Filomicrobium insigne sp. nov., isolated from an oil-polluted saline soil

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Strain SLG5B-19^T, isolated from an oil-polluted saline soil in Gudao in the coastal Shengli Oilfield, eastern China, was Gram-negative with monoprosthecae or bipolar prosthecae and buds on the prosthecal tips. Growth occurred at NaCl concentrations between 0 and 7% (w/v), at temperatures between 4 and 45 °C, and at pH 6.0–9.0. Strain SLG5B-19^T had Q-9 as the major respiratory quinone and unsaturated C_{18:1}ω7c as the predominant cellular fatty acid. The G + C content of the genomic DNA was 59.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain SLG5B-19^T belonged to a clade with the genera *Filomicrobium* and *Hyphomicrobium* in the class *Alphaproteobacteria*. However, 16S rRNA gene sequence similarities of strain SLG5B-19^T to the phylogenetically most closely related strains, i.e. the type strains of *Filomicrobium fusiforme* and *Hyphomicrobium zavarzinii*, were 95.8 and 94.5%, respectively. In addition, the 16S rRNA gene sequence of strain SLG5B-19^T had 24 signature nucleotides that were identical to those of the type strain of *F. fusiforme*. Based on phylogenetic analysis of 16S rRNA gene sequences, strain SLG5B-19^T could be allocated to the genus *Filomicrobium*. However, distinct phenotypic differences were observed between strain SLG5B-19^T and the type strain of *F. fusiforme*. It is therefore proposed that strain SLG5B-19^T represents a novel species in the genus *Filomicrobium*, *Filomicrobium insigne* sp. nov. The type strain is SLG5B-19^T (=CGMCC 1.6497^T=LMG 23927^T).

Bacteria that are able to form buds and/or possess prosthecae have been investigated in detail in terms of their chemotaxonomic characteristics and by phylogenetic analysis (Sittig & Hirsch, 1992; Stackebrandt *et al.*, 1988). These organisms belong mainly to the genera *Hyphomicrobium*, *Hyphomonas*, *Pedomicrobium* and *Filomicrobium*. The genus *Filomicrobium* was created in 1987 and comprises only one species, *Filomicrobium fusiforme*, which was isolated from the brackish water of the Kiel Bight in Germany, which is part of the Baltic Sea (Schlesner, 1987). *F. fusiforme* produced poor growth on solid media. Microcolonies of 0.1–0.2 mm in diameter with a rough surface were observed after 3 weeks incubation at 25 °C. *Filomicrobium* strains are non-motile and do not use C₁ compounds as carbon sources, characteristics that enable them to be differentiated from members of the genus *Hyphomicrobium* (Rainey *et al.*, 1998). The genus *Hyphomicrobium* currently contains 12

recognized species and can be divided into two phylogenetic clusters by the genus *Filomicrobium* in the phylogenetic tree based on 16S rRNA gene sequence analysis (Rainey *et al.*, 1998; also shown in Fig. 1). Species of the genus *Hyphomicrobium* are distributed widely in diverse environments (Morgan & Dow, 1985; Poindexter, 1992), including soil and groundwater (Hirsch & Rades-Rohkohl, 1983), freshwater ponds and lakes, brackish water, marine environments (Montreal Biodome) (Labbe *et al.*, 2003), hypersaline Antarctic lakes (Hirsch & Siebert, 1991), sewage treatment plants (Meyers & Meyers, 1986) and even in a fluidized bed reactor of a drinking water treatment plant (Liessens, 1993).

In this study, the characterization of a novel strain, SLG5B-19^T, isolated from an oil-polluted saline soil in a coastal oilfield in eastern China is reported. Results indicate that the strain represents a novel species of the genus *Filomicrobium*.

An oil-polluted soil was sampled at Gudao Oil-Product in the coastal Shengli Oilfield, Shandong Province, eastern China (Gu *et al.*, 2007). The soil sample was incubated in

Abbreviations: EPS, exopolysaccharide; PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DBBJ accession number for the 16S rRNA gene sequence of strain SLG5B-19^T is EF117253.

Fig. 1. Phylogenetic position of strain SLG5B-19^T according to 16S rRNA gene sequence analysis. The topology shown was obtained by using the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Bar, 0.01 nt substitutions per nt position. Clusters I and II were defined by Rainey *et al.* (1998). Other algorithms including maximum-parsimony and maximum-likelihood were also used to compare and determine the topological stability of the phylogenetic trees; these algorithms gave nearly the same topology as shown in this figure.

inorganic salts medium (Gu *et al.*, 2007) amended with 2 % crude oil at 30 °C for 2 weeks. Strain SLG5B-19^T was isolated from this enrichment by a 10-fold dilution plating technique with inorganic salts agar. The isolate was then purified by restreaking on inorganic salts agar plates incubated for 7–10 days at 30 °C.

