Undibacterium pigrum gen. nov., sp. nov., isolated from drinking water

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Two Gram-negative, rod-shaped, oxidase-positive, non-spore-forming, non-motile bacteria (strains CCUG 49009^T and CCUG 49012), both isolated from drinking water, were characterized. On the basis of chemotaxonomic data [major ubiquinone, Q-8; predominant polyamines, putrescine and 2-hydroxyputrescine; major polar lipids, phosphatidylethanolamine, moderate amounts of diphosphatidylglycerol and phosphatidylglycerol and minor amounts of three aminolipids and phosphatidylserine; major fatty acids, C_{16:0} and summed feature 3 (C_{16:1}ω7c/C_{15:0} iso 2-OH)] and 16S rRNA gene sequence similarities, both strains clearly belong to the family *Oxalobacteraceae* of the *Betaproteobacteria*. 16S rRNA gene sequence similarities with members of the most closely related genera of this group (*Hermiimonas*, *Massilia*, *Duganella*, *Telluria*, *Herbaspirillum*, *Janthinobacterium*, *Naxibacter* and *Paucimonas*) were less than 96.5% for both strains. The two strains also shared a relatively low 16S rRNA gene sequence similarity (96.8%). Although phylogenetic analysis based on 16S rRNA gene sequence similarities clearly showed that the two organisms formed a separate branch, their phenotypes (including chemotaxonomic features) were hardly distinguishable and showed high similarities to those reported for the most closely related genera. On the basis of DNA–DNA hybridization results, the two strains were shown to represent separate species (sharing only 20% DNA–DNA relatedness), but they could not be clearly differentiated phenotypically from each other. It is evident that these organisms represent a new genus, *Undibacterium* gen. nov., with one species, *Undibacterium pigrum* sp. nov. The type strain of *Undibacterium pigrum* is strain CCUG 49009^T (= CIP 109318^T). Strain CCUG 49012 (= CIP 108976) probably represents a second species of this genus, but is described here as a second genomovar of this species because of the lack of differentiating characters.

In recent years, several new taxa have been added to the family *Oxalobacteraceae* (Garrity *et al.*, 2005). In addition to the genera *Oxalobacter*, *Collimonas*, *Massilia*, *Duganella*, *Janthinobacterium*, *Herbaspirillum*, *Oxalicibacterium* and *Telluria*, the genera *Naxibacter* (Xu *et al.*, 2005) and *Hermiimonas* (Fernandes *et al.*, 2005) have recently been

described. The majority of these organisms form yellowish-pigmented colonies on nutrient-rich media such as nutrient agar. However, they show very few phenotypic differences, and their allocation to separate genera is based largely on 16S rRNA gene sequence analyses.

Strains CCUG 49009^T and CCUG 49012 were isolated from drinking water on R2A agar (Oxoid) at 22 °C. Both strains showed beige-coloured colonies on R2A agar. Subcultivation was done on R2A agar at 25 °C for 7 days. On this agar, both organisms were able to grow at 4–30 °C, but not at temperatures above 36 °C. Strain CCUG 49009^T was able to

Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains CCUG 49009^T and CCUG 49012 are AM397630 and AM397629.

grow on tryptone soy agar (TSA), nutrient agar and MacConkey agar (all from Oxoid), whereas strain CCUG 49012 was not able to grow on TSA and grew only slowly on nutrient agar.

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at $\times 1000$, with cells grown for 7 days at 25 °C on R2A agar. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple sequence alignment employing CLUSTAL X (Thompson *et al.*, 1997). The sequenced lengths of the 16S rRNA gene were 1434 and 1442 bp, respectively, for strains CCUG 49009^T and CCUG 49012. Nucleotide sequence similarities were below 96.5 % with all established species of the genera *Herminiimonas*, *Massilia*, *Duganella*, *Telluria*, *Herbaspirillum*, *Janthinobacterium*, *Naxibacter* and *Paucimonas*. The two strains shared a 16S rRNA gene similarity of 96.8 %. The phylogenetic tree shown in Fig. 1 results from a neighbour-joining reconstruction. A maximum-parsimony tree revealed the same branching (not shown). In all calculations, the two sequences were placed on a separate phylogenetic branch, indicating their independent affiliation.

