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BIOGEOCHEMICAL FEATURES OF HYDROCARBONS IN CULTURED CYANOBACTERIA AND GREEN ALGAE FROM ANTARCTICA

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Abstract: Hydrocarbons in cultured cyanobacteria *(Lyngbya murrayi, Oscillatoria priestleyi, Phormidium foveolarum, Phormidium fragile, Phormidium frigidum, Phormidium laminosum, Phormidium priestleyi and Phormidium unicinatum*) and green algae (*Chlorella vulgaris, Chlorococcum* sp., *Cosmarium crenatum, Cosmarium speciosum, Cosmarium subcrenatum, Cosmarium* sp. and a species of Chlorococcales) from Antarctica were analyzed by the use of a gas chromatograph-mass spectrometer to clarify their geochemical features and discuss their biogeochemical significance. Short-chain ($<C_{20}$) *n*-alkanes [*n*-$C_{17:0}$ (total carbon numbers per molecule: number of double bonds)] and *n*-alkenes (*n*-$C_{17:1}$, *n*-$C_{17:2}$, *n*-$C_{18:1}$ and/or *n*-$C_{19:1}$) were found in some cyanobacteria and green algae, but branched alkenes [(4-methyl-$C_{18:1}$, 3-methyl-$C_{18:1}$, 3-methyl-$C_{18:2}$, 4-methyl-$C_{19:1}$, 3-methyl-$C_{19:1}$ and/or 3-methyl-$C_{19:2}$] were detected only in cyanobacteria (*L. murrayi, P. fragile* and/or *P. laminosum*). Phytadienes were the predominant hydrocarbons in a cyanobacterium (*P. frigidum*) and green algae (*C. vulgaris*, and 4 species of *Cosmarium*). The presence of long-chain ($>C_{19}$) *n*-alkanes and *n*-alkenes in some cyanobacteria and green algae strongly suggests that these organisms are important sources of long-chain ($>C_{19}$) hydrocarbons in sedimentary and soil environments of Antarctica. The abundance of alkenes in cyanobacteria (75.7–98.0%) and green algae (88.2–100%) is probably due to the cultured low incubation temperatures of cultures (10 or 15°C) and/or to adaptation to an extremely cold climate in Antarctica. The branched alkenes could be useful in chemotaxonomy of cyanobacteria.

1. Introduction

Hydrocarbons are widely distributed organic compounds in various modern and ancient environments on the planet, and are useful biomarkers to estimate sources, migration and maturation of organic matter in these environments. Antarctica is characterized by a paucity of vascular plants, except in the northern part of the Antarctic Peninsula where two few vascular plants are present. Thus, Antarctica is an excellent region for the study of microbial biomarkers. Interestingly, long-chain ($>C_{19}$) *n*-alkanes and/or *n*-alkenes are often major hydrocarbons in lake sediments and soils in the ice-free areas (oases), such as Syowa and McMurdo Oases of Antarctica, in

spite of the complete lack of vascular plants in these regions (MATSUMOTO *et al.*, 1979, 1990, 1993a; MATSUMOTO, 1993). Also, branched and normal alkenes are major hydrocarbons in cyanobacterial mats, which are widely distributed in lakes, ponds and meltwater streams in Antarctica, and are thought to be main sources of organic components in the continent (MATSUMOTO *et al.*, 1993b). However, specific source organisms of these hydrocarbons are not yet clear, because cyanobacterial mats are a mixture of various species of cyanobacteria and other microorganisms. Thus, the information of hydrocarbons in pure cultured organisms is very important to clarify specific source organisms in sedimentary and soil environments of antarctic oases. Here we report analytical results of hydrocarbons in cultured cyanobacteria and green algae from Antarctica to elucidate specific source organisms of these hydrocarbons in the oases, and discuss their biogeochemical significance.

