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Polar Lipids and Fatty Acids of Three Wild Cyanobacterial Strains of the genus *Chroococcidiopsis*

T. ŘEZANKA[a], I. VÍDEN[b], J.V. GO[c], I. DOR[d], V.M. DEMBITSKY[c]

[a]*Institute of Microbiology, 142 20 Prague, Czechia*
fax +420 241 062 347
e-mail rezanka@biomed.cas.cz

[b]*Laboratory of Mass Spectrometry, Institute of Chemical Technology, 166 28 Prague, Czechia*
[c]*Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University of Jerusalem, 91120 Israel*
[d]*Division of Environmental Sciences, Graduate School of Applied Science, The Hebrew University of Jerusalem, 91904 Israel*

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ABSTRACT. The occurrence of *n*-saturated, branched, and unsaturated fatty acids of 3 wild terrestrial strains of the genus *Chroococcidiopsis* (Order *Chroococcales*): *C. supralittoralis*, *C. umbratilis*, and *C. versatilis* collected from Lake Kinneret, Dead Sea, and Ein Kerem (Jerusalem) was investigated and individual compounds identified by gas chromatography–mass spectrometry. Polar lipids also were examined. Among polar lipids (studied using two-dimensional thin-layer chromatography) were as major glycolipids isolated: monogalactosyl-diacylglycerols, digalactosyl-diacylglycerols, 6-sulfoquinovosyl-diacylglycerols and phosphatidylglycerol. Nonphosphorus betaine-containing lipid, *viz. N*,*N*,*N*-trimethylhomoserin-4-*O*-yl-diacylglycerol, was found for the first time in cyanobacterial species.

Abbreviations

MGDG	monogalactosyl-diacylglycerol	PG	phosphatidylglycerol
DGDG	digalactosyl-diacylglycerol	GC-MS	gas chromatography mass spectrometry
SQDG	6-sulfoquinovosyl-diacylglycerol	2D-TLC	two-dimensional thin-layer chromatography
DGTS	*N*,*N*,*N*-trimethylhomoserin-4-*O*-yl-diacylglycerol		

Cyanobacteria belonging to the genus *Chroococcidiopsis* are widespread in nature. Thus, species of this genus have been discovered in extreme environments, such as thermal springs of Aedipsos, Hellas in Greece (Anagnostidis and Pantazidou 1988), India (Vasishta 1968) and Indonesia (Geitler 1933). Also they have been isolated from shady caves in Israel (Friedman 1961), rocks, walls and paddy field soils in India (Tiwari 1972), from the supralittoral rocks of Lake Kinneret, from a shaded stonewall and from a hypersaline pond in Israel (Dor *et al.* 1991), in hot deserts (Billi *et al.* 2001; Caiola *et al.* 1993; Stolz 1990), and also in Ross Desert, Antarctica (Friedmann *et al.* 1988).

The primitive characteristics of the cyanobacterium *Chroococcidiopsis* suggest that it represents a very ancient type of the group (Fewer *et al.* 2002). Its morphology is simple but shows a wide range of variability, and it resembles certain Proterozoic microfossils (Friedmann and Ocampo-Friedmann 1995). *Chroococcidiopsis* is probably the most desiccation-resistant cyanobacterium, the sole photosynthetic organism in extreme arid habitats. It is also present in a wide range of other extreme environments, from Antarctic rocks to thermal springs and hypersaline habitats, but it is unable to compete with more specialized organisms. Genetic evidence suggests that all forms belong to a single species. The hypolithic microbial growth form (which lives under stones of a desert pavement) could be used as a model for development of technologies for exploring arid areas (Friedmann and Ocampo-Friedmann 1995).

Caudales *et al.* (2000) studied the cellular fatty acids of five genera of unicellular cyanobacteria (*Dermocarpa*, *Xenococcus*, *Dermocarpella*, *Myxosarcina*, *Pleurocapsa*) containing high proportions of saturated straight-chain fatty acids (26–41 % of the total) and unsaturated straight chains (40–67 %). Isomers of 16:1 were the main monounsaturated acid component (11–59 %). Oligounsaturated acids were present at trace levels (0–1 %) in *Xenococcus* and *Myxosarcina*, at a concentration of <7 % in *Dermocarpa*, *Dermocarpella*, *Pleurocapsa*, and at a high concentration (≥35 %) in *Chroococcidiopsis* spp. This genus was also different in terms of the percentage of 16:1 isomers (10–12 %) compared to other genera (30–59 %), and in terms of total C_{16} and C_{18} fatty acids.

