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Comparison of fatty acid content and DNA homology of the filamentous gliding bacteria *Vitreoscilla, Flexibacter, Filibacter*

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Abstract. DNA hybridization experiments showed that there was a high degree of homology among *Vitreoscilla* strains but not with DNA from *Filibacter limicola. Flexibacter* spp were much more heterogeneous indicating a low genetic similarity. These results were also reflected in the membrane fatty acids of the bacteria. The *Vitreoscilla* strains were very similar with the 16:1ω7c fatty acid being dominant. The membrane fatty acids of *F. limicola* were dominated by a15:0 and a17:0 components which provided additional support for its relatedness to the genus *Bacillus.* There was much greater diversity in the fatty acid patterns of the *Flexibacter* spp. *F. aurantiacus, F. ruber* and *F. elegans* shared the common dominant fatty acids 16:1ω7c with the *Vitreoscilla* strains, but this was replaced by the 16:1ω6c acid in *F. flexilis. F. ruber* was distinguished by the absence of branched odd-chain monounsaturated fatty acids and *F. elegans* by the dominance of the β-OH i15:0 acid. Precise determination of fatty acid double bond positions and geometry are essential for correct interpretation of increasingly complex ecological and taxonomic data sets.

Key words: Filamentous gliding bacteria – DNA homology – Membrane fatty acids

Filamentous gliding bacteria are usually found in the benthic communities of freshwater systems. Their gliding movement is one which is, essentially, associated with solid substrata whereas planktonic organisms rely on other forms of motility. Although numbers of filamentous bacteria in sediments may be much lower than those of unicellular forms, they are more important in terms of biomass (Jørgensen 1977) and can account for as much as 50% of the bacterial mass in eutrophic lake sediments (Godinho-Orlandi and Jones 1981). Among the filamentous bacteria most frequently encountered in the sediments of lakes in the English Lake District are members of the genera *Vitreoscilla* and *Flexibacter* although many others remain to be isolated and characterized. A new taxon, *Filibacter limicola* was isolated recently from lake sediment. Although the results indicated that it was more similar to the genus *Flexibacter* than to *Vitreoscilla* (Maiden and Jones 1984), a more recent analysis demonstrated its closest relatedness to the genus *Bacillus* (Clausen et al. 1985).

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Measurement of DNA homology and analysis of cell fatty acid profiles have become standard tools in chemotaxonomy (Goodfellow and Minnikin 1985). Bacterial fatty acids are also used as biomarkers in microbial ecology and often provide valuable information on the structure of the microbial community when other methods fail (White 1983). Finally, fatty acids provide the geochemist with an historical record of the sediment biota (Cranwell 1982). Filamentous bacteria present a challenge to both ecologists and taxonomists. Their role in aquatic sediments and their taxonomic relationships are imperfectly understood. The detailed fatty acid profiles of *Vitreoscilla, Flexibacter* and *Filibacter* which are presented in this paper should, therefore, be of interest to the ecologist, the taxonomist and the geochemist.

Materials and methods

Bacterial strains and culture conditions. The *Vitreoscilla* strains were *V. stercoraria* (ATCC 15218) and *Vitreoscilla* spp strains 389 and 390 (Costerton et al. 1961) which were a gift from R. G. E. Murray. *Filibacter limicola* (NCIB 11923) was isolated from the profundal sediments of Blelham Tarn (Maiden and Jones 1984) and the strains of *Flexibacter* were *F. ruber* (NCMB 1436), *F. aurantiacus* (NCMB 1455), *F. flexilis* (NCMB 1377) and *F. elegans* (NCMB 1385). The mineral medium described by Maiden and Jones (1984) was used for all cultures. Trypticase soy (27 g l^{-1}) was added for growth of the *Vitreoscilla* spp and *F. limicola* (TS Medium) and 2 g l^{-1} Tryptone, 2 g l^{-1} Peptone and 1 g l^{-1} Yeast Extract for growth of *Flexibacter* spp (TnPY Medium). *Vitreoscilla* strains 389 and 390 were also grown on TnPY to determine whether medium composition affected the results obtained. Cultures were harvested by centrifugation (30 min, 10,000 × *g*) after 24 h incubation at 20° C. Harvested cells were washed twice in STE buffer (Maniatis et al. 1982) for DNA analysis and in mineral medium for fatty acid extraction.

