Pseudomonas deceptionensis sp. nov., a psychrotolerant bacterium from the Antarctic

Ornella Carrión, David Miñana-Galbis, M[a] Jesús Montes and Elena Mercadé

Correspondence
Elena Mercadé
mmercade@ub.edu

Laboratori de Microbiologia, Facultat de Farmacia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain

During the taxonomic investigation of cold-adapted bacteria from samples collected in the Antarctic area of the South Shetland Islands, one Gram-reaction-negative, psychrotolerant, aerobic bacterium, designated strain M1^T, was isolated from marine sediment collected on Deception Island. The organism was rod-shaped, catalase- and oxidase-positive and motile by means of a polar flagellum. This psychrotolerant strain grew at temperatures ranging from −4 °C to 34 °C. Phylogenetic studies based on 16S rRNA gene sequences confirmed that Antarctic isolate M1^T was a member of the genus *Pseudomonas* and was located in the *Pseudomonas fragi* cluster. 16S rRNA gene sequence similarity values were >98 % between 13 type strains belonging to the *Pseudomonas fluorescens* lineage. However, phylogenetic analysis of *rpoD* gene sequences showed that strain M1^T exhibited high sequence similarity only with respect to *Pseudomonas psycrophila* (97.42 %) and *P. fragi* (96.40 %) and DNA–DNA hybridization experiments between the Antarctic isolate M1^T and the type strains of these two closely related species revealed relatedness values of 58 and 57 %, respectively. Several phenotypic characteristics, together with the results of polar lipid and cellular fatty acid analyses, were used to differentiate strain M1^T from related pseudomonads. Based on the evidence of this polyphasic taxonomic study, strain M1^T represents a novel species, for which the name *Pseudomonas deceptionensis* sp. nov. is proposed. The type strain is M1^T (=LMG 25555^T =CECT 7677^T).

In recent years, attention has been increasingly devoted to cold-adapted micro-organisms and their enzymes (Antranikian *et al.*, 2005). Antarctica has become a great source of novel psychrophilic and psychrotolerant strains, some of them belonging to the genus *Pseudomonas* (Kriss *et al.*, 1976; Shivaji *et al.*, 1989; Ma *et al.*, 2006; Maugeri *et al.*, 1996; Bruni *et al.*, 1999; Reddy *et al.*, 2004). During a taxonomic investigation of cold-adapted bacteria, soil, water and sediment samples were collected on Deception Island in the Antarctic area of the South Shetland Islands. From these samples, eight strains that were able to grow at 0–30 °C were isolated. Two of these strains were classified as *Pseudomonas guineae* in previous studies (Bozal *et al.*, 2007), two were classified as a novel species of the genus *Marinobacter*, *Marinobacter guineae* (Montes *et al.* 2008), and another two strains were classified as *Shewanella vesiculosa* (Bozal *et al.* 2009). Another isolate, designated strain M1^T, was able to grow at −4 to 34 °C and was characterized further in this study. The taxonomic status of strain M1^T was investigated by using a combination of phenotypic characterization, 16S rRNA and *rpoD* gene sequencing, DNA G+C content determination, DNA–DNA hybridization experiments and cellular fatty acid analysis. The data obtained showed that strain M1^T represented a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas deceptionensis* sp. nov. is proposed.

Strain M1^T was isolated from a marine sediment sample collected from Deception Island (South Shetland Islands, Antarctica). Sample aliquots were removed with a platinum loop and diluted in a saline solution (pH 7) containing the following salts (g l^{-1}): NaCl (0.56), KCl (0.027), $CaCl_2$ (0.03) and $NaHCO_3$ (0.01). Tryptic soy agar (TSA) plates (Oxoid) were inoculated with loopfuls of several sample dilutions by using the streak-plate method to obtain isolated colonies. The plates were incubated for 4 days at 15 °C. Isolates were maintained aerobically on TSA slopes at 4 °C and also at −80 °C in cryo-beads (AES Laboratoire).

Phenotypic characteristics of strain M1^T and its most closely related species, *Pseudomonas psychrophila* DSM 17535^T, *Pseudomonas fragi* DSM 3456^T, *Pseudomonas*

Abbreviations: DPG, diphosphatidylglycerol; MLSA, multilocus sequence analysis; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *rpoD* gene sequences of strain M1^T are GU936597 and GU936596, respectively.

