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DETECTION OF A MICROBIAL CONSORTIUM, INCLUDING TYPE II METHANOTROPHS, BY USE OF PHOSPHOLIPID FATTY ACIDS IN AN AEROBIC HALOGENATED HYDROCARBON-DEGRADING SOIL COLUMN ENRICHED WITH NATURAL GAS

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Abstract—The phospholipid ester-linked normal and lipopolysaccharide layer hydroxy fatty acids from microbes in a natural gas (85% methane)-stimulated soil column capable of degrading halogenated hydrocarbons were analyzed in detail by capillary column GC-MS. Microbial biomass, calculated from phospholipid fatty acid (PLFA) concentrations to be 5.6×10^9 bacteria/g (dry weight), was greater in the hydrocarbon-degrading column than in either an azide-inhibited soil column or an untreated surface soil. Microbial community structure information, using GC-MS analysis of derivatized monounsaturated PLFA, indicated that the major component (16 to 28%) of the PLFA in the hydrocarbon-degrading column was the PLFA 18:1Δ10c. This novel PLFA has been reported as a major component in type II methanotrophs. The high relative proportions of C_{18} components relative to C_{16} fatty acids indicated that type II rather than type I methanotrophs were the most abundant microbial flora present in the active soil column. Fatty acids from other bacterial groups and microeukaryotes also were detected in the hydrocarbon-degrading soil column. Differences between the relative proportions of these metabolic groups of microorganisms were quantified and compared among the three soils analyzed. Based on these differences, the potential exists to use these methods to monitor shifts in microbial biomass and community structure in aquifers where indigenous bacteria are stimulated to biotransform pollutant compounds.

Keywords—Microbial consortium Halogenated hydrocarbons Methanotrophic bacteria Phospholipid fatty acids

INTRODUCTION

Bacteria capable of growth using methane as their sole carbon and energy source are known as methanotrophs [1,2]. The physiology and ecology of methanotrophic bacteria have been described in recent reviews [1–5]. Methane monooxygenase (MMO) is used by these bacteria to oxidize methane to methanol [1,2,4]. MMO can also produce primary or secondary alcohols upon oxidation of alkanes up to octane [6,7] and, in addition, can oxidize halogenated one-carbon compounds [6]. Halogenated one- and two-carbon compounds are commonly detected in contaminated subsurface environments and ground water [7]. Wilson and Wilson [8] used natural gas (77% methane, 10% ethane, 7% propane, remainder containing four to seven hydrocarbons) to enrich a population of soil bacteria capable of degrading trichloroethylene; which was degraded to carbon dioxide. It appears, therefore, that methanotrophic bacteria may be useful in the removal of halogenated one- or two-carbon compounds from contaminated environments by direct metabolism or by stimulation of

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other microbes. An estimate of their biomass in natural systems would thus be useful in optimizing conditions for their growth and activity. Prior to this study, methods for the direct measurement, other than isolation and identification, of methanotrophic bacteria in microcosms or field samples did not exist.

Methanotrophic bacteria are grouped into two divisions, types I and II, based on differences in intracytoplasmic membrane organization and carbon metabolism [1,2]. Type I methanotrophic bacteria contain esterified fatty acids, predominantly 16 carbons in length, and with saturated (16:0) and monounsaturated (16:1) fatty acids present [9,10]. Type II methanotrophic bacteria have monounsaturated 18-carbon (18:1) fatty acids as the predominant phospholipid fatty acid (PLFA) [9,10].

Analysis of cellular fatty acid profiles has become a standard tool in chemotaxonomy [11]. Fatty acids are also used as biomarkers or signature lipids in microbial ecology to provide valuable information about the structure of the microbial community [12]. If membrane fatty acids are to be used as biomarkers by taxonomists, ecologists and geochemists, precisely determining the double-bond positions and geometry will be essential for correctly interpreting increasingly complex data sets. Several relatively simple and rapid procedures have recently been reported that allow such determinations to be performed routinely [13]. The differences in carbon chain length and, more important, position and geometry of unsaturation [9,14] suggest that the results of analysis of extractable PLFAs from an ecosystem would indicate the presence or absence of methanotrophic bacteria. Similarly, PLFA profiles may be useful for characterizing other microbial groups, capable of degrading short-chain hydrocarbons, present in soils.

The phospholipid ester-linked and lipopolysaccharide-layer normal and hydroxy fatty acid profiles from a soil column stimulated with natural gas and capable of degrading halogenated hydrocarbons, such as trichloroethylene, *cis*- and *trans*-1,2-dichloroethylene, chloroform, dichloromethane, 1,1- and 1,2-dichloroethane and 1,2-dibromoethane [15], are reported here. The overall aim of this study was to identify specific lipid components that can be used to monitor for methanotrophic bacteria. These lipid biomarkers can be used for interpretation not only of the manipulated laboratory microcosms analyzed here but also of samples taken in field experiments.

