Phylogenetic analysis and intrageneric structure of the genus *Hyphomicrobium* and the related genus *Filomicrobium*

Frederick A. Rainey,¹ Naomi Ward-Rainey,¹ Christian G. Gliesche² and Erko Stackebrandt¹

Author for correspondence: Erko Stackebrandt. Tel: +49 531 26 16 352. Fax: +49 532 26 16 418.
e-mail: erko@gbf-de

¹ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, D-38124 Braunschweig, Germany

² Institut für Allgemeine Mikrobiologie, Universität Kiel, Am Botanischen Garten 1–9, D-24118 Kiel, Germany

Almost complete 16S rDNA sequences from the type strains of seven species of the genus *Hyphomicrobium* and of *Filomicrobium fusiforme* have been determined. The *Hyphomicrobium* species form two phylogenetic clusters that are only moderately related to each other. While cluster I contains the type species *Hyphomicrobium vulgare*, *Hyphomicrobium aestuarii*, *Hyphomicrobium hollandicum* and *Hyphomicrobium zavarzinii*, cluster II comprises *Hyphomicrobium facilis*, *Hyphomicrobium denitrificans* and *Hyphomicrobium methylovorum*. Within the two species clusters, the species are highly related. Phylogenetically, *Filomicrobium fusiforme* clusters moderately with *Hyphomicrobium* species. The lack of distinguishing phenotypical properties presently excludes the possibility of describing cluster II as a new genus.

Keywords: *Hyphomicrobium*, *Filomicrobium*, intrageneric structure

INTRODUCTION

Hyphomicrobia are appendaged bacteria that reproduce by budding and have a dimorphic life cycle involving non-motile prosthecae mother cells and motile swarmer cells (Hirsch, 1989). In contrast to morphologically similar taxa such as *Hyphomonas* (Moore & Weiner, 1989), *Pedomicrobium* (Gebers, 1989), *Dichotomicrobium* (Hirsch & Hoffmann, 1989), *Filomicrobium* (Schlesner, 1987) or *Rhodomicrobium* (Imhoff & Trüper, 1989), hyphomicrobia are restricted facultative methylotrophs capable of growth on reduced C₁ compounds such as methanol, methylated amines or formate (Harder & Attwood, 1978). In recent years, hyphomicrobia became of special interest because of their versatility and ability to use toxic waste compounds that are not metabolized by other methylotrophs (Hanson, 1992). They can be used in the denitrification of sewage (Nyberg *et al.*, 1992) or drinking water (Liessnes, 1993) or in the bioremediation of C₁ compounds such as halomethanes, methyl sulphates and methylated phosphates (Large & Bamforth, 1988) and, consequently, many strains have

been isolated and included in these and in taxonomic studies (Gebers *et al.*, 1986; Gliesche *et al.*, 1988; Stackebrandt *et al.*, 1988; Roggentin & Hirsch, 1989; Holm *et al.*, 1996).

The genus *Hyphomicrobium* presently contains nine species (Hirsch, 1989; Urakami *et al.*, 1995), one of which, *H. coagulans* (Takada, 1975), is not available from any culture collection, while *H. indicum* has been discussed in the literature to be a non-authentic member of the genus (Hirsch, 1989; Urakami *et al.*, 1995). Of the other species, only a few strains have been included in chemotaxonomic studies (Guckert *et al.*, 1991; Sittig & Hirsch, 1992) and in the phylogenetic analysis of 16S rRNA (Stackebrandt *et al.*, 1988; Roggentin & Hirsch, 1989; Tsuji *et al.*, 1990) and 5S RNA sequences (Stackebrandt *et al.*, 1988; Boulygina *et al.*, 1993). These studies have indicated that the genus belongs to the alpha subclass of the class *Proteobacteria*. However, according to the results of DNA dot-blot hybridization studies on hundreds of *Hyphomicrobium* strains (Holm *et al.*, 1996), 19 DNA similarity clusters were identified, which points towards the presence of a significantly higher number of species than presently described.

