Adhaeribacter aquaticus gen. nov., sp. nov., a Gram-negative isolate from a potable water biofilm

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A Gram-negative bacterium was isolated from a freshwater biofilm developed on a stainless steel surface under a fluid velocity of 0.26 m s^{-1} . The strain, MBRG1.5^T, was cultivated on R2A agar and formed pink colonies. Light microscopy and negative staining in a transmission electron microscope showed that the cells were rod-shaped, approximately $2.8\text{--}4.1 \text{ }\mu\text{m}$ long by $0.9\text{--}1.7 \text{ }\mu\text{m}$ wide in size and produced large quantities of extracellular fibrillar material. Additionally, following growth in batch culture, transmission electron microscopy showed that many cells plasmolysed. Stationary-phase cells were more variable in size and shape. The DNA G+C content was 40.0 mol%. The most abundant fatty acids were 15:0 iso (22.5%), followed by 16:1 ω 5c (16.9%) and 15:0 iso 2-OH (16.5%). Phylogenetic analysis of the 16S rRNA gene showed that the strain was a member of the family 'Flexibacteraceae' of the *Cytophaga-Flavobacterium-Bacteroides* group. Phenotypic and genotypic analyses indicated that the strain could not be assigned to any recognized genus; therefore a novel genus and species, *Adhaeribacter aquaticus* gen. nov., sp. nov., is proposed, with MBRG1.5^T (=DSM 16391^T = NCIMB 14008^T) as the type strain.

In nature, bacteria predominantly exist in close association with surfaces, forming multispecies communities called biofilms (Costerton *et al.*, 1995). In potable water systems, biofilms form readily at all solid-liquid interfaces, including the inner surfaces of pipes and storage vessels (Kerr *et al.*, 2003). Many bacterial species, including members of the *Proteobacteria*, the *Actinobacteria*, low-G+C Gram-positive bacteria and members of the *Cytophaga-Flavobacterium-Bacteroides* (CFB) group, co-exist within potable water biofilms (Schmeisser *et al.*, 2003; Norton & Lechevallier, 2000). The ability of these taxonomically diverse organisms to attach to surfaces and co-develop within multispecies biofilms is essential for their survival and persistence within such flowing freshwater environments (Bott & Grant, 2001; Purevdorj *et al.*, 2002; Stoodley *et al.*, 2002).

In the last 20 years, evidence has emerged that biofilms in potable water systems may pose a significant hazard to public health (Burke *et al.*, 1984). In particular, potable water biofilms have been linked to outbreaks of

gastrointestinal illness (Williams & Braun-Howland, 2003; Zalmum *et al.*, 1998) and outbreaks of legionellosis (Steinert *et al.*, 2002). Other problems that are associated with potable water biofilms include microbially induced corrosion of pipe surfaces and unpleasant taste and odour (Kerr *et al.*, 2003). In general, biofilms in potable water systems are problematic because they are composed of numerous species of bacteria. Collectively, these generate multispecies biofilm communities that possess properties (such as a decreased susceptibility to antimicrobial agents and the ability to corrode metal) that are not displayed by the individual species (Gilbert *et al.*, 2002). As a consequence, there is increasing interest in studies on the microbial diversity of freshwater biofilms and the physiological properties of the component organisms.

A recent study on the influence of water velocity on the community diversity of potable water biofilms demonstrated that the taxonomic diversity of biofilms developed at high velocities was much lower than that of biofilms developed under conditions of minimal velocity (Rickard *et al.*, 2004). Some strains that were isolated on R2A agar (Reasoner & Geldreich, 1985) at the high velocities possessed unusual morphological and biochemical characteristics. In particular, strain MBRG1.5^T possessed a low partial 16S rRNA gene sequence identity (92.7%) when compared with sequences from species with validly published names

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Abbreviations: CFA, cellular fatty acid; CFB, *Cytophaga-Flavobacterium-Bacteroides*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MBRG1.5^T is AJ626894.

in the EMBL database (Rickard *et al.*, 2004). In this study, strain MBRG1.5^T is described using morphological and physiological characterization, fatty acid methyl ester analysis and full 16S rRNA-based phylogenetic classification. On the basis of these data and the significant lack of information available on the closest relatives, we propose a novel genus and species, *Adhaeribacter aquaticus* gen. nov., sp. nov., with MBRG1.5^T (=DSM 16391^T=NCIMB 14008^T) as the type strain.

