Pseudomonas yamanorum sp. nov., a psychrotolerant bacterium isolated from a subantarctic environment

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A psychrotolerant strain, 8H1^T, was isolated from soil samples collected in Isla de los Estados, Ushuaia, Argentina. Cells were Gram-negative, aerobic, straight rods, occurring singly or in pairs, non-spore-forming and motile by means of two polar flagella. The isolate was able to grow in the range 4–35 °C, with optimum growth at 28 °C. The predominant cellular fatty acids were summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{16:0}$ and summed feature 8 ($C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$). The polar lipid pattern of strain 8H1^T comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid. Ubiquinone 9 (Q-9) was the predominant lipoquinone. The DNA G+C content was 59.8 mol%. 16S rRNA gene sequence-based phylogeny suggested the affiliation of strain 8H1^T to the '*Pseudomonas fluorescens* group', displaying ⩾98.5 % sequence similarity to 29 type strains. A multilocus sequence analysis (MLSA) study performed by concatenating 16S rRNA, *gyrB*, *rpoD* and *rpoB* gene sequences showed that isolate 8H1^T could be discriminated from closely related species of the genus *Pseudomonas* and placed in the '*Pseudomonas gessardii* subgroup', including the species with the highest MLSA sequence similarities: *Pseudomonas brenneri* (96.2 %), *P. gessardii* (96.1 %), *P. proteolytica* (96.0 %), *P. meridiana* (96.0 %) and *P. mucidolens* (95.4 %). DNA–DNA hybridization analysis between 8H1^T and the type strains of these closely related species revealed relatedness values of 27.0, 8.8, 41.2, 39.7 and 46.1 %, respectively. These results, together with differences in several phenotypic features, support the classification of a novel species, for which the name *Pseudomonas yamanorum* sp. nov. is proposed. The type strain is 8H1^T (=DSM 26522^T=CCUG 63249^T=LMG 27247^T).

The genus *Pseudomonas* Migula 1894 was originally described to include Gram-negative, strictly aerobic rods that are motile by polar flagella; the name was included on the Approved Lists of Bacterial Names (Skerman *et al.*, 1980). The genus is characterized by a high level of metabolic diversity and it is one of the most diverse and ubiquitous bacterial genera, species of which have been isolated worldwide in many environments, from Antarctica to the Tropics, from sediments, water, soil, the sea, deserts, the plant rhizosphere, fungi, diseased animal specimens and human clinical samples (Peix *et al.*, 2009). The genus *Pseudomonas* (*sensu lato*) has been reclassified on the basis of phenotypic features (Lysenko, 1961; Stanier *et al.*, 1966; Sneath *et al.*, 1981), rRNA–DNA hybridization (Palleroni *et al.*, 1973), 16S rRNA gene sequence similarity (Anzai *et al.*, 2000) and chemotaxonomic data (Oyaizu & Komagata, 1983; Vancanneyt *et al.*, 1996). Currently, the species belonging to the genus *Pseudomonas sensu stricto* are restricted to rRNA similarity group I of Palleroni (1984), which belongs to the class *Gammaproteobacteria*, and they make up close to 140 species with names that have standing in nomenclature according to the LPSN (http://www.bacterio.net/pseudomonas.html; Euzéby, 1997). Since the ad hoc committee for the re-evaluation of the species definition in bacteriology (Stackebrandt *et al.*, 2002) recommended the evaluation and application of protein-coding gene sequence analysis to 'genomically circumscribe the taxon species and in order to differentiate it from

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gyrB*, *rpoB* and *rpoD* gene sequences of strain 8H1^T are respectively EU557337, EU557346, JX987893 and JX987895.

Nine supplementary figures and a supplementary table are available with the online Supplementary Material.

Abbreviations: ML, maximum-likelihood; MLSA, multilocus sequence analysis; MP, maximum-parsimony.

neighbouring species', phylogenetic analysis of sequences of several housekeeping genes, defined as multilocus sequence analysis (MLSA), has become a common method of genotypic characterization. Based on this approach, Mulet *et al.* (2010) provided an updated study for the phylogeny of the genus *Pseudomonas*, splitting it into two main lineages or intrageneric groups (IG), named IG *Pseudomonas aeruginosa* and IG *Pseudomonas fluorescens*. In this way, MLSA has been applied to recent descriptions of novel species of the genus *Pseudomonas* (Campos *et al.*, 2010; Hirota *et al.*, 2011; López *et al.*, 2012; Pascual *et al.*, 2012; Ramos *et al.*, 2013). However, identification of novel species not only requires genetic data, but must also be supported by phenotypic and chemotaxonomic data (the polyphasic approach; Kämpfer & Glaeser, 2012).

