Phospholipid Ester-linked Fatty Acid Biomarkers of Acetate-oxidizing Sulphate-reducers and Other Sulphide-forming Bacteria

By NICHOLAS J. E. DOWLING,¹* FRIEDRICH WIDDEL²
AND DAVID C. WHITE¹

¹Department of Biological Science, Florida State University, Tallahassee,
Florida 32306-3043, USA

²Fakultät für Biologie, Universität Konstanz, Postfach 5560 D-7750, Konstanz, FRG

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The phospholipid ester-linked fatty acids were examined in four *Desulfobacter* strains (2ac9, AcBa, 3ac10 and 4ac11), a *Desulfobacter*-like 'fat vibrio' (AcKo) and *Desulfotomaculum acetoxidans* (5575), which are all sulphate-reducing bacteria that oxidize acetate. A thermophilic sulphate reducer, *Desulfovibrio thermophilus*, and two sulphur-reducing bacteria, *Desulfuromonas acetoxidans* (11070) and a *Campylobacter*-like spirillum (5175), were also studied. The *Desulfobacter* spp. were characterized by significant quantities of 10-methylhexadecanoic acid. Other 10-methyl fatty acids were also detected in *Desulfobacter* spp. No 10-methyl fatty acids were detected in the other organisms examined, supporting the use of 10-methylhexadecanoic acid as a biomarker for *Desulfobacter*. High levels of cyclopropyl fatty acids, including two isomers of both methylenehexadecanoic (cy17:0) and methyleneheptadecanoic (cy18:0) acids, were also characteristic of *Desulfobacter* spp. The influence of the volatile fatty acids (VFA) propionate, isobutyrate, isovalerate and 2-methylbutyrate on the lipid fatty acid distribution was studied with two *Desulfobacter* strains (2ac9, AcBa) and *Desulfotomaculum acetoxidans*. Although these sulphate reducers cannot oxidize the VFA, their presence in the acetate growth medium caused a shift in the fatty acid distribution in favour of odd-numbered and branched chains by apparent direct incorporation into the fatty acids as chain initiators. The *Desulfobacter* strains were distinguished from other sulphide-forming bacteria by the percentage of unsaturated and the percentage of branched fatty acids.

INTRODUCTION

Sulphate-reducing bacteria perform the terminal process in anaerobic degradation of organic matter in aquatic environments. These bacteria oxidize low- M_r compounds formed by fermentative bacteria and use sulphate as terminal acceptor, the sulphate being reduced to sulphide. In sulphate-rich habitats such as marine sediments, more than half of the incoming detritus may be mineralized via sulphate reduction (Jørgensen, 1977, 1982; Sansone & Martens, 1982). If sulphate is absent or limiting, as in most freshwater sediments, the terminal degradation step is taken over by methanogenic bacteria and their syntrophic partners (Banat & Nedwell, 1983; Winfrey & Zeikus, 1977; Stieb & Schink, 1985). Molecular hydrogen and acetate are the two key intermediates via which dead biomass (necromass) is channelled into either sulphate reduction or methanogenesis (Loveley *et al.*, 1982; Sørensen *et al.*, 1981; Winfrey & Zeikus, 1977). *Desulfovibrio* spp. and *Desulfobulbus* spp. are potentially hydrogen-scavenging sulphate reducers in nature (Brandis & Thauer, 1981; Kristjansson *et al.*, 1982; Taylor & Parkes, 1985). In the laboratory, *Desulfovibrio* spp. are usually cultivated on lactate (Postgate, 1984), which is probably not a major anaerobic intermediate under natural conditions. Acetate is

Abbreviations: FAME, fatty acid methyl esters; VFA, volatile fatty acids.

oxidized by various sulphate-reducing bacteria (Pfennig *et al.*, 1981); however, only a few species of these, namely *Desulfobacter* spp. and *Desulfotomaculum acetoxidans*, really grow well on acetate (Widdel & Pfennig, 1977, 1981*a, b*) and may, therefore, be significant acetate oxidizers under natural conditions. *Desulfobacter postgatei* exhibits a high affinity for acetate (Schönheit *et al.*, 1982; Ingvorsen *et al.*, 1984). *Desulfobacter* spp. usually do not utilize hydrogen, whereas *Desulfovibrio* spp. and *Desulfobulbus* spp. cannot oxidize acetate. Thus, there are apparently functionally distinctive groups of sulphate-reducing bacteria (Banat *et al.*, 1981).

