Desertibacter roseus gen. nov., sp. nov., a gamma radiation-resistant bacterium in the family *Rhodospirillaceae*, isolated from desert sand

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A Gram-negative, rod-shaped, strictly aerobic bacterium, strain 2622^T, was isolated from gamma-irradiated soil sampled from the Taklimakan desert in Xinjiang, China. Phylogenetic analyses showed that strain 2622^T formed a distinct lineage in the family *Rhodospirillaceae* and shared 91.7 and 90.1 % 16S rRNA gene sequence similarity with its closest relatives, the type strains of *Skermanella xinjiangensis* and *Skermanella aerolata*, respectively. The DNA G+C content of strain 2622^T was 71.4 mol% and the isoprenoid quinone was ubiquinone Q-10. Based on phenotypic and chemotaxonomic data and phylogenetic analysis, strain 2622^T is considered to represent a novel species of a new genus in the family *Rhodospirillaceae*, for which the name *Desertibacter roseus* gen. nov., sp. nov. is proposed. The type strain of *Desertibacter roseus* is strain 2622^T (=CCTCC AB 208152^T =KCTC 22436^T).

At the time of writing, the family *Rhodospirillaceae* encompassed 26 genera (http://www.bacterio.cict.fr/classif generafamilies.html#Rhodospirillaceae) including bacteria isolated from, for example, marine habitats, freshwater, activated sludge biomass, air, soil, the rhizosphere and deserts (Mack *et al.*, 1993; Pfennig *et al.*, 1997; López-López *et al.*, 2002; Garrity *et al.*, 2005; Liu *et al.*, 2007; Weon *et al.*, 2007; Yoon *et al.*, 2007; Kodama *et al.*, 2008; Zhang *et al.*, 2008; An *et al.*, 2009). None, however, has been reported to withstand gamma radiation (Cox & Battista, 2005). Radiation-resistant bacteria can survive severe damage from gamma radiation, which implies that they have high DNA repair efficiency (Sghaier *et al.*, 2008) and are adept at detoxifying reactive oxygen species (ROS) (Zhang *et al.*, 2007). It is widely acknowledged that carotenoids play beneficial roles in scavenging electrons from ROS and, because of the close link between ROS and various diseases, there is continuing interest in finding antioxidants, particularly carotenoids, that can act as preventive or therapeutic drugs. Therefore, in an attempt to explore novel radiation-resistant bacteria, numerous bacteria were isolated from the Taklimakan desert, Xinjiang, China, as there is known to be a close link between desiccation and radiation resistance (Fredrickson *et al.*, 2008; Mattimore & Battista, 1996). A pink-pigmented bacterial strain was isolated, designated 2622^T, belonging to the family *Rhodospirillaceae*. The aim of the present study was to determine the exact taxonomic position of this strain, which was isolated from a gamma-irradiated sand sample.

Sand was sampled from the desert, and 1 g samples were exposed to 10 kGy radiation at a dose of 300 Gy min^{-1} at room temperature. After exposure, the samples were serially diluted in water (0.85 %, w/v, NaCl) and plated on different media: tenfold-diluted trypticase soy agar (0.1× TSA; Difco), twofold-diluted trypticase soy agar (0.5× TSA; Difco), twofold-diluted R2A agar (0.5× R2A; Difco), PTYG agar (Fredrickson *et al.*, 2008), nutrient agar (Difco) and R2A agar (Difco). After incubation at 30 °C for 20 days, single colonies on the plates were purified. Strain 2622^T was isolated on R2A agar and stored by lyophilization.

Genomic DNA extraction and amplification of the 16S rRNA gene of strain 2622^T were carried out as described by Rainey *et al.* (1996); PCR products were sequenced by Invitrogen Biotechnology Co. Ltd. Similarity searches with reference sequences were performed with the EzTaxon database (Chun *et al.*, 2007). Phylogenetic analysis was performed by using MEGA version 4.0 (Tamura *et al.*, 2007), after multiple alignment of the data via CLUSTAL X (Thompson *et al.*, 1997). Distances were obtained by using options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining and maximum-parsimony methods

Abbreviations: PHB, poly-β-hydroxybutyrate; ROS, reactive oxygen species.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 2622^T is EU833987.

Two supplementary tables are available with the online version of this paper.