2. Materials and Methods

Cyanobacteria and microalgae were collected from lakes, ponds, meltwater streams, soils and moss surfaces in the McMurdo Oasis, Syowa Oasis and King George Island, Antarctica (Fig. 1, Table 1). Cyanobacteria (*Lyngbya murrayi, Oscillatoria priestleyi, Phormidium foveolarum, Phormidium fragile, Phormidium frigidum, Phormidium laminosum, Phormidium priestleyi* and *Phormidium unicinatum*) and green algae (*Chlorella vulgaris, Chlorococcum* sp., *Cosmarium crenatum, Cosmarium speciosum, Cosmarium subcrenatum, Cosmarium* sp. and a species of Chlorococcales) were cultured at University of Canterbury (BG-11 medium for 85 days, 16-h light and 8-h dark cycle at 15°C, 100 μmol/m^{2}s), Shimane University (BBM agar medium for 30 days, 12-h light

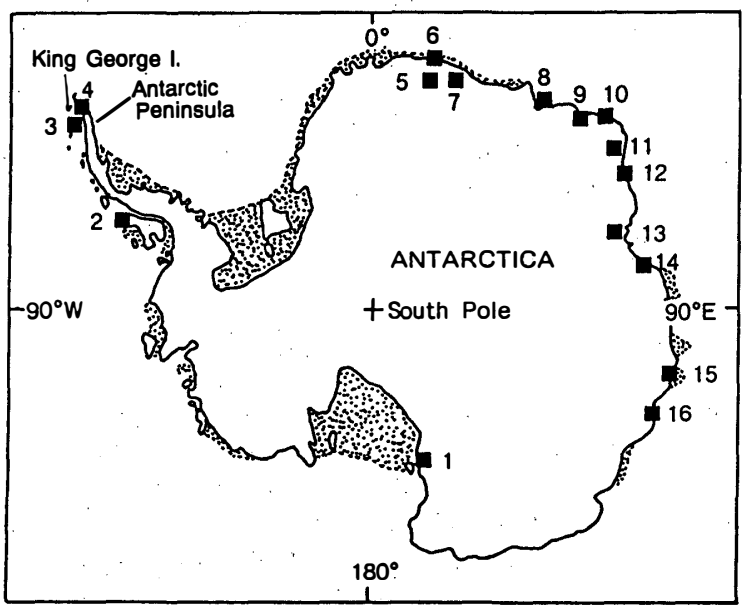

Fig. 1. Antarctic oases and sampling locations of cyanobacteria and green algae. Oasis. 1, McMurdo; 2, Alexander; 3, Bellingshausen; 4, Snow Hill; 5, Zimmermann; 6, Shirmacher; 7, Insel; 8, Syowa; 9, Molodezhnaya; 10, Thule; 11, Øygarden; 12, Stefansson; 13, Amery; 14, Vestfold; 15, Bunger; 16, Greason.

Table 1. Sources and habitats of cultured cyanobacteria and green algae from Antarctica.

Organism	Source	Habitat
Cyanobacteria		
Lyngbya murrayi (O-2)	McMurdo Oasis	ND
Oscillatoria priestleyi (O-17)	McMurdo Oasis	ND
Phormidium foveolarum (KG106)	King George I.	Moss epiphyte
Phormidium fragile (O-43)	McMurdo Oasis	ND
Phormidium frigidum (SO35)	Syowa Oasis	Moss epiphyte
Phormidium laminosum (O-23)	McMurdo Oasis	ND
Phormidium priestleyi (SO29)	Syowa Oasis	Moss epiphyte
Phormidium unicinatum (KG6)	King George I.	Edaphic
Green algae		
Chlorella vulgaria (SO26)	Syowa Oasis	Moss epiphyte
Chlorococcum sp. (SO25)	Syowa Oasis	Moss epiphyte
Cosmarium crenatum (KG76)	King George I.	Moss epiphyte
Cosmarium speciosum (KG104)	King George I.	Moss epiphyte
Cosmarium subcrenatum (KG82)	King George I.	Meltwater stream
Cosmarium sp. (KG86)	King George I.	Submerged moss surface in a pond
A species of Chlorococcales (KG23)	King George I.	Pool water

ND: No data.

and 12-h dark cycle at 15℃, *ca.* 50 μmol/m^{2}s, BISCHOFF and BOLD, 1963) and Science University of Tokyo (medium C for 30 days, 12-h light and 12-h dark cycle at 10℃, 150-180 μE/m^{2}s, ICHIMURA, 1971). The samples cultured at University of Canterbury were freeze-dried and air-transported to Otsuma Women's University. The samples cultured at Shimane University and Science University of Tokyo were kept frozen at −20℃ until analysis.

