Polaromonas aquatica sp. nov., isolated from tap water

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Two Gram-negative, rod-shaped, non-spore-forming bacteria (CCUG 39402^T and CCUG 39797), isolated from different water sources, were investigated in a polyphasic study. The two isolates shared 100 % 16S rRNA gene sequence similarity and it was shown that they belonged to the *Betaproteobacteria*, most closely related to *Polaromonas vacuolata* (97.8 %) and *Polaromonas naphthalenivorans* (97.8 %). A polyamine pattern with 2-hydroxyputrescine and putrescine, as well as ubiquinone Q-8, were in agreement with characteristics of *Betaproteobacteria*. The presence of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, and major fatty acids C_{16:1}ω7c, C_{16:0} and C_{17:0} cyclo supported the affiliation of the two strains to the genus *Polaromonas*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of the two isolates from the two *Polaromonas* species with validly published names. They therefore represent a novel species, for which the name *Polaromonas aquatica* sp. nov. is proposed, with the type strain CCUG 39402^T (= CIP 108776^T).

The genus *Polaromonas* was proposed by Irgens *et al.* (1996) and until 2004 contained only one species, *Polaromonas vacuolata*. A second species, *Polaromonas naphthalenivorans*, was recently described by Jeon *et al.* (2004).

In a polyphasic taxonomic study, isolates CCUG 39402^T and CCUG 39797 were investigated to assess their taxonomic position. Strain CCUG 39402^T was isolated in 1998 on blood agar at 30 °C from tap water from a paper mill in Sweden. Strain CCUG 39797 was recovered from municipal drinking water in Sweden. Both isolates showed beige-coloured colonies on nutrient agar at 30 °C. Subcultivation was done on tryptone soy agar (TSA) at 30 °C for 24 h. Growth at 37 °C was also observed on nutrient agar and R2A agar, but not on SS agar (all from Oxoid).

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was studied under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene was analysed as described by

Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the ARB software package (Strunk *et al.*, 2000) and also MEGA version 2.1 (Kumar *et al.*, 2001), after multiple alignment 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 and maximum-parsimony were performed by using bootstrap values based on 1000 replications (results not shown). The 16S rRNA gene sequences of strains CCUG 39402^T and CCUG 39797 were continuous stretches of 1406 and 1407 bp, respectively. Sequence similarity calculations, following neighbour-joining analysis, indicated that the two sequences were identical. The closest relatives of the two strains were *P. vacuolata* (97.8 %) and *P. naphthalenivorans* (97.8 %). Lower sequence similarities (<97.0 %) were found with described species with validly published names from other genera of the *Betaproteobacteria* (Fig. 1).

Analyses of the fatty acids (Kämpfer & Kroppenstedt, 1996) of the novel strains and other *Polaromonas* species are detailed in Supplementary Table S1 in IJSEM Online. The fatty acid profiles of the two strains were very similar and were composed mainly of C_{16:1}ω7c, C_{16:0} and C_{17:0} cyclo; in addition, the hydroxylated fatty acid C_{8:0} 3-OH was detected. For *P. naphthalenivorans*, C_{10:0} 3-OH was detected, which is in accordance with the report of Jeon *et al.* (2004). However, for *P. vacuolata*, C_{8:0} 3-OH was found,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CCUG 39402^T and CCUG 39797 are AM039830 and AM039831.

Contents of major fatty acids of *P. aquatica* sp. nov. and other *Polaromonas* species are available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses) constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997) as outlined in the text. Bootstrap values based on 1000 replications are listed as percentages at the branching points. The name '*Aquamonas fontana*' has not been validly published. Bar, 0.01 nucleotide substitutions per nucleotide position.

which was not detected in the original report of Irgens *et al.* (1996). It should be noted that the fatty acid profiles (Supplementary Table S1) were obtained on the basis of whole-cell extracts, grown on different culture media. This prevents a quantitative comparison of the results, but does give an indication of the presence or absence of diagnostic fatty acids.

Quinones, polar lipids, polyamines and cell-wall diamino acid were extracted from biomass which was grown on PYE medium (l^{-1} : 3 g peptone from casein, 3 g yeast extract, pH 7.2). Quinone system and polar lipid profiles were analysed as described by Tindall (1990) and Altenburger *et al.* (1996). CCUG 39402^T and CCUG 39797 showed a quinone system with ubiquinone Q-8 predominant and traces of Q-9. Strains CCUG 39402^T (Fig. 2) and CCUG 39797 exhibited a polar lipid profile consisting of the major lipid phosphatidylethanolamine, moderate amounts of diphosphatidylglycerol and phosphatidylglycerol, and minor to trace amounts of phosphatidylserine, six unknown polar lipids, three unknown phospholipids and three unknown aminolipids. The presence of phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol and lack of glycolipids were in agreement with the characteristics reported for *P. naphthalenivorans* (Jeon *et al.*, 2004). Polyamines were analysed as described previously (Busse & Auling, 1988; Busse *et al.*, 1997). Both strains were characterized by the presence of the *Betaproteobacteria*-specific diamine 2-hydroxyputrescine and putrescine. The content of 2-hydroxyputrescine in the two strains was significantly higher [69–77 $\mu\text{mol (g dry weight)}^{-1}$] than in any other species analysed so far (Busse & Auling, 1988; Auling *et al.*, 1991) and might be a characteristic of this species.

