concentrations of the *Chlorella* sp. due to absence of nitrate in the medium.

Concentration $NaNO_3$	12 days			18 days			24 days		
	C_a+ C_b (µg/ml)	*Car* (µg/ml)	*C/Car*	C_a+ C_b (µg/ml)	*Car* (µg/ml)	*C/Car*	C_a+ C_b (µg/ml)	*Car* (µg/ml)	*C/Car*
0	0.132	0.088	1.5	0.111	0.109	1.018	0.094	0.116	0.810
0.8	2.224	0.836	2.66	1.799	1.078	1.687	1.415	1.071	1.321

Values represent mean of three replications and results were significant at P< 0.001

Table 3

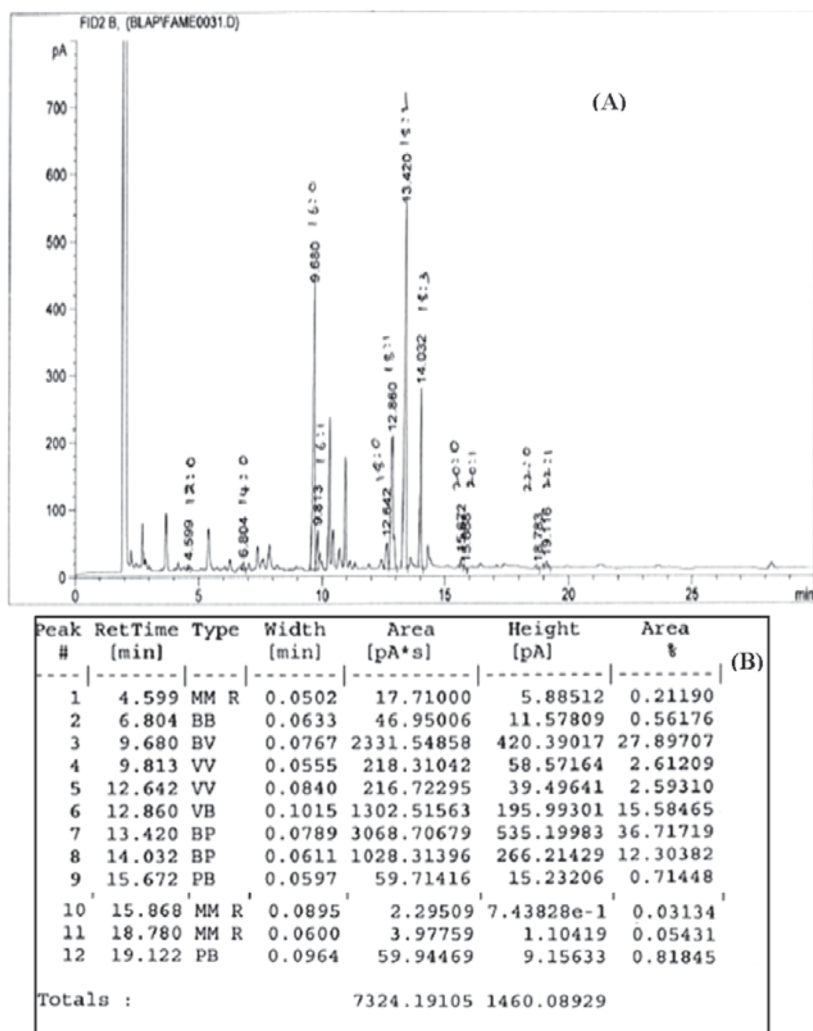
Fatty acids profile of *Chlorella* sp. JD-2016 under normal and nitrates stress conditions.

