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Fatty acid profiling of filamentous non-heterocystous cyanobacteria from Loktak Lake, the largest freshwater lake in North-Eastern region of India

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Abstract

In the present study, twenty-one (21) strains of cyanobacteria isolated from freshwater habitats of Loktak Lake were characterized for total lipid and fatty acid composition. Total lipid content ranged between 0.20% to 7.00%. Maximum lipid content was observed in Phormidium tenue BTA-1073 followed by Phormidium corium BTA-64 and Phormidium tenue BTA-63 while Phormidium corium BTA-1065 showed the minimum. Profiling of fatty acid composition showed that capric acid (10:0) and lauric acid (C12:0) were only the predominant component. Plectonema sp. BTA-65 and Phormidium tenue BTA-1076 showed high capric acid content as 21.03% and 17.26%. Caprylic acid (C8:0) was maximum in Phormidium fragile BTA-1020 (10.08%). Lauric acid (C12:0) content was maximum in Plectonema sp. BTA-65 (16.23%) and minimum in Phormidium corium BTA-1065 (2.37%). Of the polyunsaturated fatty acid, α-linolenic acid (C18:3n3) and arachidic acid (C20:0) were present only in Lyngbya putealis BTA-1013. Linoleic acid (C18:2n6) was observed only in Phormidium tenue BTA-63 (0.48%) and Plectonema sp. BTA-65 (0.65%). One of the pharmaceutically potential componenet, Eicosapentaenoic acid (C20:5n3) was occurred in Phormidium tenue BTA-63 (0.44%) only. Palmitoleic acid (C16:1) and oleic acid (C18:1n9) were the most abundant MUFAs in all the strains ranging between 0.05% to 6.85% for palmitoleic acid and 0.24% to 8.71% for oleic acid except Phormidium tenue BTA-1076 and Lyngbya birgei BTA-1080. The levels of erucic acid (C22:1n9) was also present in low quantity in most strains except in few cases. The goal of the present study was to profile the fatty acid components of cyanobacteria isolated from Loktak Lake where potent strains could be used for the production of commercially important desired products.

Keywords: Cyanobacteria, Fatty acids, Gas chromatography, Lipids, Loktak Lake, North-East India.

Introduction

Cyanobacteria are the photosynthetic, prokaryotic organisms capable to store lipids as reserve cellular constituents. Microalgae, being the important organisms in the production of food products, fine chemicals and compounds with industrial applications, an increasing awareness has been focussed on microalgal biotechnology[1,2].

Cyanobacteria contain significant quantities of lipids and some of them are also quite high in linoleic and γ-linolenic acids content. There are some health benefits of polyunsaturated fatty acids (PUFA) for aquatic organisms which has spurred interest in their commercial production[3]. They store reserve food materials which can be used as the source of pigments, lipids, vitamins, proteins and certain secondary metabolites[4,5]. *Spirulina platensis* accumulating large amount of γ-linolenic acid was also reported[6]. Linoleic, γ-linolenic, eicosapentaenoic and arachidonic are also the suitable for nutritionally product for aquaculture[7].

The surface activity properties of cyanobacterial lipids can be applied as biosurfactants in emulsification and flocculation process. Fatty acids from microalgae such as linoleic acid, α-linolenic acid, γ-linolenic acid, arachidonic acid and eicosapentaenoic acid are in large demand in the paharmaceutical market[8]. γ-linolenic acid has occupy a globally potential applications in healthcare industries. This fatty acid has been used in the treatment of inflammatory diseases, Parkinson's disease, multiple sclerosis, pre-menstrual syndrome, heart disease and other health disorders[9-12]. *Spirulina*, a potential microalgae accumulates high amount of γ-linolenic acid has been utilized as a health food[13-15]. Under the availability of adequate nutrients and growth conditions, cyanobacteria especially filamentous forms can produced high volume of biomass and desired products.

Earlier contributions have also made on the diversity of cyanobacteria from Loktak Lake[16-18]. This present research reports for the first time, the fatty acid profiling of filamentous non-heterocystous cyanobacterial strains isolated from natural freshwater habitats of Loktak Lake.

Material and Methods

Cyanobacterial strains and growth conditions: The present

studied cyanobacterial strains were obtained from Freshwater Cyanobacterial and Microalgal Repository (National facility created by the Department of Biotechnology, Government of India with reference No. BT/PR 11323/PBD/26/171/2008 dated 31-03-2009), Institute of Bioresources and Sustainable Development (IBSD), Imphal, Manipur, India. These strains were previously isolated from Loktak Lake, the only largest freshwater lake in the North-Eastern region of India.