For quinone and polar lipid analysis, cells of CCUG 49009^T and CCUG 49012 were grown on PYE medium (Hauser *et al.*, 2005). The content of respiratory quinones was determined as described previously (Tindall, 1990; Altenburger *et al.*, 1996) but using an HPLC consisting of

a JASCO PU 2080 Plus Pump and JASCO UV-2075 Plus UV/Vis detector. In both strains, ubiquinone Q-8 was detected exclusively, which is a characteristic trait of all members of the *Betaproteobacteria* (Yokota *et al.*, 1992). For polyamine analyses, cells of CCUG 49009^T were grown under standardized conditions as described by Busse & Auling (1988). Because of its very poor growth on PYE medium, strain CCUG 49012, which grew well on R2A, was cultivated in a medium of new composition which consisted of the carbon sources of PYE medium and the salts of R2A, here designated R2-PYE (1^{-1} : 0.75 g peptone from casein, 0.75 g yeast extract, 0.3 g K_2HPO_4 , 0.024 g $MgSO_4$, pH 7.2). In this medium, like in PYE medium, CCUG 49012 showed flocculent growth. This growth characteristic made it impossible to measure the optical density, and it was therefore not possible to determine 70 % of the maximum optical density, as recommended for harvesting of cells to be subjected to polyamine analysis (Busse & Auling, 1988). In order to obtain biomass from the exponential growth phase, two Erlenmeyer flasks containing identical volumes of R2-PYE were inoculated separately with 0.5 and 3.0 ml of a 2 day culture and growth was followed visually. After 2 days of cultivation at room temperature on a rotary shaker, when cell density was still visibly lower in the low-inoculum flask than in the high-inoculum flask, cells from the former flask were harvested and used for polyamine analysis. Polyamines were analysed as described by Busse & Auling (1988) and Busse *et al.* (1997) except that a JASCO PU 2080 Plus pump was employed. Strains CCUG 49009^T and CCUG 49012 exhibited polyamine patterns that were in accordance with their affiliation to the *Betaproteobacteria* (Busse & Auling,

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers are given in parentheses) constructed after multiple alignments of data by CLUSTAL X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.005 nucleotide substitutions per nucleotide position.

1988; Auling *et al.*, 1991; Hamana & Takeuchi, 1998), consisting of the predominant compounds putrescine [respectively 79.8 and 80.1 $\mu\text{mol (g dry weight)}^{-1}$ in strains CCUG 49009^T and CCUG 49012] and 2-hydroxyputrescine [39.8 and 44.1 $\mu\text{mol (g dry weight)}^{-1}$], moderate to minor amounts of spermidine [8.8 and 0.7 $\mu\text{mol (g dry weight)}^{-1}$], minor to trace amounts of spermine [1.4 and <0.1 $\mu\text{mol (g dry weight)}^{-1}$] and traces of 1,3-diaminopropane in CCUG 49012 [<0.1 $\mu\text{mol (g dry weight)}^{-1}$].

The polar lipid patterns of strains CCUG 49009^T and CCUG 49012 were almost identical. Both strains contained the predominant lipid phosphatidylethanolamine (PE), moderate amounts of diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) and minor amounts of three aminolipids and phosphatidylserine (Fig. 2). In a second analysis from independently grown biomass, an additional phospholipid was detected in the extract of CCUG 49012 that showed chromatographic behaviour similar to PE in the first dimension, but the migration distance was almost twice that of PE in the second dimension. Since this finding could not be reproduced, its presence might be related to not highly standardized growth conditions and hence it appears not to be useful for differentiation between the two strains. The lipids PG, PE and DPG have been already reported for other betaproteobacteria, such as species of the *Alcaligenaceae* and *Janthinobacterium* and *Polaromonas aquatica* and *Herminiimonas aquatilis* (Lincoln *et al.*, 1999; Stolz *et al.*, 2005; Kämpfer *et al.*, 2006a, b, c) and *Massilia timonae* CCUG 45783^T, but within a much more complex profile (H.-J. Busse, unpublished results). Thus, polar lipid profiles of newly examined strains consisting of the components

