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Fatty Acid Composition Reveals Morphological Heterogeneity of Freshwater *Anabaena* strains

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In this study, ten isolates of freshwater filamentous heterocystous *Anabaena* spp. were selected for comparing their fatty acid composition with the morphological properties. Morphologically the investigated strains could be discriminated in to two groups. One important feature for specific identity of the taxa is the proximity of the akinetes to heterocyst, whether adjacent to or away from. Fatty acids and their chemo-taxonomic values were investigated in *Anabaena*. Totally 21 fatty acids were found all the ten isolates. A dendrogram for the ten strains are shown in relation to fatty acid composition and resulted in two major clusters. The major clusters consisted of seven strains. The other clusters had a maximum of two morphoforms. There was a close correlation between fatty acid composition and morphological characters for six species among the ten *Anabaena* species tested.

Key words: Cyanobacteria, *Anabaena*, Fatty acids, Morphological Heterogeneity.

Cyanobacteria (Blue-green algae) are unique photosynthetic organisms of cosmopolitan distribution in terrestrial and aquatic habitats and are known to exist since the Precambrian Era (3.5 billion years ago)[1, 2, 3]. Cyanobacteria are also involved in symbiotic associations with an exceptionally broad range of representatives within the plant kingdom[4]. The classification scheme given in Geitler[5] serves as the basis for the later treatments of Elenkin[6], Desikachary[7]. Cyanobacteria are among the most widespread, morphologically distinct and abundant prokaryotes known[8]. Cyanobacteria are morphologically diverse group of oxygenic photosynthetic prokaryotes[9] traditionally classified on the basis of their morphology into five orders representing Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales as given in Bergey's Manual[10].

Over 70 isolates of *Anabaena* in cultures have been studied by Anand[11]. A review of the cyanobacterial flora of the rice fields of Tamil Nadu by Anand[12] stresses the importance of the study of the forms in its native habitat for correct classification and revision of the pre existing ones. Three *Anabaena* species with spores on either side

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of the heterocyst, two *Anabaena* sp. and one *Cylindrospermum* sp. with spore away from the heterocyst were investigated in various inorganic nitrogen sources[13]. Prasanna *et al.*[14] suggested that in a selected set of 13 *Anabaena* strains from diverse geographical regions, significant differences were observed among the *Anabaena* strains with regard to the shape and size of trichomes and individual cells within a filament, besides biochemical attributes, and molecular marker profiles.

Fatty acids were analysed from unicellular and filamentous cyanobacteria. The concept of using a fatty –acid profile in chemotaxonomy was initiated by Kenyon[15], Kenyon, Stanier[16], Kenyon *et al*.[17]. Fatty acid profiling could be a useful approach in the taxonomic or phylogenetic study of the genus *Nostoc*. *Nostoc* species was assigned to Group II due to the presence of C18:2n3 and C18:3n3 and the absence of C18:3n6[18]. Cellular fatty acids a chemotaxonomic marker has been used as a tool for classifying genus and species level[19]. The lipid profiles of the genus *Spirulina* was studied indicating the presence of mono-, di-glycosyl, sulphoquinovolosyl and phosphatidyl (MGDG, DGDG, SQDG and PG)[20].

Planktonic species of *Anabaena* with straight and coiled trichomes were investigated by analyzing the correlations of fatty acid content and fatty acid composition with morphological properties .The fatty acids in planktonic *Anabaena* were classified as Type 2 according to the Kenyon – Murata system. The strains were divided into 2 subtypes: 18 species belonging to Type 2A, which contains 16:2 and 16:3, and 6 strains to Type 2B, which lacks 16:2 and 16:3. In the dendrogram, the 24 strains are shown in relation to fatty acid compositions of Type 2A and 2B[21,22]. Cellular fatty acid composition of 22 cyanobacterial strains was analysed[23]. Six cyanobacterial species from different habitats belonging to genus *Nostoc* were studied for low molecular, hydroxyl, dioic, saturated and unsaturated fatty acids [24]. Highest degree of unsaturation of the major cellular fatty acid content was observed and correlated with the morphology and physiology based classification of the cyanobacteria[23].

