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Variability of Lipid Constituents of the Soil Cyanobacterium *Microcoleus vaginatus* from the Dead Sea Basin and Negev Desert

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Abstract—A study of lipids of the soil cyanobacterium *Microcoleus vaginatus*, which was isolated from microbial crusts collected in the Dead Sea basin and in the Negev desert, was performed. Twenty-six hydrocarbons and fatty acids were separated and identified by GC/MS using serially coupled capillary columns of different polarity. Changes in the lipid composition were evaluated by comparison of samples collected from different locations. Heptadecane, 1-heptadecene, 6- and 7-methylheptadecane, hexadecanoic and 9(Z)-octadecenoic acids were identified as the major constituents. Biochemical mechanisms of production of the different lipid compounds under UV irradiation are proposed.

Key words: heptadecane, fatty acids, *Microcoleus vaginatus*, soil cyanobacterium, Dead Sea, Negev desert, UV irradiation

There has been considerable interest by biochemists in the hydrocarbons and fatty acids of cyanobacteria and, in particular, their abundance in soils [1-11]. It has been reported that cyanobacteria constitute an ancient group of photosynthetic procaryotes present since the early evolution of the biosphere [12, 13]. Their fossil representatives have been detected in Precambrian stromatolitic rocks, about three billion years old [14, 15], giving further interest for biogeochemical investigations [16, 17]. Recent descendants of cyanobacteria thrive in various aquatic and terrestrial environments and exhibit adaptive biochemical, morphological, and metabolic characteristics [18]. Some of them exert prominent effects on their corresponding habitats. It is known that certain cyanobacteria species form water blooms in eutrophic lakes, excreting secondary metabolites toxic to aquatic animals and humans [19, 20]. Some cosmopolitan species thrive in desert soils and form surface crusts rich in polysaccharides, which contribute to the soil's stability [21, 22]. Microbial soil crusts dominate in the northwestern part of the Negev desert and the Dead Sea basin, and their biota has been described [23, 24].

Microcoleus vaginatus is a common species found in many parts of the world and is typical of desert soil [21, 24-26]. Several isolates of *Microcoleus* were prepared from crust samples in the Negev desert and the Dead Sea basin [26, 27]. Cyanobacteria belonging to the genus *Microcoleus* contain polysaccharides [23, 24, 27, 28], carotenoids [29], and mycosporine-like amino acids [30]. Volatile constituents of soil containing *Microcoleus chichonoplastes* were studied by Grimalt et al. [6]. There is no information on the composition of hydrocarbons and fatty acids of the cyanobacterium *Microcoleus vaginatus*.

This report is part of our investigation of cyanobacteria [31] in the framework of a comprehensive program on the ecology and biochemistry of soil cyanobacteria.

MATERIALS AND METHODS

Cyanobacterial samples. Microbial crust samples were collected in the Hatzeva, Sede Boqer (both are located in the Negev desert), and Almog (Dead Sea basin) in September 1996 and again in 1998 [24, 32, 33]. It is noteworthy that soil surface temperatures reach very high values during the day in summer: for instance, the maximal average noon temperature for July 1992 was 60°C [26]. *Microcoleus vaginatus* Gom. was isolated from crust samples [34] and cultivated in the Laboratory of Hydrobiology (Environmental Division). Cultures A, B, and D (Table 1) were grown on dry agar and then on medium BG-11 at 27°C as described by Dor [34] under cold white fluorescent lights of 15 $\mu E/m^2$ per sec at the surface of the flasks for 100 h. Culture B of *M. vaginatus* (Almog) was additionally cultivated at 35°C using a

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monochromatic UV-B lamp (312 nm, Vilber-Laurmat, T-15M 7C) at 16 W/m^2 for 100 (II), 200 (III), 300 (IV), and 400 h (V), respectively (see Fig. 3 and Table 2) in a special UV protected box. Cells were harvested by centrifugation, lyophilized, and stored at −20°C.

