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Extensimonas vulgaris gen. nov., sp. nov., a member of the family *Comamonadaceae*

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A novel strain, named S4^{T}, was obtained from industrial wastewater in Xiaoshan, Zhejiang Province, China. Cells were Gram-negative, neutrophilic and non-spore-forming and moved by means of a polar flagellum. Normal cells were 0.8–0.9×1.3–1.9 μm and the cells elongated to 10–25 μm when cultivated at high temperatures. Strain S4^{T} grew at 15–50 °C (optimum at 48 °C), pH 5.5–8.5 (optimum 7.0–7.5) and 0–2 % (optimum 0.5 %) (w/v) NaCl. Ubiquinone-8 was the predominant respiratory quinone. $C_{16:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and $C_{17:0}$ cyclo were the major cellular fatty acids. The major 3-OH fatty acid was $C_{10:0}$ 3-OH. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an unknown aminoglycolipid. The genomic DNA G+C content was 68.8 mol%. Based on 16S rRNA gene sequences alignment, the most closely related strains were members of the genera *Comamonas* (94.6–95.6 % similarities), *Giesbergeria* (94.9–95.6 %), *Acidovorax* (94.8–95.4 %), *Brachymonas* (94.1–95.2 %) and *Macromonas* (95.1 %). Phylogenetic analysis showed the closest relatives of strain S4^{T} were members of the genus *Macromonas*. Based on phenotypic and phylogenetic characteristics, we suggest that strain S4^{T} represents a novel species of a new genus of the family *Comamonadaceae*, for which the name *Extensimonas vulgaris* gen. nov., sp. nov. is proposed. The type strain of *Extensimonas vulgaris* is S4^{T} (=CGMCC 1.10977^{T}=JCM 17803^{T}).

The family *Comamonadaceae*, which belongs to the class *Betaproteobacteria*, was first described by Willems *et al.* (1991). This family contained numerous genera, including *Acidovorax* (Willems *et al.*, 1990), *Brachymonas* (Hiraishi *et al.*, 1995), *Comamonas* (Tamaoka *et al.*, 1987), *Giesbergeria* (Grabovich *et al.*, 2006) and *Macromonas* (Dubinina & Grabovich, 1984). Most isolates of this family were obtained from soil, freshwater, wastewater, activated sludge, pond water and so on (Dubinina & Grabovich, 1984; Grabovich *et al.*, 2006; Heylen *et al.*, 2008; Hiraishi *et al.*, 1995; Spring *et al.*, 2005; Yu *et al.*, 2011), which indicated that this evolutionary cluster had a wide spectrum of habitat and varied metabolic pathways. Some isolates were capable of degrading hydrocarbons (Rouvière & Chen, 2003), accumulating phosphorous (Blackall *et al.*, 2002; Lee *et al.*, 2003), oxidizing ammonia (Juretschko *et al.*, 2002; Purkhold *et al.*, 2000) and performing denitrification (Ginige *et al.*, 2004). Here we report the characterization of strain S4^{T}, which was isolated from industrial wastewater in China.

The wastewater sample was collected from Xiaoshan, Zhejiang Province, China, in December 2009. About 10 ml wastewater sample was filtered through a 50 μm pore size filter to remove the impurities and then diluted and spread onto CM plates at 28 °C. The CM medium contained (l^{-1} distilled water) 0.5 g NaCl, 0.5 g yeast extract, 0.5 g beef extract, 1 g peptone and 1.0 g glucose, the pH was adjusted to 7.0–7.2. After 3 days of incubation, a colourless colony was picked and purified by repeated restreaking. The isolate was routinely cultured on CM medium and maintained at −80 °C with 25 % (v/v) glycerol.

Colonies of strain S4^{T} on CM medium after 2 days incubation were 0.1–0.5 mm in diameter, non-pigmented, circular, elevated and transparent. Cell morphology and motility were determined by using an optical microscope (BX40; Olympus) and transmission electron microscopy

Abbreviations: AGL, aminoglycolipid; DPG, diphosphatidylglycerol; FAME, fatty acid methyl ester; GL, glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S4^{T} is HQ596491.

Five supplementary figures are available with the online version of this paper.

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(JEM-1230; JEOL) (Huo *et al.*, 2010). Gram staining was performed during all growth phases. Cells were Gram-negative, short rod-shaped, neutrophilic and non-spore-forming. The strain moved by means of a polar flagellum. Normal cells were $0.8–0.9 \times 1.3–1.9$ μm (Fig. S1 available in IJSEM Online) and the cells elongated when cultivated at higher temperature (Fig. S2).