MATERIALS AND METHODS

Soil and column description

The soil columns were prepared using Lincoln fine sand obtained in the late fall near the Robert S. Kerr Environmental Research Laboratory in Ada, Oklahoma, as described previously [8,16]. Column A had a headspace containing 0.6% natural gas in air to stimulate bacteria capable of growth using gaseous hydrocarbons. Column B was inhibited by the addition of 0.1% sodium azide to the water and was not exposed to natural gas. The natural gas was composed of 85% methane, 10% ethane and 3% propane. Column A was exposed to natural gas for three weeks before an aqueous solution of the halogenated hydrocarbons was applied; a similar solution was also applied to column B [15]. After three months of operation at 22 to 25°C, the columns were unpacked, and increments from 0 to 10 and 148 to 150 cm were lyophilized prior to lipid extraction.

Surface soil samples (0 to 10 cm) were acquired from the same site (in early summer rather than late fall) at the same location near Ada and were lyophilized as above. For samples from all depths, the pH ranged from 6.6 to 6.1 and the cation exchange capacity from 4.8 to 2.3 meq/100 g; sand constituted from 95 to 89% of the sample, silt from 8.8 to 4.0% and clay from 3.5 to 1.5%. Organic carbon contents of the 0 to 10 cm and 140 to 150 cm samples were 0.20 to 0.22% and 0.02%, respectively.

Lipid extraction and fractionation

Soil samples, in duplicate, were placed into 250-ml separatory funnels and the lipids were quantitatively extracted with a modified [17] one-phase chloroform-methanol extraction [18]. The recovery and fractionation of the lipids were performed as described previously [19,20]. The mild alkaline methanolysis procedure [17] to produce fatty acid methyl esters (FAMEs) from the phospholipids was modified in that hexane:chloroform (4:1, v/v) replaced chloroform in the extraction. Normal and hydroxy fatty acids from the lipopolysaccharide layer (LPS) were recovered by acidification of the lipid-extracted residue, chloroform extraction, methylation of the fatty acids and conversion to trimethylsilyl ethers as described elsewhere [21].

Analytical techniques

The techniques for separation, quantitation and tentative peak identification for the fatty acid com-

ponents were as described elsewhere [22]. GC-MS analysis for confirmation of identification and configuration was as described elsewhere [22]. Mass spectral data were acquired and processed with a Hewlett-Packard RTE-6/VM data system.

Determination of fatty acid double-bond configuration

The dimethyldisulfide adducts of monounsaturated FAMEs were formed to locate double-bond positions using the method of Dunkelblum et al. [13], as described previously [22].

Fatty acid nomenclature

Fatty acids are designated by total number of carbon atoms: number of double bonds, followed by the position of the double bond from the Δ (carboxylic) end of the molecule. The suffixes "c" and "t" indicate *cis* and *trans* geometry. The prefixes "i" and "a" refer to iso and anteiso branching, respectively, and the prefix "OH" indicates a hydroxy group at the position indicated. Other methyl branching is indicated as position of the additional methyl carbon from the carboxylic acid (Δ) end, i.e., 10 methyl 16:0. Cyclopropane fatty acids are designated with the prefix "cy," with the ring position relative to the carboxylic end of the molecule in parentheses.

RESULTS

PLFA biomass estimates

The PLFA concentration data (Table 1) were converted into number of bacteria per gram of soil using the following approximations: 5.9×10^{12} bacteria/g (dry weight) of bacteria [17], with an average methanotroph containing 57 μmol PLFA/g (dry.weight) [14]. Biomass estimates of 5.6×10^9 (range 4.1×10^9 to 7.7×10^9), 9.3×10^8 and 2.4×10^9 were determined for the upper 10 cm of the natural gas-enriched column (column A), the azide-inhibited column (column B) and the untreated surface soil, respectively (Table 1). Soil taken from the bottom (148 to 150 cm) of the two columns contained at least two orders of magnitude less biomass than the upper layers in each column.

Table 1. Phospholipid ester-linked fatty acid (PLFA) content and calculated bacterial numbers for a natural gas-enriched soil column (A), a soil column not exposed to natural gas (B) and untreated surface soil

Sample	nmol PLFA (g dry wt.)	Calculated number of bacteria[a] (g dry wt.)
Column A		
0 to 10 cm[b]	53.8	5600×10^6
148 to 150 cm[c]	0.61	63×10^6
Column B		
0 to 10 cm[c]	8.93	930×10^6
148 to 150 cm[c]	0.04	4×10^6
Untreated soil		
0 to 10 cm[c]	22.8	2400×10^6

[a]Calculated using factors previously reported [12,14,17].
[b]Mean of ten samples.
[c]Mean of two samples.

Fatty acid profiles

A total of 40 normal PLFAs were positively identified in the columns and soil sample (Table 2). Sixteen monounsaturated components were present, and characteristic ion fragments of the derivatized products formed by reaction with dimethyldisulfide (DMDS) are shown in Table 3. Interpreting these data provided the primary information for assigning double-bond position and geometry.