Strain 8H1^T was isolated from a soil sample collected from the Isla de los Estados Reservation, Ushuaia, Argentina, at the Observatory Island location (54° 39′ S 64° 08′ W) and selected for antimicrobial production (Sánchez *et al.*, 2009). The island rises to 800 m above sea-level and its mountainous geography could be considered as the south-eastern end of the Fuegian Andes. The climate is humid due to abundant rains (>2000 mm year^{-1}) and the horizontal contribution of marine winds, recording a mean temperature of 11 °C during summer; whereas the mean temperature is around 1 °C in winter.

For isolation of antimicrobial producers, 1 g defrosted soil sample was inoculated into 50 ml of either Luria–Bertani (LB) or M9 media and incubated at 8 and 20 °C with shaking at 200 r.p.m. for 24 h. Cultures were serially diluted and 0.1 ml aliquots were plated onto the same solid media and incubated at the same temperatures for 2 days or longer when necessary. Colonies were counted and their morphological characteristics recorded. Duplicate plates were made by randomly picking over 8000 colony morphotypes. Antimicrobial producers were identified by the deferred antagonism procedure of Gratia (1946) and Fredericq (1948) and also by a modified agar-well diffusion assay as described by Portrait *et al.* (1999).

In order to assess the taxonomic position of strain 8H1^T, a polyphasic approach was applied including phenotypic characterization, chemotaxonomic analysis (fatty acid methyl esters, polar lipids and respiratory quinones), DNA G+C content determination, phylogenetic analysis based on 16S rRNA, *gyrB*, *rpoD* and *rpoB* gene sequences individually and concatenated, and DNA–DNA hybridization experiments. The data obtained showed that isolate 8H1^T represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas yamanorum* sp. nov. is herein proposed.

Strain 8H1^T was routinely cultured aerobically in LB broth or on LB agar as basal medium (~pH 7) at 25 °C. Cell suspensions were prepared in 20 % (w/v) glycerol in LB medium for long-term storage at −80 °C.

The colony characteristics of strain 8H1^T were studied after growth on LB agar at 25 °C after 48 h incubation. Cell morphology and flagellation were examined by scanning electron microscopy at the Centro Integral de Microscopía Electrónica (LAMENOA), Tucumán, Argentina. Scanning electron microscope observation was conducted using a Zeiss Supra 55 VP scanning electron microscope (Carl Zeiss) at ×10 000–50 000 magnification (7.0 kV). For this, cells were harvested after 24 h of growth on LB agar at 25 °C, fixed overnight at 4 °C with the fixative of Karnovsky (1965), washed three times with 0.1 M phosphate buffer (pH 7.0) and increasing concentrations of ethanol (from 50 to 100 %, v/v) followed by a wash with 100 % acetone, mounted on 12 mm coverslips, dried to a critical point in CO_2 and finally coated with gold/palladium (80 : 20).

Gram staining was performed using a Difco Gram stain set. Catalase and oxidase activities were assayed as described previously (Smibert & Krieg, 1994). Acid production from carbohydrates, their assimilation as carbon sources and enzymic activities were determined by using the API 50 CH, API 20 NE, API 20 E and API ZYM systems (bioMérieux) according to the manufacturer's instructions with adaptation for the strictly aerobic metabolism of members of the genus *Pseudomonas*. The adaptation consisted of avoiding sealing with mineral oil in carbohydrate acidification tests. Susceptibility to antibiotics was determined by the disc diffusion assay using Gram-negative 1, 2, 3 and *Staphylococcus* A and B series discs (Laboratorios Britania). All determinations were performed at 25 °C in duplicate.

