Polaromonas hydrogenivorans sp. nov., a psychrotolerant hydrogen-oxidizing bacterium from Alaskan soil

Maria Sizova and Nicolai Panikov

Stevens Institute of Technology, Arthur E. Imperatore School of Sciences and Arts, Castle Point on Hudson, Hoboken 07030, NJ, USA

Correspondence

Nicolai Panikov

npanikov@stevens.edu

Psychrotolerant (0–25 °C), chemolithotrophic Gram-negative cocci were isolated from Alaskan forest soil. The novel isolate was found to grow autotrophically on H₂:CO₂ mixtures and to switch to heterotrophic growth on media containing organic substrates. The novel strain utilized a wide range of organic acids, some simple sugars and alcohols. Naphthalene vapour did not support growth. On the basis of 16S rRNA gene sequence similarity, the novel strain is affiliated to the genus *Polaromonas*, of the class *Betaproteobacteria*, and is related to *Polaromonas naphthalenivorans* (99.6 % gene sequence similarity), *Polaromonas aquatica* (97.4 %) and *Polaromonas vacuolata* (96.1 %). The membrane phospholipids contained 16:1 ω 7c/16:1 ω 6c, 16:0 and 18:1 ω 7c, similar to the fatty acids found for *P. naphthalenivorans*, *P. aquatica* and *P. vacuolata*. On the basis of DNA–DNA hybridization, physiological and biochemical properties, the hydrogen-oxidizing mixotrophic isolate represents a novel species, for which the name *Polaromonas hydrogenivorans* sp. nov. is proposed. The type strain is DSM 17735^T (=NRRL B-41369^T).

The genus *Polaromonas* was first described by Irgens *et al.* (1996) and at present the genus comprises three species, *Polaromonas vacuolata* (Irgens *et al.*, 1996), *Polaromonas naphthalenivorans* (Jeon *et al.*, 2004) and *Polaromonas aquatica* (Kämpfer *et al.*, 2006). A few other representatives of this genus were recently isolated from the polar environment and have been shown to play an important role in the degradation of organic pollutants (Foght *et al.*, 2004; Futamata *et al.*, 2005).

A novel isolate, provisionally called *Polaromonas* sp. 'hydrogenivorans', was obtained from soil over permafrost at the Smith Lake field site of University of Alaska Fairbanks, USA. At this site, the soil is coarse-loamy histoturbel under white spruce with permafrost at 50 cm. The soil sample was taken at a depth of 10–20 cm. The novel isolate was enriched on liquid mineral medium containing (l⁻¹): 2 g K₂HPO₄, 1.5 g NaH₂PO₄, 1 g (NH₄)₂SO₄, 0.1 g CaCl₂, 0.4 g MgSO₄, 0.01 g Na-EDTA, 1 × 10⁻³ g FeCl₃·6H₂O, 2 × 10⁻⁴ g KI, 2 × 10⁻⁴ g CoCl₂·6H₂O, 8 × 10⁻⁴ g MnCl₂·4H₂O, 8 × 10⁻⁴ g ZnSO₄, 1 × 10⁻⁴ g H₃BO₃, 1 × 10⁻⁴ g Na₂MoO₄·2H₂O, 1 × 10⁻⁴ g CuCl₂ and 2 × 10⁻⁴ g NiCl₂·6H₂O. The gases

H₂ and CO₂ served as the energy and carbon source. Incubation temperatures were 0.1–1.0 °C. Isolation was performed aerobically on agar mineral medium with an atmosphere of O₂:N₂:H₂:CO₂ of 10:40:40:10 in the gas phase.

