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EXPERIMENTAL ARTICLES

Deinococcus soli sp. nov., a Gamma- and UV-Radiation-Resistant Bacterium from North–West China[1]

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Abstract—An ionizing- and UV-radiation-resistant bacterial strain, designated ZLM-202^T, was isolated from an arid soil sample collected from Xinjiang Province, north-west China. The soil sample was irradiated before serial dilution plating was performed using twofold-diluted marine agar. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain ZLM-202^T was a member of the genus *Deinococcus*, exhibiting sequence similarities of 86.3–92.2% to the type strains of recognized *Deinococcus* species. Strain ZLM-202^T was strictly aerobic and showed optimum growth at 30–37°C and pH 7.0. The major respiratory menaquinone was MK-8. The major fatty acids were 16:1 ω7c, 16 : 0, 15 : 1 ω6c, 15 : 0 *iso* and 16 : 1 ω5c. *L*-ornithine was detected in its peptidoglycan. The polar lipid profile consisted mainly of various unknown phosphoglycolipids, aminophospholipids, glycolipids and phospholipids. The DNA G + C content was 65.5 mol. %. The strain was shown to be extremely resistant to gamma radiation (>10 kGy) and UV light (>600 J m^{-2}). On the basis of the phylogenetic, chemotaxonomic and phenotypic data, strain ZLM-202^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus* soli sp. nov. is proposed. The type strain is ZLM-202^T (=CCTCC AB 208223^T=KCTC 13419^T).

Keywords: *Deinococcus soli* sp. nov., north–west China, phylogenetic analysis, gamma radiation.

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The genus *Deinococcus* was first described in 1981, with *Deinococcus radiodurans* DSM 20539^T as the type species [1]. At the time of writing, it comprises 45 recognized species, including the recently described species *D. depolymerans* [2] and *D. xibeiensis* [3]. These species were recovered from various environments such as soils, hot springs, foods, faeces, air dust and rhizosphere. The majority of them have shown resistance to high doses of ionizing radiation to which they would never be exposed in the natural environment.

In a study of the ionizing-radiation-resistant bacterial communities of high-altitude area, surface soil samples from an arid area in Xinjiang Province of China were exposed to various doses of ionizing radiation and the surviving bacterial populations were isolated and identified. Here we report on the taxonomic characterization of a pink-pigmented bacterial strain, 202^T, which was isolated from soil exposed to 10 kGy gamma radiation.

MATERIALS AND METHODS

Isolation of bacterial strains and culture conditions. Strain ZLM-202^T was isolated from an arid soil sample collected from Xinjiang Province, north-west China. The soil sample was irradiated with ^{60}Co γ-rays at a dose of 600 Gy h^{-1}, and then serial dilution plating was performed using twofold-diluted marine broth 2216 agar plates (MB/2; 18.7 g l^{-1}) (Difco). Plates were incubated at 28°C for up to 15 days and colonies with different morphologies were selected to obtain pure cultures. After primary isolation and purification, all strains were cultivated at 28°C on the same medium and stored as a glycerol suspension (20%, v/v) at –80°C. The reference strains *Deinococcus radiodurans* R1^T (=DSM 20539^T) and *Escherichia coli* DH5α (=ATCC 35607) were obtained from the China Center for Type Culture Collection (CCTCC), China. *D. radiodurans* R1^T was grown on R2A agar (Difco) at 30°C and *E. coli* DH5α was grown on LB (Luria–Bertani) agar (1% tryptone, 0.5% yeast extract, 1% NaCl, 1.5% agar, pH 7.0) at 37°C.

Phenotypic characteristics. Cell morphology was examined by using phase-contrast microscopy (BX51 microscope; Olympus).

The Gram reaction was carried out according to the classical Gram procedure described in [4]. Gliding motility was determined as described in [5]. The temperature range (4, 7, 10, 17, 25, 30, 37, 42, 45 and 50°C) and pH range (pH 4.0–11.0 at intervals of 1.0 pH unit) for growth and requirement for NaCl (0, 1, 2, 3 and 5%, w/v) were determined using R2A medium. Growth un-

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der anaerobic condition was determined after 1 week incubation in an anaerobic chamber (Oxoid AnaeroJar System, England) on R2A agar. Conventional biochemical tests were performed as described in [6], including tests for catalase, cytochrome oxidase, nitrate reduction, H_2S production and casein hydrolysis. Phenotypic features of strain ZLM-202^T were also determined using the API 20E, API 20NE, API ID 32GN and API ZYM test kits (bioMérieux) according to the manufacturer's instructions. Antimicrobial susceptibility testing was performed by the agar-diffusion method using antibiotic-impregnated disks, as described in [7].