Several features were apparent when the fatty acid profiles in Table 2 were compared: (a) Several relatively novel monounsaturated fatty acids, 16:1Δ8c, 16:1Δ10c, and 18:1Δ10c, were present in column A but absent in column B and the surface soil sample. The latter fatty acid was the major component in all column A samples from 2 to 10 cm and showed an increase with depth relative to the more common bacterial PLFA, 18:1Δ11c [23] (Fig. 1). (b) A series of 10 methyl-branched fatty acids was detected in all 0 to 10 cm samples. A higher relative abundance of these components occurred in column B and untreated surface soil than in soil from column A. (c) The combined relative levels of the PLFAs 16:0 and 16:1 isomers and 18:0 and 18:1 isomers showed minor changes within the upper 10 cm of column A. The sum of the C_{18} PLFAs was generally 1.5 to 2 times that of the C_{16} PLFAs in these samples (Fig. 2). In contrast, both the surface soil and column B contained higher relative proportions of the C_{16} PLFAs. (d) C_{18} and C_{20} polyunsaturated fatty acids (PUFA) were detected in all samples from the upper 10 cm. The untreated surface soil contained the highest relative level of the PUFA 18:2Δ9, whereas the C_{20} PUFAs, 20:4Δ5 and 20:5Δ5, were present at similar relative levels in column A and the untreated surface soil. The latter two PLFAs were not detected in soil from the bottom of column A and occurred at a reduced

Table 2. Percent composition of phospholipid ester-linked fatty acids from a natural gas-enriched soil column (A), a soil column not exposed to natural gas (B) and untreated surface soil

	Percent total fatty acid methyl esters (FAMEs)							
	Column A (cm)[a]						Column B (cm)[a]	Surface soil (cm)[a]
Fatty acid	0–2	2–4	4–6	6–8	8–10	148–150	0–10	0–10
12:0	0.22	TR	TR	TR	TR	1.4	—	—
13:0	TR	TR	TR	TR	TR	1.2	—	—
i14:0	0.27	0.29	0.27	0.32	0.32		0.20	0.20
14:0	1.3	1.5	1.6	1.4	1.3	1.4	1.1	0.37
i15:1[b]	0.20	0.37	0.20	0.19	0.2	—	TR	TR
i15:0	2.5	2.9	2.7	2.8	3.0	2.6	5.4	5.9
a15:0	1.2	1.5	1.4	1.4	1.7	2.1	2.3	2.6
15:0	0.73	0.45	0.44	0.46	0.42	TR	0.68	0.53
i16:1Δ9c	0.59	0.36	0.33	0.23	0.28	—	0.32	0.48
10Me15:0	0.20	0.19	0.15	0.16	0.14	—	0.31	0.29
i16:0	2.1	1.5	2.1	1.5	1.6	3.7	3.8	3.8
16:1Δ7c	0.50	1.1	0.53	0.44	1.0	—	TR	1.1
16:1Δ8c	NAQ	NAQ	2.4	3.3	2.4	TR	—	—
16:1Δ9c	9.9	13.7	11.7	9.4	7.6	6.4	10.3	3.5
16:1Δ9t[c]	1.4	1.3	1.5	1.3	1.1	2.6	1.9	TR
16:1Δ11c	2.7	4.3	5.0	4.4	4.4	2.3	5.3	5.1
16:1Δ11t	TR	0.35	0.13	0.10	TR	—	—	—
16:0	9.2	8.8	8.6	8.4	8.0	62.1	13.7	11.2
i17:1Δ9c	0.82	1.3	1.5	1.2	1.4	1.1	2.1	2.7
10Me16:0	1.6	2.0	2.0	2.0	2.3	2.3	5.4	5.7
i17:0	0.92	1.0	1.1	1.1	1.2	TR	2.8	2.9
a17:0	2.0	1.5	1.4	1.5	1.5	1.1	2.8	3.3
cy17:0	1.5	1.9	2.3	2.2	2.0	1.3	4.8	2.3
17:0	0.62	0.41	0.36	0.26	0.36	1.1	0.81	0.66
10Me17:0	0.37	0.47	0.47	0.48	0.47	—	1.2	1.1
18:4Δ6,9,12,15	2.5	1.9	1.5	1.9	2.3	—	0.38	0.24
18:2Δ9,12	1.3	1.1	0.8	1.3	0.86	TR	0.90	4.8
18:1Δ9c	4.9	NAQ	3.2	0.63	NAQ	—	5.7	6.9
18:1Δ10c	16.1	21.5	20.3	25.6	27.9	1.6	—	—
18:1Δ11c	22.5	17.7	16.4	15.7	13.5	1.8	6.7	9.1
18:1Δ11t	0.22	0.23	0.46	0.32	0.30	TR	0.69	0.41
18:1Δ13c	0.61	1.2	0.94	0.93	1.1	TR	0.64	1.5
18:0	2.1	1.8	1.8	2.0	1.8	1.8	3.2	2.6
10Me18:0	1.4	0.82	0.92	0.99	1.0	—	2.2	1.8
cy19:0	1.9	2.2	2.3	2.2	2.6	2.1	7.5	8.4
20:4Δ5,8,11,14	1.4	1.5	1.5	1.3	1.3	—	0.20	1.2
20:5Δ5,8,11,14,17	0.21	0.26	0.19	0.22	0.27	—	TR	0.36
20:3	—	—	—	—	—	—	—	0.72
20:1Δ11c	—	—	—	—	—	—	0.38	0.33
20:0	TR	TR	TR	TR	TR	TR	0.90	0.39
Others	4.0	2.6	2.4	2.3	4.4	—	5.4	7.6
Total FAMEs[d]	42	61	50	74	42	0.6	8.9	23