DNA extraction and analysis. Cells were lysed in STE buffer in which the EDTA concentration had been increased to 50 mM and to which had been added sucrose (25% w/v) lysozyme (0.1% w/v) and Na dodecyl sulfate (2% w/v). The total incubation time was 3 h at 37° C. Chromosomal DNA was prepared by repeated spooling after addition of two volumes of ethanol at −20° C. Labelling and hybridization of chromosomal DNA followed the procedure of Stulp and Stam (1984) except that [α-^{32}P] deoxycytidine 5′ triphosphate was used as the labelled nucleotide. Radioactivity was

determined by Cerenkov counting in a Packard Tricarb Model 2425 liquid scintillation spectrometer.

Fatty acid extraction and analysis. Lyophilized cultures were extracted by a modification (White et al. 1979) of the single phase chloroform-methanol method of Bligh and Dyer (1959). Phospholipids in the solvent phase were separated on a silicic acid column (King et al. 1977) and the fractions collected and evaporated down under a stream of nitrogen. Fatty acids bound to the lipid extracted residue were subject to mild alkaline hydrolysis followed by hexane:chloroform (4:1, v:v) extraction (Moss 1981). This fraction would include components of LPS lipid A (Parker et al. 1982). The phospholipid and bound lipid fractions were subject to mild alkaline methanolysis (White et al. 1979) and the methyl esters stored at −20° C. The esters were analyzed directly by glass capillary gas liquid chromatography (GC) (Bobbie and White 1980; Nichols et al. 1985a). Fatty acid methyl esters were taken up in hexane with methylnonadecanoate (19:0) as the internal injection standard. The esters were analyzed on a Hewlett Packard 5880 GC at 50° C in the splitless mode using a nonpolar cross-linked methyl silicone fused silica capillary column (50 m by 0.2 mm inner diameter). The oven was temperature programmed from 50° C to 160° C at 5° C per minute and then at 2° C per minute to 300° C. Hydrogen was used as the carrier gas (1.0 ml/min). Uniform response has been assumed for all components and peak areas were quantified using a Hewlett Packard 3350 Series programable laboratory data system operated with an internal standard program. Methyl esters of the total β-hydroxy-fatty acid were converted to their corresponding trimethylsilyl ethers using N, 0-bis-(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co., Rockford, IL, USA). After heating for 1 h at 60° C, excess reagent was removed under a stream of nitrogen, and the sample was dissolved in hexane for GC and GC-Mass Spectrometry (GCMS), the latter being performed on a Hewlett Packard 5995A system fitted with a direct capillary inlet. The column described above was used for the analyses. Samples were, once again, injected in the splitness mode at 100° C with a 0.5 min venting time after which the oven was temperature programmed from 100° C to 300° C at 4° C/min. MS operating parameters were: electron multiplier 1600 V, 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 position and geometry were determined by capillary GC-MS analysis of the Diels-Alder adducts formed by reaction of the fatty acid methyl esters with the diene 5,5-dimethoxyl-1,2,3,4-tetrachlorocyclopentadiene (Aldrich Chemical Co, Milwaukee, WI, USA). Detailed descriptions of the derivatization procedure and interpretation of the mass spectra of the adducts have been reported elsewhere (Kidwell and Bieman 1982; Nichols et al. 1985b).

Results

Eight strains of filamentous gliding bacteria, representatives of genera common in freshwater systems, were compared with regard to their DNA homology and their membrane fatty acid content. The results of DNA hybridization experiments showed that there was an extremely high degree of homology among the *Vitreoscilla* strains (Table 1) but not with DNA from *Filibacter limicola*. The *Flexibacter* spp were, on the other hand, much more heterogeneous and the degree of hybridization indicated low genetic similarity.