Chemotaxonomy. The respiratory quinone system was extracted and determined by HPLC as described earlier [8]. Polar lipids were extracted and analysed as described in [9] and [10]. For the determination of the cellular fatty acid methyl ester content, strain ZLM-202^T and *D. radiodurans* R1^T were grown on R2A agar (Difco) at 30°C for 48 h. FAME analysis was carried out according to the standard protocol of the Sherlock Microbial Identification System [11]. The peptidoglycan was prepared and analysed as described in [12].

DNA for DNA base composition analysis was prepared according to the procedure of [13]. The DNA G + C content was determined by HPLC according to the method of [14].

16S rRNA gene sequencing and phylogenetic analysis. For 16S rRNA gene sequencing and phylogenetic analysis, DNA was extracted by using Bacteria Genomic DNA Isolation Kit (ChaoShi-Bio; China). The primer pair 27f (5'-GAGTTTGATCCTGGCTCAG-3') and 1527r (5'-AGAAAGGAGGTGATCCAGCC-3') was used for amplification of the 16S rRNA gene [15]. PCR and 16S rRNA gene sequencing were carried out as described by [16]. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server [17] (http://www.eztaxon.org/). Phylogenetic analysis was performed by using MEGA, version 4.1 [18], after multiple alignment of the data via CLUSTAL_X [19]. A distance matrix method (distance options according to the Kimura two-parameter model), including clustering by neighbour-joining, and a discrete character-based maximum-parsimony method were used. In each case, bootstrap values were calculated based on 1000 replications.

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain ZLM-202^T is available from the GenBank nucleotide database at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov) under accession number FJ176238.

Measurement of cell survival rate. To determine resistance of the culture to gamma radiation, cells in the exponential phase were harvested by centrifugation, washed twice and resuspended with 0.067 M potassium phosphate buffer (pH 7.0). Three millilitres of the suspension was exposed to different doses (0, 5, 10 and 15 kGy) of gamma radiation from a ^{60}Co source (600 Gy h^{-1}) in a 10 ml glass tube. Irradiated cultures were serially diluted and 100 μl aliquots were spread on triplicate R2A agar plates. After incubation at 30°C for one week, the total c.f.u. ml^{-1} was determined. To determine the UV radiation resistance, aliquots of the prepared cells in potassium phosphate buffer as above were spread on R2A agar plates; the plates with their lids open were immediately exposed to UV light (UV-C, 254 nm) from a germicidal lamp with a calibrated dose rate of 3.0 J m^{-2} s^{-1}. The irradiated plates were carefully wrapped in aluminium foil to avoid any photorepair processes, and incubated at 30°C for one week. For both experiments, *D. radiodurans* R1^T (=DSM 20539) and *E. coli* DH5α (=ATCC 35607) were tested as positive and negative controls. Relative survival was determined by comparing with unirradiated cultures.

RESULTS AND DISCUSSION

Phenotypic characteristics. Strain ZLM-202^T was strictly aerobic, Gram-positive, non-motile and rod-shaped. It grew well on R2A and MB/2 agar (Difco) and grew weakly on trypticase soy agar and marine agar 2216 (Difco), but did not grow on MacConkey agar (Difco) or brain-heart infusion (BHI) agar (Becton Dickinson). The temperature range for growth was 10–45°C (optimum 30–37°C). The pH range for growth was pH 6.0–10.0 (optimum pH 7.0). The physiological and biochemical characteristics of strain ZLM-202^T are summarized in the species description and a comparison of selective characteristics with related type strains is given in Table 1.

Chemotaxonomy. Strain ZLM-202^T contained menaquinone 8 (MK-8) as the major respiratory quinone, which is a characteristic feature of the genus *Deinococcus*. The polar lipid profile of strain ZLM-202^T contained two unknown glycolipids, two unknown phosphoglycolipids, six unknown phospholipids and five unknown aminophospholipids (Fig. 1). The predominant component was an unknown phosphoglycolipid (PGL2), which is consistent with our previously reported result for *D. radiodurans* R1^T [20]. Strain ZLM-202^T exhibited three unknown phospholipid components (PL1-PL3) and four unknown aminophospholipids APL1, APL2, APL3 and APL5, which were not detected in *D. radiodurans* R1^T. The peptidoglycan of strain ZLM-202^T contained L-ornithine. The predominant fatty acids of strain ZLM-202^T were 16:1 ω7*c* (33.3%) and 16:0 (11.4%), which were also predominant in *D. radiodurans* R1^T and *D. peraridilitoris* KR-200^T (Table 2). The fatty acid profile of strain ZLM-202^T also contained moderate amounts of 15 : 1 ω6*c* (4.9%), 15 : 0 *iso* (4.9%) and 16:1 ω5*c* (4.8%). The DNA G + C content of strain ZLM-202^T was 65.5 mol. %.

