# Proposals of *Curvibacter gracilis* gen. nov., sp. nov. and *Herbaspirillum putei* sp. nov. for bacterial strains isolated from well water and reclassification of [*Pseudomonas*] *huttiensis*, [*Pseudomonas*] *lanceolata*, [*Aquaspirillum*] *delicatum* and [*Aquaspirillum*] *autotrophicum* as *Herbaspirillum huttiense* comb. nov., *Curvibacter lanceolatus* comb. nov., *Curvibacter delicatus* comb. nov. and *Herbaspirillum autotrophicum* comb. nov.

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Two strains of curved bacteria, 7-1$^T$ and 7-2$^T$, isolated from well water, were phylogenetically examined to determine their taxonomic position. Strain 7-1$^T$ is a Gram-negative, slightly curved rod. Analysis of the 16S rRNA gene sequence showed that strain 7-1$^T$ formed a cluster with [*Aquaspirillum*] *delicatum* and [*Pseudomonas*] *lanceolata*. It has some similar characteristics to [*A.*] *delicatum* and [*P.*] *lanceolata*, but has sufficient distance to separate it from other genera. DNA–DNA hybridization analysis, as well as chemotaxonomic and morphological studies, demonstrated that strain 7-1$^T$, [*A.*] *delicatum* and [*P.*] *lanceolata* belong to a new genus, *Curvibacter* gen. nov. Strain 7-1$^T$ (=IAM 15033$^T$=ATCC BAA-807$^T$) is classified as the type strain of *Curvibacter gracilis* gen. nov., sp. nov., and [*A.*] *delicatum* and [*P.*] *lanceolata* are classified as *Curvibacter delicatus* comb. nov. and *Curvibacter lanceolatus* comb. nov., respectively. Strain 7-2$^T$ is a Gram-negative spirillum. Phylogenetic study based on the 16S rRNA gene sequences showed that it formed a cluster with the members of the genus *Herbaspirillum*, [*Pseudomonas*] *huttiensis* and [*Aquaspirillum*] *autotrophicum*. The classification is therefore proposed of strain 7-2$^T$ (=IAM 15032$^T$=ATCC BAA-806$^T$) as the type strain of *Herbaspirillum putei* sp. nov., and [*P.*] *huttiensis* and [*A.*] *autotrophicum* are transferred to the genus *Herbaspirillum* as *Herbaspirillum huttiense* comb. nov. and *Herbaspirillum autotrophicum* comb. nov., respectively.

## INTRODUCTION

In the genus *Aquaspirillum*, 14 species and four subspecies are included, and they have all been isolated from fresh water (Krieg, 1984). However, DNA–rRNA hybridization, chemotaxonomic analysis and 16S rRNA gene sequence studies (Pot *et al.*, 1992; Sakane & Yokota, 1994; Hamana *et al.*, 1994; Wen *et al.*, 1999; Ding & Yokota, 2002) have shown that the genus is taxonomically heterogeneous. Among the species of this genus, some are closely related to species of the genera *Herbaspirillum*, *Magnetospirillum* and *Pseudomonas*.

The genus *Herbaspirillum* was established by Baldani *et al.* (1986). The organisms of this genus show nitrogen-fixing activities (Baldani *et al.*, 1996; Kirchhof *et al.*, 2001). Anzai *et al.* (2000) reported that the phylogenetic position of [*Pseudomonas*] *huttiensis* is close to that of the genus *Herbaspirillum*. [*Pseudomonas*] *lanceolata* was indicated to belong to the family *Comamonadaceae*, and is considered a close relative of [*Aquaspirillum*] *delicatum* (Anzai *et al.*, 2000).

In this study, we carried out the characterization and identification of two novel strains, 7-1$^T$ and 7-2$^T$, isolated from well water, based on phenotypic characterization,

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chemotaxonomic analysis, 16S rRNA gene sequence analysis and DNA–DNA hybridization analysis. For these two strains, we propose the names *Curvibacter gracilis* gen. nov., sp. nov. and *Herbaspirillum putei* sp. nov., respectively. In addition, we propose to transfer [*A.*] *delicatum*, [*P.*] *lanceolata*, [*Aquaspirillum*] *autotrophicum* and [*P.*] *huttiensis* as *Curvibacter delicatus* comb. nov., *Curvibacter lanceolatus* comb. nov., *Herbaspirillum autotrophicum* comb. nov. and *Herbaspirillum huttiense* comb. nov., respectively.

