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The budding bacteria, *Pirellula* and *Planctomyces*, with atypical 16S rRNA and absence of peptidoglycan, show eubacterial phospholipids and uniquely high proportions of long chain beta-hydroxy fatty acids in the lipopolysaccharide lipid A

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Abstract. Fatty acids of twelve strains of budding bacteria (*Planctomyces* and *Pirellula* spp.), which have atypical 16S rRNA and do not contain peptidoglycan cell walls, were shown to contain typical diacyl polar lipids with no indication of isoprenoid ether lipids suggestive of a relationship with the archaebacteria. The major ester-linked fatty acids of the phospholipids were palmitic, palmitoleic and oleic acids, which are more typical of microeukaryotes than of eubacteria. Lipopolysaccharide lipid A (LPS) was detected; it contained major proportions of long chain normal 3-OH fatty acids (3-OH eicosanoic at 23% and 17% of the total in two strains of *Planctomyces*, and 3-OH octadecanoic at 18%, and 3-OH palmitic at 11% of the total in one strain of *Pirellula*). Major portions of long chain 3-OH fatty acids in the LPS are extremely unusual and provide another atypical property of these organisms. Each strain investigated showed a specific total fatty acid composition, reflecting the diversity in 16S rRNA nucleotide catalogues.

Key words: Fatty acid composition – *Pirellula* – *Planctomyces* – Non-prosthecate, budding bacteria – Phylogeny of eubacteria – Lipids – Fatty acids – Lipopolysaccharides

The budding, non-prosthecate bacteria are easily distinguished from all other bacteria by their morphological features and by their mode of new cell formation and multiplication. The following genera have been listed as belonging to this artifical group: *Blastobacter* (Hirsch 1974), *Gemmobacter* (Rothe et al. 1987), *Gemmata* (Franzmann and Skerman 1984), *Isosphaera* (Giovannoni et al. 1987), *Planctomyces* and *Pirellula* (Schlesner and Hirsch 1984, 1987). Bacteria of the genera *Planctomyces* and *Pirellula* are of special interest because they were found to be resistant to cell wall antibiotics and they lack a peptidoglycan (König et al. 1984; Liesack et al. 1986). Instead, their proteinaceous walls were found to be unusually rich in proline and cystine. Studies of their phylogenetic position by means of oligonucleotide cataloguing of 16S rRNA indicated a very low similarity to all bacteria investigated thus far. The S_{AB} values to eubacteria were 0.06–0.22 and those to archaebacterial strains were 0.04–0.12 (Stackebrandt et al. 1984). The S_{AB} values between the two genera were also low, they were initally interpreted as an early separation from each other during evolution. Recent investigations show that the large differences in the 16S rRNA sequences are due to rapid evolution of this lineage (Woese 1987). Stackebrandt et al. (1986) recognized specific oligonucleotide signatures that defined the *Planctomyces-Pirellula* group; their catalogues also indicated a closer relationship with the eubacterial kingdom, which was confirmed by the diphtheria toxin test carried out by F. Klink (Stackebrandt et al. 1984). Woese et al. (1985) suggested that these budding, peptidoglycan-less bacteria be placed in a special "phylum" or "division": the "planctomycetes and their relatives". Thus, Schlesner and Stakebrandt (1986) proposed the new order of *Planctomycetales* for this group.

