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-0^T, 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 $C_{16:1}\omega7c$ and $C_{16:0}$. Its polar lipid profile contained several unidentified glycolipids, phosphoglycolipids, phospholipids, pigments and an aminophospholipid. The peptidoglycan type was Orn–Gly_2 (A3β) and the predominant respiratory quinone was MK-8. The DNA G+C content was 65.4 mol%. DNA–DNA relatedness between strain I-0^T and *Deinococcus radiodurans* ACCC 10492^T 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-0^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus gobiensis* sp. nov. is proposed. The type strain is I-0^T (=DSM 21396^T =CGMCC 1.7299^T).

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.*, 2005), aquifers (Suresh *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-0^T, which was obtained from a mixed sand sample. After exposure of the sample to 10 kGy gamma radiation from a ^{60}Co 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-0^T is EU427464.

Micrographs of colonies and cells of strain I-0^T, a 16S rRNA gene sequence-based maximum-parsimony tree, the fatty acid profile of strain I-0^T and a table of 16S rRNA gene sequence similarities to related strains are available as supplementary material with the online version of this paper.

method of Cowan (1974). Physiological characterization and additional biochemical tests were performed as described by Anderson *et al.* (1956).

Whole-cell hydrolysates were prepared as described by Hasegawa *et al.* (1983). Fatty acid methyl esters were analysed by using the trimethyl sulfonium hydroxide method of Butte (1983). Polar lipid analysis, cell-wall peptidoglycan analysis and quinone analysis were carried out according to the methods outlined by Zhang *et al.* (2007) and performed at the Institute of Microbiology of Yunnan Province.

Genomic DNA of strain I-0^T was extracted using a TIANamp bacteria DNA kit (Tiangen) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR with bacterial universal primers F27 and R1492, which were adapted from primers fD1 and rP1 (Weisburg *et al.*, 1991), and then ligated into the pSURE-T vector (Galen), followed by sequencing by Takala Co. (Dalian, China). The DNA G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962) and was calculated by using the equation of Owen & Lapage (1976). *Escherichia coli* K-12 CGMCC 1.3065 (DNA G+C content 50.6 mol%) was used as a control. DNA–DNA hybridization studies were carried out by using the fluorometric microdilution plate method (Ezaki *et al.*, 1988; Sawabe *et al.*, 1998).

To determine resistance of the culture to gamma radiation, cells were harvested by centrifugation at 5000 ***g*** for 5 min and then washed twice with 100 mM potassium phosphate buffer (pH 7.0). Four millilitres of the suspension was exposed to different doses (0, 5, 10 and 15 kGy) of gamma radiation from a ^{60}Co source (6 kGy h^{-1}; BNU) in a 15 ml EP tube. Irradiated cultures were serially diluted and 100 µl aliquots were spread on TGY agar plates. After incubation at 30 °C for 3–4 days, the total c.f.u. ml^{-1} was determined. To determine the resistance of the isolate to UV light, UV survival curves were obtained according to the method of Liu *et al.* (2008). For both experiments, *E. coli* K-12 CGMCC 1.3065 served as a negative control and *Deinococcus radiodurans* ACCC 10492^T served as a positive control.

Strain I-0^T was a Gram-positive, non-spore-forming coccus. It grew well on TGY agar in an incubator and could grow on nutrient agar in an anaerobic chamber, but did not grow on Luria–Bertani agar. Colonies on TGY plates were pink–red, circular, opaque and convex with regular edges (Supplementary Fig. S1, available in IJSEM Online). Cells were 1.0–2.2 µm in diameter, occurring singly and in tetrad forms and usually in pairs, and did not form spores (Supplementary Fig. S2). The ultrastructure of strain I-0^T was similar to that of *Deinococcus murrayi* ALT-1b^T (Ferreira *et al.*, 1997), which showed a cell wall with several layers and a large electron-dense granule (Supplementary Fig. S3). Strain I-0^T 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-0^T 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-0^T 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 $C_{16:1}\omega7c$ (42.07 %) and $C_{16:0}$ (35.06 %) (Table 1, Supplementary Table S1). This combination allows strain I-0^T 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-0^T was MK-8, as in all recognized *Deinococcus* species.

