Deinococcus antarcticus sp. nov., isolated from soil

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A pink-pigmented, non-motile, coccoid bacterial strain, designated G3-6-20^T, was isolated from a soil sample collected in the Grove Mountains, East Antarctica. This strain was resistant to UV irradiation (810 J m^{-2}) and slightly more sensitive to desiccation as compared with *Deinococcus radiodurans*. Phylogenetic analyses based on the 16S rRNA gene sequence of the isolate indicated that the organism belongs to the genus *Deinococcus*. Highest sequence similarities were with *Deinococcus ficus* CC-FR2-10^T (93.5 %), *Deinococcus xinjiangensis* X-82^T (92.8 %), *Deinococcus indicus* Wt/1a^T (92.5 %), *Deinococcus daejeonensis* MJ27^T (92.3 %), *Deinococcus wulumuqiensis* R-12^T (92.3 %), *Deinococcus aquaticus* PB314^T (92.2 %) and *Deinococcus radiodurans* DSM 20539^T (92.2 %). Major fatty acids were $C_{18:1}\omega7c$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), anteiso-$C_{15:0}$ and $C_{16:0}$. The G+C content of the genomic DNA of strain G3-6-20^T was 63.1 mol%. Menaquinone 8 (MK-8) was the predominant respiratory quinone. Based on its phylogenetic position, and chemotaxonomic and phenotypic characteristics, strain G3-6-20^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus antarcticus* sp. nov. is proposed. The type strain is G3-6-20^T (=DSM 27864^T=CCTCC AB 2013263^T).

The genus *Deinococcus*, which was proposed by Brooks & Murray (1981), comprises, at the time of writing, 48 species with validly published names (http://www.bacterio.net/deinococcus.html). Species of this genus have been isolated from a wide range of environments (Asker *et al.*, 2009; Chen *et al.*, 2012), for example airborne dust (Weon *et al.*, 2007), hot springs (Ferreira *et al.*, 1997), aquifers (Suresh *et al.*, 2004) and continental Antarctica (Hirsch *et al.*, 2004). The genus includes cells that are aerobic, non-spore-forming, non-motile, spherical or rod-shaped (Zhang *et al.*, 2007; Wang *et al.*, 2010; Srinivasan *et al.*, 2012a) and is notable for its resistance to gamma, UV and ionizing radiation, and to desiccation (Lai *et al.*, 2006; Callegan *et al.*, 2008; Wang *et al.*, 2010; Srinivasan *et al.*, 2012b). Most members of the genus are Gram-stain-positive (Srinivasan *et al.*, 2012b), but cells of *Deinococcus indicus*, *Deinococcus grandis*, *Deinococcus deserti* and *Deinococcus yunweiensis* are Gram-stain-negative (Oyaizu *et al.*, 1987; Suresh *et al.*, 2004; de Groot *et al.*, 2005; Zhang *et al.*, 2007). They have L-ornithine in the cell-wall peptidoglycan but lack teichoic acids (Srinivasan *et al.*, 2012a). In this paper, we report the results of a polyphasic taxonomic study on a novel strain, designated G3-6-20^T, isolated from Antarctic soil, which indicated that this isolate represents a novel species within the genus *Deinococcus*.

Characterized by physicochemically extreme conditions of low temperatures, high UV radiation and desiccation (Convey *et al.*, 2008), Antarctic soils harbour a large variety of extremotolerant bacteria, and among them are representatives of the genus *Deinococcus* (Hirsch *et al.*, 2004). Strain G3-6-20^T was originally isolated by the spread plate method as described by Lester *et al.* (2007) during the course of a study on the diversity of culturable bacteria in a soil sample of the Grove Mountains, East Antarctica (72° 53′ 01″ S 75° 1′ 01″ E). This strain was maintained and subcultured on trypticase soy agar (TSA; Difco) plates at 28 °C and was preserved as a suspension in trypticase soy broth (TSB; Difco) with 20 % glycerol (w/v) at −80 °C. *Deinococcus ficus* CC-FR2-10^T obtained from the China Center for Type Culture Collection (CCTCC), *Deinococcus xinjiangensis* X-82^T from CCTCC and *Deinococcus radiodurans* DSM 20539^T from the German Collection of Microorganisms and Cell Cultures (DSMZ) were used as reference strains.

We tested growth of strain G3-6-20^T on nutrient agar (Difco), TGY [1 % tryptone, 0.1 % glucose, 0.5 % yeast extract (pH 7.0±0.2), 1.5 % agar], Luria–Bertani (LB; Difco) agar, R2A agar (Difco) and TSA (Difco). Unlike some deinococcal isolates from the Sonoran Desert (Rainey

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain G3-6-20^T is KC494323.