## METHODS

**Bacterial strains and isolation.** Strains 7-1$^T$ and 7-2$^T$ were isolated from well water in Osaka, Japan. Strains of the genera *Herbaspirillum* and *Aquaspirillum* and related organisms were obtained from the IAM Culture Collection (The University of Tokyo, Japan). Strains 7-1$^T$, 7-2$^T$, [*A.*] *autotrophicum* IAM 14942$^T$ and [*A.*] *delicatum* IAM 14955$^T$ were maintained by stab culture in medium B104 (IAM, 1998) containing (per litre distilled water) 10·0 g Polypepton, 2·0 g yeast extract and 1·0 g $MgSO_4.7H_2O$, pH 7·0. Strains were incubated at 30 °C. *Herbaspirillum seropedicae* IAM 14977$^T$, *Herbaspirillum rubrisubalbicans* IAM 14976$^T$, *Herbaspirillum frisingense* IAM 14974$^T$, [*P.*] *huttiensis* IAM 14941$^T$ and [*P.*] *lanceolata* IAM 14947$^T$ were incubated in medium B1 (IAM, 1998), composed of the following: 0·5 % polypeptone (Difco), 0·3 % yeast extract (Difco), 3 % NaCl and 1·5 % agar. The incubation temperature was 25 °C. $N_2$-free medium was composed (per litre distilled water) of 10 g glucose, 0·1 g $CaCl_2.H_2O$, 0·1 g $MgSO_4.7H_2O$, 0·9 g $K_2HPO_4$, 0·1 g $KH_2PO_4$, 5 g $CaCO_3$, 0·01 g $FeSO_4.7H_2O$ and 0·005 g $Na_2MoO_4.2H_2O$, pH 7·3.

**Morphology.** Cell size and morphology were determined by optical microscopy and scanning electron microscopy of cells grown on the culture media listed above. Cells grown on solid medium were fixed in 1 % glutaraldehyde in 0·01 M phosphate buffer (pH 7·2) for 2 h at room temperature and dehydrated through a graded ethanol series and then in a Hitachi model HCP-2 critical point drying apparatus. The preparation was sputter-coated with platinum under a vacuum. Samples were observed with a scanning electron microscope (model Hitachi S4500).

**Physiological and biochemical characteristics.** Oxidase activity was determined by oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by bubble formation in a 3 % (v/v) $H_2O_2$ solution. Biochemical tests were performed with API 20NE and API 50CH test strips (bioMérieux).

**Phylogenetic analysis based on 16S rRNA gene sequence comparisons.** Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA genes and purification of PCR products were carried out using previously described procedures (Hiraishi, 1992; Uchino *et al.*, 1997). The universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1510R (5′-GGCTACCTTGTTACGA-3′) were used for PCR amplification. The PCR products were purified using the GFX PCR DNA and Gel Band Purification kit (Amersham Pharmacia Biotech). Primers 8F, 520F, 926F, 350R, 700R, 1100R and 1510R were used in the 16S rRNA gene sequencing reactions. The 16S rRNA gene sequences obtained from the DNA database were aligned using CLUSTAL W, version 1.74 (Thompson *et al.*, 1994). Nucleotide substitution rates ($K_{nuc}$ values) were calculated. All of the sequences used were almost full-length and the sequences were derived from the type strain of each species. The phylogenetic tree was constructed using the neighbour-joining algorithm (Saitou & Nei, 1987).

**Detection of the *nifH* and *nifD* genes.** PCR for amplification of the *nifH* gene was carried out by using a cell lysate extracted from the organisms. A 360 bp fragment of the *nifH* gene was amplified using the forward primer 5′-TGCGAYCCSAARGCBGACTC-3′ and the reverse primer 5′-ATSGCCATCATYTCRCCGGA-3′ (Y = C or T; S = G or C; R = A or G; B = C or G or T) (Stoltzfus *et al.*, 1997). For the *nifD* gene, primers *nifD* Fdb261 (5′-TGGGGICCIRTIAARGAYATG-3′) and *nifD* Fdb 260 (5′-TCRTTIGCIATRTGRTGNCC-3′) were used. The conditions for PCR amplification were: 1 min at 94 °C and then 30 cycles of 40 s at 94 °C, 40 s at 55 °C and 1 min at 72 °C, followed by a final step for 2 min at 72 °C. The PCR products were purified as outlined above. *nifH* and *nifD* gene sequencing of all strains was performed as described previously by Stoltzfus *et al.* (1997), Zehr & McReynolds (1989), Reinhold-Hurek & Hurek (1998) and Kirchhof *et al.* (2001).

