

Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species

Anders Edlund,* Peter D. Nichols,** Roger Roffey,* and David C. White**

Division of Microbiology, National Defense Research Institute (FOA 4),* S-901 82 Umeå, Sweden, and Department of Biological Science, Florida State University,** Tallahassee, FL 32306

Abstract An analysis of the phospholipid ester-linked and the lipopolysaccharide (LPS) fatty acids and hydroxy fatty acids of six lactate-utilizing *Desulfovibrio*-type sulfate-reducing bacteria (SRB) has been performed using capillary gas-liquid chromatography-mass spectrometry (GLC-MS). The concentrations of normal fatty acids were essentially similar, with the possible exception of a high content of normal fatty acids in the LPS of *Desulfovibrio gigas*. Determination of monounsaturated acid double bond configuration was performed by GLC-MS analysis of the derivatized fatty acids. A total of nine branched chain and eight straight chain monounsaturated fatty acids was detected in the *Desulfovibrio* species analyzed. The major component detected in five *Desulfovibrio* was the 17-carbon iso-branched monoenoic acid which showed *cis* unsaturation [i17:1(n-7)*c*] seven carbons from the terminal methyl group of the fatty acid chain. *D. gigas*, in contrast, contained almost no unsaturated fatty acids and was greatly enriched in iso-branched 15:0. Major differences between strains were found in the phospholipid and LPS hydroxy fatty acids. These components, in addition to the i17:1(n-7)*c* and other characteristic branched chain unsaturated acids, can possibly be utilized as signatures of the lactate-utilizing SRB. —Edlund, A., P. D. Nichols, R. Roffey, and D. C. White. Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. *J. Lipid Res.* 1985. 26: 982-988.

Supplementary key words sulfate-reducing bacteria • fatty acids • phospholipids • taxonomy • capillary gas-liquid chromatography-mass spectrometry

The sulfate-reducing bacteria (SRB) constitute a morphologically diverse group of anaerobic bacteria that share the unique ability of dissimilatory sulfate reduction. Through this process, sulfate is utilized as a terminal electron acceptor for respiration. Until recently, the SRB have been classified into two different genera, *Desulfovibrio* (1), and *Desulfotomaculum* (2). The application of new isolation techniques has greatly expanded the morphological and biochemical diversity of the SRB and has led to suggestions that their systematics should be revised (3-5).

Cellular fatty acid composition has been analyzed in a number of bacterial species and now provides a useful criterion for bacterial taxonomy (6-8). The analysis of

fatty acid composition has also been used in the fields of microbial ecology (9-11) and organic geochemistry (12, 13). The necessity for precise structural determinations (positional and geometrical isomers) of key biological marker fatty acids has been stressed in order to understand aspects of their biosynthesis and to increase their taxonomical value (14, 15). Although isomer separation and characterization techniques have been known for some time, microbiologists have rarely applied them to extracts of microorganisms. Two relatively simple methods for determination of fatty acid double bond position and geometry in extracts of microorganisms and sediments have recently been reported (16-18). The cellular fatty acids of a number of *Desulfovibrio* species have been previously reported (15, 19-21) and the species were divided into two major groups based on the profiles (19, 20). In addition to saturated fatty acids, branched-chain monounsaturated fatty acids accounted for significant proportions of the fatty acid profile. Of these previous studies, double bond positions were only determined by Boon et al. (15) for *Desulfovibrio desulfuricans* and by Taylor and Parkes (21). One aim of this study is to determine whether the specific branched chain monoenoic fatty acids, i17:1(n-7), i15:1(n-7), and i19:1(n-7), detected in *D. desulfuricans* are, in fact, present in other *Desulfovibrio* species.

In the present study, the ester-linked phospholipid and lipopolysaccharide (LPS) fatty acid and hydroxy fatty acid composition of *Desulfovibrio* species are examined. Double bond configuration of the major monounsaturated fatty acids has been determined. The fatty acid profiles obtained will provide information that can be utilized to recognize and quantify the contribution of this group of SRB in environmental samples. Such samples include petroleum storage tanks where SRB play a role in

Abbreviations: LPS, lipopolysaccharide; SRB, sulfate-reducing bacteria; GLC-MS, gas-liquid chromatography-mass spectrometry; FAME, fatty acid methyl esters.

the biodeterioration of the fuel and for stimulating microbial corrosion processes (22). The significance of the fatty acid profiles as a taxonomical criterion is discussed.