Cell morphology was observed with a LEO 982 FEG scanning electron microscope and a Leica DMLB light microscope equipped with phase-contrast and a mercury short arc photo optic lamp. The K3 filter was used to visualize 5-[(4,6-dichlorotriazin-2-yl)amino]-fluorescein (DTAF)-stained microbial cells. For scanning electron microscopy, microbial cells were fixed with 4 % paraformaldehyde, dehydrated by successive passages through 50, 80 and 100 % ethanol and then air-dried. The Gram reaction was determined using the Difco Gram stain kit according to the manufacturer's recommendations. Resistance to various antibiotics was tested with Difco susceptibility test disks and evaluated in accordance with 'zone diameter interpretive standards'. Oxidase and catalase activities were tested with 1 % *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride in dimethyl sulfoxide and 3 % hydrogen peroxide. Carbon source utilization was analysed with Biolog GN2 microplates. Growth in the presence of naphthalene vapour as a sole carbon source was investigated in liquid and agar mineral media at 15–20 °C. Whole-cell fatty acids were methylated, extracted and analysed by GC using the Microbial Identification System at Microbial ID, Inc. DNA base content (mol% G + C) was determined by HPLC after acid hydrolysis (Kirk, 1967) with calf thymus

Abbreviations: ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Polaromonas hydrogenivorans* DSM 17735^T is DQ094183.

A photomicrograph of cells of strain DSM 17735^T, details of fatty acid contents and phylogenetic trees are available as supplementary material in IJSEM Online.

and *Escherichia coli* DNA as standards. DNA–DNA hybridization experiments were performed as described by De Ley *et al.* (1970) and Huß *et al.* (1983) at the German Collection of Microorganisms and Cell Cultures (DSMZ).

The 16S rRNA gene was analysed as described previously (Sizova *et al.*, 2003) and compared with available 16S rRNA gene sequences using BLAST and RDP-II, release 9 (Cole *et al.*, 2003). Phylogenetic analysis (Hall, 2001) was performed using the PHYLIP software package, version 3.65 (Felsenstein, 2005; Tuimala, 2005), after multiple alignments of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (Kimura two-parameter model) and clustering were determined with the neighbour-joining (NJ) and maximum-likelihood (ML) methods. The stability of relationships was determined by using bootstrap analysis of 1000 datasets.

Strain DSM 17735^T was recovered on mineral agar with H₂ and CO₂ in the gas phase, showing 0.5–2 mm circular, convex white colonies with smooth surfaces and entire edges. On nutrient agar, the isolate formed slimy 2–5 mm shiny beige colonies. Microscopic examination with negative ink-staining revealed extracellular capsules. Similar capsules were formed on liquid glucose media, but were not found in cultures grown chemolithotrophically on mineral medium with H₂+CO₂. No diffusible pigments were observed.

Cells of the novel strain were non-spore forming, Gram-negative, non-motile cocci. Cell diameter varied from 0.8 to 2.8 µm (see Supplementary Fig. S1). Ellipsoid and swollen cells of irregular shape were observed occasionally on mineral medium with H₂ and CO₂ and more frequently on nutrient agar. Growth occurred between 0 and 25 °C at pH 6.0–7.0, but not at 28 °C. Optimum temperature for growth was 15–20 °C. Strain DSM 17735^T did not grow on either liquid or agar mineral media with naphthalene vapour as a sole source of carbon and energy in contrast to its closest relative, *P. naphthalenivorans* DSM 15660^T (Jeon *et al.*, 2004). Our comparative studies of strain DSM 17735^T and *P. naphthalenivorans* DSM 15660^T revealed that both organisms were able to grow chemolithotrophically on mineral medium with H₂+CO₂ as an energy and carbon source. The major difference was that the novel strain, DSM 17735^T, grew as a homogeneous suspension while *P. naphthalenivorans* DSM 15660^T produced large spherical aggregates of 1–3 mm in diameter. Aggregates were observed in cultures grown on all tested liquid media: with glucose, pyruvate and H₂+CO₂. Another distinction was that cells of strain DSM 17735^T were 2–6 times smaller than those of *P. naphthalenivorans* DSM 15660^T. Most cells of strain DSM 17735^T were represented by cocci, while *P. naphthalenivorans* DSM 15660^T displayed much greater morphological variability with the cell population being represented by cocci, ovals and swollen rods.