Cyanobacterial strains were inoculated in Hoffmann's flasks containing BG-11(+N) broth medium[19]. The flasks were kept in the culture room under light:dark cycles of 14:10h conditions maintained at 28±2ºC under illumination provided by cool white fluorescent tubes of 54-67 µmol photons $m^{-2}s^{-1}$. The flasks were shaken manually for 5 min daily to prevent cell clumping.

Lipid extraction and transesterification: The total lipids were extracted using the protocol[20]. Dried cyanobacterial biomass (250 mg) was taken into 100 ml round bottom flask and added 15 ml of methanolic-sulphuric acid. The flask along with the content was refluxed for 4 h at 60°C. Then the content was filtered and collected into a separating funnel. The filtrate was separated using ethyl acetate followed by washing with distilled water until pH of the filtrate shows neutral. Once pH becomes neutral, the organic phase was collected and anhydrous Na_2SO_4 was added to remove the impurities. Solvents were removed by vacuum rota evaporation (Buchi Rotavapor R-215) at a pressure of 175 mbar with temperature of 60°C. Then to it, 400-500 µl of dichloromethane was added to dissolve the organic phase followed by injecting 1 µl of the sample using syringe (Hamilton 701N) in GC for fatty acid profiling.

Gas chromatography of FAME: Fatty acid profiling of cyanobacterial strains was carried out using GC-FID having SGE forte capillary column (60 m × 0.32 mm I.D. × 0.25 µm film thicknesses). During analysis, temperature of the injector and detector were kept at 240°C and 250°C. On the other hand, oven temperature was adjusted to 140°C at 5 min and raised to 240°C at 4°C min^{-1} and later it was kept at 240°C at 52 min. Nitrogen gas was used as carrier gas which was maintained at the flow rate of 1.0 ml min^{-1}. Sample of 1 µl was used for analysis with a split ratio of 100:1. Supelco™ 37 component FAME mix (Sigma-Aldrich) was used as standard. Retention time was recorded for each sample from which each component of fatty acid can be known. Each concentration of different FAMEs was calculated by the percentage area method comparing the peak areas of their corresponding concentrations of standard using Chemito Chrom-card software version 2.6.

Total lipid content of twenty-one (21) of the strains was also presented in table-1. Total lipid ranged between 0.20% to 7.00% (% of total lipid content). Maximum lipid content was observed in *Phormidium tenue* BTA-1073 followed by *Phormidium corium* BTA-64 and *Phormidium tenue* BTA-63 while *Phormidium corium* BTA-1065 showed the minimum. The fatty acid profile of the strains were summarized in table-2a and b.

Results and Discussion

Photomicrographs of non-heterocystous cyanobacteria which were characterized for fatty acid composition was shown in figure-1. Total lipid content of twenty-one (21) of the strains was also presented in table-1. Total lipid ranged between 0.20% to 7.00% (% of total lipid content). Maximum lipid content was observed in *Phormidium tenue* BTA-1073 followed by *Phormidium corium* BTA-64 and *Phormidium tenue* BTA-63 while *Phormidium corium* BTA-1065 showed the minimum. The fatty acid profile of the strains were summarized in table-2a and b.

Table-1
Total lipid content of non-heterocystous cyanobacteria isolated from freshwater habitats of Loktak Lake, Manipur

Name of strains	% of total lipid content
Phormidium sp. BTA-52	1.70 ± 0.12
Phormidium tenue BTA-63	2.20 ± 0.23
Phormidium corium BTA-64	2.50 ± 0.08
Plectonema sp. BTA-65	2.00 ± 0.21
Lyngbya aestuarii BTA-66	1.90 ± 0.11
Phormidium sp. BTA-71	1.20 ± 0.28
Phormidium sp. BTA-75	1.90 ± 0.09
Plectonema sp. BTA-79	1.90 ± 0.07
Limnothrix redekei BTA-987	1.70 ± 0.10
Lyngbya putealis BTA-1013	1.90 ± 0.06
Phormidium fragile BTA-1020	2.10 ± 0.18
Phormidium fragile BTA-1042	1.30 ± 0.16
Phormidium tenue BTA-1045	1.60 ± 0.06
Phormidium sp. BTA-1048	1.70 ± 0.06
Limnothrix vacuolifera BTA-1051	2.10 ± 0.14
Phormidium fragile BTA-1052	0.30 ± 0.21
Phormidium tenue BTA-1064	0.50 ± 0.08
Phormidium corium BTA-1065	0.20 ± 0.09
Phormidium tenue BTA-1073	7.00 ± 0.04
Phormidium tenue BTA-1076	0.30 ± 0.12
Lyngbya birgei BTA-1080	1.50 ± 0.15

All experiments were replicated three times and results are presented as mean ±SD.