TR, trace (<0.1%); (—), not detected; NAQ, not accurately quantitated.
[a]Depth from top of column.
[b]Position of unsaturated not determined because of insufficient sample. Components coelute with i15:1Δ9c.
[c]The FAME 16:1Δ10c was present in several column A samples but was not quantified.
[d]nmol/g (dry wt.).

relative level in column B soil. (e) Cyclopropyl fatty acids, cy17:0 and cy19:0, were detected in all samples, with the highest relative proportions present in the samples not enriched with natural gas. (f) A series of saturated FAMEs, tentatively identified as dimethyl-branched components, was detected in all samples. These components are included with the designation "Others" components in Table 2.

Table 3. Monounsaturated fatty acids from a natural gas-enriched column: Gas chromatographic (GC) retention data and characteristic ion fragments of derivatized products formed by reaction of the fatty acids with dimethyldisulfide (DMDS)

Fatty acid	RT[a]	Ion fragments (m/z) of DMDS adducts		
		M+	ω fragment[b]	Δ fragment[c]
i15:1[d]	17.79	—	—	—
i16:1Δ9c	19.74	—	—	217
16:1Δ7c[d]	20.34	—	—	—
16:1Δ8c	20.40	—	159	203
16:1Δ9c	20.44	362	145	217
16:1Δ9t	20.52	362	145	217
16:1Δ10c	20.52	—	131	231
16:1Δ11c	20.64	362	117	245
16:1Δ11t	20.77	362	117	245
i17:1Δ9c	21.69	—	159	217
17:1Δ9c	—	—	159	217
18:1Δ9c	24.51	—	173	217
18:1Δ10c	24.57	390	159	231
18:1Δ11c	24.64	390	145	245
18:1Δ11t	24.76	390	145	245
18:1Δ13c	24.86	390	117	273

(—), not detected in GC-MS analysis because of insufficient sample.
[a]Retention time.
[b]ω fragment indicates fragment including aliphatic end of the molecule.
[c]Δ fragment indicates fragment including carboxylic end of the molecule.
[d]Identification based on GC retention data alone.

Hydroxy fatty acids

The LPS hydroxy fatty acid composition for samples from column A are presented in Table 4. A total of seven β-hydroxy (β-OH) acids was detected, with even-carbon numbered components predominating. Approximately 80% of the total hydroxy acid content consisted of β-OH 14:0, β-OH 16:0 and β-OH 18:0. Differences were apparent in the relative levels of these three components.

Table 4. Lipopolysaccharide layer β-hydroxy acids from a natural gas-enriched column

β-Hydroxy acid	Percentage compositions[a]					
	0–2[b]	2–4[b]	4–6[b]	6–8[b]	8–10[b]	148–150[b]
β-OH 10:0	6.5	4.6	10.5	4.7	5.5	ND
β-OH 12:0	15.9	10.8	11.8	16.0	10.9	TR
β-OH 13:0[c]	TR	TR	TR	TR	5.6	ND
β-OH 14:0	32.5	21.4	22.3	25.6	30.2	TR
β-OH 15:0[c]	TR	TR	TR	TR	4.7	ND
β-OH 16:0	21.9	35.0	30.3	33.0	29.4	TR
β-OH 18:0	23.1	33.1	25.1	20.7	13.5	TR
nmol/g[d]	3.1	3.1	2.7	3.0	4.5	0.1

TR, trace; ND, not determined.
[a]Expressed in terms of the total β-hydroxy acids.
[b]Sediment depth (cm).
[c]Branched component.
[d]Dry weight basis.

DISCUSSION

Biomass

Bacterial biomass assessments for the untreated surface soil (0 to 10 cm) and the top 10 cm from both column A and column B were close to values obtained by the acridine orange direct count (AODC) method from a similar Oklahoma surface soil (7.0 to 8.2×10^8 cells/g [wet weight]; D.L. Balkwill, personal communication). These findings support the use of PLFA as a biomass assessment tool [12]. The cell number estimates for columns A and B and the untreated surface soil were considerably higher than those reported using the AODC method [24,25] for three shallow aquifers in Oklahoma (2.9 to 9.8×10^6 cells/g [dry weight]). The data presented here showed that the utilization of short-chain hydrocarbons in natural gas was accompanied by an increase in microbial biomass.

Fatty acids

It is recognized that certain fatty acids are specific to bacteria and that different groups of bacteria can have different fatty acid compositions [11,26]. As a result, PLFA profiles have been used previously to determine microbial community structure [12,19,27–31]. At present, an increasing proportion of complex microbial consortia and environmental samples can be rationalized on the basis of their fatty acid profiles into metabolic subgroups when detailed analysis, including the determination of double-bond configuration and position, is performed. The fatty acid profiles obtained in this study are discussed from this standpoint.