To determine the growth conditions of strain $S4^T$, we used various NaCl concentrations (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 %, w/v) which were added to CM medium. The pH range for growth was determined at pH 4.0–10.0 (at intervals of 0.5 units) in CM medium with the following buffers: ammonium acetate (pH 4.0–5.0), MES (pH 5.5–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.0) at a concentration of 30 mM. The temperature range for growth was determined by incubation at 4, 10, 15, 20, 28, 32, 37, 40, 45, 48, 50 and 55 °C. As the results showed that strain $S4^T$ grew at the ranges of 0–2 % (w/v) NaCl, pH 5.5–8.5 and 15–50 °C with optimal growth at 48 °C, pH 7.0–7.5 and 0.5 % (w/v) NaCl.

The utilization of single carbon sources was tested on basal Macro medium containing (l^{-1} distilled water) 0.3 g NH_4Cl, 0.3 g $MgSO_4 . 7H_2O$, 0.2 g $CaCl_2$, 0.3 g $Na_2S_2O_3 . 5H_2O$ and 1 ml concentrated trace element solution which included (l^{-1} distilled water) 4.4 g $Na_2EDTA . 2H_2O$, 3.16 g $FeCl_3 . 6H_2O$, 0.012 g $CoSO_4 . 7H_2O$, 0.021 g $ZnSO_4 . 7H_2O$, 0.18 g $MnCl_2 . 4H_2O$, 0.007 g $CuSO_4 . 5H_2O$, 0.007 g $NaMoSO_4 . 2H_2O$, 0.024 g $NiCl_2 . 6H_2O$, 0.006 g H_3BO_3 and 10 ml HCl (7.7 M). Biochemical and nutritional tests were performed in the modified Macro medium to which 1 g yeast extract l^{-1}, 1 g sodium succinate l^{-1} and 1 g Casamino acids l^{-1} were added. Organic acids and complex proteinaceous substrates such as yeast extract, Casamino acids and peptone were autoclaved for 20 min at 121 °C. Sugars, alcohols and amino acids were sterilized by UV. The concentration of each added carbon source was 0.2 % (w/v). All tests were performed in triplicate. To analyse the use of accessory electron acceptors, sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM), sodium nitrate (20 mM) or $FeCl_3$ (20 mM) was added from autoclaved stock solutions to the medium (l^{-1} distilled water) 0.3 g NH_4Cl, 0.3 g $MgCl_2 . 6H_2O$, 0.2 g $CaCl_2$, 5 g yeast extract, 2 g sodium succinate and 1 g Casamino acids, 0.6 g L-cysteine, 0.001 g resazurin and 1 ml concentrated trace element solution. Oxygen was removed as described by Grishchenkov *et al.* (2000). The same medium lacking L-cysteine and resazurin was used under aerobic conditions for the blank control. Oxidase and catalase activity, nitrate and nitrite reduction were tested according to the protocol of Dong & Cai (2001). Indole, methyl red, Voges–Proskauer test, H_2S production and hydrolysis of starch, casein, gelatin and urea were tested as described by Shen & Chen (2008). Additional enzyme activities were determined using API ZYM kits (bioMérieux). Strain $S4^T$ was able to use a few substrates as sole carbon sources, such as alanine, glutamate, asparagine, succinate, citrate, malonate, fumarate and salicylate, and was not able to use sodium thiosulfate, sodium sulfite, sodium sulfate, sodium nitrite, sodium nitrate and $FeCl_3$ as accessory electron acceptors. No growth was detected under anaerobic conditions on modified Macro medium even after 8 days. Hydrolysis of urea, nitrate reduction, catalase and oxidase activity were positive. The presence of alkaline phosphatase, C4, C8, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphohydrolase and naphthol-AS-BI-phosphohydrolase were shown according to the API ZYM kits tests.

