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BIOGEOCHEMICAL FEATURES OF HYDROCARBONS IN CYANOBACTERIAL MATS FROM THE MCMURDO DRY VALLEYS, ANTARCTICA

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Abstract: Hydrocarbons in 9 cyanobacterial mat samples from the Labyrinth ponds and Lake Canopus in the Wright Valley of the McMurdo Dry Valleys in southern Victoria Land, Antarctica were studied to clarify their features in relation to source organisms and biogeochemical significance. The major hydrocarbons in cyanobacterial mats were all alkenes, such as n-$C_{17:1}$ (total carbon number per molecule:number of double bonds), 3-, 4- and 5-methyl-$C_{18:1}$, n-$C_{18:1}$, 3-methyl-$C_{19:1}$, 5-methyl-$C_{20:1}$ and/or hop-22(29)-ene. These hydrocarbons are mainly produced by cyanobacteria, such as *Phormidium* spp. which are major organisms of the cyanobacterial mats. The predominance of alkenes is probably ascribed to the influence of extremely low air temperatures in Antarctica. Cyanobacterial mats may be important sources of organic components in lakes and ponds in the McMurdo Dry Valleys, and other inland aquatic environments in Antarctica.

1. Introduction

There are 16 major ice-free areas in the coastal regions of Antarctica, although more than 97% of the area of Antarctica is covered with thick ice sheet approximately 2450 m in thickness. A large number of lakes and ponds are distributed in the ice-free areas. The McMurdo Dry Valleys extending 2500 km^2 are the largest ice-free area in Antarctica. Cyanobacterial mats are widely distributed in lakes, ponds and meltwater streams in the valley depressions, and thus can be expected to be important sources of organic components in aquatic and soil environments of the valleys (MATSUMOTO *et al.*, 1990a, b).

Hydrocarbons are widely distributed in natural environments, and are used as biomarkers of sources, maturation and alteration of organic components in biogeochemical studies. However, little is known on the features of organic components, including hydrocarbons in cyanobacterial mats. MATSUMOTO *et al.* (1979) preliminarily reported hydrocarbons, fatty acids and phenolcarboxylic acids in cyanobacterial mats from the McMurdo Dry Valleys. The major hydrocarbons in the cyanobacterial

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mats are alkenes such as $C_{17:1}$ (total carbon number per molecule:number of double bonds), $C_{18:1}$ and $C_{19:1}$, but their structures are not yet clear. Here we report features of hydrocarbons, including normal and methyl-branched alkenes and alkanes as well as hop-22(29)-ene in cyanobacterial mats of the Labyrinth ponds and Lake Canopus from the Wright Valley of the McMurdo Dry Valleys in southern Victoria Land, Antarctica, and discuss upon source organisms and their biogeochemical significance. Also, microbial species in cyanobacterial mats will be given.

2. Materials and Methods

2.1. Sampling sites and samples

Generally, the surface of cyanobacterial mats is orange or brown in coloration which may be due to strong UV radiation in this region. During the austral summers 1976–1986, 9 cyanobacterial mat samples were collected from the shores of ponds in the Labyrinth and Lake Canopus in the Wright Valley of the McMurdo Dry Valleys (Fig. 1). These samples are comprised of living and dead cells of microorganisms, containing coarse and fine sands. These samples were kept frozen at –20°C until analyzed in 1991.

2.2. Analytical methods

Analytical methods of hydrocarbons are reported elsewhere, except for hydrolyzed conditions (Matsumoto *et al.*, 1979, 1989). Wet cyanobacterial mat samples (1–8 g) were hydrolyzed with 0.5 M potassium hydroxide in methanol (70°C, 4 h), and extract-