Fig. 2. Two-dimensional thin-layer chromatogram of polar lipids of strain CCUG 49009^T. DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; AL1–3, unknown aminolipids; L, unknown polar lipid.

detected in CCUG 49009^T and CCUG 49012 may indicate a relationship at the genus level, whereas more complex profiles may be indicative of a more distant relationship.

The cellular fatty acid profiles of strains CCUG 49009^T and CCUG 49012 are given in detail in Table 1. Because the strains were not able to grow on TSA, they were grown on R2A agar for 48 h prior to fatty acid analysis. Methods for analysis have been described previously (Kämpfer & Kroppenstedt, 1996). The major fatty acids were C_{16:0}, summed feature 3 (C_{16:1}ω7c/C_{15:0} iso 2-OH) and C_{18:1}ω7c. In addition, C_{10:0} 3-OH was the only hydroxylated fatty acid detected. These fatty acids were detected in the type strains of all species studied comparatively under the same conditions (Table 1). The fatty acid C_{17:0} cyclo was not detected in strains CCUG 49009^T and CCUG 49012, in contrast to *Herminiimonas fonticola* S-94^T, *Telluria mixta* CCUG 35206^T, *Janthinobacterium agaricidamnorum* CCUG 43140^T and *Janthinobacterium lividum* CCUG 2344^T, in which this fatty acid accounted for >15%. This fatty acid profile was also reported for *Herminiimonas arsenicoxydans* (Muller *et al.*, 2006) and for other *Herminiimonas fonticola* strains (Fernandes *et al.*, 2005), but not for *Herminiimonas aquatilis* (Kämpfer *et al.*, 2006b), when grown on TSA (Oxoid) prior to fatty acid extraction. In addition, C_{17:0} cyclo has been reported for *Naxibacter alkalitolerans* (Xu *et al.*, 2005), all *Janthinobacterium* species and all *Herbaspirillum* species (Lincoln *et al.*, 1999; Ding & Yokota, 2004) and *Telluria* species (P. Kämpfer, unpublished) when grown on TSA (Oxoid) prior to fatty acid extraction. The only hydroxylated fatty acid of strains CCUG 49009^T and CCUG 49012 was C_{10:0} 3-OH; this hydroxylated fatty acid was found in all comparatively studied strains (Table 1) and has been reported in the fatty acid profiles of *Naxibacter* (Xu *et al.*, 2005), *Herminiimonas* (Fernandes *et al.*, 2005; Kämpfer *et al.*, 2006b; Muller *et al.*, 2006), *Massilia* (La Scola *et al.*, 1998; Lindquist *et al.*, 2003; Zhang *et al.*, 2006), *Duganella* (Li *et al.*, 2004), *Telluria* (P. Kämpfer, unpublished) and *Paucimonas* (Jendrossek, 2001). Additional hydroxylated fatty acids were detected in *Telluria mixta* CCUG 35206^T, *Janthinobacterium agaricidamnorum* CCUG 43140^T, *Janthinobacterium lividum* CCUG 2344^T and *Massilia timonae* CCUG 45783^T (Table 1). The presence of C_{12:0} 3-OH has been reported for *Paucimonas* (Jendrossek, 2001), *Janthinobacterium* (Lincoln *et al.*, 1999) and *Herbaspirillum* (Lincoln *et al.*, 1999; Ding & Yokota, 2004) and C_{12:0} 2-OH has been reported to be present in *Naxibacter* and *Duganella* (Xu *et al.*, 2005), *Janthinobacterium* (Lincoln *et al.*, 1999), *Herbaspirillum* (Lincoln *et al.*, 1999; Ding & Yokota, 2004) and *Massilia timonae* (La Scola *et al.*, 1998). The hydroxylated fatty acid C_{14:0} 2-OH was found in this study in *Telluria mixta* CCUG 35206^T and *Massilia dura* CCUG 52213^T. This compound has also been reported for *Paucimonas*, *Duganella*, *Telluria* and *Massilia* (La Scola *et al.*, 1998; Lincoln *et al.*, 1999; Jendrossek, 2001; Li *et al.*, 2004; Zhang *et al.*, 2006).