The fatty acid content of strain DSM 17735^T and other members of the genus *Polaromonas* is shown in Supplementary Table S1. The major fatty acid profiles of strains

DSM 17735^T, *P. vacuolata* and *P. naphthalenivorans* were very similar, being composed of 16:0, 16:1 ω 7c or 16:1 ω 6c and 18:1 ω 7c. These three species were clearly differentiated from *P. aquatica*, which contained 17:0 cyclo and twice as much 16:0. The hydroxylated fatty acid 10:0 3-OH was present only as a minor component (2.5–3 %) in strains DSM 17735^T and *P. naphthalenivorans* DSM 15660^T. The content of other minor fatty acids varied significantly, depending on cultivation conditions. When cells of strain DSM 17735^T were grown autotrophically, the fatty acids 17:0 and 17:0 cyclo were found. Fatty acids 12:0, 14:0, 16:0 N alcohol and 17:1 anteiso ω 9c were present only in cells grown heterotrophically on nutrient broth (NB).

All strains were positive for catalase and oxidase activities. Strain DSM 17735^T, *P. vacuolata* and *P. naphthalenivorans* were positive in tests for the assimilation of citric and pyruvic acids and negative for L-ornithine. *P. vacuolata*, *P. aquatica* and strain DSM 17735^T were positive in tests for the assimilation of D- and L-lactic acid. *P. vacuolata* and strain DSM 17735^T were positive for the assimilation of D-sorbitol, 2-oxoglutarate, succinic acid, L-glutamic acid, L-asparagine and L-proline and were negative for the assimilation of D-cellobiose, L-fucose, α -D-lactose, maltose, D-mannose, methanol, i-erythritol, L-histidine, D-serine and thymidine. In addition to the carbon sources indicated in Table 1, the following carbon sources were utilized by strain DSM 17735^T: D-galactose, D-psicose, D-raffinose, turanose, α -cyclodextrin, glycogen, Tween 40, Tween 80, xylitol, 2,3-butanediol, *cis*-aconitic acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, α - and γ -hydroxybutyric acids, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketovaleric acid, quinic acid, sebamic acid, bromosuccinic acid, succinamic acid, γ -aminobutyric acid, pyruvic acid methyl ester, succinic acid monomethyl ester, D-glucose 6-phosphate and methyl β -D-glucoside. The following carbon sources were not utilized: gentiobiose, lactulose, dextrin, adonitol, D-arabitol, *myo*-inositol, 2-aminoethanol, D-glucosaminic acid, D-saccharic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-pyroglutamic acid, urocanic acid and D-galactonic acid.

Phylogenetic analysis with NJ and ML methods indicated that strain DSM 17735^T belongs to the genus *Polaromonas* in the class *Betaproteobacteria* (see the phylogenetic trees in Supplementary Figs S2 and S3). A 16S rRNA gene sequence similarity calculation revealed that the closest relative of strain DSM 17735^T was *P. naphthalenivorans* (99.6 and 99.9 % similarity after NJ and ML methods, respectively). When compared with DSM 17735^T, *P. aquatica* showed 97.4 and 97.8 % and *P. vacuolata* showed 96.1 and 95.9 % gene sequence similarity with the NJ and ML methods, respectively.

DNA–DNA hybridization of strain DSM 17735^T was performed against *P. naphthalenivorans* DSM 15660^T. The two organisms showed hybridization values of 40.6 % (reciprocal analysis 45.9 %), clearly indicating that they diverge too far to be considered as members of the same species. By convention, DNA–DNA relatedness between

Table 1. Comparison of strain DSM 17735^T with known members of the genus *Polaromonas*

Unless indicated, data for other species are taken from Jeon *et al.*, 2004, Irgens *et al.*, 1996, and Kämpfer *et al.*, 2006. +, Positive; –, negative; ND, not determined.

