Phylogenetic relationships among members of the *Comamonadaceae*, and description of *Delftia acidovorans* (den Dooren de Jong 1926 and Tamaoka *et al.* 1987) gen. nov., comb. nov.

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The phylogenetic relationships among members of the family *Comamonadaceae* and several unclassified strains were studied by direct sequencing of their PCR-amplified 16S rRNA genes. Based on the 16S rRNA gene sequence analysis, members of the family formed a coherent group. The closest relatives are species of the *Rubrivivax* sub-group: *Leptothrix discophora*, *Ideonella dechloratans* and *Rubrivivax gelatinosus*. The genus *Hydrogenophaga* formed two subclusters, as did the species of *Acidovorax*, whereas the five species of the genus [*Aquaspirillum*] were polyphyletic. *Comamonas acidovorans* was phylogenetically distant from the type species of *Comamonas*, *Comamonas terrigena*. On the basis of this work and previous studies, *Comamonas acidovorans* is removed from the genus *Comamonas* and renamed as *Delftia acidovorans* gen. nov., comb. nov. Descriptions of the new genus *Delftia* and of the type species *Delftia acidovorans*, for which the type strain is ATCC 15668ᵀ, are presented.

Keywords: phylogeny, 16S rDNA, taxonomy, *Comamonadaceae*, *Delftia acidovorans*

INTRODUCTION

Members of the *Comamonadaceae*, originally the acidovorans rRNA complex (De Vos *et al.*, 1985; Willems *et al.*, 1987, 1989, 1990), belong to rRNA superfamily III (De Ley 1978) or the β subclass of the *Proteobacteria* (Stackebrandt *et al.*, 1988). The family *Comamonadaceae* was described by Willems *et al.* (1991a) to include the genera *Comamonas*, *Acidovorax*, *Hydrogenophaga*, *Xylophilus* and *Variovorax*, as well as a number of phylogenetically misnamed [*Aquaspirillum*] and phytopathogenic [*Pseudomonas*] species based on relationships determined by extensive DNA–rRNA hybridization data. Later, Willems *et al.* (1992b) assigned the phylogenetically misnamed phytopathogenic *Pseudomonas* species ([*Pseudomonas*] *avenae*, [*Pseudomonas*] *rubrilineans*, '[*Pseudomonas*] *setariae*', [*Pseudomonas*] *cattleyae*, [*Pseudomonas*] *pseudoalcaligenes* subsp. *citrulli* and [*Pseudomonas*] *pseudoalcaligenes* subsp. *konjaci*) to the genus *Acidovorax*. The nearest phylogenetic relatives of the *Comamonadaceae* are [*Pseudomonas*] *saccharophila*, *Rubrivivax gelatinosus*, *Leptothrix* and *Sphaerotilus* (Willems *et al.*, 1991a).

Subsequently, several other species have been assigned to the *Comamonadaceae*. On the basis of 16S rRNA sequences, Hiraishi (1994) demonstrated that *Rhodoferax fermentans* was most closely related to *Comamonas testosteroni*, and later Hiraishi *et al.* (1995) assigned *Rhodoferax fermentans* and the newly described *Brachymonas denitrificans* to this family. Two strains from activated sludge and the Baltic Sea were found to be related to *Comamonas testosteroni* using *in situ* hybridization with oligonucleotide probes (Koivula & Hantula, 1997), but their phylogenetic position and taxonomic status are not known. Recently, the causative organism of bacterial leaf spot on *Anthurium* sp. (Prior & Rott, 1989) was found to belong to the genus *Acidovorax* (Saddler *et al.*, 1995). However, its relationship to the existing species of *Acidovorax* is not clear.

Current knowledge of the phylogenetic relationship of the members of the *Comamonadaceae* is based on

The GenBank accession numbers for the 16S rDNA sequences determined in this study are AF078753–AF078774.

rRNA cistron similarities (Pot *et al.*, 1992b; Willems *et al.*, 1991a, b, 1992b), which was the most valid and useful parameter for drawing taxonomic conclusions at the generic and suprageneric levels before 16S rRNA sequence analysis was available (Tamaoka *et al.*, 1987). Based on the DNA–rRNA hybridization results (Pot *et al.*, 1992b; Willems *et al.*, 1991a, b, 1992b), sixteen rRNA branches within the *Comamonadaceae* were delineated: five *Comamonas* rRNA branches, one *Hydrogenophaga* rRNA branch, one *Xylophilus* rRNA branch, one *Variovorax* rRNA branch, two *Acidovorax* rRNA branches and six [*Aquaspirillum*] rRNA branches. Willems *et al.* (1987) and Vandamme *et al.* (1996) concluded that each of the sub-branches in the acidovorans rRNA complex (i.e. the *Comamonadaceae*) deserves a generic rank, provided that sufficient phenotypic and genotypic data are available to describe them. Because of the limit of resolution of DNA–rRNA hybridization data and the expanding number of members, the phylogenetic relationships within the family *Comamonadaceae* are yet to be fully elucidated. The sixteen rRNA branches do not correspond to the generic classification. For example, the genus *Comamonas* comprises five separate rRNA branches (Willems *et al.*, 1991b) and the genus *Acidovorax* consists of two rRNA branches (Willems *et al.*, 1992b).

Comparative sequence analysis of the 16S rRNA gene is currently one of the most powerful and reliable methods to estimate phylogenetic relationships among bacterial taxa. Prior to the commencement of this study, only four 16S rDNA sequences of bacteria belonging to the family *Comamonadaceae* were available (Hiraishi, 1994; Hiraishi *et al.*, 1995; Yang *et al.*, 1985). The purpose of this study was to use 16S rDNA sequence similarity analysis to elucidate the precise phylogenetic relationships among members of the *Comamonadaceae* and to provide a more complete set of 16S rDNA sequences for future taxonomic studies.