With their unique morphology and reproduction, the different construction of their cell wall and the isolated phylogenetic position, the *Planctomycetales* have recently been given much attention. Avirulence was studied by Famurewa et al. (1983); occurrence in groundwater was noted by Hirsch and Rades-Rohkohl (1983a, b), and Gebers et al. (1985) investigated G+C base ratios and nucleotide distribution in their DNA. Genome sizes were determined by Kölbel-Boelke et al. (1985). *Planctomyces limnophilus* and *Pirellula marina* were described as new species (Hirsch and Müller 1985; Schlesner 1986). Methods for enrichment and isolation were given by Hirsch and Müller (1986). Possible early separation of *Planctomycetales* from the other eubacteria has been discussed by Stackebrandt et al. (1986), and thus a comparison with archaebacteria became interesting to us. For this reason, we undertook a study of their fatty acid composition, since fatty acids have been considered as differentiating markers for bacterial taxa. In an initial study, three strains were investigated by T. Langworthy. Six additional strains were then examined by M. Sittig, and three further cultures were analyzed in greater detail in the laboratory of D. C. White. The results presented below clearly indicate the presence of phospholipid ester-linked fatty acids, which is as expected in eubacteria. The fatty acids of various lipid types showed differences among the genera *Planctomyces* and *Pirellula*, but there were also "signature" fatty acids for the *Planctomycetales*, such as 18:1ω9c.

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Materials and methods

Organisms and growth conditions. The bacterial cultures investigated and their origin are listed in Table 1. The strains were cultivated as described earlier by König et al. (1984). All bacteria were harvested in the stationary phase (i.e. after 8–10 days). Cultures were centrifuged for 15 min at 13,000 × *g* and 10° C. They were then lyophilized and stored dry and cold (4° C) until further use.

Fatty acid composition of polar lipids. The preparation and analysis of polar lipid fatty acids of *Pirellula marina* IFAM 1313, *Pirellula* sp. 1441 and *Planctomyces* sp. 1448 was carried out as follows: lipids were extracted from freeze-dried cells (0.7–1.0 g dry wt) by the method of Bligh and Dyer (1959). Total lipids were fractionated into lipid classes by chromatography on 1 × 10 cm columns of silicic acid (Unisil, Clarkson Chemical Co., Williamsport, PA, USA) by elution with 15 column volumes each of chloroform for neutral lipids and methanol for polar lipids (glycolipids plus phospholipids). Polar lipids were degraded by acid methanolysis employing anhydrous 1 M methanolic HCl at 100° C for 18 h; the released hydrophobic residues were extracted into *n*-hexane (Langworthy 1982).

Thin-layer chromatography (TLC) of the *n*-hexane-soluble methanolysis products was carried out on 0.25 mm layers of silica gel H developed in *n*-hexane-diethylether-acetic acid (70:30:1, by vol.). Components were detected by exposure to iodine vapors or by charring after spraying plates with 50% methanolic H_2SO_4. Quantitative estimates of components revealed by charring were assessed using a Zeinih soft laser scanning densitometer. For further analysis, components detected by iodine vapors were scraped from the plates and eluted with chloroform-methanol (2:1, by vol.). For gas chromatographic (GLC) analysis of fatty acid methyl esters, a Hewlett-Packard F and M model 402 instrument was employed which was equipped with flame ionization detectors (FJD) and a model 3370A digital electronic integrator. Analysis was done isothermally at 205° C on glass columns (1.8 m × 4.0 mm) packed with either 5% SP2100 or 10% OV-11 on 100/120 mesh Supelcoport. Fatty acid methyl esters were identified using appropriate standards (Supelco, Bellafonte, PA, USA). Unsaturated fatty acids were distinguished by treatment with bromine and by hydrogenation with H_2 in methanol over platinum oxide.

Fatty acid composition of phospholipids and glycolipids of *Pirellula* strains IFAM 1358 and 1735 was investigated as follows: lipids of freeze-dried cells (100 mg dry wt) were extracted by chloroform-methanol (2:1, by vol.). This crude lipid extract was fractionated into lipid classes by chromatography on silicic acid (column 0.8 × 5.0 cm; about 1 g silicic acid; Serva Feinbiochemica GmbH, Heidelberg, FRG), by elution with 10 column volumes each of chloroform (for neutral lipids), acetone (for glycolipids) and methanol (for phospholipids). Glycolipid and phospholipid fractions were hydrolyzed in methanolic 2 M KOH at 70° C for 4 h. After methylation the resulting fatty acid methylesters were analyzed by GLC with a Packard Model 419 Becker Gas Chromatograph, equipped with a flame ionization detector (FID) and an Autolab Digital Integrator Model 6300-02. Samples were chromatographed at 200° C in glass columns (4 m × 2 mm) on Chromosorb G, AW/DMCS, 70/80 mesh, coated with 5% DEGS. The peaks were identified by comparison with analytical grade standard fatty acid methyl esters (Applied Science Labs, Inc.).