Other physiological and phenotypic features of strain 8H1^T were studied in parallel with type strains of closely related species by using commercial and conventional tests, all performed at 25 °C in duplicate. The API 20 Strep system was used to assess acid production from carbohydrates and some enzymic activities with the adaptation described above. The ability of each strain to utilize carbon compounds as sole carbon sources was determined as described by Shivaji *et al.* (1989). Hydrolysis of starch, gelatin (gelatinase) and tributyrin (tributyrin esterase), tolerance of 1 % (w/v) 2,3,5-triphenyltetrazolium chloride and production of fluorescent pigments on King's A and King's B media were assessed as described by Gavini *et al.* (1989). The optimum temperature for growth was determined by incubation at 4, 10, 15, 20, 22, 25, 28, 30, 35, 37 and 41 °C both in nutrient broth (NB; Merck) and on nutrient agar (NA; Merck), over a pH range of 3–13 (adjusted with HCl or NaOH). The optimal growth temperature was estimated by assessing changes in OD_{600} over the incubation period. Halotolerance was tested in NB containing 0–10 % (w/v) NaCl. All tests were read within 2–8 days of incubation.

Cells of 8H1^T were Gram-negative, aerobic, straight rods (0.40–0.45 × 1.3–1.8 μm), occurring singly and in pairs, non-spore-forming and motile by means of two polar flagella (Fig. 1 and Fig. S1, available in the online Supplementary Material). Colonies of the novel isolate grown on LB agar at 25 °C for 48 h were 1.5–2.5 mm in diameter, greenish and mucoid with irregular edges, and production of fluorescent pigments on King's A and B media was observed under UV light (365 nm).

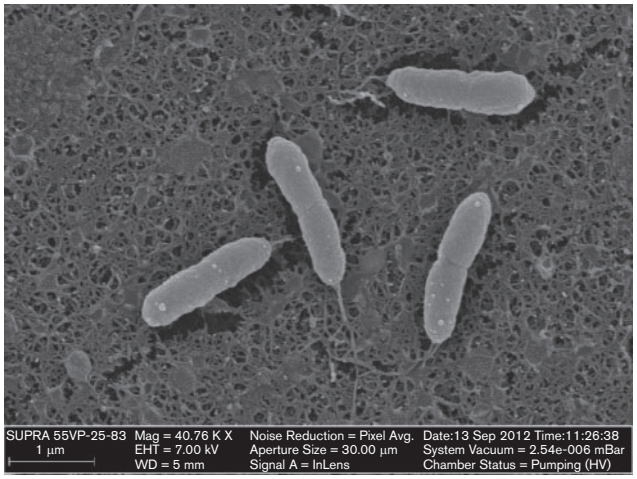

Fig. 1. Scanning electron micrograph of gold/palladium-coated cells of strain 8H1^T onto an agar background, showing the polar flagellation. Bar, 1 μm.

Strain 8H1^T was able to grow at 4–35 °C, with an optimum at 28 °C, and at pH 4.0–9.5 in NB and on NA. Tolerance of NaCl was observed at concentrations up to 6 % (w/v) in NB. Isolate 8H1^T was negative for the Voges–Proskauer test, indole and hydrogen sulfide production, hydrolysis of hippuric acid, nitrate reduction and denitrification. The novel strain 8H1^T displayed features consistent with those reported for members of the genus *Pseudomonas* and, as shown in Table 1, the isolate can be readily differentiated from other phylogenetically related species by several phenotypic properties. Optimum temperature for growth, tolerance of up to 6 % (w/v) NaCl, production of fluorescent pigment on King's A medium and assimilation of D-sorbitol distinguish strain 8H1^T from closely related species.

To characterize strain 8H1^T further, analyses of cellular fatty acid methyl esters, polar lipids, respiratory quinones and DNA G+C content were carried out by the Identification Service of the DSMZ, Braunschweig, Germany. Analyses of fatty acids, polar lipids, respiratory quinones (freeze-dried biomass) and DNA G+C content were carried out from cell biomass growing on trypticase soy broth (TSB) agar (30 g TSB and 15 g agar l^{-1}) at 28 °C for 24 h.

Fatty acids were extracted and prepared according to standard protocols described for the MIDI Microbial Identification System (Sasser, 1990). Cellular fatty acid content was analysed by GC with an Agilent 6890N gas chromatograph, with the MIDI Microbial Identification System using the TSBA6 method (MIDI, 2008) and Microbial Identification Sherlock software package version 6.1. The fatty acid profile of strain 8H1^T contained summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; 38.0 %), $C_{16:0}$ (35.4 %) and summed feature 8 ($C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$; 9.5 %) as the major fatty acids, small proportions of $C_{10:0}$ 3-OH (3.0 %), $C_{12:0}$ (2.4 %), $C_{12:0}$ 2-OH (3.7 %), $C_{12:0}$ 3-OH (3.7 %) and $C_{17:0}$ cyclo (2.2 %) and traces of other fatty acids (<1 %). The complete fatty acid profile is given in the species description. A comparative analysis between the fatty acid profile of strain 8H1^T and those in the database of the TSBA6 (version 6.10) library of MIDI showed the highest similarity indexes with *Pseudomonas mandelii* (0.798) and *P. taetrolens* (0.784). The presence of high levels of $C_{16:1}\omega7c$, $C_{16:0}$ and $C_{18:1}\omega7c$, together with the presence of $C_{10:0}$ 3-OH and $C_{12:0}$ 3-OH, in strain 8H1^T agreed with the fatty acid patterns of members of the genus *Pseudomonas* (Vancanneyt *et al.*, 1996).