MATERIALS AND METHODS

Microorganisms and cell preparations

The bacteria analyzed in this study were *Desulfovibrio desulfuricans* NCIB 8307, *D. vulgaris* NCIB 8303, *D. africanus* NCIB 8401, and *D. gigas* ATCC 29494. Two unidentified strains of SRB, 3801 and 3794, isolated from sediment samples from rock caverns used for long-term storage of jet aviation fuel, were also included in the study. Purification and identification of these strains as SRB were conducted as described by Postgate (23). Bacteria were cultivated at 30°C for 5 days using Postgate's medium B (23). The cells were harvested from stationary phase by centrifugation, washed three times with 0.9% (v/w) saline, and lyophilized.

Lipid extraction

Fifty mg of lyophilized cultures were placed in a 250-ml separatory funnel and lipids were quantitatively extracted with the modified one-phase chloroform-methanol system of the Bligh and Dyer (24) extraction procedure (25). The lipids were recovered in the chloroform phase, the solvents were removed in vacuo, and the lipids were stored under nitrogen at -20°C. Duplicate extractions of each bacterial sample were performed.

Column chromatography

The lipid was transferred in a minimal volume of chloroform to a silicic acid column (Unisil, 100-200 mesh, Clarkson Chemical Co., Inc., Williamsport, PA) and fractionated into neutral lipids, glycolipids, and phospholipids by elution with 5 ml of chloroform, acetone, and methanol, respectively (26). The fractions were collected in test tubes equipped with Teflon-lined screw-caps and dried under a stream of nitrogen. The phospholipids were used for further studies.

Acid methanolysis

For methanolysis, the phospholipid fraction from the silicic acid column was dissolved in 1 ml of methanol-chloroform-concentrated HCl 10:1:1 (v/v/v) and then heated for 1 hr at 100°C. After addition of 1 ml of chloroform and 1 ml of water with thorough mixing, the chloroform layer was recovered and evaporated under a stream of nitrogen, and the fatty acid methyl esters (FAME) were stored at -20°C.

Thin-layer chromatography

The FAME mixture was spotted, chromatographed (Whatman K6 silica gel, 250 µm thick, 20 × 20 cm) with

development in hexane-diethyl ether 1:1 (v/v). The bands containing the methylated fatty acids and hydroxy fatty acids were collected and were eluted with 5 ml of chloroform and 5 ml of chloroform-methanol 1:1 (v/v), respectively. (Authentic standards of methyl nonadecanoate and 12-hydroxystearate were developed separately on both sides of the plate and visualized under ultraviolet light after spraying with 0.1% rhodamine 6G.) The solvent was removed under a stream of nitrogen, and the methyl esters were dissolved in hexane for gas-liquid chromatographic analysis. Methyl nonadecanoate was added as internal standard.

Isolation of LPS lipids

Fatty acids bound to the lipid-extracted residue of the Bligh-Dyer extraction were recovered by acidifying the residue in 250 ml of 1 N HCl. After refluxing at 100°C for 3 hr and cooling, the contents were transferred to a separatory funnel with washes of 25 ml, and 2 × 5 ml of chloroform. The two phases were allowed to separate; the chloroform phase was drawn off and the solvent was removed. The LPS fatty acids were then methylated and separated into FAME and hydroxy FAME as described above.

Gas-liquid chromatography (GLC)

A Varian model 3700 gas chromatograph equipped with a flame ionization detector was used for analyses of fatty acid methyl esters. Samples were injected at 80°C in the splitless mode on a nonpolar cross-linked methyl silicone fused silica capillary column (50 m × 0.2 mm i.d., Hewlett Packard). The oven temperature was programmed from 80°C to 140°C at 20°C/min, then at 4°C/min to 260°C which was maintained for 7 min. Hydrogen was used as the carrier gas and the injector and detector temperatures were 250°C and 290°C, respectively. Hydroxy fatty acids were analyzed as above with the exception that the final temperature was 270°C.

Formation of trimethylsilyl ethers

Hydroxy FAME were converted to their corresponding trimethylsilyl ethers using N,O-bis-(trimethylsilyl) trifluoroacetamide (Pierce Chemical Co., Rockford, IL). After heating for 1 hr at 60°C, excess reagent was removed under a stream of nitrogen, and the sample was dissolved in hexane for GLC and GLC-MS analysis.

