Deinococcus xinjiangensis sp. nov., isolated from desert soil

Fang Peng, Lei Zhang, Xuesong Luo, Jun Dai, Hongli An, Yali Tang and Chengxiang Fang

A Gram-positive-staining, spherical-shaped and faintly pink-pigmented bacterial strain, X-82\textsuperscript{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\textsuperscript{T} is member of a novel species belonging to the genus Deinococcus, with Deinococcus hopiensis KR-140\textsuperscript{T} as its closest relative (93.5 % similarity). The DNA G+C content (60 mol%) of this new species is very close to those of other Deinococcus species. The DNA G+C content of strain X-82\textsuperscript{T} is 68.7 mol%, while those of Deinococcus radiodurans KR-140\textsuperscript{T} and Deinococcus xinjiangensis sp. nov. are 68.5 mol% and 66.3 mol%, respectively. It was 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.

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.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain X-82\textsuperscript{T} is EU026561.

Fatty-acid profiles of strain X-82\textsuperscript{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.

Deinococcus xinjiangensis sp. nov. is proposed, with the type strain X-82\textsuperscript{T} (=CCTCC AB 207226\textsuperscript{T} =NRRL B-51287\textsuperscript{T}).

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\textsuperscript{-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\textsuperscript{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, H\textsubscript{2}S 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... (Mattimore & Battista, 1996; Minton, 1994). Recently, a UV-radiation-resistant but gamma-radiation- and desiccation-sensitive bacterium, strain X-82\textsuperscript{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.
(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 agar plates. At the same time, triplicate on TGY agar plates. At the same time, other physiological and biochemical properties of strain X-82T are listed in Table 1 or in the species description.

A 16S rRNA gene sequence of 1381 nt was determined for strain X-82T. 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-82T and other *Deinococcus* species were in the range 89–93%, the highest sequence similarities being found with *Deinococcus hopiensis* KR-140T (93.5%), *D. ficus* CC-FR2-10 (93.2%), *D. mumbaiensis* CON-1T (93.1%), *D. indicus* Wt/1aT (92.6%) and *D. radiodurans* DSM 20539T (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-82T were monounsaturated and straight-chain saturated fatty acids, such as C16:1ω7c (22.59%) and C16:0 (11.05%), which were also predominant in most other *Deinococcus* species. Specifically, larger amounts of iso-branched fatty acids were also found, such as C17:1ω9c (24.06%) and C17:0ω9c (6.21%), which were also found in *Deinococcus murrayi* and *Deinococcus radiopugnans* (Ferreira et al., 1997) (Supplementary Table S1). The peptidoglycan of strain X-82T contained ornithine, glutamic acid, alanine and glycine at a molar ratio of 1:1:2:2 (Orn–Gly; type Aβ), as reported for *D. radiodurans* DSM 20539T. The major respiratory quinone was menaquinone 8 (MK-8), as in all recognized *Deinococcus* strains. Strain X-82T 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-[(9S)-galactosyl]-N-d-glycerol alkylamine (Anderson & Hansen, 1985), was also detected. The chromatographic behaviour of the polar lipids aminophospholipid (APL) and PGL1 of strain X-82T 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., 2007). D. alpinitundrae, D. radiomollis and D. claudiosum, the four ionizing radiation-sensitive
species reported recently by Callegen 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 aquaticus (Kämpfer et al., 2008), D. indicus (Ferreira et al., 1997), D. marmoris (Callegen 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-82T appeared to be somewhat resistant to these stresses, but less so than D. radiodurans DSM 20539T (Supplementary Fig. S3). Exposure of cultures to 5 kGy gamma radiation resulted in survival of 1–0.5 % for X-82T, compared with 90–85 % for D. radiodurans DSM 20539T. At the higher dose tested (10 kGy), X-82T 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-82T culture decreased to 6–7 % after 6 weeks of desiccation, which is approximately 10-fold more sensitive than D. radiodurans DSM 20539T. As for UV radiation tolerance, 1.7 and 12 % survival was observed for X-82T and D. radiodurans DSM 20539T, respectively, when they were exposed to a UV dose of 810 J m⁻².

Although strain X-82T 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-82T 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 : 1ω9c. The peptidoglycan contains L-ornithine (Orn–Gly₂; type A3β). MK-8 is the predominant lipoquinone. The polar lipid profile contains two unknown phosphoglycolipids, three glycolipids, one unknown aminophospholipid and one unknown phospholipid. One of the phosphoglycolipids 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,

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

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
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<td>Spherical</td>
<td>Rod</td>
<td>Rod</td>
<td>Spherical</td>
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<tr>
<td><strong>Pigmentation</strong></td>
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<td>Pale pink</td>
<td>Red</td>
<td>Pink</td>
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<tr>
<td>L-Arabinose</td>
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<tr>
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<td>ND</td>
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<td>N-Acetyl-d-glucosamine</td>
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<td>Valine arylamidase</td>
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<td>–</td>
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<tr>
<td>β-Galactosidase</td>
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<td>Nitrate reduction</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
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</table>

Strains: 1, strain X-82T; 2, D. radiodurans DSM 20539T (data from this study); 3, D. ficus CC-FR2-10T (data from Lai et al., 2006); 4, D. indicus Wt/1aT (Suresh et al., 2004; Weon et al., 2007); 5, D. hopiensis KR-140T (Rainey et al., 2005; this study).

+ , Positive result or growth; –, negative result or growth; ND, no data available.
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-82T (5CCTCC AB 207226T = NRRL B-51287T), was isolated from soil of the Taklimakan desert, Xinjiang, China.

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References


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