One supplementary table and three supplementary figures are available with the online Supplementary Material.

et al., 2005) and dust of the stratosphere (Yang *et al.*, 2009), which grow slowly and need almost 2 weeks to harvest on these plates, strain G3-6-20^T grows well after 3 days of cultivation on all the above plates. The cell morphology and motility of strain G3-6-20^T were examined with a Nikon eclipse 80i microscope. Colonial morphology was observed on TSA plates after 2 days of incubation at 28 °C. Scanning electron microscopy of this strain was conducted as described by Yang *et al.* (2009) and using an EDAX XL-30 environmental scanning electron microscope operated at 15 kV (Fig. S1, available in the online Supplementary Material).

To test the Gram reaction, a Gram-stain kit and the KOH lysis test (Buck, 1982) were used. Growth temperatures were determined by spreading exponentially grown cells on TSA plates, followed by incubation at 4, 10, 12, 20, 25, 28, 30, 35, 37, 40 and 45 °C. Plates were incubated for 4 weeks and growth was indicated by visible colonies. The pH range for growth was measured on LB agar at pH 5.0–10.0 (0.5 pH unit intervals) after incubation for 4 weeks at 28 °C; the pH of the medium was adjusted as described by Srinivasan *et al.* (2012a). Tolerance to NaCl [0.5, 1, 2, 3, 4, 5 and 10 % (w/v)] was also studied using LB plates. Substrate utilization patterns and enzyme activities of strain G3-6-20^T and the three reference strains were tested by using the commercial API 50CH, API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions. Acid production from carbohydrates was determined with API 50CH strips using the API 50CHB/E medium. All of the tests were performed in duplicate. Catalase activity was assessed through observing bubble production in 3 % (v/v) H_2O_2 (Smibert & Krieg, 1994). Resistance to UV irradiation and to desiccation were determined with reference to the methods outlined by Peng *et al.* (2009), under a 254 nm UV lamp; *Escherichia coli* DH5α (=ATCC 35607) and *D. radiodurans* DSM 20539^T served as negative and positive controls, respectively. Regarding UV radiation tolerance, 5 and 13 % survival was observed for strain G3-6-20^T and *D. radiodurans* DSM 20539^T, respectively, when they were exposed to a UV dose of 810 J m^{-2}. The viability of the G3-6-20^T culture decreased to 2–3 % after 6 weeks of desiccation, which equates to approximately four-fold greater sensitivity than *D. radiodurans* DSM 20539^T. Morphological, physiological and biochemical characteristics of strain G3-6-20^T are given in Table 1 and the species description (see below).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were carried out using the methods described by Li *et al.* (2010). The nearly complete 16S rRNA gene sequence (1465 bp) of strain G3-6-20^T was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim *et al.*, 2012). According to the 16S rRNA gene sequence similarity values, the closest relatives

Table 1. Phenotypic differences among strain G3-6-20^T and related members of the genus *Deinococcus*

Strains: 1, G3-6-20^T; 2, *D. ficus* CC-FR2-10^T; 3, *D. xinjiangensis* X-82^T; 4, *D. radiodurans* DSM 20539^T. All data were obtained during this study unless indicated otherwise. +, Positive result or growth; −, negative result or growth; ND, no data available.

Characteristic	1	2	3	4
Morphology*	Spherical	Rod	Spherical	Spherical
Pigmentation	Faint pink	Pale pink	Faint pink	Red
Growth temperature range (°C)	20–40	10–40	10–37	25–40
Growth pH range	6.5–8.0	5.5–10	5.0–8.0	7.0–8.5
Tolerance to NaCl (%, w/v)	0–1	0–1	0–1	0–7
Utilization of:				
L-Arabinose	−	+	−	−
Lactose	−	+	−	−
D-Xylose	−	+	−	−
D-Mannose	+	+	−	−
Melibiose	−	+	−	−
N-Acetyl-D-glucosamine	−	+	−	+
D-Sorbitol	−	+	−	−
Raffinose	−	+	−	−
API ZYM test results				
Valine arylamidase	+	−	+	−
α-Glucosidase	+	+	+	−
Trypsin	+	−	−	−
β-Glucosidase	+	−	−	−
DNA G+C content (mol%)*	63.1	ND	60.0	67.0

*Data for strains 2, 3 and 4 were taken from Lai *et al.* (2006), Peng *et al.* (2009) and Srinivasan *et al.* (2012a), respectively.

