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

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A novel strain, named S4T, 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 S4T 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. C16 : 0, summed feature 3 (C16 : 1 6 and/or iso-C15 : 0 2-OH) and C17 : 0 cyclo were the major cellular fatty acids. The major 3-OH fatty acid was C10 : 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 S4T were members of the genus Macromonas. Based on phenotypic and phylogenetic characteristics, we suggest that strain S4T 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 S4T (=CGMCC 1.10977T=JCM 17803T).

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 S4T, 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 S4T 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 S4T is HQ596491.

Five supplementary figures are available with the online version of this paper.
H2S production and hydrolysis of starch, casein, gelatin and nitrite reduction were tested according to the protocol of for the blank control. Oxidase and catalase activity, nitrate reduction, 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 S4T and five type strains of related genera, Comamonas zongliandii DSM 22523T, Macromonas bipunctata DSM 12705T, Giesiergeria voronezhensis DSM 12825T, Acidovorax facilis LMG 2193T and Brachynonas dentrifricas DSM 15123T, 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 S4T were C16:0 (35.5 %), summed feature 3 (22.9 %), C17:0 cyclo (10.3 %), C18:1ω9c (9.2 %) and C10:0 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 S4T 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.
Moreover, strain S4T shared higher sequence similarities (94.1–95.2%) and three sequences mentioned above, together with that of related genera (94.9–95.6%), *Giesbergeria* the following isomers: C19:1 compound with the equivalent chain-length 18.846.

Based on the result of sequence alignment, strain S4T was clustered with the genus *Macromonas* which had only one available species *M. bipunctata* DSM 12705T (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 S4T should be classified into the family *Comamonadaceae*. The cellular fatty acid profiles showed great differences between strain S4T and other type strains of five related genera. The amount of C16:0 in strain S4T was larger than in all other type strains of five related genera. But the amount of summed feature 3 and C18:1ω7c in strain S4T was lower than that for all other type strains except *G. voronezhensis* DSM 12825T which contained little C18:1ω7c too. C17:0 cyclo was detected in strain S4T (10.3%) and *C. zonglianii* DSM 22523T (6.6%) but was present only as a trace in *A. facilis* DSM 2193T and not detected in the other type strains. Furthermore, C19:0 cyclo ω7c and summed feature 7 were only detected in strain S4T. C14:0 was obviously detected in four type strains while only a trace was present in strain S4T and *B. denitrificans* DSM 15123T. C17:0 cyclo was detected in strain S4T while it was present at trace levels in all other type strains except *G. voronezhensis* DSM 12825T, in which it was not detected. Strain S4T 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 S4T and other type strains of related genera. *A. facilis* DSM 2193T contained only PG, DPG, PE and GL1 as its polar lipids but strain S4T contained more types of lipids like PL1, PL2 and AGL1. Compared with strain S4T, *C. zonglianii* DSM 22523T 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 12825T, which possessed small amounts of AGL2, GL4 and PL2. *B. denitrificans* DSM 15123T contained more PE and PG than strain S4T, lacked PL2 and contained GL3. *M. bipunctata* DSM 12705T possessed more types of lipids than strain S4T such as GL1, GL2 and an unknown lipid (L), contained less DPG than strain S4T and lacked GL5. The detailed differences are shown in Fig. S3.

There were also some differences in phenotypic characteristics between strain S4T and other strains of five related genera. Strain S4T 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 S4T elongated when cultivated at higher temperature. Nitrate could be reduced to nitrite. Polyphosphates were stored intracellularly as reserve material. Neither strain S4T 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 S4T as a novel species representing a new genus of *Comamonadaceae*, for which

<table>
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Extensimonas vulgaris gen. nov., sp. nov.

Description of Extensimonas vulgaris gen. nov.

Extensimonas [Ex.ten.si.mo’nas. L. part. extensis 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\textsubscript{16:0}, summed feature 3 and C\textsubscript{17:0}cyclo. C\textsubscript{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.gar’is. 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\textsubscript{2}S production, hydrolysis of starch, casein and gelatin are negative. The activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine 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-glucoronidase, 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\textsuperscript{T} (=CGMCC 1.10977\textsuperscript{T} = JCM 17803\textsuperscript{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 S4T and other related genera of the family Comamonadaceae

Strain: 1, S4T; 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: glycerophosphatidylethanolamine; PG: phosphatidylglycerol; DPG: diphosphatidylglycerol; AGL, unknown aminoglycolipids; PL, unknown phospholipids.

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<td>V</td>
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<td>C16:1ω7c, C18:0</td>
<td>C16:1ω7c, C18:1ω7c</td>
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<td>60–65</td>
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*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.


References