METHODS

Bacterial strains and cultivation. The 22 bacterial strains used in this study are listed in Table 1. Several species studied are assigned to incorrect genera and these combinations are indicated by enclosing the genus name in square brackets. The [*Aquaspirillum*] species were grown on PSS agar (Pot *et al.*, 1992b), *Xylophilus ampelinus* was grown on GYCA medium (Willems *et al.*, 1987), and all other species were grown on medium B of King *et al.* (1954). *Xylophilus ampelinus* was incubated at 24 °C, [*Aquaspirillum*] *psychrophilum* was grown at 20 °C, and all other species were grown at 28 °C, for 48–72 h.

PCR amplification of 16S rRNA genes. A bacterial suspension (10^8 c.f.u. ml^{-1}) was boiled for 10 min to release the DNA and centrifuged for 5 min in a microcentrifuge. The supernatant was used as DNA template for PCR amplification of the 16S rRNA gene. PCR amplification was performed in a 100 µl reaction volume containing PCR buffer [67 mM Tris/HCl (pH 8·8), 16·6 mM $(NH_4)_2SO_4$, 0·45 % (v/v) Triton X-100, 200 µg gelatin ml^{-1}], 1·5 mM $MgCl_2$, each deoxynucleoside phosphate at a concentration of 200 µM, 0·25 µM primer 27f (Lane, 1991), 0·25 µM primer 1525r (Lane, 1991), 5 µl lysed cells and 2 U *Tth* Plus DNA polymerase (Biotech International). A negative control was always included in each experiment, which contained all of the ingredients described above except for the template DNA.

All PCR amplifications were performed in a Perkin-Elmer Cetus model 480 thermal cycler (Applied Biosystems). The PCR conditions consisted of an initial denaturation step at 96 °C for 5 min; 28 cycles of 48 °C for 1 min, 72 °C for 2 min and 94 ° for 1 min; and one additional cycle at 48 °C for 1 min and 72 °C for 5 min to allow all extension products to be completed. The PCR products were purified by using the Promega Wizard Minipreps DNA purification system according to the manufacturer's instructions (Promega).

16S rDNA sequencing. The purified PCR product was used as the template for sequencing. *Taq* DyeDeoxy Terminator Cycle or ABI PRISM Dye Terminator Cycle sequencing kits (both from Applied Biosystems) were used following procedures recommended by the manufacturer. The following nine 16S rDNA sequencing primers were used in the sequencing reactions: 27f, 342r, 357f, 519r, 530f, 907r, 1114f and 1525r (Lane, 1991); and 803f (Stackebrandt & Charfreitag, 1990). The sequencing products were purified according to the manufacturer's instructions. The sequences were determined on an Applied Biosystems 373A DNA sequencer.

Phylogenetic analysis. The 16S rDNA sequences were manually aligned using the ae2 editor program (Maidak *et al.*, 1997) against the existing sequences obtained from GenBank for *Comamonas testosteroni* and other reference species (*Burkholderia cepacia*, *Brachymonas denitrificans*, *Variovorax paradoxus*, *Rhodoferax fermentans*, *Ideonella dechloratans*, *Leptothrix discophora*, *Rubrivivax gelatinosus* and *Spirillum volutans*) belonging to the *β-Proteobacteria*. The phylogenetic tree shown in Fig. 1 was constructed by comparison of 1309 nucleotide positions. Ambiguous nucleotide positions, where alignment was uncertain, were excluded from the analysis. The sequence of *Burkholderia cepacia* ATCC 25416^T was used as the outgroup in the phylogenetic analyses. The construction of the phylogenetic trees was computed using two different methods of phylogenetic analysis (parsimony and distance) available in the PHYLIP software package, version 3.5 (Felsenstein, 1993). Pairwise evolutionary distances were calculated by the method of Jukes & Cantor (1969) using the DNADIST program, and the parsimony analysis was performed using DNAPARS. A dendrogram was constructed from evolutionary distance values by using the neighbour-joining method of Saitou & Nei (1987) contained in the NEIGHBOR program of PHYLIP. The tree topologies and statistical significance of branch points of the distance and parsimony trees were tested by 100 bootstrap resamplings of the data (Felsenstein, 1985).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have GenBank accession numbers AF078753–AF078774 (Table 1). The strain numbers and the nucleotide sequence accession numbers for the reference strains are as follows: *Burkholderia cepacia* ATCC 25416^T, M22518; *Comamonas testosteroni* RH1104 (= ATCC 11996^T), M11224; *Brachymonas denitrificans* AS-P1 (= JCM 9216^T), D14320; *Variovorax paradoxus* IAM 12373^T, D30793; *Rhodoferax fermentans* FR 2 (= JCM 7819^T), D16211; *Polaromonas vacuolata* strain 34-P^T (= ATCC 51984^T), U14585; *Ideonella dechloratans* CCUG

Table 1. Strains used in 16S rRNA gene sequencing

ACM, Australian Collection of Microorganisms, Department of Microbiology, The University of Queensland, St Lucia, Australia; ATCC, American Type Culture Collection, Manassas, VA, USA; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; Hantula, J. Hantula, University of Helsinki, Finland; ICMP, Culture Collection of the Plant Disease Division, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand; IMI, International Mycological Institute, Egham, Surrey, UK; LMG, Culture Collection Laboratorium voor Microbiologie, State University of Ghent, Ghent, Belgium; NICB, National Collection of Industrial Bacteria, Aberdeen, UK; NCPPB, National Collection of Plant-pathogenic Bacteria, Central Science Laboratory, York, UK.