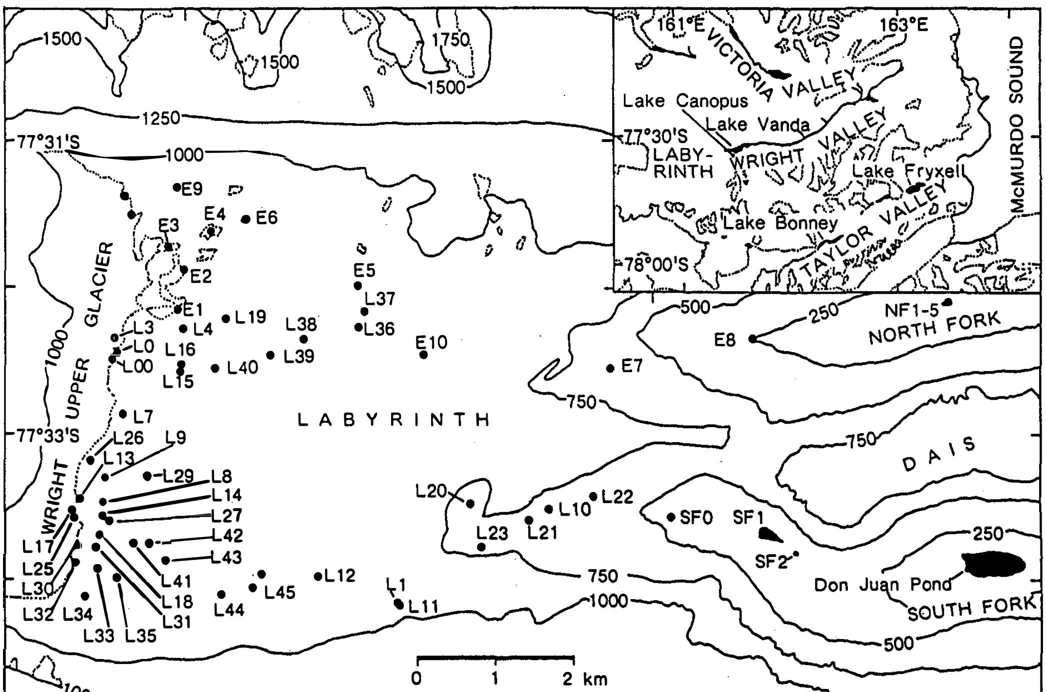

Fig. 1. Sampling locations of cyanobacterial mats from the Wright Valley of the McMurdo Dry Valleys in southern Victoria Land, Antarctica.

ed with ethyl acetate after acidification. The ethyl acetate extracts were chromatographed on a silica gel column (100 mesh, 160 × 5 mm i.d., 5% water). Hydrocarbons were eluted with 2 column volumes of hexane. Selected samples were hydrogenated with hydrogen gas with platinum dioxide catalyzer (MATSUMOTO *et al.*, 1989).

Hydrocarbons were analyzed by the use of a JEOL JMS-Automass 150 gas chromatograph-mass spectrometer (GC-MS), Shimadzu QP2000 GC-MS or Hewlett-Packard HP5971A GC-MS, equipped with a fused silica capillary column (J&W Sci. DB-5, 30 m × 0.32 mm i.d., film thickness 0.25 μm). The column temperature was programmed from 70 to 120°C at 25°C/min, and then from 120 to 300°C at 6°C/min. The temperatures of injection block and ion source were maintained at 320, and 180 or 250°C, respectively. The identification of hydrocarbons was made by the comparison of retention sequences and mass spectra with those of authentic compounds and published literature (MATSUMOTO *et al.*, 1979; SHIEA *et al.*, 1990; ROBINSON and EGLINTON, 1990).

Microorganisms in cyanobacterial mat communities were identified by microscopic observations (× 1000).

3. Results

Capillary gas chromatogram of the hydrocarbon fraction obtained from a cyanobacterial mat sample (L1 Pond) from the McMurdo Dry Valleys is shown in Fig. 2 (top). Alkenes and alkanes ranging from C_{17} to C_{21} were found in the chromatogram, with the major hydrocarbons of $C_{17:1}$, $C_{18:1}$, $C_{19:1}$ and $C_{20:2}$, and hop-22(29)-ene, although the branched positions were not clear. After hydrogenation, hop-22(29)-ene was reduced into 17ß(H), 21ß(H)-hopane. Also, the branched-$C_{18:1}$ alkenes were resolved into major three peaks (Fig. 2, bottom). The mass spectra of these peaks had intense peaks at M-57 (*m/z* 197), M-43 (*m/z* 211) and M-29 (*m/z* 225) due to alpha-cleavage of the alkanes, and thus were identified to be 5-, 4- and 3-methylheptadecanes, respectively (Fig. 3). In the similar manner, 3-methyloctadecane and 5-methylnonadecane were identified in the chromatogram (Fig. 2, bottom). Thus, the alkenes found in the chromatogram before hydrogenation were identified to be *n*-$C_{17:1}$, *n*-$C_{18:1}$, 3-, 4- and 5-methyl-$C_{18:1}$, *n*-$C_{19:1}$, 3-methyl-$C_{19:1}$, 5-methyl-$C_{20:2}$ and *n*-$C_{21:2}$.