Table 1. Hybridization rate of DNA isolated from filamentous gliding bacteria

DNA Hybridized[a]	Relative binding (%)
Vitreoscilla sp Str 389 × *Vitreoscilla* sp Str 390	103
Vitreoscilla sp Str 389 × *Vitreoscilla stercoraria*	97
Vitreoscilla sp Str 389*Filibacter limicola*	20
Flexibacter ruber × *Flexibacter aurantiacus*	29
Flexibacter ruber × *Flexibacter flexilis*	34
Flexibacter ruber × *Flexibacter elegans*	20
Flexibacter elegans × *Flexibacter ruber*	12
Flexibacter elegans × *Flexibacter aurantiacus*	12
Flexibacter elegans × *Flexibacter flexilis*	20

[a] The organism listed first in each hybridization was that from which the radio-labelled tracer DNA was prepared

The apparent similarity of the *Vitreoscilla* strains was also reflected in their phospholipid fatty acid content (Table 2). In all three the 16:1ω7c acid was dominant with significant quantities of 16:0, 18:1ω7c and 14:0 acids. These components accounted for ca. 95% of the total fatty acids. In contrast the phospholipid fatty acid profile of *F. limicola* was dominated by a15:0 and a17:0 acids (accounting for ca. 70% of the total), but with significant contributions from 16:0, branched 17:1 (two isomers) and 16:1ω7c components. This consistency was observed in the bound fatty acids and the total β-hydroxy fatty acids of the *Vitreoscilla* strains (Table 3). Once again 16:1ω7c and 16:0 fatty acids were present in the largest quantities but on this occasion concentrations of 12:0 and 14:0 acids were significantly higher than the 18:1ω7c component. Growth of *Vitreoscilla* strains 389 and 390 on TnPY medium did not alter the pattern of fatty acid dominance (Tables 2 and 3) although the absolute quantities of fatty acids obtained did change. The dominant bound fatty acids of *F. limicola* were similar to those found in the phospholipid fraction except that i17:0 replaced a17:0 in the former. The β-OH 12:0 fatty acid was dominant in all four strains. Quantities of phospholipid and particularly of bound fatty acids were significantly less in *F. limicola* than in the *Vitreoscilla* strains.

There was far less consistency in the phospholipid fatty acid profiles of the *Flexibacter* strains (Table 4). Three of the strains shared the common dominant fatty acids 16:1ω7c and 16:0 with the *Vitreoscilla* strains but these were replaced by 16:1ω5c and i15:0 in *F. flexis*. *F. ruber* may be distinguished from the other strains by the absence of branched odd-chain monounsaturated fatty acids. It is similar to the *Vitreoscilla* spp in phospholipid fatty acids and the dominance of the 12:0 component in the total β-hydroxy fatty acids, but can be distinguished from them by the greater quantities of 18:1ω9c than of 18:1ω7c, and the absence of β-OH 10:0 acid. *F. aurantiacus* and *F. elegans* are not readily distinguished on the basis of their phospholipid fatty acid profiles, although *F. elegans* differs from all the other organisms tested here in that the total β-OH fatty acids are dominated by β-OH i15:0 (53%) and β-OH i17:0 (25%).

Table 2. Phospholipid fatty acid content of *Vitreoscilla* spp and *Filibacter limicola*

Fatty acid	Fatty acid content (mol % of total)			
	Vitreoscilla spp[a]		*V. stercoraria*	*F. limicola*
	Str 389	Str 390		
12:0	0.3 (0.27)[b]	0.1 (0.6)	0.1	0.3
i 14:0	0.09 (0.06)	0.09 (0.1)	tr[d]	tr
14:1	0.7 (0.09)	0.6 (1.4)	0.3	0.06
14:1ω5	nd[c] (tr)	nd (nd)	nd	0.06
14:0	10.0 (8.4)	7.4 (11.5)	3.4	1.8
i 15:0	0.05 (nd)	nd (nd)	nd	1.4
a 15:0	0.06 (tr)	nd (tr)	tr	48.8
15:0	0.07 (0.16)	0.07 (0.1)	0.1	0.08
i 16:0	0.05 (nd)	0.05 (nd)	nd	0.3
16:1ω9c	0.7 (0.9)	1.2 (nd)	0.3	5.4
16:1ω7c	48.8 (54.1)	53.4 (53.9)	52.9	2.8
16:1ω7t	0.8 (1.1)	1.0 (0.9)	1.0	0.09
16:1ω5c	0.4 (0.2)	0.8 (0.2)	nd	0.1
16:0	29.2 (25.0)	26.2 (24.4)	31.1	9.2
br 17:1[e]	nd (nd)	nd (nd)	nd	0.2
br 17:1[e]	nd (nd)	nd (nd)	nd	6.5
i 17:0	nd (nd)	nd (nd)	nd	0.2
a 17:0	nd (nd)	nd (nd)	nd	21.3
17:0	nd (nd)	nd (nd)	nd	tr
18:1ω9c	0.15 (0.4)	0.4 (0.5)	0.3	0.1
18:1ω7c	8.6 (7.5)	7.4 (5.7)	8.9	0.3
18:1ω7t	0.1 (0.08)	0.1 (0.07)	0.1	0.08
18:0	1.0 (0.6)	0.8 (0.7)	1.3	0.2
20:1ω9	0.09 (0.06)	0.07 (tr)	0.1	nd
Total fatty acids (nmol g^{-1} dry wt)	24,400 (29,000)	23,600 (42,700)	44,100	10,100