Phylogeny

Almost the entirety of the 16S rRNA gene of MBRG1.5^T was amplified by using a PCR with the universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3'; *Escherichia coli* positions 8–27) (Edwards *et al.*, 1989) and 1492r (5'-TACGGTTACCTTGTACGACTT-3'; positions 1492–1512) (Weisberg *et al.*, 1991). The PCR was carried out as described by Glockner *et al.* (1996) except that the annealing temperature was 55 °C. Sequencing of the amplified product was carried out using the primers 27f, 1492r, 518f (5'-CCAGCAGCCGCGTAAT-3'; positions 518–534) and 1070r (5'-AGCTGACGACAGCCAT-3'; positions 1070–1085) according to the method of Lane (1991). Similarity searches with the 16S rRNA gene sequence derived were done in the EMBL database using the program FASTA (<http://www.ebi.ac.uk/fasta33/>) (Pearson & Lipman, 1988). Comparisons against 16S rRNA gene sequences in the EMBL database showed that strain MBRG1.5^T possessed the highest identities with an unsequenced 'Taxeobacter' strain (SAFR-033; 92.1%) and an uncultured member of the *Cytophagales* (*Cytophagales* bacterium clone FBP292; 88.8%). The two sequenced organisms with the highest identities to strain MBRG1.5^T were *Flexibacter aggregans* (88.8%) and *Hymenobacter actinosclerus* (87.5%). The sequence identities to other members of the CFB group

ranged from 85 to 87%. Such low similarity to species with validly published names is indicative that the organism under study is likely to be a member of a novel genus (Stackebrandt & Goebel, 1994). To examine the phylogenetic relationships between strain MBRG1.5^T and validated and closely related strains, CLUSTAL X (version 1.81) (Thompson *et al.*, 1997) was used to align 1350 unambiguously identified base pairs of partial 16S rRNA gene sequences from strain MBRG1.5^T against sequences from related strains in the EMBL database. Neighbour-joining analysis was conducted with the correction of Jukes & Cantor (1969), using TREECON (version 1.3b) (Van de Peer & De Wachter, 1997). The stability of the groupings was estimated by using bootstrap analysis (1000 replications). *Flavobacterium aquatile* (M62797) was used as the outgroup (Fig. 1). The tree clearly showed that strain MBRG1.5^T formed a separate and distinct lineage within the family 'Flexibacteraceae' of the CFB group. Furthermore, it is clear that strain MBRG1.5^T is unrelated to members of the genera *Hymenobacter*, 'Taxeobacter' and *Flexibacter*. The robustness of the phylogenetic relationships and the low sequence similarities between strain MBRG1.5^T and the other validated strains within the CFB group strongly suggest that the strain represents a novel genus in the family 'Flexibacteraceae'.

Phenotypic characteristics

Cell morphology, size and motility were examined using phase-contrast microscopy (Axioskop 2-MOT; Zeiss) and transmission electron microscopy (Technai 12 Biotwin; Philips). To study strain MBRG1.5^T by transmission electron microscopy, cells were prepared for negative staining using the method of Handley *et al.* (1985). Cells in exponential phase (2-day liquid R2A batch cultures) were rod-shaped (typically 2.8–4.1 × 0.9–1.7 µm), produced

Fig. 1. Neighbour-joining phylogenetic tree derived from comparative analysis of 16S rRNA gene sequences from members of the 'Flexibacteraceae' and the *Sphingobacteriaceae*. Database accession numbers are shown in parentheses. Bootstrap values above 50% from 1000 bootstrap resamplings from the distance analysis are presented at nodes. *Flavobacterium aquatile* was used as the outgroup. Bar, 0.1 nucleotide substitution per nucleotide position.