Species	Strain	Source	Other designation(s)	GenBank no.
Acidovorax avenae subsp. *avenae*	ATCC 19860^T	ATCC	NCPPB 1011^T, LMG 2117^T, CCUG 15838^T	AF078759
Acidovorax avenae subsp. *citrulli*	ATCC 29625^T	ATCC	CCUG 1739^T,LMG 5376^T, ICMP 7500^T	AF078761
Acidovorax avenae subsp. *cattleyae*	NCPPB 961^T	NCPPB	LMG 2364^T, LMG 5286^T, ATCC 33619^T, CCUG 21975^T	AF078762
Acidovorax konjaci	ATCC 33996^T	ATCC	CCUG 17394^T, LMG 5691^T, ICMP 7733^T	AF078760
Acidovorax facilis	CCUG 2113^T	CCUG	ATCC 11228^T, LMG 2193^T	AF078765
Acidovorax temperans	CCUG 11779^T	CCUG	LMG 7169^T	AF078766
Acidovorax delafieldii	ATCC 17505^T	ATCC	LMG 5943^T, CCUG 1779^T	AF078764
Acidovorax sp.	IMI 357678	IMI		AF078763
Acidovorax sp.	7087	Hantula		AF078767
[*Aquaspirillum*] *gracile*	ATCC 19624^T	ATCC	LMG 4333^T, LMG 8201^T	AF078753
[*Aquaspirillum*] *metamorphum*	LMG 4339^T	LMG	ATCC 15280^T, LMG 4338^T, NCIB 9509^T, CCUG 13974^T	AF078757
[*Aquaspirillum*] *delicatum*	LMG 4328^T	LMG	ATCC 14667^T, LMG 4327^T, CCUG 15846^T, NCIB 9419^T	AF078756
[*Aquaspirillum*] *psychrophilum*	LMG 5408^T	LMG	ATCC 33335^T	AF078755
[*Aquaspirillum*] *sinuosum*	LMG 4393^T	LMG	NCIB 9010^T, ATCC 9786^T, CCUG 4347^T, CCUG 13728^T	AF078754
Comamonas acidovorans	ACM 489^T	ACM	ATCC 15668^T, CCUG 14481^T, LMG 1226^T, Stanier 14^T	AF078774
Comamonas terrigena	IMI 359870^T	IMI	ATCC 8461^T, LMG 1253^T, NCIB 8193^T, CCUG 15327^T, CCUG 2185^T (= LMG 5929^T)	AF078772
Comamonas sp.	12022	Hantula		AF078773
Hydrogenophaga flava	CCUG 1658^T	CCUG	ATCC 33667^T, LMG 2185^T, DSM 619^T	AF078771
Hydrogenophaga pseudoflava	ATCC 33668^T	ATCC	CCUG 13799^T, LMG 5945^T, GA3^T	AF078770
Hydrogenophaga palleronii	CCUG 20334^T	CCUG	ATCC 17724^T (= CCUG 1780^T), LMG 2366t1^T, Stanier 362t1^T	AF078769
Hydrogenophaga taeniospiralis	ATCC 49743^T	ATCC	LMG 7170^T, CCUG 15921^T, DSM 2082^T	AF078768
Xylophilus ampelinus	ATCC 33914^T	ATCC	NCPPB 2217^T, LMG 5856^T, CCUG 21976^T	AF078758

30898^T (= ATCC 15173^T), X72724; *Leptothrix discophora* SS-1 (= ATCC 43182), Z18533; *Rubrivivax gelatinosus* strain A3 (= ATCC 17011^T), D16213; and *Spirillum volutans* ATCC 19554, M34131.

RESULTS AND DISCUSSION

Nearly complete 16S rDNA sequences (> 1500 nucleotides) were determined for the 22 strains listed in Table 1. The phylogenetic relationships among members of the family *Comamonadaceae*, determined by a neighbour-joining distance analysis of their 16S rDNA sequences (Fig. 1), were by and large in agreement with the relationships deduced from rRNA cistron similarities (Pot *et al.*, 1992b; Willems *et al.*, 1991a, b, 1992b). The relationships inferred from a parsimony analysis of the data were similar to those obtained by the distance analysis. A more detailed phylogenetic picture at the generic and intrageneric levels was obtained with the 16S rDNA sequence analysis than