Only few papers deal with fatty acid composition of cyanobacteria belonging to the order *Chroococcales* (Caudales *et al.* 2000; Řezanka *et al.* 2003*a*,*b*). Here we describe the polar lipid and fatty acid

composition of 3 strains of *Chroococcidiopsis* isolated from the supralittoral rocks of Lake Kinneret, from a shaded wall limestone in Jerusalem, and from a hypersaline sublittoral solar pond in the Dead Sea (Dor *et al.* 1991).

MATERIALS AND METHODS

Cyanobacterial samples. The supralittoral strain *Chroococcidiopsis supralittoralis* sp. *nova* DOR was isolated from the basalt rocky shore of Lake Tiberias (Sea of Galilee) about 2 m a.s.l., at a site exposed to direct sunlight. The stone wall strain *C. umbratilis* sp. *nova* DOR was found in the proximity of Mary's Spring in Ein Kerem (Jerusalem), where it formed dark patches on the limestone wall in the shade, beyond the zone of the water spray. The strain *C. versatilis* sp. *nova* DOR was collected in a sublittoral rock of a hypersaline solar pond in Dead Sea area. All cyanobacterial species were isolated by seeding small samples of collected material on Chu-11 enriched agar medium followed by a 7-d incubation of the preparatory cultures and the transfer of small aggregates, recognizable due to the presence of a characteristic multiple fission pattern, to a fresh, sterile agar medium. After several transfers, monospecific, stock cultures were obtained. A part of each stock was maintained on freshwater agar at 22–23 °C, under low illumination of 10–20 μE m^{-2} s^{-1} (*Lab-Line* Biotronette Mark III Environmental Chamber) and a part was preserved on dry agar in darkness (Dor 1987). All strains were photoautotrophically cultivated and grown aseptically on dry agar, and then in 6-L batch cultures of BG11 growth medium containing nitrate (Stanier *et al.* 1971). Cultures were sparged with sterile air and maintained at 22–25 °C under constant illumination provided by cool white fluorescent tubes at an irradiance of ≈20 μE m^{-2} s^{-1} incident on the surface of the growth vessels (Dor *et al.* 1991). Cells were harvested during the stationary phase by centrifugation (4000 ***g***, 20 min), lyophilized and stored at –20 °C until required. Specimens of all strains are kept at the *Laboratory of Hydrobiology* (*Division of Environmental Sciences, Graduate School of Applied Science, The Hebrew University of Jerusalem*, Israel). Microphoto of *C. supralittoralis* is shown in Fig. 1.

For *extraction and identification of fatty acids* see Řezanka *et al.* (2003*a*,*b*); briefly: cells of each strain (50–130 mg) were added to 50 mL of MeOH–H_2O–HCl (30 : 3 : 1, *V*/*V*) mixture and held at 55 °C for