If elemental sulphur is present, this may be used as terminal acceptor by sulphur-reducing bacteria (Jørgensen, 1982; Smith & Klug, 1981). Mesophilic, eubacterial sulphur reducers that oxidize hydrogen are represented by *Campylobacter*-like spirilla (Wolfe & Pfennig, 1977; Pfennig & Biebl, 1981), and sulphur reducers that oxidize acetate by *Desulfuromonas* spp. (Pfennig & Biebl, 1976, 1981).

Quantitative assay of bacterial biomass and community structure in marine or other sediments requires methods that avoid the problems associated with quantitative recovery of bacteria from surfaces and selective culturing procedures (White, 1983). Recent work has shown that fatty acid analysis may be a powerful tool in the interpretation of microbial community structure (Bobbie & White, 1980) as well as in microbial taxonomy (Minnikin *et al.*, 1978). Few articles detail the lipids of the sulphide-forming eubacteria, most being concerned with the lactate- or hydrogen-utilizing sulphate-reducing bacteria (i.e. *Desulfovibrio* spp. and *Desulfotomaculum* spp.), which commonly exhibit branched monoenoic 17-carbon fatty acids as major components (Makula & Finnerty, 1974, 1975; Ueki & Suto, 1979; Edlund *et al.*, 1985; Boon *et al.*, 1977). Taylor & Parkes (1983) investigated the cellular fatty acids of a *Desulfobacter* sp. (strain 3ac10) utilizing acetate, a *Desulfobulbus* sp. (strain 3pr10) able to utilize propionate, hydrogen or lactate, and a *Desulfovibrio desulfuricans* strain. The *Desulfobacter* strain exhibited high proportions of 10-methylhexadecanoic acid (10Me16:0), which had previously been observed in anoxic marine sediments (Volkman *et al.*, 1980; Parkes & Taylor, 1983; Perry *et al.*, 1979). Because of its absence in *Desulfobulbus* and *Desulfovibrio*, 10Me16:0 was proposed as a biomarker for *Desulfobacter* spp. in these environments. Hitherto, saturated 10-methyl fatty acids have only been observed in actinomycetes and related taxa (Kroppenstedt & Kutzner, 1978).

In this study, we examined other *Desulfobacter* strains for 10Me16:0 to determine if it could be of use as a more general biomarker for that genus. The hydrogen- or lactate-utilizing thermophilic *Desulfovibrio thermophilus* (Rozanova & Khudyakova, 1974) was included in the study in order to compare its phospholipid fatty acids with those of mesophilic *Desulfovibrio* spp. Two representatives of sulphur reducers were also examined: the hydrogen-oxidizing *Campylobacter*-like spirillum 5175 (Wolfe & Pfennig, 1977) and the acetate-oxidizing *Desulfuromonas acetoxidans* (Pfennig & Biebl, 1976). The effects of supplementing the culture medium with volatile fatty acids (not required for growth) on the phospholipid fatty acids of three acetate-utilizing strains were also investigated.

METHODS

Bacterial strains and culture methods. The origins and nutritional capacities of all the strains examined are listed in Table 1.

The 'fat vibrio' strain AcKo is a new isolate from an ammonia-limited freshwater enrichment on acetate. Strain AcKo resembles *Desulfobacter postgatei* in that it uses only acetate as electron donor and its growth is stimulated in brackish media; however, AcKo has motile fat vibrio-shaped cells, whereas *Desulfobacter* cells are oval to rod-shaped and scarcely motile in pure cultures.

The strains were grown in anaerobic 2 litre batch cultures in bicarbonate-buffered media reduced with sulphide and dithionite as described by Pfennig *et al.* (1981). The rubber-sealed culture bottles had a small gas phase of 10–20% (v/v) CO₂ in N₂. The media were supplemented with vitamins, but contained no yeast extract, chelating agents or redox indicator. For *Desulfovibrio thermophilus*, bicarbonate was omitted and the phosphate concentration was increased from 0.2 to 0.8 g KH₂PO₄ l⁻¹. The strains required different concentrations of NaCl and MgCl₂·6H₂O in their media for optimal growth; the respective concentrations were as follows (g l⁻¹): 1.0 and 0.5 for *Desulfotomaculum acetoxidans*, *Desulfovibrio thermophilus* and spirillum 5175; 7.0 and 1.2 for *Desulfobacter postgatei* and the fat vibrio AcKo; 20.0 and 3.0 for *Desulfuromonas acetoxidans* and the *Desulfobacter* strains AcBa,

Table 1. *Sulphate-reducing and sulphur-reducing bacteria analysed: origin of strains and physiological characteristics*

Culture numbers 1–7 are sulphate-reducing and 8 and 9 are sulphur-reducing bacteria. Growth substrates used in the present study are given in italics.