Four supplementary figures and two supplementary tables are available with the online version of this paper.

lundensis DSM 6252^T and *Pseudomonas taetrolens* DSM 21104^T, were investigated simultaneously. The morphology of cells grown on TSA at 15 °C was determined by means of negative staining and transmission electron microscopy (JEOL 1010) of ultrathin sections after freeze fixation and freeze-substitution (Nevot *et al.*, 2006). Cell motility was determined by phase-contrast microscopy. Oxidase, catalase and urease activities, nitrate reduction and hydrolysis of casein, lecithin, gelatin, DNA, starch and Tween 80 were determined according to Barrow & Feltham (1993). The presence of fluorescent pigments was tested under UV light after 8 days on King's B medium (King *et al.*, 1954). Acid production from carbohydrates, enzyme production and additional characteristics were determined by using API 50 CH, API ZYM and API 20 NE strips (bioMérieux) and Biolog GENIII MicroPlates (Biolog) according to the instructions of the manufacturer. Tolerance of NaCl was determined by growth on nutrient agar (Cultimed) containing 0–7 % (w/v) NaCl after 20 days of incubation at 20 °C. Growth at −4 to 42 °C was determined on TSA after 14 days of incubation and growth at pH 3.5–11.0 (increments of 0.5 pH units) was determined in tryptic soy broth (TSB; Difco) after 10 days of incubation at 20 °C. Anaerobic growth was determined on TSB plus 1.5 % agar and on Marine agar (MA; Difco) after incubation in an anaerobic chamber at 20 °C for 14 days.

Cells of strain M1^T were Gram-reaction-negative, rod-shaped (0.8 × 1.5–2.0 μm) and motile by means of a single polar flagellum (Supplementary Fig. S1, available in IJSEM Online). Colonies of the novel isolate grown on TSA at 20 °C for 72 h were white, round, mucous, slightly convex and 1.5–2 mm in diameter and did not produce fluorescent pigments on King's B medium. The isolate grew at temperatures ranging from −4 to 34 °C and tolerated NaCl concentrations of up to 6 % (w/v) on nutrient agar. The novel isolate was negative for the hydrolysis of lecithin, casein, starch, Tween 80 and DNA. Other phenotypic characteristics of strain M1^T and its closest phylogenetic relatives are shown in Table 1. These phenotypic studies showed that the novel isolate displayed characteristics consistent with those of members of the genus *Pseudomonas* and could be clearly differentiated from the its most closely related species.

Cellular fatty acids from strain M1^T and its closest phylogenetic neighbours were prepared from 40 mg wet cell material harvested from a TSB agar (30 g TSB l^{-1}, 15 g agar l^{-1}) culture after 24 h of incubation at 28 °C. Whole-cell fatty acids were determined as described previously by Bozal *et al.* (2002). The mean fatty acid composition of strain M1^T, together with those of the type strains of the closest phylogenetic neighbours, are shown in Supplementary Table S1. The most abundant fatty acids were $C_{16:0}$ (34.9 %), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ ω7*c*; 21.5 %) and $C_{17:0}$ cyclo (16.1 %). This profile was similar to that of other phylogenetically related strains except for that of *P. lundensis* DSM 6252^T, which showed a remarkably high proportion of hydroxy fatty acids.

Polar lipids were analysed as described by Tindall (1990). The polar lipid profile of strain M1^T consisted of phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), and phosphatidylglycerol (PG) as the major components, moderate amounts of unknown aminolipids (AL1–3), unknown phospholipids (PL1, PL2) and unknown polar lipids (L3, L7, L9), and minor to trace amounts of unknown polar lipids (L1–2, L4–6, L8, L10–13) (Supplementary Fig. S4). Strain M1^T and its closest phylogenetic relative *Pseudomonas psychrophila* DSM 17535^T exhibited almost identical polar lipid profiles.

Genomic DNA of strain M1^T was prepared according to the protocol of Niemann *et al.* (1997). A 1452 nt portion of the 16S rRNA gene was sequenced as described previously by Bozal *et al.* (2002). PCR amplification and sequencing of the *rpoD* gene of strain M1^T were carried out as described previously (Yamamoto & Harayama, 1998). Multiple sequence alignments, distance matrix calculations and phylogenetic analysis of 16S rRNA and *rpoD* gene sequences from strain M1^T and related species, taken from GenBank, were performed using MEGA version 4.0 (Tamura *et al.*, 2007). Phylogenetic trees were reconstructed using the maximum-parsimony and neighbour-joining methods and their topological robustness was evaluated by bootstrap analysis based on 1000 replicates. For DNA–DNA hybridization experiments and the determination of G + C content, total DNA was prepared according to the procedure of Wilson (1987) with modifications. The G + C content was determined by using the HPLC technique as described by Mesbah *et al.* (1989). DNA–DNA hybridizations were performed at 47 °C according to the method described by Ezaki *et al.* (1989) with the modifications of Goris *et al.* (1998) and Cleenwerck *et al.* (2002).