Gas-liquid chromatography-mass spectrometry (GLC-MS)

GLC-MS analyses were performed on a Hewlett-Packard 5995A system fitted with a direct capillary inlet. The same column type as described above was used for analyses. Samples were injected in the splitless mode at 100°C with a 0.5 min venting time, after which the oven temperature was programmed from 100°C to 300°C at

4°C/min. Hydrogen was used as the carrier gas. MS operating parameters were: electron multiplier 1600 volts, transfer line 300°C, source and analyzer 250°C, autotune file DFTPP normalized, optics tuned at m/z 502, MS peak detect threshold = 300 triggered on total ion abundance, electron impact energy = 70 eV.

Double bond configuration

Double bond position and geometry were determined by capillary GLC-MS analysis of the Diels-Alder adducts formed by reaction of the FAME with diene 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene (Aldrich Chemical Co., Milwaukee, WI). Detailed descriptions of the derivatization procedure and interpretation of the mass spectra of the adducts have been reported elsewhere (17, 18, 27).

Fatty acid designation

Fatty acids are designated as number of carbon atoms:number of double bonds. The position (x) of the double bond nearest the terminal methyl group of the fatty acid chain is indicated in the form (n-x) where n indicates the number of carbons in the chain. The prefixes "i" and "a" refer to iso and anteiso branching, respectively, and the prefix OH indicates a hydroxyl group at the position indicated from the carboxyl end.

RESULTS

Phospholipids and LPS fatty acid concentrations

The concentrations of phospholipid ester-linked normal and hydroxy fatty acids in the *Desulfovibrio* species are essentially equivalent (Table 1). LPS normal fatty acids were 4-12% of the total fatty acids with one exception, *D. gigas*, where the molar concentration of LPS normal fatty acids was 27% of the total acids. The LPS hydroxy acids in the six bacteria, in contrast, ranged from 0.05% to as much as 5% of the total acids. The concen-

trations of the fatty acids in the two unidentified isolates were comparable to the other species analyzed.

Fatty acids

The phospholipid and LPS fatty acid components of the pure cultures of four different *Desulfovibrio* species and of the two unidentified SRB were in the C14-C19 range (Table 2 and Table 3) commonly found in bacteria (6, 7). Three of the *Desulfovibrio* species, *D. desulfuricans*, *D. vulgaris*, and *D. africanus*, showed similar profiles of both phospholipid and LPS fatty acids. These bacteria contained a high proportion of monounsaturated acids in the phospholipid fraction with i17:1(n-7)c, 16:1(n-7)c, and 18:1(n-7)c as the most abundant acids. A total of nine branched chain and eight straight chain monounsaturated fatty acids were detected in these bacteria (Tables 2 and 3). There was insufficient material for a number of the minor components for positive identification of the double bond configuration. The major saturated fatty acids were i15:0, 16:0, and i17:0. In the bound fatty acid fraction, an increase of saturated acids was observed with 16:0, 18:0, and i17:0 as the major acids. The two unidentified SRB showed acid profiles comparable to the three *Desulfovibrio* listed above. These data indicate that they are probably related to these species. *D. gigas*, in contrast to the other *Desulfovibrio* species, exhibited a markedly different fatty acid profile with almost only saturated fatty acids in both the phospholipid and LPS fatty acid fractions (Tables 2 and 3). The major fatty acids detected were i15:0, a15:0, 16:0, and i17:0.

Hydroxy fatty acids

The hydroxy fatty acid compositions for the six strains of sulfate-reducing bacteria are presented in Table 4. A total of six hydroxy fatty acids was detected in these *Desulfovibrio*. Differences between the LPS and phospholipid hydroxy fatty acid compositions of each of the SRB are apparent, as well as differences in the hydroxy fatty acid compositions between the strains.

TABLE 1. Concentration of phospholipid and lipopolysaccharide fatty acids in *Desulfovibrio* species

Organism	Phospholipid		Lipopolysaccharide	
	Normal	Hydroxy	Normal	Hydroxy
	<i>nmol/g dry wt</i>			
<i>Desulfovibrio desulfuricans</i>	15640 (1300)	30 (2)	650 (178)	9 (2)
<i>Desulfovibrio vulgaris</i>	7450 (1110)	21 (1)	650 (43)	89 (15)
<i>Desulfovibrio africanus</i>	10350 (985)	46 (13)	840 (200)	340 (6)
Isolate 3801	8050 (143)	25 (4)	405 (150)	103 (14)
Isolate 3794	8700 (360)	44 (8)	995 (170)	499 (16)
<i>Desulfovibrio gigas</i>	9800 (1010)	7 (1)	2650 (135)	11 (1)

Values are averages of two separate analyses and are given as the mean (SD).

