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Screening and evaluation of non-heterocystous filamentous cyanobacteria for lipid and commercially viable fatty acids

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ABSTRACT

Thirty eight unialgal non-heterocystous filamentous cyanobacteria were isolated from rice fields of Manipur, India; cultured as unialgal, deposited to the national repository of cyanobacteria and microalgae and obtained accession number. All these strains were screened and investigated for the production of total lipid and commercially viable fatty acids in culture condition. Equal amount of total lipid (3%) was produced by *Limnothrix vacuolifera* BTA05, *Plectonema boryanum* BTA16, *Plectonema nostocorum* BTA47, *Lyngbya laxespiralis* BTA85 and *Lyngbya norgardhii* BTA184 in exponential growth phase. The commercially viable fatty acids, namely; palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1n9c), linoleic acid (C18:2n6c) and γ- linolenic acid (C18:3n6) were focused in present study. The investigation revealed that *Plectonema notatum* BTA88 yielded high content of palmitic acid (27.9%); *Oscillatoria agardhii* BTA170 of palmitoleic acid (8.90%); *Lyngbya martensiana* BTA640 of oleic acid (56.2%); *Phormidium faveolarum* BTA20 (11.8%) in linoleic acid and *Phormidium boryanum* BTA16 of γ- linolenic acid (8.82%). These organism were considered as the potential candidates for fatty acids profiling, however palmitic acid C16:0 was common and recorded in all 38 examined strains.

1. INTRODUCTION

Cyanobacteria, a large group of microorganisms in the prokaryotic kingdom, perform oxygenic photosynthesis using two photosystems that resemble those in the chloroplasts of eukaryotic plants. Cyanobacteria contain three glycolipids, monogalactosyldiacylglycerol, digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol, and a phospholipid, phosphatidylglycerol, as major glycerolipids. The lipid composition of most cyanobacteria is similar to that of the inner envelope membranes and thylakoid membranes of the chloroplasts of higher plants, and it is different from that of the membranes of most bacteria, which contain phospholipids as major glycerolipids. Besides nutritional value, the fatty acids of cyanobacteria are generally used to address taxonomical problems [1]. According to Kenyon [2] four types of fatty acids exist in cyanobacteria i.e. n-saturated, long chain saturated, unsaturated and long chain unsaturated and are linked to morphological characteristics. Lipids are esters of long fatty acids and alcohols that comprise a large group of structurally distinct organic compounds including fats, waxes, phospholipids, glycolipids etc. Cyanobacteria may contain significant quantities of lipids (fat and oil) with compositions similar to those of vegetable oils. The lipids of some cyanobacterial species are also rich in essential fatty acids such as the C18 linoleic (18:2ω6) and γ-linolenic (18:3ω3) acids and their C20 derivatives, eicosapentaenoic acids (20:5ω3) and arachidonic acid (20:4ω6). These fatty acids are essential components of the diet of humans and animals and are becoming important feed additives in aquaculture [3]. The lipids of cyanobacteria are generally esters of glycerol and long fatty acids. They may be either saturated or unsaturated. Some of the filamentous cyanobacteria tend to have large quantities (25 to 60 % of the total) of polyunsaturated fatty acids [4-6]. Lipids (accumulated in the thylakoid membranes) are associated with high levels of photosynthesis and rapid growth rate and are of particular interest, since they can be used for biodiesel production [7-8]. Microalgae accumulate large amounts of lipids as reserve material, with especially in conditions of stress and slow growth [9]. The fatty acid composition of marine microalgae has been studied more extensively [10]; [11]; [12]; [13] than the freshwater forms [14]; [15]; [16]. Thus there is need to screen fresh water cyanobacteria for lipids of commercial value. Keeping this in mind the present work was undertaken.

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Table 1: Amounts of lipids and content of commercially viable fatty acids from non-heterocystous cyanobacteria.