The strain was grown on Luria–Bertani (LB) agar for about 7 days at 30 °C to late exponential phase; cell morphology and hyphal type were then examined using transmission and scanning electron microscopy. Gram staining and poly- β -hydroxybutyrate (PHB) staining with Sudan black (according to Smibert & Krieg, 1994) and cell motility (Dong & Cai, 2001) were determined. Optimum pH and temperatures for growth and NaCl requirements/tolerance were tested using medium 166 containing 1.0 g (NH₄)₂SO₄, 0.2 g MgSO₄·7H₂O, 0.5 g NaH₂PO₄·H₂O, 1.55 g K₂HPO₄, 3.4 g methylamine hydrochloride, 0.2 ml trace element solution, 15.0 g agar and 1 l distilled water, pH 7.2. Inorganic salts medium was used to determine carbon source assimilation. Each filter-sterilized carbon source was added at a concentration of 0.2 % (w/v) after the mineral base solution had been autoclaved and growth was then examined after incubation at 30 °C for 1, 7, 10 and 14 days. Oxidase activity of strain SLG5B-19^T was tested according to Smibert & Krieg (1994) and catalase activity was determined by using 3 % (v/v) hydrogen peroxide solution (Dong & Cai, 2001). Denitrification was

tested by growing the strain anaerobically in the presence of NO₃⁻ (Zumft, 1992). Hydrolysis of starch, gelatin and Tween 80, and urease activity were examined according to the procedures of Williams *et al.* (1983). Methyl red and Voges–Proskauer tests were performed by using methyl red and Barritt's reagent (Barritt, 1936; Mata *et al.*, 2002). Nitrate and nitrite reduction were assessed as described by Lányi (1987). The following tests were carried out according to the procedures recommended by Mata *et al.* (2002): exopolysaccharide (EPS) production, ONPG test, indole production, oxidation/fermentation of D-glucose, hydrolysis of casein and DNA, phenylalanine deaminase, and lysine and ornithine decarboxylases. Sensitivity to 5 µg rifampicin, 10 µg amoxicillin, kanamycin, carbenicillin, tobramycin and streptomycin, 15 µg erythromycin, 30 µg cefoxitin, cefotaxime, chloramphenicol, kanamycin and nalidixic acid, 300 µg nitrofurantoin, polymyxin B, sulfamethoxazole and streptomycin, and 23.75/1.25 µg sulfamethoxazole/trimethoprim was tested on LB plates by using the method described by Cho & Giovannoni (2003).

Strain SLG5B-19^T was Gram-negative and motile. Cells were bean-shaped, rod-shaped or oval, 0.5–1.0 µm in width and 1.0–2.5 µm in length with monopolar or bipolar prosthecae and buds at the end of the prosthecae (Fig. 2). Small milky white colonies were formed with a diameter of 0.1–0.2 mm on LB agar and clumps and pellicles with wall-growth were observed in medium 166 liquid culture. Cells

Fig. 2. Electron micrographs of cells of strain SLG5B-19^T. (a) Scanning electron micrograph; bar, 2 µm. (b) Electron micrograph of negatively stained cells; bar, 0.5 µm.

of strain SLG5B-19^T accumulated PHB. Growth occurred at 4–45 °C (optimum 28–30 °C), at pH 6.0–9.0 (optimum pH 7.0–7.5) and in 0–7% (w/v) NaCl (optimum 1–2%). Strain SLG5B-19^T was positive for oxidase, catalase, nitrate reduction activity, PHB production, hydrolysis of aesculin and oxidation of D-glucose, but negative for hydrolysis of gelatin, Tween 80, starch, casein and DNA, urease, phenylalanine deaminase, nitrite reduction activity, and lysine, ornithine and arginine decarboxylases, as well as the ONPG, Voges–Proskauer and methyl red tests, and EPS and indole production. Growth was observed with methanol, formate, methylamine.HCl, acetate, ethanol, D-fructose, cellobiose and trehalose as sole carbon and energy sources. The following organic compounds were not utilized as carbon sources: maltose, L-arginine, succinate, D-erythrose, L-sorbose, D-sorbitol, malonate, sucrose, myo-inositol, D-mannitol, α-D-lactose, pyruvate, D-ribose, propionate, glycine, L-alanine, D-arabinose, D-glucose, lactate, L-glutamic acid and dextrin. Strain SLG5B-19^T could grow on methanol, formate and