For fatty acid methyl esters (FAMEs) measurement, late-exponential-phase cells of strain $S4^T$and five type strains of related genera, *Comamonas zonglianii* DSM 22523^T, *Macromonas bipunctata* DSM 12705^T, *Giesbergeria voronezhensis* DSM 12825^T, *Acidovorax facilis* LMG 2193^T and *Brachymonas denitrificans* DSM 15123^T, were harvested from modified Macro medium. FAMEs were analysed by using GC–MS (Kuykendall *et al.*, 1988). Respiratory quinones were extracted and analysed by using reversed-phase HPLC as described previously (Komagata & Suzuki, 1987). Total lipids were extracted by using the modified method of Kamekura & Kates (1988). Polar lipids were separated by two-dimensional silica-gel (10×10 cm, Merck 5554) TLC and further analysed as described by Minnikin *et al.* (1984). Total lipids were revealed by spraying the plate with molybdatophosphoric acid (5 g molybdatophosphoric acid hydrate in 100 ml ethanol) before heating at 120 °C for 10 min. The DNA G+C content was determined as previously described (Mesbah & Whitman, 1989; Xu *et al.*, 2011). The predominant fatty acids of strain $S4^T$ were $C_{16:0}$ (35.5 %), summed feature 3 (22.9 %), $C_{17:0}$ cyclo (10.3 %), $C_{18:1}\omega7c$ (9.2 %) and $C_{10:0}$ 3-OH (3.9 %), and the complete fatty acid profiles are summarized in Table 1. Ubiquinone-8 was the predominant respiratory quinone. The polar lipids included phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), an unknown aminoglycolipid (AGL1), an unknown glycolipid (GL5) and two unknown phospholipids (PL1 and PL2) (Fig. S3). The genomic DNA G+C content of strain $S4^T$ was 68.8 mol%.

The 16S rRNA gene was amplified and analysed as described previously (Xu *et al.*, 2007). PCR products were cloned into vector pMD19-T (TaKaRa) and then sequenced. The 1454 nt sequence was compared with closely related sequences of reference organisms by the EzTaxon services (Chun *et al.*, 2007) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequences were aligned with CLUSTAL W1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), the maximum-parsimony (Fitch, 1971) and the maximum-likelihood (Felsenstein, 1981) methods with the MEGA 5 program package (Tamura *et al.*, 2011). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method.

Table 1. Cellular fatty acids of strain S4[T] and type strains of related genera

Taxa: 1, strain S4[T]; 2, *Macromonas bipunctata* DSM 12705 [T]; 3, *Comamonas zonglianii* DSM 22523[T]; 4, *Giesbergeria voronezhensis* DSM 12825[T]; 5, *Acidovorax facilis* LMG 2193[T]; 6, *Brachymonas denitrificans* DSM 15123[T]. Values are percentages of the total fatty acid content. cyclo, cyclopropane; TR, trace component (<1 %); –, not detected (all data were obtained in this study under the same experimental conditions). Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso-$C_{16:1}$ 2-OH. Summed feature 7 contained one or more of the following isomers: $C_{19:1}\omega6c$, $C_{19:1}$ cyclo and/or an unknown compound with the equivalent chain-length 18.846.

Fatty acid	1	2	3	4	5	6
Saturated fatty acids						
$C_{12:0}$	3.9	–	3.7	3.6	3.8	10.0
$C_{14:0}$	TR	3.6	1.2	4.4	3.8	TR
$C_{15:0}$	1.8	TR	1.8	–	TR	–
$C_{16:0}$	35.5	8.2	31.3	16.1	26.3	14.4
$C_{17:0}$	1.4	TR	TR	–	TR	TR
Unsaturated fatty acids						
$C_{17:1}\omega6c$	–	5.7	–	–	–	TR
$C_{18:1}\omega7c$	9.2	15.9	14.1	2.2	16.4	26.6
Hydroxy fatty acids						
$C_{16:0}$ 2-OH	1.7	–	TR	–	–	1.0
$C_{16:1}$ 2-OH	1.5	–	1.7	–	–	–
$C_{18:1}$ 2-OH	1.6	–	–	–	–	1.0
$C_{8:0}$ 3-OH	–	2.1	–	TR	TR	–
$C_{10:0}$ 3-OH	3.9	–	4.6	3.3	3.0	1.9
$C_{12:0}$ 3-OH	–	–	–	–	–	2.6
Cyclo propane acids						
$C_{17:0}$ cyclo	10.3	–	6.6	–	TR	–
$C_{19:0}$ cyclo $\omega8c$	1.2	–	–	–	–	–
Summed features						
3	22.9	61.0	33.9	69.4	45.5	38.9
7	2.3	–	–	–	–	–

Based on the result of sequence alignment, strain S4[T] was affiliated with the family *Comamonadaceae*, and the genera with validly published names that shared high sequence similarities to strain S4[T] were *Comamonas* (94.6–95.6 %), *Giesbergeria* (94.9–95.6 %), *Acidovorax* (94.8–95.4 %), *Brachymonas* (94.1–95.2 %) and *Macromonas* (95.1 %). Moreover, strain S4[T] shared higher sequence similarities to two uncultured bacterium clones (clone 26-H, AB274848, 98.1 % and clone FD_17, DQ984534, 97.1 %) from contaminated soil at oilfields and oil-contaminated soil respectively, and to strain CHX (AY275432; 98.1 %), which was isolated from the wastewater plant of a petroleum refinery by Rouvière & Chen (2003). All these three sequences mentioned above, together with that of strain S4[T], formed an independent cluster on the phylogenetic trees (Figs 1, S4 and S5), which clearly showed that strain S4[T] belonged to the family *Comamonadaceae* and represented a novel genus. Phylogenetic trees also indicated that the strain S4[T] clustered with the genus *Macromonas* which had only one available species *M. bipunctata* DSM 12705[T] (the type species of the genus, *Macromonas mobilis*, is not available in pure culture).