**DNA–DNA hybridization.** DNA was prepared according to the method of Meyer & Schleifer (1978). DNA–DNA hybridization analysis was carried out in microplate wells (Black Maxisorp; Nunc) using a fluorometric method (Ezaki *et al.*, 1989). The fluorescence intensity was detected by a fluorescence multiwell plate reader (Cytofluor Series 4000; PerSeptive Biosystems). DNA–DNA hybridization was carried out at 53 °C with photobiotin-labelled DNA and microplates (Ezaki *et al.*, 1989).

**Cellular fatty acid profiles.** Cellular fatty acids were extracted according to the protocol of the MIDI system. Bacterial strains were grown on TSBA medium for 48 h at 30 °C. Analysis by gas chromatography was controlled by MIS software (Microbial ID Inc.) and the peaks were automatically integrated and identified by the Microbial Identification software package (Sasser, 1990).

**Respiratory quinone analysis.** Isoprenoid quinones were extracted from freeze-dried cells with chloroform/methanol (2 : 1, v/v) and were purified by TLC by using n-hexane/diethyl ether (85 : 15, v/v) as the solvent. The ubiquinone fraction was extracted with acetone, dried under a nitrogen gas stream and then analysed by HPLC (model LC-10A apparatus; Shimadzu) with a Nacarai ODS 5C18 column (4·6 × 150 mm).

**Determination of the DNA G+C content.** Genomic DNA was prepared according to the method of Sambrook *et al.* (1989). Total DNA was digested with P1 nuclease using a Yamasa GC kit (Yamasa Shoyu). The G+C content of the total DNA was measured by HPLC according to the method described by Mesbah *et al.* (1989).

## RESULTS AND DISCUSSION

### Morphological and growth characteristics

The two isolates were Gram-negative, concave-shaped, non-motile and non-spore-forming organisms. The cell morphology is shown in Fig. 1. On B104 agar incubated at 30 °C for 3 days, young colonies were circular, smooth, convex and yellow–brown, with a diameter of 1–2 mm. The pH range for growth was 6–7 for both strains. The optimum growth temperature range was 25–30 °C for strain 7-1$^T$ and 25–37 °C for strain 7-2$^T$.

### Physiological and biochemical characteristics

The results of biochemical tests using the API system (API 20NE and API 50CH) are shown in Tables 1 and 2.

### Phylogenetic analysis

The almost complete 16S rRNA gene sequences of strains 7-1$^T$ and 7-2$^T$ were determined directly, following PCR

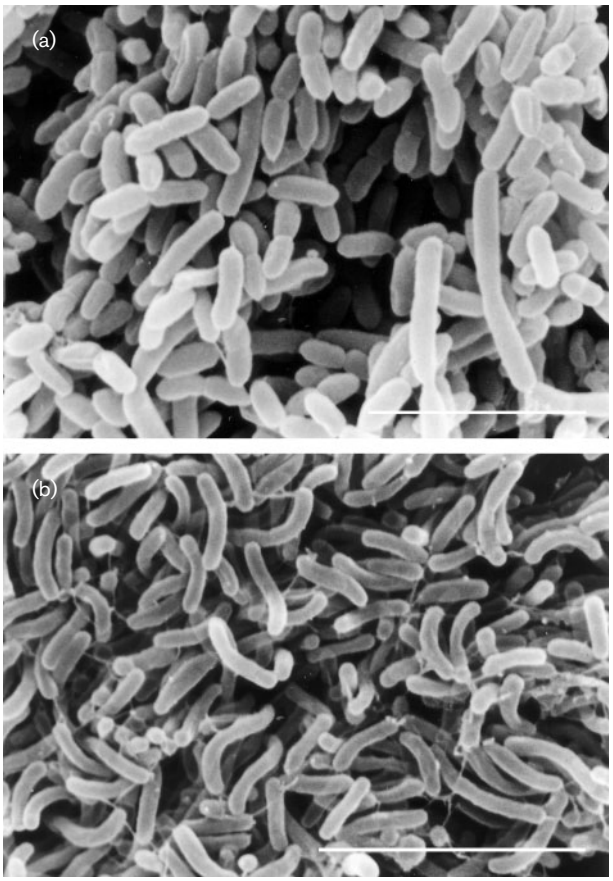


**Fig. 1.** Electron micrographs of cells of *Curvibacter gracilis* gen. nov., sp. nov. strain 7-1$^T$ (a) and *Herbaspirillum putei* sp. nov. strain 7-2$^T$ (b). Bars, 5 μm.