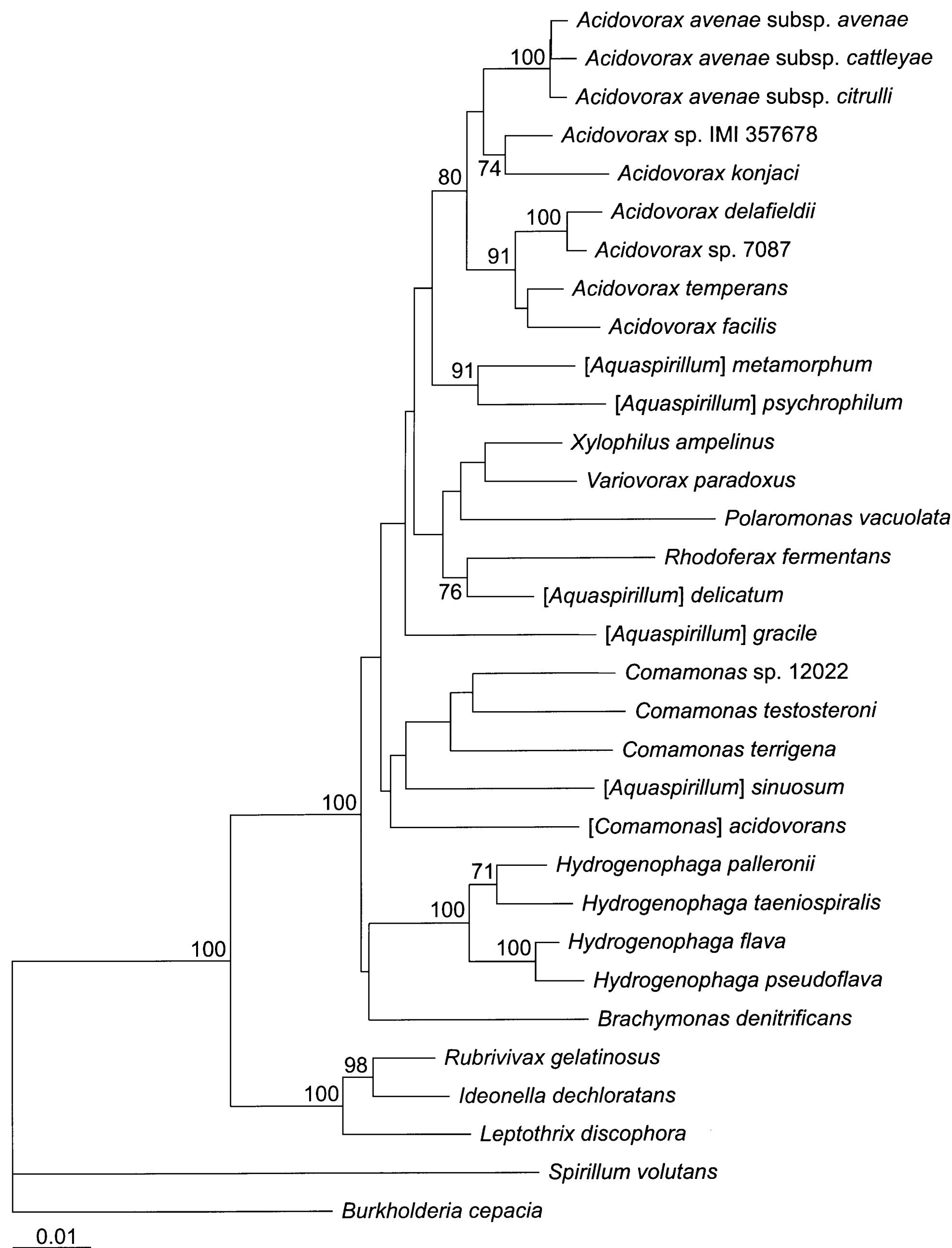

Fig. 1. Neighbour-joining tree showing phylogenetic relationships among members of the family *Comamonadaceae* and related species based on 1309 nucleotide positions of their 16S rDNA sequences. *Burkholderia cepacia* was used as the outgroup. Scale bar represents 1 nucleotide substitution per 100 nucleotides. Bootstrap values of 100 resamplings are shown at the branch points.

had been obtained from the previous rRNA cistron similarities. Each of the rRNA branches within the *Comamonadaceae* determined by rRNA cistron analysis (Pot *et al.*, 1992b; Willems *et al.*, 1991a, b, 1992b) for the genera *Comamonas*, *Acidovorax*, *Variovorax*, *Xylophilus*, *Hydrogenophaga* and [*Aquaspirillum*] have been confirmed by 16S rDNA sequence analysis. In addition the polyphyletic nature of the genus *Comamonas* and the misclassified [*Aquaspirillum*] species has been confirmed.

The genus *Comamonas*

The three species of the type genus *Comamonas* (*Comamonas terrigena*, *Comamonas testosteroni* and *Comamonas acidovorans*) belong to deep, poorly supported branches, a finding in agreement with DNA–rRNA hybridization results of Willems *et al.* (1991b). These workers demonstrated that there were five rRNA branches within the genus *Comamonas* which were linked to other rRNA branches in the family

Comamonadaceae at a T_m(e) value of 76±1·1 °C. The five rRNA branches consisted of one *Comamonas acidovorans* branch, one *Comamonas testosteroni* branch and three *Comamonas terrigena* branches. We included only the type strain IMI 359870^T of *Comamonas terrigena* (genotype 1) in our study. The results of our 16S rRNA gene sequence analysis (Fig. 1) showed that *Comamonas testosteroni* and *Comamonas terrigena* formed a deeply branched cluster. However, *Comamonas acidovorans* occurs on a very deep branch of uncertain phylogenetic position and cannot be considered a member of the genus *Comamonas*. This result confirms previous reports based on DNA–rRNA hybridization (Willems *et al.*, 1991b), 16S rRNA cataloguing (Woese *et al.*, 1984a, b) and conventional and chemotaxonomic methods (Tamaoka *et al.*, 1987). A numerical analysis of morphological, physiological and biochemical characters, protein gel electrophoresis and immunotyping experiments (Willems *et al.*, 1991b) also suggested that the species *Comamonas acidovorans* should be removed from the genus *Comamonas*. The strain 12022 from the Baltic sea (Koivula & Hantula, 1997) clustered with *Comamonas testosteroni*, although the bootstrap value for this branch point was only 61% for the neighbour-joining tree and the sequence similarity was 96·3%. The similarity value of strain 12022 to the existing species of *Comamonas*, inferred from the 16S rDNA sequence analysis, ranged from 93·1 to 96·3%, which suggests it does not belong to any of the existing species of the genus *Comamonas* (Stackebrandt & Goebel, 1994). The taxonomic position of strain 12022 requires further investigation.

The genus *Acidovorax*

Within the genus *Acidovorax*, the validly described species (*Acidovorax avenae*, *Acidovorax konjaci*, *Acidovorax facilis*, *Acidovorax temperans*, *Acidovorax delafieldii*), the strain 7087 from activated sludge (Koivula & Hantula, 1997) and the *Anthurium* pathogen strain IMI 357678 (Saddler *et al.*, 1995) clustered together. The level of the 16S rDNA sequence similarity of the species of *Acidovorax* and strains 7087 and IMI 357678 ranged from 95·8 to 99·5%, which revealed that the genus *Acidovorax* is phylogenetically homogeneous. However, the species of *Acidovorax* diverged into two sub-groups. The first sub-group consisted of all the phytopathogens including *Acidovorax avenae*, *Acidovorax konjaci* and the *Anthurium* pathogen strain IMI 357678, and the second sub-group comprised the non-phytopathogenic species *Acidovorax facilis*, *Acidovorax temperans*, *Acidovorax delafieldii* and the strain 7087 from activated sludge. The two sub-groupings deduced from 16S rDNA sequence analysis support the results obtained by rRNA cistron similarities (Willems *et al.*, 1992b) which showed that *Acidovorax avenae* and *Acidovorax konjaci* formed one rRNA branch, and *Acidovorax facilis*, *Acidovorax temperans*, *Acidovorax delafieldii* formed another rRNA branch; the two rRNA branches were linked at a T_m(e) value of 77 °C. In our results there is high bootstrap support for the branch containing the non-phytopathogens, but little support (bootstrap value of 47·0% for the distance tree) for the phytopathogen branch. The three subspecies of *Acidovorax avenae* are very closely related, sharing sequence similarities of over 99·5%. The *Anthurium* pathogen strain IMI 357678 was most closely related to *Acidovorax konjaci*, a relationship which was not revealed previously by random-amplified polymorphic DNA analysis (RAPD) (Saddler *et al.*, 1995). Strain IMI 357678 shared a sequence similarity of 98·1% with *Acidovorax konjaci*; this strain appears to be a new species but its taxonomic standing will need to be resolved by DNA–DNA hybridization. The activated sludge strain 7087 has a sequence similarity of 99·3% with *Acidovorax delafieldii*. However, cells of this strain were non-motile short rods or coccobacilli, unlike the cells of *Acidovorax delafieldii* which are straight rods (0·5 × 1·8–2·6 μm), and are motile by means of a single polar flagellum (Palleroni, 1984). The taxonomic identity of strain 7087 needs to be defined by DNA–DNA hybridization and the availability of more phenotypic data.