Strains name with assigned code from national repository	Content of total lipids and important fatty acids					
	Total lipid of dry weight	Palmitic acid (C16:0)	Palmitoleic acid (C16:1)	Oleic acid (C18:1n9c)	Linoleic acid (C18:2n6c)	γ- linolenic acid (C18:3n6)
Phormidium mucosum BTA02	1.00	2.89	2.89	ND	2.45	ND
Limnothrix vacuolifera BTA05	3.00	1.36	2.59	ND	ND	ND
Plectonema radiosum BTA09	1.70	1.34	3.06	ND	2.56	ND
Plectonema boryanum BTA10	2.00	0.42	0.18	11.6	2.61	ND
Phormidium tenue BTA13	1.00	4.56	0.78	13.5	ND	ND
Phormidium boryanum BTA16	3.00	1.49	2.55	14.6	ND	8.82
Phormidium faveolarum BTA20	1.00	3.78	ND	11.8	11.8	ND
Plectonema radiosum BTA32	1.00	6.90	3.10	12.7	ND	7.89
Plectonema litorale BTA33	2.00	3.89	0.34	11.6	3.45	ND
Phormidium arthurensis BTA42	2.00	4.56	2.45	12.8	ND	ND
Plectonema nostocorum BTA47	3.00	1.15	0.44	14.6	ND	ND
Phormidium fragile BTA48	1.00	8.90	1.24	11.2	2.67	6.78
Phormidium purpurenscens BTA81	1.00	5.78	1.87	13.7	ND	ND
Limnothrix redekei BTA82	2.00	1.46	2.67	ND	6.89	ND
Phormidium kuetzingianum BTA83	1.00	3.67	2.89	ND	ND	5.56
Lyngbya laxespiralis BTA85	3.00	7.90	1.90	8.91	1.23	ND
Phormidium tenue BTA86	1.00	4.56	1.89	15.7	ND	ND
Plectonema notatum BTA88	2.00	27.9	1.12	14.7	2.65	ND
Plectonema notatum BTA108	2.00	7.66	ND	3.95	9.49	3.45
Phormidium incrustratum BTA118	1.00	2.91	3.43	3.56	ND	3.56
Limnothrix redekei BTA123	2.00	2.11	ND	ND	ND	2.56
Phormidium valderianum BTA162	1.00	0.85	2.82	2.67	ND	ND
Oscillatoria agardhii BTA170	1.00	0.90	8.90	2.34	ND	ND
Spirulina platensis BTA174	2.00	6.89	ND	4.45	ND	7.89
Lyngbya connectens BTA178	2.50	1.01	1.66	0.37	ND	0.64
Lyngbya norgardhii BTA184	3.00	1.34	1.23	9.91	8.09	ND
Phormidium tenue BTA194	2.00	2.32	4.56	ND	ND	ND
Plectonema notatum BTA199	1.00	6.17	2.41	4.90	9.67	ND
Limnothrix mirabilis BTA222	2.00	2.12	2.46	0.72	ND	ND
Phormidium tenue BTA436	2.00	0.46	2.68	2.16	ND	ND
Lyngbya martensiana BTA436	2.00	0.98	ND	ND	ND	ND
Phormidium fragile BTA421	1.00	0.77	1.81	45.0	ND	ND
Plectonema notatum BTA565	2.00	0.51	ND	ND	ND	8.41
Phormidium autumnale BTA587	1.00	2.98	3.45	32.6	ND	ND
Lyngbya aestuarii BTA597	1.00	1.45	2.11	34.3	2.68	ND
Lyngbya allorgei BTA606	1.00	1.22	1.31	45.9	ND	ND
Lyngbya martensiana BTA640	2.00	1.34	4.79	56.2	2.90	ND
Phormidium stagnina BTA855	1.00	0.81	1.83	23.8	1.20	1.34

*ND: not detected

2. MATERIALS AND METHOD

2.1 Cyanobacterial strains isolation and cultivation

A total of thirty-eight non-heterocystous filamentous cyanobacteria were investigated in this study. All strains were isolated from rice fields of Manipur, India. The strains were subjected to purification by plating, subculturing in sterile nitrogenous BG-11 agar medium [17].

Cultures were inoculated in 1000 ml Erlenmeyer flasks containing 400 ml of BG-11 nitrogenous medium and incubated at 28±2ºC, 14/10 h light/dark cycle with light intensity of 50 µmol photons $m^{-2}s^{-1}$ under static conditions.