The predominant respiratory quinone, the DNA G+C content and the phylogenetic trees supported the hypothesis that strain S4[T] should be classified into the family *Comamonadaceae*. The cellular fatty acid profiles showed great differences between strain S4[T] and other type strains of five related genera. The amount of $C_{16:0}$ in strain S4[T] was larger than in all other type strains of five related genera. But the amount of summed feature 3 and $C_{18:1}\omega7c$ in strain S4[T] was lower than that for all other type strains except *G. voronezhensis* DSM 12825[T] which contained little $C_{18:1}\omega7c$ too. $C_{17:0}$ cyclo was detected in strain S4[T] (10.3 %) and *C. zonglianii* DSM 22523[T] (6.6 %) but was present only as a trace in *A. facilis* LMG 2193[T] and not detected in the other type strains. Furthermore, $C_{19:0}$ cyclo $\omega7c$ and summed feature 7 were only detected in strain S4[T]. $C_{14:0}$ was obviously detected in four type strains while only a trace was present in strain S4[T] and *B. denitrificans* DSM 15123[T]. $C_{17:0}$ was detected in strain S4[T] while it was present at trace levels in all other type strains except *G. voronezhensis* DSM 12825[T], in which it was not detected. Strain S4[T] contained three kinds of 2-OH fatty acids but other type strains had fewer kinds or even had no 2-OH fatty acids (Table 1). Great differences in the polar lipid profile existed between strain S4[T] and other type strains of related genera. *A. facilis* LMG 2193[T] contained only PG, DPG, PE and GL1 as its polar lipids but strain S4[T] contained more types of lipids like PL1, PL2 and AGL1. Compared with strain S4[T], *C. zonglianii* DSM 22523[T] lacked PL2 but possessed GL3 and GL4 and possessed lower amounts of DPG. PE and PG were the major polar lipids of *G. voronezhensis* DSM 12825[T], which possessed small amounts of AGL2, GL4 and PL2. *B. denitrificans* DSM 15123[T] contained more PE and PG than strain S4[T], lacked PL2 and contained GL3. *M. bipunctata* DSM 12705[T] possessed more types of lipids than strain S4[T] such as GL1, GL2 and an unknown lipid (L), contained less DPG than strain S4[T] and lacked GL5. The detailed differences are shown in Fig. S3.

There were also some differences in phenotypic characteristics between strain S4[T] and other strains of five related genera. Strain S4[T] had a polar flagellum, no pigment and could be cultivated in medium without growth factors. The optimum temperature was 48 °C and the cells of strain S4[T] elongated when cultivated at higher temperature. Nitrate could be reduced to nitrite. Polyphosphates were stored intracellularly as reserve material. Neither strain S4[T] nor strain CHX (Rouvière & Chen, 2003) were able to use sugars as single carbon sources (Table 2).

Based on the genotypic and phenotypic characteristics described above, we identified strain S4[T] as a novel species representing a new genus of *Comamonadaceae*, for which