Culture no.	Strain	DSM no.*	Origin	Optimum growth temp. (°C)	Electron donor	Electron acceptors
1	<i>Desulfobacter postgatei</i> 2ac9	2034	Brackish ditch	30–32	<i>Acetate</i>	<i>Sulphate</i> , sulphite, thiosulphate
2	<i>Desulfobacter</i> sp. AcBa	–	Oil tank	30–34	<i>Acetate</i>	<i>Sulphate</i>
3	<i>Desulfobacter</i> sp. 3ac10	2035	Marine mud	30–32	<i>Acetate</i> , ethanol	<i>Sulphate</i>
4	<i>Desulfobacter</i> sp. 4ac11	2057	Marine mud	28–31	<i>Acetate</i>	<i>Sulphate</i>
5	'Fat vibrio' sp. AcKo	–	Sewage ditch	22–25	<i>Acetate</i>	<i>Sulphate</i>
6	<i>Desulfotomaculum acetoxidans</i> 5575	771	Piggery manure	34–36	<i>Acetate</i> , butyrate, isobutyrate, ethanol	<i>Sulphate</i>
7	<i>Desulfovibrio thermophilus</i>	1276	Petroleum deposit	65	<i>Pyruvate</i> , hydrogen, lactate	<i>Sulphate</i>
8	<i>Desulfuromonas acetoxidans</i> 11070	684	Marine mud	30	<i>Acetate</i> , propionate, ethanol	<i>Malate</i> , sulphur, fumarate
9	<i>Spirillum</i> 5175	–	Freshwater mud	30	Hydrogen, formate	<i>Fumarate</i> , sulphur, malate, nitrate, oxygen (<0.05 atmos.)†

* Deutsche Sammlung von Mikroorganismen, Göttingen, FRG.

† The *Spirillum* may also grow by fumarate or malate fermentation without hydrogen or formate.

3ac10 and 4ac11. Media for sulphate reducers contained 25 mM-sulphate. *Desulfovibrio thermophilus* was grown on pyruvate (filter-sterilized; pore size 0.2 µm; final concentration 20 mM). In order to facilitate harvesting of the sulphur reducers, they were grown on alternative, purely organic energy sources: spirillum 5175 was cultivated on fumarate (40 mM, gradually added during growth), *Desulfuromonas acetoxidans* on acetate (10 mM) plus malate (40 mM, gradually added). *Desulfobacter postgatei*, *Desulfobacter* strain AcBa and *Desulfotomaculum acetoxidans* were grown in duplicate cultures on acetate, one of each pair containing, in addition, the following VFA (final concentrations in mM): propionate (2), isobutyrate (1), isovalerate (1), 2-methylbutyrate (1). All strains were grown in the dark at their respective optimum temperature (Table 1) and were briefly shaken twice a day. Cells were harvested by centrifugation at the end of exponential growth phase and lyophilized for lipid extraction.

Lipid extraction and separation. Lipids were extracted by a modified version (White *et al.*, 1979) of the procedure of Bligh & Dyer (1959). After recovery in chloroform, the lipids were fractionated on a silicic acid column (Gehron & White, 1983). Unisil (1 g, 100–200 mesh) silicic acid (activated at 100 °C, 1 h) was slurried into glass columns with chloroform. Total extractable lipid was then applied to the silicic acid in 2 ml chloroform. Neutral lipids were eluted with 10 ml chloroform, glycolipids with 10 ml acetone and phospholipids with 10 ml methanol. Phospholipid solutions were evaporated to dryness under a stream of nitrogen and stored at –70 °C.

Methanolysis of phospholipid fatty acids. Phospholipids were dissolved in 1 ml methanol/toluene (1:1, v/v), 1 ml 0.2 M-KOH was added and the samples were heated at 37 °C for 15 min. Sequential additions of 2 ml hexane, 0.3 ml 1 M-acetic acid and 2 ml water were made. The biphasic mixture was vortexed, and the hexane (upper) layer, containing the fatty acid methyl esters (FAME), was removed. The partition against 2 ml hexane was repeated. Combined hexane fractions were dried under a stream of nitrogen and stored at –70 °C.

Thin layer chromatography (TLC). Crude FAME were loaded onto a thin layer silica gel (Whatman KG, 0.25 mm, 20 × 20 cm) plate. Standard methyl nonadecanoate was spotted onto end lanes on each plate. After development of the TLC plates in hexane/diethyl ether (1:1, v/v) the end lanes were sprayed with 0.1% (w/v) Rhodamine to detect the standard. Areas at R_f values corresponding to the standards were scraped off and the FAME eluted from the silica with hexane. The solvent was dried under a stream of nitrogen and the FAME taken up in an appropriate volume of hexane for gas chromatography. Methyl nonadecanoate was added as an internal injection standard.

