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 49009T 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, C16 : 0 and summed feature 3 (C16 : 1ω7c/C15 : 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 (Herminiimonas, 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 49009T (= CIP 109318T). 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 Herminiimonas (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 49009T 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 49009T was able to...
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 ×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 49009T 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 49009T 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 49009T 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 g−1: 0.75 g peptone from casein, 0.75 g yeast extract, 0.3 g K2HPO4, 0.024 g MgSO4, 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-inoculums flask than in the high-inoculum flask, cells from the former flask were harvested and used for polyamine analysis. Polymamines 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 49009T and CCUG 49012 exhibited polyamine patterns that were in accordance with their affiliation to the Betaproteobacteria (Busse & Auling, 1988).
of strain CCUG 49009T. DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; AL1–3, unknown aminolipids; L, unknown polar lipid.

The polar lipid patterns of strains CCUG 49009T 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 phosholipid 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 45783T, but within a much more complex profile (H.-J. Busse, unpublished results). Thus, polar lipid profiles of newly examined strains consisting of the components detected in CCUG 49009T 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 49009T and CCUG 49012 are given in detail in Table 1. Because the strains were not able to grow on TSA, they were grown on RA2 agar for 48 h prior to fatty acid analysis. Methods for analysis have been described previously (Kämpfer & Kroppenstedt, 1996). The major fatty acids were C16:0, summed feature 3 (C16:1o7c/C15:0 iso 2-OH) and C18:1o9c. In addition, C10:0 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 C17:0 cyclo was not detected in strains CCUG 49009T and CCUG 49012, in contrast to Herminiimonas fonticola S-94T, Telluria mixta CCUG 35206T, Janthinobacterium agaricidamnosum CCUG 43140T and Janthinobacterium lividum CCUG 2344T, in which this fatty acid accounted for >15 %. This fatty acid profile was also reported for Herminiimonas arsenicoxidans (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, C17:0 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 49009T and CCUG 49012 was C10:0 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 35206T, Janthinobacterium agaricidamnosum CCUG 43140T, Janthinobacterium lividum CCUG 2344T and Massilia timonae CCUG 45783T (Table 1). The presence of C12:0 3-OH has been reported for Paucimonas (Jendrossek, 2001), Janthinobacterium (Lincoln et al., 1999) and Herbaspirillum (Lincoln et al., 1999; Ding & Yokota, 2004) and C12:0 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 C14:0 2-OH was found in this study in Telluria mixta CCUG 35206T and Massilia dura CCUG 52213T. 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 49009T 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

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*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C16:1ω7c and/or C15:0 iso 2-OH. Summed feature 5 contains C18:2ω6,9c and/or C18:0 anteiso.

Naxibacter, Janthinobacterium and Telluria and from Duganella violaceinigra by the absence of hydroxylated fatty acids other than C10:0 3-OH, by the absence of C17:0 cyclo and unsaturated fatty acids other than C16:1ω7c (in summed feature 3) and C18:1ω7c. Duganella violaceinigra 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, Herminiimonas, Telluria, Massilia and Janthinobacterium were included.

The G+C contents for strains CCUG 49009T 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 49009T and CCUG 49012 using the method described by Ziemke et al. (1998), except that, for nick translation, 2 μg DNA was labelled during a 3 h incubation at 15 °C. Strain CCUG 49009T 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 49009T 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.bact.er.i.um. L. n. unde’ 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 only hydroxylated fatty acid detected is C17:0 cyclo. C16:0 summed feature 3 (C16:1o7t/C15:0 iso 2-0H) and C18:1o7t. C10:0 3-0H is absent. The G+C content of the type strain 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, phosphatidylethanolamine 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-glycopyranoside, pNP β-D-glucopyranoside, pNP β-D-xlyopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphocholine, L-α-aniline pNA, γ-L-glutamate pNA and L-proline pNA. The following compounds are not used as sole sources of carbon: D-glucosone, acetate, propionate, cis-aconitate, trans-aconitate, 4-ammonobutyrate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, 2-oxoglutarate, pyruvate, L-α-alanine, β-alanine, L-aspartate, L-leucine, L-ornithine, L-proline, L-serine, N-acetylgalactosamine, N-acetylgulcosamine, 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-xylose, D-xylose, adonitol, myo-inositol, malitol, 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 49009T (=CIP 109318T), 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 rDNA gene sequence similarity with strain CCUG 49009T and a DNA–DNA relatedness of 20%. Phenotypically, it is very similar to strain CCUG 49009T. The strain represents a genomovar of U. pigrum.

Acknowledgements

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References