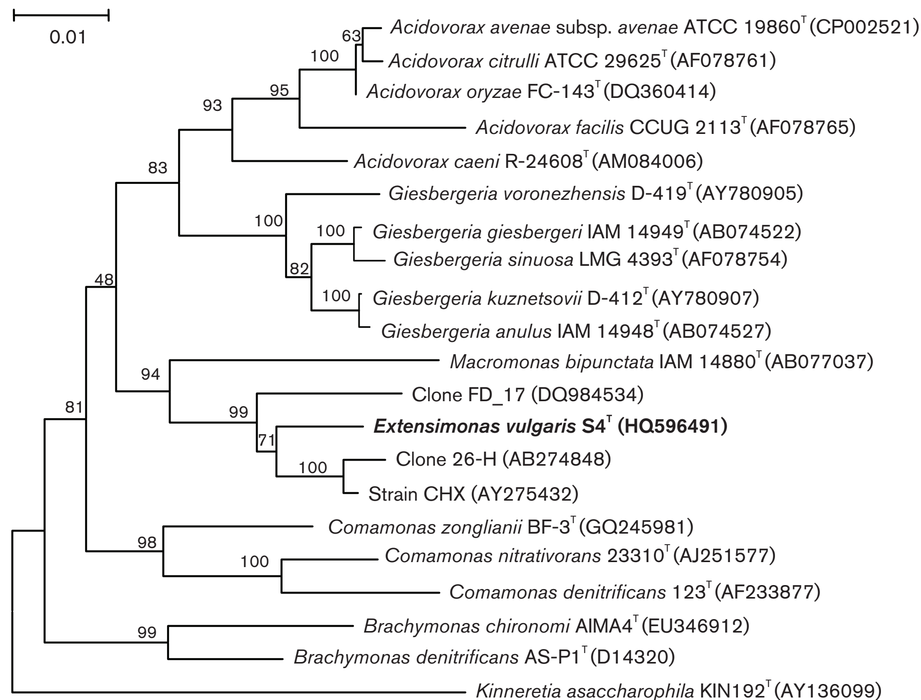

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences, showing the phylogenetic relationships of novel isolates and related taxa. Bootstrap values are based on 1000 replicates. Bar, 0.01 substitution per nucleotide positions.

the name *Extensimonas vulgaris* gen. nov., sp. nov. is proposed.

Description of *Extensimonas* gen. nov.

Extensimonas [Ex.ten.si.mo′nas. L. part. *extensus* extended; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Extensimonas* extended unit (bacterium)].

Gram-negative, short rod-shaped, neutrophilic and motile. Aerobic. The cells elongate when cultivated at higher temperatures. Growth factors are not required. Not able to use sugars as sole carbon sources. Catalase- and oxidase-positive. The predominant respiratory quinone is ubiquinone-8. The major cellular fatty acids (>10 % of total fatty acids) include $C_{16:0}$, summed feature 3 and $C_{17:0}$ cyclo. $C_{10:0}$ 3-OH is the major 3-OH fatty acid. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an unknown aminoglycolipid and two unknown phospholipids. Phylogenetically, the genus is a member of the family *Comamonadaceae* of the class *Betaproteobacteria*. The type species is *Extensimonas vulgaris.*

Description of *Extensimonas vulgaris* sp. nov.

Extensimonas vulgaris (vul.ga′ris. L. fem. adj. *vulgaris* common, referring to the lack of specific characteristics).

Cells are 0.8–0.9 × 1.3–1.9 µm. Motile by polar flagellum, non-spore-forming. Colonies on CM medium are 0.1–0.5 mm in diameter, circular, elevated and transparent after 2 days at 28 °C. Growth occurs at NaCl concentrations of 0–2 % (w/v) (optimum, 0.5 %), at 15–50 °C and pH 5.5–8.5 (optimum, 48 °C; pH 7.0–7.5). Able to use complex proteinaceous substrates and some organic acids but not sugars and alcohols as sole carbon sources. Substrates used include yeast extract, peptone, Casamino acids, alanine, glutamate, asparagine, succinate, citrate, malonate and salicylate. Hydrolysis of urea, nitrate reduction, catalase and oxidase activity are positive. Nitrite reduction, indole, methyl red, Voges–Proskauer test, H_2S production, hydrolysis of starch, casein and gelatin are negative. The activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphohydrolase and naphthol-AS-BI-phosphohydrolase are positive. Lipase (C14), β-galactosidase and α-glucosidase are weakly positive. Trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. The DNA G+C content is 68.8 mol% (HPLC).

The type strain is $S4^T$ (=CGMCC 1.10977^T=JCM 17803^T), isolated from industrial wastewater taken from Xiaoshan in China. The DNA G+C content of the type strain is 68.8 mol% (HPLC).

Table 2. Differential characteristics of strain S4^T and other related genera of the family *Comamonadaceae*

Strain: 1, S4^T; 2, *Macromonas* (Dubinina & Grabovich, 1984; Spring *et al.*, 2005); 3, *Comamonas* (Chang *et al.*, 2002; Chou *et al.*, 2007; Gumaelius *et al.*, 2001; Mechichi *et al.*, 2003; Tago & Yokota, 2004; Wauters *et al.*, 2003; Willems *et al.*, 1991; Young *et al.*, 2008; Yu *et al.*, 2011); 4, *Acidovorax* (Choi *et al.*, 2010; Gardan *et al.*, 2000; Gardan *et al.*, 2003; Heylen *et al.*, 2008; Li *et al.*, 2011; Mechichi *et al.*, 2003; Willems *et al.*, 1990, 1992); 5, *Brachymonas* (Halpern *et al.*, 2009; Hiraishi *et al.*, 1995; Mechichi *et al.*, 2003); 6, *Giesbergeria* (Grabovich *et al.*, 2006). +, Positive; −, negative; V, variable; ND, no data. PE: glycphosphatidylethanolamine; PG: phosphatidylglycerol; DPG: diphosphatidylglycerol; AGL, unknown aminoglycolipids; PL, unknown phospholipids.