Saturated and unsaturated FAME were separated on TLC plates as before, but the plates were predeveloped in 20 g AgNO₃, 60 ml water and 120 ml absolute ethanol in the dark. Development and recovery of saturated FAME using an external standard of methyl nonadecanoate was as described above.

Determination of fatty acid double bond position. This was done by gas chromatography–mass spectrometry (GC–MS) analysis of their Diels–Alder adducts (Kidwell & Bleman, 1982), as modified by Nichols *et al.* (1985), or of dimethyl disulphide derivatives (Dunkelblum *et al.*, 1985).

Determination of fatty acid cyclopropyl position. This procedure was modified from McCloskey & Law (1967), and Kaneshiro & Marr (1961). Cyclopropyl FAME (approximately 5 mg) were hydrogenated in the presence of PtO₂ (2 mg) and glacial acetic acid (0.2 ml), under 275 kPa H₂, while stirring with a glass coated magnetic stirring bar for 20 h. The solvent was removed under nitrogen, and the FAME were analysed by GC–MS.

GC and GC–MS. Gas chromatography was done on a Varian model 3700 using a flame ionization detector and a non-polar cross-linked methyl silicone fused silica capillary column (50 m × 0.2 mm i.d., Hewlett Packard). Injector and detector temperatures were 250 °C and 290 °C respectively. The oven temperature was programmed from 80 °C to 140 °C at 20 °C min^{–1}, then at 4 °C min^{–1} to 270 °C, and then isothermal for 10 min. Hydrogen was used as carrier gas at 30 cm sec^{–1} (linear velocity). Peak areas were integrated by a Hewlett Packard 3350 series programmable laboratory data system operated in an internal standard programme mode.

Initial GC–MS analysis was done on a Hewlett Packard 5995A system fitted with a direct capillary inlet (Nichols *et al.*, 1985). Several samples were also analysed with the following GC–MS parameters: splitless injection at 100 °C; the oven programmed from 100 °C to 300 °C at 4 °C min^{–1}; helium as the carrier gas; electron multiplier 1300–1400 V; transfer line 300 °C; source and analyser 250 °C; autotune file DFTPP normalized; optics tuned at m/z 502; MS peak threshold = 300 triggered on total ion abundance; electron impact energy = 70 eV. Mass spectral data were processed with a Hewlett Packard RTE-6/VM data system.

Criteria for the identification of FAME. Identification of individual fatty acids relied upon GC retention time; argentation TLC separation of unsaturated and saturated FAME; derivatization of monoenoic acids for the determination of double bond positions; and the hydrogenation of cyclopropyl groups for mass spectral analysis of the resultant branched FAME. Original FAME were also examined by GC–MS. Interpretation of mass spectral data and component identification was achieved by comparison with published data (Dinh-Nguyen *et al.*, 1961; Ryhage & Stenhagen, 1958; Rohwedder *et al.*, 1965; Campbell & Naworal, 1969).

RESULTS

The phospholipid ester-linked fatty acid distributions of the *Desulfobacter* spp. and of all the other sulphide-forming bacteria analysed are listed in Tables 2 and 3, respectively. For ease of

Table 2. *Percentage phospholipid ester-linked fatty acids of Desulfobacter spp. grown on acetate*

Cultures are numbered according to Table 1. Fatty acids are characterized by chain length and position of substituents from the methyl (ω) end. Thus 16:1 ω 7c is ω 7-cis-hexadecenoic acid. Iso and anteiso FAME are methyl-branched one and two carbons from the methyl end, respectively. Unsaturation may occur in either cis (c) or trans (t) configurations. Other notation: tr, trace quantities (<0.1%); br, multiple branching; SAT, unknown saturated fatty acid; a, FAME detected by double bond analysis only, and not quantified; *, positional data for double bond, methyl branch or cyclopropyl ring (cy) based on GC retention time only (insufficient sample was available for analysis). Cultures + VFA were grown with additional volatile fatty acid (final concentration mM): propionate (2), isobutyrate (1), isovalerate (1), 2-methylbutyrate (1).