Characteristic	DSM 17735 ^T	<i>P. vacuolata</i>	<i>P. naphthalenivorans</i>	<i>P. aquatica</i>
Morphology	Cocci	Rods	Pleomorphic cocci	Rods
Cell size (µm)	0.8–2.8	0.8 × 2–3	1–4 (1.6–10)*	1–2
Flagella	–	+	–	+
Gas vesicles	–	+	–	–
Growth temperature range (°C)	0–25	0–12	4–25	> 37
Chemolithotrophic growth	H ₂ + CO ₂	–	(H ₂ + CO ₂)	–
Growth on naphthalene as a sole carbon and energy source	–	–	+	–
DNA G + C content (mol%)	62.5	52	61.5 (62.5)	ND
Antibiotics:				
Resistant to	Neomycin, novobiocin	Bacitracin, streptomycin, gentamicin	ND	ND
Susceptible to	Tetracycline, gentamicin, streptomycin	Novobiocin, tetracycline, neomycin, kanamycin	ND	ND
Assimilation tests:				
Acetic acid	+	+	ND	–
D-Alanine	+	+	ND	–
L-Arabinose	–	ND	+	ND
DL-Arginine	ND	–	+	ND
L-Aspartic acid	+	+	ND	–
Ethanol	+	–	ND	ND
Formic acid	+	–	ND	ND
D-Fructose	+	–	ND	ND
Glucose	+	+	+	–
Glycerol	–	+	ND	ND
β-Hydroxybutyric acid	+	+	ND	–
Malonic acid	+	–	ND	ND
D-Mannitol	–	ND	+	ND
D-Melibiose	+	–	ND	ND
Oxaloacetic acid	–	+	ND	ND
L-Rhamnose	+	–	ND	ND
L-Serine	+	–	ND	ND
Sucrose	+	–	+	ND
L-Threonine	+	–	ND	ND
D-Trehalose	+	–	ND	ND

*Data in parentheses refer to tests performed in this study with type strains requested from culture collections.

members of the same species should be more than 70 % (Gevers *et al.*, 2005; Wayne *et al.*, 1987).

Table 1 summarizes the physiological properties that can be used to differentiate strain DSM 17735^T from other species of the genus *Polaromonas*. Strain DSM 17735^T and *P. naphthalenivorans* share the following common features that distinguish them from other *Polaromonas* species: the ability to grow chemolithotrophically on H₂:CO₂, coccoid cell morphology, lack of motility and a similar DNA G + C content (62 ± 0.5 mol%). In contrast to *P. naphthalenivorans*, strain DSM 17735^T did not utilize naphthalene, had

lower temperature limits for growth (0 °C as compared with +4 °C for *P. naphthalenivorans*) and cells that were 2–3 times smaller and less pleomorphic. Finally, a low DNA–DNA relatedness, 40–46 %, indicates considerable differences in their genomic structures that would probably become evident as differences in phenotypic properties if tested.

On the basis of its physiological, biochemical and molecular properties, it is proposed that the hydrogen-oxidizing, chemolithotrophic strain DSM 17735^T represents a novel species, for which the name *Polaromonas hydrogenivorans* sp. nov. is proposed.

Description of *Polaromonas hydrogenivorans* sp. nov.

Polaromonas hydrogenivorans (hy.dro.ge.ni.vo'rans. N.L. n. *hydrogenum* hydrogen; L. part. adj. *vorans* consuming; N.L. part. *hydrogenivorans* hydrogen-consuming).

Cells are non-motile, non-flagellated, non-spore forming, Gram-negative, aerobic cocci of variable size (0.8–2.8 µm). Some swollen polymorphic cells can occasionally be observed. Cells are able to grow chemolithotrophically with H₂ and CO₂ or organotrophically with a wide range of organic acids, some simple sugars and alcohols. The growth temperature range is 0–25 °C. Growth does not occur at 28 °C. The optimum temperature for growth is 15–20 °C; optimal pH for growth is 6.0–7.0. Colonies are white, dull (on mineral agar) or slimy, shiny beige (on nutrient agar), circular and convex with smooth surfaces and entire edges. Oxidase- and catalase-positive. Naphthalene vapour is not utilized. The fatty acid content is 16:1ω7c/16:1ω6c (60 %), 16:0 (20.5 %), 18:1ω7c (12.5 %), 10:0 3-OH (2.2 %) and 16:1 ω5c (1.9 %). The DNA G+C content is 62.5 mol%. Susceptible to tetracycline (30 µg per disc), gentamicin (10 µg per disc) and streptomycin (10 µg per disc). Resistant to neomycin (30 µg per disc) and novobiocin (30 µg per disc).

The type strain, DSM 17735^T (=NRRL B-41369^T), was isolated from seasonally frozen soil from an Alaskan forest, Fairbanks, USA.

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