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Fatty Acid Composition of Six Freshwater Wild Cyanobacterial Species

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ABSTRACT. Hydroxy, *n*-saturated, branched, dioic, and unsaturated fatty acids in six freshwater wild cyanobacteria (*Chroococcus minutus*, *Lyngbya ceylanica*, *Merismopedia glauca*, *Nodularia sphaerocarpa*, *Nostoc linckia*, and *Synechococcus aeruginosus*) collected from different lakes and springs of Israel have been identified by gas chromatography–mass spectrometry (GC–MS).

Cyanobacteria constitute an ancient group of photosynthetic procaryotes, which have existed continuously since the early evolution of the biosphere (Barghoorn 1992). Recent descendants of cyanobacteria thrive in variable aquatic and terrestrial environments, exhibiting adaptive morphological, biochemical, and metabolic properties (Agrawal and Singh 2000, 2002). Many freshwater cyanobacterial species form water blooms in eutrophic lakes, excreting secondary metabolites toxic to aquatic animals, cattle, and man (Carmichael 1992; Carmichael *et al*. 2001).

Fatty acid and hydrocarbon compositions are a useful analytical tool in bacterial taxonomy (Řezanka *et al.* 1982; Welch 1991; Embley and Wait 1994). Some authors (Kenyon 1972; Kenyon *et al*. 1972; Murata *et al.* 1992) proposed that four types of fatty acid composition exist in cyanobacteria and demonstrated some correlations with morphological properties. Caudales and Wells (1992) investigated the cellular fatty acids of benthic cyanobacteria belonging to the genera *Anabaena* and *Nostoc* and found significant differences between these genera. Krüger *et al*. (1995) evaluated the taxonomic importance of fatty acid composition at the genus and subgenus level by analyzing the fatty acid composition of different *Microcystis* isolates and other members of the order *Chroococcales*. However, there is little information about fatty acid composition and chemotaxonomy in freshwater cyanobacterial species living in different habitats in Israel.

This report is a part of our investigation of cyanobacterial species in the framework of a comprehensive program on the biochemistry and toxicity of freshwater and terrestrial cyanobacteria (Dembitsky *et al*. 1999, 2000*a*,*b*, 2001*a*,*b*).

MATERIALS AND METHODS

Cyanobacterial samples. The cyanobacteria *Synechococcus aeruginosus* NAGELI, *Nodularia sphaerocarpa* BORN. et FLAH., and *Lyngbya ceylanica* WILLE, have been isolated from Ein Fashkha Springs; *Nostoc linckia* (ROTH) BORN. was isolated from Ein Boqeq Springs; *Merismopedia glauca* (EHRENBERG) NAGELI was isolated from Lake Kinneret (= Lake Tiberias = Sea of Galilee) and *Chroococcus minutus* (KUTZ.) NAGELI from En Hemed Springs (all samplings in Israel). The cyanobacterial species were cultivated in the *Laboratory of Hydrobiology* (*Division of Environmental Sciences, Graduate School of Applied Science, The Hebrew University of Jerusalem*, Israel), and grown aseptically and photoautotrophically on dry agar (Dor 1987), and then in 6-L batch cultures of BG-11 growth medium with nitrate (Stanier *et al.* 1971). Cultures were sparged with sterile air and maintained at 22–27 °C under constant illumination provided by cool white fluorescent tubes at an irradiance of about 20 μmol m^{-2} s^{-1} incident on the surface of the growth vessels. Cells were harvested during the stationary phase by centrifugation (4000 ***g***, 20 min), lyophilized and stored at –20 °C until required.

Extraction of fatty acids. Cells of each sample of cyanobacteria (50–130 mg) were added to 50 mL of an MeOH–H_2O–HCl (30 : 3 : 1, *V/V/V*) mixture and held at 55 °C for 6 h. After cooling to 10 °C, 150 mL

of cold water–*n*-pentane (2 : 1, *V*/*V*) mixture was added. The mixture was stirred for 1 h and then filtered through *Whatman* no.1 filter paper. The pentane layer was concentrated to dryness under vacuum at 10 °C. The residue was extracted three times with 50 mL of CH_2Cl_2, filtered and concentrated to dryness under vacuum at 10 °C. The residues of pentane and CH_2Cl_2 extracts were combined and dissolved in 1.5 mL of cold pentane–CH_2Cl_2 (1 : 1, *V*/*V*) mixture and used for GC–MS analysis.