FAME	Culture no.					
	1	1 + VFA	2	2 + VFA	3	4
13:0	—	—	tr	—	tr	—
iso14:0	tr	0.2	0.1	1.1	0.2	tr
anteiso14:0	—	—	—	4.5	—	—
14:1 ω 7	0.3	0.2	0.4	—	0.3	tr
14:1 ω 5	—	—	tr	—	—	tr
14:0	9.5	2.4	31.4	9.4	8.8	11.3
br15:1*	—	0.8	tr	—	0.8	tr
iso15:0	1.1	8.9	1.6	2.6	8.7	2.1
anteiso15:0	0.1	4.0	0.1	1.9	0.2	0.1
15:1 ω 8	—	—	—	a	—	a
15:1 ω 6	—	1.5	0.1	4.6	tr	—
15:1*	—	—	—	0.2	—	tr
15:0	1.3	12.1	0.7	23.7	0.8	0.4
10Me15:0	—	1.4	—	—	—	—
br16:1 ω 6c*	—	—	—	1.9	0.3	—
iso16:0	0.1	1.2	0.4	0.9	0.6	0.2
anteiso16:0	—	—	—	5.3	—	—
16:1 ω 9	1.4	0.6	0.9	—	1.4	0.3
16:1 ω 7c	4.9	2.1	5.9	1.8	7.1	1.1
16:1 ω 7t	0.3	—	0.2	—	0.2	0.1
16:1 ω 5	2.0	1.3	1.8	6.3	1.8	1.4
cy16:0*	—	1.8	—	—	—	—
16:0	30.7	11.9	18.6	7.4	25.8	32.7
iso17:1 ω 7	0.1	1.5	0.1	0.1	1.4	0.2
10Me16:0	16.5	9.1	9.4	6.3	24.7	11.2
iso17:0	0.2	1.3	tr	tr	1.4	0.2
anteiso17:0	0.2	1.3	—	—	0.1	—
17:1 ω 8	—	2.0	—	0.7	—	a
17:1 ω 7	a	—	—	—	—	a
17:1 ω 6	a	8.9	—	3.6	—	a
cy17:0a(ω 7/8)	24.4	10.0	27.3	11.8	7.0	30.1
cy17:0b*	2.6	0.8	—	0.2	—	2.6
brSAT*	—	—	0.5	—	—	—
17:0	0.2	2.9	tr	1.0	0.2	0.2
br18:1*	0.2	1.4	—	0.3	tr	tr
10Me17:0	0.1	2.4	—	2.1	0.4	0.1
18:1 ω 9	0.2	0.2	tr	tr	0.4	0.1
18:1 ω 7c	0.6	0.5	0.1	—	3.8	0.4
18:1 ω 7t	0.1	—	—	—	tr	—
cy18:0a*	—	0.8	—	0.9	—	0.1
cy18:0b*	0.4	4.5	—	0.8	—	0.2
18:0	0.3	0.3	0.1	0.4	0.3	0.2
br19:1*	0.8	0.4	tr	—	1.6	1.0
10Me18:0	—	—	—	—	0.5	—
anteiso19:0	0.1	0.1	tr	—	tr	tr
cy19:0*	1.1	0.6	0.2	—	0.4	3.4
Trace components	0.2	0.1	0.1	0.2	0.2	0.3
Branched FAME	19.5	34.5	12.2	27.0	41.5	15.1
Unsaturated FAME	10.9	21.4	9.5	19.5	19.1	4.6
μ mol (g dry wt) ⁻¹	102.5	77.3	58.5	40.8	83.5	64.4

Table 3. *Percentage phospholipid ester-linked fatty acids of a Desulfobacter-like 'Fat vibrio', non-Desulfobacter sulphate reducers and sulphur reducers*

For definition of abbreviations see Table 2.