TABLE 2. Extractable nonhydroxy fatty acid composition (mol %) of *Desulfovibrio* species

Fatty Acid	<i>D. desulfuricans</i>	<i>D. vulgaris</i>	<i>D. africanus</i>	Isolate 3801	Isolate 3794	<i>D. gigas</i>
i14:0	Tr	Tr	Tr	0.3 (0.1)	Tr	0.2 (0.1)
14:0	0.2 (0.1)	0.3 (0.1)	0.4 (0.1)	0.2 (0.1)	0.2 (0.1)	4.3 (0.1)
i15:0	20.6 (0.8)	12.5 (1.2)	15.8 (0.7)	10.0 (0.8)	7.3 (0.1)	58.1 (0.5)
a15:0	3.0 (0.1)	2.2 (0.2)	5.2 (0.3)	15.0 (1.2)	3.8 (0.1)	9.1 (0.4)
15:0	0.6 (0.1)	0.6 (0.1)	0.4 (0.1)	0.3 (0.1)	0.1 (0.1)	2.7 (0.1)
i16:0	0.8 (0.1)	0.8 (0.1)	1.2 (0.1)	5.5 (0.1)	1.0 (0.1)	0.1 (0.1)
16:0	17.5 (0.5)	22.1 (0.4)	14.5 (0.1)	9.1 (0.4)	10.7 (0.1)	12.0 (0.2)
i17:0	8.5 (0.2)	8.1 (0.2)	8.1 (0.1)	4.2 (0.2)	5.6 (0.1)	9.7 (0.4)
a17:0	1.5 (0.1)	2.3 (0.1)	3.8 (0.1)	2.9 (0.1)	4.8 (0.1)	1.1 (0.1)
17:0	1.3 (0.1)	1.9 (0.1)	0.8 (0.1)	0.8 (0.1)	1.6 (0.1)	0.7 (0.1)
i18:0	Tr	Tr	Tr	Tr	Tr	Tr
18:0	0.9 (0.1)	3.1 (0.1)	5.2 (0.1)	0.5 (0.1)	14.1 (0.1)	0.4 (0.1)
i19:0	Tr	Tr	Tr	ND	ND	ND
a19:0	Tr	ND	Tr	ND	Tr	ND
i20:0	Tr	Tr	Tr	ND	ND	Tr
20:0	ND	ND	Tr	ND	Tr	ND
22:0	0.4 (0.4)	0.2 (0.2)	Tr (0.1)	0.1 (0.1)	0.1 (0.1)	Tr
i15:1(n-7) _c	0.5 (0.1)	0.4 (0.1)	1.1 (0.1)	0.1 (0.1)	0.9 (0.1)	ND
a15:1(n-7) _c	Tr	Tr	0.2 (0.1)	Tr	0.1 (0.1)	ND
i16:1 ^a	ND	ND	Tr	Tr	Tr	Tr
i16:1(n-6) _c	0.5 (0.1)	0.6 (0.1)	1.0 (0.1)	7.8 (0.1)	1.2 (0.1)	ND
16:1(n-7) _c	8.3 (0.1)	11.1 (0.1)	6.1 (0.1)	7.7 (0.1)	6.3 (0.1)	0.3 (0.1)
16:1(n-5) _c	0.2 (0.1)	0.4 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	ND
16:1 ^a	Tr	0.2 (0.1)	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)	Tr
i17:1(n-7) _c	32.9 (0.2)	27.9 (0.6)	25.2 (0.1)	23.1 (0.7)	20.7 (0.1)	0.2 (0.1)
a17:1(n-7) _c	0.7 (0.1)	1.8 (0.1)	4.5 (0.1)	7.9 (0.2)	5.3 (0.1)	Tr
17:1(n-6) _c	0.2 (0.1)	0.5 (0.1)	0.3 (0.1)	0.9 (0.1)	0.9 (0.1)	ND
i18:1(n-6) _c	Tr	Tr	Tr	0.9 (0.1)	0.2 (0.1)	ND
18:1(n-9) _c	Tr	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.4 (0.1)	0.8 (0.1)
18:1(n-7) _c	0.8 (0.1)	2.6 (0.1)	5.2 (0.1)	1.6 (0.1)	13.4 (0.2)	Tr
18:1 ^a	Tr	Tr	Tr	Tr	0.2 (0.1)	ND
i19:1(n-7) _c ^e	Tr	Tr	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	ND
a19:1(n-7) _c ^e	ND	ND	Tr	Tr	0.1 (0.1)	ND
19:1 ^a	0.1 (0.1)	0.1 (0.1)	Tr	ND	0.2 (0.1)	Tr