Gas chromatographic–mass spectrometric analysis. A Hewlett Packard 6890 (series II) gas chromatograph modified for glass-capillary work and a HP-GC mass selective detector (5973B MSD) were used. Fatty acid-methyl esters were prepared according to Metcalfe and Wang (1981) and analyzed by GC on serially coupled capillary columns (Dembitsky *et al*. 1999): the RTX 1 column (30 m, ID 0.32 mm, film thickness 0.25 μm; *Restek*, USA) was coupled with a second capillary column (RTX 1701, 30 m, 0.32 mm, 0.25 μm film; *Restek*). The GC oven was programmed for 40 °C per 2 min, increase 2 K/min to 300 °C, 20 min at 300 °C. The injector temperature was kept at 180 °C. The flow rate of the carrier gas (helium) was 25 mL/s. The MS detector operated at 194 °C, ionization energy 11 aJ (70 eV). The scan range was from 30 to 700 *m/z* at 0.9 scan per s. Solvent delay was 10 min. Fatty acid-methyl esters were identified using mass electronical spectral library search (*Wiley Registry of Mass Spectral Data* 2000).

RESULTS AND DISCUSSION

Micrographs of isolated six freshwater cyanobacterial species are shown in Fig. 1.

Most of the fatty acids were unsaturated (33–62%) but *n*-saturated fatty acids were also identified (32–49 %, *W*/*W*; Table I). All the species of cyanobacteria appear to synthesize short (C_3–C_{10}) carboxylic acids, whose content is usually smaller than that of long-chain fatty acids (C_{14}–C_{18}). The major fatty acids were 16:0 ranging from 24 to 40 % (of total fatty acids), (*Z*)-9-18:1, (*Z,Z*)-9,12-18:2, and also (*Z,Z,Z*)-9,12,15-18:3. The predominant unsaturation (39 % of the total mono-unsaturated fatty acids) was at (*Z*)-9-18:1 (up to 21 %). Other monounsaturation was observed at (*Z*)-9-16:1 and 11-16:1 (*e.g.*, (*Z*)-9-hexadecenoic and 11-hexadecenoic acids). The principal fatty acids in the investigated cyanobacteria (Table I) did not essentially differ from those of most previously studied cyanobacteria (Schneider *et al.* 1970; Murata and Nishida 1987).

Table I. Comparative fatty-acid composition of some freshwater cyanobacterial species[a]

Fatty acid	*C.m.*	*L.c.*	*M.g.*	*N.s.*	*N.l.*	*S.a.*
Hydroxy						
Total	0.42	0.72	1.05	0.71	0.69	0.59
2-Hydroxypropanoic (lactic; 2-OH-3:0)	0	0.21	0.46	0	0.15	0
3-Hydroxybutanoic (3-OH-4:0)[b]	0.18	0.33	0	0.32	0.24	0.59
2-Hydroxy-4-methylpentanoic (2-OH-4-Me-5:0)	0	0.18	0	0.39	0.12	0
2-Hydroxy-3-methylpentanoic (2-OH-3-Me-5:0)	0.24	0	0.59	0	0.18	0
***n*-Saturated**						
Total	32.41	40.49	43.64	49.10	40.29	44.44
Butanoic (4:0)[b]	0.27	0.09	0	0.13	0.11	0
Pentanoic (5:0)[b]	0.21	0.08	0	0	0.06	0
Hexanoic (6:0)[b]	0.37	0.16	0.39	0	0.14	0.10
Heptanoic (7:0)[b]	0.15	0.13	0.11	0	0.08	0
Octanoic (8:0)[b]	0.41	0.21	0.43	0.23	0.19	0.21
Nonanoic (9:0)	0.19	0.34	0.21	0	0.09	0
Decanoic (10:0)	0.53	0.41	0.78	0.37	0.21	0.18
Undecanoic (11:0)	0.27	0.14	0.10	0	0.11	0
Dodecanoic (12:0)	0.62	0.46	0.88	0.72	0.31	0.58
Tridecanoic (13:0)	0.34	0.27	0.64	0.36	0.24	0.39
Tetradecanoic (14:0)	1.76	2.40	1.23	0.89	1.30	2.79
Pentadecanoic (15:0)	0.94	1.38	1.32	1.13	1.13	1.19
Hexadecanoic (16:0)	24.13	31.28	34.07	39.72	33.03	37.49

