Deinococcus gobiensis sp. nov., an extremely radiation-resistant bacterium

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A Gram-positive, non-motile, spherical, red-pigmented and facultatively anaerobic bacterium, designated strain I-0T, was isolated from a sand sample of the Gobi desert in Xinjiang Autonomous Region, China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that this isolate represents a novel member of the genus Deinococcus, with low sequence similarities (<94%) to recognized Deinococcus species. The major cellular fatty acids were C16:0, C17:0 and C18:0. Its polar lipid profile contained several unidentified glycolipids, phosphoglycolipids, phospholipids, pigments and an aminophospholipid. The peptidoglycan type was Orn–Gly2 (A3β) and the predominant respiratory quinone was MK-8. The DNA G+C content was 65.4 mol%. DNA–DNA relatedness between strain I-0T and Deinococcus radiodurans DSM 21396T was 37%. The strain was shown to be extremely resistant to gamma radiation (>15 kGy) and UV light (>600 J m⁻²). On the basis of the phylogenetic, chemotaxonomic and phenotypic data presented, strain I-0T represents a novel species of the genus Deinococcus, for which the name Deinococcus gobiensis sp. nov. is proposed. The type strain is I-0T (=DSM 21396T =CGMCC 1.7299T).

The genus Deinococcus, which was described by Brooks & Murray (1981), comprises 31 species with validly published names at the time of writing (http://www.bacterio.cict.fr/d/deinococcus.html). These species have been isolated from a wide range of environments, e.g. desert soil (Rainey et al., 2005; de Groot et al., 2004), aquifers (Suressh et al., 2004), the plant rhizosphere (Lai et al., 2006), hot springs (Ferreira et al., 1997) and airborne dust (Shashidhar & Bandekar, 2006; Weon et al., 2007). Most members of the genus are strictly aerobic, have optimum growth temperatures in the range 25–35 °C and form red, pink, light-pink or reddish colonies. Their extreme resistance to ionizing radiation (10 kGy), UV light (600 J m⁻²) and desiccation (years) is a distinctive characteristic of this genus (Makarova et al., 2007). This resistance has been attributed to a highly proficient DNA repair system, and it seems likely that radiation resistance evolved as a consequence of chronic exposure to non-radioactive forms of DNA damage, such as desiccation (Makarova et al., 2001).

In the course of the study of stress-resistant bacteria from arid environments, a novel Deinococcus isolate was obtained from the upper sand layers of the Gobi desert, Xinjiang, China, where bacteria are exposed to cycles of high and low temperatures and to prolonged desiccation. In this paper, we report on the taxonomic characterization of this radiation-resistant, red-coloured strain, designated I-0T, which was obtained from a mixed sand sample. After exposure of the sample to 10 kGy gamma radiation from a 60Co source (CAIC), it was enriched in 50 ml TGY medium (1.0 % peptone, 0.5 % yeast extract, 0.1 % glucose) at 30 °C with shaking at 200 r.p.m. for up to 5 days, followed by isolation of surviving red-colony-forming bacteria on TGY agar plates (TGY medium with 1.5 % agar).

Morphology of cells grown for 24–48 h on TGY agar was examined by zoom stereo microscopy (model SZX7; Olympus), light microscopy (model BX-51; Olympus), scanning electron microscopy (model S-570; Hitachi) and transmission electron microscopy (model H-7500; Hitachi). Gram staining was carried out using the modified

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain I-0T is EU427464.

Micrographs of colonies and cells of strain I-0T, a 16S rRNA gene sequence-based maximum-parsimony tree, the fatty acid profile of strain I-0T and a table of 16S rRNA gene sequence similarities to related strains are available as supplementary material with the online version of this paper.
To determine resistance of the culture to gamma radiation, fluorometric microdilution plate method (Ezaki et al., 1988; Sawabe et al., 1998). Strain I-0T was positive for several layers and a large electron-dense granule (Supplementary Fig. S3). Strain I-0T was positive for catalase, oxidase and urease and reduction of nitrate to nitrite, but negative for arginine dihydrolase, indole production and the Voges–Proskauer test. Strain I-0T could degrade gelatin, starch and casein and utilized a number of substrates as sole carbon sources for growth. These sole carbon source tests showed distinct results for strain I-0T and other Deinococcus species (Table 1 and the species description).

The whole-cell sugars contained mainly glucose and small quantities of ribose. The major cellular fatty acids were straight-chain C_{16:0} (42.07 %) and C_{16:1} (35.06%) (Table 1, Supplementary Table S1). This combination allows strain I-0T to be distinguished from recognized species of the genus Deinococcus. Cell-wall peptidoglycan analysis showed that the cell wall contained l-ornithine as the diamino acid (A3β). The major respiratory quinone in strain I-0T was MK-8, as in all recognized Deinococcus species.