Fig. 2. Electron micrographs of negatively stained cells of *Adhaeribacter aquaticus* MBRG1.5^T harvested after 72 h from batch culture. Cells were stained with 1.5% (w/v) methylamine tungstate. (a) Rod-shaped cells are surrounded by an extensive fibrillar matrix composed of fibrillar bundles (FB) extending out into fine individual thin fibres (TF). Every cell possessed a clearly visible electron-dense 'halo' (EDH) and an inner membrane (IM). An outer membrane (OM) was visible on some cells. Features of cells 'A' and 'B' are described in the text. (b) Higher magnification, showing the overlapping and intertwining of individual fibrils into fibrillar bundles that radiate out into thin fibrils. Bars, 1 µm (a) and 200 nm (b).

copious amounts of extracellular polymeric substance (Fig. 2) and possessed a capsule (also confirmed by the addition of the acidic dye nigrosin). In stationary-phase cultures (10-day liquid R2A cultures), cells were much more variable in size ($1.9\text{--}6.7 \times 0.8\text{--}1.9$ µm) (Fig. 3). Mesosomes were observed in stationary-phase cells. Reproduction occurred by binary fission; the separating daughter cells were half the size of the parent cells. Negative staining showed that all cells from exponential phase and stationary phase were completely covered in a dense fibrillar matrix, probably composed of extracellular polysaccharide (Fig. 2). The fibrillar matrix appeared to fuse between cells where the fibrils overlapped, and thicker bundles of fibrillar material were conspicuous (Fig. 2). The individual fibres of the matrix were extremely thin and radiated out from the cells. The majority of the cells showed evidence of plasmolysis, as the inner membrane had clearly shrunk

away from the outer membrane in some cells (Fig. 2a, cell 'A'). In other cells, the inner membrane was seen to be blebbing and shrinking inwards (Fig. 2a, cell 'B') but the outer membrane could not be detected. Around every cell, an electron-dense 'halo' was present, appearing to be underneath the fibrillar matrix and outside the cell membranes (Fig. 2).

Gram staining was performed using the method described by Smibert & Krieg (1994). Other biochemical investigations included tests for oxidase, catalase, nitrate reduction, urease activity and indole production. Growth on various standard bacteriological media was assessed by using CM3 nutrient agar (Oxoid), water agar (Reichenbach, 1992) and marine agar (Oxoid). Growth at different temperatures (4, 20, 25, 28, 30, 37, 42, 45, 50 °C) was investigated on R2A agar plates. Tolerance towards NaCl, at 30 °C, was studied on R2A agar plates supplemented with 0.5, 1, 2.5, 4, 5, 10, 20 and 25% (w/v) NaCl (pH 7.4). Carbon-source utilization tests were done by supplementing 50 ml aliquots of minimal medium (Cohen-Bazire *et al.*, 1957) with different carbon sources and testing for growth in 100 ml conical flasks that were subjected to shaking (250 r.p.m.) at 30 °C. Growth was determined by following the OD₆₅₀ over 7 days. On R2A agar, strain MBRG1.5^T grew at 30 °C, generating pink, gelatinous, convex, entire colonies 4 ± 1 mm in diameter. Colonies were not easily emulsified in distilled water. No growth was detected on CM3 nutrient agar but growth could be detected on water agar and marine agar. Incubation of agar plates at 30 °C resulted in the generation of visible colonies after 2–4 days. Similar colony types were observed on water agar and marine agar; gliding motility was not observed. Strain MBRG1.5^T was able to grow on R2A agar at temperatures from 4 to 37 °C. The maximum growth rate was observed at 30 °C with shaking at 250 r.p.m. Under these conditions, the mean generation time in R2A broth was between 1.5 and 2.5 h. If the speed of shaking or the temperature was altered, the mean generation time

Fig. 3. Digital light micrograph of cells harvested from a 10 day (240 h) batch culture, showing the variable cell size and shape of strain MBRG1.5^T. Bar, 10 µm.

