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HYDROCARBONS AND FATTY ACIDS IN TWO STRAINS OF THE HOT SPRING ALGA *CYANIDIUM CALDARIUM*

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Key Word Index—*Cyanidium caldarium*; hot spring alga; chemotaxonomy; hydrocarbon; fatty acid.

Abstract—Normal alkanes (n-C_{14}–n-C_{30}) and a diene ($C_{19:2}$) were found in *Cyanidium caldarium* RK-1 type with predominance of n-C_{17} (46.3%), while *Cyanidium* M-8 type contained n-alkanes (n-C_{15}–n-C_{25}) and alkenes ($C_{19:1}$, $C_{21:1}$) with an abundance of $C_{19:1}$ (43.4%). Normal alkanoic acids of odd-carbon numbers, n-C_{15} (8.6%) and n-C_{17} (10.5%), as well as of even-carbon numbers, n-C_{16} (12.7%) and n-C_{18} (9.8%), and $C_{18:2}$ alkenoic acid (53.1%) were found in *Cyanidium* RK-1, while *Cyanidium* M-8 mainly contained n-C_{16} (33.5%) alkanoic acids, $C_{18:1}$ (33.3%) and $C_{18:2}$ (23.7%) alkenoic acids. The acid $C_{18:3}$ was present only in *Cyanidium* M-8. These results indicate a considerable difference between the two strains of *Cyanidium caldarium*, and *Cyanidium* RK-1 type may be unique among microalgae because it has a significant amount of odd-carbon numbered n-alkanoic acids.

INTRODUCTION

The unicellular alga *Cyanidium caldarium* (Tilden) Geitler is widely distributed in acidic hot springs throughout the world [1]. This is a blue–green, eukaryotic alga, the systematic position of which is unclear [2]. Two strains of *C. caldarium* have been found in acidic fumaroles or hot springs in Italy [3], North and Central America [4] and Japan [5]: RK-1 type [5] (corresponding to form A [3]), 2.3 μm (av.) in cell diameter, multiplying by four endospore formation, and with a one ovule chloroplast; and M-8 type [5] (corresponding to form B [3]) 4.1 μm (av.) in cell diameter, multiplying by 4, 8 and 16 endospore formation, and with a multi-lobed chloroplast and a single vacuole. *Cyanidium* M-8 type (form B) may in fact belong to a different genus from *Cyanidium* [4, 5], and both algae may be closely related to red algae [5–7].

Cyanidium RK-1 and M-8 strains differ in carbohydrate content [6, 7]. Lipids such as fatty acids of *C. caldarium* have been examined [8–12] but it is not clear which of the two strains these results relate to. Boenzi *et al.* [13] showed that forms A and B were similar in fatty acid composition, apart from the absence of linolenic acid from form A. No one has, however, reported on the hydrocarbons in *Cyanidium*.

In previous papers [14, 15], we reported that *Cyanidium* RK-1 type and M-8 type differed in their 2-hydroxy- and 3-hydroxy-acid components. This paper deals with hydrocarbon and fatty acid compositions of the two types.

RESULTS AND DISCUSSION

Hydrocarbons and fatty acids extracted from *Cyanidium* cultured at 38° were analysed by GC/MS. The retention times and mass spectra of hydrocarbons and fatty acid methyl esters were compared with those of authentic standards. *Cyanidium* RK-1 type contains n-C_{14} to n-C_{30} n-alkanes and a diene ($C_{19:2}$) with the n-C_{17} alkane as major constituent (46.3%). Normal alkanes (n-C_{15}–n-C_{25}) and alkenes ($C_{19:1}$, $C_{21:1}$, $C_{23:1}$) are found in *Cyanidium* M-8 type with an abundance of $C_{19:1}$ (43.4%) (Table 1). No C_{18} branched hydrocarbon occurs in *Cyanidium* RK-1, but it is possible that small amounts of branched hydrocarbons occur in both types. The ratio of n-alkanes to alkenes is 3.9 in RK-1 type and 0.31 in M-8 type. The ratio of odd-to-even hydrocarbons is 3.7 in RK-1 type and 29 in M-8 type. These data indicate that two types of *Cyanidium* are very different in hydrocarbon content.

Although the conditions of culture [16] and the physiological state [17] can affect the hydrocarbon composition in some green algae, Weete [18] has shown that the hydrocarbon pattern in algae is related to taxonomy. Most algae have n-C_{17} as the major hydrocarbon, but all members of the brown algae examined have n-C_{15} as principal alkane. Some blue–green algae [19, 20] have n-C_{17}, $C_{19:1}$ or branched C_{18}, and certain green algae [19] have n-C_{17}, unsaturated C_{17}, C_{23} or unsaturated C_{27}, and red algae have n-C_{17} as major hydrocarbons. From our data, it is clear that *Cyanidium* RK-1 type does not fit into any of these classes. However *C. caldarium* M-8 type containing $C_{19:1}$ resembles blue–green algae such as *Coccochloris elabens* and *Agmenellum quadruplicatum* [20].