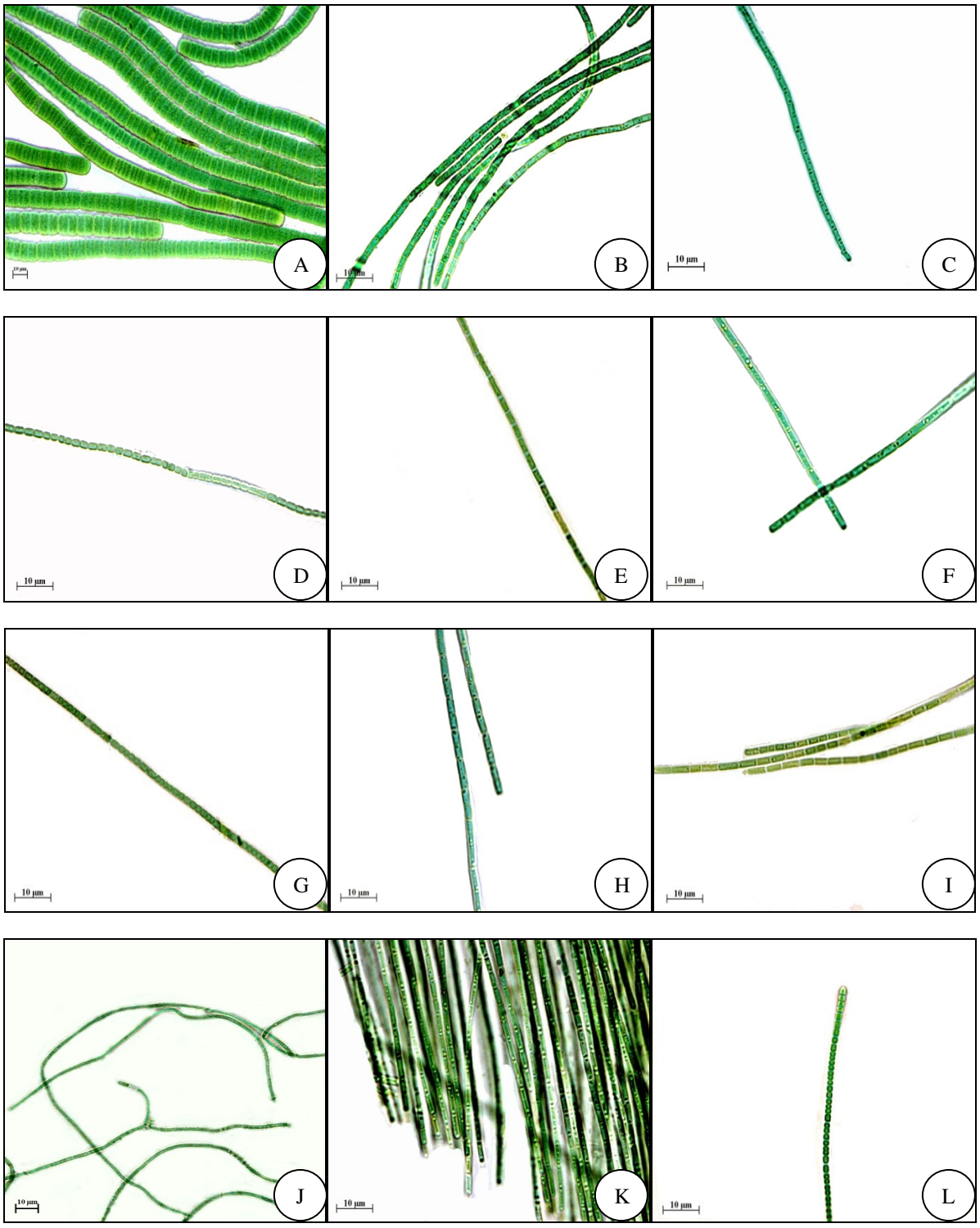

Figure-1

Photomicrographs of non-heterocystous cyanobacteria isolated from freshwater habitats of Loktak Lake, Manipur showing high fatty acid composition

