FEMS Microbiology Ecology 73 (1990) 231–238
Published by Elsevier



FEMSEC 00255

# Unusual and very-long-chain fatty acids in *Desulfotomaculum*, a sulfate-reducing bacterium

T. Řezanka [1], M.Yu. Sokolov [2] and I. Víden [3]

[1] *Institute of Microbiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia,* [2] *Institute of Microbiology, U.S.S.R. Academy of Sciences, Moscow, U.S.S.R., and* [3] *Institute of Chemical Technology, Prague, Czechoslovakia*

Received 10 July 1989
Revision received 31 October 1989
Accepted 2 November 1989

Key words: Gas chromatography-mass spectrometry; Sulfate bacteria; Very-long-chain fatty acids

## 1. SUMMARY

Fatty acids of two strains of sulfate-reducing, sporeforming bacteria *Desulfotomaculum ruminis* 21 and *D. species* 43 were analysed. In addition, the common fatty acids, very-long-chain fatty acids up to $C_{34}$, including saturated acids with straight chains were identified. α-Hydroxy and α,ω-dicarboxylic acids with more than 20 carbon atoms were also detected. None of the above-mentioned compounds have so far been described in sulfate-reducing bacteria and, therefore, their presence offers a new concept of biosynthesis of these compounds and chemotaxonomy of the sulfate-reducing bacteria.

## 2. INTRODUCTION

Sulfate-reducing bacteria are an interesting group of microorganisms capable of dissimilatory sulfate respiration, a process whereby inorganic sulfur compounds are utilized as terminal acceptors of respiration. As a result of this electron transport, sulfides are the normal end products.

In order to determine chemotaxonomic relationships among individual bacterial species, the structure of compounds used for their taxonomic classification (i.e. fatty acids in this case) must be precisely established. Thus, the chain length and branching, as well as the number and position of the unsaturated bonds must be determined. Gas chromatography-mass spectrometry (GC-MS) with a capillary column is needed for their identification.

In a sulfate-reducing bacteria of the species *Desulfovibrio,* fatty acids (FA) ($C_{15-19}$), iso-, anteiso- and straight-chain both saturated and monoenoic [1] were found. Position isomers of the straight-chain and branched monoenoic FA could be detected. $C_{15-18}$ β-hydroxy acids, straight-chain, iso- and anteiso, were also present.

In *D. desulfuricans* branched monoenoic FA were found; ai-17:1 is thought to be a biological marker of the presence of some sulfate-reducing bacteria in sediments [2]. In addition, β-hydroxy and branched monoenoic FA were reported to

*Correspondence to*: T. Řezanka, Institute of Microbiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czechoslovakia.

occur in the genus *Desulfovibrio*; none of the FA was longer than $C_{19}$ [3].

In the genus *Desulfobulbus*, acids with an odd and even number of carbon atoms (saturated and monoenoic) were found; surprisingly, however, no branched acids (saturated or monoenoic) could be detected [4].

A few authors have studied the content of FA in the genus *Desulfotomaculum* and found that it did not differ very much from that of other genera and species of sulfate-reducing bacteria [5,6].

The occurrence of very-long-chain fatty acids (VLCFA) in sulfate-reducing bacteria has not been reported. Although these acids were detected in sediments, they were assumed to be higher plant residues [6–9]. In bacterial samples isolated from these sediments, no FA longer than $C_{19}$ were found in the cells after subsequent growth. The work of Taylor and Parkes [7] who showed a chromatogram with peaks having a retention time longer that of $C_{18}$ is an exception in this respect. Therefore, we tried to demonstrate VLCFA in two species of the genus *Desulfotomaculum* by means of the capillary GC-MS.

