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

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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 M1T, 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 M1T 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 M1T exhibited high sequence similarity only with respect to Pseudomonas psychrophila (97.42%) and P. fragi (96.40%) and DNA–DNA hybridization experiments between the Antarctic isolate M1T 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 M1T from related pseudomonads. Based on the evidence of this polyphasic taxonomic study, strain M1T represents a novel species, for which the name Pseudomonas deceptionensis sp. nov. is proposed. The type strain is M1T (=LMG 25555T = CECT 7677T).

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 (Kris 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 M1T, was able to grow at −4 to 34 °C and was characterized further in this study. The taxonomic status of strain M1T 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 M1T represented a novel species of the genus Pseudomonas, for which the name Pseudomonas deceptionensis sp. nov. is proposed.

Strain M1T 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), CaCl2 (0.03) and NaHCO3 (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 M1T and its most closely related species, Pseudomonas psychrophila DSM 17535T, Pseudomonas fragi DSM 3456T, Pseudomonas...
*P. lundensis* DSM 6252\textsuperscript{T} and *Pseudomonas taetrolens* DSM 21104\textsuperscript{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 (Nevo [et al.](#), 2006). Cell motility was determined by phase-contrast microscopy. Oxidase, catalase and urease activities, nitrate reduction and hydrolysis of casein, 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\textsuperscript{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\textsuperscript{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\textsuperscript{T} and its closest phylogenetic neighbours were prepared from 40 mg wet cell material harvested from a TSB agar (30 g TSB l\textsuperscript{–1}, 15 g agar l\textsuperscript{–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\textsuperscript{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\textsubscript{16:0} (34.9%), summed feature 3 (iso-C\textsubscript{15:0} 2-OH and/or C\textsubscript{16:1} \textbf{o7c} 21.5%) and C\textsubscript{17:0} cyclo (16.1%). This profile was similar to that of other phylogenetically related strains except for that of *P. lundensis* DSM 6252\textsuperscript{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\textsuperscript{T} consisted of phosphatidyethanolamine (PE), diphasatidylglycerol (DPG), and phosphatidylglycerol (PG) as the major components, moderate amounts of unknown aminolipids (A1L–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\textsuperscript{T} and its closest phylogenetic relative *Pseudomonas psychrophila* DSM 17535\textsuperscript{T} exhibited almost identical polar lipid profiles.

Genomic DNA of strain M1\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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.](#), 2002), 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. psychrophila* (97.42%) and *P. fragi* (96.40%) exhibited high *rpoD* gene sequence similarities with respect to strain M1\textsuperscript{T},
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 M1T, DNA–DNA hybridizations were performed with \textit{P. psychrophila} LMG 24276T and \textit{P. fragi} LMG 2191T. The resultant low DNA–DNA reassociation values (58% and 57% with the type strains of \textit{P. psychrophila} and \textit{P. fragi}, respectively), along with the results of 16S rRNA and \textit{rpoD} gene sequence analyses, showed that strain M1T occupied a distinct position in the genus \textit{Pseudomonas} (Wayne et al., 1987). The DNA G+C content of strain M1T was 58.3 mol%, which lies within the range described for members of genus \textit{Pseudomonas}.

The morphological, physiological, chemotaxonomic and phylogenetic data obtained in this study showed that strain M1T belongs to the genus \textit{Pseudomonas}. DNA–DNA hybridization analyses clearly distinguished strain M1T from its closest relatives, \textit{P. psychrophila} LMG 24276T and \textit{P. fragi} LMG 2191T. On the basis of the data from this