(Saitou & Nei, 1987). The topologies of the neighbour-joining and maximum-parsimony phylogenetic trees were evaluated by using bootstrap resampling (Felsenstein, 1985) with 1000 replications (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online).

Cell morphology was examined by phase-contrast microscopy (Olympus) and by transmission electron microscopy. A number of key characteristics identified via standard procedures (Gerhardt *et al.*, 1994) were also tested: KOH string test (Gram stain), oxidase, catalase (3 % H_2O_2), nitrate reduction and hydrolysis of aesculin, casein, tyrosine, starch and gelatin. Poly-β-hydroxybutyrate (PHB) accumulation was observed by light microscopy after staining the cells with Sudan black (Smibert & Krieg, 1994). Growth at 4, 12, 18, 25, 30, 37, 42 and 45 °C and with 0, 0.5, 1.0, 1.5 and 2.0 % NaCl was tested on R2A agar; growth at pH 6–11 (at intervals of 1 pH unit) was determined in R2A broth. Anaerobic growth was assessed on R2A agar (both with and without KNO_3) incubated in air-tight jars containing an AnaeroPack (Oxoid). In addition, NFb and M media (Eckert *et al.*, 2001; Xie & Yokota, 2005) were used for assays of acetylene reduction, which were carried out as described by Mehnaz & Lazarovits (2006). Bacteriochlorophyll *a* content was determined as described by Biebl *et al.* (2005). Antibiotic resistance was determined with the disc diffusion method (Buczolits *et al.*, 2002) on R2A agar incubated for up to 3 days at 30 °C. Strain 2622^T was also characterized by using the whole test spectra of the API ZYM, API 20NE and API 20E systems (bioMérieux) according to the manufacturer's instructions.

To investigate the chemotaxonomic characteristics of strain 2622^T, it was grown on R2A agar at 30 °C for 3 days. Determination of whole-cell fatty acid profiles and analysis of fatty acid methyl esters were carried out according to the standard protocol of the Sherlock Microbial Identification System (MIDI). Respiratory quinones were analysed according to the protocol of Xie & Yokota (2003). DNA was extracted from cells grown in R2A broth by using a modified version of the method described by Wilson (1987). The HPLC method of Mesbah *et al.* (1989) was used to determine the G+C content of the extracted DNA.

Phylogenetic analysis of the 16S rRNA gene sequence of strain 2622^T showed that it formed a distinct lineage within the family *Rhodospirillaceae* (Fig. 1). The nearest phylogenetic neighbours of strain 2622^T were species of the genus *Skermanella*; strain 2622^T shared 91.7 and 90.1 % 16S rRNA gene sequence similarity with the type strains of *Skermanella xinjiangensis* and *Skermanella aerolata*, respectively, 89.8–88.1 % similarity with the type strains of species in the genus *Azospirillum* and 84.5–89.5 % with the type strains of other type species of the family *Rhodospirillaceae*. In the neighbour-joining phylogenetic tree, strain 2622^T clearly formed a basal branch of the sister clade containing *Skermanella parooensis* ACM 2042^T; nonetheless, it formed a distinct line which was clearly separated from the genus *Skermanella*.

More importantly, strain 2622^T could be differentiated from members of the family *Rhodospirillaceae* based on chemotaxonomic characteristics. The cellular fatty acid profile of strain 2622^T included $C_{18:1}\omega7c$ (49.8 %), $C_{16:0}$ (11.1 %), $C_{18:0}$ (9.3 %), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$, 8.6 %), $C_{16:1}\omega5c$ (5.6 %), $C_{18:1}\omega9c$ (4.1 %), summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I, 2.9 %) and $C_{16:0}$ 3-OH (1.1 %). Although $C_{18:1}\omega7c$ was commonly found as a major component in members of the family *Rhodospirillaceae* (Choi *et al.*, 2009; Díaz-Cárdenas *et al.*, 2010; Lakshmi *et al.*, 2011; Lai *et al.*, 2009a, b; Liu *et al.*, 2010; Urios *et al.*, 2008; Wang *et al.*, 2009; Zhang *et al.*, 2008), significant amounts of $C_{16:1}\omega5c$ and $C_{18:0}$