In this study, we present a phylogenetic analysis on all available type strains of the genus *Hyphomicrobium*, which will allow subsequent affiliation of environ-

This paper is dedicated to Dr Peter Hirsch on the occasion of his 70th birthday.

The EMBL accession numbers for the sequences reported in this paper are Y14302–Y14313.

Table 1. Bacterial strains analysed in this study

Strain*	Other designation(s)*	Reference/source
<i>H. aestuarii</i> IFAM NQ-521gr ^T	ATCC 27488	Hirsch (1989)
<i>H. denitrificans</i> DSM 1869 ^T	TK 0415 = IFAM HA-905	Urakami <i>et al.</i> (1995)
<i>H. facilis</i> subsp. <i>facilis</i> IFAM H-526 ^T	DSM 1565, ATCC 27485	Hirsch (1989)
<i>H. facilis</i> IFAM B-522		Hirsch (1989)
<i>H. facilis</i> subsp. <i>tolerans</i> IFAM I-551 ^T	ATCC 27489	Hirsch (1989)
<i>H. facilis</i> subsp. <i>ureaphilum</i> IFAM CO-582 ^T	ATCC 27492	Hirsch (1989)
<i>H. hollandicum</i> IFAM KB-677 ^T	ATCC 27498	Hirsch (1989)
<i>H. methylovorum</i> DSM 5458 ^T	ATCC 35216, KM-146	Hirsch (1989)
<i>H. vulgare</i> IFAM MC-750 ^T	ATCC 27500	Hirsch (1989)
<i>H. zavarzinii</i> IFAM ZV-622 ^T	ATCC 27496	Hirsch (1989)
<i>H. zavarzinii</i> IFAM ZV-580		Hirsch (1989)
<i>F. fusiforme</i> DSM 5304 ^T		Schlesner (1987)

* ATCC, American Type Culture Collection, Rockville, MD, USA; DSM, DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany; IFAM, Institut für Allgemeine Mikrobiologie, University of Kiel, Germany.

mental isolates to known species and the recognition of novel genomic nuclei that may represent novel species.

METHODS

Bacterial strains. Strains analysed in this study are listed in Table 1. Growth media and culture conditions followed described procedures for the cultivation of *Hyphomicrobium* strains (DSM catalogue of strains, 1993, 1996).

16S rDNA sequence determination and analysis. Extraction of genomic DNA, PCR-mediated amplification of the 16S rDNA and sequence analysis of the purified PCR products were performed as described previously (Rainey *et al.*, 1996), and the sequence reactions were electrophoresed using a model 373A automatic DNA sequencer (Applied Biosystems).

To determine the closest relatives of strains of hyphomicrobia, their phylogenetic position was determined initially using the database ARB (Strunk & Ludwig, 1995). A fine resolution of the relatedness between hyphomicrobia and their closest relatives was performed using the ae2 editor (Maidak *et al.*, 1994). Phylogenetic dendrograms were reconstructed using treeing algorithms contained in the PHYLIP package (Felsenstein, 1993). Bootstrap values were determined using the PHYLIP package (Felsenstein, 1993).

Nucleotide sequence accession numbers. The accession numbers of the 16S rDNAs of reference strains were: *Agrobacterium tumefaciens*, X67223; *Methylobacterium organophilum*, M29028; *Methylobacterium extorquens*, M29027; *Phyllobacterium myrsinacearum*, D12789; *Mesorhizobium loti*, D12791; *Rhodobium marinum*, D30790; *Rhodobium orientis*, D30792; and *Rhodomicrobium vannielii*, M34127.