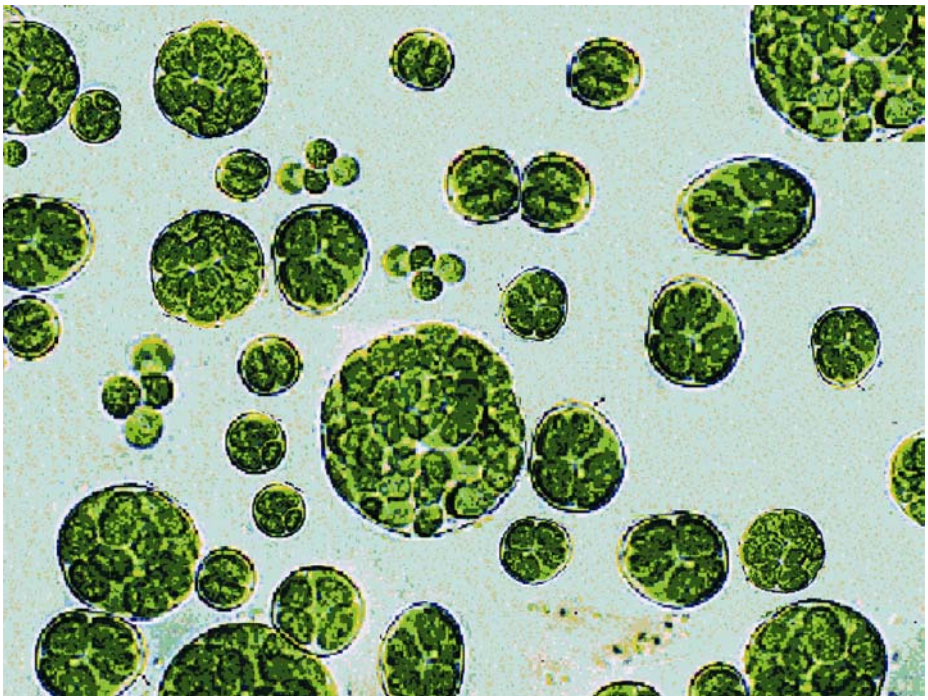

Fig. 1. Microphotograph of the strain *C. versatilis* sp. *nova* DOR, originally isolated from the sublittoral hypersaline solar pond at the Dead Sea.

6 h. After cooling to 10 °C, 150 mL of cold pentane–H_2O (1 : 2, *V*/*V*) mixture was added. The mixture was stirred for 1 h and then filtered.

Two-dimensional thin-layer chromatography. For separation of total lipids silica-gel plates 100 × 100 mm (no. 5715; *Merck*, Germany) were used with chloroform–methanol–water (65 : 25 : 4, *V*/*V*) in the 1st dimension and chloroform–methanol–isopropylamine–NH_3 conc. (65 : 35 : 0.3 : 4.5, *V*/*V*) for the 2nd dimension. Spots were visualized with 2′,7′-dichlorofluorescein (100 ppm in ethanol), and also 8 % sulfuric acid in methanol.

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 2 serially coupled capillary columns (Dembitsky *et al*. 1999) RTX 1 and RTX 1701 (30 m × 0.32 mm, film thickness 0.25 μm; *Restek*, USA).

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 30–700 *m*/*z* at 0.9 scan per s, solvent delay being 10 min. Fatty-acid methyl esters were identified using mass electronically spectral library search (*Wiley Registry of Mass Spectral Data*).

RESULTS AND DISCUSSION

We found by 2D-TLC (Fig. 2) that the genus *Chroococcidiopsis* contains major polar lipids SQDG, MGDG, DGDG, PG, nonphosphorous betaine lipid DGTS and also an unidentified minor glycolipid (Table I). DGTS has not been found previously in cyanobacterial species but betaine lipids are widespread in nature from bacteria *Rhodobacter sphaeroides* (Benning *et al.* 1995) or *Sinorhizobium melitoti* (Geiger *et al.* 1999) through algae, fungi, lichens, and higher plants (Dembitsky 1996; Rozentsvet *et al.* 2000).

Feeding experiments with methionine labeled at a specific carbon atom in algae and moss consistently suggest that S-adenosylmethionine donates the 3-amino-3-carboxypropyl portion of the DGTS head group as well as three methyl groups giving rise to the quarternary ammonium (Sato 1988; Sato and Kato 1988; Vogel and Eichenberger 1992). Because similar results were obtained with *R. sphaeroides* (Hofman and Eichenberger 1996), it seems plausible that the pathway for DGTS biosynthesis is universal in eukaryotes and prokaryotes (Klug and Benning 2001). According to this conclusion it is not surprising that the cyanobacterium *Chroococcidiopsis* synthesized DGTS.