Heptadecanoic (17:0)	0.46	0.88	1.11	0.69	0.75	0.37
Octadecanoic (18:0)	1.76	2.26	2.37	4.86	2.54	1.14
Branched saturated						
Total	3.35	6.86	9.07	12.79	6.25	3.20
2-Methylbutanoic (2-Me-4:0)[b]	0	0	0.73	0	0.13	0
3-Methylbutanoic (3-Me-4:0)[b]	0	0	0.48	0	0.14	0
4-Methylhexanoic (4-Me-6:0)[b]	0	0.35	0.52	0.25	0.18	0
8-Methyldecanoic (8-Me-10:0)[b]	0.25	0.41	0.29	0.68	0.21	0
11-Methyldodecanoic (11-Me-12:0)[b]	0.11	0.75	0.66	0.57	0.23	0.11
12-Methyltridecanoic (12-Me-13:0)	0.31	0.46	0.71	0.32	0.31	0.23
12-Methyltetradecanoic (12-Me-14:0)	0.27	0.38	1.12	0.89	0.41	0.26
13-Methyltetradecanoic (12-Me-14:0)	0.34	0.52	0.79	1.18	0.38	0.37
14-Methylpentadecanoic (14-Me-15:0)	0.47	0.67	0.99	1.36	0.52	0.44
2-Methylhexadecanoic (2-Me-16:0)	0.11	0.35	0	0	0.63	0
14-Methylhexadecanoic (14-Me-16:0)	0.36	0.49	0.67	1.34	0.34	0.22
15-Methylhexadecanoic (15-Me-16:0)	0.27	0.83	0.72	1.21	0.46	0.48
10-Methylheptadecanoic (10-Me-17:0)	0.10	0.37	0.46	1.11	0.54	0
16-Methylheptadecanoic (16-Me-17:0)	0.18	0.42	0.32	0.93	0.37	0.28
15-Methylheptadecanoic (15-Me-17:0)	0.22	0.21	0.39	0.78	0.51	0.34
2-Hexylcyclopropaneoctanoic (Cyc-17:0)	0.36	0.65	0.22	2.17	0.89	0.47
Dioic						
Total	1.60	2.01	4.32	4.40	3.54	2.67
Propane-1,3-dioic[b]	0.36	0	0.42	0	0	0
Butane-1,4-dioic[b]	0.43	0	0.53	0.77	0.35	0
2-Hydroxybutane-1,4-dioic[b]	0.28	0	0.45	0.65	0	0
Pentane-1,5-dioic[b]	0.10	0	0.72	0	0.43	0
Hexane-1,6-dioic[b]	0.18	0.56	0.44	0.48	0.24	0.87
Heptane-1,7-dioic[b]	0	0.38	0.23	0.41	1.51	0.24
Octane-1,8-dioic	0	0.33	0.67	0.67	0.25	0.65
Nonane-1,9-dioic	0.25	0.74	0.86	1.42	0.76	0.91
Unsaturated						
Total	62.22	49.92	41.92	33.00	49.23	49.10
9-Hexadecenoic (9-16:1)	3.58	10.82	1.94	2.58	7.12	13.49
11-Hexadecenoic (11-16:1)	0.34	1.89	0.48	0.21	2.16	1.94
9,12-Hexadecadienoic (9,12-16:2)	0	2.46	0.23	0.59	1.36	0.32
9-Octadecenoic (9-18:1)	20.83	9.12	19.12	12.49	15.32	11.23
9,12-Octadecadienoic (9,12-18:2)	9.47	10.31	11.33	13.46	11.21	9.11
10,13-Octadecadienoic (10,13-18:2)	3.51	1.38	1.54	1.29	1.86	3.24
12,15-Octadecadienoic (12,15-18:2)	0.85	0.47	0	0	0.87	1.19
9,12,15-Octadecatrienoic (9,12,15-18:3)	23.64	13.47	7.28	2.38	9.33	8.49

[a]*C.m.* – *Chroococcus minutus* *N.s.* – *Nodularia sphaerocarpa*
L.c. – *Lyngbya ceylanica* *N.l.* – *Nostoc linckia*
M.g. – *Merismopedia glauca* *S.a.* – *Synechococcus aeruginosus*

[b]Not previously isolated from cyanobacteria.

Hydroxy (0.42–1.05 %) and dioic fatty acids (1.6–4.4 %) were found as minor components. These acids were previously isolated by GC–MS from freshwater cyanobacteria belonging to the genus *Aphanizomenon* (Dembitsky *et al*. 2001*a*) and *Nostoc* sp. (Dembitsky *et al*. 1999). Branched saturated fatty acids that were identified accounted for 3.35–12.8 %. The fatty-acid composition of cyanobacteria was studied by Holton *et al.* (1964) in *Anacystis nidulans* and by Lennarz (1966) in *Anabaena variabilis.* Major fatty acids thus far known to be present in cyanobacteria are hexadecanoic (16:0), 9-hexadecenoic (16:1), hexadecadienoic (16:2), octadecanoic (18:0), and 9-octadecenoic (18:1). Aliphatic dicarboxylic acids surprisingly afforded potent cytotoxicity and antineoplastic activity (Hall *et al.* 1999), and could serve as lipidoic markers for

identification of some human and animal diseases (Ma *et al.* 1999; Martin 1998). These acids are of major interest for medical specialists and biochemists.