Table 1. Biochemical characteristics of strain MBRG1.5^T and related strains

Strains: 1, MBRG1.5^T; 2, *Hymenobacter actinosclerus* CCUG 39621^T (data from Collins *et al.*, 2000); 3, *Flexibacter aggregans* ATCC 23162^T (Lewin & Lounsbury, 1969); 4, *Algoriphagus ratkowskyi* ACAM 646^T (Bowman *et al.*, 2003); 5, *Hongiella mannitolivorans* LMG 22067^T (Yi & Chun, 2004); 6, *C. marinum* DSM 745^T (Raj & Maloy, 1990). +, Positive; –, negative; v, variable; ND, not determined; pNP, *para*-nitrophenyl. All strains gave the same results for the following: growth at 25 °C (+), aerobic growth (+), anaerobic growth (–), gliding motility (–) and H₂S production (–).

Characteristic	1	2	3	4	5	6
Cell morphology	Rods	Rods	Rods	Rods	Rods	Ring-like, spiral
Cell size (µm)	0.9–1.7 × 2.8–4.1	0.5–0.6 × 2.0–3.6	1.2–1.8 × 2.2–2.7	0.3–0.4 × 0.3–0.9	0.4–0.5 × 1.1–1.7	0.3–0.7 × 0.8–1.5
Colony colour	Pink	Red	Pink	Pink	Orange	Pink
DNA G + C content (mol%)	40	62	35	35–36	42	34–38
Growth at:						
4 °C	+	–	+	+	–	+
28 °C	+	+	+	–	+	+
37 °C	+	+	+	–	+	+
42 °C	–	+	–	–	–	+
Growth on:						
2.5 % (w/v) NaCl	+	–	–	+	+	+
10.0 % (w/v) NaCl	–	–	–	–	+	+
Oxidase	+	+	+	+	+	+
Catalase	+	+	–	–	+	+
Indole	–	–	–	ND	+	–
Nitrate reduction	–	–	–	–	v	–
Organic acids and sugars assimilated:						
Adonitol	+	ND	ND	–	ND	–
Arabinose	+	ND	ND	–	–	ND
Citrate	–	+	ND	ND	–	+
D-Fructose	+	–	ND	+	ND	ND
Fumarate	–	+	ND	ND	ND	ND
Galactose	–	ND	+	+	ND	ND
D-Gluconate	+	+	ND	+	ND	+
D-Glucose	+	+	+	+	+	ND
Inositol	+	ND	ND	+	–	–
D-Lactate	–	+	ND	–	ND	ND
Lactose	–	ND	–	+	+	ND
Malonate	–	–	ND	+	ND	+
D-Maltose	–	–	ND	+	ND	ND
D-Mannitol	–	–	ND	+	ND	–
D-Mannose	–	+	ND	+	+	+
N-Acetyl D-glucosamine	+	+	ND	+	+	ND
Pyruvate	+	+	ND	–	ND	ND
D-Rhamnose	–	–	ND	+	ND	+
D-Ribose	+	+	ND	ND	–	ND
Salicin	–	–	ND	+	+	ND
Sorbitol	–	ND	ND	+	–	–
Suberate	–	+	ND	–	ND	ND
Sucrose	+	–	+	+	+	ND
D-Trehalose	+	–	ND	+	+	ND
Xylose	–	ND	ND	+	+	ND
Amino acids assimilated:						
Arginine	–	ND	–	–	–	–
Alanine	+	+	ND	–	ND	ND
Glutamic acid	–	ND	+	–	ND	ND
Histidine	+	ND	ND	–	ND	ND

Table 1. cont.

