Deinococcus xinjiangensis sp. nov., isolated from desert soil

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A Gram-positive-staining, spherical-shaped and faintly pink-pigmented bacterial strain, X-82^T, was isolated from soil samples collected from a desert in Xinjiang, China. The organism was found to be resistant to UV radiation but sensitive to gamma radiation and desiccation. The optimum growth pH, NaCl concentration and temperature were pH 7.0, 0–1 % NaCl and 30 °C. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain X-82^T is member of a novel species belonging to the genus *Deinococcus*, with *Deinococcus hopiensis* KR-140^T as its closest relative (93.5 % similarity). The DNA G+C content (60 mol%), quinone type (MK-8), major cellular fatty acids (16:1 ω 7c, 16:0 and 17:1 iso ω 9c), the presence of peptidoglycan with L-ornithine and the dominant polar lipid (phosphoglycolipids and glycolipids) support the affiliation of strain X-82^T with the genus *Deinococcus*. A novel species is proposed, for which the name *Deinococcus xinjiangensis* sp. nov. is proposed, with the type strain X-82^T (=CCTCC AB 207226^T =NRRL B-51287^T).

The genus *Deinococcus*, which represents a deep-branching lineage within the *Bacteria*, comprises 26 species with validly published names at the time of writing, isolated from various environments such as desert soils, foods, faeces, dust, hot springs, Antarctic environments and the plant rhizosphere (Rainey *et al.*, 2005; Ferreira *et al.*, 1997; Lai *et al.*, 2006; Weon *et al.*, 2007; Anderson *et al.*, 1956; Davis *et al.*, 1963; Lewis, 1973; Masters *et al.*, 1991; Oyaizu *et al.*, 1987; Hirsch *et al.*, 2004). The most striking characteristic of species of the genus is their extreme resistance to UV light, gamma radiation and desiccation. *Deinococcus radiodurans*, the type species of the genus, has been shown to survive exposure to doses of gamma radiation greater than 20 kGy (Daly *et al.*, 2004; Ito *et al.*, 1983; Anderson *et al.*, 1956). However, around the time of writing, four psychrophilic, ionizing-radiation-sensitive *Deinococcus* species were described (Callegan *et al.*, 2008). It has been suggested that resistance to unnaturally large doses of ionizing radiation is a consequence of the ability to repair desiccation-induced double-strand breaks in the DNA, similar to those produced by ionizing radiation, while UV resistance is conferred by two nucleotide excision-repair pathways acting simultaneously

(Mattimore & Battista, 1996; Minton, 1994). Recently, a UV-radiation-resistant but gamma-radiation- and desiccation-sensitive bacterium, strain X-82^T, was isolated from non-irradiated soil samples collected from a desert in Xinjiang, China. The faintly pink-pigmented bacterium was identified as a novel member of the genus *Deinococcus*.

In the course of a study on the diversity of culturable bacteria in the Taklimakan desert of Xinjiang, China, a soil sample collected from the desert was suspended in 1.0 ml sterile water and homogenized for 5 min in a sterile mortar. The resulting supernatant was serially diluted and spread on tenfold-diluted trypticase soy broth (TSB/10; 3 g l⁻¹) agar plates (Bacto; Becton Dickinson). Plates were incubated at 28 °C for 7 days and colonies with different morphologies were subcultured to obtain pure cultures. Among the organisms isolated in this study (about 600 pure cultures), we selected randomly 80 pure cultures for 16S rRNA gene sequencing and further chemotaxonomic and phenotypic study. Strain X-82^T was isolated during the sampling campaign and was observed in the lag, exponential and stationary phases of growth under phase-contrast microscopy (Olympus). To test the Gram reaction, a Gram-stain kit and the KOH lysis test (Buck, 1982) were used. Conventional biochemical tests were performed as described by Smibert & Krieg (1994) such as gelatinase, catalase and cytochrome oxidase activities, H₂S production and casein hydrolysis. Biolog GP2 plates and API 20 NE strips, API ID 32 GN and API ZYM (bioMérieux) were used for classical and phenotypic tests according to the manufacturers' instructions. The temperature range

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain X-82^T is EU626561.