The genus *Hydrogenophaga*

The four species of *Hydrogenophaga* formed a tight group with very high bootstrap support (100% for the distance tree and 95% for the parsimony tree). The 16S rDNA sequences of the four species exhibited levels of similarity in the range from 97·0 to 99·1%. There is some evidence of a bifurcation within the genus with *Hydrogenophaga flava* and *Hydrogenophaga pseudoflava* forming one sub-group, and *Hydrogenophaga taeniospiralis* and *Hydrogenophaga palleronii* forming a second sub-group. This finding is in good agreement with results obtained by rRNA cistron similarity (Willems *et al.*, 1989).

The *Polaromonas–Variovorax–Xylophilus* branch

The grapevine pathogen *Xylophilus ampelinus* clustered with *Variovorax paradoxus* and *Polaromonas vacuolata*. The closeness of the relationship between *Xylophilus ampelinus* and *Variovorax paradoxus* was not previously revealed by DNA–rRNA hybridization studies (Willems *et al.*, 1987). The 16S rDNA sequence similarity of 97·9% between these two taxa indicates a closer relationship than is expected between two separate genera. The type species of the two taxa exhibit 20% DNA–DNA reassociation indicating their separate species status (Willems *et al.*, 1987). Phenotypically, these taxa exhibit similarities in cell morphology, positive reaction for catalase and production of a yellow non-diffusible pigment, whilst exhibiting differences in oxidase reaction, flagella arrangement, growth temperature, chemolithotrophic growth with hydrogen, nitrate reduction, habitat and utilization of carbon sources (Willems *et al.*, 1987, 1991a, b). The taxonomic status of these two taxa may

need to be revised following a more polyphasic characterization.

The newly described species *Polaromonas vacuolata* clusters with *Xylophilus ampelinus* and *Variovorax paradoxus* and has 94·5% and 94·8% sequence similarity, respectively, although the grouping is not well supported by bootstrap values. *Polaromonas vacuolata* was previously shown (Irgens *et al.*, 1996) to be most closely related to *Variovorax paradoxus*, but in the absence of a sequence for *Xylophilus ampelinus*, the relationship with this species was not revealed. *Polaromonas vacuolata* is a psychrophilic chemo-organotroph from marine Antarctic waters, and the first gas vacuolate member of the *Comamonadaceae* and the β-*Proteobacteria* (Irgens *et al.*, 1996).

The *Brachymonas* branch

The newly described species *Brachymonas denitrificans* falls on a separate deep branch of the *Comamonadaceae* and is the only known member of the branch. Hiraishi *et al.* (1995) found *Brachymonas denitrificans* to group with *Comamonas testosteroni* but used too few reference sequences to adequately infer the relationship of this species with other members of the *Comamonadaceae*. The cells of this species are non-motile coccobacilli or short rods (Hiraishi *et al.*, 1995). They have a similar cell morphology to strains 7087 and 12022 (results not shown), but the three strains are not phylogenetically related according to their 16S rRNA gene sequences.

Misclassified [*Aquaspirillum*] species

Based on DNA–rRNA hybridization, Pot *et al.* (1992b) determined that seven species of [*Aquaspirillum*] belong to the family *Comamonadaceae* whereas the type species of the genus *Aquaspirillum*, *Aquaspirillum serpens*, belongs to the *Aquaspirillum serpens* rRNA branch in rRNA superfamily III. The species [*Aquaspirillum*] *anulus*, [*Aquaspirillum*] *delicatum*, [*Aquaspirillum*] *metamorphum*, [*Aquaspirillum*] *sinuosum*, [*Aquaspirillum*] *giesbergeri*, [*Aquaspirillum*] *gracile* and [*Aquaspirillum*] *psychrophilum* are phylogenetically distinct and cannot be considered as members of this genus. Pot *et al.* (1992b) showed that among the seven misclassified *Aquaspirillum* species, only [*Aquaspirillum*] *giesbergeri* and [*Aquaspirillum*] *sinuosum* are closely related; all other species occupy separate positions in the acidovorans rRNA complex. Each distinct rRNA branch is indicative of generic rank (Vandamme *et al.*, 1996). We determined the 16S rDNA sequences for five of these species, but were unable to obtain viable cultures of [*Aquaspirillum*] *anulus* and [*Aquaspirillum*] *giesbergeri* which therefore have not been included in this study. Our 16S rDNA sequence analysis revealed that the five species of [*Aquaspirillum*] examined are deeply branching in accordance with the results demonstrated by rRNA cistron similarities (Pot *et al.*, 1992b; Willems *et al.*, 1991a). The results showed that the species [*Aquaspirillum*] *psychrophilum* and [*Aquaspirillum*] *metamorphum* are closely related, sharing a sequence similarity of 97·2%, and that [*Aquaspirillum*] *delicatum* and *Rhodoferax fermentans* clustered together on a separate deep branch with a sequence similarity of 96·8%. The precise phylogenetic positions of [*Aquaspirillum*] *sinuosum* and [*Aquaspirillum*] *gracile* are unclear due to deep branching with low bootstrap support, but they show no close relationship to any other well-supported branches within the *Comamonadaceae*. In general, the deep branchings and low bootstrap values make it difficult to confirm the relationship between the misclassified species of [*Aquaspirillum*] and the genus *Acidovorax*, which was revealed by Willems *et al.* (1992b). Using rRNA cistron similarity, Willems *et al.* demonstrated that the [*Aquaspirillum*] strains have significantly higher T_m(e) values versus rRNA from either *Acidovorax avenae* NCPPB 1011^T or *Acidovorax facilis* ATCC 11228^T than to the other taxa of the *Comamonadaceae*. Recently, the chemotaxonomic characterizations of the genus [*Aquaspirillum*] were reported (Hamana *et al.*, 1994; Sakane & Yokota, 1994). Based on polyamine patterns (Hamana *et al.*, 1994), the seven species of [*Aquaspirillum*] are separated into two groups: 2-hydroxyputrescine-putrescine type containing [*Aquaspirillum*] *anulus*, [*Aquaspirillum*] *delicatum*, [*Aquaspirillum*] *gracile*, [*Aquaspirillum*] *giesbergeri*, [*Aquaspirillum*] *metamorphum* and [*Aquaspirillum*] *sinuosum*, and 2-hydroxyputrescine-putrescine-spermidine type containing [*Aquaspirillum*] *psychrophilum* only. This grouping does not agree with our 16S rRNA gene sequence analysis, nor with the result of DNA–rRNA hybridization (Pot *et al.*, 1992b). Studies on the fatty acid compositions have shown that the seven [*Aquaspirillum*] species were heterogeneous in 3-hydroxy fatty acid: [*Aquaspirillum*] *anulus*, [*Aquaspirillum*] *giesbergeri*, [*Aquaspirillum*] *metamorphum* and [*Aquaspirillum*] *sinuosum* have 3-OH 10:0; [*Aquaspirillum*] *delicatum* has 3-OH 8:0; [*Aquaspirillum*] *psychrophilum* has 3-OH 10:0 and 8:0; and [*Aquaspirillum*] *gracile* has 3-OH 10:0 and 12:0 as major 3-hydroxy fatty acid (Sakane & Yokota, 1994). Grouping using this chemotaxonomic marker does not correlate with our results either.

