Polaromonas jejuensis sp. nov., isolated from soil in Korea

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A Gram-negative, rod-shaped, non-motile bacterial strain, designated JS12-13^T, was isolated from soil from Halla Mountain on Jeju Island, Korea. Analysis of the 16S rRNA gene sequence of strain JS12-13^T revealed that it was a member of the genus *Polaromonas*, sharing 96.9–98.4 % sequence similarity with type strains of the genus *Polaromonas* and being most closely related to *Polaromonas aquatica* CIP 108776^T. The major fatty acids of strain JS12-13^T were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 38.3 %), $C_{16:0}$ (28.4 %), $C_{17:0}$ cyclo (15.9 %) and $C_{18:1}\omega7c$ (9.1 %). The major quinone found was ubiquinone-8. The G+C content of the genomic DNA of strain JS12-13^T was 63.7 mol%. On the basis of phenotypic and genotypic characteristics, strain JS12-13^T represents a novel species of the genus *Polaromonas*, for which the name *Polaromonas jejuensis* sp. nov. is proposed. The type strain is JS12-13^T (=KACC 12508^T =DSM 19351^T).

The genus *Polaromonas* was first proposed by Irgens *et al.* (1996) for a psychrophilic, marine bacterium isolated from Antarctica, *Polaromonas vacuolata*. Since then, three more species with validly published names have been included: *Polaromonas naphthalenivorans*, from freshwater sediment (Jeon *et al.*, 2004), *Polaromonas aquatica*, isolated from tap water (Kämpfer *et al.*, 2006), and *Polaromonas hydrogenivorans*, from Alaskan soil (Sizova & Panikov, 2007). In this work, a polyphasic taxonomic approach was used to characterize a bacterial strain, JS12-13^T, isolated from a soil sample from Halla Mountain on Jeju Island, Republic of Korea; this strain was found to represent a novel species of the genus *Polaromonas*.

For the isolation of strain JS12-13^T, a 1 g soil sample was suspended in 9 ml 0.85 % NaCl (w/v) and mixed in a shaker for 30 min. After serial dilution, aliquots of the serial diluents were spread on R2A agar plates (Reasoner & Geldreich, 1985) and incubated at 28 °C for 5 days. Isolate JS12-13^T was cultured routinely and maintained on R2A agar at 28 °C. It also grew on nutrient agar (Difco). However, no growth was observed on tryptic soy agar (Difco) and MacConkey agar (Difco).

Gram staining and catalase and oxidase determinations and tests for the hydrolysis of casein, DNA, starch and Tween 80 were conducted according to the methods of Smibert & Krieg (1994). The hydrolysis of CM-cellulose (Sigma) (0.1 %), chitin (1 %, w/v), hypoxanthine (0.5 %, w/v), pectin (0.5 %, w/v), tyrosine (0.5 %, w/v) and xanthine (0.5 %, w/v) was also tested. Cell morphology was observed by means of transmission electron microscopy (model 912AB; LEO) and phase-contrast microscopy (AXIO; Zeiss), using cells grown for 2 days at 28 °C on R2A. Anaerobic growth was tested on R2A incubated in an anaerobic jar (BBL). Growth at different temperatures (5, 15, 20, 25, 30, 35 and 40 °C) and various pH values (pH 4.0–10.0, in increments of 1.0 pH units) was assessed after 14 days incubation on R2A. Salt tolerance was tested on R2A agar supplemented with 0–5 % (w/v) NaCl. Other physiological and biochemical properties, including carbon-source utilization patterns and enzyme activities, were tested by using the API 20NE, API ID 32GN and API ZYM test kits (bioMérieux).

The 16S rRNA gene was amplified by using a PCR with two universal primers, as described previously (Kwon *et al.*, 2003). The sequence of the amplified 16S rRNA gene was analysed using an Applied Biosystems DNA sequencer (ABI3100). 16S rRNA gene sequences were aligned using CLUSTAL W software (Thompson *et al.*, 1994). Nucleotide

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JS12-13^T is EU030285.

similarity values were calculated from the alignment. Phylogenetic trees for the datasets were inferred from neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) analyses using MEGA, version 3.0 (Kumar *et al.*, 2004). The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings.

DNA–DNA hybridization was carried out using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). Chemotaxonomic characteristics were determined using cells grown at 28 °C for 3 days on R2A. Isoprenoid quinones were analysed using HPLC (Shimadzu) as described by Groth *et al.* (1996). The DNA G+C content (mol%) was determined by HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco). Analysis of the fatty acid methyl esters was performed using GLC according to the instructions of the Microbial Identification System (MIDI).

Cells of strain JS12-13^T were aerobic, Gram-negative, non-motile, non-spore-forming rods that were 0.6 μm wide and 1–3 μm long. Colonies on R2A agar were pale yellow, circular and convex with entire margins. The differential phenotypic properties for JS12-13^T and recognized *Polaromonas* species are shown in Table 1.