Characteristic	1	2	3	4	5	6
Isoleucine	+	ND	—	ND	ND	ND
Leucine	+	+	—	—	+	ND
Lysine	+	ND	—	ND	—	ND
Methionine	+	ND	—	ND	ND	ND
Tryptophan	—	ND	—	ND	ND	ND
Ornithine	+	ND	ND	V	ND	ND
Proline	+	+	ND	ND	ND	ND
Phenylalanine	—	+	ND	—	ND	ND
Glycine	+	ND	—	ND	ND	ND
Enzymic hydrolysis of:						
pNP α -D-glucoside	+	+	ND	+	—	ND
pNP β -D-glucoside	+	—	ND	+	—	ND
pNP bisphosphate	+	+	ND	+	ND	ND
pNP N-acetylglucosamine	+	ND	ND	+	+	ND
pNP phosphate	+	+	ND	+	ND	ND
pNP phosphorylcholine	+	+	ND	ND	ND	ND
pNP α -galactoside	+	—	ND	+	+	ND
pNP β -galactoside	+	—	ND	ND	ND	ND
Starch	—	+	—	+	ND	+
Urea	—	—	ND	—	—	ND

increased. Strain MBRG1.5^T grew in the absence of NaCl and in the presence of 0.5, 1.0 and 2.5 % (w/v) NaCl. No growth was observed in the presence of >4.0 % (w/v) NaCl. No growth was detected in cultures incubated under anaerobic conditions. Strain MBRG1.5^T grew equally well in the presence or absence of light. Starch hydrolysis and nitrate reduction were not detected. Catalase was detected (weak activity) and the strain was oxidase-positive. Using minimal medium as a base, the requirement for, and utilization of, 26 simple sugars and organic acids and 14 amino acids were tested (Table 1). Additionally, the enzymic hydrolysis of eight substrates was tested using BBL Crystal test kits (Becton Dickinson) according to the manufacturer's instructions. The biochemical and physiological characteristics of strain MBRG1.5^T were compared with those of members of the two genera that were most closely related to strain MBRG1.5^T (according to 16S rRNA gene sequence analysis, described below). A high degree of dissimilarity was evident with respect to both *Hymenobacter actinosclerus* (12 different characteristics) and *Flexibacter aggregans* (nine different characteristics). Unfortunately, in the case of the comparison between strain MBRG1.5^T and *Flexibacter aggregans*, many of the characteristics could not be compared because many current techniques and tests were not available to the original investigators (Lewin & Lounsbery, 1969) (Table 1). Three other species with validly published names (*Cyclobacterium marinum*, *Hongiella mannitolivorans* and *Algoriphagus ratkovskyi*) were also included as representative members of the CFB group and were compared with strain MBRG1.5^T. Strain MBRG1.5^T is clearly distinct from all these representative members of the CFB group (Table 1).

Antibiotic-susceptibility tests were performed by using the agar-diffusion method with antibiotic-impregnated discs (Oxoid). Briefly, 100 μ l bacterial suspension with an OD₆₅₀ of 1.0 was plated onto R2A agar and left for 1 h. The following antibiotics were tested (μ g antibiotic per disc shown in parentheses): amikacin (30), carbenicillin (100), cefotaxime (30), cefoperazone (75), cefsulodin (30), ciprofloxacin (5), erythromycin (5), fusidic acid (10), gentamicin (10 and 30), imipenem (10), methicillin (10), penicillin G (1 U), piperacillin (100), ticarcillin (75), tobramycin (10), trimethoprim (5) and vancomycin (5). After incubation at 30 °C for 5 days, plates were examined and any sign of growth inhibition was scored as sensitivity to that antibiotic. Strain MBRG1.5^T was susceptible to methicillin, imipenem, gentamicin, trimethoprim, cefoperazone, vancomycin, erythromycin, carbenicillin, ciprofloxacin, cefotaxime, fusidic acid, cefsulodin, piperacillin, penicillin G, tobramycin, amikacin and ticarcillin. A comparison between these results and those obtained for *Hymenobacter actinosclerus* showed that susceptibilities to different antibiotics were identical.