Analytical methods for hydrocarbons were essentially similar to those of previous studies (MATSUMOTO *et al.*, 1979, 1989, 1993b). Shortly, cultured organisms were saponified with 0.5 M potassium hydroxide in methanol (70℃, 4 h). Hydrocarbons were extracted with ethyl acetate after acidification. The ethyl acetate extracts were chromatographed on a silica gel column (160 mm×5 mm i.d., 100 mesh, 5% water). Hydrocarbons were eluted with hexane and analyzed by a JEOL JMS Automass 150 gas chromatograph-mass spectrometer (GC-MS) equipped with a fused silica capillary column (J & W Scientific, DB225 and/or DB5, 30 m×0.32 mm i.d., film thickness 0.25 μm). The column oven temperature for DB225 column was programmed from 60 to 100℃ at 20℃/min, from 100 to 240℃ at 5℃/min and then maintained at 240℃ for 10 min. The temperatures of the injection block, GC-MS interface and ion source were maintained at 270, 240 and 250℃, respectively. The ionization energy, filament current and detector gain were 70 eV, 0.30 mV and −0.65 kV, respectively. These samples were hydrogenated with hydrogen gas and platinum dioxide catalyst to determine the structure of alkenes, and analyzed again by the GC-MS system.

Identification of hydrocarbons was carried out by the elucidation of mass spectra, and the comparison of mass spectra with those of authentic standards and/or literature (*e.g.*, MATSUMOTO *et al.*, 1979, 1993b; PHILP, 1985; ROBINSON and EGLINTON, 1990; SHIEA *et al.*, 1990). Quantitation of hydrocarbons was carried out by the measurement of peak heights in gas chromatogram.

3. Results and Discussion

3.1. Identification

Capillary gas chromatograms (before and after hydrogenation) of the hydrocarbon fraction of a cyanobacterium (*Phormidium laminosum*) from the McMurdo Oasis, Antarctica are shown in Fig. 2. Before hydrogenation, *n*-$C_{17:0}$ (total carbon numbers per molecule: number of unsaturation) and alkenes, such as $C_{17:1}$, $C_{18:1}$, $C_{18:2}$, $C_{19:2}$ and $C_{20:2}$ were detected in the chromatogram. The structures of these alkenes were clarified after hydrogenation. The mass spectra of the resulting alkanes can be interpreted to be *n*-$C_{17:0}$, 3-methyl-$C_{18:0}$, 3-methyl-$C_{19:0}$ and phytane (*e.g.*, MATSUMOTO *et al.*, 1993b). Thus, the peaks before hydrogenation were identified to be *n*-$C_{17:0}$, *n*-$C_{17:1}$, 3-methyl-$C_{18:1}$, 3-methyl-$C_{18:2}$, 3-methyl-$C_{19:2}$ and phytadiene. Normal and branched alkenes in other samples were identified by a similar method. The analytical results of hydrocarbons in cyanobacteria and green algae are summarized in Tables 2 and 3, respectively.