Fatty acid composition of whole cells. The total fatty acids of *Planctomyces* strain IFAM 1190 and the *Pirellula* strains IFAM 1310, 1318, 1319, and 1358 were investigated by extracting and separating whole cell lipids with chloroform-methanol-0.74% KCl (4:2:1.5; by vol.) and by hydrolyzing the lipid extract in methanolic 2 M KOH. After methylation with 50 ml methanol and 0.1 ml conc. H_2SO_4 for 2 h at 70° C the resulting fatty acid methyl esters were analyzed by GLC as described above for strains IFAM 1358 and 1735.

Detailed investigation of phospholipid fatty acid methyl esters. Methods employed for sample extraction, fractionation of total lipids, and methylation of the phospholipid ester-linked fatty acids of strains IFAM 1008, 1189 and 1317 have been reported previously (Guckert et al. 1985). The GC-MS analyses were performed on a Hewlett-Packard 5996 A system fitted with a direct capillary inlet. The column was a 50 m nonpolar, cross-linked, methyl silicone fused silica capillary column (0.2 mm i.d., Hewlett-Packard). Samples were injected in the splitless mode at 100° C with an 0.5 min venting time, after which the oven temperature was programmed to 280° C at either 3 or 4° C per minute. Helium was used as the carrier gas. MS operating conditions were: electron multiplier, between 1400 and 1600 V; transfer line, 300° C;

Table 1. Cultures of *Planctomycetales* investigated and their origin

IFAM[a] No.	Name	ATCC-Nr.	Origin and Reference
1008	*Planctomyces limnophilus*[T]	43296[T]	Lake Plussee (Holstein), Hirsch and Müller 1985
1190	*Planctomyces maris*[T]	29201[T]	Ocean water, Bauld and Staley 1976
1317	*Planctomyces maris*		Kiel Fjord, Baltic Sea: Schlesner 1983
1448	*Planctomyces* sp.		Lagoa Vermelha, Brazil: Schlesner (unpublished work)
1189	*Pirellula staleyi*[T] (= *Pasteuria ramosa* sensu Staley 1973)	27377[T]	Lake Lansing (Mich.): Staley 1973
1313	*Pirellula marina*[T]		Kiel Fjord, Baltic Sea: Schlesner 1983
1310	*Pirellula* sp.		Kiel Fjord, Baltic Sea: Schlesner 1983
1318	*Pirellula* sp.		Kiel Fjord, Baltic Sea: Schlesner 1983
1319	*Pirellula* sp.		Kiel Fjord, Baltic Sea: Schlesner 1983
1358	*Pirellula* sp.		Kiel Fjord, Baltic Sea: Schlesner 1983
1441	*Pirellula* sp.		Lagoa Vermelha, Brazil: Schlesner (unpublished work)
1735	*Pirellula* sp.		Kiel Fjord, Baltic Sea: Schlesner (unpublished work)

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source and analyzer, 250°C; autotune file, DETPP normalized; optics tuned at m/z 502; electron impact energy, 70 eV. Mass spectral data were acquired and processed with a Hewlett-Packard RTE-6/VM data system.

Determination of fatty acid double-bond configuration. The dimethyl disulphide adducts of monounsaturated fatty acid methyl esters were formed according to methods similar to those described by Dunkelblum et al. (1985) and Nichols et al. (1986) to locate the double-bond positions. A higher temperature (50°C) than reported for the monounsaturated acetates (Dunkelblum et al. 1985) was required to achieve complete reaction.