Fatty-acid profiles of strain X-82^T and related type strains, polar-lipid profiles following 2D TLC separation, representative survival curves in response to UV and gamma radiation and 16S rRNA gene sequence-based UPGMA, maximum-parsimony and minimum-evolution trees are available as supplementary material with the online version of this paper.

(4–50 °C) and pH range (pH 4–10) for growth and the requirement for NaCl (0.2–5 %) were determined using TSB/10.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were carried out as described by Lin *et al.* (2004). Pairwise alignment with sequences of a broad selection of related species and calculations of levels of sequence similarity were carried out using the BLAST facility at the EzTaxon website (<http://www.eztaxon.org/search>; Chun *et al.*, 2007). Phylogenetic analyses were performed using the MEGA 3 program (Kumar *et al.*, 2004). Phylogenetic dendrograms were constructed using the neighbour-joining, UPGMA, minimum-evolution and maximum-parsimony methods with bootstrap values based on 1000 replications.

Respiratory quinones were extracted and separated by HPLC (Ultimate 3000) as described by Xie & Yokota (2003). Fatty acid methyl esters were extracted and analysed by GC (Hewlett Packard 6890N) according to the standard protocol of the Microbial Identification System (MIDI) using cells grown on TSB/10 agar plates for 48 h at 30 °C. The peptidoglycan was prepared and analysed as described by Schleifer & Kandler (1972). The DNA G+C content was determined by HPLC according to the method of Mesbah *et al.* (1989). Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990).

To determine the tolerance of the culture to UV radiation, strains were grown in the appropriate liquid medium to the exponential phase. The cells were recovered by centrifugation, washed and resuspended in 0.067 M potassium phosphate buffer at pH 7.0. Aliquots were spread on TGY (0.5 % tryptone, 0.3 % yeast extract, 0.1 % glucose, pH 7.0) agar plates; the plates, with their lids open, were exposed immediately to UV (UV-C, 254 nm) for the desired dose, monitored by using a Spectroline DRC-100H digital radiometer (Bioblock Scientific), and incubated at 30 °C for 1 week. Aliquots of cells prepared as described above were exposed to gamma radiation from a cobalt-60 source at a dose of 600 Gy h⁻¹ for gamma-radiation-resistance determination, while further aliquots were dried over CaSO₄ and kept at 5 % relative humidity until rehydration in order to determine the desiccation resistance of the culture. After exposure to gamma radiation or desiccation and rehydration, suspensions were dilution-plated in triplicate on TGY agar plates. At the same time, *D. radiodurans* DSM 20539^T and *Escherichia coli* DH5 α (=ATCC 35607) were tested as positive and negative controls. Relative survival was determined by comparing with unirradiated cultures.

Strain X-82^T stained Gram-positive and the cells were aerobic, non-motile and spherical (2.0–3.0 μ m in diameter). The colonies were opaque, circular with entire edges and faintly pink-pigmented on TGY agar plates. The temperature range for growth was 10–37 °C, with optimum growth at 30 °C. The pH range for growth was

pH 5.0–8.0, with optimum growth at pH 7.0. The strain was able to grow in the presence of 1 % (w/v) NaCl. The strain was positive for cytochrome oxidase and catalase activities, H₂S production and hydrolysis of casein and gelatin. Other physiological and biochemical properties of strain X-82^T are listed in Table 1 or in the species description.

A 16S rRNA gene sequence of 1381 nt was determined for strain X-82^T. Comparative sequence and phylogenetic analysis grouped the strain within the radiation of species that currently comprise the genus *Deinococcus* (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online). The levels of 16S rRNA gene sequence similarity between strain X-82^T and other *Deinococcus* species were in the range 89–93 %, the highest sequence similarities being found with *Deinococcus hopiensis* KR-140^T (93.5 %), *D. ficus* CC-FR2-10^T (93.2 %), *D. mumbaiensis* CON-1^T (93.1 %), *D. indicus* Wt/1a^T (92.6 %) and *D. radiodurans* DSM 20539^T (92.4 %). These values are significantly below the level generally regarded to demarcate species (97 %). The DNA G+C content was 60 mol%, a value that is within the range found for other species of the genus *Deinococcus* (59.4–70.0 mol%; Hirsch *et al.*, 2004).