methylamine.HCl as sole carbon and energy source, which is similar to species of the genus *Hyphomicrobium*, but differs compared with other *Filomicrobium* species. Strain SLG5B-19^T was sensitive to 5 µg rifampicin, 10 µg amoxicillin, kanamycin, carbenicillin, tobramycin and streptomycin, 15 µg erythromycin, 30 µg cefoxitin, cefotaxime, chloramphenicol and kanamycin, and 300 µg polymyxin B and streptomycin. It was resistant to 30 µg nalidixic acid, 300 µg nitrofurantoin and sulfamethoxazole, and 23.75/1.25 µg sulfamethoxazole/trimethoprim. The main characteristics that differentiate strain SLG5B-19^T from species of the genera *Filomicrobium* and *Hyphomicrobium* are listed in Table 1.

Cellular fatty acid methyl esters of strain SLG5B-19^T and *F. fusiforme* IFAM 1315^T were prepared after growth in medium 166 broth at 30 °C for 4 days and analysed using GC according to the instructions of the Microbial Identification System (MIDI). Fatty acid profiles were analysed using the Sherlock system (Microbial ID). Isoprenoid quinones were extracted from fresh cells with methyl chloride/methanol (2:1, v/v) and analysed by reverse-phase HPLC (Shim-pack, VP-ODS; Shimadzu). Strain SLG5B-19^T contained C_{18:1}ω7c (76.7%), C_{16:0} (9.26%), C_{19:0} cyclo ω8c (4.4%) and C_{14:0} 3-OH (3.2%) as the main fatty acids; *Hyphomicrobium zavarzinii* DSM 1566^T contained C_{18:1}ω7c (80.6%), C_{16:0} (4.5%) and C_{14:0} 3-OH (3.5%), but lacked C_{19:0} cyclo ω8c. Cells of strain SLG5B-19^T contained ubiquinone-9 (Q-9) as the major quinone.

Genomic DNA was extracted and purified by the method of Marmur (1961). The DNA G+C content was determined by thermal denaturation (Marmur & Doty, 1962) using DNA from *Escherichia coli* K-12 as a control. The DNA G+C content was 59.5 mol%. The 16S rRNA gene was amplified using the bacterial universal primer pair 8f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991). PCR products were purified using a QIAquick PCR purification kit (Qiagen) and cloned into pGEM-T Easy plasmid (Promega) according to the manufacturers' protocols. Positive clones were screened by PCR using the vector-specific primers SP6 (5'-ATTTAGGTGACACTATAGAA-3') and T7 (5'-TAATACGACTCACTATAGGG-3') and the reaction conditions 5 min at 95 °C, 30 cycles of 30 s at 94 °C, 30 s at 56 °C and 1 min at 72 °C, with a final extension comprising 7 min at 72 °C, and the sequence was determined. Multiple alignments of the 16S rRNA gene sequence were performed with the CLUSTAL_X program version 1.64b (Thompson *et al.*, 1997). The phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) and evaluated by bootstrap analysis (based on 1000 resampling replicates) with the SEQBOOT, DNADIST, NEIGHBOR and CONSENSE programs of the PHYLIP package version 3.6 (Felsenstein, 2004). The maximum-parsimony and maximum-likelihood algorithms were also used to compare and determine the topological stability of the phylogenetic trees. Nearly complete 16S rRNA gene

Table 1. Differential characteristics of strain SLG5B-19^T and related species of the genera *Hyphomicrobium* and *Filomicrobium*

Strains: 1. SLG5B-19^T (data from this study); 2. *F. fusiforme* IFAM 1315^T (Schlesner, 1987); 3. *H. vulgare* IFAM MC-750 (Gliesche *et al.*, 2005); 4. *H. denitrificans* TK 0415^T (Urakami *et al.*, 1995); 5. *H. zavarzinii* IFAM ZV-622^T (Gliesche *et al.*, 2005). All strains are positive for catalase, oxidase and utilization of acetate. –, Negative; +, positive; ND, not described; 0, no growth.