FAME	Culture no.					
	5	6	6 + VFA	7	8	9
iso14:0	tr	—	0.3	—	—	—
14:1 ω 7	0.2	—	—	—	0.1	0.3
14:1*	0.1	—	—	—	0.1	—
14:0	5.5	1.3	0.6	—	2.1	3.1
br15:1*	0.3	—	—	—	tr	—
iso15:0	3.5	0.5	0.9	0.3	4.4	tr
anteiso15:0	tr	—	—	0.2	0.4	tr
15:1 ω 8	—	—	a	—	—	—
15:1 ω 6	tr	—	0.4	—	0.8	0.1
15:1 ω 5	—	—	a	—	—	a
15:0	0.1	0.3	4.5	—	—	1.0
br16:1 ω 6*	tr	—	3.2	—	—	—
iso16:0	0.2	—	2.0	23.4	0.1	—
16:1 ω 9	0.8	5.9	3.5	—	—	—
16:1 ω 7c	23.9	24.4	13.4	—	37.3	50.1
16:1 ω 7t	0.4	0.6	tr	—	0.5	1.1
16:1 ω 5	1.6	4.8	2.6	—	2.6	1.3
16:0	41.5	34.0	22.2	4.1	47.3	31.8
iso17:1 ω 7	1.0	—	—	—	0.2	—
iso17:0	0.6	—	—	9.6	0.7	—
anteiso17:0	tr	—	—	39.6	—	—
17:1 ω 8	—	—	5.2	—	0.4	tr
17:1 ω 6	—	—	13.3	—	0.6	0.5
cy17:0a(ω 7/8)	18.2	—	—	—	—	—
cy17:0b*	1.1	—	—	—	—	—
17:0	tr	—	7.0	0.8	0.8	0.2
iso18:0	—	—	—	12.9	—	—
18:1 ω 9	0.1	1.0	0.9	—	tr	—
18:1 ω 7c	0.5	24.1	17.0	—	0.8	9.7
18:1 ω 7t	—	0.4	—	—	—	0.2
18:1 ω 5	—	1.4	0.9	—	tr	0.1
18:0	tr	1.2	1.2	7.7	0.4	0.2
iso19:0	—	—	—	0.3	—	—
anteiso19:0	tr	—	0.8	0.9	0.2	tr
cy19:0*	0.3	—	—	—	—	—
Trace components	0.1	0.1	0.1	0.2	0.2	0.3
Branched FAME	5.6	0.5	7.2	87.2	6.0	tr
Unsaturated FAME	28.9	62.6	60.4	0.0	43.4	63.4
μ mol (g dry wt) ⁻¹	81.5	19.8	9.8	13.8	61.5	49.2

interpretation of major differences, the total percentages of branched and unsaturated fatty acids occurring in each strain are presented at the bottom of each table. Due to the constraints of time and materials only one batch culture of each strain and set of conditions was analysed. Previously this laboratory has shown that phospholipid fatty acid profiles of duplicate batch cultured bacteria usually have a standard deviation of less than 1% for each fatty acid (e.g. Edlund *et al.*, 1985).

The sulphide-forming bacteria in this study are clearly distinguished by the presence or absence of 10-methyl fatty acids. Among the strains examined, 10-methyl fatty acids occur exclusively and consistently in the *Desulfobacter* spp. In *Desulfobacter* spp. grown on acetate, 10Me16:0 ranged from 24.7% in *Desulfobacter* sp. 3ac10 to 9.4% in *Desulfobacter* sp. AcBa.

VFA were added to cultures of *Desulfobacter postgatei* 2ac9, *Desulfobacter* sp. AcBa and *Desulfotomaculum acetoxidans* 5575. In these cultures, all even-numbered fatty acids were

Fig. 1. Graph illustrating the distribution of the sulphidogenic bacteria based on percentage unsaturation and percentage branching of their fatty acids. Numbers refer to the culture numbers in Table 1 except for: 10, *Desulfovibrio desulfuricans*; 11, *Desulfovibrio vulgaris*; 12, *Desulfovibrio africanus*. Strains 10–12 are shown for reference; the data for them is from Edlund *et al.* (1985), by kind permission of the authors. Broken lines: sulphur-reducing bacteria.

present in decreased proportions. Those of strain 2ac9 decreased from 67 to 39% with respect to the total, those of strain AcBa from 69 to 47% and those of strain 5575 from 99 to 67%. Simultaneously the branched chain fatty acids increased (2ac9, from 19 to 34%; AcBa, from 12 to 27%; 5575, from 0.5 to 7%).

All the *Desulfobacter* spp. grown on acetate were characterized by even-numbered fatty acid distributions ranging from 77% (3ac10) to 59% (4ac11). 10-Methyl fatty acids other than 10Me16:0 were detected, including 10Me15:0 (2ac9 + VFA), 10Me17:0 (2ac9 ± VFA; AcBa + VFA; 3ac10 – VFA; 4ac11 – VFA) and 10Me18:0 (3ac10 – VFA). Components in insufficient quantity for structural confirmation by GC–MS (2ac9 – VFA; AcBa + VFA; 3ac10) had identical retention times to 10Me15:0; these components are not listed in Table 2.

Cyclopropyl fatty acids are also characteristic of *Desulfobacter* spp. The major component was ω 7/ ω 8 methylenehexadecanoic acid (cy17:0a), which ranged from 30% in 4ac11 to 7% in 3ac10 when the bacteria were grown on acetate without the VFA. The position of the cyclopropyl group in a second methylenehexadecanoic acid (cy17:0b) was not identified; this fatty acid ranged from 0% in AcBa to 3% in 4ac11 and 2ac9 under the same conditions. *Desulfobacter* spp. 2ac9 and AcBa showed a decrease in proportion of cyclopropyl fatty acids when VFA were included in the media (2ac9, from 28 to 18%; AcBa, from 27 to 13%). Other cyclopropyl FAME were also detected including cy16:0 (2ac9 + VFA) and two cyclopropyl 18:0 FAME designated cy18:0a and cy18:0b.