The major fatty acids of strain X-82^T were monounsaturated and straight-chain saturated fatty acids, such as C_{16:1} ω 7c (22.59 %) and C_{16:0} (11.05 %), which were also predominant in most other *Deinococcus* species. Specifically, larger amounts of iso-branched fatty acids were also found, such as C_{17:1} iso ω 9c (24.06 %) and C_{17:0} iso (6.21 %), which were also found in *Deinococcus murrayi* and *Deinococcus radiopugnans* (Ferreira *et al.*, 1997) (Supplementary Table S1). The peptidoglycan of strain X-82^T contained ornithine, glutamic acid, alanine and glycine at a molar ratio of 1:1:2:2 (Orn–Gly₂; type A3 β), as reported for *D. radiodurans* DSM 20539^T. The major respiratory quinone was menaquinone 8 (MK-8), as in all recognized *Deinococcus* strains. Strain X-82^T had a complex polar lipid profile consisting of different unidentified glycolipids, phospholipids and phosphoglycolipids (Supplementary Fig. S2) but which did not include phosphatidylglycerol, consistent with previously reported results for other *Deinococcus* species (Embley *et al.*, 1987; Counsell & Murray, 1986; Thompson *et al.*, 1980). A predominant phosphoglycolipid (PGL2), which was identified in *D. radiodurans* as 2'-O-(1,2-diacyl-*sn*-glycero-3-phospho)-3'-O-(α -galactosyl)-N-D-glyceroyl alkylamine (Anderson & Hansen, 1985), was also detected. The chromatographic behaviour of the polar lipids aminophospholipid (APL) and PGL1 of strain X-82^T was similar to polar lipid spots reported for *D. radiodurans*, *D. ficus* (Lai *et al.*, 2006), *D. deserti* (Weon *et al.*, 2007), *D. geothermalis* (Ferreira *et al.*, 1997) and *D. aquiradiocola* (Asker *et al.*, 2009). However, the significantly larger amounts of GL1–GL3 clearly distinguished X-82^T from other members of the genus *Deinococcus*. In addition, it differed from *Deinococcus altitudinis*, *D. alpinitundrae*, *D. radiomollis* and *D. claudionis*, the four ionizing radiation-sensitive

Table 1. Phenotypic differences between strain X-82^T and related members of the genus *Deinococcus*

Strains: 1, strain X-82^T; 2, *D. radiodurans* DSM 20539^T (data from this study); 3, *D. ficus* CC-FR2-10^T (data from Lai *et al.*, 2006); 4, *D. indicus* Wt/1a^T (Suresh *et al.*, 2004; Weon *et al.*, 2007); 5, *D. hopiensis* KR-140^T (Rainey *et al.*, 2005; this study).
+, Positive result or growth; –, negative result or growth; ND, no data available.

Characteristic	1	2	3	4	5
Morphology	Spherical	Spherical	Rod	Rod	Spherical
Pigmentation	Faint pink	Red	Pale pink	Red	Pink
Utilization of:					
L-Arabinose	–	–	+	+	+
Lactose	–	–	+	+	–
Trehalose	+	+	+	ND	+
D-Xylose	–	–	+	ND	+
Melibiose	–	–	+	+	–
N-Acetyl-D-glucosamine	–	+	+	+	+
L-Alanine	+	–	–	–	–
D-Mannitol	+	–	+	–	–
D-Galactose	+	–	+	–	+
L-Rhamnose	–	–	+	+	+
Raffinose	–	–	+	+	–
API ZYM test results					
Valine arylamidase	+	–	–	–	–
β-Galactosidase	–	–	+	+	–
β-Glucosidase	–	–	+	–	+
Gelatinase	+	+	+	+	+
Casein hydrolysis	–	+	ND	+	+
Nitrate reduction	–	+	ND	+	–

species reported recently by Callegan *et al.* (2008), and *D. cellulolyticus* by the presence of PGL1 and by the absence of APGL for *D. altitudinis* and *D. alpinitundrae*. The lack of APL in *Deinococcus aquatilis* (Kämpfer *et al.*, 2008), *D. indicus* (Ferreira *et al.*, 1997), *D. marmoris* (Callegan *et al.*, 2008), *D. aquaticus* and *D. caeni* (Im *et al.*, 2008) distinguished them from the isolate.