amplification. A phylogenetic tree, generated using the neighbour-joining algorithm, showed that strain 7-1$^T$ fell within a cluster comprising genera of the family *Comamonadaceae*. Strain 7-1$^T$ formed a coherent cluster with [*A.*] *delicatum* and [*P.*] *lanceolata* (Fig. 2) and showed 16S rRNA gene sequence similarity of 99·0 and 97·3 % to [*P.*] *lanceolata* and [*A.*] *delicatum*, respectively. Strain 7-2$^T$ fell within the radiation of the cluster comprising [*P.*] *huttiensis*, [*A.*] *autotrophicum* and species of the genus *Herbaspirillum* in the family *Oxalobacteriaceae*. [*A.*] *autotrophicum* showed 16S rRNA gene sequence similarity of 96·8–98·4 % to *Herbaspirillum* species. However, 16S rRNA gene sequence similarity with *Oxalobacter formigenes* and *Paucimonas lemoignei* was respectively 94·2 and 95·8 %. Strain 7-2$^T$ showed 16S rRNA gene sequence similarity of 99·0, 97·9, 98·1, 98·3, 96·6 and 96·5 % to [*P.*] *huttiensis*, *H. seropedicae*, *H. rubrisubalbicans*, *H. frisingense*, *Herbaspirillum lusitanum* and [*A.*] *autotrophicum*, respectively.

## DNA–DNA hybridization

Table 3 shows DNA–DNA binding values for strains 7-1$^T$ and 7-2$^T$. The values of DNA–DNA hybridization between strain 7-1$^T$ and [*P.*] *lanceolata* and [*A.*] *delicatum* were respectively 42–52 and 7–9 %. DNA–DNA hybridization of strain 7-2$^T$ with [*P.*] *huttiensis*, [*A.*] *autotrophicum*, *H. seropedicae*, *H. rubrisubalbicans* and *H. frisingense* revealed reassociation values of 40–45, 14, 6–17, 18–21 and 14–18 %, respectively.

## Nitrogen-fixing ability

Since strain 7-2$^T$, [*P.*] *huttiensis* and [*A.*] *autotrophicum* were found to be phylogenetically close to the species of

**Table 1.** Differential characteristics of strain 7-1$^T$ and related species

+, Positive; −, negative; (+), weakly positive. Biochemical data and G+C contents were from this study; other data for reference species were taken from Krieg (1984) (*A. delicatum*) and Leifson (1962) (*P. lanceolata*).

| Characteristic | 7-1$^T$ | [*A.*] *delicatum* | [*P.*] *lanceolata* |
|---|---|---|---|
| Cell size (μm) | 0·5 × 1·4 | 0·3 × 0·7 | 0·6 × 1·8 |
| Optimal temperature for growth (°C) | 25–30 | 30–32 | 20–30 |
| Optimal pH for growth | 5·0–8·0 | 5·5–8·5 | Neutrophilic |
| API 20 NE test: | | | |
| L-Arginine | + | − | − |
| Urea | + | − | + |
| Aesculin | − | + | − |
| Gelatin | − | + | − |
| *p*-Nitrophenyl β-D-galactopyranoside | − | (+) | − |
| Glucose | (+) | (+) | − |
| D-Mannose | − | − | (+) |
| Mannitol | − | (+) | − |
| Maltose | − | (+) | − |
| Gluconate | + | − | − |
| DNA G+C content (mol%) | 66 | 62 | 66 |