Characteristic	1	2	3	4	5
Cell size (µm)	0.5–1.0 × 1.0–2.5	0.5–0.7 × 1.0–4.0	0.3–1.2 × 1.0–3.0	0.3–0.6 × 1.0–3.0	0.5–0.9 × 0.7–2.5
Bean-shaped mother cells	+	–	–	–	–
Cell clumping in liquid medium	+	ND	+	+	+
Temperature range for growth (°C)	4–45	ND	15–37	ND	ND
Optimal growth temperature (°C)	28–30	20–28	ND	30	ND
Growth at:					
4 °C	+	–	–	ND	–
37 °C	+	–	+	–	+
45 °C	+	–	–	–	–
Maximum growth at pH>7.5	+	–	–	–	–
Optimum pH	7.0–7.5	ND	6.5–7.5	6.0–8.0	ND
Growth in 7 % NaCl	+	–	–	–	–
PHB production	+	ND	+	+	+
Fermentation of D-glucose	–	–	ND	–	ND
Hydrolysis of casein and DNA	–	0	ND	ND	ND
Indole production	–	ND	ND	–	ND
Methyl red test	–	ND	ND	–	ND
Voges–Proskauer	–	ND	ND	–	ND
Hydrolysis of:					
Starch	–	–	ND	–	ND
Aesculin	+	0	+	ND	+
Gelatin	–	0	ND	–	–
Tween 80	–	0	ND	ND	ND
Urease	–	+	–	+	–
Nitrate reduction	+	–	+	+	+
Utilization of:					
D-Arabinose	–	ND	+	ND	ND
Cellobiose	+	–	ND	ND	ND
Dextrin	–	ND	+	ND	ND
Ethanol	+	ND	ND	+	+
Formate	+	–	+	+	+
D-Fructose	+	–	–	ND	ND
Glycerol	–	–	+	–	+
myo-Inositol	–	ND	ND	–	ND
Lactate	–	ND	–	–	ND
α-D-Lactose	–	ND	+	–	ND
Maltose	–	ND	+	–	ND
D-Mannitol	–	ND	+	–	ND
Methanol	+	–	+	+	+
Methylamine . HCl	+	–	+	+	+
Propionate	–	–	+	–	ND
Pyruvate	–	–	+	ND	ND
D-Sorbitol	–	ND	ND	–	ND
Succinate	–	ND	+	–	ND
Sucrose	–	ND	–	–	ND
Trehalose	+	–	ND	–	ND
DNA G + C content (mol%; T _m)	59.5	61.9	61	60–61	65

sequence (1445 bp) analysis revealed that strain SLG5B-19^T was a member of the class *Alphaproteobacteria* and had a close phylogenetic relationship with the type strains of species of the genera *Filomicrobium* and *Hyphomicrobium*

(Fig. 1); it was in a clade with the type strain of *F. fusiforme* (95.8 % 16S rRNA gene sequence similarity). In comparison, the 16S rRNA gene sequence similarities of strain SLG5B-19^T with the type strains of *Hyphomicrobium*

sulfonivorans and *Hyphomicrobium* species in cluster II and cluster I were 90.7%, 92.2–92.6% and 93.7–94.5%, respectively. There are 27 'signature nucleotides' in the 16S rRNA gene that are reported to define *Hyphomicrobium* species and *F. fusiforme* (Rainey *et al.*, 1998). Among them, 24 'signature nucleotides' of strain SLG5B-19^T were the same as those of the type strain of *F. fusiforme*; less than 14 signature nucleotides were identical between strain SLG5B-19^T and the *Hyphomicrobium* species in clusters I and II (Table 2). These results also support the placement of strain SLG5B-19^T in the genus *Filomicrobium*, rather than the genus *Hyphomicrobium*.

Although the phenotypic characteristics of strain SLG5B-19^T and *F. fusiforme* were distinctly different, phylogenetic analysis based on 16S rRNA gene sequences suggested that strain SLG5B-19^T could be allocated to the *Filomicrobium* clade. A novel species of the genus *Filomicrobium* is

Table 2. 16S rRNA gene signature nucleotides that define clusters I and II of species of the genus *Hyphomicrobium* and their occurrence in the 16S rRNA gene sequences of the type strain of *F. fusiforme* and strain SLG5B-19^T

The positions given correspond to those of the *Escherichia coli* gene sequence as determined by Brosius *et al.*, 1978.