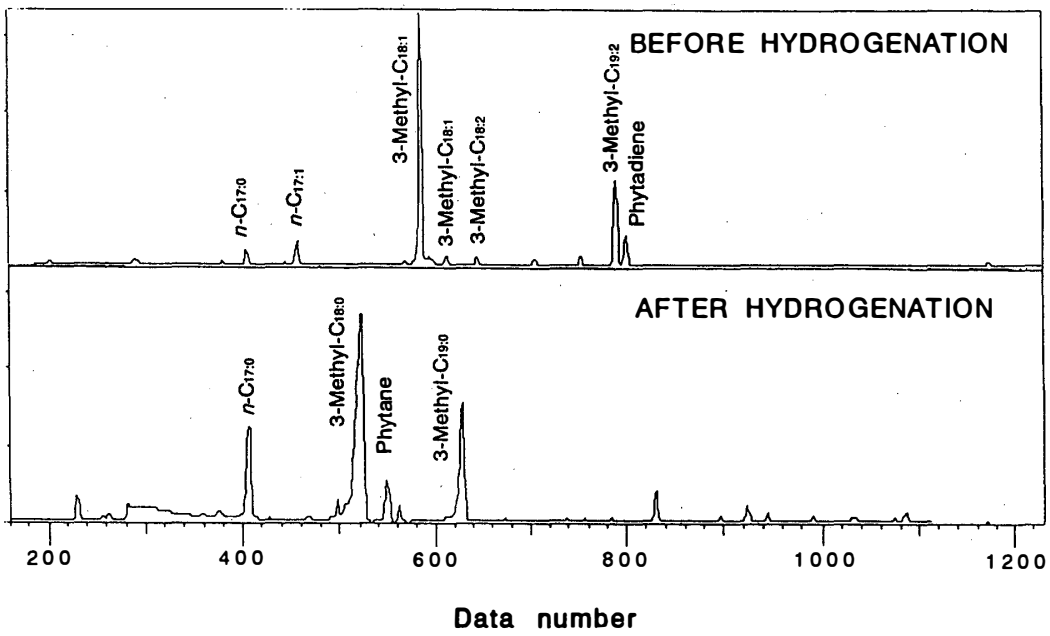

Fig. 2. Capillary gas chromatograms (before and after hydrogenation) of the hydrocarbon fraction of a cyanobacterium (Phormidium laminosum) *from King George Island, Antarctica.* $C_{m:n}$ *= Total carbon numbers per molecule: number of double bonds.*

Branched alkanoic acids (*iso*- and *anteiso*-) are only synthesized by bacteria (*e.g.*, O'LEARY, 1982), and are useful markers of bacterial contamination of cultured organisms. Very small amounts of branched alkanoic acids were detected in some cultured samples (YAMADA *et al.*, unpublished). Thus the contamination of bacteria in cultured organisms was negligible.

3.2. Biogeochemical features

Normal-alkanes (*e.g.*, *n*-$C_{17:0}$) and *n*-alkenes (*e.g.*, *n*-$C_{17:1}$, *n*-$C_{17:2}$, *n*-$C_{19:1}$) were found in some cyanobacteria, whereas branched-alkenes (*e.g.*, 4-methyl-$C_{18:1}$, 3-methyl-$C_{18:1}$, 3-methyl-$C_{18:2}$, 4-methyl-$C_{19:1}$, 3-methyl-$C_{19:1}$ and/or 3-methyl-$C_{19:2}$) were detected in

Table 2. Hydrocarbons found in cultured cyanobacteria from Antarctica.

	Lyngbya murrayl	*Oscillatoria priestleyi*	*Phormidium foveolarum*	*Phormidium fragile*	*Phormidium frigidum*	*Phormidium laminosum*	*Phormidium piestleyi*	*Phormidium unicinatum*
Compound (%)*1								
n-$C_{16:0}$	0.0	0.0	0.0	0.0	0.4	0.0	0.7	0.7
n-$C_{16:1}$	0.0	0.7	0.0	1.5	0.0	0.0	0.3	0.0
n-$C_{17:0}$	6.1	3.3	12.5	2.0	1.6	3.7	10.8	23.6
n-$C_{17:1}$	48.1	33.0	26.0	73.7	0.0	6.4	9.5	56.2
n-$C_{17:2}$	19.7	63.0	0.0	5.9	0.0	0.0	0.0	0.0
n-$C_{18:0}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-$C_{18:1}$	0.0	0.0	32.4	0.0	0.0	0.0	20.2	0.0
n-$C_{18:2}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4-Methyl-$C_{18:1}$	13.9	0.0	0.0	15.0	0.0	0.0	0.0	0.0
3-Methyl-$C_{18:1}$	0.0	0.0	0.0	0.0	0.0	60.4	0.0	0.0
3-Methyl-$C_{18:2}$	8.3	0.0	0.0	0.0	0.0	2.7	0.0	0.0
n-$C_{19:1}$	0.0	0.0	18.2	0.6	24.2	0.0	19.3	0.0
4-Methyl-$C_{19:1}$	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0
3-Methyl-$C_{19:1}$	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0
3-Methyl-$C_{19:2}$	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
n-$C_{20:1}$	0.0	0.0	4.0	0.0	0.0	0.0	15.3	0.0
Phytadienes	3.9	0.0	6.9	0.0	73.8	6.8	18.6	0.0
n-$C_{21:1}$	0.0	0.0	0.0	0.0	0.0	0.0	2.2	19.5
n-$C_{21:2}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-$C_{22:1}$	0.0	0.0	0.0	0.0	0.0	0.0	3.1	0.0
n-$C_{25:0}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Composition (%)								
n-Alkanes	6.1	3.3	12.5	2.0	2.0	3.7	11.5	24.3
n-Alkenes	67.8	96.7	80.6	81.7	24.2	6.4	69.9	75.7
Branched-alkanes	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Branched-alkenes	22.2	0.0	0.0	16.3	0.0	83.1	0.0	0.0
Phytadienes	3.9	0.0	6.9	0.0	73.8	6.8	18.6	0.0
Alkenes (%)*2	93.9	96.7	87.5	98.0	98.0	96.3	88.5	75.7