**Table 2.** Differential phenotypic characteristics of strain 7-2$^T$ and *Herbaspirillum* species

Strains: 1, strain 7-2$^T$; 2, *H. seropedicae* IAM 14977$^T$ (unless indicated, data were from Baldani *et al.*, 1986); 3, *H. frisingense* IAM 14974$^T$ (Baldani *et al.*, 1996); 4, *H. rubrisubalbicans* IAM 14976$^T$ (Kirchhof *et al.*, 2001); 5, [*P.*] *huttiensis* IAM 14941$^T$ (Leifson, 1962); 6, [*A.*] *autotrophicum* IAM 14942$^T$ (Aragno & Schlegel, 1978); 7, *H. lusitanum* LMG 21710$^T$ (Valverde *et al.*, 2003). Biochemical data and G+C contents for all strains except *H. lusitanum* were from this study. +, Positive; −, negative; (+), weakly positive; NA, information not available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Cell size (μm) | 0·4–0·5 × 1·4–1·8 | 0·6–0·7 × 1·5–5·0 | 0·5–0·7 × 1·4–1·8 | 0·6–0·7 × 1·5–5·0 | 0·4 × 1·8 | 0·6–0·8 × 2·0–5·0 | 0·5 × 1·6 |
| Optimal temperature for growth (°C) | 25–37 | 34 | 30–37 | 30 | 25–30 | 28 | 28 |
| Optimal pH for growth | 6·0–7·0 | 5·3–8·0 | 6·0–7·0 | 6·7–6·8 | Neutrophilic | 5·0–8·0 | NA |
| API 20NE (50CH) test: | | | | | | | |
| Nitrate reduction | − | + | + | + | − | − | − |
| Nitrite reduction | + | − | − | − | + | + | − |
| Growth on: | | | | | | | |
| Glucose | − | − | + | + | − | − | + |
| Arginine | + | (+) | + | + | + | − | − |
| Mannose | (+) | − | (+) | − | − | − | + |
| *N*-Acetyl-D-glucosamine | + | + | + | − | + | + | + |
| Erythritol | − | − | − | + | + | − | − |
| Rhamnose | − | − | + | − | − | − | + |
| Inositol | − | + | − | − | − | − | − |
| D-Arabinose | − | + | − | + | + | + | + |
| Galactose | − | − | + | + | − | − | − |
| DNA G+C content (mol%) | 62·9 | 64–65 | 61–65 | 62–63 | 63·3 | 60–62 | 57·0 |

the genus *Herbaspirillum*, which is known to be a nitrogen-fixing genus, we examined its nitrogen-fixing ability. Strains of *Herbaspirillum* species grew on $N_2$-free medium, but strain 7-2$^T$, [*P.*] *huttiensis* and [*A.*] *autotrophicum* did not (data not shown). However, it should be noted that the *nifH* gene was detected in strain 7-2$^T$; in contrast, the *nifH* and *nifD* genes were not detected in [*P.*] *huttiensis* or [*A.*] *autotrophicum*.

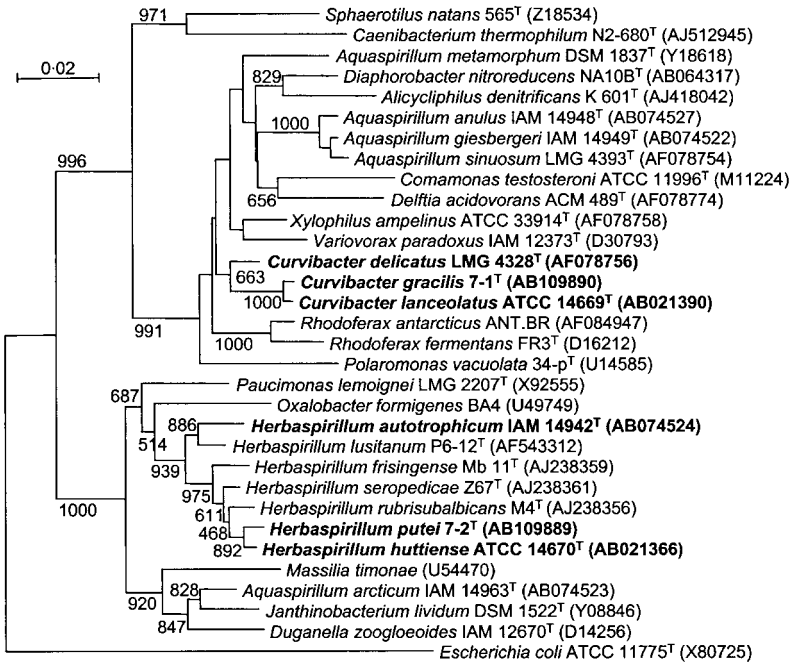


**Fig. 2.** Phylogenetic tree based on 16S rRNA gene sequences displaying the relationships among strains 7-1$^T$ and 7-2$^T$ and members of the families *Oxalobacteriaceae* and *Comamonadaceae*. The tree was constructed by the neighbour-joining method using 1296 positions. Bootstrap values from 1000 resamplings are shown at branch points. Bar, 2 nucleotide substitution per 100 nucleotides.