As shown in Fig. 1, the polar lipid profile of strain I-0^T consisted of three unidentified glycolipids, four phosphoglycolipids, three phospholipids, two pigment spots and an aminophospholipid. The polar lipid profile of strain I-0^T 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-0^T was similar to that of the lipid spots PL, APL, PGL1–2 and GL3 and PIG1–2 reported for *D. radiodurans* AS 1.633^T (Zhang *et al.*, 2007). The presence of these lipids extracted from strain I-0^T confirms that it should be assigned to the genus *Deinococcus*.

An almost-complete 16S rRNA gene sequence (1470 bp) was determined for strain I-0^T. 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 dendrograms, 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-0^T and *D. radiodurans* ACCC 10492^T was 37 %.

Survival rates after exposure to various doses of gamma radiation and UV light were analysed for strain I-0^T, *D. radiodurans* ACCC 10492^T 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 10492^T, strain I-0^T showed higher resistance to gamma radiation and UV light.

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

Strains: 1, strain I-0^T (data from this study); 2, *D. radiodurans* R1^T [data for carbon utilization, cell morphology, pigmentation and Gram reaction from this study and data for fatty acids from Zhang *et al.* (2007) using strain ACCC 10492^T; other data from Brooks & Murray (1981)]; 3, *D. hohokamensis* KR-40^T; 4, *D. navajonensis* KR-114^T (data in columns 3 and 4 from Rainey *et al.*, 2005); 5, *D. indicus* Wt/1a^T (Suresh *et al.*, 2004); 6, *D. deserti* VCD115^T (de Groot *et al.*, 2005); 7, *D. grandis* DSM 1963^T (Oyaizu *et al.*, 1987; Suresh *et al.*, 2004). +, Positive; −, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Pigmentation	Pink–red	Pink–red	Light pink	Pink	Red	Faintly pink	Red or pink
Cell morphology	Coccus	Coccus	Coccus	Rod	Rod	Rod	Rod
Gram reaction	+	+	+	+	−	−	−
Demand for oxygen	Facultative anaerobe	Strict aerobe	Aerobe	Aerobe	Strict aerobe	Strict aerobe	Aerobe
Cytochrome oxidase	+	+	+	+	−	ND	−
Catalase	+	+	−	−	−	+	+
Carbon source utilization							
D-Fucose	−	+	−	+	ND	ND	ND
Raffinose	+	W	−	−	+	ND	−
D-Xylose	−	−	−	−	ND	−	ND
Fructose	+	+	−	+	−	+	ND
Glucose	+	+	−	−	−	+	−
Lactose	+	−	−	−	+	−	−
L-Arabinose	−	−	+	+	+	−	−
L-Tryptophan	−	+	−	−	+	−	−
Major fatty acids (%)	16 : 1ω7*c* (42.1), 16 : 0 (35.1)	16 : 1ω7*c* (39.9), 16 : 0 (14.1), 17 : 1ω8*c* (11.8)	16 : 1ω7*c* (ND), 17 : 1ω8*c* (ND), 17 : 1ω7*c* iso (ND)	16 : 1ω7*c* (ND), 17 : 1ω8*c* (ND), 17 : 1ω7*c* iso (ND)	15 : 1 (12.5), 16 : 1 (33.7), 17 : 1 (10.7)	16 : 1ω7*c* (29.4), 17 : 1ω8*c* (14.1)	15 : 0 (13), 15 : 1 (23), 16 : 1 (18)
DNA G+C content (mol%)	65.4	67	67.9	66.4	65.8	60	68.7

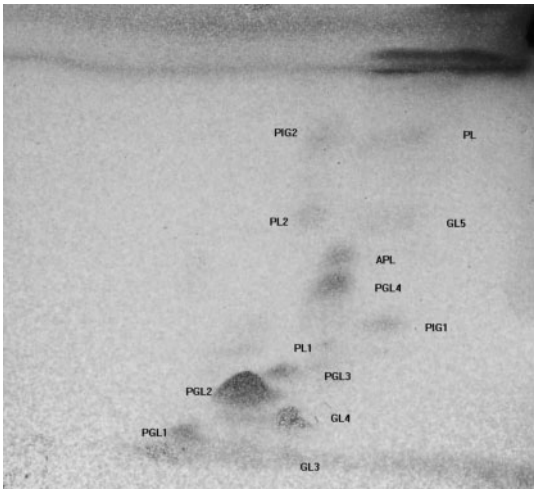

Fig. 1. Polar lipid profile of strain I-0^T. GL3–GL5, Unidentified glycolipids; PGL1–PGL4, unidentified phosphoglycolipids; PL, PL1 and PL2, unidentified phospholipids; APL, unidentified aminophospholipid; PIG1 and PIG2, pigments.