Determination of lipopolysaccharide hydroxy fatty acids. The 3-hydroxy fatty acids of lipopolysaccharides of the three strains IFAM 1008, 1189, and 1317 were obtained by extraction methods and mild acid hydrolysis etc. described in detail by Parker et al. (1982).

Identification of phospholipid fatty acid and lipopolysaccharide components. The structures of the PLFA and LPS components have been confirmed by comparing their retention times with those of authentic compounds and previously identified laboratory standards, and by GC-MS analysis. Data are expressed as the mean of two or three replicate analyses. Standard deviations for individual components were in the range 0–30%, typically less than 10%.

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 ω (methyl) 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; the prefix OH indicates a hydroxyl group at the position indicated. Other methyl-branching is indicated by the position of the additional methyl carbon from the carboxyl end, e.g. 10 methyl 16:0.

Results

General lipid composition and presence of phospholipid ester-linked fatty acids

The lipids of *Planctomyces* strain 1448 and *Pirellula* strains 1441 and 1313 were initially examined to determine their general composition and then, in view of the unusual composition of the cell walls, to judge whether the organisms are more closely related to eubacteria or archaebacteria (Langworthy et al. 1982). Total extractable lipids represented 10% of the cell dry weight of *Planctomyces* 1448 and 6.5% of the two *Pirellula* strains. Of the total lipids of the three organisms, the polar lipids (glycolipids plus phospholipids) accounted for about 93% and neutral lipids for 7%. Thin-layer chromatography of the polar lipid acid methanolysis products indicated that fatty acid esters represented 97% of the hydrophobic residues. No archaebacterial isoprenoid ether lipids or other unusual components could be detected (Langworthy et al. 1982). The composition of the fatty acids of *Planctomyces* sp. 1448 and the two *Pirellula* strains is shown in Table 2. In *Planctomyces* 1448 the major fatty acids were 16:0, 16:1, 18:1, and 20:1, whereas the two principal fatty acids of the *Pirellula* strains were 16:0 and 18:1. Branched fatty acids could not be detected.

Table 2. Fatty acid composition of the polar lipids of *Planctomyces* strain IFAM 1448 and *Pirellula* strains IFAM 1441 and 1313

Fatty acid	Fatty acids (rel. %)		
	Planctomyces strain 1448	*Pirellula* strain 1441	*Pirellula* strain 1313
14:0	4.0	0.5	1.1
15:0	8.4	0.4	1.3
16:1	25.0	0.7	6.5
16:0	30.7	50.2	49.2
17:1	1.8	0.6	2.6
17:0	1.3	0.4	1.4
18:1	13.1	45.2	37.2
18:0	5.6	1.6	0.7
20:1	9.9	0.4	tr[a]

[a] Trace, >0.1%

Table 3. Phospholipid and glycolipid fatty acid composition of two *Pirellula* strains. Data in relative percentages

Fatty acid	*Pirellula* sp. IFAM 1358		*Pirellula* sp. IFAM 1735	
	Phospholipid fatty acids	Glycolipid fatty acids	Phospholipid fatty acids	Glycolipid fatty acids
14:0	0.5	1.6	1.2	2.5
a15:0	n.d.	0.2	n.d.	0.5
15:0	2.1	4.9	0.8	1.9
i16:0	n.d.[a]	0.2	0.4	0.5
16:0	25.4	38.5	32.0	31.3
16:1	8.6	11.6	5.6	5.3
17:0	8.3	8.6	2.2	3.7
17:1	13.2	11.5	3.2	2.6
18:0	1.7	2.0	11.1	15.5
18:1	39.1	20.0	41.5	28.5
19:0	0.3	0.2	0.4	1.1
19:1	n.d.	tr[b]	tr	0.3
cy19:0	1.4	0.7	0.7	0.6
20:0	n.d.	n.d.	0.2	4.2

[a] Not detected
[b] Trace, <0.1%

The phospholipid and glycolipid fatty acids of two additional Pirellula strains

The polar lipid composition of *Pirellula* sp. IFAM 1358 and IFAM 1735 was investigated in order to differentiate between the fatty acids of phospholipids and glycolipids. The data in Table 3 show significant differences between the two *Pirellula* strains, especially with respect to 16:1, 17:0, 17:1, and 18:0. There were also significant differences between the fatty acids of phospholipids and glycolipids within each of the two strains. Both organisms had cy 19:0, and *Pirellula* IFAM 1358 lacked the 20:0 acid.