(A) ***Lyngbya aestuarii*** **(B)** ***Phormidium corium*** **(C)** ***Phormidium*** **sp. (D)** ***Phormidium fragile*** **(E)** ***Phormidium tenue*** **(F)** ***Limnothrix vacuolifera*** **(G)** ***Phormidium fragile*** **(H)** ***Phormidium corium*** **(I)** ***Phormidium tenue*** **(J)** ***Limnothrix redekei*** **(K)** ***Plectonema*** **sp. (L)** ***Phormidium fragile***

Table-2
Fatty acid composition (% of total fatty acid) of non-heterocystous cyanobacteria

Strain	Saturated fatty acid content (% of total fatty acid)						
	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$
1	0.45	8.82	6.04	0.77	0.91	1.09	nil
2	0.48	0.77	9.35	0.79	2.61	nil	nil
3	1.80	10.56	7.60	nil	3.09	nil	nil
4	7.48	21.03	16.23	1.31	6.58	0.42	nil
5	0.12	7.56	5.33	0.25	2.67	nil	nil
6	1.14	15.26	10.84	0.62	0.38	nil	nil
7	4.26	5.39	3.90	nil	1.13	nil	nil
8	3.58	7.07	5.12	nil	3.66	nil	nil
9	0.19	6.96	4.79	0.19	0.38	nil	nil
10	0.56	7.83	6.21	0.58	0.56	nil	0.51
11	10.08	5.57	3.97	nil	2.07	nil	nil
12	0.33	8.82	5.96	0.25	4.08	nil	nil
13	0.94	15.77	12.94	0.94	0.94	nil	nil
14	nil	9.48	7.08	nil	2.65	nil	nil
15	0.13	4.97	3.50	0.25	2.04	nil	nil
16	0.51	7.06	4.86	0.13	1.94	nil	nil
17	2.77	3.61	2.38	0.08	1.06	nil	nil
18	1.62	3.99	2.37	nil	3.02	nil	nil
19	0.55	7.19	5.22	0.24	0.40	nil	nil
20	1.02	17.26	6.60	nil	nil	nil	nil
21	nil	12.15	7.64	nil	nil	nil	nil
Strain	**Monounsaturated and polyunsaturated fatty acid content (% of total fatty acid)**						
	$C_{16:1}$	$C_{18:1n9}$	$C_{22:1n9}$	$C_{18:2n6}$	$C_{18:3n3}$	$C_{18:3n6}$	$C_{20:5n3}$
1	3.18	1.18	1.59	nil	nil	nil	nil
2	3.20	1.19	0.21	0.48	nil	0.59	0.44
3	2.44	0.90	0.90	nil	nil	nil	nil
4	6.85	2.21	1.68	0.95	nil	0.73	nil
5	0.19	0.68	0.87	nil	nil	0.12	nil
6	3.80	0.24	nil	nil	nil	nil	nil
7	0.96	3.74	2.35	nil	nil	nil	nil
8	2.44	0.33	nil	nil	nil	0.16	nil
9	1.79	3.58	nil	0.38	nil	nil	nil
10	0.05	0.62	nil	0.47	0.08	0.60	nil
11	1.45	nil	nil	nil	nil	nil	nil
12	1.72	0.49	0.49	nil	nil	0.33	nil
13	5.80	6.37	0.12	0.17	nil	0.44	nil
14	2.02	1.26	nil	0.76	nil	nil	nil
15	1.34	3.57	nil	nil	nil	nil	nil
16	0.21	0.42	0.21	0.13	nil	0.42	nil
17	1.10	8.71	1.58	0.88	0.40	nil	nil
18	1.72	1.19	nil	nil	nil	nil	nil
19	1.90	5.22	nil	nil	nil	nil	nil
20	nil	nil	nil	nil	nil	nil	nil
21	nil	2.43	nil	nil	nil	nil	nil