## 3. MATERIALS AND METHODS

### *3.1. Bacterial isolates and culture conditions*

The sulfate-reducing sporeforming bacteria *Desulfotomaculum ruminis* strain 21 and *Desulfotomaculum sp.* strain 43 were isolated from saline soils in Kazakhstan (U.S.S.R.). The strains were cultivated on a growth medium containing (g/l): $Na_2SO_4$ (3); $KH_2PO_4$ (0.3); $CaCl_2 \cdot 2H_2O$ (0.225); $NH_4Cl$ (0.3); KCl (0.2); NaCl (1); $MgCl_2 \cdot 6H_2O$ (0.5); trace element solution containing Fe, Zn, Mn, B, Ni, Co, Cu, Mo [10]; vitamin solution containing *p*-amino-benzoic acid, biotin, nicotinamide, pantothenic acid, thiamine, pyridoxine, folic acid [11] and sodium lactate (20 mmol/l). The initial pH was 7 and was adjusted by sodium carbonate. In addition, sodium dithionite (20 mg/l) and rezazurin (indicator of $O_2$) were also added. The cultures were grown in 10-l vessels at 30°C.

The cells were harvested by centrifugation at various time intervals and washed with a phosphate buffer (0.01 mol/l, pH 7). After lyophilization, fatty acids were prepared by alkaline hydrolysis and reaction of free acids with $BF_3$-methanol [12]. Pyrrolidides were prepared according to Andersson [13].

### *3.2. GC-MS identification*

Methyl esters were identified in a Shimadzu QP-1000 apparatus equipped with a fused silica capillary column (60 m × 0.32 mm ID/SPB-1; Supelco, U.S.A.); splitless injection, carrier gas He. Oven temperature was programmed from 100 to 320°C at a rate of 4°C/min; Ionization energy 70 eV, electron multiplier voltage 2.5 kV. Pyrrolidides were chromatographed under identical conditions.

### *3.3. Gas chromatography*

Position isomers were separated on an 120-m capillary column (two linked 60-m Supelcowax 10 columns, ID 0.25 mm) in a Hewlett-Packard 5890 apparatus (Hewlett-Packard, U.S.A.) separation conditions: carrier gas hydrogen; linear velocity 35 cm/s; injector temperature (splitless) 270°C; detector temperature, 280°C; oven temperature was programmed from 150 to 250°C at 1°C per min.

## 4. RESULTS AND DISCUSSION

The two strains used (21 and 43) were grown under identical conditions. Table 1 shows growth yields at various cultivation times. The amounts of biomass and fatty acids (both absolute and relative) increased with increasing cultivation time. The content of fatty acids reached 20 to 25% of dry biomass in the late stationary phase. This amount is higher than that reported for the total lipids in the literature (14.3%, [3]; 3.0%, [6]). The difference can be partially explained by differences in treatment of the biomass samples; alkaline hydrolysis liberates all FA bound by the ester bond.

We were able to separate and identify more than 70 peaks representing both the usual FA, typical of sulfate-reducing bacteria, and acids that have not yet been reported in these bacteria. First

Table 1

Content of dry weight, fatty acids and morphological exterior

| Time of harvest (h) | *D. species* 43 | | | *D. ruminis* 21 | | |
|---|---|---|---|---|---|---|
| | Dry weight (mg) | FA (mg) | % | Dry weight (mg) | FA (mg) | % |
| 24 [a] | 270 | 55 | 20.3 | 220 | 35 | 15.9 |
| 48 [b] | 440 | 95 | 21.6 | 670 | 110 | 16.4 |
| 72 [c] | 1140 | 315 | 27.6 | 630 | 125 | 19.8 |

[a] Mid-exponential growth phase, dividing exponential cells in pairs.

[b] Early stationary phase, single non-dividing cells some of them with prospores.