Position	<i>Hyphomicrobium</i> species		<i>F. fusiforme</i> SLG5B-19 ^T	
	Cluster I	Cluster II		
99	A	G	G	G
131–231	U–A	C–G	C–G	C–G
137–226	U–A	C–G	U–A	U–A
138–225	A–U	U–A	A–U	A–U
139–224	U–A	A–U	U–U	U–A
140–223	C–G	U–A	U–A	U–G
141–222	G–C	A–U	G–C	G–C
144–178	A–C	A–U	A–U	G–C
153–168	C–G	C–A	C–G	C–G
154–167	U–G	C–G	U–U	U–U
Loop 187–190	UUCG	GA[A/G]A	GAGA	GAGA
240–286	C–G	A–U	C–G	C–G
295–302	C–G	U–A	C–G	C–G
241–285	U–A	G–C	G–C	G–C
418–425	U–A	C–G	C–G	C–G
422	C	A	A	A
501–544	C–G	U–A	C–G	C–G
661–744	G–C	A–U	A–U	A–U
670–736	A–U	G–C	A–U	A–U
818	U	G	G	G
849	G	U	G	G
1007–1022	U–U	C–G	G	G
1008–1021	U–U	C–G	U–U	U–U
1254–1283	G–U	C–G	U–U	U–U
1264–1271	C–G	U–A	G–C	C–G
1304	G	A	G	G
1310–1327	G–C	A–U	G–C	G–C

therefore proposed, *Filomicrobium insigne* sp. nov., to accommodate strain SLG5B-19^T.

Description of *Filomicrobium insigne* sp. nov.

Filomicrobium insigne (in.sig'ne. L. neut. adj. *insigne* distinguished by a mark, remarkable, extraordinary, referring to the remarkable phylogenetic relationship between the type strain and members of the genera *Hyphomicrobium* and *Filomicrobium*).

Cells are Gram-negative, aerobic, motile, non-pigmented, 0.5–1.0 µm in width and 1.0–2.5 µm long with mono-prosthecae or bipolar prosthecae and buds on the prosthecal tips. Colonies on LB agar are milky white and 0.1–0.2 mm in diameter after incubation at 30 °C for 5–7 days. Accumulates PHB and produces clumps and pellicles with wall-growth, but does not produce EPS after incubation in medium 166 broth at 30 °C for 5–7 days. Growth occurs at pH 6.0–9.0 (optimum pH 7.0–7.5), at NaCl concentrations between 0 and 7% (w/v) (optimum 1–2%), and at 4–45 °C (optimum 28–30 °C). Positive for oxidase, catalase, nitrate reduction activity, PHB production, hydrolysis of aesculin and oxidation of D-glucose, but negative for hydrolysis of gelatin, Tween 80, starch, casein and DNA, urease, phenylalanine deaminase, nitrite reduction activity, indole production, lysine, ornithine and arginine decarboxylases, and the ONPG, Voges–Proskauer and methyl red tests. Grows on methanol, formate, methylamine.HCl, acetate, ethanol, D-fructose, cellobiose and trehalose as sole carbon and energy sources. The following organic compounds are not utilized as carbon sources: maltose, L-arginine, succinate, D-erythrose, L-sorbose, D-sorbitol, malonate, sucrose, *myo*-inositol, D-mannitol, α-D-lactose, pyruvate, D-ribose, propionate, glycine, L-alanine, D-arabinose, D-glucose, lactate, L-glutamic acid and dextrin. The major quinone is Q-9 and the major fatty acids are C_{18:1}ω7c, C_{16:0}, C_{19:0} cyclo ω8c and C_{14:0} 3-OH. Inhibited by 5 µg rifampicin, 10 µg amoxicillin, kanamycin, carbenicillin, tobramycin and streptomycin, 15 µg erythromycin, 30 µg cefoxitin, cefotaxime, chloramphenicol and kanamycin, and 300 µg polymyxin B and streptomycin, but resistant to 30 µg nalidixic acid, 300 µg nitrofurantoin and sulfamethoxazole, and 23.75/1.25 µg sulfamethoxazole/trimethoprim.

The type strain, SLG5B-19^T (=CGMCC 1.6497^T=LMG 23927^T), was isolated from an oil-polluted saline soil at Gudao Oil-Product in the coastal Shengli Oilfield, Shandong Province, eastern China. The DNA G+C content of the type strain is 59.5 mol%.