Extraction of hydrocarbons and fatty acids. Cells of each sample of cyanobacteria (390 mg) were added to 50 ml of MeOH–H_2O–HCl (90 : 9 : 3 v/v) mixture and held at 55°C for 6 h. After cooling to 10°C, 150 ml of cold H_2O–C_5H_{12} (100 : 50 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 with 50 (×3) ml of CH_2Cl_2, then filtered and concentrated to dryness under vacuum at 10°C. The dried C_5H_{12} and CH_2Cl_2 extracts were combined and dissolved in 1.5 ml of cold C_5H_{12}–CH_2Cl_2 (1 : 1 v/v) mixture and used for GC-MS analysis.

Gas chromatographic–mass spectrometric analysis. A Hewlett-Packard 5890 (series II) gas chromatograph modified for glass capillary work and a HP GC-mass selective detector (5971B MSD) were used. Fatty acid methyl esters and hydrocarbons were analyzed by gas chromatography on serially coupled capillary columns [35]: a RTX-1 (Restek, USA) column (30 m, ID 0.32 mm, film thickness 0.25 μm) was coupled with a second capillary column (Restek RTX-1701, 30 m, 0.32 mm, 0.25 μm film). The GC oven was programmed as follows: 40°C per 2 min, 2°C/min to 300°C, 20 min at 300°C. The injector

Table 1. Hydrocarbons and fatty acids of *Microcoleus vaginatus*

Compound (peak number)	M.W.	A	B	C	D
1. 8-Heptadecene	238	0.35	1.94	1.13	1.34
2. 1-Heptadecene	238	0.28	21.53	14.40	1.70
3. Heptadecane	240	24.58	14.37	7.49	23.01
4. 2-Methylhexadecane	240	0.41			
5. Tetradecanoic acid	242	0.22	0.58	0.41	1.75
6. 7-Methylheptadecane	254	16.02	0.34	0.51	9.04
7. 6-Methylheptadecane	254	3.16		0.62	1.67
8. 9-Methyltetradecanoic acid	256	1.96	0.11	0.64	1.31
9. Pentadecanoic acid	256	0.48	0.29	0.76	1.42
10. 3-Hydroxytetradecanoic acid	258	0.59		9.84	0.47
11. Neophytadiene	278	0.32		1.94	
12. 7,10,13-Hexadecatrienoic acid	264	4.78	7.69	0.97	5.44
13. 7,10-Hexadecadienoic acid	266	0.98	4.61	4.16	3.24
14. 11-Hexadecenoic acid	268	0.96			2.01
15. 9(Z)-Hexadecenoic acid	268	11.33	1.19	1.15	6.01
16. 7(Z)-Hexadecenoic acid	268	1.66	3.73	0.55	2.79
17. Hexadecanoic acid	270	13.48	19.85	15.26	18.33
18. 6,9,12-Octadecatrienoic acid	292	0.45			0.27
19. 9,12,15-Octadecatrienoic acid	292	1.82	0.32	0.31	1.61
20. 9(Z),12(Z)-Octadecadienoic acid	294	0.52	3.09	23.92	3.57
21. 9(Z)-Octadecenoic acid	296	5.96	9.78	11.03	9.15
22. 11-Octadecenoic acid	296	0.39		0.73	1.25
23. Octadecanoic acid	298	0.41	0.11	0.54	0.89
Compounds not indicated in Fig. 1					
Tetradecane	198	0.21	0.33	0.10	0.19
Phytol	296	0.21	0.11	0.30	0.23
Squalene	410	1.04	2.18	1.64	1.19
Minor and other volatile compounds		7.47	7.85	1.60	2.12

Note: M.W., molecular weight; A, B, and D are strains collected from different locations.

temperature was kept at 180°C. The flow rate of the carrier gas (helium) was 25 ml/sec. The MS detector was operated at 194°C, ionization energy 70 eV. The scan range was from 30 to 650 m/z at 0.9 scan/sec. Solvent delay was 10 min. Hydrocarbons and fatty acid methyl esters were identified using mass spectral library search (NBS75, Wiley 138 & Wiley 275).