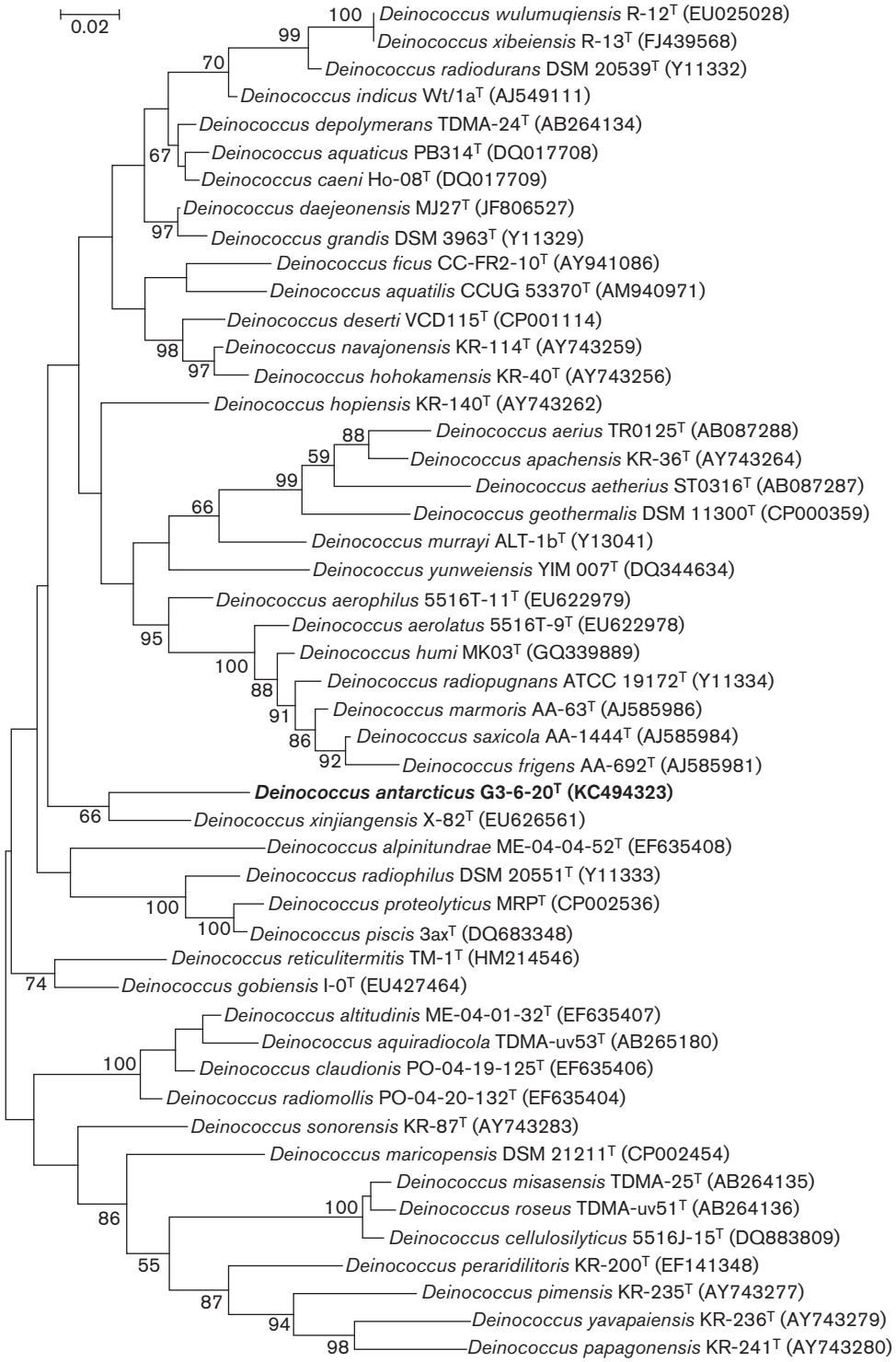

Fig. 1. Maximum-likelihood phylogenetic dendrogram based on 16S rRNA gene sequences showing the relationships between strain G3-6-20^T and the type strains of recognized species of the genus *Deinococcus*. Percentage bootstrap values based on 1000 replications are shown at branch points; only values $\geqslant$50 % are shown. GenBank accession numbers are shown in parentheses. Bar, 0.02 substitutions per nucleotide position. Neighbour-joining and maximum-parsimony trees are shown in Fig. S2.

of strain G3-6-20^T were *D. ficus* CC-FR2-10^T (93.5 %), followed by *D. xinjiangensis* X-82^T (92.8 %), *D. indicus* Wt/1a^T (92.5 %), *Deinococcus daejeonensis* MJ27^T (92.3 %), *Deinococcus wulumuqiensis* R-12^T (92.3 %), *Deinococcus aquaticus* PB314^T (92.2 %) and *D. radiodurans* DSM 20539^T (92.2 %). The partial 16S rRNA gene sequence of strain G3-6-20^T and the sequences of type strains of described species of the genus *Deinococcus* were aligned for phylogenetic analysis using CLUSTAL X 2.0 (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed by using the maximum-likelihood method in the PHYLIP 3.69 package (Felsenstein, 2009). The relationships among taxa were also established with the neighbour-joining and maximum-parsimony methods with Kimura's two-parameter analysis in MEGA, version 5.0 (Tamura *et al.*, 2011). The topologies of the trees were evaluated by bootstrap analysis of 1000 replications. Results showed that strain G3-6-20^T was phylogenetically most closely related to species of the genus *Deinococcus* (Figs 1 and S2).