In conclusion, strains CCUG 49009^T and CCUG 49012 can be distinguished from species of the genera *Herminiimonas*,

Table 1. Major fatty acids (%) of the *Undibacterium* strains and the type strains of selected species of the most closely related genera

Strains: 1, CCUG 49009^T; 2, CCUG 49012; 3, *Duganella zoogloeoides* IAM 12670^T; 4, *Hermiimonas fonticola* S-94^T; 5, *Telluria mixta* CCUG 35206^T; 6, *Janthinobacterium agaricidamnorum* CCUG 43140^T; 7, *Janthinobacterium lividum* CCUG 2344^T; 8, *Massilia timonae* CCUG 45783^T; 9, *Massilia dura* CCUG 52213^T. All strains were grown on R2A agar at 25 °C for 48 h prior to fatty acid analysis. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated fatty acids									
C _{10:0}	1.0	3.0	0.6		0.4	1.3	0.4	0.4	0.3
C _{12:0}	5.3	3.2	5.8		3.1	3.0	3.9	3.7	6.8
C _{14:0}	1.5	0.8	0.8	4.5	1.6	0.6	0.5	0.4	
C _{16:0}	22.6	23.2	26.0	20.3	27.8	34.8	30.6	28.7	30.7
C _{18:0}					0.7				
Unsaturated fatty acids									
C _{18:1} ω 7 <i>c</i>	9.0	2.1	8.0	11.0	11.0	4.5	6.7	9.5	8.6
Hydroxy fatty acids									
C _{10:0} 3-OH	4.7	5.2	8.7	7.9	6.5	7.8	5.7	5.0	7.2
C _{12:0} 2-OH						2.2	2.6	2.3	
C _{14:0} 2-OH					3.6				4.3
Summed features*									
Summed feature 3	55.7	62.5	50.0	38.0	29.4	9.7	23.8	41.2	42.1
Summed feature 5				1.6					
Cyclopropane acids									
C _{17:0} cyclo				15.2	15.0	34.2	25.0	0.5	
C _{19:0} cyclo ω 8 <i>c</i>				1.5					
Branched-chain fatty acids									
C _{16:0} iso					0.5				
C _{19:0} iso					0.5	0.7	0.8		

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1} ω 7*c* and/or C_{15:0} iso 2-OH. Summed feature 5 contains C_{18:2} ω 6,9*c* and/or C_{18:0} anteiso.

Naxibacter, *Janthinobacterium* and *Telluria* and from *Duganella violaceinigra* by the absence of hydroxylated fatty acids other than C_{10:0} 3-OH, by the absence of C_{17:0} cyclo and unsaturated fatty acids other than C_{16:1} ω 7*c* (in summed feature 3) and C_{18:1} ω 7*c*. *Duganella zoogloeoides* has a similar fatty acid pattern (Table 1) but is clearly different on the basis of the physiological characteristics.

Results of the physiological characterization are given in the species description. Methods used were described previously (Kämpfer *et al.*, 1991). Both organisms showed negative results for all carbon substrate utilization tests. Completely negative profiles were not found in a comparative study on the basis of the same method in which the type strains of all species of the genera *Naxibacter*, *Duganella*, *Hermiimonas*, *Telluria*, *Massilia* and *Janthinobacterium* were included.