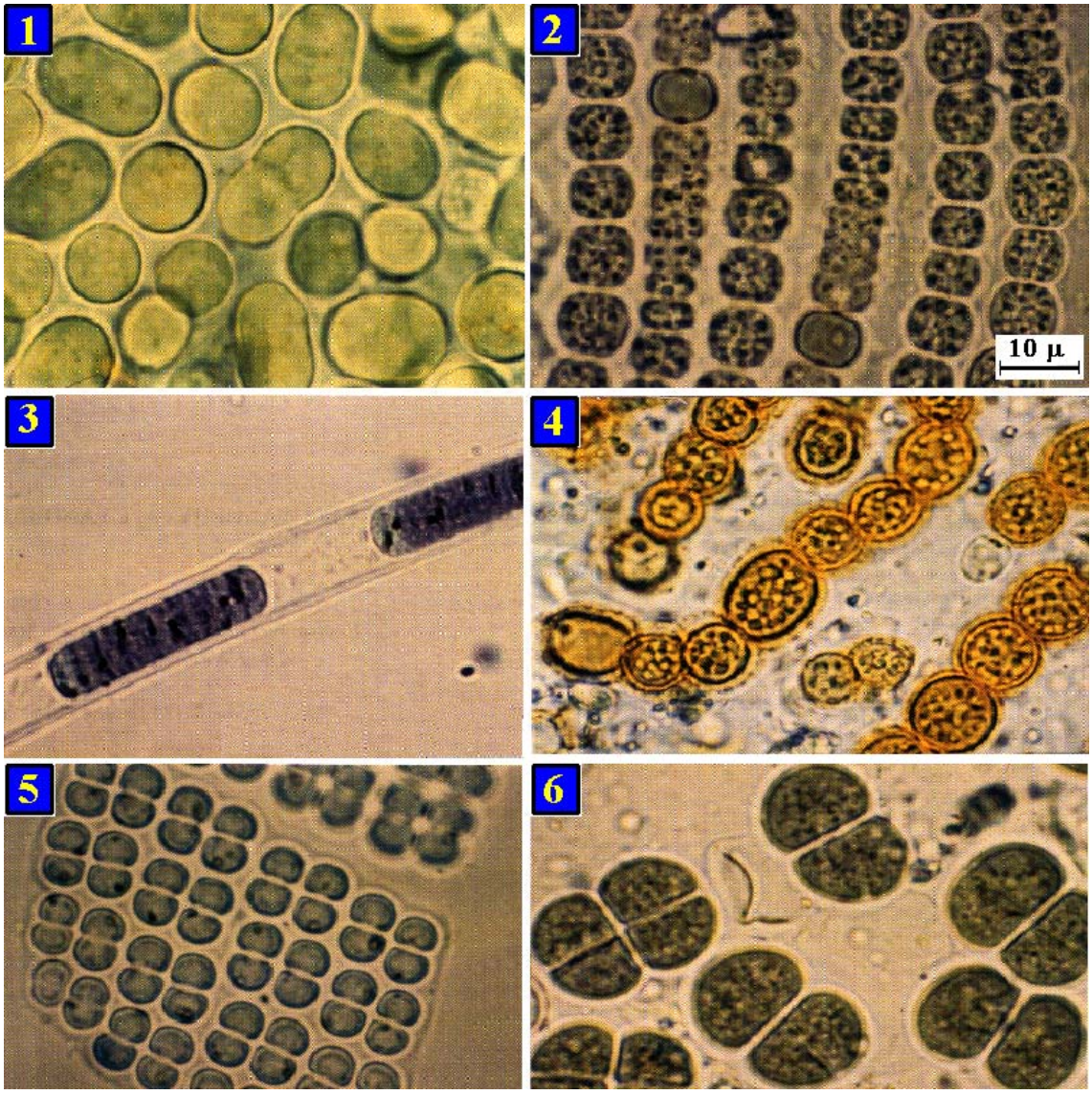

Fig. 1. Microphotographs of some wild freshwater cultivated cyanobacteria isolated in Israel. **1** – *Synechococcus aeruginosus* NAGELI (isolated in Ein Fashkha Springs), **2** – *Nodularia sphaerocarpa* BORN. et FLAH. (Ein Fashka Springs), **3** – *Lyngbia ceylanica* WILLE (Ein Flashka Springs), **4** – *Nostoc linckia* (ROTH) BORN. (Ein Boqeq Springs), **5** – *Merismopedia glauca* (EHRENBERG) NAGELI (Lake Kinneret = Lake Tiberias = Sea of Galilee), **6** – *Chroococcus minutus* (KUTZ.) NAGELI (En Hemed Springs).

It is known that particular groups of organisms are characterized by particular fatty acids (Kerwin 1994) that can be used as their biological markers. Our chromatographic assay allowed us to discover previously unknown acids, such as, *e.g.*, methyl-branched-saturated and dioic acids (*see* footnote *b* in Table I), in some freshwater wild cyanobacteria and showed the presence of 51 fatty acids in some cyanobacterial species.

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