Characteristic	**1**	**2***	**3**	**4**	**5**	**6**
Number of species (n)	1	2	14	14	2	5
Cell morphology	Short rods	Pear-shaped or cylindrical	Rods or spirilla	Straight to slightly curved rods	Coccobacilli or rods	Spirilla curved rods
Flagella	1, polar	Polar tuft	Polar or bipolar tufts or none	0–3, polar	None	Bipolar tufts
Anaerobic growth	Aerobic	Aerobic	Aerobic	Aerobic or anaerobic	Aerobic or anaerobic	Aerobic
Motility	+	+	V	V	−	+
Pigments	−	−	V	+	+	+
Growth factors	−	+	V	−	−	+
Urease	+	+	V	−	−	+
Nitrate reduction	+	−	V	+	+	−
Carbon source used for growth						
D-Fructose	−	−	−	V	−	−
D-Glucose	−	−	−	V	V	−
Formate	−	+	ND	V	−	+
Acetate	−	+	V	V	+	+
Fumarate	+	+	ND	ND	+	+
DL-Lactate	−	+	+	+	+	+
Glycerol	−	−	V	V	−	V
Alanine	+	−	V	V	+	V
Malonate	+	−	−	V	−	ND
Major quinone system	Q-8	Q-8	Q-8	Q-8	Q-8,RQ-8	Q-8
Major fatty acids	$C_{16:0}$, Summed feature 3, $C_{17:0}$ cyclo, $C_{18:1}\omega7c$	Summed feature 3, $C_{18:1}\omega7c$, $C_{16:0}$	Summed feature 3, $C_{16:0}$, $C_{18:1}$/$C_{18:1}$ $\omega7c$	Summed feature 3, $C_{16:0}$, $C_{18:1}$ $\omega7c$	$C_{16:1}\omega7c$, $C_{16:0}$, $C_{18:1}\omega7c$	$C_{16:0}$, $C_{16:1}$
Major 3-OH fatty acids	$C_{10:0}$ 3-OH	$C_{8:0}$ 3-OH	$C_{10:0}$ 3-OH	$C_{10:0}$ 3-OH $C_{8:0}$ 3-OH	$C_{10:0}$ 3-OH $C_{12:0}$ 3-OH	$C_{10:0}$ 3-OH
Major polar lipids	PE, PG, DPG, AGL, PL1, PL2	PE, PG, AGL	PE, PG, DPG, AGL	PE, PG	PG, PE	PE, PG
DNA G+C content (mol%)	68.8	68	59.7–68.7	62–66	60–65	56.5–60

*Data for this genus are based on the type strain of the only available species, *Macromonas bipunctata*. The type species of the genus, *Macromonas mobilis*, is not available in pure culture.

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References

Blackall, L. L., Crocetti, G. R., Saunders, A. M. & Bond, P. L. (2002). A review and update of the microbiology of enhanced biological phosphorus removal in wastewater treatment plants. *Antonie van Leeuwenhoek* **81**, 681–691.

Chang, Y. H., Han, J. I., Chun, J., Lee, K. C., Rhee, M. S., Kim, Y. B. & Bae, K. S. (2002). *Comamonas koreensis* sp. nov., a non-motile species from wetland in Woopo, Korea. *Int J Syst Evol Microbiol* **52**, 377–381.

Choi, J.-H., Kim, M.-S., Roh, S. W. & Bae, J.-W. (2010). *Acidovorax soli* sp. nov., isolated from landfill soil. *Int J Syst Evol Microbiol* **60**, 2715–2718.

Chou, J.-H., Sheu, S.-Y., Lin, K.-Y., Chen, W.-M., Arun, A. B. & Young, C.-C. (2007). *Comamonas odontotermitis* sp. nov., isolated from the gut of the termite *Odontotermes formosanus*. *Int J Syst Evol Microbiol* **57**, 887–891.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Dong, X.-Z. & Cai, M.-Y. (editors) (2001). Determination of biochemical properties. In *Manual for the Systematic Identification of General Bacteria*, pp. 370–398. Beijing: Science Press (in Chinese).

Dubinina, G. A. & Grabovich, M. Y. (1984). Isolation, cultivation, and characteristics of *Macromonas bipunctata*. *Microbiology* (*English translation of Microbiologiia*) **53**, 610–617.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Gardan, L., Dauga, C., Prior, P., Gillis, M. & Saddler, G. S. (2000). *Acidovorax anthurii* sp. nov., a new phytopathogenic bacterium which causes bacterial leaf-spot of anthurium. *Int J Syst Evol Microbiol* **50**, 235–246.