[a] Values are means of duplicate determinations
[b] Values in parentheses refer to organisms grown on TnPY broth
[c] nd = not detected
[d] tr = trace < 0.5% total fatty acid class content
[e] Double bond configuration of branched 17:1 components in *F. limicola* were not determined

Discussion

It would appear that the genera *Vitreoscilla*, *Filibacter* and *Flexibacter* can be distinguished on the basis of their membrane fatty acids. The differences may be summarized two dimensionally in terms of the percent unsaturation and branching in the groups (Fig. 1). Whereas the strains of *Vitreoscilla* showed remarkable similarity in terms of DNA homology and fatty acid content, the *Flexibacter* spp comprised an heterogeneous group which could be readily distinguished. The three strains of *Vitreoscilla* may not be representative of the genus but all clearly belong to that genus, and according to the results of the DNA hybridization experiments probably were isolates of the same species. The *Flexibacter* spp tested were clearly not related to any degree and the status of the genus requires further examination, including that of its inadequate separation from the genus *Cytophaga* (Christensen 1980).

Woese et al. (1984) clearly distinguished the genus *Vitreoscilla* from other gliding bacteria by placing it in the Beta subdivision of the purple bacteria. The results obtained here support such a distinction. The dominance of *Filibacter* fatty acids by the a 15:0 component was of particular interest since this is also found as a major component of the *Bacillus subtilis* group (Daron 1970; Harwood and Russell 1984; Minnikin and Goodfellow 1981). A recent analysis of the 16S ribosomal RNA and the cell wall structure of *F. limicola* showed that it was Gram-positive and that its closest known relationship was with that of the genus *Bacillus* (Clausen et al. 1985). The same authors indicate a low degree of relatedness between several genera of gliding bacteria, results which are born out by the findings of this paper and which question the value of gliding motility as a major taxonomic character. The three *Flexibacter* spp grouped together can be distinguished readily upon closer examination of their constituent fatty acids. The following features of the fatty acid profiles were considered worthy of further comment.

Increased membrane fluidity is considered to be an attribute of gliding bacteria and this fluidity is considered to be enhanced by the presence of branched and unsaturated (particularly polyunsaturated) fatty acids. Unsaturation at the ω7 and ω9 positions is considered to be particularly important in this respect (Jantzen and Bryn 1985). We found no significant quantities of polyunsaturated acids, particularly the C_{18} and C_{20} acids reported in *Flexibacter polymorphus* by Johns and Perry (1977). Lack of poly-unsaturated acids in benthic filamentous cyanobacteria was attributed to the fact that the organisms may encounter occasional periods of anoxia and that oxygen was required for the synthesis of these acids (Oren et al. 1985). This would appear to be an unlikely explanation for the results obtained

Table 3. Bound fatty acids and total β-hydroxy fatty acids of *Vitreoscilla* spp and *Filibacter limicola*