RESULTS

The almost complete primary structure of the 16S rDNA of eight type strains of *Hyphomicrobium* species and of *Filomicrobium fusiforme* as well as of four

additional strains of *Hyphomicrobium* were sequenced. Their EMBL accession numbers are: *Hyphomicrobium vulgare* IFAM MC-750^T (Y14302), *Hyphomicrobium hollandicum* IFAM KB-677^T (Y14303), *Hyphomicrobium aestuarii* IFAM NQ-521gr^T (Y14304), *Hyphomicrobium zavarzinii* IFAM ZV-622^T (Y14305), *Hyphomicrobium zavarzinii* IFAM ZV-580 (Y14306), *Hyphomicrobium methylovorum* DSM 5458^T (Y14307), *Hyphomicrobium denitrificans* DSM 1869^T (Y14308), *Hyphomicrobium facilis* subsp. *facilis* IFAM H-526^T (Y14309), *Hyphomicrobium facilis* subsp. *ureaphilum* IFAM CO-582^T (Y14310), *Hyphomicrobium facilis* subsp. *tolerans* IFAM I-551^T (Y14311), *Hyphomicrobium facilis* IFAM B-522 (Y14312) and *Filomicrobium fusiforme* DSM 5304^T (Y14313).

The length of sequences ranged between 1410 and 1446 bases, which corresponded to 91 and 94% of the *Escherichia coli* sequence (Brosius *et al.*, 1978), respectively. The position of the type strain of the type species of *Hyphomicrobium* was searched for in the ARB database (Strunk & Ludwig, 1995), and the position within the '*Rhodomicrobium vannielii* assemblage' (Maidak *et al.*, 1994) could be confirmed. Subsequent phylogenetic analysis was carried out using the ae2 editor. The sequences of *Hyphomicrobium* strains and of *Filomicrobium fusiforme* were aligned with each other and with representatives of neighbouring taxa. A total of 1360 nucleotides were used in the analysis, and phylogenetic trees were generated using the algorithms of De Soete (1983) and those included in the PHYLIP package (Felsenstein, 1993). All trees showed very similar topologies, in that all hyphomicrobia as well as *F. fusiforme* formed a phylogenetically coherent group, which was most closely related to *Rhodomicrobium vannielii* (around 90% 16S rDNA sequence similarity), while the other reference organisms were more distantly related (between 87 and 89.5% similarity). The only differences in

Fig. 1. Dendrogram showing the phylogenetic position of the genera *Hyphomicrobium* and *Filomicrobium* among members of the alpha-2 subclass of the class *Proteobacteria*. The tree was constructed by the neighbour-joining method (Saitou & Nei, 1987), using corrected distance values (Jukes & Cantor, 1969). The sequences of *Methylobacterium extorquens* and *Methylobacterium organophilum* were used to root the dendrogram. The position of *H. zavarzinii* ZV-580 corresponds fully to that of strain ZV-622^T. Bootstrap values (expressed as percentages of 500 replications) of 65% or more are indicated at the branch points. Bar, 5% sequence divergence.

the tree topologies were observed in the branching point of *Filomicrobium fusiforme*.

The type strains of the seven *Hyphomicrobium* species formed two 16S rDNA clusters in which the species showed a high degree of relatedness (Fig. 1). Bootstrap values of 100% indicated that these clusters are of statistical significance. Cluster I contained the type species of the genus *H. vulgare*, as well as *H. hollandicum*, *H. aestuarii* and *H. zavarzinii*. The 16S rDNA sequence similarities ranged between 97.8 and 98.8%. Strains ZV-622^T and ZV-580 had identical 16S rDNA sequences. Cluster II contained the species *H. methylovorum*, *H. denitrificans* and *H. facilis* and its two subspecies *H. facilis* subsp. *tolerans* IFAM I-551^T and *H. facilis* subsp. *ureaphilum* IFAM CO-582^T. The last three strains, as well as strain IFAM B-522, shared 99.9% sequence similarity. The sequence similarities of the type strains of the three species ranged from 97.3 to 98.8%. Members of clusters I and II were separated by 6.4–8.0% sequence differences. The two clusters can be differentiated clearly by a set of cluster-specific nucleotides, which can be considered a molecular signature. The positions of these nucleotides in the 16S rDNA sequence are indicated in Table 2.