Values are expressed as mol % of total extractable fatty acids and are given as the mean (SD) of two separate analyses. ND, not detected; Tr, <0.1%.

^aDouble bond position not confirmed.

DISCUSSION

Fatty acids

D. desulfuricans, *D. vulgaris*, and *D. africanus* and the two unidentified *Desulfovibrio* strains contained high proportions of unsaturated fatty acids with i17:1(n-7)_c, 16:1(n-7)_c, and 18:1(n-7)_c as the most abundant acids. Other branched odd chain fatty acids identified included i15:1(n-7)_c and i19:1(n-7)_c. An increase in saturated fatty acids is seen in the LPS fatty acid fraction. The dominance of i17:1(n-7)_c for the *Desulfovibrio* species, apart from *D. gigas*, is in agreement with analyses reported for *D. desulfuricans* strains isolated from the Dutch Wadden Sea (15) and by Scottish workers (21). The similarity of the fatty acid profiles for this range of *Desulfovibrio* indicates that these specific components have the potential to be used as signature lipids in environmental studies. These data also emphasize the need for derivatization procedures to be performed in order that fatty acid biosynthesis and taxonomic aspects can be better understood.

The two unidentified sulfate-reducing isolates showed fatty acid compositions similar to *D. desulfuricans*, *D. vulgaris*, and *D. africanus*. The fatty acid profile of isolate 3794 resembled the composition of *D. africanus* suggesting, based on fatty acid data alone, that this isolate may belong to this species. Further morphological and biochemical studies on this strain must, however, be performed before this relationship can be confirmed.

The influence of substrate on the fatty acid composition of *Desulfobulbus sp.* and *Desulfovibrio desulfuricans* has been determined (21). Although changes in the medium of *Desulfobulbus sp.* produced highly significant variations in the fatty acid profiles, a similar trend was not observed for *D. desulfuricans* (21). The fatty acid distribution of *D. desulfuricans* also seems to be independent of growth phase, as the fatty acid distribution reported for batch growth is almost identical to that observed for the organism in continuous culture (21, 28). It was not the purpose of this study to monitor the effects of variation in growth substrate or phase of isolation, however, the existing litera-

TABLE 3. Lipopolysaccharide nonhydroxy fatty acid composition (mol %) of *Desulfovibrio* species