\begin{table}[h]
\centering
\caption{Characteristics of strain M1T and its closest phylogenetic neighbours}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Characteristic} & \textbf{1} & \textbf{2} & \textbf{3} & \textbf{4} & \textbf{5} \\
\hline
Cell length (\text{\textmu}m) & 1.5–2.0 & 2.0–2.7 & 1.3–2.6 & 1.2–1.8 & 1.5–3.4 \\
Cell diameter (\text{\textmu}m) & 0.8 & 0.7 & 0.8 & 0.7 & 0.6 \\
\hline
Growth at: & & & & & \\
\quad pH 4.5 & – & – & + & – & – \\
\quad –4 °C & + & + & + & w & + \\
\quad 36 °C & – & – & – & + & – \\
\hline
Pigments on King’s B medium & – & – & + & – & – \\
\hline
Enzyme activities: & & & & & \\
\quad Gelatinase & – & – & + & + & – \\
\quad Esterase lipase C8 & – & w & w & – & + \\
\quad Trypsin, \textalpha\text{-}chymotrypsin & – & – & + & – & – \\
\quad Valine arylamidase & – & w & + & w & w \\
\hline
Acid production from (API 50 CH): & & & & & \\
\quad D-Arabitol, D-lyxose & – & – & – & – & + \\
\quad Inositol & + & – & – & + & + \\
\quad L-Rhamnose & – & + & – & – & + \\
\quad D-Mannitol & + & + & – & – & + \\
\quad Lactose & + & + & – & – & w \\
\quad Sucrose, trehalose & + & + & – & – & – \\
\quad Raffinose & + & + & – & – & – \\
\quad Gentiobiose & + & + & w & – & + \\
\quad L-Fucose & + & + & w & + & – \\
\hline
Oxidation of (Biolog GENIII): & & & & & \\
\quad D-Mannose & – & + & + & – & + \\
\quad D-Galacturonic acid & – & + & – & – & – \\
\quad Glycerol, \textalpha\text{-}glutamic acid, \textalpha\text{-}histidine & – & + & – & + & + \\
\quad L-Pyrogulatmic acid & – & + & – & – & – \\
\quad Quinic acid & + & – & + & – & – \\
\hline
Growth in the presence of (Biolog GENIII): & & & & & \\
\quad 1 % Sodium lactate & + & – & + & + & + \\
\quad Troleandomycin & + & – & + & – & + \\
\quad Lincomycin, fusidic acid, D-serine & + & – & + & – & + \\
\quad Tetrazolium blue & + & – & + & – & + \\
\hline
DNA G+C content (mol\%): & 58.3 & 57.2* & 59.2 & 60† & 59.8‡ \\
\hline
16S rRNA sequence similarity to strain M1T (%): & 100 & 99.4 & 99.4 & 99.3 & 98.6 \\
\hline
rpoD sequence similarity to strain M1T (%): & 100 & 97.4 & 96.4 & 90.4 & 93.5 \\
\hline
DNA–DNA hybridization with strain M1T (%): & 100 & 58 & 57 & ND & ND \\
\hline
\end{tabular}
\end{table}

*Data from Yumoto et al. (2001).
†Data from Molin et al. (1986).
‡Data from De Vos et al. (1989).
polyphasic study, M1T 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-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 24 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, x-chymotrypsin, x- and β-galactosidase, β-glucuronidase, x- and β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase and x-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-xyllose. In Biolog GENIII MicroPlates, positive for oxidation of D-galactose, D-glucuronic acid, D-fucose, glucuronamide, acetocetate acid, L-fucose and quinic acid; also positive for growth in the presence of 1 % sodium lactate, treleandomycin, lincomycin, vancomycin, aztreonam, fusid acid, rifampicin SV, tetrazolium violet, D-serine, niaprof 4 and potassium telurite. Fatty acid profile contains C16:0 (34.9 %), summed feature 3 (iso-C15:0 2-OH and/or C16:1; 21.5 %) and C17:0 cyclo (16.1 %), as the major fatty acids with smaller proportions of C18:1ω7c (4.9 %), C10:0 3-OH (5.4 %), C12:0 2-OH (5.8 %), C12:0 3-OH (5.6 %), C13:0 3-OH (3.6 %) and C14:0 (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, M1T (=LMG 25555T =CECT 7677T), 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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**References**


**Fig. 1.** Compressed neighbour-joining phylogenetic tree based on rpoD sequences of strain M1T 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.