The analytical results of hydrocarbons are summarized in Table 1. Alkanes and alkenes ranging from C_{16} to C_{21} and hop-22(29)-ene were found in cyanobacterial mat samples. The major hydrocarbons were all alkenes, such as *n*-$C_{17:1}$, *n*-$C_{18:1}$, 2-, 3- and 4-methyl-$C_{18:1}$, b-$C_{19:1}$, b-$C_{20:2}$ and/or hop-22(29)-ene. The predominance of alkenes in these cyanobacterial mats is generally similar to those found in the previous study (MATSUMOTO *et al.*, 1979). 2,6-dimethylhexadecane was found in a cyanobacterial mat sample (E4 Pond).

Cyanobacterial mats from the McMurdo Dry Valleys were composed of *Phormidium laminosum*, *P. tenue* and *Phormidium* spp., with small amounts of other cyanobacteria, green alga (unidentified) and diatoms (*Hantzschia amphioxys* and *Navicula muticopsis*; Table 2). These cyanobacteria are commonly distributed in freshwater habitats of the McMurdo Dry Valleys, and other ice-free areas in Antarctica

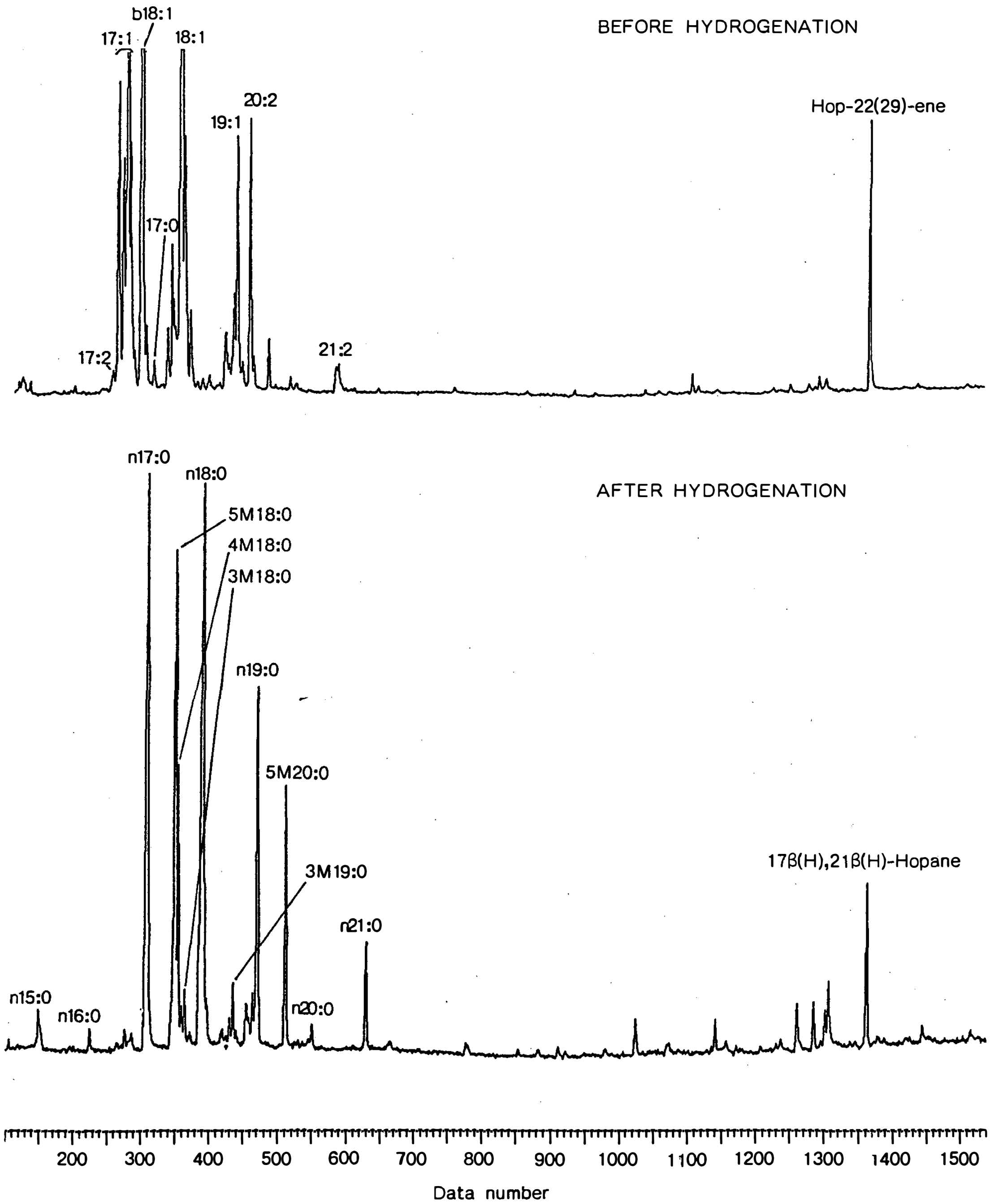