Closest neighbours of the family *Comamonadaceae*

The 16S rDNA sequence analysis determined that the species *Rubrivivax gelatinosus*, *Ideonella dechloratans* and *Leptothrix discophora* are the closest relatives of the family *Comamonadaceae*, which confirmed results previously obtained by DNA–rRNA hybridization (Willems *et al.*, 1991a). No 16S rRNA sequence is available for [*Pseudomonas*] *saccharophila*, which was therefore not included in the analysis.

Taxonomic considerations

The 16S rDNA sequence analysis of members of the *Comamonadaceae* has confirmed that this is a coherent phylogenetic group and has extended knowledge of the

relationships at intrageneric and generic levels. We have resolved several taxonomic issues, some of which have been identified by other workers. However, resolution of other issues will require further research. The major questions to be addressed in the future are whether the phytopathogenic and non-phytopathogenic species of *Acidovorax* belong to separate genera, the transfer of [*Comamonas*] *acidovorans* to a new genus, the transfer of [*Aquaspirillum*] *psychrophilum* and [*Aquaspirillum*] *metamorphum* to a new genus, and the transfer of [*Aquaspirillum*] *gracile* and [*Aquaspirillum*] *sinuosum* to new genera. Further work is also required to clarify the relationship between [*Aquaspirillum*] *delicatum* and *Rhodoferax fermentans*, and between *Variovorax paradoxus* and *Xylophilus ampelinus*. At this time, a start can be made on the taxonomic revision of the genus *Comamonas*. There is sufficient phylogenetic and phenotypic evidence to remove the species [*Comamonas*] *acidovorans* from the genus *Comamonas*, and for its transfer to a new genus for which we propose the name *Delftia*.

Description of *Delftia* gen. nov.

Delftia (Delf.′tia. M.L. fem. n. *Delftia* referring to the city of Delft, the site of isolation of the type species, and in recognition of the pioneering role of Delft research groups in the development of bacteriology). The description is based on data from previous research (Busse & Auling, 1988; De Vos *et al.*, 1985; Ikemoto *et al.*, 1978; Palleroni, 1984; Tamaoka *et al.*, 1987; Willems *et al.*, 1989, 1991b, 1992a), and the present 16S rRNA gene sequence analysis.

Cells are straight to slightly curved rods, 0·4–0·8 × 2·5–4·1 μm (occasionally up to 7 μm), which occur singly or in pairs. Motile by means of polar or bipolar tufts of one to five flagella. Gram-negative. Oxidase and catalase-positive. Endospores are not produced, and no fluorescent pigments are produced. Poly-*β*-hydroxybutyrate is accumulated in cells. Strictly aerobic, nonfermentative and chemo-organotrophic. Hydrolyses acetamide, nitrate reduced to nitrite, no levan formation from sucrose, gelatin-liquefaction-negative, no starch hydrolysis, lipase (Tween 80 hydrolysis)-positive, arginine-dihydrolase-negative, *meta* cleavage of protocatechuate. Unable to denitrify. Unable to grow autotrophically with hydrogen. Cells grow well on media containing organic acids, amino acids, peptone and carbohydrates (but not glucose). Putrescine and 2-hydroxyputrescine are the main polyamine components. Ubiquinone Q-8 is the main quinone, Q-7 and Q-9 are minor quinones, but menaquinone is not produced. Major fatty acids are hexadecanoic acid (16:0), hexadecenoic acid (16:1) and octadecenoic acid (18:1); 3-hydroxy fatty acids (3-OH 10:0 and 8:0) are present, but 2-hydroxy fatty acid is not present. The mean G+C content of the DNA ranges from 67 to 69 mol% (as determined by the thermal denaturation method). The genus belongs to the family *Comamonadaceae* according to DNA–rRNA hybridization (Willems *et al.*, 1991a) and 16S rRNA gene sequence analysis. The characteristics differentiating *Delftia* from related genera are listed in Table 2. The type species of the genus is *Delftia acidovorans*.