1-*Phormidium* sp. BTA-52; 2-*Phormidium tenue* BTA-63; 3-*Phormidium corium* BTA-64; 4-*Plectonema* sp. BTA-65; 5-*Lyngbya aestuarii* BTA-66; 6-*Phormidium* sp. BTA-71; 7- *Phormidium* sp. BTA-75; 8-*Plectonema* sp. BTA-79; 9-*Limnothrix redekei* BTA-987; 10-*Lyngbya putealis* BTA-1013; 11-*Phormidium fragile* BTA-1020; 12-*Phormidium fragile* BTA-1042; 13-*Phormidium tenue* BTA-1045; 14-*Phormidium* sp. BTA-1048; 15- *Limnothrix vacuolifera* BTA-1051; 16-*Phormidium fragile* BTA-1052; 17-*Phormidium tenue* BTA-1064; 18- *Phormidium corium* BTA-1065; 19-*Phormidium tenue* BTA-1073; 20-*Phormidium tenue* BTA-1076; 21-*Lyngbya birgei* BTA-1080

In the present analysis, the concentrations of fatty acids were found to vary. The result showed that seven types of saturated fatty acid (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0 and C20:0), four types of polyunsaturated fatty acid (PUFA) (C18:2n6, C18:3n3, C18:3n6, C20:5n3) and three monounsaturated fatty acid (MUFA) (C16:1, C18:1n9 and C22:1n9) were observed. In this study, most of the strains had C8:0, C10:0, C12:0, C14:0 and C16:0 as the dominating fatty acid. Small quantities of C18:2n6, C18:3n3, C18:3n6 and C20:5n3 were also present. The content of C16:1, C18:1n9 and C22:1n9 were moderate. *Plectonema* sp. BTA-65 and *Phormidium tenue* BTA-1076 showed high content of capric acid as 21.03% and 17.26%. Caprylic acid (C8:0) content was maximum in *Phormidium fragile* BTA-1020 (10.08%). Lauric acid (C12:0) content was maximum in *Plectonema* sp. BTA-65 (16.23%) but minimum in *Phormidium corium* BTA-1065 (2.37%). Of the polyunsaturated fatty acids, α-linolenic acid (C18:3n3) and arachidic acid (C20:0) were present only in *Lyngbya putealis* BTA-1013 (0.08% and 0.51%). Linoleic acid (C18:2n6) was observed only in *Phormidium tenue* BTA-63 (0.48%) and *Plectonema* sp. BTA-65 (0.65%). *Phormidium tenue* BTA-63 was the only strain which showed Eicosapentaenoic acid (C20:5n3) with 0.44%. This fatty acid has a great value in pharmaceutical applications. The levels of SAFAs was also high in all the strains except myristic acid (C14:0) and palmitic acid (C16:0). Capric acid (10:0) and lauric acid (C12:0) were the most abundant fatty acids which were present in all the strains. Palmitoleic acid (C16:1) and oleic acid (C18:1n9) were the most abundant MUFAs in all the strains ranging between 0.05% to 6.85% for palmitoleic acid and 0.24% to 8.71% for oleic acid except *Phormidium tenue* BTA-1076 and *Lyngbya birgei* BTA-1080. The levels of erucic acid (C22:1n9) was also present in low amount in most strains except a few cases. Low levels of fatty acids which was longer than 18C-atoms were reported in cyanobacteria[1]. Major fatty acids 16:0, 16:1, 18:2, and ALA, which are present in cyanobacteria are also differs even among same species[22,23]. The four strains of *Spirulina* were analyzed for their total lipid content[24] and values obtained were within the range 2.7-6.8%.

In the present study, the levels of saturated fatty acids were high in all the strains. On the contrary, monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) contents were generally low. Similar results were also obtained[25]. High cis-palmitoleic acid content (54.5 and 54.4% of total fatty acid) in two marine species of *Phormidium* and *Oscillatoria* were also reported[26].

The total lipid and fatty acid composition against certain external environmental factors like light, temperature and salinity some species of cyanobacterial species have also been reported by many workers[27,28,29]. PUFA play an important role in regulating cell membrane properties, precursors for production in animal hormones, maintaining high growth, survival and reproductive rates, aquaculture studies[30]. Cyanobacteria have been applied for the production of a range of value-added pharmacological and industrial desired products. However, understanding the lipid content is not sufficient for its applications in the industries. They should be analyzed at the molecular levels or genetically modified for providing better product yield.

Conclusion

From the above investigation, it was concluded that these strains which showed the presence of essential fatty acids of biotechnologically importance could be useful in mass production. Since Loktak Lake lies in one of the remote place in North-East India, more biotechnological research should be focussed to explore the potential organisms from this lake. These potential cyanobacterial strains would be useful in the production of high value-added biomolecules and would gain much importance in the biotech industries.

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