Fig. 2. Two-dimensional chromatographic analysis of the polar lipids of CCUG 39402^T. Polar lipids were detected with molybdatophosphoric acid. Abbreviations: PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; APL, phosphatidylserine; PL1–3, unknown phospholipids; AL1–2, unknown aminolipids; L, unknown polar lipids. Another amino-lipid is present, but could not be detected here, that exhibits an R_f value similar to that of PE in the first dimension (horizontal) and an R_f value similar to that of PL3 in the second dimension (vertical).

Other polyamines were only detected in minor to trace amounts. The cell-wall diamino acid of the two strains, which was analysed according to Schleifer (1985), was *meso*-diaminopimelic acid.

Results of the physiological characterization are given in Table 1 and the species description, with methods as described previously (Kämpfer *et al.*, 1991). Production of acid from sugars was examined in phenol red broth (Merck) according to the instructions of the manufacturer at room temperature for 7 days. Neither strain exhibited acid production with any sugar tested (see species description below). The presence of catalase was tested with H₂O₂.

DNA–DNA hybridization experiments were performed with the two strains and type strains of both established *Polaromonas* species 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. The two isolates showed DNA–DNA hybridization values of 97.8 % (reciprocal analysis 89.7 %), clearly indicating that the two strains belong to the same species. Strain CCUG 39402^T showed relatively low DNA–DNA hybridization values to *P. vacuolata* 34-P^T (9 %, reciprocal 8 %) and *P. naphthalenivorans* DSM 15660^T (5 %, reciprocal 14 %). The hybridization values of strain CCUG 39797 to *P. vacuolata* 34-P^T (18 %, reciprocal 17 %) and *P. naphthalenivorans* DSM 15660^T (8 %, reciprocal 10 %) were also very low. On the basis of these genotypic and phenotypic results, the two strains represent a novel species of the genus *Polaromonas*. Comparison of the genomic fingerprints of CCUG 39402^T and CCUG 39797 after ERIC-PCR, which was analysed as reported previously (Wieser & Busse, 2000), showed two bands of identical sizes at approximately 600 bp in each fingerprint (results not shown). This observation indicates that representatives of this species might be rapidly identified using ERIC-PCR.

Table 1. Physiological characteristics of strains of *Polaromonas* species

Strains: 1, CCUG 39402^T; 2, CCUG 39797; 3, *P. vacuolata* 34-P^T (results read after 5 days incubation at 4 °C); 4, *P. naphthalenivorans* DSM 15660^T. +, Positive; –, negative; (+), weakly positive.

Assimilation of:	1	2	3	4
D-Glucose	–	–	+*	–
Acetate	–	–	+*	(+)
Propionate	–	–	+*	–
Fumarate	–	–	+*	–
Glutarate	(+)	(+)	+	–
DL-3-Hydroxybutyrate	–	–	+	–
DL-Lactate	+	+	+*	–
L-Malate	+	+	+*	–
L-Alanine, β-alanine, L-aspartate	–	–	+*	–

*The test (based on a different method) was also performed by Irgens *et al.* (1996) and gave congruent results.

Description of *Polaromonas aquatica* sp. nov.

Polaromonas aquatica (a.qua'ti.ca. L. fem. adj. *aquatica* aquatic, from water).

Cells are motile, non-spore-forming rods (approx. 1–2 µm in length). Gram-negative, oxidase- and catalase-positive, showing an oxidative metabolism. Good growth occurs on R2A, TSA, PYE and nutrient agar at 25–30 °C; beige, translucent and shiny colonies with entire edges form within 24 h, with a diameter of approximately 2 mm. The quinone system is ubiquinone Q-8 with traces of Q-9. The fatty acid profile is largely composed of C_{16:1}ω7c, C_{16:0} and C_{17:0} cyclo, and C_{8:0} 3-OH. The polar lipid profile consists of the major component phosphatidylethanolamine, moderate amounts of diphosphatidylglycerol and phosphatidylglycerol and minor to trace amounts of phosphatidylserine, six unknown polar lipids, three unknown phospholipids and three unknown aminolipids. The polyamine pattern contains the major components 2-hydroxyputrescine [69–77 µmol (g dry weight)^{–1}] and putrescine [58–76 µmol (g dry weight)^{–1}] and traces of spermidine [<1 µmol (g dry weight)^{–1}]. The cell-wall diamino acid is *meso*-diaminopimelic acid. Only a few carbon sources are utilized. Carbon source utilization (including differentiating characteristics for all *Polaromonas* species) is indicated in Table 1. Negative for production of acid from D-fructose, mannose, D-glucose, L-arabinose, raffinose and rhamnose.

The type strain is strain CCUG 39402^T (=CIP 108776^T). The type strain and a reference strain (CCUG 39797) were isolated from tap water in Sweden.

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