As shown in Fig. 1, the polar lipid profile of strain I-0T consisted of three unidentified glycolipids, four phosphoglycolipids, three phospholipids, two pigment spots and an aminophospholipid. The polar lipid profile of strain I-0T was dominated by phosphoglycolipids, which co-migrated with those found in other Deinococcus species (Embley et al., 1987; Ferreira et al., 1997; Suresh et al., 2004; Lai et al., 2006; Rainey et al., 2007; Zhang et al., 2007). The chromatographic behaviour of the polar lipids PL, APL, PGL1–2 and GL3 and pigments PIG1–2 of strain I-0T was similar to that of the lipid spots PL, APL, PGL1–2 and GL3 and PIG1–2 reported for D. radiodurans AS 1.6335 (Zhang et al., 2007). The presence of these lipids extracted from strain I-0T confirms that it should be assigned to the genus Deinococcus.

An almost-complete 16S rRNA gene sequence (1470 bp) was determined for strain I-0T. A FASTA search of the EMBL nucleotide sequence database using this sequence showed relatively low similarity (<94%) to sequences from other Deinococcus species (Supplementary Table S2), which indicated that this strain might represent a novel species. Phylogenetic analyses were performed using MEGA version 4 (Tamura et al., 2007). Phylogenetic dendograms, which showed slightly different phylogenetic topologies, were conducted by the neighbour-joining (Fig. 2) and maximum-parsimony (Supplementary Fig. S4) methods with bootstrap values based on 1000 replications. The G+C content of the DNA was 65.4 mol%. DNA–DNA hybridization tests indicated that the relatedness between strain I-0T and D. radiodurans ACCC 10492T was 37%.

Survival rates after exposure to various doses of gamma radiation and UV light were analysed for strain I-0T, D. radiodurans ACCC 10492T and E. coli K-12 CGMCC 1.3065 (Fig. 3). The gamma radiation and UV light survival curves of E. coli K-12 CGMCC 1.3065 dropped most sharply, while the two Deinococcus strains were significantly resistant to gamma radiation and UV light. Compared with D. radiodurans ACCC 10492T, strain I-0T showed higher resistance to gamma radiation and UV light.
In summary, the results of 16S rRNA gene sequence comparison and the chemotaxonomic data clearly demonstrate that strain I-0 T is a member of the genus *Deinococcus*. On the basis of its distinct phylogenetic position, the presence of the combination of fatty acids C16 : 1<sup>v</sup> and C16 : 0 and its phenotypic characteristics (Table 1), strain I-0T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus gobiensis* sp. nov. is proposed.

**Description of Deinococcus gobiensis** sp. nov.

*Deinococcus gobiensis* (go.bi.en' sis. N.L. masc. adj. *gobiensis* pertaining to the Gobi, a great bare-rock desert in Xinjiang Autonomous Region, China, the source of the type strain).

Cells are facultatively anaerobic, Gram-positive, non-spore-forming cocci, 1.0–2.2 μm in diameter. Catalase- and oxidase-positive. Reduces nitrate to nitrite. Positive for urease and negative for arginine dihydrolase and indole production. Negative in the Voges–Proskauer test. Grows well on TGY agar at 15–35 °C (optimum 30 °C) and pH 7–8; does not grow on LB agar. The reddish colonies are circular, opaque and convex with regular edges. Glucose, sucrose, lactose, fructose, L-aspartic acid and L-histidine