The 'fat vibrio' strain AcKo grown on acetate had 75% even-numbered and 94% straight-chain fatty acids, with high levels of cy17:0 (cy17:0a, 18%; cy17:0b, 1%) similar to the *Desulfobacter* spp.; however, no 10-methyl FAME could be detected.

Desulfotomaculum acetoxidans 5575 grown on acetate gave a distribution of 99% even-numbered and 99% straight-chain fatty acids. Incorporation of VFA into the medium decreased the proportions of even-numbered and straight-chain fatty acids to 68 and 93%, respectively. High levels of 16:1 ω 7c (24%) and 18:1 ω 7c (24%) were characteristic of this organism. Monoenoic fatty acids represented 62% (– VFA) and 60% (+ VFA) of the total. Neither 10-methyl nor cyclopropyl FAME were detected.

Desulfovibrio thermophilus grown on pyruvate had no detectable unsaturated fatty acids. Odd-numbered and branched-chain fatty acids predominated (51 and 87% respectively) in this

species, in contrast to the other organisms. The major fatty acids were iso16:0 and anteiso17:0.

Desulfuromonas acetoxidans 11070, a sulphur-reducing bacterium grown on acetate plus fumarate, exhibited a distribution of 91% even-numbered and 94% straight-chain fatty acids. This organism showed the highest proportions of the fatty acids 16:0 (47%) and 16:1 ω 7c (37%), which are common among all the strains examined except the *Desulfovibrio thermophilus* strain, which lacked 16:1 ω 7c; otherwise, no distinctive components were detected.

The *Campylobacter*-like spirillum strain 5175, which also utilized elemental sulphur as an electron acceptor, was grown on fumarate. It had a distribution of predominantly even-numbered (98%) and straight-chain (99%) fatty acids. The major fatty acids were 16:1 ω 7c (50%) and 16:0 (32%).

Fig. 1 shows a graphic representation of all the organisms examined based on the percentage unsaturation and percentage branching in their fatty acids. Organisms showing homology of morphological and physiological parameters (e.g. the *Desulfobacter* spp.) are grouped. The sulphur-reducing bacteria (*Desulfuromonas* and the spirillum) are encircled with broken lines to distinguish them from the sulphate-reducing bacteria. No other homology of characters in the sulphur reducers is implied.

DISCUSSION

All the strains examined could be differentiated by their respective distributions of phospholipid ester-linked fatty acids.

The *Desulfobacter* spp. exhibited homology of fatty acid biomarkers. High levels of cyclopropyl and 10Me16:0 fatty acids were observed with largely an even-numbered, straight-chain fatty acid distribution after growth on acetate. This is in agreement with Taylor & Parkes (1983), who examined the fatty acids of *Desulfobacter* sp. 3ac10. Our profile for 3ac10 also included low levels of 10Me17:0 and 10Me18:0 (Table 2). These unusual fatty acids, as well as 10Me15:0, were also found in other *Desulfobacter* spp. No other genera contained 10-methyl fatty acids. Kroppenstedt & Kutzner (1978) showed that 10-methyl fatty acids occur in actinomycetes and increase as a function of age; however, unlike *Desulfobacter* spp., 10Me17:0 and 10Me18:0 tend to dominate in these filamentous bacteria.

Some bacterial fatty acid profiles vary in composition according to external stimuli (temperature, pH, nitrogen source, salinity, etc.; Lechevalier, 1976). In order to use specific fatty acid biomarkers to interpret environmental community structure, micro-organisms should be examined for fatty acid patterns and their variation under different conditions. Taylor & Parkes (1983) showed that fatty acid profiles in some sulphate-reducing bacteria can be influenced by carbon source; however, in all cases major fatty acid biomarkers were identifiable. With three separate cultures of *Desulfobulbus* sp. 3pr10 it was shown that growth on propionate produced an odd carbon number straight-chain profile, CO₂/H₂ produced an even carbon number straight-chain profile and lactate an approximately equal distribution of odd and even carbon number and of straight and branched chains. These changes could be explained in terms of the carbon sources being used as fatty acid chain initiators. This study is concerned with the induced variation in fatty acid profiles due to the presence of propionate and branched-chain volatile fatty acids. The latter are formed in sediments and sludges by fermentative breakdown of branched-chain amino acids. With the exception of isobutyrate being oxidized by *Desulfotomaculum acetoxidans* (Widdel & Pfennig, 1981*b*), none of the added VFA could be utilized as electron donor by the sulphate reducers. The present study has shown that interpretation of environmental data using lipids should consider the potential effect of a variety of VFA produced by fermentative bacteria, which may change the fatty acid distributions of various micro-organisms present. Ingram *et al.* (1977) showed that exogenous propionate can be incorporated as a chain initiator into *Escherichia coli* fatty acids during growth on glucose broth, giving rise to odd-numbered chains. It seems likely that the VFA incorporated into strains 2ac9, AcBa and 5575 were used as chain initiators, because all three cultures displayed increased proportions of odd carbon number and branched fatty acids (see Tables 2 and 3). *Desulfobacter* sp. AcBa grown in media containing VFA synthesized anteiso14:0 and anteiso16:0; the