Fatty acid compositions of the two *Cyanidium* strains are illustrated in Table 2. Mass spectra of n-alkanoic acid methyl esters are characterized by the intense ions at m/z 74 (base peak) $[MeOC(OH)CH_2]^+$,

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Table 1. Hydrocarbons found in *Cyanidium caldarium* RK-1 and M-8 types

	Composition (%)	
	Cyanidium RK-1	*Cyanidium* M-8
n-Alkane		
C_{14}	0.1	—
C_{15}	1.2	0.9
C_{16}	4.2	—
C_{17}	46.3	8.7
C_{18}	9.8	0.7
C_{19}	1.9	4.0
C_{20}	0.3	0.3
C_{21}	0.6	1.4
C_{22}	0.6	0.6
C_{23}	3.4	2.5
C_{24}	3.6	1.7
C_{25}	3.4	2.6
C_{26}	1.9	—
C_{27}	1.0	—
C_{28}	0.6	—
C_{29}	0.4	—
C_{30}	0.3	—
Alkene*		
$C_{17:1}$	—	3.3
$C_{19:1}$	—	43.4
$C_{19:2}$	20.4	—
$C_{21:1}$	—	19.6
$C_{23:1}$	—	7.2
$C_{25:1}$	—	3.1
n-Alkane/alkene	3.9	0.31
Odd/even†	3.7	29
Total concn (mg/g)	0.28	0.022

*$C_{m:n}$: m and n are carbon chain length and the number of unsaturation, respectively.

†Ratios of odd-to-even carbon numbered hydrocarbons.

—, Peak area was less than twice that of the blank.

Table 2. Fatty acids found in *Cyanidium caldarium* RK-1 and M-8 types

	Composition (%)	
	Cyanidium RK-1	*Cyanidium* M-8
n-Alkanoic		
C_{13}	0.1	—
C_{14}	0.1	0.3
C_{15}	8.6	0.4
C_{16}	12.7	33.5
C_{17}	10.5	0.2
C_{18}	9.8	1.7
C_{19}	0.9	—
C_{20}	0.1	—
Alkenoic		
$C_{16:n}$	—	—
$C_{18:n}$*	57.2	63.9
n-Alkanoic/alkenoic	0.75	0.56
Even/odd†	4.0	170
Total concn (mg/g)	11	6.5

*The further analysis by GC packed with 10% DEGS indicates that *Cyanidium* RK-1 contains $C_{18:1}$ (4.8%), $C_{18:2}$ (53.1%) and $C_{18:3}$ (0.0%), and *Cyanidium* M-8 contains $C_{18:1}$ (33.3%), $C_{18:2}$ (23.7%) and $C_{18:3}$ (7.3%) (see Experimental).

†Ratios of even-to-odd carbon numbered fatty acids.

—, Peak area was less than twice that of the blank.

87 $[MeOC(OH)CHCH_2]^+$, 101 $[MeOCO(CH_2)_3]^+$, 143 $[MeOCO(CH_2)_6]^+$, 157, 171, 185, etc., $[MeOCO(CH_2)_{7,8,9}]^+$ etc., $[M-43]^+$, $[M-29]^+$, and $[M-31]^+$ (M−OMe) [21]. The relative abundances of major ions for methylpentadecanoate and methylheptadecanoate from *Cyanidium* RK-1 were m/z 74 (100 and 100), 87 (59 and 64), 143 (12 and 14), $[M-43]^+$ (7 and 8), $[M-31]^+$ (5 and 4), and $[M]^+ = 256$ and $[M]^+ = 284$ (6 and 9), respectively. Alkenoic acids were also assigned by their retention times and mass spectra; no branched fatty acids were found.