Methanotrophs

The most significant feature of the fatty acid profiles of column A when compared with those of column B and of the untreated surface soil was the presence of the relatively novel monounsaturated PLFAs 18:1Δ10c, 16:1Δ8c, 16:1Δ10c. These PLFAs were absent in the samples not enriched with natural gas. The amounts of these PLFAs increased with depth in column A (Fig. 1), constituting 28% of the PLFAs at the 8 to 10 cm depth.

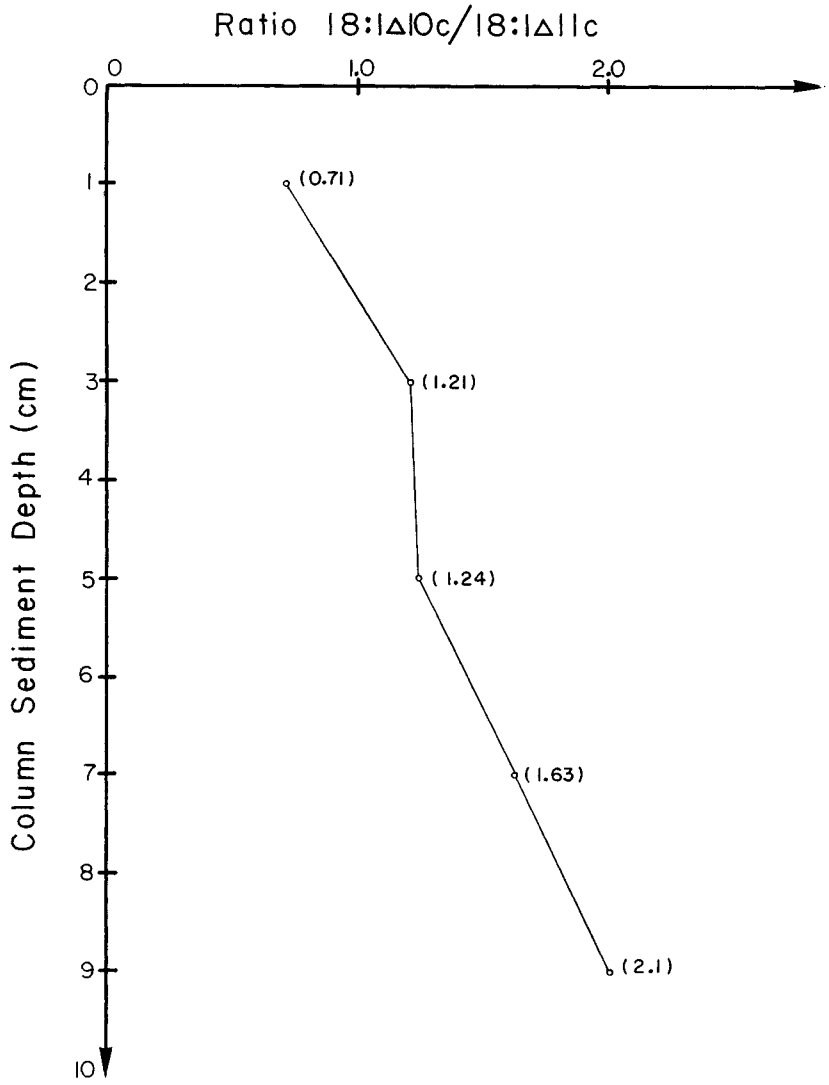

Fig. 1. Ratio of major fatty acids 18:1Δ10c to 18:1Δ11c versus depth in a natural gas-enriched soil column.

The PLFA 18:1Δ10c has been reported to be a major component only in methanotrophic bacteria, including *Methylosinus trichosporium* [9,14]. This PLFA constituted approximately 50% of the total PLFAs in *M. trichosporium* and 37% and 51% of the total PLFAs in two related but unclassified methanotrophs. Thus, from the data obtained for *M. trichosporium*, it can be calculated that this bacterium, or related bacteria, accounts for 32 to 56% of the total microbial biomass in column A. As this novel signature fatty acid was detected in neither the untreated surface soil nor column B, these data indicate that a significant change in the community structure occurred in the column A soil relative to the other soils analyzed.

The second most abundant PLFA detected in column A, 18:1Δ11c, was detected by Makula [9] as a minor component (11 to 18% of the total PLFAs) in type II methanotrophs. In an analysis of the PLFAs of four methanotrophs [14], 18:1Δ11c was the dominant component (84 to 89% of the total PLFAs) in two strains of the type II methanotroph *Methylobacterium organophilum*. Thus, it appears that *M. organophilum* or related type II methanotrophs may also contribute a substantial proportion of this fatty acid and the