There was no growth for *E. coli* DH5α at a dose of 3.0 kGy gamma radiation, after 6 weeks of desiccation or after exposure to a UV dose of 30 J m^{–2}. Strain X-82^T appeared to be somewhat resistant to these stresses, but less so than *D. radiodurans* DSM 20539^T (Supplementary Fig. S3). Exposure of cultures to 5 kGy gamma radiation resulted in survival of 1–0.5 % for X-82^T, compared with 90–85 % for *D. radiodurans* DSM 20539^T. At the higher dose tested (10 kGy), X-82^T did not survive, whereas most *Deinococcus* species have been shown to survive. Increased sensitivity to DNA damage was also observed when the desiccation resistance of these strains was compared. The viability of the X-82^T culture decreased to 6–7 % after 6 weeks of desiccation, which is approximately 10-fold more sensitive than *D. radiodurans* DSM 20539^T. As for UV radiation tolerance, 1.7 and 12 % survival was observed for X-82^T and *D. radiodurans* DSM 20539^T, respectively, when they were exposed to a UV dose of 810 J m^{–2}.

Although strain X-82^T was somewhat more sensitive to gamma radiation and desiccation than other species of the genus *Deinococcus*, the UV-radiation resistance and the

combination of chemotaxonomic and phenotypic characteristics corroborate the results of the 16S rRNA gene sequence analysis, indicating that strain X-82^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus xinjiangensis* sp. nov. is proposed.

Description of *Deinococcus xinjiangensis* sp. nov.

Deinococcus xinjiangensis (xin.jiang.en'sis. N.L. masc. adj. *xinjiangensis* pertaining to Xinjiang, an autonomous region in north-western China, where the type strain was isolated).

Cells stain Gram-positive and are non-motile, aerobic and spherical. Colonies on TGY agar medium are opaque, circular with entire edges, faintly pink-pigmented and 3–4 mm in diameter. The optimum growth pH, NaCl concentration and temperature are pH 7.0, 0–1 % and 30 °C. Sensitive to gamma radiation (<10 kGy) and desiccation but resistant to UV irradiation (>810 J m^{–2}). The major cellular fatty acids are 16:1ω7c, 16:0 and 17:1 iso ω9c. The peptidoglycan contains L-ornithine (Orn-Gly₂; type A3β). MK-8 is the predominant lipoquinone. The polar lipid profile contains two unknown phosphoglycerolipids, three glycolipids, one unknown aminophospholipid and one unknown phospholipid. One of the phosphoglycerolipids and the three glycolipids are the major polar lipids. DNA of the type strain has a G + C content of 60 mol%. Positive for activities of urease, gelatinase, catalase, cytochrome oxidase, alkaline phosphatase, butyrate esterase, caprylate esterase, leucine arylamidase,

Fig. 1. Phylogenetic dendrogram showing the phylogenetic position of strain X-82^T based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses), constructed from distance matrices (according to the Kimura-2 model) and clustered with the neighbour-joining method by using the software package MEGA, version 3.1 (Kumar *et al.*, 2004). Bootstrap values, expressed as percentages of 1000 replications, are given at branching points. *Flavobacterium aquatile* ATCC 11947^T (Weeks, 1955) was used as the outgroup. Bar, 0.05 K_{nuc} value. UPGMA, maximum-parsimony and minimum-evolution trees are available as Supplementary Fig. S1.

valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase. The following compounds are utilized as sole carbon sources (i.e. produce positive results in the Biolog system): dextrin, glycogen, Tween 40, Tween 80, D-fructose, D-galactose, α -D-glucose, maltose, maltotriose, D-mannitol, palatinose, D-psicose, sucrose, trehalose, turanose, α -ketovaleric acid, pyruvic acid methyl ester, succinic acid monomethyl ester, L-alanine, L-asparagine, glycerol, adenosine, 2'-deoxyadenosine, thymidine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, D-fructose 6-phosphate, α -D-glucose 1-phosphate, D-glucose 6-phosphate and DL- α -glycerol phosphate. The type strain assimilates sucrose, maltose, glycogen, mannitol and glucose.

The type strain, X-82^T (=CCTCC AB 207226^T =NRRL B-51287^T), was isolated from soil of the Taklimakan desert, Xinjiang, China.

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