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 $C_{16:1}\omega7c$ and $C_{16:0}$ and its phenotypic characteristics (Table 1), strain I-0^T 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

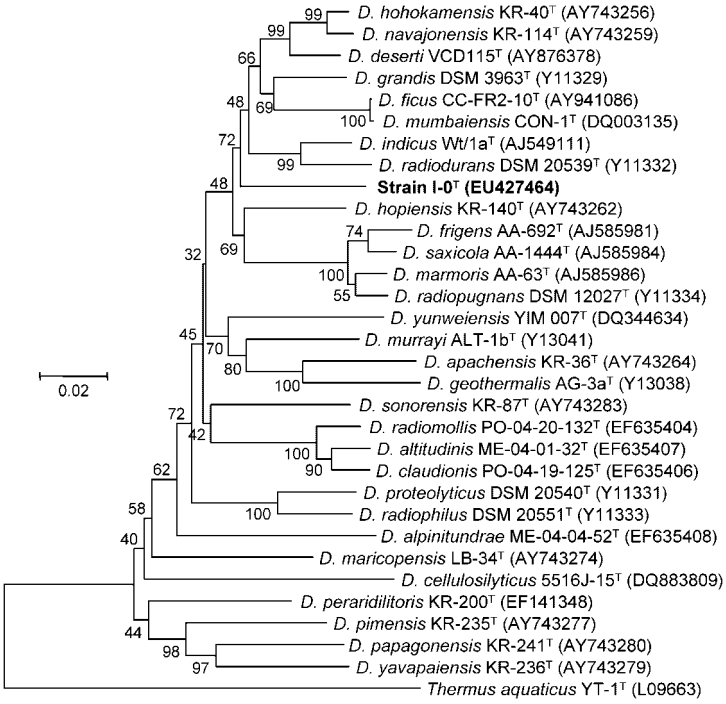

Fig. 2. Neighbour-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain I-0^T with other *Deinococcus* strains. Numbers on branch nodes are percentage bootstrap values. Bar, 0.02 substitutions per nucleotide position. The sequence of *Thermus aquaticus* YT-1^T was used as an outgroup.

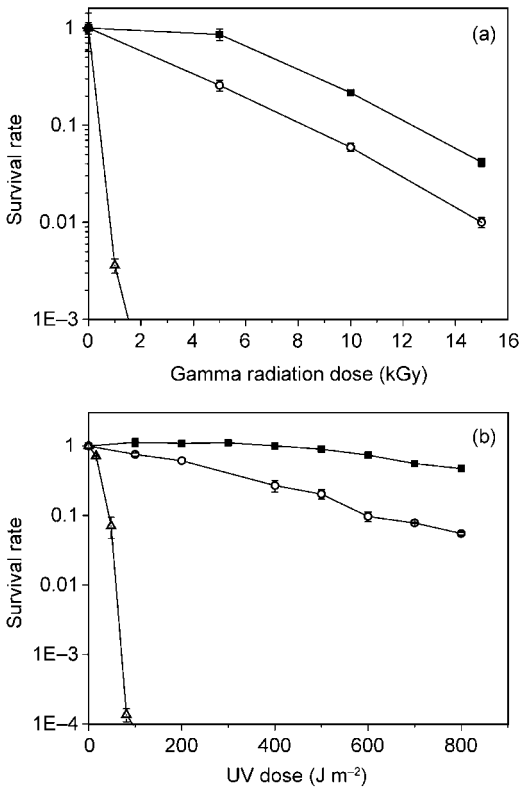

Fig. 3. Survival curves following exposure to gamma radiation (a) and UV light (b) of strain I-0^T (■), *D. radiodurans* ACCC 10492^T (○) and *E. coli* K-12 CGMCC 1.3065 (△).

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 $C_{16:1}\omega7c$ and $C_{16:0}$. Peptidoglycan type is Orn–Gly_2 (A3β). 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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