Whole cell lipid fatty acids of a Planctomyces and four Pirellula strains

The major fatty acids of whole-cell lipid of *Planctomyces* sp. IFAM 1190 and four *Pirellula* strains were 16:0, 18:0, and

Table 4. Whole cell lipid fatty acids of *Planctomyces* IFAM 1190 and four *Pirellula* strains. Data in relative percentages

Fatty acids	*Planctomyces* 1190	*Pirellula* spp. 1310	1318	1319	1358
14:0	1.8	9.1	1.0	4.4	8.2
a15:0	0.4	2.9	n.d.[a]	3.7	3.3
15:0	2.6	4.7	0.7	5.0	6.6
i16:0	0.7	3.1	0.5	n.d.	3.6
16:0	37.7	17.3	34.6	20.3	16.1
16:1	4.4	5.2	7.8	6.0	6.1
17:0	5.9	5.1	2.1	4.2	5.2
17:1	3.0	3.8	1.0	5.0	3.8
18:0	7.0	9.4	4.4	10.0	8.4
18:1	7.7	16.2	44.4	18.9	16.4
19:0	0.6	5.0	0.7	4.5	5.2
19:1	0.4	3.9	tr[b]	4.7	3.2
cy19:0	n.d.	5.0	tr	4.8	5.6
20:0	0.8	5.3	0.4	3.7	5.6

[a] Not detected
[b] Trace, <0.1%

18:1 (Table 4). All strains contained a long-chain fatty acid, 20:0. Also present were some iso- and anteiso branched short-chain acids.

Detailed analysis of the phospholipid fatty acids of Pirellula staleyi and two Planctomyces strains

Planctomyces limnophilus IFAM 1008, *Planctomyces* sp. 1317, and *Pirellula staleyi* 1189 were subjected to a more detailed phospholipid fatty acid (PLFA) analysis using gas liquid chromatography and mass spectrometry. The data (Table 5) indicate the presence of a number of different monounsaturated fatty acids in the *Planctomyces* strains and also in *P. staleyi*. The total concentrations of PLFA in these strains were similar to those found in other eubacteria, which supports the placement of *Planctomycetales* with eubacteria rather than archaebacteria.

All three strains were distinguishable from each other, based on the PLFA profiles. IFAM 1008 contained significantly more (5.6×) 18:1ω9c than IFAM 1317 and significantly more (18.4×) 16:1ω7c than IFAM 1189. Strain IFAM 1317 contained significantly more 17:0 than the other two strains, and IFAM 1189 had a higher proportion of 20:1ω9c. The presence of the monounsaturated fatty acids and their different proportions in these bacteria made it necessary to confirm their double-bond configurations. This was achieved with derivatization by reaction with dimethyl disulphide as described in Materials and methods. Table 6 lists the characteristic ion fragments of these derivatives.

Two trans acids were found as minor components in *Planctomyces* IFAM 1317 with low trans to cis ratios observed (0.06). This is taken as an indication for optimal growth conditions of these organisms, since a high trans/cis ratio occurs and increases under starvation conditions of pure cultures (Guckert et al. 1986). Strain 1317 also contained the mid-chain methyl-branched fatty acid 10 Me 16:0 as a minor component. This acid has been reported as a major component of *Desulfobacter* spp. (Dowling et al. 1986; Nichols et al. 1986) and in actinomycetales (Kroppenstedt and Kutzner 1978) and was absent in the other two strains.

Table 5. Phospholipid fatty acid content of two *Planctomyces* strains and *Pirellula staleyi*. Data given as relative abundances (mol percent).