Fatty Acid	<i>D. desulfuricans</i>		<i>D. vulgaris</i>		<i>D. africanus</i>	Isolate 3801	Isolate 3794	<i>D. gigas</i>	
i14:0	0.1	(0.1)	0.2	(0.1)	Tr	0.2 (0.1)	0.2 (0.1)	0.2	(0.1)
14:0	2.1	(1.2)	4.4	(0.1)	1.7 (1.0)	4.1 (1.1)	2.1 (0.4)	4.4	(0.1)
i15:0	1.7	(0.2)	1.6	(0.1)	1.5 (0.1)	4.7 (1.2)	2.5 (1.0)	45.4	(2.4)
a15:0	1.0	(0.3)	2.1	(0.5)	1.5 (0.5)	3.7 (0.9)	1.9 (0.5)	10.6	(0.6)
15:0	2.6	(1.5)	3.6	(0.3)	1.8 (0.7)	3.5 (1.5)	1.7 (0.1)	2.7	(0.1)
i16:0	0.6	(0.1)	0.6	(0.1)	0.8 (0.1)	1.5 (0.3)	1.2 (0.4)	0.1	(0.1)
16:0	24.2	(1.7)	28.8	(4.4)	27.1 (4.8)	34.6 (3.0)	26.6 (2.1)	13.9	(0.1)
i17:0	32.8	(9.6)	10.2	(3.0)	7.7 (2.8)	4.5 (0.4)	6.0 (2.4)	11.8	(1.2)
a17:0	2.3	(0.2)	2.5	(0.2)	2.8 (0.2)	2.0 (0.1)	2.9 (0.7)	1.5	(0.1)
17:0	2.1	(0.1)	1.9	(0.2)	1.8 (0.1)	2.4 (0.2)	1.9 (0.4)	1.1	(0.2)
i18:0	Tr		Tr		0.2 (0.1)	ND	0.1 (0.1)	0.1	(0.1)
18:0	6.6	(1.1)	10.9	(3.7)	18.1 (3.2)	16.1 (3.0)	15.9 (0.3)	1.9	(1.0)
i19:0	0.9	(0.5)	ND		0.7 (0.2)	ND	0.5 (0.2)	ND	
a19:0	ND		ND		0.2 (0.1)	ND	0.2 (0.1)	ND	
i20:0	0.4	(0.1)	0.9	(0.2)	0.5 (0.2)	1.4 (0.4)	0.5 (0.2)	Tr	
20:0	0.5	(0.1)	0.6	(0.1)	0.6 (0.1)	0.7 (0.1)	2.0 (0.9)	Tr	
22:0	0.8	(0.1)	3.4	(0.7)	2.5 (1.3)	3.0 (0.1)	3.5 (1.3)	ND	
i15:1(n-7) ^c	1.5	(0.5)	2.3	(1.5)	4.2 (1.6)	3.0 (1.7)	1.8 (0.4)	0.7	(0.4)
a15:1(n-7) ^c	0.3	(0.2)	0.7	(0.1)	0.1 (0.1)	ND	0.3 (0.1)	ND	
i16:1 ^a	ND		ND		1.1 (0.5)	0.7 (0.4)	0.4 (0.1)	0.1	(0.1)
i16:1(n-6) ^c	ND		0.9	(0.2)	0.3 (0.1)	ND	Tr	ND	
16:1(n-7) ^c	6.0	(2.2)	7.0	(0.5)	1.8 (1.0)	1.8 (0.8)	2.2 (0.7)	0.4	(0.2)
16:1(n-5) ^c	0.4	(0.1)	0.4	(0.1)	0.5 (0.3)	ND	0.5 (0.1)	Tr	
16:1 ^a	Tr		0.2	(0.1)	0.1 (0.1)	ND	0.1 (0.1)	Tr	
i17:1(n-7) ^c	4.4	(1.3)	8.1	(2.9)	10.6 (3.7)	4.7 (1.1)	5.2 (1.8)	1.4	(0.5)
a17:1(n-7) ^c	1.7	(0.2)	2.0	(0.4)	1.0 (0.6)	0.9 (0.4)	2.0 (1.2)	0.2	(0.1)
17:1(n-6) ^c	0.1	(0.1)	0.4	(0.1)	0.2 (0.1)	0.3 (0.1)	0.1 (0.1)	Tr	
i18:1(n-6) ^c	ND		0.3	(0.1)	ND	ND	ND	ND	
18:1(n-9) ^c	4.6	(1.9)	4.3	(0.4)	3.0 (1.1)	3.6 (1.7)	4.6 (1.9)	2.0	(0.1)
18:1(n-7) ^c	1.3	(0.1)	1.2	(0.6)	5.0 (1.6)	2.7 (1.3)	6.0 (2.3)	1.0	(0.3)
18:1 ^a	0.7	(0.3)	0.5	(0.3)	1.0 (0.6)	ND	0.6 (0.1)	0.1	(0.1)
i19:1(n-7) ^c	ND		ND		ND	ND	0.1 (0.1)	ND	
a19:1(n-7) ^c	ND		ND		ND	ND	0.9 (0.1)	ND	
19:1 ^a	0.2	(0.1)	ND		Tr	ND	0.5 (0.1)	Tr	

Values are expressed as mol % of total lipopolysaccharide fatty acids and are given as the mean (SD) of two separate analyses. ND, not detected; Tr, <0.1%.

^aDouble bond position not confirmed.