The G+C contents for strains CCUG 49009^T and CCUG 49012 were determined as described by Ziemke *et al.* (1998) and resulted in values of 52.3 mol% (SD 1 mol%) and 50.6 mol% (SD 1 mol%), respectively. DNA–DNA

hybridization experiments were performed with CCUG 49009^T and CCUG 49012 using the method described by Ziemke *et al.* (1998), except that, for nick translation, 2 μ g DNA was labelled during a 3 h incubation at 15 °C. Strain CCUG 49009^T showed relatively low DNA–DNA relatedness to strain CCUG 49012 (20 %, reciprocal analysis 18 %).

From the results of 16S rRNA gene sequencing, it is evident that strains CCUG 49009^T and CCUG 49012 are different from each other and from members of all other genera of the family *Oxalobacteraceae*. Phenotypic differentiation from all other genera of the family is possible by the combination of growth characteristics, fatty acid and polar lipid profiles and physiological properties.

Description of *Undibacterium* gen. nov.

Undibacterium (Un'di.bac.te'ri.um. L. n. *unda* water; L. neut. n. *bacterium* rod; N.L. neut. n. *Undibacterium* a rod of water).

Cells are non-motile, non-spore-forming rods (approx. 2 µm in length). Gram-negative and oxidase-positive, showing an oxidative metabolism. The polyamine pattern consists of the predominant compounds putrescine and 2-hydroxyputrescine. The quinone system is ubiquinone Q-8 and the polar lipid profile consists of the predominant compound phosphatidylethanolamine with moderate amounts of diphosphatidylglycerol and phosphatidylglycerol. The major fatty acids are C_{16:0}, summed feature 3 (C_{16:1}ω7c/C_{15:0} iso 2-OH) and C_{18:1}ω7c. C_{10:0} 3-OH is the only hydroxylated fatty acid detected. C_{17:0} cyclo is absent. The G + C content of the type strain of the type species is 52.3 mol%. The type species is *Undibacterium pigrum*.

Description of *Undibacterium pigrum* sp. nov.

Undibacterium pigrum (pig'rum. L. neut. adj. *pigrum* inactive).

Shares all characteristics listed in the genus description. Polyamine pattern, quinone system, polar lipid profile and fatty acid patterns are those listed in the genus description. Additionally, phosphatidylserine and three aminolipids are present in the polar lipid profile. Good growth occurs on R2A agar. No growth on TSA, nutrient agar or MacConkey agar at 25–30 °C. Beige, translucent and shiny colonies with entire edges form within 24 h, with a diameter of approximately 2 mm. L-Alanine *p*-nitroanilide (pNA) is hydrolysed on the basis of the method described by Kämpfer *et al.* (1991). The following compounds are not hydrolysed: *p*-nitrophenyl (pNP) β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xylopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, L-aniline pNA, γ-L-glutamate pNA and L-proline pNA. The following compounds are not used as sole sources of carbon: D-gluconate, acetate, propionate, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, 2-oxoglutarate, pyruvate, L-alanine, β-alanine, L-aspartate, L-leucine, L-ornithine, L-proline, L-serine, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, α-D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, adipate, azelate, suberate, L-histidine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate and phenylacetate on the basis of the method described by Kämpfer *et al.* (1991). No acids are produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol or D-mannose.

The type strain is CCUG 49009^T (= CIP 109318^T), which was isolated by one of us (F.P.) from drinking water on 10 March 2004 in Göteborg, Sweden. A second strain, CCUG 49012 (= CIP 108976), is also allocated to this genus

at present. The G + C content of the DNA of this strain is 50.6 mol%. This strain shares 96.8% 16S rRNA gene sequence similarity with strain CCUG 49009^T and a DNA–DNA relatedness of 20%. Phenotypically, it is very similar to strain CCUG 49009^T. The strain represents a genomovar of *U. pigrum*.

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