Fig. 2. Capillary gas chromatogram of the hydrocarbon fraction of a cyanobacterial mat sample from L1 Pond in the McMurdo Dry Valleys. n, b and nM are normal, branched and methyl-branched hydrocarbons, respectively. m:n = total carbon number per molecule:number of double bonds.

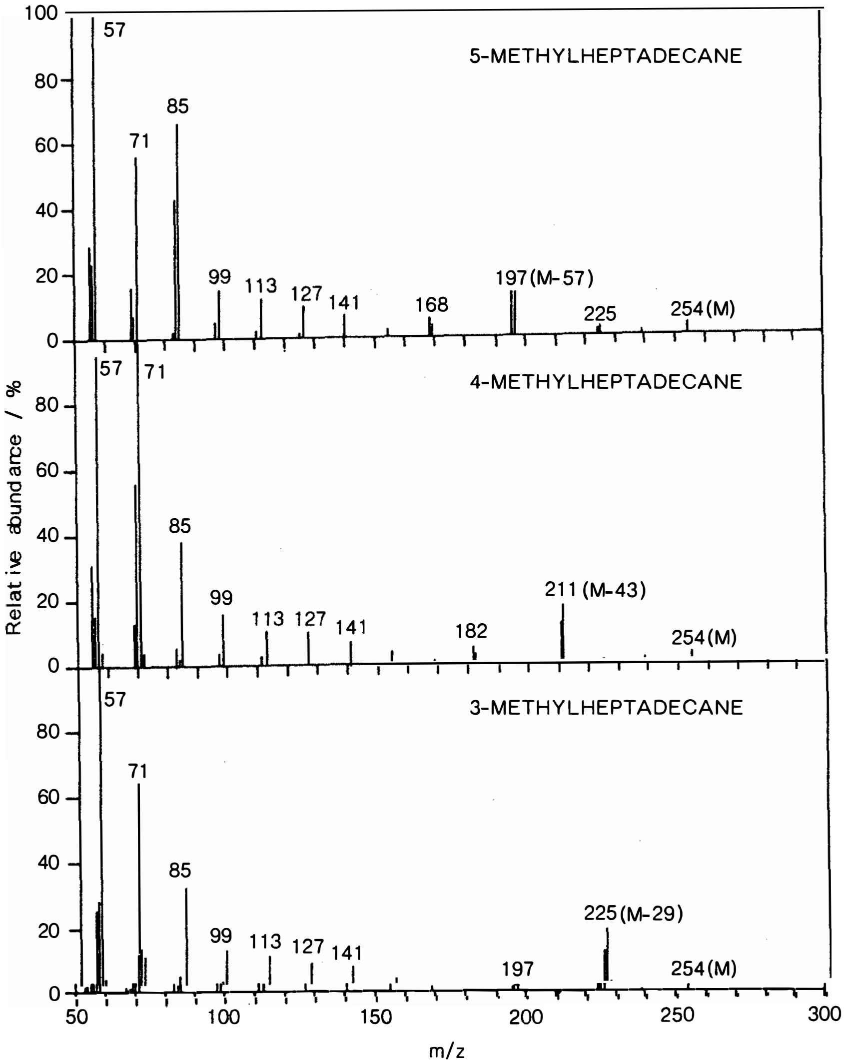

Fig. 3. The mass spectra of 5-, 4- and 3-methylheptadecanes of a cyanobacterial mat sample from L1 Pond in the McMurdo Dry Valleys.

(FRITSCH, 1912; BROADY, 1981).

4. Discussion

Cyanobacteria are the only microorganisms known to produce mid-chain branched monomethyl-alkanes in the C_{15}–C_{20} range, especially, 7- and 8-methylheptadecanes (SHIEA *et al.*, 1990). Various C_{18}–C_{20} monomethyl-alkanes and C_{19}–C_{20} dimethyl-alkanes are identified in Icelandic hot spring microbial mats (ROBINSON and EGLINTON, 1990). Also, SHIEA *et al.* (1990) reported mid-chain branched alkanes in the C_{16}–C_{18} range as well as several C_{19} dimethyl-alkenes and C_{20} multi-branched alkanes in hot spring cyanobacterial mats from the Yellowstone National Park, U.S.A.