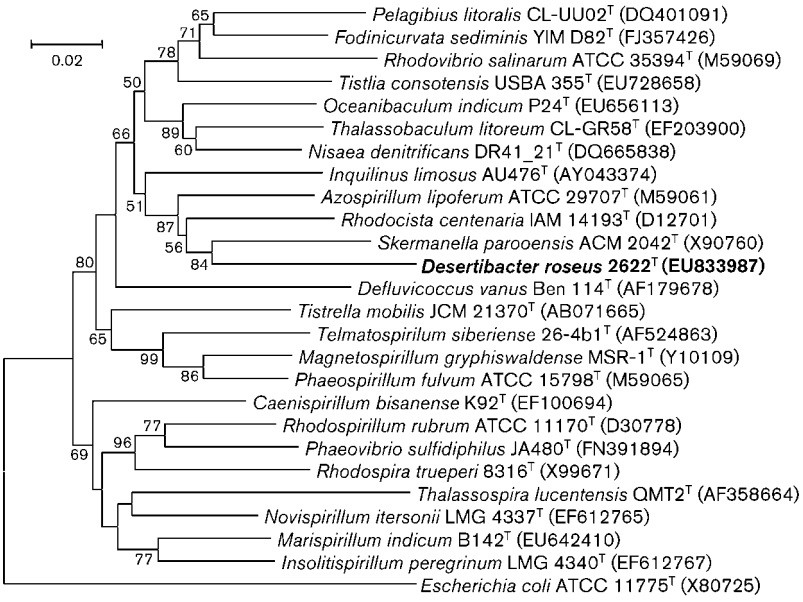

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain 2622^T and related taxa. Bootstrap values (expressed as percentages of 1000 replications) $\geqslant$50 % are given at nodes. Bar, 0.02 substitutions per nucleotide position. The sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup.

3-OH were found in strain 2622^T only, and not in *Azospirillum lipoferum* ATCC 29707^T or *S. parooensis* DSM 9257^T (Table 1). Moreover, the sole isoprenoid quinone in strain 2622^T was ubiquinone 10 (Q-10), which is also found in the genera *Skermanella* and *Azospirillum*; however, in *S. parooensis* DSM 9257^T and *A. lipoferum* ATCC 29707^T, small amounts of menaquinone 8 (MK-8; 20.9 %) and Q-9 (4.5 %), respectively, were also found (Table 2).

Cells of strain 2622^T were strictly aerobic, Gram-negative rods that were motile by means of a single polar flagellum (Supplementary Fig. S2). PHB granules were also detected. Colonies were pink, circular and convex with regular margins after growth on R2A agar at 37 °C for 4 days. Growth was observed at 12–42 °C, with good growth between 37 and 40 °C. Growth was observed with 0–1.5 % NaCl (optimum 0.5 % NaCl) and at pH 7–10 (optimum pH 8). Strain 2622^T was unable to reduce acetylene in either NFb or M media and was unable to fix nitrogen. This latter characteristic can be used to distinguish strain 2622^T from species of the genus *Azospirillum*. Strain 2622^T was positive for oxidase, catalase and nitrate reductase. Aesculin and gelatin were hydrolysed, but casein, tyrosine and starch were not. The results of API ZYM, API 20E, API 20NE and antibiotic susceptibility tests are detailed in the species description.

Strain 2622^T is phylogenetically closely related to the genera *Skermanella* and *Azospirillum* (Fig. 1). The lack of strong clustering, however, suggested that strain 2622^T belonged to a new genus, which was supported by physiological features, fatty acid profile and quinone composition. Based on the phenotypic, chemotaxonomic and phylogenetic data presented, we therefore suggest that strain 2622^T represents a novel species of a new genus, for which the name *Desertibacter roseus* gen. nov., sp. nov. is proposed.

Table 1. Cellular fatty acid profiles of strain 2622^T and the type strains of phylogenetically related type species of the family *Rhodospirillaceae*

Strains: 1, 2622^T; 2, *Azospirillum lipoferum* ATCC 29707^T; 3, *Skermanella parooensis* DSM 9257^T. All data were obtained in this study. *A. lipoferum* ATCC 29707^T and *S. parooensis* DSM 9257^T were grown on R2A agar (Difco) at 30 °C for 3 days. −, Not detected or <1 %.