Fatty acid compositions were compared in the marine *Synechococcus* species and fresh water *Synechocystis* species. All the marine strains contained myristic acid [14:0] as the major fatty acid with only traces of polyunsaturated fatty acids similar to one of the fresh water species of *Synechocystis*. The major lipids classes of non motile marine strain were identified as mono-, di-glycosyl, sulphoquinovolosyl and phosphatidyl (MGDG, DGDG, SQDG and PG) which was identical to those found in other cyanobacteria[25]. Sato *et al*.[26] and Nicholov *et al*.[27] found four major membrane acylglycerols with similar fatty acid compositions *viz*., monogalactosyldiacylglycerol [MGDG], diacylglycerol [DGDG], phosphotidylglycerol [PG] and sulphoquinovosyldiacylglycerol [SQDG)] in *Anacystis nidulans*. The filamentous heterocystous strains of *Nostoc* and *Anabaena* were distinguished on the basis of the cellular fatty acids present in them[28].

In the present study the genus *Anabaena* Bory was chosen for taxonomic analysis. The morphological criteria traditionally used for identification of *Anabaena* species are: biometric characters of vegetative cells, heterocysts and spores. One important feature for specific identity of the taxa is the proximity of the akinetes to heterocyst, whether adjacent to or away from. Fatty acid analyses have been employed for the species level variations of *Anabaena*. Genetic distances between strains were calculated using the algorithm of Nei and Li[29] as provided in the RAPDistance software package developed by Armstrong *et al*.[30].

MATERIAL AND METHODS

Cultures and Culture conditions

In the study, cultures were chosen from the Culture Collection of Algae, Centre for Advanced Studies in Botany, University of Madras. Axenic cultures of *Anabaena* species were grown in BG 11_o medium[31]. Liquid cultures were grown at 25°C± 1°C in a growth chamber under fluorescent illumination of 40 $\mu Em^{-2}s^{-1}$, with 12 hrs dark/12 hrs light conditions. The growth chamber was fitted with a Sangmo Weston Ltd., S656 313 model automatic timer. Gentle shaking of the flasks was done to reduce the clumping of cells. The cultures used in the present study are listed and described (Tables 1 and 2).

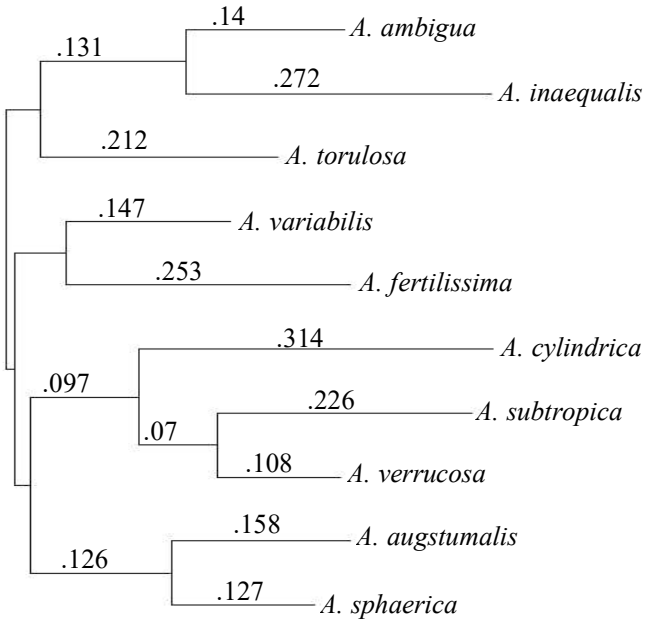

Fig. 1.

Analyses of fatty acids

Total lipids were extracted from 1 gram each of fresh and desiccated cells using chloroform/methanol (2:1 v/v) according to Subramanian *et al*.[32]. One ml of saponification reagent (45 g of sodium hydroxide dissolved in 30 ml of 1:1 v/v methanol water mixture)was added to aliquots (100 µl) of the lipid extract, and the contents were vortexed and boiled. Then, 2 ml of methylation reagent (325 ml of 6 M hydrochloric acid mixed with 275 ml of methanol) was added, mixed thoroughly, and boiled in a water bath at 80°C for 20 min. After cooling to room temperature, 1.25 ml of the extraction solvent was added, mixed for 10 min and the aqueous lower phase was discarded. Base wash (10.8 g of sodium hydroxide dissolved in 900 ml of distilled water) was then added and mixed for