	TYPES	% FATTYA CID	
		+ N	- N
Saturated	12:0	0.3	0.7
	14:0	0.6	0.9
	16:0	27.7	28.2
	18:0	2.6	4.6
	20:0	0.7	1.5
	22:0	0.1	0.2
		32	36.1 (12.8 % more)
Monounsatureted (MUFA)	16:1	2.5	1.5
	18:1	15.6	18.6
	20:1	0.1	0.1
	22:1	0.7	0.1
		18.9	20.3 (7.4 % more)
Polyunsaturated (PUFA)	18:2	36.8	35.4
	18:3	12.3	8.2
		49.1	43.6 (11.2 % less)

4. Discussion

Out of the selected ten isolates in this study based on morphological features and absorption spectroscopy of the whole organisms, only one eukaryotic alga (isolate S-8) was screened out among all other cyanobacteria. Absorption spectra of algae provide the information of different types of pigments and their concentrations. Such useful information in photosynthesis is also used for phylogenetic and taxonomic purposes (Govindjee and Braun, 1974). The same analogy was used here with absorption spectroscopy to differentiate between prokaryotic cyanobacteria and the singled out eukaryotic alga.

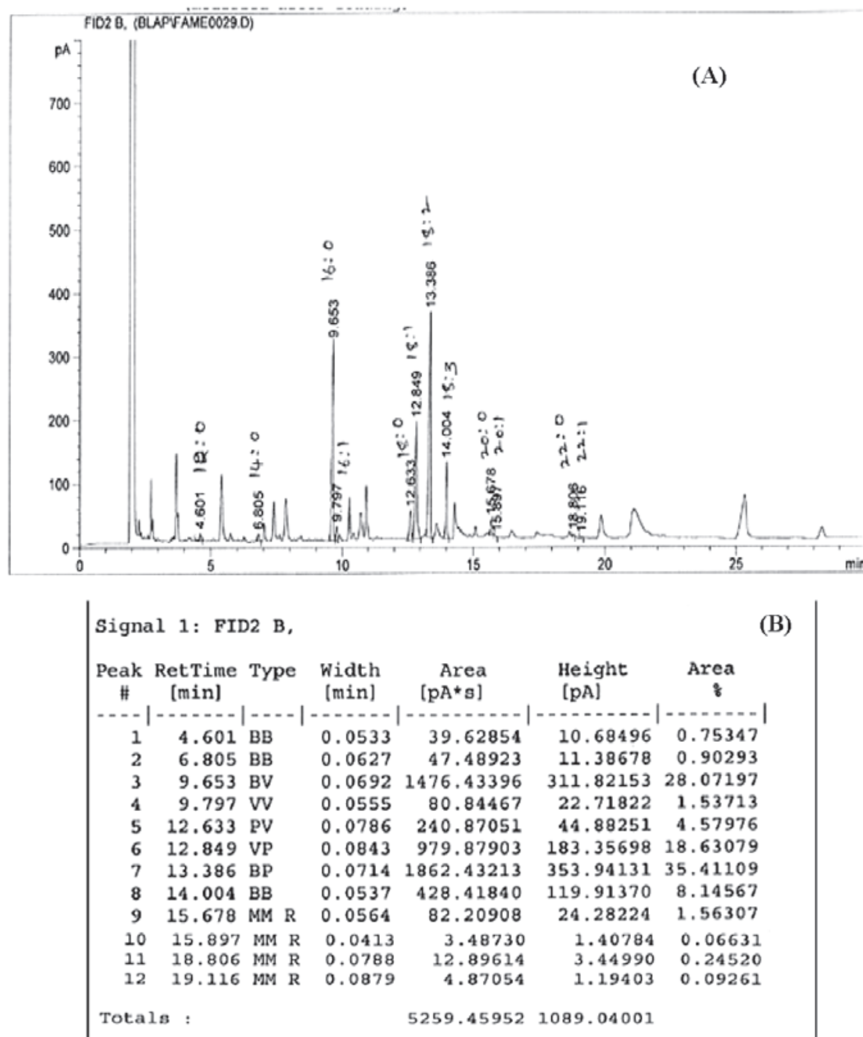
While the cyanobacterial group of algae under stress condition could produce large amount polar lipids like diacylglycerols (DAG), in eukaryotic algae these are stored as triacylglycerols (TAG). Saha *et al.* (2003) reported reduced synthesis of lipids and fatty acids in *Oscillatoria willie* BDU 130511 under nitrogen starvation.



Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	4.599	MM R	0.0502	17.71000	5.88512	0.21190
2	6.804	BB	0.0633	46.95006	11.57809	0.56176
3	9.680	BV	0.0767	2331.54858	420.39017	27.89707
4	9.813	VV	0.0555	218.31042	58.57164	2.61209
5	12.642	VV	0.0840	216.72295	39.49641	2.59310
6	12.860	VB	0.1015	1302.51563	195.99301	15.58465
7	13.420	BP	0.0789	3068.70679	535.19983	36.71719
8	14.032	BP	0.0611	1028.31396	266.21429	12.30382
9	15.672	PB	0.0597	59.71416	15.23206	0.71448
10	15.868	MM R	0.0895	2.29509	7.43828e-1	0.03134
11	18.780	MM R	0.0600	3.97759	1.10419	0.05431
12	19.122	PB	0.0964	59.94469	9.15633	0.81845
Totals :				7324.19105	1460.08929	

Fig. 3. Chromatogram obtained for fatty acids profile of *Chlorella* sp. JD-2016 grown in optimum nitrogen medium (A) and the area percent report (B).

After growing the *Chlorella* sp. JD-2016 in nitrate plus and minus media it was found that, growth was reduced in the nitrate deprived medium heavily with low specific growth rate, biomass productivity, fewer divisions per day besides a prolonged generation time followed by nitrate starvation. Similar observations under nitrogen starvation experiments were made by Illman *et al.* (2000) and Solovchenko *et al.* (2008) in their respective studies. In response to the lack of nitrogen in microalgae almost complete cell proliferation inhibition was reported (Phadwal and Singh, 2003; Cakmak *et al.,* 2012). Nitrogen is required by photosynthetic organisms to synthesize proteins, nucleic acids, and chlorophylls, among other important cell molecules. The estimated chlorophyll levels were lower in N-starved cells. This is a commonly observed phenomenon in N-starved algal cell (Berges *et al.,* 1996). The limiting nitrogen in N-starved cultures might affect the cells' ability to synthesize amino acids like glycine and glutamate, limiting the synthesis of 5-aminolevulinic acid, a precursor of chlorophyll that in turn leads to lower chlorophyll levels in the algal cells (Ellis *et al.,* 1975). Similar to chlorophyll, carotenoid levels were also found lower in *Chlorella* sp. cultured in lower nitrate, suggesting that the availability of nitrogen affects the level of carotenoids in the cells. This finding corroborates similar other reports of lower carotenoids levels under nitrogen limitation (Li *et al.,* 2012; Kim *et al.,* 2013). However, the chlorophyll / carotenoids ratio was lower in the N-starved cultures and is consistent with most of the nitrogen limitation studies with algae (Berges *et al.,* 1996).The decrease in carotenoid level may be due to the limiting step of dimerization of geranyl-pyrophosphate as suggested by Richmond (1986). Kim *et al.* (2013) clearly showed that higher light intensities accompanied by N limitation usually lead to higher carotenoid levels, whereas lower light intensitithis experiment (100 µmol/m^{2}S) was not high enough to induce higher photo-



Signal 1: FID2 B,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	4.601	BB	0.0533	39.62854	10.68496	0.75347
2	6.805	BB	0.0627	47.48923	11.38678	0.90293
3	9.653	BV	0.0692	1476.43396	311.82153	28.07197
4	9.797	VV	0.0555	80.84467	22.71822	1.53713
5	12.633	PV	0.0786	240.87051	44.88251	4.57976
6	12.849	VP	0.0843	979.87903	183.35698	18.63079
7	13.386	BP	0.0714	1862.43213	353.94131	35.41109
8	14.004	BB	0.0537	428.41840	119.91370	8.14567
9	15.678	MM R	0.0564	82.20908	24.28224	1.56307
10	15.897	MM R	0.0413	3.48730	1.40784	0.06631
11	18.806	MM R	0.0788	12.89614	3.44990	0.24520
12	19.116	MM R	0.0879	4.87054	1.19403	0.09261
Totals :				5259.45952	1089.04001	

Fig. 4. Chromatogram obtained for fatty acids profile of *Chlorella* sp. JD-2016 grown in nitrogen deprived medium (A) and the area percent report (B).

protective carotenoid production (Couso *et al.,* 2012). The observed decrease in photosynthetic pigments in N- starved cells as compared to normal cells growing in nitrate supply, clearly indicates the importance of nitrogen for chlorophyll and carotenoid synthesis.