Gardan, L., Stead, D. E., Dauga, C. & Gillis, M. (2003). *Acidovorax valerianellae* sp. nov., a novel pathogen of lamb's lettuce [*Valerianella locusta* (L.) Laterr]. *Int J Syst Evol Microbiol* **53**, 795–800.

Ginige, M. P., Hugenholtz, P., Daims, H., Wagner, M., Keller, J. & Blackall, L. L. (2004). Use of stable-isotope probing, full-cycle rRNA analysis, and fluorescence in situ hybridization-microautoradiography to study a methanol-fed denitrifying microbial community. *Appl Environ Microbiol* **70**, 588–596.

Grabovich, M., Gavrish, E., Kuever, J., Lysenko, A. M., Podkopaeva, D. & Dubinina, G. (2006). Proposal of *Giesbergeria voronezhensis* gen. nov., sp. nov. and *G. kuznetsovii* sp. nov. and reclassification of [*Aquaspirillum*] *anulus*, [*A.*] *sinuosum* and [*A.*] *giesbergeri* as *Giesbergeria anulus* comb. nov., *G. sinuosa* comb. nov. and *G. giesbergeri* comb. nov., and [*Aquaspirillum*] *metamorphum* and [*A.*] *psychrophilum* as *Simplicispira metamorpha* gen. nov., comb. nov. and *S. psychrophila* comb. nov. *Int J Syst Evol Microbiol* **56**, 569–576.

Grishchenkov, V. G., Townsend, R. T., McDonald, T. J., Autenrieth, R. L., Bonner, J. S. & Boronin, A. M. (2000). Degradation of petroleum hydrocarbons by facultative anaerobic bacteria under aerobic and anaerobic conditions. *Process Biochem* **35**, 889–896.

Gumaelius, L., Magnusson, G., Pettersson, B. & Dalhammar, G. (2001). *Comamonas denitrificans* sp. nov., an efficient denitrifying bacterium isolated from activated sludge. *Int J Syst Evol Microbiol* **51**, 999–1006.

Halpern, M., Shakèd, T. & Schumann, P. (2009). *Brachymonas chironomi* sp. nov., isolated from a chironomid egg mass, and emended description of the genus *Brachymonas*. *Int J Syst Evol Microbiol* **59**, 3025–3029.

Heylen, K., Lebbe, L. & De Vos, P. (2008). *Acidovorax caeni* sp. nov., a denitrifying species with genetically diverse isolates from activated sludge. *Int J Syst Evol Microbiol* **58**, 73–77.

Hiraishi, A., Shin, Y. K. & Sugiyama, J. (1995). *Brachymonas denitrificans* gen, nov, sp, nov, an aerobic chemoorganotrophic bacterium which contains rhodoquinones, and evolutionary relationships of rhodoquinone producers to bacterial species with various quinone classes. *J Gen Appl Microbiol* **41**, 99–117.

Huo, Y. Y., Xu, X. W., Cui, H. L. & Wu, M. (2010). *Gracilibacillus ureilyticus* sp. nov., a halotolerant bacterium from a saline–alkaline soil. *Int J Syst Evol Microbiol* **60**, 1383–1386.

Juretschko, S., Loy, A., Lehner, A. & Wagner, M. (2002). The microbial community composition of a nitrifying–denitrifying activated sludge from an industrial sewage treatment plant analyzed by the full-cycle rRNA approach. *Syst Appl Microbiol* **25**, 84–99.

Kamekura, M. & Kates, M. (1988). Lipids of halophilic archaebacteria. In *Halophilic Bacteria II*, pp. 25–54. Edited by F. Rodriguez Valera. Boca Raton, FL: CRC Press.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Lee, N., Nielsen, P. H., Aspegren, H., Henze, M., Schleifer, K. H. & la Cour Jansen, J. (2003). Long-term population dynamics and *in situ* physiology in activated sludge systems with enhanced biological phosphorus removal operated with and without nitrogen removal. *Syst Appl Microbiol* **26**, 211–227.

Li, D., Rothballer, M., Schmid, M., Esperschütz, J. & Hartmann, A. (2011). *Acidovorax radicis* sp. nov., a wheat-root-colonizing bacterium. *Int J Syst Evol Microbiol* **61**, 2589–2594.

Mechichi, T., Stackebrandt, E. & Fuchs, G. (2003). *Alicycliphilus denitrificans* gen. nov., sp. nov., a cyclohexanol-degrading, nitrate-reducing β-proteobacterium. *Int J Syst Evol Microbiol* **53**, 147–152.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr A* **479**, 297–306.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Purkhold, U., Pommerening-Röser, A., Juretschko, S., Schmid, M. C., Koops, H. P. & Wagner, M. (2000). Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys. *Appl Environ Microbiol* **66**, 5368–5382.