The branching point of the *F. fusiforme* sequence was close to the bifurcation point of the two *Hyphomicrobium* lineages. Although in the algorithm of De Soete (1983) this species grouped with members of cluster II, it branched with members of cluster I in the neighbour-joining analysis (Saitou & Nei, 1987). The finding that 16S rDNA similarity values for *F. fusiforme* were about 2% higher for members of cluster I than for members of cluster II may be indicative of a specific relatedness to the former cluster as indicated in Fig. 1. This relationship is also expressed in the presence of 13 signature nucleotides that are shared between *F. fusiforme* and members of cluster I, while nine signatures are common to *F. fusiforme* and members of cluster II. Bootstrap values of the branching points (65% significance for cluster I members compared with 23% significance for cluster II members) are too low to make a decision about the phylogenetic neighbour of *F. fusiforme*. The primary

Table 2. 16S rDNA signature nucleotides that define clusters I and II of *Hyphomicrobium* species and their occurrence in the 16S rDNA sequence of *F. fusiforme*

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

* *E. coli* position (Brosius *et al.*, 1978).

structure of the 16S rDNA of *F. fusiforme*, unlike those of *Hyphomicrobium* species, lack a stretch between positions 1257 and 1277. This position is also missing in *Rhodobium marinum*, while it is present in its closest relative *Rhodobium orientis* and in the sequence of the other reference organisms of the alpha-2 subclass included in Fig. 1.

DISCUSSION

The membership of *Hyphomicrobium* species in the alpha-2 subclass of the class *Proteobacteria* confirms earlier findings that were based on the analysis of 5S rDNA (Stackebrandt *et al.*, 1988; Nikitin *et al.*, 1990) and 16S rDNA (Stackebrandt *et al.*, 1988; Tsuji *et al.*, 1990), determination of 16S rRNA cistron similarities (Moore, 1977; Roggentin & Hirsch, 1989) and membrane fatty acids (Gluckert *et al.*, 1991; Sittig & Hirsch, 1992), but not more than five species were included in a single study. The coherency of the genus was supported by phage typing (Gliesche *et al.*, 1988), analysis of low-molecular-mass RNA patterns (Höfle, 1990) and 16S rDNA cistron similarity studies (Roggentin & Hirsch, 1989), while 16S rRNA cataloguing showed *Filomicrobium* to branch within the radiation of *Hyphomicrobium* (Stackebrandt *et al.*, 1988). The intragenetic structure was elucidated by the latter two approaches, which clearly showed the phylogenetically separate position of *H. vulgare*, *H. aestuarii* and *H. zavarzinii* on the one side and *H. facilis* on the other. *H. hollandicum* and *F. fusiforme* grouped intermediate to these clusters in the studies on RNA cistron similarities (Roggentin & Hirsch, 1989) and 16S rRNA cataloguing (Stackebrandt *et al.*, 1988), respectively.

The distinctness of *Hyphomicrobium* species is demonstrated by differences of more than 1.2% in 16S rDNA sequence found for the most closely related species. These data confirm results of DNA-DNA hybridization studies (Moore & Hirsch, 1972; Gebers *et al.*, 1986) in which the separate species status was demonstrated for *H. vulgare*, *H. aestuarii*, *H. zavarzinii* and *H. facilis*. The three subspecies of *H. facilis*, showing almost identical 16S rDNA sequences, showed more than 85% DNA similarities as determined by hybridization, and these data confirm their membership of the same species. The molecular percentage G+C content of DNA has also been used to separate members of *H. vulgare* (61.4 mol%), *H. facilis* (60 mol%) and the pair *H. aestuarii* and *H. zavarzinii* (63–64 mol% each) (Gebers *et al.*, 1986), and the presence of species-specific low-molecular-mass RNA patterns was demonstrated for each of the five species studied (Höfle, 1990).