Chemotaxonomy

Strain MBRG1.5^T was subcultured onto R2A agar, incubated for 4 days at 30 °C and harvested by washing with distilled water. Cells (40 mg) were harvested from triplicate cultures. Lysis of cells (saponification), methylation of fatty acids and extraction of the methyl esters into the organic phase were achieved using the methods of Ghanem *et al.* (1991). Fatty acid methyl ester profiles were generated using a model 5898A Microbial Identification system (Microbial ID) consisting of a Hewlett Packard model 6890

Table 2. Identifiable CFAs from MBRG1.5^T and related strains

Strains: 1, MBRG1.5^T; 2, *Hymenobacter actinosclerus* CCUG 39621^T; 3, *Algoriphagus ratkowskyi* ACAM 646^T; 4, *Hongiella manitolivorans* LMG 22067^T. Values shown are percentages of total fatty acids. Only fatty acids representing more than 0.2% of the total fatty acids of at least one of the strains are shown.

CFA	1	2	3	4
10:0 iso	1.2	—	—	—
11:0 anteiso	—	—	2.1	0.6
Summed feature 2*	—	2.3	—	—
14:0 iso	—	—	1.4	2.3
15:0	—	—	1.6	3.0
15:0 iso	22.5	22.3	30.5	32.7
15:0 anteiso	4.4	25.8	3.6	3.7
15:0 iso 2-OH	16.5	0.8	—	—
15:0 iso 3-OH	3.1	1.6	2.9	2.7
15:1 ω 6c	—	0.8	2.1	4.1
15:1 iso G	—	—	1.1	0.4
16:0	—	—	2.9	0.2
16:1 iso H	—	1.5	—	—
Summed feature 4*	—	13.1	22.3	7.4
16:1 ω 5c	16.9	3.7	5.8	0.6
16:0 3-OH	—	—	1.9	—
16:0 iso	—	—	3.4	6.5
16:0 iso 3-OH	—	—	2.8	4.7
16:1 iso H	—	—	1.7	3.5
Summed feature 5*	11.2	19.9	—	—
17:0 iso	—	1.8	—	—
17:0 anteiso	—	0.7	—	—
17:0 2-OH	—	—	—	0.4
17:0 iso 3-OH	12.1	3.1	9.2	6.4
17:0 iso 2-OH	—	2.0	—	—
17:1 ω 6c	5.1	0.7	0.5	7.0
17:1 iso ω 9c	—	—	1.5	6.0
17:1 anteiso ω 9c	—	—	—	—
Summed feature 6*	—	—	0.9	2.5

*Summed features consist of one or more fatty acids that cannot be separated by the Microbial Identification system. Summed features consisted of: 2, 15:1 iso I and/or 13:0 3-OH; 4, 16:1 ω 7c, 15:0 iso 2-OH and/or 16:1 ω 7t; 5, 17:1 iso I, 17:1 anteiso B and/or 17:1 anteiso B/iso I; 6, 17:1 iso I and/or 17:1 anteiso B.

