Pseudomonas xinjiangensis sp. nov., a moderately thermotolerant bacterium isolated from desert sand

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A yellow-pigmented, moderately thermotolerant bacterial strain, designated S3-3^T, was isolated from desert sand in Xinjiang province, China, and subjected to a polyphasic taxonomic characterization. This isolate was Gram-negative, aerobic and motile. Phylogenetic analyses based on the 16S rRNA gene sequence showed that strain S3-3^T is phylogenetically affiliated to the genus *Pseudomonas*. Strain S3-3^T exhibited levels of 16S rRNA gene sequence similarity of less than 96.3% to the type strains of all *Pseudomonas* species, making it clear that strain S3-3^T represents a species that is separate from previously recognized *Pseudomonas* species. Its major fatty acids were summed feature 8 (C_{18:1}ω6c and/or C_{18:1}ω7c), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and C_{19:0} cyclo ω8c. The major isoprenoid quinone was Q-9. The DNA G + C content was 60.9 mol%. On the basis of phylogenetic, phenotypic and chemotaxonomic analysis, strain S3-3^T should be classified within a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas xinjiangensis* sp. nov. is proposed. The type strain is S3-3^T (=CCTCC AB 207151^T =NRRL B-51270^T).

Pseudomonas species are important members of natural microbial communities. The genus was first created by Migula (1894) and, at the time of writing, 181 species with validly published names have been described (<http://www.bacterio.cict.fr/p/pseudomonas.html>). Most species in the genus *Pseudomonas* are motile by one or several polar flagella, fail to grow under acid conditions, contain C_{10:0} 3-OH, C_{12:0} and C_{12:0} 2-OH as major fatty acids and do not accumulate polyhydroxybutyrate granules (Palleroni, 2005). *Pseudomonas* species have been isolated from diverse environments such as soils, plants, fresh water, clinical specimens and marine environments (Palleroni, 1992; Romanenko *et al.*, 2005). However, few species have been recovered from dry environments; the characteristics of pseudomonads isolated from deserts have not been reported.

In the present study, strain S3-3^T was isolated from a sand sample from a desert in Xinjiang province, China, using tenfold-diluted tryptic soy broth (TSB/10; Difco) agar. After 1 week of incubation at 30 °C, colonies were yellow, circular and convex (<1 mm in diameter) with entire edges. Cell morphology was examined by light microscopy and electron microscopy. Motility was observed by using motility agar. Cells were rod-shaped and motile. The strain

could grow on marine agar 2216 (MA; Difco) and R2A agar (Difco).

Genomic DNA for amplification of the 16S rRNA gene was extracted with a Bacteria Genomic DNA Isolation kit (Shanghai Chaoshi Bio Technologies). The 16S rRNA gene was amplified by PCR with bacterial universal primers 27F and 1492R (Lane, 1991) and the PCR products were sequenced by Invitrogen Biotechnology Co. Ltd. Similarity searches with the derived sequences were done by BLAST at NCBI (<http://www.ncbi.nlm.nih.gov/>). Phylogenetic analysis was performed by using MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of the data via CLUSTAL_X (Thompson *et al.*, 1997). Distances were obtained using options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony method (MEGA 3.1; data not shown). The topology of the neighbour-joining phylogenetic tree was evaluated by using bootstrap resampling (Felsenstein, 1985) with 1000 replications (Fig. 1).

The 16S rRNA gene sequence of strain S3-3^T showed the highest similarity to those of *Pseudomonas plecoglossicida* FPC951^T (96.23%), followed by *Pseudomonas pachastrellae* KMM 330^T (96.16%), *Pseudomonas mosselii* CIP 105259^T (GenBank accession no. AF072688, not shown in Fig. 1; 96.16%) and *Pseudomonas monteilli* CIP 104883^T (AF064458, not shown in Fig. 1; 96.06%), and showed

Abbreviation: PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain S3-3^T is EU286805.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain S3-3^T and other species of the genus *Pseudomonas*. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are given at nodes. Bar, 0.5 % sequence difference.

less than 96 % sequence similarity to other species of the genus *Pseudomonas*. A global phylogenetic analysis indicates that the strain cannot be affiliated to any of the intrageneric groups (the *P. syringae*, *P. chlororaphis*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *P. aeruginosa* and *P. pertucinogena* groups) proposed by Anzai *et al.* (2000) within *Pseudomonas sensu stricto* (data not shown), forming a distinct branch between *P. pachastrellae* and the *P. pertucinogena* group. The sequences from its close neighbours and representative species (*Pseudomonas syringae*, *P. chlororaphis*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *P. aeruginosa* and *P. pertucinogena*) from each of the *Pseudomonas* intrageneric groups were selected to build the phylogenetic tree (Fig. 1).

Fatty acid methyl esters were analysed by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's instructions (MIDI, 2005) after 48 h incubation on TSB agar at 30 °C. The strain included summed feature 8 (*C*_{18:1}ω6*c* and/or *C*_{18:1}ω7*c*) (24.48 %), summed feature 3 (*C*_{16:1}ω7*c* and/or iso-*C*_{15:0} 2-OH) (19.70 %), *C*_{19:0} cyclo ω8*c* (10.78 %), *C*_{17:0} cyclo (7.54 %), *C*_{16:0} (6.58 %), *C*_{12:0} (5.57 %) and *C*_{13:0} 3-OH (4.45 %) as major fatty acids. The respiratory quinone system was extracted and determined by HPLC as described by Xie & Yokota (2003). Analysis of respiratory lipoquinones indicated that the isolate contained ubiquinone-9 (Q-9), corresponding to the characteristic feature of members of the genus *Pseudomonas* (Oyaizu & Komagata, 1983). The DNA G+C content of strain S3-3^T, determined using an HPLC method (Mesbah *et al.*, 1989), was 60.9 mol%.