**Table 3.** DNA–DNA hybridization results

| Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1. *H. frisingense* IAM 14974$^T$ | 100 | 24 | 14 | 13 | 18 | 10 | – | – | – |
| 2. *H. seropedicae* IAM 14977$^T$ | 34 | 100 | 8 | 21 | 17 | 15 | – | – | – |
| 3. *H. rubrisubalbicans* IAM 14976$^T$ | 13 | 16 | 100 | 24 | 21 | 12 | – | – | – |
| 4. [*P.*] *huttiensis* IAM 14941$^T$ | 18 | 22 | 26 | 100 | 40 | 11 | – | – | – |
| 5. Strain 7-2$^T$ | 14 | 16 | 18 | 45 | 100 | 14 | – | – | – |
| 6. [*A.*] *autotrophicum* IAM 14942$^T$ | 21 | 19 | 11 | 12 | 14 | 100 | – | – | – |
| 7. [*A.*] *delicatum* IAM 14955$^T$ | – | – | – | – | – | – | 100 | 24 | 9 |
| 8. [*P.*] *lanceolata* IAM 14947$^T$ | – | – | – | – | – | – | 27 | 100 | 52 |
| 9. Strain 7-1$^T$ | – | – | – | – | – | – | 7 | 42 | 100 |

## Chemotaxonomic characteristics

The results of the analysis of G+C content, quinone type and fatty acid composition are shown in Tables 1, 2 and 4. The G+C content of the DNA of strain 7-1$^T$ was 66·2 mol% and the strain contained Q-8 as an isoprenoid quinone, 16 : 0, 16 : 1 and 18 : 1 as major cellular fatty acids and 3-OH 8 : 0 as a major cellular 3-hydroxy fatty acid. The G+C content of the DNA, isoprenoid quinone composition and the major fatty acids of [*A.*] *delicatum* and [*P.*] *lanceolata* were 62·2 and 66·0 mol%, respectively, Q-8, 16 : 0, 16 : 1 and 18 : 1 and 3-OH 8 : 0. The G+C content of the DNA of strain 7-2$^T$ was 62·9 mol%. Strain 7-2$^T$ contained Q-8 as the isoprenoid quinone. The major cellular fatty acids were 16 : 0, 16 : 1, 18 : 0 and 18 : 1 and 3-OH 10 : 0 and 3-OH 12 : 0 were the major cellular 3-hydroxy fatty acids. The G+C DNA content and quinone compositions of species of the genus *Herbaspirillum* and [*P.*] *huttiensis* were 57·9–65 and 63·3 mol%, respectively, and Q-8 (in both cases). The major fatty acids of the species of the genus *Herbaspirillum* and [*P.*] *lanceolata* were 16 : 0, 16 : 1 and 18 : 1 and the major 3-hydroxy fatty acids were 3-OH 10 : 0 and 3-OH 12 : 0. The extracted fatty acid composition for strain 7-2$^T$ and related species are shown in Table 4. Tables 1 and 2 show differential characteristics among strains 7-1$^T$, 7-2$^T$ and other closely related species.

**Table 4.** Fatty acid compositions of strain 7-2$^T$ and related species

Species/strains: 1, strain 7-2$^T$; 2, [*P.*] *huttiensis*; 3, *H. seropedicae*; 4, *H. rubrisubalbicans*; 5, *H. frisingense*; 6, [*A.*] *autotrophicum* (data from Sakane & Yokota, 1994). Unless indicated, data were from this study. +, Present.

| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| $C_{10:0}$ | 0·2 | | 0·2 | 0·2 | 0·3 | |
| $C_{12:0}$ | 3·5 | 4·0 | 1·1 | 3·1 | 1·6 | 3·0 |
| $C_{14:0}$ | 0·5 | | 4·6 | 1·1 | 3·2 | |
| $C_{16:0}$ | 24·6 | 18·0 | 24·3 | 24·2 | 29·4 | 33·0 |
| $C_{17:0}$ | 0·2 | | | | | 3·0 |
| $C_{17:1}$ | | | | | | 3·0 |
| $C_{18:0}$ | 1·7 | 4·0 | 1·1 | 1·5 | 1·7 | 1·0 |
| $C_{19:0}$ | | | | | | 6·0 |
| $C_{16:1}\omega7c/C_{15:0}$ iso 2-OH | 38·0 | 28·8 | 38·1 | 32·4 | 29·3 | |
| $C_{16:1}$ | | | | | | 33·0 |
| $C_{18:1}\omega7c$ | 22·5 | 30·0 | 17·1 | 24·4 | 21·9 | 19·0 |
| 11-Methyl $C_{18:1}\omega7c$ | 0·5 | | 0·2 | 0·1 | 0·5 | |
| $C_{12:0}$ 2-OH | 0·3 | | 1·4 | 0·9 | 1·4 | |
| $C_{14:0}$ 2-OH | 2·4 | 2·8 | 2·1 | 1·7 | 2·5 | |
| $C_{10:0}$ 3-OH | 1·6 | 1·8 | 1·8 | 1·5 | 1·2 | + |
| $C_{12:0}$ 3-OH | 3·3 | 3·9 | 3·8 | 3·9 | 3·5 | + |
| $C_{17:0}$ cyclo | 0·9 | 6·7 | 3·2 | 4·1 | 2·1 | |

In the present study, strain 7-1$^T$ was shown to represent a distinct species closely related to [*P.*] *lanceolata* and [*A.*] *delicatum*, two genetically misclassified species belonging to the family *Comamonadaceae* (Anzai *et al.*, 2000; Ding & Yokota, 2002). Table 5 shows the similarity among the three species and the differential characteristics from other closely related species. We propose to classify these three species in a new genus, *Curvibacter* gen. nov., as *Curvibacter gracilis* gen. nov., sp. nov., *Curvibacter delicatus* comb. nov. and *Curvibacter lanceolatus* comb. nov.

Similarly, strain 7-2$^T$ was shown to represent a novel species closely related to *Herbaspirillum* species, [*P.*] *huttiensis* and [*A.*] *autotrophicum*, two genetically misclassified species (Kersters *et al.*, 1996; Anzai *et al.*, 2000). In addition, the *nifH* gene was found in strain 7-2$^T$, although it did not show $N_2$-fixing activity. We propose to reclassify this novel species, [*P.*] *huttiensis* and [*A.*] *autotrophicum* in the genus *Herbaspirillum* as *Herbaspirillum putei* sp. nov., *Herbaspirillum huttiense* comb. nov. and *Herbaspirillum autotrophicum* comb. nov.

## Description of *Herbaspirillum putei* sp. nov.

*Herbaspirillum putei* (pu.te′i. L. gen. n. *putei* of a well, from which the type strain was isolated).

Gram-negative, curved rods or spirilla, 0·5–0·7 × 2·1–3·4 μm. The optimum growth temperature is 25–37 °C. The optimum pH is 6–7. Catalase- and oxidase- positive. Contains the *nifH* gene. The G+C content is 66·2 mol% and the quinone system is ubiquinone Q-8. The major

**Table 5.** Differential characteristics of the genus *Curvibacter* gen. nov. and related genera

Data for *Curvibacter* were taken from Krieg (1984) and this study. Data for *Polaromonas* and *Rhodoferax* were taken from Irgens *et al.* (1996) and Hiraishi *et al.* (1991), respectively. +, Positive; −, negative; ND, not detected.

| Characteristic | *Curvibacter* | *Polaromonas* | *Rhodoferax* |
|---|---|---|---|
| Cell shape | Slightly curved rods | Rods | Curved rods |
| Gas vesicles | ND | + | − |
| Flagella arrangement | Polar or none | Polar | Polar |
| $O_2$ requirement | Aerobe or microaerobic | Obligate aerobe | Facultative aerobe |
| Temperature relationship | Mesophilic | Psychrophilic | Mesophilic |
| Colony pigmentation | Yellow–brown | White | Peach–brown |
| Quinone* | Q-8 | ND | Q-8 + RQ-8 |
| DNA G+C content (mol%) | 62·2–66·0 | 52–57 | 60 |

*Q, Quinone; RQ, rhodoquinone.

cellular fatty acids are 16 : 0, 16 : 1, 18 : 0 and 18 : 1. The major cellular 3-hydroxy fatty acids are 3-OH 10 : 0 and 3-OH 12 : 0.

The type strain, strain 7-2$^T$ (=IAM 15032$^T$=ATCC BAA-806$^T$), was isolated from well water in Osaka, Japan.