Polar lipids and respiratory quinones of strain 8H1^T were analysed as described by Tindall (1990a, b). Polar lipid analysis of strain 8H1^T was performed by two-dimensional TLC. The polar lipids detected in strain 8H1^T were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid (Fig. S2). Strain 8H1^T contained respiratory quinones Q-9 (~91 %) and Q-8 (~5 %). The presence of Q-9 as a major ubiquinone is in agreement with the results obtained for species of the genus *Pseudomonas* (Palleroni, 2005; Toro *et al.*, 2013; Amoozegar *et al.*, 2014).

In order to determine the G+C base content, total DNA was prepared by cell disruption with a French pressure cell and purified on hydroxyapatite (Cashion *et al.*, 1977). The DNA was hydrolysed with P1 nuclease and dephosphorylated with bovine alkaline phosphatase. G+C content was determined by HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) according to the method of Mesbah *et al.* (1989). The DNA G+C content of strain 8H1^T was 59.8 mol%, which is in agreement with the range described for members of the genus *Pseudomonas* (Palleroni, 1984; 2005).

Genomic DNA was extracted and purified as described by Sambrook *et al.* (1989). Universal primers 27F/1492R and UP1/UP2r, respectively corresponding to the *rrnB* operon (Brosius *et al.*, 1978) and positions 316–1479 of the sequence of *Escherichia coli* K-12 (Yamamoto & Harayama, 1995), were used for amplification and partial sequencing of the 16S rRNA (1436 bp) and *gyrB* (1124 bp) genes, respectively, as described previously (Sánchez *et al.*, 2009).

Amplification and partial sequencing of the *rpoB* gene (1140 bp) were carried out using universal primers LAPS5/LAPS27 covering the *rpoB* sequence of *P. aeruginosa* PAO1 (GenBank accession no. AE004091) from position 1531 to 2760 as described by Ait Tayeb *et al.* (2005). In the same way, the *rpoD* (658 bp) gene was amplified by using universal primers PsEG30F/PsEG790R covering the *rpoD* sequence of *Pseudomonas alcaligenes* from position 28 to 767 as described by Mulet *et al.* (2009).

PCR products were purified by using the AccuPrep PCR Purification kit (Bioneer) according to the manufacturer's instructions and direct sequence determination was performed in an Applied Biosystems automated sequencer (ABI/Hitachi Genetic Analyzer 3130) at the Centro de Referencia para Lactobacilos (CERELA), Tucumán, Argentina. Other

Table 1. Differential characteristics of strain 8H1^T and type strains of related species of the '*P. gessardii* subgroup'

Strains: 1, 8H1^T; 2, *P. brenneri* CIP 106646^T (unless indicated, data from Baïda *et al.*, 2001); 3, *P. proteolytica* DSM 15321^T; 4, *P. gessardii* CIP 105469^T (unless indicated, data from Verhille *et al.*, 1999); 5, *P. mucidolens* DSM 19186^T; 6, *P. meridiana* CIP 108465^T. Data are from this study unless indicated otherwise. +, Positive; W, weakly positive; −, negative; ND, no data available; TTC, 2,3,5-triphenyltetrazolium chloride. Data in brackets indicate that the result differs among strains.