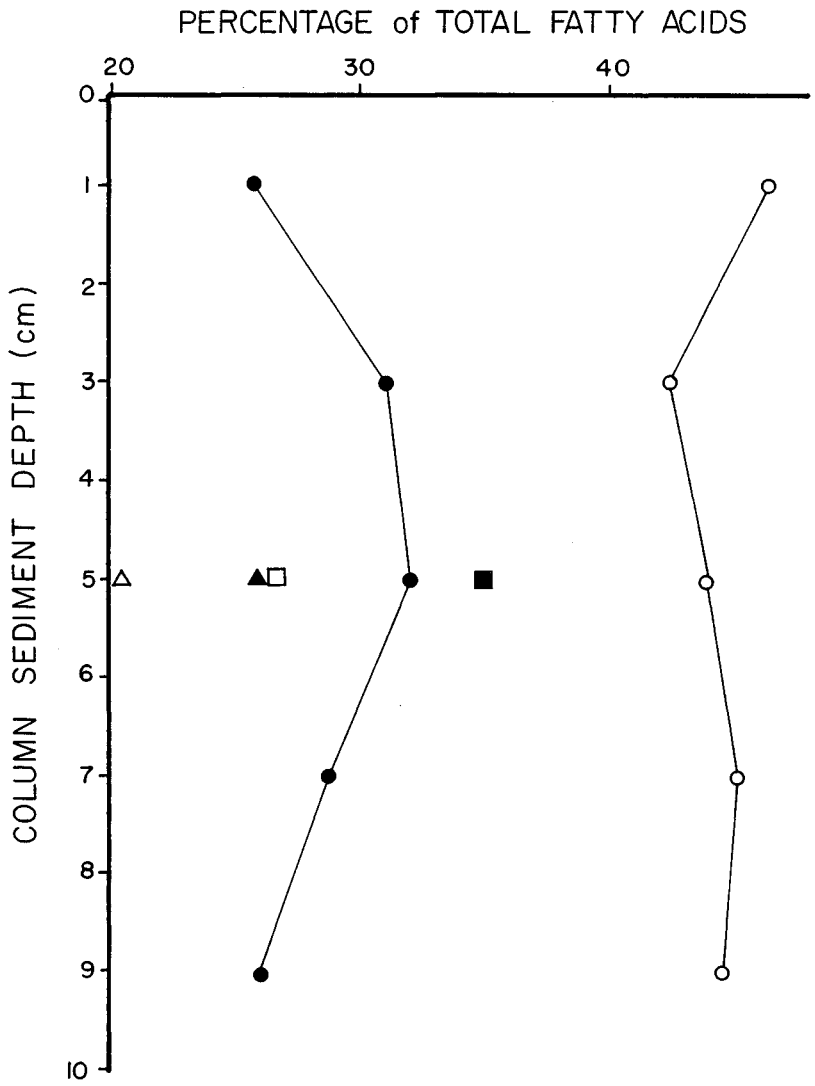

Fig. 2. Relative proportions of 16:0 and 16:1 isomers (closed symbols) and of 18:0 and 18:1 isomers (open symbols). Circles: natural gas-enriched column; squares: column B (exposed to sodium azide); triangles: untreated surface soil.

overall microbial biomass in column A. This PLFA is the most commonly detected bacterial C_{18} monounsaturated PLFA in other environments [19,28,29], and thus sources additional to type II methanotrophic bacteria may also be possible. At present, however, the significant increases in both the absolute and relative proportions of 18:1Δ11c when column A is compared with column B and the untreated surface soil seem consistent with a large increase in the biomass of *M. organophilum* or related type II methanotrophs.

The C_{18} monounsaturated PLFAs were found in greater concentrations than were the C_{16} PLFAs of type I methanotrophs, indicating that type II methanotrophs were more abundant than type I methanotrophs (Fig. 2).

Other microbial groups

Actinomycetes are commonly found in soils and have been reported to constitute 13 to 30% of the total microbial flora, depending on the season of the year [32]. Members of *Arthrobacter, Nocardioides* and other genera contain a number of 10-methyl fatty acids [33]. Similarly, cyclopro-

pane fatty acids have been detected only in *Actinomycetes* [34].

The presence of a series of 10-methyl branched fatty acids in all soil samples analyzed in this study (Table 2) is consistent with the presence of members of the Actinomycetes. The relative proportion of these components is significantly lower in soil column A (20% total PLFA) than in either column B or the untreated surface soil (50% total PLFA). Since *Desulfobacter* spp. also contain 10Me16:0 [35], these bacteria may have contributed a small amount of this PLFA.

Several fatty acids reported in sulfate-reducing bacteria [35–37], were present in all samples (Table 2). The branched chain monoenoic fatty acids, i17:1Δ9c and i15:1Δ9c, are common to *Desulfovibrio* spp. [36,37], and 10 methyl 16:0 is a major component in *Desulfobacter*, as noted above. i17:1Δ9c and i17:1Δ11c have also been detected in several *Flexibacter* (unpublished data). As i17:1Δ11c was not detected in this study, the contribution from *Flexibacter* is probably minimal. Assuming that all the i17:1Δ9c detected can be attributed to *Desulfovibrio* spp., this bacterial group contributed approximately 5, 8 and 10% of the total PLFA in column A, column B and the untreated surface soil, respectively.

A minor contribution from microeukaryotic organisms occurred in all surface samples, as the C_{20} PUFAs 20:4Δ5 and 20:5Δ5 are specific to microeukaryotes [27,31,38].

LPS-hydroxy fatty acids

Even-numbered β-hydroxy acids dominated the LPS profile for column A (Table 4). Differences in the upper 10 cm of the column can be assumed to be due to variations in the microbial, in particular methanotrophic, community structure. The three major components, β-OH 14:10, β-OH 16:0 and β-OH 18:0, were the only LPS components detected in four methanotrophs [14]. These data further support the view, based on the ester-linked PLFA profiles, that methanotrophic bacteria constitute a major proportion of the microbial biomass in the soil of column A.