presence of these fatty acids could not be interpreted in terms of any of the VFA being used as chain initiator (Kaneda, 1977).

Carbon sources influenced 10-methyl fatty acids and their straight-chain equivalents in similar ways; their proportion was lowered when odd-numbered and branched VFA were incorporated into the growth medium. As Kroppenstedt & Kutzner (1978) suggest, this implies that 10Me16:0 is formed by methylation at the 10th carbon after initial synthesis of a mono-unsaturated 16-carbon acid. If so, this almost certainly takes place *in situ* to the esterified phospholipid in the membrane (Akamatsu & Law, 1970).

The 'fat vibrio' strain AcKo exhibits both nutritional and fatty acid profile similarities to *Desulfobacter* spp.; however, strain AcKo has no 10-methyl fatty acids.

Desulfotomaculum acetoxidans 5575 grown on acetate exhibited a very different fatty acid distribution compared with that of the *Desulfobacter* spp. or the 'fat vibrio' AcKo. Neither cyclopropyl nor 10-methyl fatty acids were detected. The predominant fatty acids were 16:1 ω 7c, 18:1 ω 7c and 16:0. The presence of substantial amounts of unsaturated fatty acid in *Desulfotomaculum acetoxidans* (62%), *Desulfuromonas acetoxidans* (44%) and the *Campylobacter*-like spirillum (63%), as compared to the other sulphide-forming bacteria, probably does not indicate a physiological or genealogical relationship. Nutritionally, these species are very different; moreover, the sporogenous *Desulfotomaculum acetoxidans* belongs to the *Clostridium* branch of the Gram-positive bacteria, whereas *Desulfuromonas* and the spirillum are Gram-negative (Fowler *et al.*, 1985). Bacteria actually assigned to the genus *Campylobacter* exhibit high levels of unsaturation (approximately 50%), with no branched fatty acids (Blaser *et al.*, 1980), and have fatty acid profiles that are similar to that of the *Campylobacter*-like spirillum 5175 (although strain 5175 exhibited some branched fatty acids).

Desulfovibrio thermophilus, unlike other *Desulfovibrio* spp. (Edlund *et al.*, 1985), exhibited no detectable unsaturated fatty acids; however, 87% were iso or anteiso branched. Silvius & McElhaney (1979a, b) have shown that iso and anteiso branching provide the same effect as unsaturation with respect to membrane fluidity. This could explain why some bacteria have a low degree of fatty acid branching with high unsaturation and others have completely the reverse. Thus the *Campylobacter*-like spirillum and *Desulfovibrio thermophilus* may achieve the same effect by different mechanisms.

The ratios of unsaturation to branching of the fatty acids of different sulphide-forming bacteria can be used to distinguish certain physiological and taxonomic groups (Fig. 1). This does not apply to *Desulfotomaculum acetoxidans* and the sulphur-reducing spirillum, but the presence or absence of certain fatty acids (Table 3) can be used to separate these two organisms.

In this study we have shown that among the sulphide-forming bacteria examined only members of the genus *Desulfobacter* (all acetate utilizers) contain the fatty acid 10Me16:0. Other genera containing 10Me16:0 in their fatty acids include members of the actinomycetales (Kroppenstedt & Kutzner, 1978). However, those members which do exhibit 10-methyl fatty acids have major quantities of 10Me18:0, but not of 10Me16:0. Preliminary attempts to assess the contribution of these actinomycetes to the microbial biomass of marine sediments appear to indicate that they are terrestrial organisms that have been washed into the sea, where they do not contribute to biomass turnover (Goodfellow & Haynes, 1984). Thus the presence of 10Me16:0 and cy17:0, without high levels of 10Me18:0, seems to have the potential to act as a biomarker for *Desulfobacter* spp. in marine sulphate-reducing environments.

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