RESULTS AND DISCUSSION

Hydrocarbons and fatty acids. Analysis of the total hydrocarbon and fatty acid compositions of the soil cyanobacterium *M. vaginatus* indicated the presence of twenty six hydrocarbons and fatty acids: the quantitative data are shown in Table 1. The major constituents were identified as 1-heptadecene, heptadecane, hexadecanoic (C16:0), 9(Z)-hexadecenoic (C16:1), and 9(Z)-octadecenoic (C18:1) acids. Partial chromatograms of volatile compounds of *M. vaginatus* cultures collected at different locations are shown in Fig. 1. The chromatograms indicate similar major components but different abundances, for example, compare 1-heptadecene, heptadecane, 6- and 7-methylheptadecane in the different samples.

According to data published by Grimalt et al. [6], a *Microcoleus* mat contained heptadecane and heptadecenes as minor components. We have studied the mass spectra of methylheptadecanes in sample A (from Hatzeva, Negev desert). Peak 6 was identified as 7-methylheptadecane, and peak 7 as 6-methylheptadecane. Mass spectra of these two hydrocarbons are shown in Fig. 2. Fragments at C_8 (m/z 112/113) and C_{12} (m/z 168/169) predominate over C_9 and C_{11} fragments in Fig. 2 (upper part) and represent a mass spectrum taken on the upward slope. The relatively high intensity of the C_7 (m/z 98/99) and C_{13} (m/z 182/183) ions in Fig. 2 (lower part) indicate the presence of the 6-methylheptadecane.

Heptadecane is widely abundant in nature and is the major *n*-hydrocarbon component in marine benthic algae and cyanobacteria [36-39]. 1-Heptadecene is considerably less abundant in nature; it has been noted in the green microalga *Chlorella vulgaris* [40], in some marine macrophytic algae [41], in the roots of the Chinese plant *Changium smyrnioides* [42], in the roots of the plant species *Centaurea scabiosa* [43], in floral oils of cacao [44], among volatile compounds of seed germination of Canadian thistle *Cirsium arvense* [45], in young leaves and green fruits of Japanese pepper [46], in essential oil from the Chinese traditional drug Haifenteng attributed to Piper [47], and in kerogen and coal [48]. 1-Heptadecene was also isolated from the marine polychaete *Arenicola marina* [49], the ascidian *Halocynthia roretzi*, and the sea urchin *Strongylocentrotus intermedius* [50]. 8-Heptadecene was isolated from gamma-irradiated shrimp [51], found among hydrocarbons of irradiated fish and Crustaceans [52], in foods [53], in virgin olive oils [54],