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References

- Barritt, M. M. (1936).** The intensification of the Voges-Proskauer reaction by the addition of α -naphthol. *J Pathol Bacteriol* **42**, 441–454.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978).** Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Cho, J.-C. & Giovannoni, S. J. (2003).** *Parvularcula bermudensis* gen. nov., sp. nov., a marine bacterium that forms a deep branch in the α -Proteobacteria. *Int J Syst Evol Microbiol* **53**, 1031–1036.
- Dong, X.-Z. & Cai, M.-Y. (2001).** *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press (English translation).
- Felsenstein, J. (2004).** PHYLIP (phylogeny inference package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Gliesche, C., Fesefeldt, A. & Hirsch, P. (2005).** Genus I. *Hyphomicrobium* Stutzer and Hartleb 1898, 76^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, pp. 476–494. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Gu, J., Cai, H., Yu, S. L., Qu, R., Yin, B., Guo, Y. F., Zhao, J. Y. & Wu, X. L. (2007).** *Marinobacter gudaonensis* sp. nov., isolated from an oil-polluted saline soil in a Chinese oilfield. *Int J Syst Evol Microbiol* **57**, 250–254.
- Hirsch, P. & Rades-Rohkohl, E. (1983).** Microbial diversity in a groundwater aquifer in Northern Germany. *Dev Ind Microbiol* **24**, 183–200.
- Hirsch, P. & Siebert, J. (1991).** Die Vielfalt der Mikroorganismen im hypersalinen Ekho Lake (Vestfold Hills, Ostantarktis). *Mitt Kieler Polarforsch* **6**, 11–14 (in German).
- Labbé, N., Juteau, P., Parent, S. & Villemur, R. (2003).** Bacterial diversity in a marine methanol-fed denitrification reactor at the Montreal Biodome, Canada. *Microb Ecol* **46**, 12–21.
- Lane, D. J. (1991).** 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lányi, B. (1987).** Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Liessens, J. (1993).** Removing nitrate with a methylotrophic fluidized bed: microbiological quality. *J Am Water Works Assoc* **85**, 155–161.
- Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Mata, J. A., Martínez-Cánovas, J., Quesada, E. & Béjar, V. (2002).** A detailed phenotypic characterisation of the type strains of *Halomonas* species. *Syst Appl Microbiol* **25**, 360–375.
- Meyers, A. J. & Meyers, C. D. (1986).** *Hyphomicrobium*-mediated sludge bulking in an industrial wastewater treatment system, abstract N-93, p. 257. In *Abstracts of the 86th Annual Meeting of the American Society for Microbiology 1986*. Washington, DC: American Society for Microbiology.
- Morgan, P. & Dow, C. S. (1985).** Environmental control of cell-type expression in prosthecate bacteria. In *Bacteria in their Natural Environments*, pp. 131–169. Edited by M. Fletcher & G. D. Floodgate. London: Academic Press.
- Poindexter, J. S. (1992).** Dimorphic prosthecate bacteria: the genera *Caulobacter*, *Asticcacaulis*, *Hyphomicrobium*, *Pedomicrobium*, *Hyphomonas*, and *Thiodendron*. In *The Prokaryotes*, 2nd edn, pp. 2176–2196. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Rainey, F. A., Ward-Rainey, N., Gliesche, C. G. & Stackebrandt, E. (1998).** Phylogenetic analysis and intrageneric structure of the genus *Hyphomicrobium* and the related genus *Filomicrobium*. *Int J Syst Bacteriol* **48**, 635–639.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schlesner, H. (1987).** *Filomicrobium fusiforme* gen. nov., sp. nov., a slender budding, hyphal bacterium from brackish water. *Syst Appl Microbiol* **10**, 63–67.
- Sittig, M. & Hirsch, P. (1992).** Chemotaxonomic investigation of budding and/or hyphal bacteria. *Syst Appl Microbiol* **15**, 209–222.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E., Fischer, A., Roggentin, T., Wehmeyer, U., Bomar, D. & Smida, J. (1988).** A phylogenetic survey of budding, and/or prosthecate, non-phototrophic eubacteria: membership of *Hyphomicrobium*, *Hyphomonas*, *Pedomicrobium*, *Filomicrobium*, *Caulobacter* and ‘*Dichotomicrobium*’ to the alpha-subdivision of purple non-sulfur bacteria. *Arch Microbiol* **149**, 547–556.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Urakami, T., Sasaki, J., Suzuki, K.-I. & Komagata, K. (1995).** Characterization and description of *Hyphomicrobium denitrificans* sp. nov. *Int J Syst Bacteriol* **45**, 528–532.
- Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983).** Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.
- Zumft, W. G. (1992).** The denitrifying bacteria. In *The Prokaryotes*, pp. 554–582. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer-Verlag.