Strain JS12-13^T showed sequence similarities of 96.9–98.4 % with respect to *P. aquatica* CIP 108776^T (98.4 %), *P. hydrogenivorans* DSM 17735^T (97.8 %), *P. naphthalenivorans* DSM 15660^T (97.6 %) and *P. vacuolata* 34-P^T (96.8 %). Strain JS12-13^T showed less than 97 % 16S rRNA gene sequence similarity with respect to the type strains of other species. In the neighbour-joining phylogenetic tree, strain JS12-13^T formed a robust cluster with members of the genus *Polaromonas*, with 100 % bootstrap support (Fig. 1). The maximum-parsimony phylogenetic tree also supported

Table 1. Differential phenotypic characteristics of strain JS12-13^T and type strains of recognized *Polaromonas* species

Strains: 1, JS12-13^T; 2, *P. aquatica* CIP 108776^T (unless indicated, data from Kämpfer *et al.*, 2006); 3, *P. hydrogenivorans* DSM 17735^T (Sizova & Panikov, 2007); 4, *P. naphthalenivorans* DSM 15660^T (Jeon *et al.*, 2004); 5, *P. vacuolata* 34-P^T (Irgens *et al.*, 1996). Assimilation data for all strains except *P. vacuolata* 34-P^T were obtained using API 20NE and API ID 32GN test strips in this study. All of the strains are positive for catalase and oxidase and all are negative for nitrate reduction, indole production and glucose fermentation. +, Positive; −, negative; ND, no data available.

Characteristic	1	2	3	4	5
Isolation source	Soil	Tap water	Soil	Freshwater sediment	Seawater
Cell shape	Rods	Rods	Cocci	Cocci	Rods
Cell size (width × length; μm)	0.6 × 1–3	ND × 1–2	0.8–2.8	1–4	0.8 × 2–3
Motility	−	+	−	−	+
Growth temperature (°C)	5–30	ND	0–25	4–25	0–12
Growth at 30 °C	+	+	−	−	−
Urease	−	−*	−*	−*	+
β-Galactosidase	+	−*	−*	−*	ND
Assimilation of:					
D-Glucose	−	−	+	−	+
Potassium gluconate	+	+	+	−	ND
Malic acid	+	+	−	−	+
Trisodium citrate	+	−	−	−	+
Itaconic acid	+	−	−	−	ND
Suberic acid	+	+	−	−	ND
Lactic acid	+	+	−	−	+
L-Alanine	+	+	+	−	+
Glycogen	−	−	−	+	ND
3-Hydroxybenzoic acid	−	+	−	−	ND
L-Serine	−	−	+	−	−
D-Sorbitol	−	−	−	−	+
Propionic acid	+	−	−	−	+
L-Histidine	+	−	−	−	−
3-Hydroxybutyric acid	+	+	−	−	+
4-Hydroxybenzoic acid	−	+	−	−	ND
L-Proline	+	+	+	−	+
DNA G+C content (mol%)	63.7	ND	62.5	61.5	52

*Data from this study.

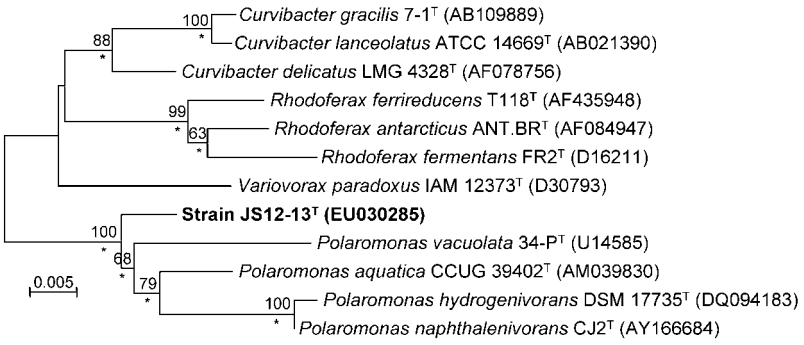

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain JS12-13^T within the genus *Polaromonas*. Asterisks indicate branches replicated with maximum-parsimony tree-making algorithms. Numbers at nodes indicate bootstrap percentages (based on 1000 resampled datasets); values below 50 % are not shown. Bar, 0.005 substitutions per site.

the inclusion of strain JS12-13^T as a member of the genus *Polaromonas* (Fig. 1).

DNA–DNA hybridization tests between strain JS12-13^T and *P. aquatica* CIP 108776^T, *P. hydrogenivorans* DSM 17735^T and *P. naphthalenivorans* DSM 15660^T revealed values of 43, 34 and 28 %, respectively. These values are below the threshold (70 %) suggested for species delineation (Stackebrandt & Goebel, 1994), indicating that strain JS12-13^T represents a novel species.