There are reports that nitrogen deprivation is the best strategy for neutral lipid induction in microalgae. In case of the tested *Chlorella* sp., it is already reported using NR that large amount of neutral lipids are accumulated during the stationary growth phase (Dash *et al*., 2016). In order to study the changes in the lipid composition of the organism under normal and under stressful conditions, the FAME analysis was performed using Gas chromatography. Although the lipid content of algal species remains congruous if grown under the similar set of conditions, the lipid profile which generally remains constant in a species changes under limiting growth conditions.

Fatty acids are long aliphatic carbon chains that vary in length, degree of unsaturation, and structure. The quantitative analysis of the lipids through determination of FAME profiles revealed that 70 % of the fatty acids belonged to C16–C18 type with USFA/SFA ratio (2:1) being in the range of 1.9–2.3% in the strains. N limitation exhibited a differential effect, it was clearly noted that during the nitrate starvation in the medium the saturated fatty acids (SFA) were many folded. The overall increase in SFA was around 13 %. The abundance of saturated fatty acids is of extreme significance for consideration as a source of biodiesel, because such oils have higher cetane number (CN), decreased NOx emissions, a shorter ignition delay time and oxidative stability (Antolin *et al.,* 2002).

Polyunsaturated fatty acids (PUFA) are of the utmost importance like antibacterial, anti-inflammatory, antioxidant, prevention of cardiac diseases and inhibition of tumor

progression. Such properties are indicative of the potential of PUFA for nutraceutical and pharmaceutical purpose (Pereira *et al.,* 2012).

Under normal growth conditions the tested *Chlorella* sp. was found to contain more of unsaturated fatty acids (68%) as compared to saturated fatty acids (32 %). The relative amounts of SFA (25% -38%) and the PUFA (37%–64%) values were found within reported value in the literature (Li *et al.,* 2002). Among all the fatty acids most abundant was linoleic acid (36.8 %) followed by palmitic acid (27.7 %) and oleic acid (15.6 %). This was in agreement with results obtained by Sahu *et al.,* 2013 in Chlorophyceae who examined 12 different strains of microalgae. Linoleic acid (LA; C18:2*n*-6) was the main PUFA of most chlorophytes. The only exception is *Ulva* sp., in which higher percentages of ALA (16%) were detected, in comparison to LA (5.7%) (Pereira *et al.,* 2012). In Chlorophyceaen members, the prominent fatty acids like C16:0, C18:0, C18:1n9c and C18:2n6 were found similar to the earlier report of Lee *et al.* (2010).

The deficiency in nitrogen did not bring significant difference in the levels of linoleic acid and palmitic acid suggesting that the tested alga can be used as a regular source of these valuable fatty acids. The alga in its optimum growth and metabolism produces very high amount of 49.1 % PUFA in which upon change of nutrient (nitrate) the internal adjustment of lipids brought about uniform decrease in PUFA and same quantity of increase in SFA. This could be a very good strategy to use this alga both the ways i.e. grow normally to use for high value metabolites (MUFA, PUFA, pigments, antioxidants such as carotenoids and phenolics) and impose nitrate stress to synthesize more of SFA to be exploited for biofuel purpose.

5. Conclusion

Microalgae grown in any habitat like south coast of Odisha are potent with so many biotechnological applications some of which are presently unexplored to a large extent. Lipid profile of the tested alga i.e. *Chlorella* sp. is greatly influenced by the change in nitrate concentration. The nutrient manipulation experiment was used as a progressive starvation strategy, instead of first growing it in N- rich medium and then imparting stress, which is generally adopted for lipid induction in case of algae. The lipid profile of the tested alga is very rich in PUFA which is also valuable nutraceutical, as well as can act as antioxidants. Depriving nitrate completely from the medium results in a double fold increase in the quantity of saturated fatty acids, which suggests that this alga could be a promising candidate to be used as biofuel.

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