The PLFA and LPS hydroxy acid profiles reported have enabled the microbial biomass in a natural gas-enriched soil column and related control samples to be determined. Bacterial numbers were similar to those obtained by the AODC method for a similar surface sample. These data thus support the use of PLFA as a method for determining microbial biomass in soil. More important, comparison of the PLFA profiles, particularly when the double-bond configurations are precisely determined, allows the differentiation of the bacterial community structure. The natural gas-enriched column was found to contain significantly higher amounts of PLFA specific to type II methanotrophs. A decrease in biomarkers for Actinomycetes and *Desulfovibrio* spp. relative to the other samples analyzed was also noted for column A.

Community structure information, as determined from PLFA, provides data on the effects of stimulating indigenous soil bacteria by adding natural gas. The findings for this model system can be drawn upon when biotransformation processes similar to the one described here are adapted to aquifers. Such a project is presently under way in collaborative studies between our laboratories.

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REFERENCES

1. **Anthony, C.** 1982. *The Biochemistry of Methylotrophs.* Academic Press, New York, NY.
2. **Higgins, I.J., D.J. Best, R.C. Hammond** and **D.C. Scott.** 1981. Methane-oxidizing microorganisms. *Microbiol. Rev.* **45**:556–590.
3. **Colby, J., H. Dalton** and **R. Whittenburg.** 1979. Biological and biochemical aspects of microbial growth on C-1 compounds. *Annu. Rev. Microbiol.* **33**:481–517.
4. **Haber, C.L., L.N. Allen** and **R.S. Hanson.** 1983. Methylotrophic bacteria: Biochemical diversity and genetics. *Science* **221**:1147–1152.
5. **Rudd, J.W.M.** and **C.D. Taylor.** 1980. Methane cycling in aquatic sediments. *Adv. Aquat. Microbiol.* **2**:77–150.
6. **Colby, J., D.F. Stirling** and **H. Dalton.** 1977. The soluble methane mono-oxygenase of *Methylococcus capsulatus* (Bath): Its ability to oxygenate n-alkanes, n-alkenes, ethers, and aliphatic, aromatic, and heterocyclic compounds. *Biochem. J.* **165**:395–402.
7. **Schwarzenbach, R.P.** and **W. Giger.** 1985. Behavior and fate of halogenated hydrocarbons in ground water. In C.H. Ward, W. Giger and P.L. McCarty, eds., *Ground Water Quality*. John Wiley & Sons, New York, NY, pp. 446-471.
8. **Wilson, J.T.** and **B.H. Wilson.** 1985. Biotransformation of trichloroethylene in soil. *Appl. Environ. Microbiol.* **49**:242–243.
9. **Makula, R.A.** 1978. Phospholipid composition of methane utilizing bacteria. *J. Bacteriol.* **134**:771–777.
10. **Urakami, T.** and **K. Komagata.** 1984. Cellular fatty

composition and quinone system in methane-utilizing bacteria and methylamine-utilizing bacteria. In R.L. Crawford and R.S. Hanson, eds., *Microbial Growth on C-1 Compounds*. American Society for Microbiology, Washington, DC, pp. 123–133.