[c] Late stationary phase, cells single not-dividing, about 80% with mature spores inside, non-synchronous, with or without prospores.

of all, straight-chain, iso-, anteiso- and mono-unsaturated FA were identified. The mono-unsaturated FA were separated on a capillary column and several position and geometric isomers were obtained. Until now two [5] or three [6] positional isomers have been reported in the genus *Desulfotomaculum*. We observed three isomers of both $C_{16}$ and $C_{18}$ FA. A further separation of $C_{18}$ monoenoic acids was accomplished on a 120-m capillary column and as many as five isomers were identified. On the basis of mass spectra of their pyrrolidides, the structures *cis* 9-, 11-, 13-18 : 1 and *trans*-11-18 : 1 were proved. The structure of the fifth isomer was not determined, however, retention characteristics give evidence in favour of 15-18 : 1. Surprisingly, no monoenoic branched FA of the type i-17 : 1 or ai-17 : 1 were found in our samples; they are considered a taxonomic marker of the genera *Desulfovibrio* and *Desulfotomaculum* [2,3,5,6]. Even the single ion monitoring (SIM) with the detection limit around 0.001% of the total injected sample and the scanning of the abundance of the ions m/z 282 ($M^+$) 253 (M-29 = M-$C_2H_5$) and 250 (M-32 = M-MeOH) (i.e. conspicuously intensive peaks in the region of the molecular ion) failed to detect these acids.

To our best knowledge, acids longer than $C_{20}$ have not yet been detected in sulfate-reducing bacteria and, therefore, the presence of very long acids (i.e. the compounds with more than 24 carbon atoms in the chain) is quite surprising. Their presence could, however, be anticipated on the basis of a chromatogram published by Taylor and Parkes [7] showing peaks with a retention time longer than that for $C_{18}$ and, also of the fact that these compounds are normally found in the sediments [8–10]. Therefore, the finding cannot be considered accidental (cf. Fig. 1). By using a previously developed technique [14], we could increase the sample amount by about one order of magnitude, which resulted in an increase of the abundance of the minor peaks shown in Table 2. Certain ions are characteristic of the acid methyl esters (base peaks). The following respective ions are specific for saturated and monoenoic FAME, and for methyl esters of $\alpha,\omega$-dicarboxylic acids: m/z 74, m/z 55 and m/z 98. FA up to $C_{34}$ (tetratriacontanoic acid) were present (Fig. 1). They were identified on the basis of their retention characteristics and their mass spectra. Iso and anteiso acids were also detected. The fact that position isomers of monoenoic acids were found is not surprising, as several isomers of their precursors were detected as well. Unfortunately, due to the chromatographic behavior of pyrrolidides, we could not identify the position of the unsaturated bond in the homologues with more than 24 carbon atoms. In spite of that, on the basis of their biosynthesis and chromatographic behavior, we predict the existence of the unsaturated bond in position w-9 or w-7 of the longer homologues.

In contrast to the papers published so far [1,3,5], no hydroxy acids up to $C_{20}$ (OH-FA) were detected in our strains. These compounds are characteristic of the lipopolysaccharides of sulfate-reducing bacteria. On the other hand, we identified very long OH-FA with a hydroxy group in position 2. The above authors identified the hydroxy group in position 3. The hydroxy groups in position 2 were found only in eukaryotic microorganisms, e.g. the yeasts, as components of sphingolipids, or in green and red algae [15]. Very long OH-FA were identified only in ocean sediments. They had the hydroxy group in position 3 [16]. The authors presented a hypothesis concerning the taxonomic origin of these compounds indicating that: "the absence of $\alpha$-hydroxy FA (above $C_{20}$) in this sediment makes a contribution of

Table 2

Content of fatty acids from *Desulfotomaculum species* strain 43 and *D. ruminis* strain 21 (% of total fatty acids)