Table 1. Anabaena strains selected for the study

Strain No	Taxonomic designation	Position of heterocysts and akinetes	Origin / Source
A485	*A.ambigua*	heterocysts adjacent to akinetes	1403/7 CCAP, UK
A525	*A. torulosa*	heterocysts adjacent to akinetes	M2/2 aS2T2 Gif Sur Yvette from, France
A621	*A. cylindrica*	hetearocysts adjacent to akinetes	Isolate175,Kantz(1403/2a),ICC, U.S.A
A802	*A. augstumalis*	heterocysts adjacent to akinetes	Czech.Jahnke 4a
A904	*A. sphaerica*	heterocysts adjacent to akinetes	1616 ICC, U.S.A
A487	*A. inaequalis*	heterocysts away from the akinetes	1403/9 CCAP, UK
A549	*A. fertilissima*	heterocysts away from the akinetes	M2/3b Gif Sur YvettePCC, France
A514	*A. variabilis*	heterocysts away from the akinetes	1403/12 CCAP, UK
A618	*A. subtropica*	heterocysts away from the akinetes	Isolate 45 Kantz Feb.71 ICC, U.S.A
A622	*A. verrucosa*	heterocysts away from the akinetes	ICC, U.S.A

CCAP: Culture Collection of Algae and Protozoa, Cambridge, U.K.
ICC: Indiana Culture Collection, U.S.A.

5 min. The organic extract was transferred to the GC vial and 1 µl of it was used for injection. Analysis was performed in a Hewlett Packard 5890 gas chromatograph with a flame ionization detector, a Diethylene glycol succinate column and nitrogen as carrier gas.

RESULTS

The ten strains of freshwater filamentous heterocystous *Anabaena* species used in the present study (Table 1) were identified based on morphological observations. Fatty acids and their chemo-taxonomic values were investigated in *Anabaena*. Ten saturated fatty acids (SAFAs) and eleven unsaturated fatty acids (UFAs) totally 21 fatty acids were found all the ten isolates. The composition and content of fatty acids in these strains are shown in Table 3.

Totally Ten acids in *Anabaena ambigua* [SAFAs (13:0, 15:0, 17:0, 18:0 and 22:0) and UFAs (18:1, 18:3, 20:2, 20:1 and 24:1)] , Nine acids in *Anabaena torulosa* [SAFAs (13:0, 15:0, 17:0, 19: 0, 21:0 and 24:1) and UFAs (14: 1, 18:1 and 24:1)] , Five acids in *Anabaena cylindrica*[SAFAs (14:0 and 24:0) and UFAs (18:1, 18:3 and 20:2,)] , Eight acids in *Anabaena augstumalis* [SAFAs (13:0,17:0, 23:0 and 24:0) and UFAs (18:1, 18:3, 20:5 and 24:1)], Six acids in *A.sphaerica*[SAFAs (13:0, 15:0, 17:0,and 24:0) and UFAs (18:3and 20:5)] , Seven acids in *A. inaequalis* [SAFAs (17:0, 21:0 and 22:0) and UFAs (18:1,20:2, 20:1 and 24:1)], The highest number of acids (13) in *A. variabilis* [SAFAs (13:0, 15:0, 17:0, 18:0 , 21:0 and 24:0) and UFAs (14:1, 16:1, 18:1, 18:2, 18:3, 20:1 and 20:2)], Seven acids in *A. fertilissima* [SAFAs (15:0, 21:0 , 22:0 and 24:0) and UFAs (18:1, 18:2 and 18:3)], Five acids in *A. subtropica* [SAFAs (13:0, 18:0 and 24:0) and UFAs (18:1, and 20:1)] and Seven acids in *A. verrucosa* [SAFAs (13:0, 18:0, 21:0and 24:0) and UFAs (16:1, 18:1 and 18:3)] were found. Fatty acids content were more in *A. variabilis* (5.9607 mg F.A/g lipid) and minimum in *A. inaequalis* (0.243 mg F.A/g lipid) compared with other eight strains both qualitatively and quantitatively (Table 1). Myristic acid (0.0994 mg F.A/g lipid) in *A. cylindrica*, Non decanoic acid (0.1786 mg F.A/g lipid) in *A. torulosa* and Tricosanoic

Table 2. Mophological characteristics of *Anabaena* spp.