Fatty acid	1	2	3
$C_{18:1}\omega7c$	49.8	39.1	42.5
$C_{16:0}$	11.1	15.0	25.6
$C_{18:0}$	9.3	6.0	8.8
$C_{16:1}\omega5c$	5.6	−	−
$C_{18:1}\omega9c$	4.1	2.2	3.5
$C_{18:0}$ 3-OH	1.5	−	−
$C_{16:0}$ 3-OH	1.1	2.0	3.0
$C_{17:1}\omega6c$	1.0	9.4	−
Summed feature 3*	8.6	3.1	5.5
Summed feature 2*	2.8	3.8	4.8

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 3 comprised iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; summed feature 2 comprised $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I.

Table 2. Characteristics that separate strain 2622^T from phylogenetically related members of the family *Rhodospirillaceae*

Strains: 1, 2622^T; 2, *Azospirillum lipoferum* ATCC 29707^T; 3, *Skermanella parooensis* DSM 9257^T. Data were obtained in this study unless indicated.

Characteristic	1	2	3
Colony colour	Pink	White	Pale pink
Cell shape	Rod	Vibrioid	Rod
Maximum growth temperature (°C)	42	40	37
pH range	7–10	6–7	6–9
Tolerance of 2 % NaCl	−	+	+
Assimilation of carbon sources			
N-Acetylglucosamine	−	+	−
L-Arabinose	−	+	+
D-Glucose	−	+	+
D-Mannose	−	+	+
D-Mannitol	−	+	+
Enzyme activities			
Gelatinase	+	−	−
Cystine arylamidase	+	−	−
Acid phosphatase	−	+	+
Major quinone(s)	Q-10	Q-10, Q-9	Q-10, MK-8
DNA G+C content (mol%)	71.4	69–70[a*]	66.4–68.0[b]

*Data for the species from: *a*, Tarrand *et al.* (1978); *b*, Sly & Stackebrandt (1999).

Description of *Desertibacter* gen. nov.

Desertibacter (De.ser.ti.bac′ter. L. n. *desertum* desert; N.L. masc. n. *bacter* rod; N.L. masc. n. *Desertibacter* a desert bacterium).

Cells are Gram-negative rods, motile by means of a single polar flagellum and strictly aerobic. Catalase- and oxidase-positive. Nitrate is reduced to nitrite. Unable to fix nitrogen. Bacteriochlorophyll *a* is not detected. The isoprenoid quinone is Q-10. The main cellular fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$, $C_{18:0}$, summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) and $C_{16:1}\omega5c$. On the basis of 16S rRNA gene sequence analysis, the genus belongs to the family *Rhodospirillaceae*. The type species is *Desertibacter roseus*.

Description of *Desertibacter roseus* sp. nov.

Desertibacter roseus (ro′se.us. L. masc. adj. *roseus* rose-coloured, pink).

Displays the following properties in addition to those given in the genus description. Colonies are pink, circular and convex with regular margins. Cells contain PHB. Growth occurs at 12–42 °C (optimum 37–40 °C), at pH 7–10 (optimum pH 8) and at NaCl concentrations of up to 1.5 % (optimum 0.5 % NaCl). Hydrolyses aesculin and gelatin but not casein, tyrosine or starch. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase (weakly) and α-glucosidase (weakly) and negative for lipase (C14), trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, β-glucosidase, α-mannosidase and α-fucosidase. In API 20NE tests, shows positive reactions for nitrate reduction, aesculin hydrolysis, gelatin hydrolysis, urease and β-galactosidase and negative reactions for arginine dihydrolase, indole production and glucose fermentation. Does not assimilate D-glucose, L-arabinose, maltose, D-mannose, D-mannitol, *N*-acetylglucosamine, adipic acid, capric acid, malic acid, potassium gluconate, trisodium citrate or phenylacetic acid. In API 20E tests, shows positive reactions for β-galactosidase, Voges–Proskauer reaction, urease and gelatin hydrolysis, but negative reactions for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, tryptophan deaminase, indole production and oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. Susceptible to (per disc) erythromycin (15 μg), vancomycin (30 μg), streptomycin (10 μg), acheomycin (30 μg) and penicillin (10 IU). The DNA G+C content of the type strain is 71.4 mol%.

The type strain, 2622^T (=CCTCC AB 208152^T =KCTC 22436^T), was isolated from sand from the Taklimakan desert in Xinjiang, China.

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