*1 $C_{m:n}$ = Total carbon numbers per molecule: number of double bonds.

*2 Normal and branched-alkenes, and phytadienes.

Lyngbya murrayi, Phormidium fragile and/or *P. laminosum* (Table 2). Also, phytadienes were found in the cyanobacteria except for *Oscillatoria priestleyi.* Interestingly, 3-methyl-$C_{18:1}$ was the most predominant hydrocarbon in *Phormidium laminosum*, while phytadienes were most abundant in *Phormidium frigidum* (Table 2).

Normal alkanes (*e.g.*, *n*-$C_{17:0}$) and *n*-alkenes (*e.g.*, *n*-$C_{17:1}$, *n*-$C_{17:2}$, *n*-$C_{18:2}$) and/or phytadienes were present in green algae (Table 3). Normal-$C_{17:1}$ was the most predominant hydrocarbon in *Chlorococcum* sp. and a species of Chlorococcales, while phytadienes were the most abundant in *Chlorella vulgaris, Chlorococcum* sp., *Cosmarium crenatum, C. speciosum, C. subcrenatum* and *Cosmarium* sp.

Of special interest is the occurrence of long-chain *n*-alkanes and *n*-alkenes in cyanobacteria (*Lyngbya murrayi* and *Phormidium priestleyi*) and the green alga (*Chlorococcum* sp.), although their percentages were low (Tables 2 and 3). These results strongly suggest that these cyanobacteria and the green alga are important sources of

Table 3. Hydrocarbons found in cultured green algae from Antarctica.

	Chlorella vulgaris	*Chlorococcum* sp. (SO25)	*Cosmarium crenatum*	*Cosmarium speciosum*	*Cosmarium subcrenatum*	*Cosmarium* sp. (KG86)	A species of Chlorococcales
Compound (%)[*2]							
n-$C_{16:0}$	0.0	3.7	3.1	0.0	2.0	4.7	4.9
n-$C_{16:1}$	0.0	0.0	0.0	0.0	0.0	3.5	0.0
n-$C_{17:0}$	1.4	4.1	4.6	0.0	3.0	7.0	6.3
n-$C_{17:1}$	7.5	78.6	0.0	0.0	0.0	0.0	74.2
n-$C_{17:2}$	11.6	0.0	0.0	0.0	0.0	0.0	0.0
n-$C_{18:1}$	0.4	0.0	0.0	0.0	0.0	0.0	0.0
n-$C_{18:2}$	0.3	6.6	0.0	0.0	0.0	0.0	14.6
4-Methyl-$C_{18:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3-Methyl-$C_{18:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3-Methyl-$C_{18:2}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-$C_{19:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4-Methyl-$C_{19:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3-Methyl-$C_{19:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3-Methyl-$C_{19:2}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-$C_{20:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phytadienes	78.8	3.0	92.3	100.0	95.0	84.8	0.0
n-$C_{21:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
n-$C_{21:2}$	0.0	4.0	0.0	0.0	0.0	0.0	0.0
n-$C_{22:1}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Composition (%)							
n-Alkanes	1.4	7.8	7.7	0.0	5.0	11.7	11.2
n-Alkenes	19.8	89.2	0.0	0.0	0.0	3.5	88.8
Branched-alkanes	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Branched-alkenes	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phytadienes	78.8	3.0	92.3	100.0	95.0	84.8	0.0
Alkenes (%)	98.6	92.2	92.3	100.0	95.0	88.3	88.8