No clear differences between major hydrocarbons and microbial communities were

Table 1. Hydrocarbons found in cyanobacterial mats from lake and ponds in the McMurdo Dry Valleys, Antarctica.

Hydrocarbon*	L1# Pond	L3# Pond	L4# Pond	L8# Pond	L9# Pond	E1# Pond	E4# Pond	SF1# Pond	Lake Canopus
n16:0	+	+	+	+	+	+	+	+	+
n17:0	+	+	+	+	+	+	+	+	+
n17:1	++	++	+++	+++	+++	++	++	+++	+
17:2	+	+–	+–	–	–	–	–	+	–
M17:0	+–	–	–	–	–	+–	+–	–	–
DM17:0	–	–	–	–	–	+–	+–	–	–
n18:0	–	–	–	+	+–	–	–	+–	+–
2,6DM18:0	–	–	–	–	–	–	++	–	–
n18:1	+++	+++	+–	+++	+	+	++	+	++
b18:1	+++	++	++	++	+++	+++	++	+++	+++
18:2	–	+	+–	–	–	–	–	–	+
19:1	+	++	–	+	+	+–	++	++	+–
20:1	–	–	–	–	–	–	–	–	+
20:2	+	–	–	++	–	+–	+++	+–	++
Long chain n-alkanes (>C_{19})	–	+–	+–	+	+	+	++	+–	+–
Long chain n-alkenes (>C_{19})	+	+–	+–	+–	–	+–	+–	+–	+–
Hop-22(29)-ene	++	+	+	+	+	+	+	+	+

* n, M, DM and b are normal, methyl-branched, dimethyl-branched, and branched hydrocarbons, respectively. $m:n$ = total carbon number per molecule : number of double bonds.
\# Unnamed ponds in the Labyrinth and South Fork (Fig. 1).
+++: Very abundant. ++: Abundant. +: Present. +–: Possibly present. –: Absent.

Table 2. Microorganisms identified in cyanobacterial mats from lake and ponds of the McMurdo Dry Valleys, Antarctica (by S. Ohtani).

Microorganisms	L1 Pond	L3 Pond	L4* Pond	L8* Pond	L9* Pond	E1 Pond	E4 Pond	SF1 Pond	Lake Canopus
Cyanophyceae									
Aphanothece castagnei	r	+	–	+	–	+	–	c	–
Lyngbya murrayi	–	rr	–	–	–	–	–	–	–
Lyngbya sp.	–	–	–	–	–	rr	–	–	–
Phormidium laminosum	cc#	–	–	–	–	c	cc#	–	cc#
Phormidium tenue	–	cc#	–	–	–	–	–	–	–
Phormidium sp.	–	–	–	cc#	cc#	–	–	cc	–
Bacillariophyceae									
Hantzschia amphioxys	–	–	–	–	–	–	–	–	r
Navicula muticopsis	–	–	–	–	–	–	–	rr	+
Others	–	–	–	–	+	–	–	–	–
Chlorophyceae									
Cocoid green alga	r	rr	r	–	–	+	rr	–	+

* Samples were considerably degraded.
cc: Very abundant. c: Abundant. +: Common. r: Rare. rr: Very rare.
\# Approximately 90% of the total cyanobacterial mat community.

observed in the cyanobacterial mats from the McMurdo Dry Valleys (Tables 1 and 2). This result suggests that the major hydrocarbon components of these cyanobacteria are similar to each other. Certain cyanobacteria probably produce 2,6-dimethylhexadecane which is a characteristic hydrocarbon in lakes and ponds of the McMurdo Dry Valleys (MATSUMOTO, 1989).

The predominant hydrocarbons in the cyanobacterial mats from the McMurdo Dry Valleys are all *n*- and methyl branched-alkenes (Table 1), and much different from those in hot spring cyanobacterial mats. It is known that the biosynthesis of *n*-alkenoic acids relative to *n*-alkanoic acids increases with decrease of environmental temperatures (*e.g.*, HOLTON *et al.*, 1964; JEFFRIES, 1970). Also, when culture temperatures were raised from 20 to 45°C, the $C_{19:1}/C_{19:0}$ hydrocarbon ratios decreased in *Cyanidium caldarium* strain M-8 (= *Galdieria sulphuraria*; NAGASHIMA *et al.*, personal communication). Hence, it is probable that the predominance of alkenes in the cyanobacterial mats of the McMurdo Dry Valleys is attributed to the influence of extremely low air temperatures.