Phylogenetic analyses based on 16S rRNA gene sequences confirmed that strain M1^T was a member of the genus *Pseudomonas* and was located in the *P. fragi* cluster with a bootstrap value of 99 % (Supplementary Fig. S2). 16S rRNA gene sequence similarity values greater than 98 %, the mean value considered to be the threshold for the identification of strains as the same species within a genus (Yarza *et al.*, 2008), were found with 13 type strains belonging to the *P. fluorescens* lineage (Supplementary Table S2). Due to the low resolution of 16S rRNA gene sequences at the intrageneric level, the *rpoD* gene, the most discriminating housekeeping gene described to date in the genus *Pseudomonas* (Mulet *et al.*, 2010), was selected for analysis. The comparison of this gene as individual datasets or as combined concatenated sequences provided a higher resolution analysis than 16S rRNA gene sequences alone and also complemented the results of DNA–DNA relatedness studies (Tindall *et al.*, 2010). Compressed and expanded *rpoD* phylogenetic trees, reconstructed using the neighbour-joining method with the Jukes–Cantor model, are shown in Fig. 1 and Supplementary Figure S3, respectively. As shown in Supplementary Table S2, only *P. psycrophila* (97.42 %) and *P. fragi* (96.40 %) exhibited high *rpoD* gene sequence similarities with respect to strain M1^T,

Table 1. Characteristics of strain M1^T and its closest phylogenetic neighbours

Strains: 1, M1^T; 2, *P. psychrophila* DSM 17535^T; 3, *P. fragi* DSM 3456^T; 4, *P. lundensis* DSM 6252^T; 5, *P. taetrolens* DSM 21104^T. All strains are Gram-reaction-negative rods that are motile by means of a polar flagellum and positive for catalase and oxidase activities. All data are from this study unless indicated otherwise. +, Positive; −, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5
Cell length (μm)	1.5–2.0	2.0–2.7	1.3–2.6	1.2–1.8	1.5–3.4
Cell diameter (μm)	0.8	0.7	0.8	0.7	0.6
Growth at:					
pH 4.5	−	−	+	−	−
−4 °C	+	+	+	W	+
36 °C	−	−	−	+	−
Pigments on King's B medium	−	−	+	+	−
Enzyme activities:					
Gelatinase	−	−	+	+	−
Esterase lipase C8	−	W	W	−	+
Trypsin, α-chymotrypsin	−	−	+	−	−
Valine arylamidase	−	w	+	w	w
Acid production from (API 50 CH):					
D-Arabitol, D-lyxose	−	−	−	−	+
Inositol	+	−	−	+	+
L-Rhamnose	−	+	−	−	+
D-Mannitol	+	+	−	−	+
Lactose	+	+	−	−	w
Sucrose, trehalose	+	+	+	−	−
Raffinose	+	+	−	−	−
Gentiobiose	+	+	w	−	+
L-Fucose	+	+	+	−	+
Oxidation of (Biolog GENIII):					
D-Mannose	−	+	+	−	+
D-Galacturonic acid	−	+	−	−	+
Glycerol, L-glutamic acid, L-histidine	−	+	−	+	+
L-Pyroglutamic acid	−	+	−	+	−
Quinic acid	+	−	+	−	+
Growth in the presence of (Biolog GENIII):					
1 % Sodium lactate	+	−	+	+	+
Troleandomycin	+	−	−	+	−
Lincomycin, fusidic acid, D-serine	+	−	+	+	−
Tetrazolium blue	+	−	+	+	−
DNA G+C content (mol%)	58.3	57.2*	59.2	60†	59.8‡
16S rRNA sequence similarity to strain M1^T (%)	100	99.4	99.4	99.3	98.6
rpoD sequence similarity to strain M1^T (%)	100	97.4	96.4	90.4	93.5
DNA–DNA hybridization with strain M1^T (%)	100	58	57	ND	ND

*Data from Yumoto *et al.* (2001).
†Data from Molin *et al.* (1986).
‡Data from De Vos *et al.* (1989).

since a 97 % sequence similarity in MLSA analysis has been proposed as the minimal value between strains of the same species (Mulet *et al.*, 2010). To further verify the taxonomic position of the Antarctic isolate M1^T, DNA–DNA hybridizations were performed with *P. psychrophila* LMG 24276^T and *P. fragi* LMG 2191^T. The resultant low DNA–DNA reassociation values (58 % and 57 % with the type strains of *P. psychrophila* and *P. fragi*, respectively), along with the results of 16S rRNA and *rpoD* gene sequence analyses, showed that strain M1^T occupied a distinct position in the genus *Pseudomonas* (Wayne *et al.*, 1987). The DNA G+C content of strain M1^T was 58.3 mol%, which lies within the range described for members of genus *Pseudomonas*.