| Fatty acids [a] | Strains | | | | | |
|---|---|---|---|---|---|---|
| | 43 [a] | 43 [b] | 43 [c] | 21 [a] | 21 [b] | 21 [c] |
| 11:0 | 0.22 | 0 | 0 | 0.23 | 0.37 | 0.31 |
| i-12:0 | 0.17 | 0.32 | 0.16 | 0.15 | 0.41 | 0.28 |
| 5-12:1 | 0.37 | 0.76 | 0.12 | 0.41 | 0.32 | 0.35 |
| 7-12:1 | 0.29 | 0 | 0.14 | 0.35 | 0.29 | 0.39 |
| 12:0 | 0.38 | 0.25 | 0.19 | 3.54 | 3.14 | 3.31 |
| 6-13:1 | 0 | 0.20 | 0 | 0 | 0 | 0 |
| 13:0 | 0.85 | 1.02 | 0.17 | 0.61 | 0.42 | 0.56 |
| i-14:0 | 0.32 | 0.67 | 0.66 | 0.42 | 0.35 | 0.28 |
| 7-14:1 | 0.32 | 0.56 | 0.29 | 0.24 | 0.22 | 0.29 |
| 14:0 | 2.38 | 5.00 | 8.64 | 6.58 | 5.76 | 5.57 |
| i-15:0 | 0.23 | 0.67 | 0.55 | 0.31 | 0.43 | 0.42 |
| ai-15:0 | 0.35 | 0.52 | 0.20 | 0.28 | 0.19 | 0.27 |
| 6-15:1 | 0.33 | 1.34 | 0.17 | 0.47 | 0.37 | 0.39 |
| 8-15:1 | 0.75 | 0 | 0 | 0.51 | 0.37 | 0.61 |
| 15:0 | 1.22 | 2.25 | 1.40 | 2.07 | 1.73 | 1.95 |
| i-16:0 | 0.13 | 0.44 | 0.10 | 0.12 | 0.13 | 0.14 |
| 7-16:1 | 22.01 | 22.37 | 15.74 | 16.18 | 17.11 | 16.15 |
| 9-16:1 | 3.10 | 1.95 | 12.66 | 4.28 | 2.56 | 3.46 |
| 11-16:1 | 0 | 0 | 0 | 1.35 | 2.24 | 1.75 |
| 16:0 | 11.97 | 14.10 | 18.10 | 15.18 | 16.33 | 15.93 |
| i-17:0 | 0.56 | 0.29 | 0.47 | 0.57 | 0.31 | 0.42 |
| ai-17:0 | 0.23 | 0.37 | 0.29 | 0.35 | 0.47 | 0.37 |
| 8-17:1 | 4.96 | 1.72 | 5.62 | 6.13 | 2.95 | 5.46 |
| 10-17:1 | 0.58 | 0 | 0 | 2.24 | 2.38 | 2.42 |
| 17:0 | 1.05 | 1.12 | 0.51 | 1.98 | 1.56 | 2.81 |
| 9-18:1 | 7.74 | 6.89 | 4.18 | 12.31 | 13.41 | 10.98 |
| 11-18:1 | 9.51 | 7.06 | 4.01 | 5.46 | 6.18 | 6.56 |
| 13-18:1 | 3.57 | 2.00 | 1.10 | 8.64 | 10.13 | 10.12 |
| 18:0 | 4.87 | 5.59 | 4.34 | 5.12 | 6.18 | 5.28 |
| 10-19:1 | 0.77 | 0.54 | 0.13 | 0.10 | 0.12 | 0.10 |
| 19:0 | 0.34 | 0.36 | 0.10 | 0.12 | 0.31 | 0.22 |
| 11-20:1 | 0.28 | 0.30 | 0.19 | 0.24 | 0.15 | 0.10 |
| 13-20:1 | 0.25 | 0.45 | 0 | 0 | 0 | 0 |
| 15-20:1 | 0.90 | 0 | 0 | 0 | 0 | 0 |
| 20:0 | 0.53 | 0.81 | 0.98 | 0.35 | 0.34 | 0.42 |
| 12-21:1 | 0 | 0.66 | 0.29 | 0 | 0 | 0 |
| 21:0 | 1.02 | 0.23 | 0 | 0 | 0 | 0 |
| i-22:0 | 0.13 | 0.19 | 0.22 | 0 | 0 | 0 |
| 13-22:1 | 1.34 | 1.56 | 1.72 | 0.71 | 0.98 | 0.70 |
| 15-22:1 | 0 | 0 | 0.36 | 0 | 0 | 0 |
| 22:0 | 1.47 | 1.42 | 2.45 | 0.98 | 1.13 | 1.02 |
| 14-23:1 | 1.20 | 1.60 | 0.49 | 0 | 0 | 0 |
| 23:0 | 0.21 | 0.27 | 0.32 | 0 | 0 | 0 |
| i-24:0 | 0.21 | 0 | 0 | 0 | 0 | 0 |
| 15-24:1 | 0 | 0.10 | 0 | 0 | 0 | 0 |
| 17-24:1 | 1.53 | 1.56 | 0.53 | 0.12 | 0.10 | 0.10 |
| 24:0 | 0.81 | 0.81 | 2.86 | 0.31 | 0.56 | 0.61 |
| ai-25:0 | 0.14 | 0 | 0 | 0 | 0 | 0 |
| 25:1 | 0.92 | 1.38 | 0.51 | 0 | 0 | 0 |
| 25:0 | 0.67 | 0.30 | 0.41 | 0 | 0 | 0 |
| i-26:0 | 0.23 | 0 | 0.10 | 0 | 0 | 0 |
| 26:1 | 0 | 0.82 | 0 | 0 | 0 | 0 |
| 26:1 | 1.34 | 2.08 | 0.58 | 0 | 0 | 0 |
| 26:0 | 0.48 | 0.42 | 1.40 | 0 | 0 | 0 |