gas chromatograph fitted with two 5% phenylmethyl silicone capillary columns (0.2 mm $\times$ 25 m), flame-ionization detectors, a Hewlett Packard model 7637A automatic sampler and a Hewlett Packard Vectra XM computer. Samples (2 μ l) were injected onto the columns with a temperature gradient rising from 170 to 270 °C, an injection port temperature of 300 °C and ultra-high-purity hydrogen as the carrier gas. Fatty acids were identified and peaks integrated automatically using MIS Library Generation Software (Microbial ID). All of the cellular fatty acids (CFAs) extracted from strain MBRG1.5^T were

identified by using the MIDI system. The fatty acid 15:0 iso represented the highest proportion (22.5%), this being followed by 16:1 ω 5c (16.9%) and 15:0 iso 2-OH (16.5%) (Table 2). A comparison with *Hymenobacter actinosclerus* (the most closely related strain to undergo CFA analysis) demonstrated that the types and amounts of CFA from strain MBRG1.5^T were completely different. A review of the literature concerning the amounts of CFA isolated from strains belonging to the CFB group also suggests that the CFA profile for strain MBRG1.5^T is unique; such a significant difference in fingerprint pattern has not been observed before.

For further comparison of MBRG1.5^T with other strains, its DNA G+C content was determined. DNA was prepared according to the method of Chun & Goodfellow (1995) and the G+C content was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) by using the thermal denaturation method (Mandel & Marmur, 1968). The G+C content of the DNA of strain MBRG1.5^T was established as 40.0 mol%. The G+C contents of the most closely related species, *Hymenobacter actinosclerus* and *Flexibacter aggregans*, are significantly different (62 and 35 mol%, respectively; Table 1).

Taxonomic conclusions

Members of the CFB group are widely distributed in nature and have previously been isolated from soil, sub-surface sediments, plants and water (Junge *et al.*, 2004; Buczolits *et al.*, 2002; Garbeva *et al.*, 2001; Hiorns *et al.*, 1997). In many environments they are present as community members of multispecies biofilms (Kindaichi *et al.*, 2004; Battin *et al.*, 2001). The widespread distribution of members of the CFB group can be explained by their ability to survive and grow at relatively low temperatures (Rickard *et al.*, 2004), at low nutrient concentrations (Junge *et al.*, 2004) and in chemically or physically adverse environments (Rickard *et al.*, 2004; Eiler *et al.*, 2003). The broad range of different environments in which bacteria belonging to this group can survive is a reflection of the variety of species- and genus-specific biochemical and physiological properties that they possess (Staley & Gosink, 1999). Unfortunately, this is a significant problem when comparing them by using a polyphasic approach and subsequently assigning genus and species identities. This is further compounded by the phylogenetic heterogeneity of the group as a whole, a lack of named genera, and the fact that much of the work that was carried out on the CFB group was done more than 30 years ago (Lewin, 1969; Lewin & Lounsbury, 1969).

It is evident from this polyphasic study of an unusual freshwater biofilm isolate that the Gram-negative rod-shaped organism described is distinct from all other currently described members of the CFB group. A phylogenetic comparison of the 16S rRNA gene sequence of strain MBRG1.5^T with sequences from established species

clearly shows that it is genetically distinct, although pairwise sequence comparisons strongly indicate that it is a member of the family 'Flexibacteraceae' (Garrrity & Holt, 2001). A comparison of the cell morphology of strain MBRG1.5^T with those of the most closely related species (*Flexibacter aggregans*, *Flexibacter elegans*, *Hymenobacter actinosclerus* and 'Taxeobacter ocellatus') further demonstrated the difference between strain MBRG1.5^T and other members of the 'Flexibacteraceae'. None of the currently identified members of the 'Flexibacteraceae' with validly published names have similar cellular characteristics and none have been reported to produce large quantities of extracellular fibrillar material, which is characteristic of strain MBRG1.5^T. Additionally, whilst only scant information is available on the biochemical/CFA characteristics of members of the 'Flexibacteraceae', strain MBRG1.5^T possesses many biochemical/CFA characteristics unique to all other members of the 'Flexibacteraceae' (Tables 1 and 2; also reviewed by Reichenbach, 1992). Thus, strain MBRG1.5^T is a novel member of the 'Flexibacteraceae' and all available evidence suggests that it is a member of a novel genus.