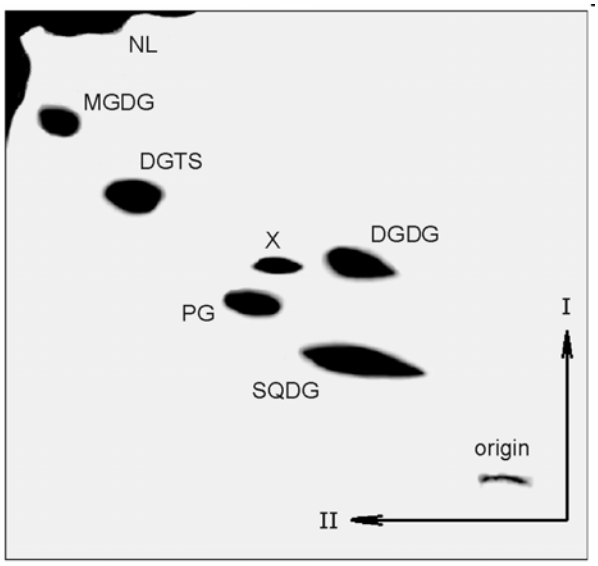

Fig. 2. 2D-TLC of polar lipids from the strain *C. versatilis* (sublittoral of hypersaline solar pond in the Dead Sea); for abbreviations *see* the text.

Polar lipids of cyanobacteria usually contain 3 classes of glycolipids such as MGDG, DGDG, SQDG, and phosphorous lipid PG (Murata and Nishida 1987).

In spite of a great amount of different polar lipids (Reshef *et al.* 1997; Son *et al.* 2000; Antonopoulou *et al.* 2002) discovered in cyanobacteria, none of them is identical with our unidentified lipid on the basis of physico-chemical methods. (For their further structure identification a large amount of biomass would have to be cultivated.)

Both *Chroococcidiopsis* spp. differ in the distribution of majority compounds (SQDG and DGTS) from *C. versatilis* (Table I). The high content of DGTS, which was detected in *Cyanophyta* for the first time, stimulated the further study of this class of compounds.

The fatty acid composition of the 3 strains of *Chroococcidiopsis* is shown in Table II. In comparison with fresh-water cyanobacterial species (Řezanka *et al.* 2003*a*,*b*) the terrestrial cyanobacteria belon-

ging to genus *Chroococcidiopsis* show the presence of 25 fatty acids with predominant unsaturated types (64–71 %, *W*/*W*). All the strains of cyanobacteria appear to synthesize C_{16}–C_{18}-families of fatty acids; the major 9,12-18:2 (18–25 % of total), (*Z*,*Z*,*Z*)-9,12,15-18:3 (9.2–14.6 %), and 2 monoenoic acids such as 8-18:1 and 9-18:1. Other unsaturation was also detected: 7-16:1, 7,10-16:2, and 7,10,13-16:3.

Table I. Composition of polar lipids of three strains of the genus *Chroococcidiopsis*[a,b]

Lipid	*C. supralittoralis*	*C. umbratilis*	*C. versatilis*
MGDG	38.4 ± 4.3	28.9 ± 2.4	14.5 ± 2.5
DGDG	19.5 ± 2.1	23.4 ± 3.2	20.6 ± 4.3
SQDG	22.1 ± 1.9	27.1 ± 2.4	31.6 ± 5.1
DGTS	4.9 ± 1.3	2.1 ± 0.9	16.5 ± 1.8
PG	13.4 ± 1.8	11.6 ± 1.6	12.4 ± 2.2
PA	0.9 ± 0.4	0.7 ± 0.4	0
Unidentified lipid	0.8 ± 0.3	1.1 ± 0.5	4.4 ± 1.3

[a]Mean ± SD of three determinations. [b]Percentage of total polar lipids.

Table II. Fatty acid composition (%) of three strains of the genus *Chroococcidiopsis*