The morphological, physiological, chemotaxonomic and phylogenetic data obtained in this study showed that strain M1^T belongs to the genus *Pseudomonas*. DNA–DNA hybridization analyses clearly distinguished strain M1^T from its closest relatives, *P. psychrophila* LMG 24276^T and *P. fragi* LMG 2191^T. On the basis of the data from this

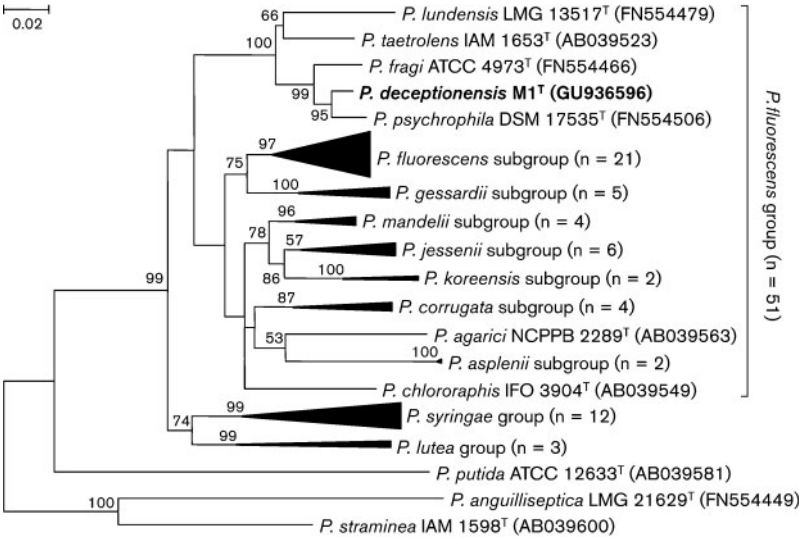

Fig. 1. Compressed neighbour-joining phylogenetic tree based on *rpoD* sequences of strain M1^T and type strains of closely related species of the genus *Pseudomonas*. Number of strains (n) in collapsed subgroups and GenBank accession numbers for individual type strains are indicated in parentheses. Bar, 0.02 substitutions per nucleotide position. Bootstrap values >50 % (based on 1000 replicates) are shown at branch points.

polyphasic study, M1^T represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas deceptionensis* sp. nov. is proposed.

Description of *Pseudomonas deceptionensis* sp. nov.

Pseudomonas deceptionensis (de.cep.tio.nen′sis. N.L. fem. adj. deceptionensis pertaining to Deception Island, Antarctic Sea).

Cells are rod-shaped (0.8 × 1.5–2.0 μm), Gram-reaction-negative, oxidase-positive, non-spore-forming and motile by means of a single polar flagella. Does not produce fluorescent pigments on King's B medium. After 72 h of incubation at 20 °C on TSA, colonies are 1.5–2.0 mm in diameter, white, round, mucous and slightly convex. Growth occurs at −4 to 34 °C and at pH 5–10. NaCl is tolerated at concentrations up to 6 % (w/v). Growth on MA is very poor under anaerobic conditions. Positive for catalase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities but negative for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. Negative for hydrolysis of aesculin, Tween 80, gelatin, casein, starch and lecithin but positive for hydrolysis of urea. Negative for indole and hydrogen sulfide production and nitrate reduction. In API 50 CH tests, acid is produced from D- and L-arabinose, D-fructose, cellobiose, D- and L-fucose, D-galactose, glycerol, D-glucose, inositol, lactose, D-mannitol, D-mannose, maltose, melibiose, raffinose, gentiobiose, D-ribose, sucrose, trehalose and D-xylose. In Biolog GENIII MicroPlates, positive for oxidation of D-galactose, D-glucuronic acid, D-fucose, glucuronamide, acetoacetic acid, L-fucose and quinic acid; also positive for growth in the presence of 1 % sodium lactate, troleandomycin, lincomycin, vancomycin, aztreonam, fusic acid, rifampicin SV, tetrazolium violet, D-serine, niaproof 4 and potassium telurite. Fatty acid profile contains $C_{16:0}$ (34.9 %), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$; 21.5 %) and $C_{17:0}$ cyclo (16.1 %), as the major fatty acids with smaller proportions of $C_{18:1}\omega7c$ (4.9 %), $C_{10:0}$ 3-OH (5.4 %), $C_{12:0}$ 2-OH (5.8 %), $C_{12:0}$ 3-OH (5.6 %), $C_{12:0}$ (3.6 %) and $C_{14:0}$ (1.7 %). The polar lipid profile consists of the major compounds PE, DPG and PG; moderate amounts of three unknown aminolipids, two unknown phospholipids and three unknown polar lipids; and minor to trace amounts of 10 unknown polar lipids.

The type strain, M1^T (=LMG 25555^T =CECT 7677^T), was isolated from a marine sediment sample collected from Deception Island, South Shetland Islands, Antarctica. The DNA G+C content of the type strain is 58.3 mol%.

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