Description of *Delftia acidovorans* (den Dooren de Jong 1926 and Tamaoka *et al.* 1987) comb. nov.

Delftia acidovorans (a.ci.do′vo.rans. L. neut. n. *acidum* acid; L. v. *voro* to devour; M.L. part. adj. *acidovorans* acid-devouring). The description is the same as that for the genus. A detailed description is given below based on information from Palleroni (1984), Tamaoka *et al.* (1987), Willems *et al.* (1991b) and the present 16S rRNA gene sequence analysis.

Characteristics of the species are as follows: growth at 30 °C, no growth at 4 °C and 41 °C, growth in the presence of 0·5 or 1·5% NaCl, no pigment production on nutrient agar.

The following characteristics are absent: growth in the presence of 6·5% NaCl; acid production in 10% lactose, in triple sugar iron medium and in oxidative-fermentative medium containing D-glucose, D-fructose, D-xylose, maltose, or adonitol; production of H_2S in triple sugar iron medium; hydrolysis of aesculin, gelatin and DNA; indole production; *β*-galactosidase activity; hydrolysis of 2-naphthylmyristate, L-valyl-2-naphthylamide, *N*-benzoyl-DL-arginine-2-naphthylamide, *N*-glutaryl-phenylalanine-2-naphthylamide, 6-bromo-2-naphthyl-*α*-D-galactopyranoside, 2-naphthyl-*β*-D-galactopyranoside, naphthol-AS-BI-*β*-D-glucuronate, 2-naphthyl-*α*-D-glucopyranoside, 6-bromo-2-naphthyl-*β*-D-glucopyranoside, 1-naphthyl-*N*-acetyl-*β*-D-glucosaminide, 6-bromo-2-naphthyl-*α*-D-mannopyranoside and 2-naphthyl-*α*-L-fucopyranoside.

The following organic compounds can be utilized as carbon and energy sources: acetate, acetamide [reported as a variable reaction by Tamaoka *et al.* (1987) and Willems *et al.* (1991b)], aconitate, adipate, D-alanine [reported as a variable reaction by Willems *et al.* (1991b)], L-alanine [reported as a variable reaction by Willems *et al.* (1991b)], 2-aminobutyrate [reported as a variable reaction by Willems *et al.* (1991b)], *δ*-aminovalerate, L-aspartate, azelate, butanol, 2,3-butylene glycol, butyrate, caproate [reported as a variable reaction by Willems *et al.* (1991b)], citraconate, citrate [reported as a variable reaction by Willems *et al.* (1991b)], ethanol, D-fructose, fumarate, gluconate, L-glutamate, glutarate, glycerate, glycine [reported as a variable reaction by Willems *et al.* (1991b)], glycolate, hippurate, L-histidine, *m*-hydroxybenzoate, *p*-hydroxybenzoate, *β*-hydroxybutyrate, hydroxymethylglutarate, isobutyrate, L-isoleucine [reported as a variable reaction by Willems *et al.* (1991b)], isovalerate, itaconate, *α*-ketoglutarate, kynurenate, L-kynurenine, lactate, levulinate, L-leucine, D-malate [reported as a variable reaction by Willems *et*

Table 2. Differential characteristics of the genus *Delftia* and other genera in the family *Comamonadaceae*

+, Present in all species; –, absent in all species; (+), weak reaction; d, 11–89 % of strains positive; D, variable reaction in different species; NA, no data available. References: *a*, De Vos *et al.* (1985), Palleroni (1984), Tamaoka *et al.* (1987) and Willems *et al.* (1991b); *b*, De Vos *et al.* (1985), Palleroni (1984), Tamaoka *et al.* (1987) and Willems *et al.* (1991b); *c*, Palleroni (1984) and Willems *et al.* (1990, 1992b); *d*, Palleroni (1984) and Willems *et al.* (1989); *e*, Kersters & De Ley (1984), Urakami *et al.* (1995) and Willems *et al.* (1991a); *f*, Bradbury (1984) and Willems *et al.* (1987, 1991a); *g*, Hiraishi *et al.* (1991); *h*, Hiraishi *et al.* (1995); *i*, Irgens *et al.* (1996); *j*, Hamana *et al.* (1994), Krieg (1984), Pot *et al.* (1992a, b) and Sakane & Yokota (1994).

Character	*Delftia*[a]	*Comamonas*[b]	*Acidovorax*[c]	*Hydrogenophaga*[d]	*Variovorax*[e]	*Xylophilus*[f]	*Rhodoferax*[g]	*Brachymonas*[h]	*Polaromonas*[i]	*[Aquaspirillum]*[j]
Cell morphology	Rods	Rods or spirilla	Rods	Rods	Rods	Rods	Curved rods	Coccobacilli or short rods	Rods	Spirilla or curved rods
Flagella	Polar or bipolar tufts	Polar or bipolar tufts	One polar	One polar	Peritrichous	One polar	One polar	–	One polar	Bipolar tufts or 1–2 flagella at only one pole
Pigments	–	–	–	+	+	+	–	(+)	–	–
Occurrence:										
Soil	+	+	+	+	+	–	–	–	–	–
Fresh water	+	+	+	+	+	–	–	–	–	+
Marine water	–	–	–	–	–	–	–	–	+	–
Infected plants	–	–	+	–	–	+	–	–	–	–
Clinical samples	+	+	+	–	–	–	–	–	–	–
Activated sludge	+	+	+	–	NA	NA	+	+	–	–
Phototrophy	–	–	–	–	–	–	+	–	–	–
Oxidase	+	+	+	+	+	–	+	+	+	+
Chemolithotrophic growth with H_2	–	–	D	+	D	–	NA	NA	–	NA
Psychrophilic growth	–	–	–	–	–	–	–	–	+	–
Growth factors	–	D	–	–	–	L-Glutamate	Biotin and thiamine	–	–	D
Denitrification	–	–	D	D	–	–	–	+	–	D
Carbon source used for growth										
Acetamide	d	–	D	–	–	NA	NA	NA	NA	NA
β-Alanine	d	–	D	–	d	NA	–	NA	NA	NA
2-Aminobutyrate	d	–	D	D	NA	NA	NA	–	NA	NA
3-Aminobutyrate	d	D	D	D	NA	NA	NA	–	NA	NA
D-Fructose	+	–	+	D	NA	–	+	–	–	–
D-Glucose	–	–	D	+	+	+	+	–	+	D
Glycerol	d	D	+	+	+	+	–	–	+	D
Malonate	d	–	D	–	d	–	NA	–	–	D
D-Mannitol	+	–	D	D	NA	–	+	–	NA	NA
Maleate	d	–	D	–	d	–	NA	NA	NA	NA
Phenylacetate	d	–	–	–	d	NA	NA	NA	NA	NA
L-(+)-Tartrate	d	–	–	D	d	NA	NA	–	NA	D
D-Tryptophan	d	D	–	–	NA	NA	NA	NA	NA	–
L-Tryptophan	d	–	D	D	d	NA	NA	–	NA	–
Major quinone system	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8 + RQ-8	Q-8 + RQ-8	NA	Q-8	
Major cellular fatty acid(s)	16:0, 16:1, 18:1	16:0, 16:1, 18:1	16:0, 16:1, 18:1	16:0, 16:1	16:1, 16:0, 18:1	NA	16:0, 16:1	16:0, 16:1	16:1ω7c, 16:0, 18:1ω7c, 18:1ω9t or 18:1ω12t	16:0, 16:1
Major 3-OH acids	10:0, 8:0	10:0	10:0, 8:0	8:0 (10:0)	10:0	NA	8:0	10:0	NA	10:0
G+C content (mol%)	67–69	63–66	67–70	65–69	66–68	68–69	59–61 (HPLC method)	63–65 (HPLC method)	52–57	56–62