Characteristic	1	2	3	4	5	6
DNA G+C content (mol%)	59.8	58	58.3[a]*	58	61[b]	63.2[a]
Genetic relatedness to strain 8H1^T (%)						
16S rRNA gene sequence similarity	(100)	99.7	99.4	99.2	98.9	98.8
MLSA sequence similarity	(100)	96.2	96.0	96.1	95.4	96.0
DNA–DNA hybridization	(100)	27.0	41.2	8.8	46.1	39.7
Temperature for growth (°C)						
Optimum	28	25	22	30	25	22
Range	4–35	4–37	4–30	4–35	4–37	4–30
pH range for growth	4.0–9.5	ND	4.5–9.5	ND	5.0–9.0	4.5–9.5
Tolerance of NaCl (%, w/v)	0–6	0.8–3	0–5	[0–0.8]†	0–5	0–3
Tolerance of 1 % (w/v) TTC	+	+	−	[−]	−	−
Starch hydrolysis	−	+[c]	−	−[c]	−	−
Gelatinase	+	+[a]	+	[−]	−	−
Tributyrin esterase	+	+	+	[−]	W	W
Fluorescent pigment on King's A medium	+	−	−	−	−	−
Assimilation of:						
L-Arabinose	+	−	−	−	+	+
D-Xylose	+	−	−	−	+	−
Inositol	+	+	−	+	+	+
D-Sorbitol	+	−	−	−	−	−
Acid produced from:						
L-Arabinose	+	ND	−	ND	+	−
D-Mannitol	−	ND	+	ND	+	+
D-Sorbitol	+	ND	−	ND	−	−
Starch	−	+	−	ND	−	−
Enzyme activities						
Arginine dihydrolase	+	+	−	ND	−	−
Pyrrolidonyl arylamidase	+	ND	+	+	−	−
Leucine arylamidase	+	+	+	+[d]	W	W
Alkaline phosphatase	+	[+]	−	ND	−	−

*Data from: *a*, Reddy *et al.* (2004); *b*, De Vos *et al.* (1989); *c*, Baïda *et al.* (2002); *d*, Park *et al.* (2005).
†Other strains of *P. gessardii* tolerate up to 3 % (w/v) NaCl.

sequences analysed in this paper were obtained from the GenBank database and their accession numbers are displayed on phylogenetic trees based on single genes.

Identification of phylogenetic neighbours was initially carried out based on analysis of the partial 16S rRNA gene sequence by using the EzTaxon server (http://www.ezbiocloud.net/eztaxon; Kim *et al.*, 2012) coupled with the RDP database (http://rdp.cme.msu.edu/seqmatch; Cole *et al.*, 2014). Pairwise 16S rRNA gene sequence similarity was calculated using the global alignment algorithm (Myers & Miller, 1988), implemented at the EzTaxon server (http://www.ezbiocloud.net/eztaxon; Kim *et al.*, 2012). Pairwise sequence similarity for the other analysed genes was calculated with the jPHYDIT program (Jeon *et al.*, 2005). Phylogenetic analysis based on 16S rRNA, *gyrB*, *rpoD* and *rpoB* nucleotide sequences, both individually and concatenated (MLSA), was performed using sequences of type strains of related species, showing 16S rRNA gene sequence similarities $\geqslant$98.5 %. Similar studies were performed previously by Ramírez-Bahena *et al.* (2014).

16S rRNA gene sequences were aligned using the secondary structure-aware Infernal aligner (Nawrocki *et al.*, 2009) available at RDP (http://pyro.cme.msu.edu), whereas nucleotide sequences of protein-coding genes were aligned individually according to translated amino acid sequences using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic analysis was performed with MEGA 5 (Tamura *et al.*, 2011) by using the maximum-likelihood (ML) and maximum-parsimony (MP) algorithms; the all sites option was selected to remove ambiguous positions from each sequence pair, using all available comparative data due to uneven lengths of sequences employed. For ML, the best-fitting substitution model was determined with the Akaike information criterion