Phylogenetic analysis. For strain ZLM-202^T, 1417 bp of the 16S rRNA gene sequence was determined. Comparative 16S rRNA gene sequence analysis showed that strain ZLM-202^T was most closely related to members of the genus *Deinococcus*. In a phylogenetic

Table 1. Phenotypic characteristics that differentiate strain ZLM-202^T from related members of the genus *Deinococcus*

Characteristic	1	2	3	4	5	6	7
Cell morphology	Rod	Spherical	Spherical/short rod	Rod	Rod	Rod	Rod
Optimum growth temperature (°C)	30–37	30	30	40	30	30	30
Cytochrome oxidase	+	+	+	+	+	–/+	+
Utilization of: L-Rhamnose	+	–	–	–	+	+	+
L-Arabinose	+	–	+	+	+	+	–
Ribose	W	+	+	–	–	+	–
D-Melibiose	+	–	–	–	–	–	–
D-Mannose	–	+	ND	+	+	+/–	+
N-acetyl-D-glucosamine	–	+	+	+	+	+	+
Alanine	W	–	–	–	+	W	–
Hydrolysis of:							
Casein	+	+	–	+	–	+	+
Gelatin	+	+	–	+	+	+	+
DNA G + C content (mol. %)	65.5	67	63.9	71.1	69.0	71.5	66.1

Strains: 1, ZLM-202^T (data from the present study); 2, *D. radiodurans* R1^T (data from the present study except the DNA G+C content, which was from [1]); 3, *D. peraridilitoris* KR-200^T [22]; 4, *D. maricopensis* LB-34^T; 5, *D. papagonensis* KR-241^T; 6, *D. pimensis* KR-235^T; 7, *D. yavapaiensis* KR-236^T (data in columns 4–7 from [23]). All strains were catalase-positive. Glucose, sucrose, maltose and proline were utilized by all of the strains. None of the strains utilized citrate. Symbols: +, positive; –, negative; W, weakly positive; ND, not determined.

tree based on the neighbour-joining algorithm, strain ZLM-202^T fell within the radiation of the cluster comprising *Deinococcus* species (Fig. 2a). Similar tree topology was also found in the tree generated by the maximum-parsimony algorithm (Fig. 2b). Strain ZLM-202^T showed the highest 16S rRNA gene sequence sim-