Table 2 (continued)

| Fatty acids * | Strains | | | | | |
|---|---|---|---|---|---|---|
| | 43 [a] | 43 [b] | 43 [c] | 21 [a] | 21 [b] | 21 [c] |
| i-27:0 | 0 | 0.20 | 0 | 0 | 0 | 0 |
| 27:1 | 1.03 | 0.18 | 0.54 | 0 | 0 | 0 |
| 27:0 | 0.17 | 0.46 | 0.25 | 0 | 0 | 0 |
| i-28:0 | 0.28 | 0 | 0 | 0 | 0 | 0 |
| 28:1 | 0.87 | 1.09 | 0.45 | 0 | 0 | 0 |
| 28:1 | 0 | 0.23 | 0 | 0 | 0 | 0 |
| 28:0 | 0.70 | 0.58 | 1.61 | 0 | 0 | 0 |
| i-29:0 | 0 | 0 | 0.27 | 0 | 0 | 0 |
| 29:1 | 0.77 | 0.58 | 0.28 | 0 | 0 | 0 |
| 29:0 | 0.55 | 0.80 | 0.19 | 0 | 0 | 0 |
| i-30:0 | 0 | 0.11 | 0 | 0 | 0 | 0 |
| 30:1 | 0.45 | 0.67 | 0.24 | 0 | 0 | 0 |
| 30:0 | 0.91 | 0.80 | 0.99 | 0 | 0 | 0 |
| i-31:0 | 0 | 0.13 | 0 | 0 | 0 | 0 |
| 31:1 | 0.19 | 0.13 | 0 | 0 | 0 | 0 |
| 31:0 | 0.16 | 0.13 | 0.18 | 0 | 0 | 0 |
| i-32:0 | 0 | 0 | 0.10 | 0 | 0 | 0 |
| 32:1 | 0.28 | 0.35 | 0 | 0 | 0 | 0 |
| 32:0 | 0.40 | 0.24 | 1.45 | 0 | 0 | 0 |

* Nomenclature of fatty acids: first number, number of carbon atoms in chain; second number, number of double bonds; number before the hyphen, position of double bond; position of double bond not localized, only degree of unsaturation is given; i = isoacid; ai-anteisoacid.

[a,b,c] See notes a, b, c under Table 1.

sphingolipids derived from higher organisms less likely".

The results referred to have shown that it is necessary to be more careful in drawing conclusions. The last very long acids were dicarboxylic. They were identified many times in sediments, such as peat [17], shales [18], or marine sediments [9,19]. $\alpha,\omega$-Dicarboxylic acids (DCA) are commonly found in waxes, cutine and suberine; their length is limited to $C_{22}$. Therefore, Gillan and Hogg [9] hypothesize that they became components of the sediments together with contaminants of plant origin. On the basis of our previous experience [20], we could identify DCA up to $C_{31}$.