corrected (AICc) option using the ML model test implemented in MEGA 5. The robustness of the tree topology was evaluated by bootstrap analysis based on 1000 replicates. The trees obtained with the ML and MP methods for the 16S rRNA, *gyrB*, *rpoD* and *rpoB* genes are shown in Figs S3, S4, S5 and S6, respectively. The MP trees showed similar topologies to their respective ML trees for the different genes analysed. Phylogenetic analysis of the 16S rRNA gene confirmed that strain 8H1^T belongs to the '*P. fluorescens* group'. However, strain 8H1^T did not form a robust clade (bootstrap values $<$70 %) with other members of the genus *Pseudomonas* that showed high sequence similarity. According to the EzTaxon server, 16S rRNA gene sequence similarity values $\geqslant$98.5 %, the mean value considered to be the threshold for the identification of strains as the same species within a genus (Stackebrandt & Ebers, 2006), were found with 29 type strains belonging to the '*P. fluorescens* group' (Table S1). The closest relatives of strain 8H1^T were found to be *Pseudomonas brenneri* DSM 15294^T (99.7 %), *P. proteolytica* CMS 64^T (99.4 %) (both belonging to the '*P. gessardii* subgroup') and *P. migulae* CIP 105470^T (99.4 %), which belongs to the '*P. mandelii* subgroup' as defined by Mulet *et al.* (2010). Phylogenetic analysis of the *rpoD* gene, the most discriminating housekeeping gene for members of the genus *Pseudomonas* (Kämpfer & Glaeser, 2012; Mulet *et al.*, 2010), placed strain 8H1^T into the '*P. gessardii* subgroup' supported by a high bootstrap value, whereas analysis of the *rpoB* gene showed 8H1^T to be grouped with *P. brenneri*, although its taxonomic position was not supported by a high bootstrap value. A different phylogenetic position of 8H1^T was observed from analysis of the *gyrB* gene, where 8H1^T and *Pseudomonas veronii* were clustered together.

Due to the low resolution of the 16S rRNA gene sequence at the intragenic level and the observation of incongruences in the phylogeny inferred from the partial *gyrB* gene sequence, the phylogenetic position of strain 8H1^T was refined by using an MLSA of 16S rRNA, *gyrB*, *rpoD* and *rpoB* genes. Phylogenetic differentiation based on MLSA of these concatenated gene sequences was applied previously by Mulet *et al.* (2010, 2012) and Pascual *et al.* (2012). Since MLSA analyses a larger number of nucleotides than a single gene analysis, it allows possible effects on phylogenetic relationships that could be generated by homologous recombination and/or lateral gene transfer to be overcome and, therefore, it could be considered that MLSA provides a more reliable evolutionary pattern among the included species. For construction of concatenated sequences, individual alignments were trimmed to match the length of the shortest ORF in the alignment and concatenated in the following order: 16S rRNA (from alignment position 49 to 1479, including gaps), *gyrB* (from 208 to 1002), *rpoD* (from 103 to 735) and *rpoB* (from 118 to 969). ML and MP phylogenetic trees and MLSA pairwise nucleotide sequence similarity values were obtained as described above.

Both ML (Fig. 2) and MP (Fig. S7) trees based on the four concatenated genes showed that strain 8H1^T is located in the '*P. gessardii* subgroup' supported by high bootstrap values. ML and MP analyses were also performed for nucleotide (Fig. S8) and translated amino acid (Fig. S9) sequences of the *gyrB*, *rpoD* and *rpoB* genes, concatenated in the above-mentioned order. The relative position of strain 8H1^T was essentially the same as that obtained with the four genes concatenated. As shown in Tables 1 and S1, only the species belonging to the '*P. gessardii* subgroup' exhibited high MLSA nucleotide sequence similarities with respect to strain 8H1^T, since a 97 % sequence similarity in MLSA analysis of these four genes has been established as the threshold value for strains in the same species for the genus *Pseudomonas* (Mulet *et al.*, 2010). In addition, the closest related species according to fatty acid methyl ester analysis, *P. mandelii* (94.2 %) and *P. taetrolens* (92.2 %), showed MLSA similarities below the boundary value. Also, *gyrB*, *rpoB* and *rpoD* gene sequence similarities were always below this threshold value, being highest mainly with members of the '*P. gessardii* subgroup' (Table S1).

In order to verify the taxonomic position of strain 8H1^T, spectroscopic DNA–DNA hybridization was performed by the Identification Service at the DSMZ, including only the type strains belonging to the '*P. gessardii* subgroup'. Cells were disrupted by using a Constant Systems TS 0.75 kW instrument (IUL Instruments) and DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) with modifications described by Huss *et al.* (1983). A Cary 100 Bio UV/Vis-spectrophotometer equipped with Peltier-thermostatted 6 $\times$ 6 multicell changer and temperature controller with *in-situ* temperature probe (Varian) was used. Mean DNA–DNA relatedness values (duplicate values) between 8H1^T and *Pseudomonas mucidolens* DSM 19186^T, *P. proteolytica* DSM 15321^T, *P. meridiana* CIP 108465^T, *P. brenneri* DSM 15294^T and *P. gessardii* DSM 17152^T were 46.1 $\pm$ 10.6, 41.2 $\pm$ 7.4, 39.7 $\pm$ 10.9, 27.0 $\pm$ 4.9 and 8.8 $\pm$ 7.6 %, respectively (Table 1). These results demonstrated a separate species status for strain 8H1^T, if the recommendation by the ad hoc committee (Wayne *et al.*, 1987) of 70 % DNA–DNA relatedness (threshold value) for the definition of bacterial species is considered.