Hop-22(29)-ene, and other hopenes, such as neohop-13(18)-ene, hop-17(21)-ene and fern-7-ene are widely distributed in various sediment samples in the world, including Antarctic marine sediments from the McMurdo Sound and Bransfield Strait (VENKATESAN, 1988). Hop-22(29)-ene is also found in Antarctic lake sediments (VOLKMAN *et al.*, 1986; MATSUMOTO *et al.*, 1989). Hopanoids are often essential components of prokaryotes (ROHMER *et al.*, 1984). Hop-22(29)-ene is, in particular, found in cyanobacteria (GELPI *et al.*, 1970). Consequently, hop-22(29)-ene found in the cyanobacterial mats is probably directly derived from cyanobacterial communities, such as *Phormidium* spp.

Cyanobacterial mats may be important sources of organic components in lakes and ponds in the McMurdo Dry Valleys and other inland aquatic environments, Antarctica. Biogeochemical and physiological studies of pure cultured Antarctic cyanobacterial mat communities should be fruitful.

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References

BROADY, P. A. (1981): Non-marine algae of Cape Bird, Ross Island and Taylor Valley, Victoria Land, Antarctica. Melbourne University Programme in Antarctic Studies. Report No. 37, 97 p.

FRITSCH, F. E. (1912): Freshwater algae. Natl Antarct. Exp. 1901–1904, Nat. Hist., **6**, Zool. Bot., 1–60.

GELPI, E., SCHNEIDER, H., MANN, J. and ORÓ, J. (1970): Hydrocarbons of geochemical significance in microscopic algae. Phytochemistry, **9**, 603–612.

HOLTON, R. W., BLECKER, H. H. and ONORE, M. (1964): Effect of growth temperature on the fatty acid composition of a blue-green alga. Phytochemistry, **3**, 595–602.

JEFFRIES, H. P. (1970): Seasonal composition of temperate plankton communities: Fatty acids. Limnol.

Oceanogr., **15**, 419–426.

MATSUMOTO, G. I. (1989): Biogeochemical study of organic substances in Antarctic lakes. Hydrobiologia, **172**, 265–289.

MATSUMOTO, G., TORII, T. and HANYA, T. (1979): Distribution of organic constituents in lake waters and sediments of the McMurdo Sound region in the Antarctic. Mem. Natl Inst. Polar Res., Spec. Issue, **13**, 103–120.

MATSUMOTO, G. I., WATANUKI, K. and TORII, T. (1989): Vertical distribution of organic constituents in an Antarctic lake: Lake Fryxell. Hydrobiologia, **172**, 291–303.

MATSUMOTO, G. I., AKIYAMA, M., WATANUKI, K. and TORII, T. (1990a): Unusual distributions of long-chain *n*-alkanes and *n*-alkenes in Antarctic soil. Org. Geochem., **15**, 403–412.

MATSUMOTO, G. I., HIRAI, A., HIROTA, K. and WATANUKI, K. (1990b): Organic geochemistry of the McMurdo Dry Valleys soil, Antarctica. Org. Geochem., **16**, 781–791.

ROBINSON, N. and EGLINTON, G. (1990): Lipid chemistry of Icelandic hot spring microbial mats. Org. Geochem., **15**, 291–298.

ROHMER, M., BOUVIER-NAVE, P. and OURISSON, G. (1984): Distribution of hopanoid triterpenes in prokaryotes. J. Gen. Microbiol., **130**, 1137–1150.

SHIEA, J., BRASSELL, S. C. and WARD, D. M. (1990): Mid-chain branched mono- and dimethyl alkanes in hot spring cyanobacterial mats: A direct biogenic source for branched alkanes in ancient sediments? Org. Geochem., **15**, 223–231.

VENKATESAN, M. I. (1988): Diploptene in Antarctic sediments. Geochim. Cosmochim. Acta, **52**, 217–222.

VOLKMAN, J. K., ALLEN, D. I., STEVENSON, P. L. and BURTON, H. R. (1986): Bacterial and algal hydrocarbons in sediments from a saline Antarctic lake, Ace Lake. Org. Geochem., **10**, 671–681.

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