The effect of the cultivation time on the quality and quantity of FA present was also studied. In both strains the time of sampling did not influence the content of FA. Although in the paper of Lechevalier [21], an extensive variability of FA in dependence on the time of harvest was described, it was not observed with VLCFA [22]. The authors reported that the relative quantities of various FA did not exhibit dramatic changes during the growth cycle. This fact is very important for taxonomists because it enables them to use FA as chemotaxonomic markers, irrespective of the time of harvest of the cells.

Several new facts were presented in this work. First, no branched monoenoic FA were found in our strains, even if a sensitivity of $10^{-3}$% was used, and, in addition, the usual $\beta$-hydroxy acids ($C_{11-17}$) were not detected as well. On the other hand, three types of unusual acids were identified, mainly VLCFA, branched or straight-chain, saturated and monoenoic. Second, $\alpha$-hydroxy and $\alpha,\omega$-dicarboxylic acids were detected, with a chain even longer than 24 atoms. The presence of these unusually-long acids in sulfate-reducing bacteria may change assumptions concerning their biosynthesis and chemotaxonomy. Until now the presence of the compounds with a very long chain in the sediments has been mainly explained by a possible contamination of these materials by the organic residues of higher plants. Our results sug-

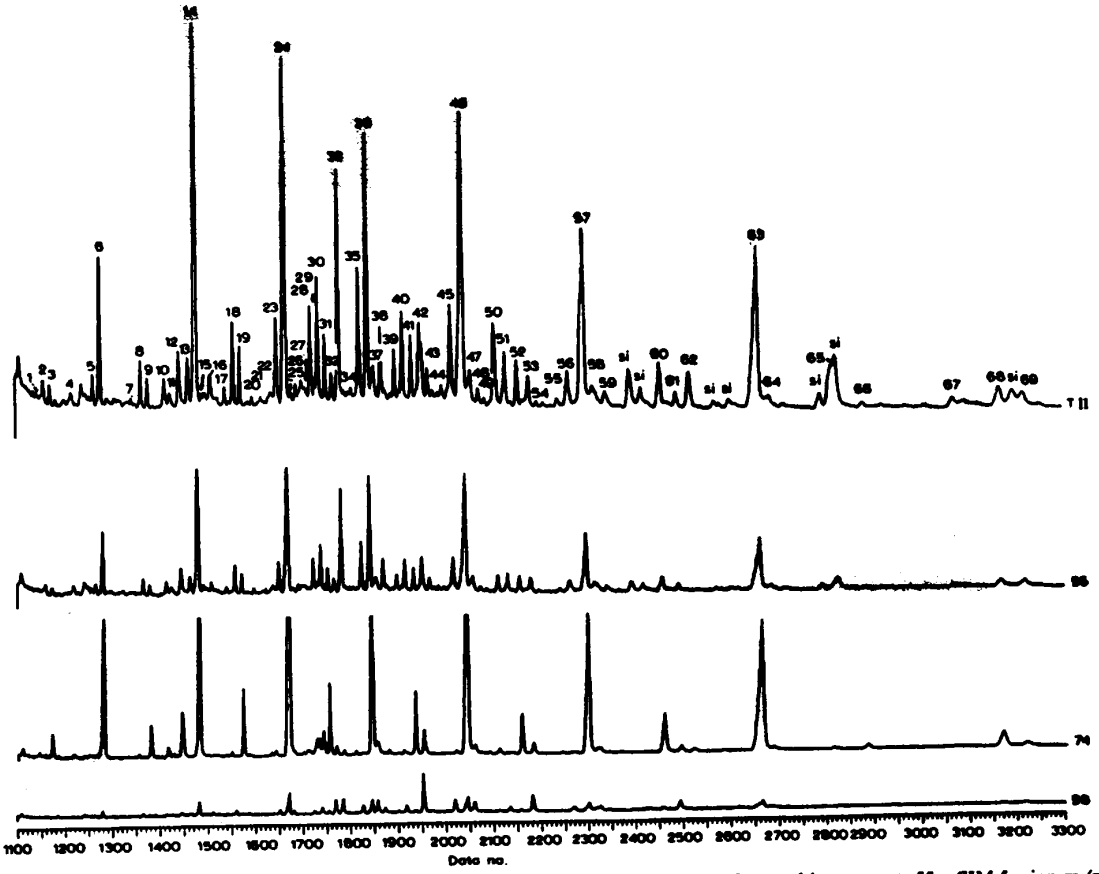


Fig. 1. GC-MS of VLCFA methylesters of *D. species* strain 43 (harvested after 72 h). TII = total ion current; 55 = SIM for ion m/z 55; 74 = SIM for ion m/z 74; 98 = SIM for ion m/z 98; Data No. = scan number expressed in seconds (1 scan/s). For conditions, see text. Peak identification: 1 = i-19:0, 2 = 19:1; 3 = 19:0; 4 = i-20:0; 5 = 20:1; 6 = 20:0; 7 = i-21:0; 8 = 21:1; 9 = 21:0; 10 = i-22:0; 11 = 22:1; 12 = 22:1; 13 = phtalate ester; 14 = 22:0; 15 = di-19; 16 = 2-OH-21:0; 17 = i-23:0; 18 = 23:1; 19 = 23:0; 20 = di-20; 21 = 2-OH-22:0; 22 = i-24:0; 23 = 24:1; 24 = 24:0; 25 = di-21; 26 = 2-OH-23:0; 27 = i-25:0; 28 = ai-25:0; 29 = 25:1; 30 = 25:1; 31 = 25:0; 32 = di-22; 33 = 2-OH-24:0; 34 = i-26:0; 35 = 26:1; 36 = 26:0; 37 = di-23; 38 = 2-OH-25:0; 39 = i-27:0; 40 = 27:1; 41 = 27:0; 42 = di-24:0; 43 = 2-OH-26:0; 44 = i-28:0; 45 = 28:1; 46 = 28:0; 47 = di-25; 48 = 2-OH-27:0; 49 = i-29:0; 50 = ai-29:0; 51 = 29:1; 52 = 29:0; 53 = di-26; 54 = 2-OH-28:0; 55 = i-30:0; 56 = 30:1; 57 = 30:0; 58 = di-27; 59 = 2-OH-29:0; 60 = 31:0; 61 = di-28; 62 = 2-OH-30:0; 63 = 32:0; 64 = di-29; 65 = i-33:0; 66 = 33:0; 67 = i-34:0, 68 = 34:0; 69-di-31; Si = methylsilicone oligomers. di-19 is $\alpha,\omega$-dicarboxylic acid with 19 carbon atoms, etc.; 2-OH-21:0 is 2-hydroxy-21:0, etc.

gest that this hypothesis should probably be revised.

## REFERENCES

[1] Edlund, A., Nichols, P.D., Roffey, R. and White, D.C. (1985) Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio species*. J. Lipid Res. 26, 982–988.

[2] Taylor, J. and Parkes, R.J. (1983) The cellular fatty acids of the sulphate-reducing bacteria, *Desulfobacter* sp., *Desulfobulbus* sp. and *Desulfovibrio desulfuricans*. J. Gen. Microbiol. 129, 3303–3309.

[3] Boon, J.J., De Leeuw, J.W., Hoek, G.J. and Vosjan, J.H. (1977) Significance and taxonomic value of iso and ante-iso monoenoic fatty acids and branched $\alpha$-hydroxy acids in *Desulfovibrio desulfuricans*. J. Bacteriol. 129, 1183–1191.

[4] Parkes, R.J. and Calder, A.G. (1985) The cellular fatty acids of three strains of *Desulfobulbus*, a propionate-utilising sulphate reducing bacterium. FEMS Microbiol. Ecol. 31, 361–363.