To conclude, the morphological, physiological, chemotaxonomic and phylogenetic data obtained in this study showed that strain 8H1^T belongs to the genus *Pseudomonas*, and it is included in the '*P. gessardii* subgroup'. The results of MLSA, DNA G+C content, DNA–DNA hybridization, physiological and phenotypic analyses distinguished strain 8H1^T from the known species of this group. Therefore, 8H1^T represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas yamanorum* sp. nov. is proposed.

Description of *Pseudomonas yamanorum* sp. nov.

Pseudomonas yamanorum (ya.ma.no′rum. N.L. gen. pl. n. *yamanorum* of the Yámanas, a tribe indigenous to the Ushuaia region, from where the type strain was isolated).

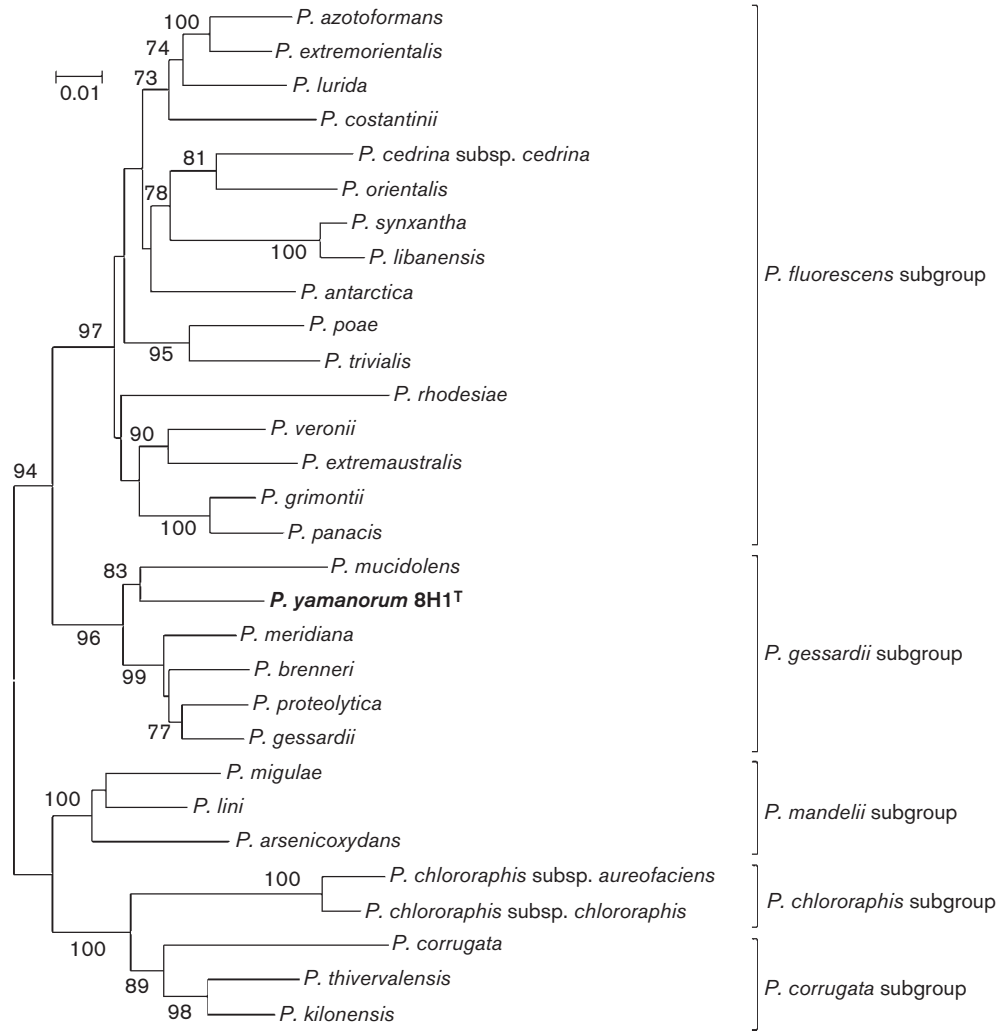

Fig. 2. Unrooted ML phylogenetic tree based on concatenated nucleotide sequences of the 16S rRNA, *gyrB*, *rpoD* and *rpoB* genes, showing the position of strain 8H1^T within the closest related type strains of the '*P. fluorescens* group'. The tree was reconstructed using the GTR model by using a discrete Gamma distribution (+G) with five rate categories and by assuming that some sites are evolutionary invariable (+I). All codon positions were considered. All ambiguous positions were removed for each sequence pair; 3711 positions were in the final dataset. GenBank accession numbers are displayed in the phylogenetic trees based on single genes (Figs S3–S6). Subgroups according to Mulet *et al.* (2010) are indicated to the right. Bar, 0.01 substitutions per nucleotide position. Bootstrap values $\geqslant$70 % (based on 1000 replicates) are shown at branch points.

Gram-negative, aerobic, straight rods (0.40–0.45 × 1.3–1.8 μm), occurring singly and in pairs, non-spore-forming and motile by two polar flagella. After 48 h of incubation at 25 °C on LB agar, colonies are 1.5–2.5 mm in diameter, greenish and mucoid with irregular edges. Growth occurs at 4–35 °C with an optimum at 28 °C, and at pH 4.0–9.5. Able to grow in the presence of 0–6 % (w/v) NaCl. Growth is observed on NA containing 1 % (w/v) 2,3,5-triphenyltetrazolium chloride, with slightly red to pink colonies. Green fluorescent pigments are produced on King's A and B media. Positive for catalase, oxidase, arginine dihydrolase, gelatinase, tributyrin esterase, leucine arylamidase, pyrrolidonyl arylamidase, valine arylamidase, trypsin, alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities, but negative for activities of lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase and α-chymotrypsin. Does not ferment glucose. Negative for the Voges–Proskauer test, production of indole and hydrogen sulfide, hydrolysis of hippuric acid and starch, nitrate reduction and denitrification. Acid is produced from D- and L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, melibiose, trehalose, D-lyxose, D- and L-fucose, gentiobiose, *N*-acetyl-D-glucosamine, potassium gluconate, potassium 2-ketogluconate, glycerol, erythritol, xylitol, inositol, D-adonitol,

D- and L-arabitol and D-sorbitol. No acid is produced from amygdalin, arbutin, aesculin ferric citrate, salicin, inulin, starch, glycogen, L-xylose, L-sorbose, cellobiose, maltose, lactose, sucrose, melezitose, raffinose, turanose, D-tagatose, dulcitol, D-mannitol, methyl β-D-xylopyranoside, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside and potassium 5-ketogluconate. Utilizes glycerol, adonitol, xylitol, inositol, D-sorbitol, D-galactose, D-xylose, D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, calcium 2-ketogluconate, gluconate, malate, citrate and capric, adipic and phenylacetic acids as sole carbon sources, but not D-mannitol, maltose, cellobiose, lactose, sucrose, melezitose, raffinose or methyl α-D-glucopyranoside. Sensitive to the antibiotics amikacin, azithromycin, aztreonam, cefepime, cefoperazone/sulbactam, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, minocycline, piperacillin, piperacillin/tazobactam and rifampicin, but resistant to ampicillin/sulbactam, cefalotin, cefotaxime, cefuroxime, ceftazidime, chloramphenicol, clindamycin, colistin, erythromycin, oxacillin, penicillin, teicoplanin, trimethoprim/sulfamethoxazole (cotrimoxazole) and vancomycin. The whole-cell fatty acid profile contains $C_{10:0}$, $C_{10:0}$ 3-OH, $C_{12:0}$, $C_{12:0}$ 2-OH, $C_{12:0}$ 3-OH, $C_{14:0}$, $C_{15:0}$, summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{16:1}\omega5c$, $C_{16:0}$, $C_{17:1}\omega8c$, $C_{17:0}$ cyclo, $C_{17:0}$, summed feature 8 ($C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$), $C_{18:0}$ and $C_{19:0}$ cyclo $\omega8c$. Polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid. Ubiquinone 9 (Q-9) is the main lipoquinone.

The type strain, 8H1^T (=DSM 26522^T=CCUG 63249^T=LMG 27247^T), was isolated from a soil sample collected in Isla de los Estados, Ushuaia, Argentina. No clinical significance is known. The DNA G+C content of the type strain is 59.8 mol%.

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