TABLE 4. Extractable and Lipopolysaccharide hydroxy fatty acid composition (mol %) of *Desulfovibrio* species

Hydroxy Fatty Acid	<i>D. desulfuricans</i>		<i>D. vulgaris</i>		<i>D. africanus</i>	Isolate 3801	Isolate 3794	<i>D. gigas</i>	
Extractable									
β-OHi15:0	12.4	(1.6)	22.1	(1.7)	30.2 (2.9)	28.8 (1.1)	35.7 (1.4)	53.3	(5.5)
β-OHa15:0	ND		ND		10.9 (0.5)	20.0 (0.8)	20.6 (0.1)	8.6	(2.8)
β-OHi16:0	31.5	(0.5)	27.5	(0.2)	16.6 (5.3)	9.2 (1.3)	13.3 (0.5)	38.1	(6.7)
β-OHi17:0	38.4	(4.8)	27.9	(1.0)	20.2 (4.2)	42.0 (0.9)	9.4 (0.6)	ND	
β-OHi17:0	13.0	(3.0)	12.1	(2.9)	16.3 (5.8)	ND	6.2 (0.5)	ND	
β-OHi18:0	4.7	(1.9)	10.5	(0.4)	5.7 (1.7)	ND	14.8 (1.6)	ND	
Lipopolysaccharide									
β-OH:15:0	ND		52.0	(0.8)	55.2 (1.5)	37.2 (1.1)	57.5 (0.1)	30.2	(1.0)
β-OHa15:0	ND		11.7	(0.8)	9.8 (0.1)	10.0 (0.5)	12.1 (0.1)	12.8	(3.4)
β-OHi16:0	ND		14.2	(1.1)	12.6 (0.2)	5.8 (0.6)	16.7 (1.3)	16.1	(4.3)
β-OHi17:0	100		22.1	(0.5)	18.7 (0.8)	47.0 (1.1)	10.8 (1.2)	17.1	(1.8)
β-OHi17:0	ND		ND		3.1 (0.6)	ND	2.8 (0.6)	23.8	(1.7)
β-OHi18:0	ND		ND		0.7 (0.1)	ND	ND	ND	

Values are expressed as mol % of total extractable hydroxy fatty acids and of total lipopolysaccharide hydroxy fatty acids and are given as the mean (SD) of two separate analyses. ND, not detected.

ture suggests that these parameters did not influence the fatty acid composition of the *Desulfovibrio*-type SRB analyzed here.

The fatty acid profiles obtained for the five organisms listed above indicate that the specific odd chain branched monounsaturated fatty acids previously found in *D. sulfuricans* are more general "biological markers" or "signatures" for *Desulfovibrio*-type SRB, apart from *D. gigas*. The phospholipid ester-linked fatty acid composition of *D. gigas* differed from the other *Desulfovibrio* species. This has been reported earlier (20) and these data are consistent with the doubtful taxonomic position of *D. gigas* as a *Desulfovibrio* (19). The absence of monounsaturated fatty acids and the high proportion of i15:0 (58% of the total phospholipid ester-linked fatty acids) is characteristic for this bacterium. The fatty acid composition of *D. gigas* reported here is in general agreement with the data of Ueki and Suto (19), although several differences were apparent. The Japanese workers reported a15:0 as the most abundant fatty acid and they also detected some straight chain unsaturated acids (25% of the total). In this study, *D. gigas* strain contained only trace amounts of monounsaturated acids.

Hydroxy fatty acids

Hydroxy fatty acids occur in the lipid A of gram-negative bacteria (29) and thus are not normally considered to be extractable lipids. Hydroxy fatty acids were found, however, in both the phospholipid and LPS fatty acid of the *Desulfovibrio* strains studied. The presence of extractable ester-linked hydroxy acids in the phospholipid of *D. desulfuricans* has been reported (15). This observation, when taken together with the detection of extractable hydroxy acids in all *Desulfovibrio* analyzed in this study, may be a more general feature of SRB. The phospholipid hydroxy acid composition of *D. desulfuricans* is similar to that reported by Boon et al. (15). This reproducibility and the fact that each species showed a different hydroxy fatty acid composition could be of taxonomic value. ■■

This work was partially supported by the National Board of Economic Defense, ÖEF, Sweden, and by contracts N 00014-82-C-0404 and N 00014-83-K0056 from the Department of the Navy, Office of Naval Research, USA. C. Mancuso and N. Dowling are thanked for helpful comments during manuscript preparation. C. Antworth is thanked for performing GLC-MS analyses.

Manuscript received 4 January 1984.

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