Fatty acid	*C. supralittoralis*	*C. umbratilis*	*C. versatilis*
n-*Saturated*	36.22	27.26	30.14
8:0	0.13	0.67	<0.1
9:0	<0.1	0.13	<0.1
10:0	0.24	0.62	1.11
11:0	0.33	<0.1	<0.1
12:0	0.80	0.92	0.94
13:0	0.36	0.28	<0.1
14:0	1.87	1.65	2.58
15:0	0.64	0.38	<0.1
16:0	24.80	18.72	20.80
17:0	0.59	0.46	<0.1
18:0	6.46	3.43	4.71
Unsaturated	62.19	69.99	66.63
7-16:1	4.37	3.76	2.47
9-16:1	1.01	1.22	4.70
7,10-16:2	0.77	1.95	1.21
7,10,13-16:3	0.90	1.60	2.12
8-18:1	8.62	5.20	23.53
9-18:1	11.95	19.75	1.65
9,12-18:2	24.43	20.67	18.23
8,11,14-18:3	0.94	1.27	2.01
9,12,15-18:3	9.20	14.57	10.71
Branched saturated	1.59	2.75	3.23
9-Me-13:0	<0.1	0.67	<0.1
10-Me-14:0	0.86	0.40	1.05
10-Me-17:0	0.14	0.49	<0.1
cyc-17:0	0.59	1.19	2.18

The principal fatty acids of the 3 strains do not essentially differ from those of most previously studied cyanobacteria belonging to the genus *Chroococcidiopsis* (Caudales *et al.* 2000). Table II contains only two octadecatrienoic acids: 6,9,12-18:3 (γ-linolenic) and 8,11,14-18:3. In contrast, the widespread 9,12,15-18:3 (α-linolenic) acid which is a constituent of higher plants was not detected. Therefore, we assume that *Cyanophyta* as very ancient microorganisms that do not contain the enzymic systems (common to higher plants) for the formation of α-linolenic acid.

We can evaluate the different distribution patterns of fatty acids in the presented *Chroococcidiopsis* spp. Considerable differences were observed for unsaturated fatty acids as a whole and especially for posi-

tional isomers of octadecenoic acid. Oleic acid (9-18:1) was detected as a majority compound in *C. supralittoralis* and *C. umbratilis*. In contrast, the majority compound in *C. versatilis* was the positional isomer 8-18:1 (Fig. 3). This difference can be attributed to the location of sample collection. The samples of *C. supralittoralis* and *C. umbratilis* were found in dry places (≈2 m above water, *i.e.* on stones or shore). On the other hand, the samples of "fresh-water" *C. versatilis* were found in a hypersaline pond. Therefore, it seems to be the reasonable explanation that the effect of intens light and differences in local temperatures (the stones could be hot near 100 °C unlike water at 40 °C) result in changes of the spectrum of synthesized fatty acids.

Besides the fact that cyanobacteria of the genus *Chroococcidiopsis* are the first pioneer of life in extreme environments as arid areas or hypersaline swamps, they may also serve as an important source of essential fatty acids for herbivorous animals.

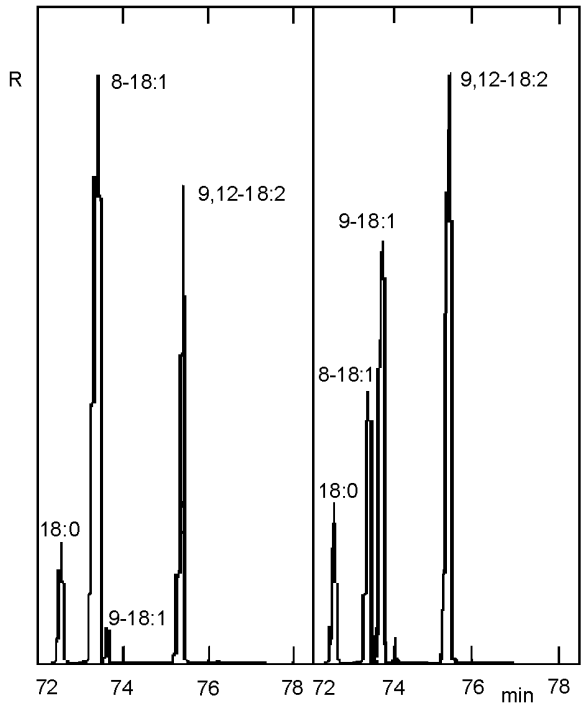

Fig. 3. GC–MS chromatographic separation of two isomers of fatty-acid methyl esters (*E*)-8-18:1 and (*Z*)-9-18:1, isolated from *C. versatilis* (Dead Sea; *left*), and *C. supralittoralis* (Lake Kinneret; *right*); R – detector response.

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