11. **Goodfellow, M.** and **D.E. Minnikin.** 1985. Introduction to chemosystematics. In M. Goodfellow and D.E. Minnikin, eds., *Chemical Methods in Bacterial Systematics*. Society for Applied Bacteriology Technical Series, No. 20. Academic Press, London, pp. 1–15.
12. **White, D.C.** 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. In J.H. Slater, R. Whittenburg and J.W.T. Wimpenny, eds., *Microbes in Their Natural Environments*. Cambridge University Press, New York, NY, pp. 37–66.
13. **Dunkelblum, E., S.H. Tan** and **P.J. Silk.** 1985. Double-bond location in monounsaturated fatty acids by dimethyldisulfide derivatization and mass spectrometry: Application to analysis of fatty acids in pheromone glands of four Lepidoptera. *J. Chem. Ecol.* **11**:265–277.
14. **Nichols, P.D., G.A. Smith, C.P. Antworth, R.S. Hanson** and **D.C. White.** 1985. Phospholipid and lipopolysaccharide normal and hydroxy fatty acids as potential signatures for methane-oxidizing bacteria. *FEMS Microbiol. Ecol.* **31**:327–355.
15. **Henson, J.M., J.C. Cochran** and **J.T. Wilson.** 1985. Aerobic biodegradation of halogenated aliphatic hydrocarbons. *Abstracts*, Sixth Annual Meeting of the Society of Environmental Toxicology and Chemistry, Rockville, MD, November 10–13, p. 100.
16. **Wilson, J.T., C.G. Enfield, W.J. Dunlap, R.L. Cosby, D.A. Foster** and **L.B. Baskin.** 1981. Transport and fate of selected organic pollutants in a sandy soil. *J. Environ. Qual.* **10**:501–506.
17. **White, D.C., R.J. Bobbie, J.D. King, J.S. Nickels** and **P. Amoe.** 1979. Methodology for biomass determinations and microbial activities in sediments. In C.D. Litchfield and P.L. Seyfried, eds., *Methodology for Biomass Determinations and Microbial Activities in Sediments*. STP 673. American Society for Testing and Materials, Philadelphia, PA, pp. 87–103.
18. **Bligh, E.G.** and **W.J. Dyer.** 1959. A rapid method of lipid extraction and purification. *Can. J. Biochem. Physiol.* **35**:911–917.
19. **Guckert, J.B., C.P. Antworth, P.D. Nichols** and **D.C. White.** 1985. Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiol. Ecol.* **31**:147–158.
20. **Gehron, M.J.** and **D.C. White.** 1983. Sensitive assay of phospholipid glycerol in environmental samples. *J. Microbiol. Methods* **1**:23–32.
21. **Moss, C.W.** 1981. Gas-liquid chromatography as an analytical tool in microbiology. *J. Chromatogr.* **203**:337–347.
22. **Nichols, P.D., J.B. Guckert** and **D.C. White.** 1986. Determination of monounsaturated fatty acid double bond position and geometry for microbial monocultures and complex consortia by capillary gas chromatography-mass spectrometry of their dimethydisulphide adducts. *J. Microbiol. Meth.* **5**:49–55.
23. **Perry, G.J., J.K. Volkman, R.B. Johns** and **H.J. Bavor, Jr.** 1979. Fatty acids of bacterial origin in contemporary marine sediments. *Geochim. Cosmochim. Acta* **43**:1715–1725.
24. **Balkwill, D.L.** and **W.C. Ghiorse.** 1985. Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. *Appl. Environ. Microbiol.* **50**:580–588.
25. **Wilson, J.T., J.F. McNabb, D.L. Balkwill** and **W.C. Ghiorse.** 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. *Ground Water* **21**:134–142.
26. **Lechevalier, M.P.** 1977. Lipids in bacterial taxonomy—A taxonomist's view. *CRC Crit. Rev. Microbiol.* **5**:109–210.
27. **Bobbie, R.J.** and **D.C. White.** 1980. Characterization of benthic microbial community structure by high-resolution gas chromatography of fatty acid methyl esters. *Appl. Environ. Microbiol.* **39**:1212–1222.
28. **Gillan, F.T.** and **R.W. Hogg.** 1984. A method for the estimation of bacterial biomass and community structure in mangrove associated sediments. *J. Microbiol. Meth.* **2**:275–293.
29. **Gillan, F.T., R.B. Johns, T.V. Verheyen, P.D. Nichols, R.J. Esdaile** and **H.J. Bavor.** 1983. Monounsaturated fatty acids as specific bacterial markers in marine sediments. In M. Bjorøy et al., eds., *Advances in Organic Geochemistry 1981*. John Wiley & Sons, New York, NY, pp. 198–206.
30. **Parkes, R.J.** and **J. Taylor.** 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. *Estuarine Coastal and Shelf Science* **16**:173–189.
31. **Volkman, J.K., R.B. Johns, F.T. Gillan, G.J. Perry** and **H.J. Bavor, Jr.** 1980. Microbial lipids of an intertidal sediment. I. Fatty acids and hydrocarbons. *Geochim. Cosmochim. Acta* **44**:1133–1143.
32. **Waksman, S.A.** 1967. *The Actinomycetes: A Summary of Current Knowledge*. Ronald Press, New York, NY.
33. **O'Donnell, A.G., M. Goodfellow** and **D.E. Minnikin.** 1982. Lipids in the classification of *Nocardioides*: Reclassification of *Arthrobacter simplex* (Jensen) Lockhead in the genus *Nocardioides* (Prauser) emend. O'Donnell *et al.* as *Nocardioides simplex* comb. nov. *Arch. Microbiol.* **133**:323–329.
34. **Kroppenstedt, R.M.** and **H.J. Kutzner.** 1978. Biochemical taxonomy of some problem Actinomycetes. *Zbl. Bakt. I. Abt.* (suppl. 6):125–133.
35. **Taylor, J.** and **R.J. Parkes.** 1983. The cellular fatty acids of the sulphate-reducing bacteria, *Desulfobacter* sp., *Desulfobulbus sp.*, and *Desulfovibrio desulfuricans*. *J. Gen. Microbiol.* **129**:3303–3309.
36. **Boon, J.J., J.W. deLeeuw, G.J. Hock** and **J.H. Vosjan.** 1977. Significance and taxonomic value of iso and anteiso monoenoic fatty acids and branched beta-hydroxy acids in *Desulfovibrio desulfuricans*. *J. Bacteriol.* **129**:1183–1191.
37. **Edlund, A., P.D. Nichols, R. Roffey** and **D.C. White.** 1985. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. *J. Lipid Res.* **26**:982–988.
38. **Erwin, J.A.** 1973. Comparative biochemistry of fatty acids in eukaryotic microorganisms. In J.A. Erwin, ed., *Lipids and Biomembranes of Eukaryotic Microorganisms*. Academic Press, New York, NY, pp. 41–143.