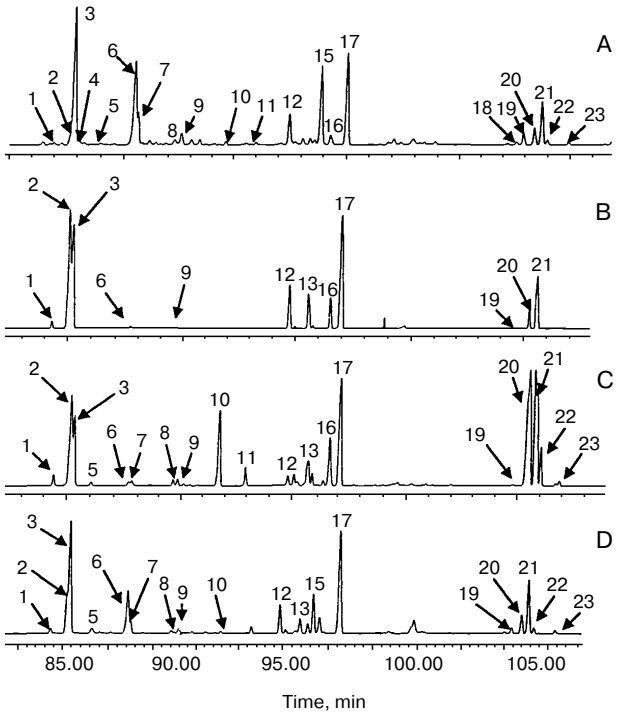

Fig. 1. GC/MS chromatogram of hydrocarbons and fatty acids of the soil cyanobacterium *Microcoleus vaginatus*. Separation of hydrocarbons and fatty acid methyl esters by gas chromatography using serially coupled capillary columns with stationary phases of different polarity. Full chromatogram (run up to 155 min). Parts of chromatogram running from 80 to 110 min. A, *M. vaginatus* sample from Hatzeva (Negev desert); B, *M. vaginatus* sample from Almog (Dead Sea basin); C, *M. vaginatus* sample from Almog and cultured in the laboratory; D, *M. vaginatus* sample from Sede Boqer (Negev desert). Peak identification refers to Table 1.

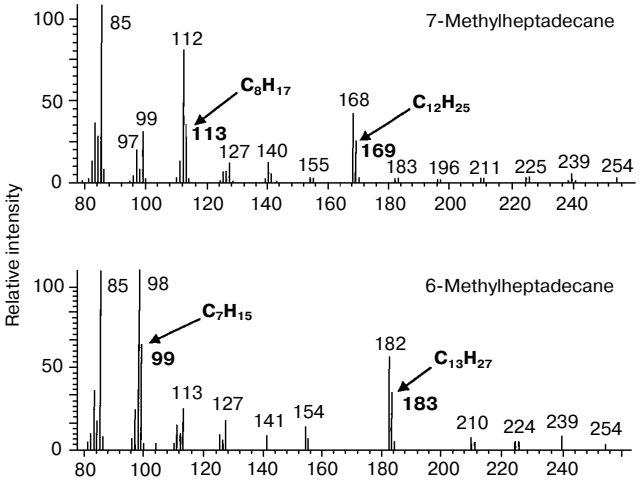

Fig. 2. Mass spectra of methylheptadecanes isolated from the soil cyanobacterium *Microcoleus vaginatus*.

Table 2. Comparative features of native and cultured samples of *Microcoleus vaginatus*

Number, compound	I(B)	II(C)	III	IV	V	VI(D)	VII(A)
2. 1-Heptadecene	21.53	14.40	8.09	6.73	4.21	1.70	0.28
3. Heptadecane	14.37	7.49	8.56	12.63	19.94	23.01	24.58
6. 7-Methylheptadecane	0.34	0.51	3.01	4.71	5.94	9.04	16.02
7. 6-Methylheptadecane		0.62	1.14	1.44	1.51	1.67	3.16
17. *n*-Hexadecanoic acid	19.85	15.26	16.34	17.87	19.09	18.33	13.48
Total of indicated compounds	56.09	38.28	37.14	43.38	50.69	53.75	57.52
Ratio							
1-Heptadecene/C16:0	1.08	0.94	0.49	0.38	0.20	0.09	0.02
Heptadecane/C16:0	0.72	0.49	0.52	0.71	1.04	1.25	1.82
Heptadecane/1-Heptadecene	0.67	0.52	1.04	1.88	4.74	13.53	87.78
7-Methylheptadecane/C16:0	0.02	0.03	0.18	0.26	0.31	0.49	1.19
6-Methylheptadecane/C16:0		0.04	0.07	0.08	0.08	0.09	0.23
7-Methyl-C_{17}/6-Methyl-C_{17}		0.82	2.64	3.27	3.93	5.41	5.07

and in organic matter collected in the Loire River estuary (France) [55].