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**Table 1.** Properties of strain I-0<sup>T</sup> useful for differentiation from type strains of related species of genus *Deinococcus*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</thead>
<tbody>
<tr>
<td>Pigmentation</td>
<td>Pink–red</td>
<td>Pink–red</td>
<td>Light pink</td>
<td>Pink</td>
<td>Red</td>
<td>Faintly pink</td>
<td>Red or pink</td>
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<tr>
<td>Cell morphology</td>
<td>Coccus</td>
<td>Coccus</td>
<td>Coccus</td>
<td>Coccus</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
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<tr>
<td>Gram reaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Demand for oxygen</td>
<td>Facultative anaerobe</td>
<td>Strict aerobe</td>
<td>Aerobe</td>
<td>Aerobe</td>
<td>Strict aerobe</td>
<td>Strict aerobe</td>
<td>Aerobe</td>
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<tr>
<td>Cytochrome oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Carbon source utilization</td>
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<tr>
<td>D-Fucose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>ND</td>
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<td>ND</td>
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<td>Raffinose</td>
<td>+</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>D-Xylose</td>
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<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>+</td>
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<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>L-Tryptophan</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Major fatty acids (%)</td>
<td>16:1&lt;sup&gt;v&lt;/sup&gt;7c (42.1), 16:0 (35.1), 16:1&lt;sup&gt;v&lt;/sup&gt;7c (39.9), 16:0 (14.1), 17:1o8c (11.8), 17:1o7c iso (12.5), 16:1o8c (13.7), 17:1o7c iso (10.7), 16:1o7c (29.4), 15:0 (13), 16:1o7c (18), 16:1o8c (14.1), 17:1o7c (16.1), 15:1 (23), 15:1 (18)</td>
<td>16:1&lt;sup&gt;v&lt;/sup&gt;7c (42.1), 16:0 (35.1), 16:1&lt;sup&gt;v&lt;/sup&gt;7c (39.9), 16:0 (14.1), 17:1o8c (11.8), 17:1o7c iso (12.5), 16:1o8c (13.7), 17:1o7c iso (10.7), 16:1o7c (29.4), 15:0 (13), 16:1o7c (18), 16:1o8c (14.1), 17:1o7c (16.1), 15:1 (23), 15:1 (18)</td>
<td>16:1&lt;sup&gt;v&lt;/sup&gt;7c (42.1), 16:0 (35.1), 16:1&lt;sup&gt;v&lt;/sup&gt;7c (39.9), 16:0 (14.1), 17:1o8c (11.8), 17:1o7c iso (12.5), 16:1o8c (13.7), 17:1o7c iso (10.7), 16:1o7c (29.4), 15:0 (13), 16:1o7c (18), 16:1o8c (14.1), 17:1o7c (16.1), 15:1 (23), 15:1 (18)</td>
<td>16:1&lt;sup&gt;v&lt;/sup&gt;7c (42.1), 16:0 (35.1), 16:1&lt;sup&gt;v&lt;/sup&gt;7c (39.9), 16:0 (14.1), 17:1o8c (11.8), 17:1o7c iso (12.5), 16:1o8c (13.7), 17:1o7c iso (10.7), 16:1o7c (29.4), 15:0 (13), 16:1o7c (18), 16:1o8c (14.1), 17:1o7c (16.1), 15:1 (23), 15:1 (18)</td>
<td>16:1&lt;sup&gt;v&lt;/sup&gt;7c (42.1), 16:0 (35.1), 16:1&lt;sup&gt;v&lt;/sup&gt;7c (39.9), 16:0 (14.1), 17:1o8c (11.8), 17:1o7c iso (12.5), 16:1o8c (13.7), 17:1o7c iso (10.7), 16:1o7c (29.4), 15:0 (13), 16:1o7c (18), 16:1o8c (14.1), 17:1o7c (16.1), 15:1 (23), 15:1 (18)</td>
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<td>16:1&lt;sup&gt;v&lt;/sup&gt;7c (42.1), 16:0 (35.1), 16:1&lt;sup&gt;v&lt;/sup&gt;7c (39.9), 16:0 (14.1), 17:1o8c (11.8), 17:1o7c iso (12.5), 16:1o8c (13.7), 17:1o7c iso (10.7), 16:1o7c (29.4), 15:0 (13), 16:1o7c (18), 16:1o8c (14.1), 17:1o7c (16.1), 15:1 (23), 15:1 (18)</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>65.4</td>
<td>67</td>
<td>67.9</td>
<td>66.4</td>
<td>65.8</td>
<td>60</td>
<td>68.7</td>
</tr>
</tbody>
</table>
can be utilized as sole carbon sources, but L-arabinose, D-fucose, D-xylose, L-rhamnose, L-tryptophan and L-arginine can not. Gelatin, starch and casein are degraded. The major cellular fatty acids are C16:1ω7c and C16:0. Peptidoglycan type is Orn−Gly2 (A3b). The major respiratory quinone is MK-8. The polar lipid profile consists of various unidentified glycolipids, phosphoglycolipids, phospholipids, pigments and an aminophospholipid. The DNA G+C content of the type strain is 65.4 mol%. The type strain is extremely resistant to gamma radiation (15 kGy) and UV light (600 J m$^{-2}$) compared with *E. coli* K-12 CGMCC 1.3065 and *D. radiodurans* ACCC 10492$^T$.

The type strain is I-0$^T$ (DSM 21396$^T$ = CGMCC 1.7299$^T$), isolated from a mixed sand sample from the Gobi desert.

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**References**

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