The most noteworthy characteristic of strain MBRG1.5^T was that the colonies were mucoid in appearance and the cells did not pellet tightly when centrifuged. These observations can be explained by the presence of a capsule (detected by light microscopy) and the presence of the extracellular fibrillar matrix (detected by negative staining). However, it is not clear whether these two components detected by the two different methods are in fact the same component. In addition, the electron-dense 'halo' could represent the capsule. Further electron microscopy using fixation and thin sectioning would resolve the structural identity of the various layers, detected by negative staining, outside the inner membrane. Strain MBRG1.5^T was isolated from a biofilm exposed to high fluid velocity (0.26 m s⁻¹) (Rickard *et al.*, 2004) and it is well known that extracellular polymeric materials provide cohesiveness in biofilms and are considered to be essential for the formation and maintenance of biofilms *in situ* (see review by Flemming *et al.*, 2000). It is therefore likely that the fibrillar matrix material seen by means of negative staining and transmission electron microscopy is the extracellular polymer that enables the cells to become established within the biofilm and to resist the high shear force.

Description of *Adhaeribacter* gen. nov.

Adhaeribacter (Ad.haer'i.bac.ter. L. v. *adhaereo*, *adhaere* to adhere to, to stick fast; N.L. masc. n. *bacter* from Gr. n. *bakterion* rod; N.L. masc. n. *Adhaeribacter* sticky rod).

Cells are Gram-negative rods that are non-motile. Obligate aerobic, chemo-organotrophic and oxidase- and catalase-positive. Changes in cell size occur during growth in batch culture. Cells produce copious amounts of extracellular fibrillar material. The predominant CFAs are 15:0 iso (22.5%), followed by 16:1 ω 5c (16.9%) and 15:0 iso 2-OH

(16.5%). As determined by 16S rRNA gene sequence analysis, the genus *Adhaeribacter* is a member of the 'Flexibacteraceae' in the CFB phylum. The type species is *Adhaeribacter aquaticus*.

Description of *Adhaeribacter aquaticus* sp. nov.

Adhaeribacter aquaticus (a.qua'ti.cus. L. masc. adj. *aquaticus* living, growing, or found in or by water, aquatic).

Displays the following properties in addition to those given in the genus description. Colonies on R2A agar are circular, entire, pink and not easily emulsified. Cells are 2.8–4.1 μ m in length and 0.9–1.7 μ m in width. No gliding motility is observed. Weak hydrolysis of starch is observed. Tests for nitrate reduction, H₂S production and indole production are negative. Temperature for growth is 4–37 °C. Growth occurs in the range 4–37 °C and the optimal temperature for growth is 30 °C. NaCl is not required for growth; up to 4.0% (w/v) NaCl is tolerated. Acid and gas are not produced from D-glucose. Adonitol, arabinose, D-fructose, D-gluconate, D-glucose, inositol, N-acetyl D-glucosamine, pyruvate, D-ribose, sucrose and D-trehalose are assimilated. Citrate, fumarate, galactose, D-lactate, lactose, malonate, D-maltose, D-mannose, D-rhamnose, salicin, sorbitol, suberate and xylose are not utilized. Hydrolysis of α -D-glucoside, β -D-glucoside bisphosphate, N-acetylglucosamine phosphate, phosphorylcholine, α -galactoside and β -galactoside detectable. Susceptible to methicillin, imipenem, gentamicin, trimethoprim, cefoperazone, vancomycin, erythromycin, carbenicillin, ciprofloxacin, cefotaxime, fusidic acid, cefsulodin, piperacillin, penicillin G, tobramycin, amikacin and ticarcillin. The DNA G + C content is 40.0 mol%.

The type strain is MBRG1.5^T (=DSM 16391^T=NCIMB 14008^T), isolated from a freshwater biofilm, developed in a model system, which was exposed to a fluid velocity of 0.26 m s⁻¹ over a 3-month period.

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