The question arises as to whether the two phylogenetically distinct clusters of *Hyphomicrobium* species represent two genera or whether a single genus, including *Filomicrobium fusiforme*, should be maintained. The degree of 16S rDNA sequence divergence between clusters I and II is as large as those found separating members of *Agrobacterium*, *Phyllobacterium* and *Mesorhizobium* (Fig. 1). The dissection of the genus *Hyphomicrobium* has been indicated before on molecular grounds (Stackebrandt *et al.*, 1988; Roggentin & Hirsch, 1989), but the lack of phenotypic properties specific for the newly emerging groups has postponed this step. The branching point of the *Filomicrobium fusiforme* line of descent should

not be taken as an argument for either decision, as it is deep in any phylogenetic tree generated, and none of them is supported with high statistical significance. Also, Sittig & Hirsch (1992), while clearly separating members of *Hyphomicrobium* and *F. filiformis* by the results of phospholipid and fatty acids analysis, could not identify specific patterns for the *Hyphomicrobium* species and, of all the morphological and physiological properties compiled by Hirsch (1989), only the formation of rosettes or cell clumping would identify members of cluster I, because these properties are absent in members of cluster II. The lack of non-molecular evidence stops us from proposing a new genus for the cluster II organisms. In addition, a comprehensive taxonomic revision of the genus *Hyphomicrobium* should await a molecular analysis of undescribed strains for which species status has been discussed on the basis of phenotypic analysis (Vedenina *et al.*, 1990; Sittig & Hirsch, 1992), DNA dot-blot hybridization studies (Holm *et al.*, 1996) and morphology (Holm *et al.*, 1996).

ACKNOWLEDGEMENTS

We wish to thank Orsola Päuker for her help in the molecular work.

REFERENCES

- Boulygina, E. S., Chumakov, K. M. & Netrusov, A. I. (1993). Systematics of Gram-negative methylotrophic bacteria based on 5S rRNA sequences. In *Microbial Growth on C₁ Compounds*, pp. 275–284. Edited by J. C. Murrell & D. P. Kelly. Andover: Intercept.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of the 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- De Soete, G. (1983). A least squares algorithm for fitting additive trees to proximity data. *Psychometrika* **48**, 621–626.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5.1. Department of Genetics, University of Washington, Seattle.
- Gebers, R. (1989). Genus *Pedomicrobium* Aristovskaya 1961, 957^{AL} emend. Gebers 1981, 313. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, 9th edn, pp. 1910–1914. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Gebers, R., Martens, B., Wehmeyer, U. & Hirsch, P. (1986). Deoxyribonucleic acid homologies of *Hyphomicrobium* spp., *Hyphomonas* spp., and other hyphal, budding bacteria. *Int J Syst Bacteriol* **36**, 241–245.
- Gliesche, C. G., Holm, N. C., Beese, M., Neumann, N., Völker, H., Gebers, R. & Hirsch, P. (1988). New bacteriophages active on strains of *Hyphomicrobium*. *J Gen Microbiol* **134**, 1339–1353.
- Guckert, J. B., Ringelberg, D. B., White, D., Hanson, D. S. & Bratina, B. J. (1991). Membrane fatty acids as phenotypic markers in the polyphasic taxonomy of methylotrophs within the *Proteobacteria*. *J Gen Microbiol* **137**, 2631–2641.
- Hanson, R. S. (1992). Introduction. In *Methane and Methanol Utilizers*, pp. 1–21. Edited by J. C. Murrell & H. Dalton. New York: Plenum Press.