Strain	Shape of trichomes	Veg. cells		Heterocystes		Akinets	
		Length (µ)	Width (µ)	Length (µ)	Width (µ)	Length (µ)	Width(µ)
A525	Straight, terminal cell conical	2.8-(4.67)-7.0	2.8-(3.71)-5.6	4.2 -(6.57)-8.4	4.2 -(5.52) -5.6	7.0-(10.02)-14.0	5.6-(7.24)-9.8
A621	Straight, terminal cell conical	2.8-(4.21)-7.0	2.8-(4.46)-5.6	5.6-(6.17)-8.4	5.6-(6.02)-7.0	9.8-(13.9)-21.0	5.6-(6.17)-8.4
A802	Straight, terminal cell conical	1.4-(3.32)-4.2	1.4-(2.86)-4.2	4.2-(5.44)-7.0	2.8-(3.86)-5.6	7.0-(12.74)-18.2	5.6-(7.41)-9.8
A904	Straight, terminal cell conical	2.8- (4.84)- 8.4	2.8-(3.37)-7.0	5.6-(7.54)-9.8	4.2-(7.13)-8.4	9.8-(15.2)-19.6	8.4-(10.8)-12.6
A487	Straight, terminal cell conical	2.8 -(4.64)- 5.6	2.8-(3.61)- 4.2	5.6-(6.38)-7.0	4.2-(5.76)-7.0	5.6-(8.1)-11.2	4.2-(5.37)-7.0
A514	Straight, terminal cell conical	2.8-(4.2)-5.6	2.8-(3.02)- 4.2	4.2-(5.36)-7.0	2.8-(3.62)-5.6	5.6-(8.62)-11.2	5.6-(7.26)-8.4
A618	Bent, terminal cell round	2.8-(4.2)-5.6	2.8-(3.5)-4.2	4.2-(4.95)- 5.6	2.8-(4.17)-5.6	5.6-(6.25)-8.4	5.6-(5.75)-8.4
A622	Straight, terminal cell conical	2.8-(3.56)- 4.2	2.8-(4.48)-5.6	5.6-(6.98)-8.4	4.2-(3.43)-5.6	7.0-(11.2)-14.0	5.6-(6.44)-7.0

Table 3. Fatty acid composition of the different species of *Anabaena* on the exponential day

No.	Fatty acids	*Anabaena* Bory (No. strains)									
		1 *Anabaena ambigua* (485)	2 *Anabaena torulosa* (A525)	3 *Anabaena cylindrica* (A621)	4 *Anabaena augstumalis* (A802)	5 *Anabaena sphaerica* (A904)	6 *Anabaena inaequalis* (A487)	7 *Anabaena variabilis* (A514)	8 *Anabaena fertilissima* (A549)	9 *Anabaena subtropica* (A618)	10 *Anabaena verrucosa* (A622)
	SAFAs										
1.	Tridecanoic acid(C13:0)	0.1383	0.1683	-	0.1096	0.1755	-	0.3289	-	0.1361	0.1416
2.	Myristic acid (C14:0)	-	-	0.0994	-	-	-	-	-	-	-
3.	Pentadecanoic acid (C15:0)	0.0635	0.2199	-	-	0.1236	-	0.1136	0.0777	-	-
4.	Heptadecanoic acid (C17:0)	0.0368	0.0555	-	0.0443	0.0687	0.0712	0.1684	-	-	-
5.	Stearic acid (C18:0)	0.0081	-	-	-	-	-	0.0032	-	0.0284	0.1023
6.	Non decanoic acid (C19:0)	-	0.1786	-	-	-	-	-	-	-	-
7.	Heneicosanoic acid (C21:0)	-	0.0017	-	-	-	0.0163	0.0015	0.0016	-	0.0059
8.	Behenic acid (C22:0)	0.0860	-	-	-	-	0.0215	-	0.0026	-	-
9.	Tricosanoic acid (C23:0)	-	-	-	0.2300	-	-	-	-	-	-
10.	Lignoceric acid (C24:0)	-	0.0286	0.0347	0.4716	0.0191	-	0.2396	0.2088	0.2283	0.1123
	UFAs										
11.	Myristoleic acid (C14:1)	-	0.5767	-	-	-	-	0.5279	-	-	-
12.	Palmitoleic acid(C16:1)	-	-	-	-	-	-	0.0099	-	-	0.1079
13.	Elaidic acid (C18: 1 tra)	-	-	-	0.0661	-	0.0846	0.9473	0.0008	-	-
14.	Oleic acid (C18:1)	0.1566	0.0002	0.0273	-	-	-	-	-	0.0462	0.1082
15.	Linoelaidic acid (C18:3)	0.0713	-	-	-	-	-	0.0445	0.0745	-	-
16.	Linolenic acid (C18:3)	-	-	0.1867	1.5794	1.4903	-	3.5633	3.1900	-	0.4338
17.	Eicosenoic acid (C20:1)	-	-	-	-	-	-	0.0087	-	0.0387	-
18.	Eicosadienoic acid (C20:2)	0.0008	-	0.1451	-	-	0.0155	0.0039	-	-	-
19.	Arachiolonic acid (C20:4)	0.0869	-	-	-	-	0.0202	-	-	-	-
20.	Eicosapentaenoic acid (C20:5)	-	-	-	0.2405	0.0230	-	-	-	-	-
21.	Nervonic acid (C24:1)	0.0362	0.0138	-	0.0982	-	0.0137	-	-	-	-
	Total	0.6845	1.2433	0.4932	2.8397	1.9002	0.243	5.9607	3.556	0.4777	1.012