## Description of *Herbaspirillum huttiense* comb. nov.

*Herbaspirillum huttiense* (hut.ti.en′se. N.L. neut. adj. *huttiense* pertaining to Lower Hutt, New Zealand). Note: Rule 61 of the Bacteriological Code prevents the correction of this epithet to '*huttense*'.

Basonym: *Pseudomonas huttiensis* Leifson 1962.

The description is identical to the description given for [*P.*] *huttiensis* by Leifson (1962). In addition, this bacterium is catalase- and oxidase-positive. The G+C content of the DNA is 63·3 mol% and its quinone system is ubiquinone Q-8. The major cellular fatty acids are 16 : 0, 16 : 1, 18 : 0 and 18 : 1. The major 3-hydroxy fatty acids are 3-OH 10 : 0 and 3-OH 12 : 0. The sample used for classification was isolated from distilled water. The type strain is IAM 14941$^T$ (=ATCC 14670$^T$).

## Description of *Herbaspirillum autotrophicum* comb. nov.

*Herbaspirillum autotrophicum* (au.to.tro′phi.cum. Gr. pron. *autos* self; Gr. adj. *trophikos* nursing, tending or feeding; N.L. neut. adj. *autotrophicum* self-nursing or self-feeding).

Basonym: *Aquaspirillum autotrophicum* Aragno and Schlegel 1978.

The description is identical to the description given for [*A.*] *autotrophicum* by Aragno & Schlegel (1978). In addition, the major cellular fatty acids are 16 : 0, 16 : 1 and 18 : 1. The major 3-hydroxy fatty acids are 3-OH 12 : 0 and 3-OH 14 : 0. The type strain is IAM 14942$^T$ (=DSM 732$^T$).

## Description of *Curvibacter* gen. nov.

*Curvibacter* (Cur.vi.bac′ter. L. adj. *curvus* curved or crooked; N.L. masc. n. *bacter* rod; N.L. masc. n. *Curvibacter* curved rod).

Gram-negative, vibrioid or slightly curved, rod-shaped cells, 0·3–0·9 × 1·1–1·8 μm, with an anticlockwise helix; may or may not have flagella. Catalase-, oxidase- and phosphatase-positive. The temperature range necessary for growth is 9–40 °C and the pH range for growth is 5·5–8·5. No growth occurs in the presence of 3 % NaCl. The G+C content of the DNA is 62·2–66·0 mol% and the quinone type is Q-8. The major cellular fatty acids are 16 : 0, 16 : 1 and 18 : 1 and the major 3-hydroxy fatty acid is 3-OH 8 : 0. The type species is *Curvibacter gracilis*.

## Description of *Curvibacter gracilis* sp. nov.

*Curvibacter gracilis* (gra′ci.lis. L. masc. adj. *gracilis* slender or thin).

Displays the following properties in addition to those given in the genus description. Slightly curved rod, 0·4–0·5 × 1·1–2·8 μm. The optimum growth temperature is 30 °C. The optimum pH for growth is 6–7. The G+C content of the DNA is 66·0 mol% and the quinone type is ubiquinone Q-8.

The type strain, strain 7-1$^T$ (=IAM 15033$^T$=ATCC BAA-807 $^T$), was isolated from well water in Osaka, Japan.

## Description of *Curvibacter lanceolatus* comb. nov.

*Curvibacter lanceolatus* (lan.ce.o.la′tus. L. masc. adj. *lanceolatus* lancet-shaped).

Basonym: *Pseudomonas lanceolata* Leifson 1962.

The description is identical to the description given for [*P.*] *lanceolata* by Leifson (1962). In addition, this bacterium is

catalase- and oxidase-positive. The G+C content of the DNA is 66·2 mol% and the quinone type is ubiquinone 8. The major cellular fatty acids are 16 : 0, 16 : 1 and 18 : 1. The major 3-hydroxy fatty acid is 3-OH 8 : 0. The type strain is IAM 14947$^T$ (=ATCC 14669$^T$).

### Description of *Curvibacter delicatus* comb. nov.

*Curvibacter delicatus* (de.li.ca′tus. L. masc. adj. *delicatus* delicate).

Basonym: *Aquaspirillum delicatum* (Leifson 1962) Hylemon *et al.* 1973.

The description is identical to the description given for [*A.*] *delicatum* by Hylemon *et al.* (1973). The type strain is IAM 14955$^T$ (=ATCC 14667$^T$).

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