Gelpi et al. [37] studied hydrocarbons of fossil microalgae including species belonging to the genus *Cyanophyta*: 7- and 8-methyl-derivatives of heptadecane are dominant (up to 38%), and 6-methylheptadecane does not exceed one percent of the total hydrocarbons. High concentrations of 7- and 8-methyl-C_{17} were discovered in the cyanobacterium *Nostoc muscorum* (16%), 4-methyl-C_{17} was detected in *Chlorogloea fritschii* (11.5%) [39], and a high level of 2-methyl-C_{17} (24.2%) was found in *Nostoc commune* [56]. 5-Methyl-C_{17} is the dominant isomer in a cyanobacterial mat from the Orakei Korako thermal basin [57]; 6- and 4-methyl-C_{17} isomers were found as minor components. Recently, Köster et al. [10] isolated a series of six methyl-C_{17} isomers including 2-, 3-, 4-, 5-, 6-, and 7-methyl-C_{17} from the cyanobacterium *Calothrix scopulorum*.

Variation of major hydrocarbons under UV-B irradiation. There is controversy as to the different major C_{17} hydrocarbons in the species *M. vaginatus*. We assume that this might be due to the intensity of UV irradiation. Thus, sample B (from the Dead Sea basin) contained minor amounts of 7-methyl-C_{17} (0.34%) and 21.53% of 1-heptadecene (Table 1 and Fig. 1). The composition of five major C_{17}-components is presented in Table 2. Figure 3 shows the variation of 1-heptadecene and heptadecane in natural and cultivated samples of *M. vaginatus*. The composition of other compounds is shown in Table 2.

It is known that photoautotrophic organisms require more exposure to visible light than the harmful solar UV irradiation [17]. The effect of UV-B (280-320 nm) irradiation on the synthesis of different lipids and mycosporine-like components in microalgae is well known [17, 58, 59]. Samples of the soil cyanobacterium *M. vaginatus* collected in the desert Negev and the Dead Sea basin are exposed to different UV radiation levels. According to Kudish et al. [60], the Dead Sea basin offers a unique site and has a low level of radiation since it is located at the lowest point on Earth (about 400 m below sea level), and the air above the Dead Sea is characterized by relatively high aerosol content due to the very high salt content in the Dead Sea. In contrast, the radiation level in the Negev desert is twice as high [60]. The considerable difference in the radiation level at the Dead Sea and in the Negev desert apparently exerts a major effect on the composition of hydrocarbons of the same species of the cyanobacterium, *M. vaginatus*, growing at the two locations.

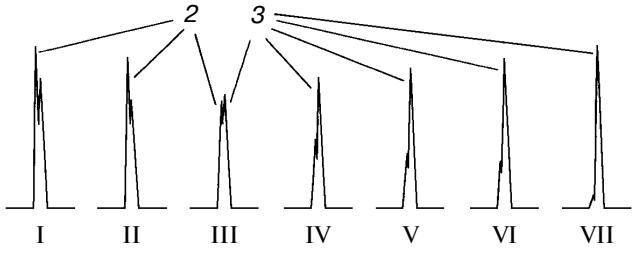

Fig. 3. Interrelation of two major hydrocarbons, 1-heptadecene (*2*) and heptadecane (*3*), in native and cultured cyanobacterium *M. vaginatus*. I, native sample from Almog (Dead Sea, B as indicated in Table 1); II-V, Almog samples cultivated during eight weeks; VI, Sede Boqer sample (Negev desert, D); VII, Hatzeva sample (Negev desert, A).

The impact of UV irradiation on lipids in algae has been reported [58, 61-64]. In some algal species, polyunsaturated fatty acid concentrations have been shown to decrease under UV-B, but can be restored to normal levels during periods of lower UV-B [63, 64].

Our results are in good agreement with the suggestions of previous researchers that heptadecane and its methyl-derivatives found in sediments are likely to be of cyanobacterial origin. In addition, 1- and 8-heptadecenes extend the range of hydrocarbons known to be synthesized by soil cyanobacteria, and they have the potential of being biomarkers of these microorganisms. For the first time, we show that the cyanobacterium *M. vaginatus* synthesizes 1-heptadecene in great amounts (up to 25% of total volatile compounds). This is probably the consequence of the unique climatic conditions in the Dead Sea basin.

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