SAFAs (Saturated Fatty acid), UFAs (Unsaturated Fatty acids), Not detected (-)

acid (0.2300 mg F.A/g lipid) in *A. augstumalis* only found in these organisms.

In the dendrogram the ten strains are shown in relation to fatty acid composition of two major clusters. The major clusters consisted of seven strains including *A. variabilis*, *A. fertilissima*, *A. subtropica* and *A. verrucosa*. The other clusters had a maximum of two morphoforms. There was a close correlation between fatty acid composition and morphological characters for 6 species among the 10 species tested.

The dendrogram of the *Anabaena* species based on the fatty acid composition grouped the ten isolates into four clusters. Two clusters had two species each and the other two had three species each. *A. augstumalis* and *A. sphaerica* had five common fatty acids and formed a cluster and Eicosa pentaenoic acid was present in these two species only. These two species had akinetes on either side of the heterocysts. *A. variabilis* and *A. fertilissima* with six common fatty acids were together and in these two akinetes were always away from the heterocysts. *A. ambigua* and *A. torulosa* had four fatty acids so as A. *subtropica* and *A. verrucosa*. *A. inaequalis* joined the former and *A.cylindrica* the later clusters.

DISCUSSION

Present study ten isolates of *Anabaena* Bory were taken up, in which strains of five species have spores adjacent to heterocysts and the other five with spores away from the heterocysts. These cultures were studied and subjected to fatty acid fingerprinting analysis. The patterns of fatty acid composition among the blue green algae could be of phylogenetic significance ranging from unicellular colonial forms to the complex heterotrichomes forms and highly unsaturated acids are present in the morphologically more complex *Hapalosiphon laminosus*[33]. Caudales and Wells[28] also demonstrate the importance of fatty acid composition in the taxonomy of cyanobacteria at the genus and subgenus level. Romono *et al*. [20] used lipid profile for the classification of a new cyanobacterium in the *Spirulina* genus. Fatty acid composition has also been effectively used to determine the diversity and evolutionary closeness between species[23,34].

In the present study, fatty acids and their chemo-taxonomic values were investigated in the strains of *Anabaena*. The dendrogram of the *Anabaena* species based on the fatty acid composition grouped the ten isolates into four clusters. Two clusters had two species each and the other two had three species each. *A. augstumalis* and *A. sphaerica* had five common fatty acids and formed a cluster and Eicosa pentaenoic acid was present in these two species only. There was a close correlation between fatty acid composition and morphological characters for 6 species among the 10 species tested. Studies on 24 species of *Anabaena* with straight trichomes assigned to 7 species were carried out by analyzing the pattern and content of their fatty acid composition with their morphological properties[19]. These strains were divided into 2 subtypes: Type2A (18 strains) and Type 2B (6 strains) and both types were present in planktonic strains of *Anabaena*[19,20].

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