It is unexpected that *Cyanidium* RK-1 contains the odd-carbon numbered *n*-alkanoic acids *n*-C_{15} (8.6%) and *n*-C_{17} (10.5%) in significant amounts, in addition to even-carbon numbered *n*-C_{16} (12.7%) and *n*-C_{18} (9.8%). Odd-carbon numbered acids have been already reported in small quantities (up to 2.4% of each acid) in *Cyanidium*, the types of which were not specified [8, 11]. However, they were not found in *Cyanidium* form A [13] corresponding to RK-1 type. $C_{18:2}$ alkenoic acid (linoleic acid) is the most abundant (53.1%), and no $C_{18:3}$ (linolenic acid) was found in *Cyanidium* RK-1 nor in *Cyanidium* from A cultured at 20° [13]. In *Cyanidium* M-8, *n*-C_{16} (33.5%) is the main *n*-alkanoic acid and odd-carbon numbered acids are almost negligible; $C_{18:1}$ (33.3%) and $C_{18:2}$ (23.7%) are predominant, and $C_{18:3}$ (7.3%) is also present. The fatty acid components of *Cyanidium* M-8 are similar to those of *Cyanidium* reported previously [8, 10–12]. The ratio of *n*-alkanoic to alkenoic acids is 0.75 in RK-1 type and 0.56 in M-8 type. The ratio of even-to-odd carbon numbered acids is 4.0 in RK-1 type and 170 in M-8 type. Thus the fatty acid compositions of the two *Cyanidium* strains are very different.

The concentrations of alkenoic acids, especially linolenic acid, are known to be affected by temperature in *Cyanidium* cultures [8, 10]. However, the fatty acid composition is stable when the algae are grown under controlled conditions, and in the present case we can compare the composition of *Cyanidium* with those of other algal samples. *Cyanidium* RK-1 type is unique because it contains significant amounts of odd-carbon numbered *n*-alkanoic acids (*n*-C_{15} and *n*-C_{17}) which have been reported only in small amounts or not at all in other algae [22, 23]. The fatty acid composition of *Cyanidium* M-8 type is quite similar to the primitive red alga *Porphyridium aeruginosa* which contains *n*-C_{16} (30%) and *n*-C_{18} (11%) alkanoic and $C_{18:1}$ (28%), $C_{18:2}$ (8%) and $C_{18:3}$ (3%) alkenoic acids [22, 23]. All these data support the idea [5, 7] that *Cyanidium* M-8 type is taxonomically distinct from *Cyanidium* RK-1 type and that *Cyanidium* M-8 is closely related to some primitive red algae.

EXPERIMENTAL

Cyanidium caldarium strain RK-1 and strain M-8 were obtained from acidic hot springs in Japan [5]. *Cyanidium* RK-1 was cultured axenically by aeration adding 5% CO_2 in the inorganic medium (pH 2.5) [24]. *Cyanidium* M-8 was also cultured in shaking flasks containing the same medium as for RK-1 strain except for addition of final 0.5% glucose. *Cyanidium* cells were grown at 38° under 2000 lux with fluorescent lamps for 1–3

weeks. They were collected and washed by centrifugation and kept frozen until analyses.

Analytical methods of hydrocarbons and fatty acids have been reported elsewhere, except for the separation of alkanes and alkenes [25, 26]. Briefly, algal samples were saponified with 0.5 M KOH–MeOH (80°, 2 hr) and centrifuged. Both supernatants and residues were extracted with EtOAc after acidification. The extracts were combined, concd and chromatographed on a silica gel column (180 × 5 mm i.d., 100 mesh, 5% H_2O). Hydrocarbons and fatty acids were eluted with hexane and C_6H_6–EtOAc (19:1), respectively. Hydrocarbons were further separated into alkane and alkene fractions on a silica gel column impregnated with 10% $AgNO_3$ (60 × 5 mm i.d., 100 mesh). Alkanes and alkenes were eluted with 3 column vols of hexane and 3 column vols of C_6H_6–EtOAc (1:1), respectively. Fatty acids were methylated with 14% BF_3–MeOH. Hydrocarbons and fatty acid methyl esters were analysed using a Shimadzu LKB 9000 EI GC/MS. A silanized glass column (2 m × 3 mm i.d.) was packed with 1% silicone OV-1 on 80–100 mesh Chromosorb W AW DMCS. The flow rate of He carrier gas was 30 ml/min. The column temp. was programmed from 100 to 285° at 8°/min. The injection block, molecular separator and ion source were maintained at 300, 310 and 330°, respectively. Mass fragmentograms and MS were obtained at ionization energies of 20 and 70 eV, respectively, with an accelerating voltage of 3.5 kV. For the analyses of alkenoic acids which could not be separated by GC as mentioned above, freeze-dried algal samples were methylated with 5% HCl–MeOH soln for 3 hr at 100°. The fatty acid methyl ester fractions extracted were analysed by GC on a glass column (1.5 × 3 mm i.d.) packed with 10% diethylene glycol succinate (DEGS); isothermal 180°; injector temp. 200°; N_2, 30 ml/min. The identification of hydrocarbons and fatty acids was carried out by the comparison of *RR*, and MS data with those of authentic standards. The quantification was performed by their peak areas. The analytical error was ± 12%.

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