- Harder, W. & Attwood, M. M. (1978). Biology, physiology and biochemistry of hyphomicrobia. *Adv Microb Physiol* **17**, 303–359.
- Hirsch, P. (1989). Genus *Hyphomicrobium* Stutzer and Hartleb 1898, 76^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 9th edn, vol. 3, pp. 1897–1904. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Hirsch, P. & Hoffmann, B. (1989). *Dichotomicrobium thermohalophilum*, gen. nov., spec. nov., budding prosthecate bacteria from the Solar Lake (Sinai) and some related strains. *Syst Appl Microbiol* **11**, 291–301.
- Höfle, M. G. (1990). Transfer RNAs as genotypic fingerprints of eubacteria. *Arch Microbiol* **153**, 299–304.
- Holm, N. C., Gliesche, C. G. & Hirsch, P. (1996). Diversity and structure of *Hyphomicrobium* populations in a sewage treatment plant and its adjacent receiving lake. *Appl Environ Microbiol* **62**, 522–528.
- Imhoff, J. F. & Trüper, H. G. (1989). Genus *Rhodomicrobium* Duchow and Douglas 1949, 415^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, 9th edn, pp. 1677–1678. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Large, P. J. & Bamforth, C. W. (1988). *Methylotrophy and Biotechnology*. New York: Longman Scientific & Technical and Wiley.
- Liessens, J. (1993). Removing nitrate with a methylotrophic fluidized bed: microbiological quality. *J Am Water Assoc* **85**, 155–161.
- Maidak, B. L., Larsen, N., McCaughey, M. J., Overbeek, R., Olsen, G. J., Fogel, K., Blandy, J. & Woese, C. R. (1994). The Ribosomal Database Project. *Nucleic Acids Res* **22**, 3485–3487.
- Moore, R. L. (1977). Ribosomal ribonucleic acid cistron homologies among *Hyphomicrobium* and various other bacteria. *Can J Microbiol* **23**, 478–481.
- Moore, R. L. & Hirsch, P. (1972). Deoxyribonucleic acid base sequence homologies of some budding and prosthecate bacteria. *J Bacteriol* **110**, 256–261.
- Moore, R. L. & Weiner, R. M. (1989). Genus *Hyphomonas* (ex Pongratz 1957) Moore, Weiner and Gebers 1984, 71^{VP}. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, 9th edn, pp. 1904–1910. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Nikitin, D. I., Yu, O., Vishnewetskaya, K., Chumakov, M. & Zlatkin, I. V. (1990). Evolutionary relationship of some stalked and budding bacteria (genera *Caulobacter*, *Hyphobacter*, *Hyphomonas*, and *Hyphomicrobium*) as studied by the new integral taxonomical method. *Arch Microbiol* **153**, 123–128.
- Nyberg, U., Aspegren, H., Andersson, B., Jansen, J. laC. & Villadsen, I. S. (1992). Full-scale application of nitrogen removal with methanol as carbon source. *Water Sci Technol* **26**, 1077–1086.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Roggentin, T. & Hirsch, P. (1989). Ribosomal RNA cistron similarities among *Hyphomicrobium* species and several other hyphal, budding bacteria. *Syst Appl Microbiol* **11**, 140–147.
- 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.
- Stackebrandt, E. R. G., Fischer, A., Roggentin, T., Wehmeyer, U. & 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.
- Strunk, O. & Ludwig, W. (1995). *ARB – a Software Environment for Sequence Data*. Munich: Department of Microbiology, Technical University of Munich.
- Takada, N. (1975). A new species of *Hyphomicrobium*. In *Proceedings of the International Symposium on Microbial Growth on C₁ Compounds*, pp. 29–33. Edited by G. Terui. Tokyo: Society for Fermentation Technology.
- Tsuji, K., Tsien, H. C., Hanson, R. S., De Palma, S. R., Scholtz, R. & Laroche, S. (1990). 16S ribosomal RNA sequence analysis for determination of phylogenetic relationship among methylotrophs. *J Gen Microbiol* **136**, 1–10.
- 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.
- Vedenina, J. Ya., N., Chernykh, A., Lebedinskii, A. V. & Andreev, L. V. (1990). Correlations of characteristics in subgroups of the genus *Hyphomicrobium*. *Mikrobiologiya* **59**, 895–902 (English translation 617–622).