The DNA G+C content was determined by HPLC (Ultimate 3000) as described by Mesbah *et al.* (1989). 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 TSA plates for 48 h at 28 °C when the cells are in the late exponential phase. Respiratory quinones were extracted and separated by HPLC according to the method of Xie & Yokota (2003). Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990). The peptidoglycan was prepared and the amino acids were analysed by using the methods described by Schleifer & Kandler (1972).

The genomic DNA G+C content of strain G3-6-20^T was 63.1 mol%. The fatty acid profile of strain G3-6-20^T is compared with those of some closely related species of the genus *Deinococcus* in Table S1. The major fatty acids of strain G3-6-20^T were $C_{18:1}\omega7c$ (24.88 %), summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 21.52 %), anteiso-$C_{15:0}$ (15.12 %) and $C_{16:0}$ (11.53 %). The fatty acid profile of strain G3-6-20^T was similar to those of its closest relatives in the genus *Deinococcus* but showed some quantitative and qualitative differences; for example, strain G3-6-20^T contained much more $C_{18:1}\omega7c$ and anteiso-$C_{15:0}$ than any of the closely related type strains with which it was compared (Table S1). Like almost all recognized strains of the genus *Deinococcus*, the predominant respiratory quinone in strain G3-6-20^T was menaquinone 8 (MK-8). The major peptidoglycan amino acids of strain G3-6-20^T were glycine, L-alanine, D-aspartate, L-glutamine and L-ornithine.

Strain G3-6-20^T displayed a complex polar lipid profile consisting of various unidentified glycolipids, phosphoglycolipids, an unknown phospholipid and an unknown polar lipid; an unknown phosphoglycolipid (PGL3), which was also identified in *D. xinjiangensis* X-82^T and *D. ficus* CC-FR2-10^T, was the predominant component in strain G3-6-20^T (Fig. S3). The lack of aminophospholipid (APL) and the presence of significantly larger amounts of glycolipids GL1–GL5 clearly distinguished G3-6-20^T from other members of the genus *Deinococcus*, such as *D. ficus*, *D. xinjiangensis* and *D. radiodurans*.

On the basis of the data presented, strain G3-6-20^T is considered to represent a novel species of the genus *Deinococcus*, for which the name *Deinococcus antarcticus* sp. nov. is proposed.

Description of *Deinococcus antarcticus* sp. nov.

Deinococcus antarcticus (ant.arc′ti.cus. L. masc. adj. *antarcticus* southern, of the Antarctic, the geographical origin of the type strain).

Cells are Gram-stain-positive, non-motile, spherical (~1.8 μm in diameter), non-spore-forming and aerobic. Colonies on TSA plates are smooth, opaque, pink and circular with entire edges and 3–4 mm in diameter; colonies become mucoid. Growth occurs on TSA, nutrient agar, LB agar, TGY and R2A agar. Growth occurs at 20–40 °C, at pH 6.5–8.0 and with 0–1 % (w/v) NaCl. Resistant to UV irradiation (810 J m^{-2}). Slightly more sensitive to desiccation as compared with *D. radiodurans*. Catalase-positive and oxidase-negative. Positive, on API ZYM and API 20NE strips, for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, α-glucosidase, β-glucosidase, naphthol-AS-BI-phosphohydrolase, gelatin, 4-nitrophenyl β-D-galactopyranoside and potassium nitrate. Positive, on API 50CH strips, for the assimilation of glycerol, D-glucose, D-fructose, D-mannose, methyl α-D-glucopyranoside, aesculin ferric citrate, salicin, cellobiose, maltose, sucrose, trehalose, starch, glycogen, turanose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 5-ketogluconate. The predominant respiratory quinone is MK-8. The major fatty acids are $C_{18:1}\omega7c$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), anteiso-$C_{15:0}$ and $C_{16:0}$. The major peptidoglycan amino acids are glycine, L-alanine, D-aspartate, L-glutamine and L-ornithine. The polar lipid profile is complex, containing various unidentified phosphoglycolipids, glycolipids, phospholipid and lipid.

The type strain, G3-6-20^T (=DSM 27864^T=CCTCC AB 2013263^T), was isolated from a soil sample collected in the Grove Mountains area, East Antarctica. The genomic DNA G+C content of the type strain is 63.1 mol%.

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