[*1]Small percentages of hydrocarbons other than phytadienes may present because the amount of analyzed sample was very small.

[*2]Abbreviations are the same as in Table 2.

long-chain *n*-alkanes and *n*-alkenes in antarctic oases. Also, it is very likely that other species of cyanobacteria and green algae are significant sources of long-chain hydrocarbons in the oases.

It is known that methyl-branches at the middle position of alkanes in the carbon chain length ranging from C_{15} to C_{20} are produced only by cyanobacteria (SHIEA *et al.*, 1990). Various mono- and dimethyl-alkanes were found in hot spring microbial mats (ROBINSON and EGLINTON, 1990; SHIEA *et al.*, 1990). Although no methyl-alkanes were detected in the cultured cyanobacteria, methyl-branched alkenes were detected in 3 species of cyanobacteria (Table 2), and cyanobacterial mats from the McMurdo Oasis (MATSUMOTO *et al.*, 1993b).

The total abundances of alkenes (*n*- and branched alkenes, and phytadienes) in cyanobacteria and green algae were very high, ranging from 75.7 to 98.0% and from 88.2 to 100%, respectively (Tables 2 and 3). These results are similar to those of cyanobacterial mats from the McMurdo Oasis (MATSUMOTO *et al.*, unpublished). The

degree of unsaturation of fatty acids in plankton and microalgae increases with decrease of ambient temperatures (HOLTON *et al.*, 1964; JEFFRIES *et al.*, 1970). Hence, the abundance of alkenes in these cultured cyanobacteria and green algae is probably due to the low incubation temperatures (10 or 15°C) of cultures and/or attributed to adaptation to an extremely cold climate in Antarctica.

No branched alkanes and alkenes except for phytadienes were detected in all green algae and some cyanobacteria (*Oscillatoria priestleyi, Phormidium foveolarum, P. frigidum*). Thus, these branched alkenes may be useful in chemotaxonomy of microalgae and cyanobacteria.

4. Conclusions

Biogeochemical features of hydrocarbons in the cultured 8 species of cyanobacteria and 7 species of green algae from Antarctica are summarized as follows:

(1) Short-chain *n*-alkanes (*e.g.*, *n*-$C_{17:0}$) and *n*-alkenes (*n*-$C_{17:1}$, *n*-$C_{17:2}$, *n*-$C_{18:1}$ and/or *n*-$C_{19:1}$) were found in some cyanobacteria and green algae, but branched alkenes (4-methyl-$C_{18:1}$, 3-methyl-$C_{18:1}$, 3-methyl-$C_{18:2}$, 4-methyl-$C_{19:1}$, 3-methyl-$C_{19:1}$ and/or 3-methyl-$C_{19:2}$) were detected only in cyanobacteria (*Lyngbya murrayi, Phormidium fragile* and *P. laminosum*).

(2) Phytadienes were the predominant hydrocarbons in cyanobacterium (*Phormidium frigidum*) and green algae (*Chlorella vulgaris* and 4 species of *Cosmarium* sp.).

(3) The presence of long-chain *n*-alkanes and *n*-alkenes in some cyanobacteria and green algae strongly suggests that cyanobacteria and green algae are important sources of long-chain hydrocarbon in antarctic oases.

(4) The abundance of alkenes in cyanobacteria (75.7–98.0%) and green algae (88.2–100%) is probably due to the low incubation temperatures of cultures (10 or 15 °C) and/or to adaptation to an extremely cold climate in Antarctica.

(5) Branched alkenes could be useful in chemotaxonomy of cyanobacteria.

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