Fatty acid	*Planctomyces* IFAM 1008	*Planctomyces* IFAM 1317	*Pirellula staleyi* IFAM 1189
14:0	0.1	1.0	2.2
i15:0	n.d.[a]	n.d.	0.1
a15:0	n.d.	n.d.	0.1
15:1ω6c	n.d.	0.5	n.d.
15:0	1.0	1.8	0.3
i16:0	0.3	tr[b]	0.1
16:1ω7c	18.4	32.6	1.0
16:1ω7t	n.d.	0.3	n.d.
16:0	46.6	35.8	43.9
10Me16:0	n.d.	0.4	n.d.
i17:0	n.d.	n.d.	1.1
a17:0	n.d.	n.d.	0.3
17:1ω8c	2.6	3.0	n.d.
17:1ω6c	n.d.	1.6	n.d.
17:0	1.7	10.5	0.4
18:1ω9c	20.6	3.7	32.5
18:1ω7c	5.5	3.1	4.8
18:1ω7t	n.d.	tr	n.d.
18:0	1.0	4.1	3.5
19:1ω10c	0.4	n.d.	n.d.
20:1ω9c	1.3	0.3	6.9
20:0	0.1	0.6	n.d.
other	0.4	0.6	2.8
total	100	100	100

[a] Not detected
[b] Trace, <0.1%

Table 6. *Planctomyces* spp. and *Pirellula staleyi*: monounsaturated components of phospholipid fatty acids and characteristic ion fragments of derivatives formed following the reaction with dimethyl disulphide (DMDS)

Fatty acid	M^+	Ion fragments of DMDS adducts	
		ω-fragment[a]	Δ-fragment[b]
15:1ω6	n.d.[c]	n.d.	217
16:1ω7	362	145	217
17:1ω8	376	159	217
17:1ω6	n.d.	131	245
18:1ω9	390	173	217
18:1ω7	390	145	245
19:1ω10	n.d.	187	217
20:1ω9	n.d.	173	245

[a] ω-Fragment indicates fragment including aliphatic end of the molecule
[b] Δ-Fragment indicates fragment including carboxylic end of the molecule
[c] Not detected, due to insufficient sample material

Hydroxy fatty acid composition of lipopolysaccharides

LPS 3-hydroxy acids are present in Gram-negative bacteria, but have not been reported for archaebacteria. The data in Table 7 show the presence of 3-hydroxy acids in *Planctomyces* IFAM 1008 and IFAM 1317, as well as in *Pirellula staleyi* IFAM 1189. There were remarkable

Table 7. Lipopolysaccharide hydroxy fatty acids of *Planctomyces* spp. and *Pirellula staleyi*

Hydroxy acid	Relative abundance (mol percent)		
	Planctomyces IFAM 1008	*Planctomyces* IFAM 1317	*Pirellula staleyi* IFAM 1189
3-OH 12:0[a]	tr[b]	39.6	65.7
3-OH 13:0	n.d.[c]	12.0	n.d.
3-OH 14:0	74.1	28.2	5.0
3-OH 16:0	n.d.	2.7	10.7
3-OH 18:0	3.4	n.d.	18.3
3-OH 20:0	22.5	17.3	n.d.

[a] 3-OH indicates the hydroxy group at the third carbon from the carboxylic end of the molecule
[c] Trace, <1.0%
[c] Not detected

differences in the relative abundance of such components. *Pirellula staleyi* did not contain 3-OH 20:0, whilst both *Planctomyces* strains possessed significant portions of it. In contrast, *P. staleyi* had 3-OH 18:0 as the second most abundant component (18.3% of the total LPS 3-OH acids), whereas the two *Planctomyces* ssp. had only 3.4% of 3-OH 18:0 among the total LPS 3-OH acids. The two *Planctomyces* strains were also differentiated by their 3-OH 13:0 and 3-OH 14:0 acids.

Normal fatty acids were also present in the LPS fatty acid fraction. The profiles observed were similar to the PLFA profiles of each strain.