Strain JS12-13^T contained summed feature 3 (comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 38.3 %), $C_{16:0}$ (28.4 %), $C_{17:0}$ cyclo (15.9 %) and $C_{18:1}\omega7c$ (9.1 %) as the major fatty acids. The fatty acid data (Table 2) were a little different from those of Kämpfer *et al.* (2006), perhaps because of differences in cultivation method and growth medium. The major quinone found in strain JS12-13^T was ubiquinone-8. The G+C content of the genomic DNA of strain JS12-13^T was 63.7 mol%.

Phenotypically, the unique characteristics of strain JS12-13^T included the presence of β-galactosidase activity, the assimilation of itaconic acid and L-histidine and the presence of fatty acid $C_{12:0}$ (2.9 %) (Tables 1 and 2). The fatty acid composition of JS12-13^T was similar to that of *P. aquatica* CIP 108776^T (Table 2). However, strain JS12-13^T could be differentiated from *P. aquatica* CIP 108776^T on the basis of the former's lack of motility and from the substrate-assimilation patterns. The genetic and phenotypic data presented above show that strain JS12-13^T represents a novel species of the genus *Polaromonas*, for which the name *Polaromonas jejuensis* sp. nov. is proposed.

Description of *Polaromonas jejuensis* sp. nov.

Polaromonas jejuensis (je.ju.en′sis. N.L. fem. adj. *jejuensis* referring to Jeju, Korea).

Cells are aerobic, Gram-negative, non-motile, non-spore-forming rods, 0.6 μm wide and 1–3 μm long. Colonies on R2A agar are pale yellow, circular and convex with entire margins. Growth occurs at 5–30 °C (optimum, 28–30 °C), pH 5–9 (optimum, pH 6–7) and 0–1 % NaCl. Hydrolyses hypoxanthine, tyrosine and xanthine, but not casein, chitin, CM-cellulose, DNA, pectin, starch or Tween 80. Assimilates potassium gluconate, malic acid, trisodium citrate, itaconic acid, suberic acid, lactic acid, L-alanine, propionic acid, L-histidine, 3-hydroxybutyric acid and L-proline, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, capric acid, adipic acid, phenylacetic acid, L-rhamnose, D-ribose, inositol, sucrose, sodium malonate, sodium acetate, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, salicin, melibiose, L-fucose, D-sorbitol, valeric acid, potassium 2-ketogluconate or 4-hydroxybenzoic acid (API 20NE and API ID 32GN test strips). Positive for the following enzyme activities: β-galactosidase, alkaline

Table 2. Fatty acid compositions of strain JS12-13^T and type strains of recognized *Polaromonas* species

Strains: 1, JS12-13^T; 2, *P. aquatica* CIP 108776^T; 3, *P. hydrogenivorans* DSM 17735^T; 4, *P. naphthalenivorans* DSM 15660^T; 5, *P. vacuolata* 34-P^T (data from Kämpfer *et al.*, 2006). Cells of strains JS12-13^T, KACC 11696^T and DSM 15660^T were harvested after growth on R2A agar at 28 °C for 3 days. Strain DSM 17735^T was grown on R2A agar at 10 °C for 4 days. Only fatty acids that represent more than 0.5 % of the total fatty acids are indicated.

Fatty acid	1	2	3	4	5
$C_{8:0}$ 3-OH	1.3	1.0	0.8	0.5	2.8
$C_{10:0}$ 3-OH	–	–	3.1	3.5	–
$C_{12:0}$	2.9	–	–	–	–
$C_{14:0}$	0.6	0.6	–	–	–
$C_{15:1}\omega6c$	0.9	0.6	–	–	–
$C_{16:0}$	28.4	33.8	14.1	28.0	9.5
$C_{16:1}\omega5c$	0.7	0.5	1.6	1.0	–
$C_{17:0}$	0.8	1.0	–	–	–
$C_{17:0}$ cyclo	15.9	30.7	–	12.9	0.8
$C_{18:0}$	0.7	0.5	–	–	–
$C_{18:1}\omega7c$	9.1	7.2	5.2	3.1	7.5
$C_{18:1}\omega7c$ 11-methyl	–	–	–	–	4.5
Summed features					
3*	38.3	23.4	75.2	49.1	73.7
7†	–	–	–	2.0	–

*Summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.
†Summed feature 7 comprises $C_{19:1}\omega6c$, $C_{17:0}$ cyclo and/or an unknown fatty acid with an equivalent chain length of 18.846.

phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and acid phosphatase (API 20NE and API ZYM). Negative for the following characteristics: nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis, gelatin hydrolysis, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API 20NE and API ZYM). Naphthol-AS-BI-phosphohydrolase activity is weak (API ZYM). The predominant fatty acids are summed feature 3, $C_{16:0}$ and $C_{17:0}$ cyclo. The major quinone is ubiquinone-8. The DNA G+C content of the type strain is 63.7 mol%.

The type strain, JS12-13^T (=KACC 12508^T =DSM 19351^T), was isolated from a soil sample collected from Halla Mountain on Jeju Island, Republic of Korea.

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