[5] Ueki, A. and Suto, T. (1979) Cellular fatty acid composition of sulfate-reducing bacteria. J. Gen. Appl. Microbiol. 25, 185–195.

[6] Fredrickson, H.L. (1984) Fatty acid characterizations of selected sulfate-reducing bacteria and sedimentary communities, PhD Thesis, The Florida State University, College of Arts and Science.

[7] Taylor, J. and Parkes, R.J. (1985) Identifying different populations of sulphate-reducing bacteria within marine sediment systems, using fatty acid biomarkers. J. Gen. Microbiol. 131, 631–642.

[8] Parkes, R.J. and Taylor, J. (1983) The relationship between fatty acid distribution and bacterial respiratory types in contemporary marine sediments. Estuar. Coast. Shelf Sci. 16, 173–189.

[9] Gillan, F.T. and Hogg, R.W. (1984) A method for the estimation of bacterial biomass and community structure in mangrove-associated sediments. J. Microbiol. Meth. 2, 275–293.

[10] Pfennig, N. and Lipperz, K.D. (1966) Ueber das Vitamin $B_{12}$-Bedürfnis phototropher Schwefelbakterien. Arch. Mikrobiol. 55, 245–256.

[11] Widdel, F. (1980) Anaerober Abfall von Fettsäuren und Benzoesäure durch neu isolierte Arten Sulfatreduzierender Bakterien. Doctoral Thesis, University of Göttingen, F.R.G.

[12] Vančura, A., Řezanka, T., Maršálek, J., Křišťan, V. and Basařová, G. (1987) Fatty acids and production of tylosin-like compounds in *Streptomyces fradiae*. J. Basic Microbiol. 27, 167–171.

[13] Andersson, B.A. (1978) Mass spectrometry of fatty acid pyrrolidides. Prog. Chem. Fats Other Lipids 16, 279–308.

[14] Tvrzická, E., Řezanka, T., Krijt, J. and Janoušek, V. (1988) Identification of very-long-chain fatty acids in rat and mouse Harderian gland lipids by capillary gas chromatography-mass spectrometry. J. Chromatogr. 431, 231–238.

[15] Matsumoto, G.I., Shioya, M. and Nagashima, H. (1984) Occurrence of 2-hydroxy acids in microalgae. Phytochemistry 23, 1421–1423.

[16] Klok, J., Baas, M., Cox, H.C., Leeuw, J.W., Rijpstra, W.I.C. and Schenck, P.A. (1988) The mode of occurrence of lipids in a Namibian Shelf diatomaceous ooze with emphasis on the $\beta$-hydroxy fatty acids. Org. Geochem. 12, 75–80.

[17] Ekman, R. and Fagernäs, L. (1983) Distribution of lipid components in peat extracts and their wax and resin fractions. Finn. Chem. Lett. 129–133.

[18] Douglas, A.G., Blumer, M., Eglinton, G. and Douraghi-Zadeh, K. (1971) Gas chromatography-mass spectrometric characterization of naturally-occuring acyclic isoprenoid carboxylic acids. Tetrahedron 27, 1071–1092.

[19] Perry, G.J., Volkman, J.K. and Johns, R.B. (1979) Fatty acids of bacterial origin in contemporary marine sediments. Geochim. Cosmochim. Acta 43, 1715–1725.

[20] Vlden, I. and Řezanka, T. (1989) Capillary gas chromatography-mass spectrometry of very-long-chain $\alpha,\omega$-dicarboxylic acid dimethyl esters from *Equisetum* (horsetail). J. Chromatogr. 465, 390–394.

[21] Lechevalier, M.P. (1982) Lipids in bacterial taxonomy in Handbook of Microbiology, 2nd edn., Vol. IV, (A.I. Laskin and H.A. Lechevalier, Eds.), CRC Press, Boca Raton, FL, U.S.A.

[22] Welch, J.W. and Burlingame, A.L. (1973) Very long-chain fatty acids in yeast. J. Bacteriol. 115, 464–466.