Discussion

Our analyses did not reveal any indication for the presence of ether lipids in the 12 strains investigated. This supports the view that they are more closely related to the eubacteria than to the archaebateria (Stackebrandt et al. 1984, 1986). The *Planctomycetales* as a group may be characterized by the presence, in their phospholipids, of the 18 carbon monounsaturated acid 18:1ω9c, which is not normally a major component in bacteria. This fatty acid is the second most abundant component in both *Planctomyces* IFAM 1008 and *Pirellula* IFAM 1189 and occurs as 3.7 mole% in *Planctomyces* IFAM 1317, as well as in variable amounts in one *Planctomyces* and six *Pirellula* strains so far investigated (Sittig, unpublished data). Therefore, it may be a useful signature fatty acid for these bacteria in environmental samples. Fatty acid signatures useful in substructuring the *Planctomycetales* group may be the 3-OH fatty acids of their lipopolysaccharides. While 3-OH 20:0 was found in two *Planctomyces* strains (1008, 1317), it was absent from the LPS of *Pirellula staleyi* 1189. In contrast, 3-OH 18:0 was low in the planctomycetes but occurred with 18.3 mole% in *P. staleyi*. Relatively low phylogenetic relationship between the genera *Planctomyces* and *Pirellula* have been deduced from 16S rRNA oligonucleotide catalogues ($S_{AB} = 0.3$; Stackebrandt et al. 1986). Even within the two genera there was considerable variation in the fatty acid profiles, which is for example demonstrated by the differences in 3-OH 14:0 and 3-OH 13:0 observed in *Planctomyces* strains 1008 and 1317. Since the 3-OH fatty acids in LPS are the least variable of the LPS components with changes in growth conditions (Saddler and Wardlaw 1980), differences among strains appear to be real. Our data also indicate that *Planctomycetales*, which have proteinaceous cell walls (Liesack et al. 1986) also contain lipopolysaccharide lipid A (LPS). However, the LPS hydroxy fatty acid pattern is unique. The LPS of two strains of *Planctomyces* contained 23% and 17% 3-OH 20:0 (Table 7). The *Pirellula*, which has a similar, atypical cell wall, contained 18% of 3-OH 18:0 and 11% of 3-OH 16:0. These LPS 3-OH fatty acid compositions in the LPS lipid A are sufficiently unusual to be possible signature biomarkers for these organisms. In a comprehensive review of LPS-lipid A, Wilkinson (1977) found no organisms with 3-OH 20:0, and one cyanophyte with a trace of 3-OH 18:0, in the LPS-lipid A of the 59 species examined. Significant proportions of 3-OH 20:1, 3-OH 20:0, 3-OH 18:0, 3-OH 22:0 from *Rhodobacter sulfidophilus* and much smaller proportions of 3-OH 20:0 from *Chlorobium vibrioforme* have been detected in lipid extracts after acid but not alkaline hydrolysis (Goossens et al. 1985). These long-chains 3-OH fatty acids are believed to be amide-linked to extractable ornithine, glyco- or amino-lipids and not a part of the LPS as described for *Pirellula* and *Planctomyces* in this study.

These findings have demonstrated an affinity, based on lipid fatty acid composition, between the budding bacteria *Planctomyces* and *Pirellula* and classical Gram-negative eubacteria. The presence or absence of ester-linked 18:1ω9c can now be determined in subsurface sediments or ground water, which does not contain significant numbers of microeukaryotes (readily determined by the absence of ester-linked polyenoic fatty acids with >20 carbons in the phospholipids). The presence of 18:1ω9c and of 3-OH 20:0 or high proportions of 3-OH 18:0 in the lipid-extracted residue after hydrolysis and a second extraction should thus be useful in assessing the ecology of these organisms in subsurface aquifer sediments or other environments. The presence of unusual hydroxy fatty acids in the LPS-lipid A has also been used to detect acid secreting *Thiobacilli* in corroded concrete samples (Kerger et al. 1987).

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