Fatty acid	Fatty acid content (mol % of total)			
	Vitreoscilla spp[a]		*V. stercoraria*	*F. limicola*
	Str 389	Str 390		
Normal fatty acids				
12:0	13.8 (8.5)[b]	5.6 (nd)	9.2	nd[c]
14:1	tr (0.9)	tr[d] (tr)	0.4	nd
14:0	9.3 (8.3)	8.3 (8.4)	3.4	nd
a 15:0	nd (nd)	nd (nd)	nd	44.7
16:1ω9c	0.8 (1.0)	1.3 (tr)	0.8	5.2
16:1ω7c	40.3 (46.6)	46.2 (51.2)	46.4	2.6
16:1ω7t	1.7 (1.2)	1.4 (1.9)	0.9	nd
16:1ω5c	tr (0.7)	0.7 (tr)	0.6	nd
16:0	26.1 (24.5)	24.9 (26.0)	29.7	15.0
br 17:1[e]	nd (nd)	nd (nd)	nd	7.9
i 17:0	nd (nd)	nd (nd)	nd	24.5
18:1ω9c	1.3 (0.7)	5.4 (1.3)	0.4	nd
18:1ω7c	5.3 (6.6)	5.2 (5.4)	7.3	nd
18:1ω7t	tr (tr)	tr (tr)	tr	nd
18:0	1.0 (1.0)	0.9 (5.7)	1.2	nd
β-Hydroxy fatty acids				
β-OH 10:0	5.7 (3.9)	5.8 (nd)	2.6	nd
β-OH 12:0	86.4 (88.5)	87.0 (100)	86.8	100
β-OH 14:0	7.9 (7.6)	7.1 (nd)	10.5	tr
Total Fatty acid content (nmol g^{-1} dry wt)				
n-fatty acids	8,890 (19,400)	5,520 (1,580)	13,260	380
β-OH fatty acids	1,370 (1,780)	447 (100)	987	20

[a] Values are means of duplicate determinations
[b] Values in parentheses refer to organisms grown on TnPY broth
[c] nd = not detected
[d] tr = trace < 0.3% total fatty acid class content
[e] Double bond configuration of branched 17:1 components in *F. limicola* were not determined

with the benthic bacteria described here since all are obligate aerobes.

The predominance of 16:1ω7c, 16:0 and 18:1ω7 fatty acids in all the isolates is typical of most bacteria including numbers of the *Flavobacterium/Cytophaga* complex and other *Flexibacter* strains (Jantzen and Bryn 1985; Poen et al. 1984; Senghas and Lingens 1985). These groups are also identified as bacterial markers in freshwater sediments (Cranwell 1982). Features which distinguished particular species of *Flexibacter* included larger quantities of 18:1ω9c than of 18:1ω7c fatty acids in *F. ruber* (this is unusual for bacteria) and is in agreement with DNA:rRNA hybridizations with other members of the *Flavobacterium-Cytophaga* complex. The results do not agree with the relatedness between *Flexibacter ruber* and *Flexibacter aurantiacus* observed by Bauwens and De Ley (1981). These authors do not, however indicate which strain of *F. aurantiacus* was examined. The dominance of the 16:1ω5c component in *F. flexilis* and the fact that it has been reported at significant levels only in *Cytophaga hutchinsonii* (Walker 1969) perhaps casts further doubt on the taxonomic distinction of the two genera (Christensen 1980). Completion of the rRNA oligonucleotide catalogues for these groups should resolve the issue.

The total β-OH fatty acids were, in most cases, dominated by the β-OH 12:0 acid in contrast to the normal

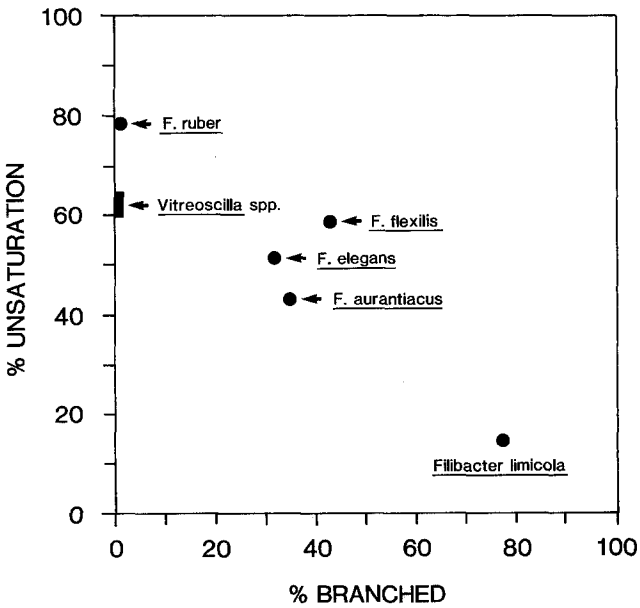

Fig. 1. Two-dimensional graph illustrating the distribution of the gliding bacteria based on percent branching of their fatty acid profiles. The data are taken from Tables 2 and 4