To investigate the physiological and biochemical characteristics of strain S3-3^T further, we used the methods described by Palleroni & Doudoroff (1972) and Smibert & Krieg (1994) for the following tests: Gram reaction, oxidase and catalase reactions, accumulation of poly-β-hydroxybutyrate (PHB), arginine dihydrolase, nitrate reduction and hydrolysis of gelatin, starch, casein and Tween 80. Production of pyocyanin and formation of fluorescent pigments were tested on King A and King B medium, respectively (King *et al.*, 1954). Temperature tolerance was tested by checking growth at 4, 12, 21, 30, 37, 42 and 45 °C and tolerance of salinity was tested with growth on TSB agar with 1, 3, 5, 7, 9, 10 and 11 % (w/v) NaCl. The pH

range (pH 4–10 at intervals of 1 pH unit) for growth was determined in marine broth (Difco). In addition, the API 20NE, API ZYM systems (bioMérieux) and Biolog GN2 MicroPlates (Biolog) were used to test biochemical properties and these tests were performed according to the manufacturers' instructions. The physiological and biochemical characteristics of strain S3-3^T are listed in the species description and in Table 1.

Strain S3-3^T showed characteristics similar to those of *P. pachastrellae* in their lack of carbohydrate utilization. However, strain S3-3^T, *P. plecoglossicida* and *P. pachastrellae* utilize different carbohydrates. Strain S3-3^T can be distinguished from *P. pertucinogena* based on the presence of intracellular pigment, accumulation of PHB and hydrolysis of Tween 80 (Table 1).

Organisms sharing 16S rRNA gene sequence similarity of less than 97 % are usually regarded as belonging to different bacterial species (Stackebrandt & Goebel, 1994). Thus, from the phylogenetic analysis and the physiological tests, it is evident that strain S3-3^T represents a distinct, previously undescribed species within the genus *Pseudomonas*. In conclusion, strain S3-3^T is phylogenetically and physiologically distinct and represents a novel species, for which we propose the name *Pseudomonas xinjiangensis* sp. nov.

Description of *Pseudomonas xinjiangensis* sp. nov.

Pseudomonas xinjiangensis (xin.jiang.en'sis. N.L. fem. adj. *xinjiangensis* pertaining to Xinjiang, in north-west China, where the type strain was isolated).

Cells are Gram-negative, motile, non-spore-forming rods, 0.9–1.8 × 0.6–0.7 μm. Colonies are yellow. Pyocyanin on King A medium and fluorescent pigments on King B medium are not produced. Catalase- and oxidase-positive. Growth occurs at 4–42 °C (optimum 37 °C), pH 6–9 (optimum pH 8) and at NaCl concentrations of up to 6 % (optimum 1–3 %). Does not hydrolyse gelatin, casein or starch. Hydrolysis of Tween 80 is positive. Shows positive reactions for nitrate reduction, but negative reactions for aesculin hydrolysis, gelatin hydrolysis, indole production, glucose fermentation and arginine dihydrolase, urease and

Table 1. Differential phenotypic characteristics of strain S3-3^T and related *Pseudomonas* species

Taxa: 1, strain S3-3^T; 2, *P. pachastrellae* KMM 330^T; 3, *P. plecoglossicida* ATCC 700383^T; 4, *P. pertucinogena* (data from Kawai & Yabuuchi, 1975). +, Positive; –, negative; ND, no data available. Data are from this study unless otherwise indicated.

Characteristic	1	2	3	4
Production of:				
Yellow/orange cellular pigment	+	–	–	–
Fluorescent pigment	–	–	+	ND
Urease	–	+	+	–
PHB accumulation	+	+	+	–
Arginine dihydrolase	–	–	+	–
Growth at:				
4 °C	+	– ^{a*}	– ^b	ND
41 °C	+	+	– ^b	+
Tolerance of 8 % NaCl	–	+	– ^b	ND
Hydrolysis of:				
Tween 80	+	+	– ^b	–
Aesculin	–	+	+	ND
Assimilation of:				
D-Glucose	–	–	+	–
Maltose	–	–	–	–
D-Mannose	–	–	+	–
D-Mannitol	–	–	–	–
Gluconate	–	–	–	ND
L-Arabinose	–	–	+	–
Potassium gluconate	–	–	+	–
Capric acid	–	+	+	ND
Adipic acid	+	–	–	ND
Malic acid	–	+	+	ND
Trisodium citrate	–	+	+	ND
Phenylacetic acid	–	–	+	ND

*Data taken from: *a*, Romanenko *et al.* (2005); *b*, Nishimori *et al.* (2000).

β -galactosidase activities. Assimilates adipic acid, but not D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, capric acid, malic acid, maltose, potassium gluconate, trisodium citrate or phenylacetic acid. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. In Biolog GN2 plates, the type strain utilizes Tweens 40 and 80, pyruvic acid methyl ester, *cis*-aconitic acid and α -ketoglutaric acid. Major fatty acids are summed feature 8 (C_{18:1} ω 6c and/or C_{18:1} ω 7c), summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH), C_{19:0} cyclo ω 8c, C_{17:0} cyclo, C_{16:0}, C_{12:0} and C_{13:0} 3-OH. Ubiquinone Q-9 is the major respiratory quinone. The DNA G + C content of the type strain is 60.9 mol%.

The type strain is S3-3^T (=CCTCC AB 207151^T =NRRL B-51270^T), isolated from desert sand in Xinjiang province, China.

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