Rouvière, P. E. & Chen, M. W. (2003). Isolation of *Brachymonas petroleovorans* CHX, a novel cyclohexane-degrading β-proteobacterium. *FEMS Microbiol Lett* **227**, 101–106.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Shen, P. & Chen, X.-D. (2008). [*Experiment of Microbiology*]. Beijing: Higher Education Press (in Chinese).

Spring, S., Wagner, M., Schumann, P. & Kämpfer, P. (2005). *Malikia granosa* gen. nov., sp. nov., a novel polyhydroxyalkanoate- and polyphosphate-accumulating bacterium isolated from activated sludge, and reclassification of *Pseudomonas spinosa* as *Malikia spinosa* comb. nov. *Int J Syst Evol Microbiol* **55**, 621–629.

Tago, Y. & Yokota, A. (2004). *Comamonas badia* sp. nov., a floc-forming bacterium isolated from activated sludge. *J Gen Appl Microbiol* **50**, 243–248.

Tamaoka, J., Ha, D. & Komagata, K. (1987). Reclassification of *Pseudomonas acidovorans* den Dooren de Jong 1926 and *Pseudomonas testosteroni* Marcus and Talalay 1956 as *Comamonas acidovorans* comb. nov. and *Comamonas testosteroni* comb. nov., with an emended description of the genus *Comamonas*. *Int J Syst Bacteriol* **37**, 52–59.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Wauters, G., De Baere, T., Willems, A., Falsen, E. & Vaneechoutte, M. (2003). Description of *Comamonas aquatica* comb. nov. and *Comamonas kerstersii* sp. nov. for two subgroups of *Comamonas terrigena* and emended description of *Comamonas terrigena*. *Int J Syst Evol Microbiol* **53**, 859–862.

Willems, A., Falsen, E., Pot, B., Jantzen, E., Hoste, B., Vandamme, P., Gillis, M., Kersters, K. & De Ley, J. (1990). *Acidovorax*, a new genus for *Pseudomonas facilis, Pseudomonas delafieldii*, E. Falsen (EF) group 13, EF group 16, and several clinical isolates, with the species *Acidovorax facilis* comb. nov., *Acidovorax delafieldii* comb. nov., and *Acidovorax temperans* sp. nov. *Int J Syst Bacteriol* **40**, 384–398.

Willems, A., Pot, B., Falsen, E., Vandamme, P., Gillis, M., Kersters, K. & De Ley, J. (1991). Polyphasic taxonomic study of the emended genus *Comamonas*: relationship to *Aquaspirillum aquaticum*, E. Falsen group 10, and other clinical isolates. *Int J Syst Bacteriol* **41**, 427–444.

Willems, A., Goor, M., Thielemans, S., Gillis, M., Kersters, K. & De Ley, J. (1992). Transfer of several phytopathogenic *Pseudomonas* species to *Acidovorax as Acidovorax avenae* subsp. *avenae* subsp. nov., comb. nov., *Acidovorax avenae* subsp. *citrulli, Acidovorax avenae* subsp. *cattleyae,* and *Acidovorax konjaci*. *Int J Syst Bacteriol* **42**, 107–119.

Xu, X. W., Wu, Y. H., Zhou, Z., Wang, C. S., Zhou, Y. G., Zhang, H. B., Wang, Y. & Wu, M. (2007). *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov., and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57**, 1619–1624.

Xu, X. W., Huo, Y. Y., Wang, C. S., Oren, A., Cui, H. L., Vedler, E. & Wu, M. (2011). *Pelagibacterium halotolerans* gen. nov., sp. nov. and *Pelagibacterium luteolum* sp. nov., novel members of the family *Hyphomicrobiaceae*. *Int J Syst Evol Microbiol* **61**, 1817–1822.

Young, C.-C., Chou, J.-H., Arun, A. B., Yen, W.-S., Sheu, S.-Y., Shen, F.-T., Lai, W.-A., Rekha, P. D. & Chen, W.-M. (2008). *Comamonas composti* sp. nov., isolated from food waste compost. *Int J Syst Evol Microbiol* **58**, 251–256.

Yu, X. Y., Li, Y. F., Zheng, J. W., Li, Y., Li, L., He, J. & Li, S. P. (2011). *Comamonas zonglianii* sp. nov., isolated from phenol-contaminated soil. *Int J Syst Evol Microbiol* **61**, 255–258.