al. (1991b)], L-malate, maleate [reported as a variable reaction by Willems *et al.* (1991b)], malonate [reported as a variable reaction by Willems *et al.* (1991b)], mannitol, mesaconate, mucate, nicotinate, DL-norleucine [reported as a variable reaction by Willems *et al.* (1991b)], L-norleucine [reported as a variable reaction by Willems *et al.* (1991b)], DL-norvaline, phenylacetate [reported as a variable reaction by Willems *et al.* (1991b)], L-phenylalanine [reported as a variable reaction by Willems *et al.* (1991b)], pimelate, L-proline, *n*-propanol, propionate, pyruvate, quinate, saccharate, sebacate, suberate, succinate, L-(+)-tartrate [reported as a variable reaction by Willems *et al.* (1991b)], *m*-tartrate, trigonelline [reported as a negative reaction by Willems *et al.* (1991b)], L-tryptophan [reported as a variable reaction by Tamaoka *et al.* (1987) and Willems *et al.* (1991b)], D-tryptophan [reported as a variable reaction by Willems *et al.* (1991b)], L-tyrosine and valerate.

The following compounds are not utilized by the species: *N*-acetylglucosamine, adonitol, 3-aminobenzoate, amylamine, anthranilate, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, L-arginine,

benzoate [reported as a variable reaction by Willems *et al*. (1991b)], benzoylformate, benzylamine, betaine, butylamine, caprylate, cellobiose, L-citrulline, creatine, L-cysteine, diaminobutane, dodecane, dulcitol, aesculin, erythritol, ethanolamine, ethylamine, ethylene glycol [reported as a positive reaction by Tamaoka *et al*. (1987)], D-fucose [reported as a variable reaction by Willems *et al*. (1991b)], D-galactose, β-gentiobiose, geraniol, glucosamine, D-glucose, methyl α-D-glucoside, glycogen, heptanoate, hexadecane, histamine, *o*-hydroxybenzoate, poly-β-hydroxybutyrate, DL-β-hydroxybutyrate, inulin, isophthalate, 2-ketogluconate, 5-ketogluconate, lactose, L-lysine, D-lyxose, maltose, D-mandelate, L-mandelate [reported as a variable reaction by Willems *et al*. (1991b)], D-mannose, methyl-α-D-mannoside, D-melezitose, D-melibiose, naphthalene, L-ornithine, oxalate, pantothenate, pelargonate [reported as a variable reaction by Willems *et al*. (1991b)], phenol, phenylethanediol, phthalate [reported as a variable reaction by Willems *et al*. (1991b)], propylene glycol [reported as a positive reaction by Tamaoka *et al*. (1987)], putrescine, L-rhamnose, D-raffinose, D-ribose, salicin, sarcosine [reported as a variable reaction by Willems *et al*. (1991b)], L-serine, sorbitol, L-sorbose, spermine, sucrose, starch, D-(−)-tartrate, terephthalate, testosterone, L-theronine [reported as a positive reaction by Tamaoka *et al*. (1987) and a variable reaction by Willems *et al*. (1991b)], D-turanose, trehalose, tryptamine, urea, L-valine [reported as a variable reaction by Willems *et al*. (1991b)], *m*-xylitol, D-xylose, L-xylose and methyl β-D-xyloside.

Variable utilizations among different strains: β-alanine, 2-aminobenzoate, 4-aminobenzoate, 3-aminobutyrate, 4-aminobutyrate, 5-aminobutyrate, α-aminovalerate, amygdalin, 2,3-butylene glycol, caprate, glycerol, *m*-erythritol, heptanoate, *m*-inositol, isobutanol, DL-kynurenine, L-methionine and tagatose.

Strains have been isolated from soil, sediment, activated sludge, crude oil, oil brine, water and various clinical samples. The G+C values of the DNA range from 67 to 69 mol% (as determined by the thermal denaturation method). Belongs to the family *Comamonadaceae* based on the 16S rRNA gene sequence analysis.

The type strain is ATCC 15668^T (den Dooren de Jong 7^T; Stanier 14^T; ACM 489^T; LMG 1226^T NCIB 9681^T). The type strain was isolated from soil enriched with acetamide in Delft in the Netherlands in 1926. The characteristics of the type strain are the same as those given above for the species. The G+C value of the DNA of strain Stanier 14^T is 67 mol% (as determined by the thermal denaturation method).

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