2.2 Lipid extraction

The most widely used derivatives were methyl esters by [18]. 15 ml of 2% H_2SO_4 in methanol solution was added to total biomass (500 mg) in round bottom (RB) flask. RB flasks which contained biomass were refluxed in heating mantle at 60°C for 4 h. FAME solution was transferred to a separating funnel then added50 ml ethyl acetate and 200 ml distilled water to the FAME solution contained inside the separating funnel. Two layers were formed; the upper phase was retained and washed with distilled water till it gave a pH 7.0 (checked through pH strip). The extract was separated into a conical flask, added 10 g sodium sulphate (Na_2SO_4) into it and kept for 20 min. Contents were transferred to 50 ml RB flask and rota-evaporated at 65 ºC. RB containing FAME was rinsed by addition of few drops of dichloro-methane and transferred the solution to a vial.

One µl of the sample was injected in GC using micro syringe (HAMILTON # 701 USA) and Supelco™ 37 FAME mix, Sigma-Aldrich was run as standard.

2.3 Gas chromatography of FAME

Methyl esterified samples were diluted with dichloro-methane in the vial with micropipettes. Fatty acid profile was determined by the capillary column gas chromatrographic method. The sample (1µl) was injected into the gas chromatograph (Thermo Chemito Ceres 800 plus).

The samples were carried out using GC equipped with FID detector and forte capillary column (60 m × 0.32 mm I.D. ×

0.25 µm film thicknesses). The carrier gas was nitrogen used at a flow rate of 1.0 ml/min. The run time of single sample was 60 min. Fatty acids were identified and quantified by comparing the retention time and area of the authentic standards SupelcoTM 37 FAME mix (Sigma-Aldrich) and relative concentrations of different FAMEs and were calculated by the percentage area method using Chemito Chrom-card software version 2.6.

3. RESULTS AND DISCUSSION

Lipid profiling and fatty acid composition of thirty-eight (38) non-heterocystous filamentous cyanobacteria are presented in table 1. Photomicrographs are shown in photoplate 1 and 2. *Plectonema notatum* BTA88 and *Oscillatoria agardhii* BTA170 showed high content of palmitic acid (27.9%) and palmitoleic acid (8.90%) respectively. *Lyngbya martensiana* BTA640 and *Phormidium faveolarum* BTA20 showed high content of oleic acid (56.2%) and linoleic acid (11.8%), respectively. *Phormidium boryanum* BTA16 yielded high amount of γ-linolenic acid (8.82%) in culture conditions (table-1). Palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1n9c), linoleic acid (C18:2n6c) and γ- linolenic acid (C18:3n6) are to be highly valuable fatty acids for nutraceutical and pharmaceutical based industry.

In the present investigation, *Phormidium faveolarum* BTA020 and *Phormidium boryanum* BTA16 showed high content of linoliec acid, gamma linolenic acid which is very valuable for nutraceutical and pharmaceutical industry. The gamma linolenic acid (GLA) is reported as a promising therapeutic agent for numerous health disorders as it acts as a precursor for prostaglandin E1 an important compound necessary for reducing inflammation and in treatment of heart disease [19]; [20]; [21]. Most cyanobacteria are a common source of a wide range of fats, oils, hydrocarbons and sterols with potential not only as a renewable source of liquid fuels but also for the production of a range of pharmacologically and industrially important products. The applications of cyanobacteria in the production of these compounds are only just being explored.

New developments in the chemical industries, particularly in the area of converting natural products to industrial value, will further enhance the range of commercially important products synthesized by cyanobacteria.

4. CONCLUSIONS

The present study was focused mainly on extraction and evaluation of total lipid and fatty acid composition from non-heterocystous filamentous cyanobacteria isolated from rice fields of Manipur, India falling under Indo-Burma biodiversity hotspots. A total of 38 unialgal cultures were screened for lipid profiling and fatty acid composition.

These freshwater cyanobacteria are a source of essential fatty acids that are of commercial interest, including linoleic, and γ- linolenic acids, among others. Further, some cyanobacteria serve as an important source of essential fatty acids for pharmaceutical and nutraceutical aspects.

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