Table 4. Phospholipid fatty acid content of *Flexibacter* spp

Fatty acid	Fatty acid content (mol % of total)			
	F. ruber	*F. aurantiacus*	*F. flexilis*	*F. elegans*[a]
12:0	tr[c]	nd[b]	nd	nd
i 13:0	nd	0.06	tr	0.06
i 14:0	nd	tr	0.27	tr
14:1ω5c	0.08	0.1	0.44	0.13
14:0	2.9	0.63	1.0	0.54
i 15:1ω6c	nd	tr	0.81	tr
i 15:1ω5c	nd	0.5	0.15	0.41
i 15:0	tr	22.8	31.1	17.1
a 15:0	tr	1.0	0.96	0.8
15:1ω6c	0.09	0.68	nd	0.9
15:0	0.2	2.0	1.4	2.7
i 16:1ω7c	nd	0.29	0.78	0.3
i 16:0	nd	0.37	3.0	0.26
16:1ω9c	nd	0.27	nd	nd
16:1ω7c	57.8	27.8	0.53	34.6
16:1ω7t	0.8	0.34	nd	nd
16:1ω5c	0.06	4.5	51.1	3.2
16:0	21.6	24.9	1.9	25.3
i 17:1ω7	nd	4.7	0.8	5.2
i 17:1ω5c	nd	2.6	3.3	2.9
i 17:0	tr	2.9	1.1	3.2
a 17:0	0.7	nd	0.23	nd
17:1ω8c	nd	1.2	tr	1.4
17:1ω6c	nd	nd	tr	1.3
17:0	0.13	0.19	tr	0.2
18:2ω6	0.21	0.09	0.06	0.13
18:3ω3	tr	tr	tr	tr
18:1ω9c	11.8	0.27	0.12	0.33
18:1ω7c	4.10	0.38	tr	0.33
18:1ω6c	tr	0.17	0.15	0.13
18:0	0.36	0.19	0.10	0.10
Total fatty acids (nmol g^{-1} dry wt)	26,700	42,500	47,000	59,600

[a] Double bond configuration of branched 15:1 components were only determined for *F. elegans*
[b] nd = not detected
[c] tr = trace < 0.05% of total fatty acid class content

dominance by the β-OH 14:0 acid associated with Gram-negative bacteria (Harwood and Russell 1984). The β-OH branched acids which were observed in *Flexibacter elegans* are considered to play a part in membrane fluidity in other gliding bacteria (Harwood and Russell 1984). Johns and Perry (1977) have previously tentatively identified i17:1ω11 in *Flexibacter polymorphus* but they did not confirm the double bond position. Two iso-branched 17:1 isomers, including double bond configurations, were positively identified in *F. limicola* and three species of *Flexibacter* in this study. The presence of the i17:1ω7c fatty acid in these bacteria could not, however, be considered as a useful marker since it is also found in sulphate reducing bacteria of the genus *Desulfovibrio* (Taylor and Parkes 1985; Edlund et al. 1985). Interestingly, the three *Flexibacter* species also contained i17:1ω5c as a significant component in addition to the i17:1ω7c acid. The former may be a biomarker for selected members of the genus *Flexibacter* as it has not, to our knowledge, been detected in other bacteria. It may also contribute to the solution of taxonomic uncertainties within the genus.

Clearly, chemotaxonomic procedures will produce results which challenge our current concepts of taxonomic relationships between bacterial groups. It is equally clear that if membrane fatty acids are to be used as biomarkers by taxonomists, ecologists and geochemists, then precise determination of double bond positions and geometry, such as those provided here, will be essential in the correct interpretation of increasingly complex data sets.

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