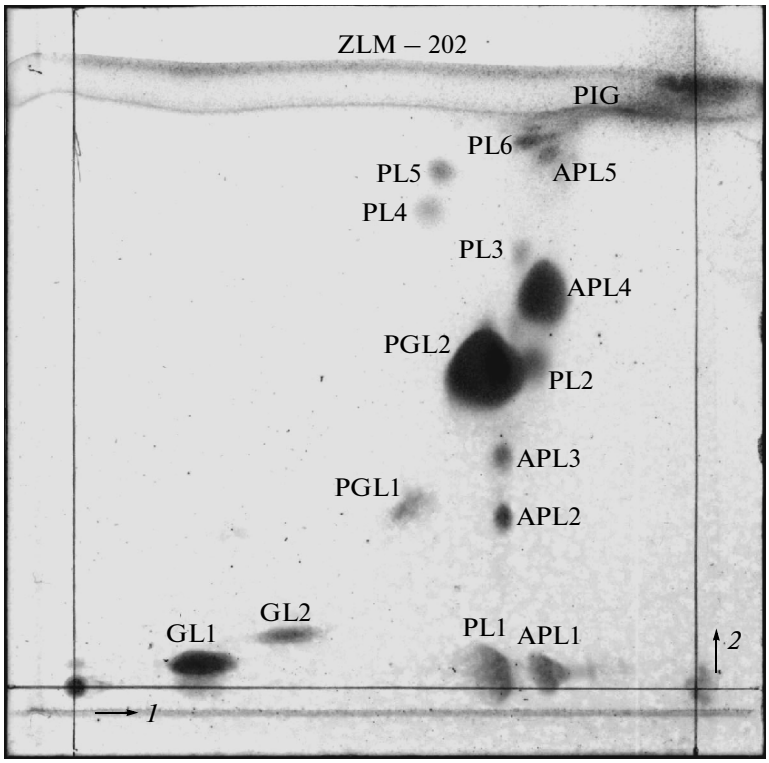

Fig. 1. Polar lipid profile of strain ZLM-202^T. GL1–GL2, unidentified glycolipids; PGL1–PGL2, unidentified phosphoglycolipids; PL1–PL6, unidentified phospholipids; APL1-APL5, unidentified aminophospholipid; PIG, pigment.

Table 2. Fatty acid compositions (%) of strain ZLM-202^T and related members of the genus *Deinococcus*

Fatty acid	1	2	3	4	5	6	7
12:0 *iso*	0.2	–	–	1.6	0.5	0.9	1.9
12:0	0.2	–	–	–	–	–	–
13:0 *iso*	0.5	–	–	7.1	1.4	9.6	7.2
13:0 *anteiso*	0.2	–	–	0.7	–	2.9	5.2
13:0	0.1	–	–	–	–	–	–
14:0 *iso*	2.0	–	–	3.1	2.1	4.1	2.5
14:0	2.3	–	0.5	–	0.5	1.9	1.3
13:0 *iso* 3OH	0.9	1.3	–	–	–	–	–
15:1 *iso* F	0.9	1.1	–	–	–	–	–
15:1 *iso* I	–	–	–	4.1	0.8	11.1	1.9
13:0 3OH/15:1 i H	2.1	0.8	–	–	–	–	–
15:0 *iso*	4.9	0.5	0.9	31.9	11.2	21.4	24.5
15:0 *anteiso*	1.6	–	–	0.7	5.6	4.8	5.7
15:1 ω8c	–	0.3	–	–	1.6	–	–
15:1 ω6c	4.9	3.7	5.0	1.5	8.8	1.1	1.3
15:0	–	–	1.5	2.8	4.8	2.1	3.8
Unknown 15.356	–	–	2.3	1.3	1.0	–	1.3
16:1 *iso* H	2.2	–	1.6	–	6.1	1.1	0.5
16:0 *iso*	3.6	0.8	1.7	8.7	10.5	6.2	5.7
16:1 ω9c	–	3.0	–	–	1.8	–	–
16:1 ω7c	33.3	38.5	36.8	4.3	10.6	3.4	5.7
16:1 ω5c	4.8	0.7	1.5	–	–	0.7	–
16:0	11.4	16.9	11.9	12.7	6.9	6.6	15.6
17:1 ω9c *iso*	0.3	–	–	–	6.0	–	–
17:1 *iso* I/17:1 *anteiso* B	0.8	–	–	1.7	0.6	1.8	1.0
17:1 ω9c *anteiso*	0.5	–	–	–	–	–	–
17:0 *iso*	3.6	–	2.6	13.8	10.8	12.9	7.4
17:0 *anteiso*	0.5	–	–	–	1.1	1.1	1.4
17:1 w8c	1.3	11.3	3.2	–	7.2	–	–
Unknown 16.833	–	–	–	–	–	–	1.0
17:1 ω6c	3.3	7.6	3.0	–	1.6	–	–
17:0	1.0	5.9	4.6	1.2	2.6	–	1.3
16:0 *iso* 3OH	0.2	–	–	–	0.6	1.1	–
16:0 3OH	1.2	–	–	–	–	–	–
18:0 *iso*	0.3	–	1.5	–	1.1	–	–
18:1 ω9c	0.9	1.5	–	–	–	0.6	1.2
18:1 ω7c	2.0	3.3	6.7	–	–	–	–
18:0	3.4	1.5	6.1	–	0.5	–	0.8
17:0 *iso* 3OH	1.2	–	–	–	3.1	4.5	–

Strains: 1, ZLM-202^T (data from the present study); 2, *D. radiodurans* R1^T (data from the present study); 3, *D. peraridilitoris* KR-200^T [22]; 4, *D. maricopensis* LB-34^T; 5, *D. papagonensis* KR-241^T; 6, *D. pimensis* KR-235ωT; 7, *D. yavapaiensis* KR-236^T (data in columns 4–7 from [23]. Values are percentages of total fatty acids. –, Not detected.

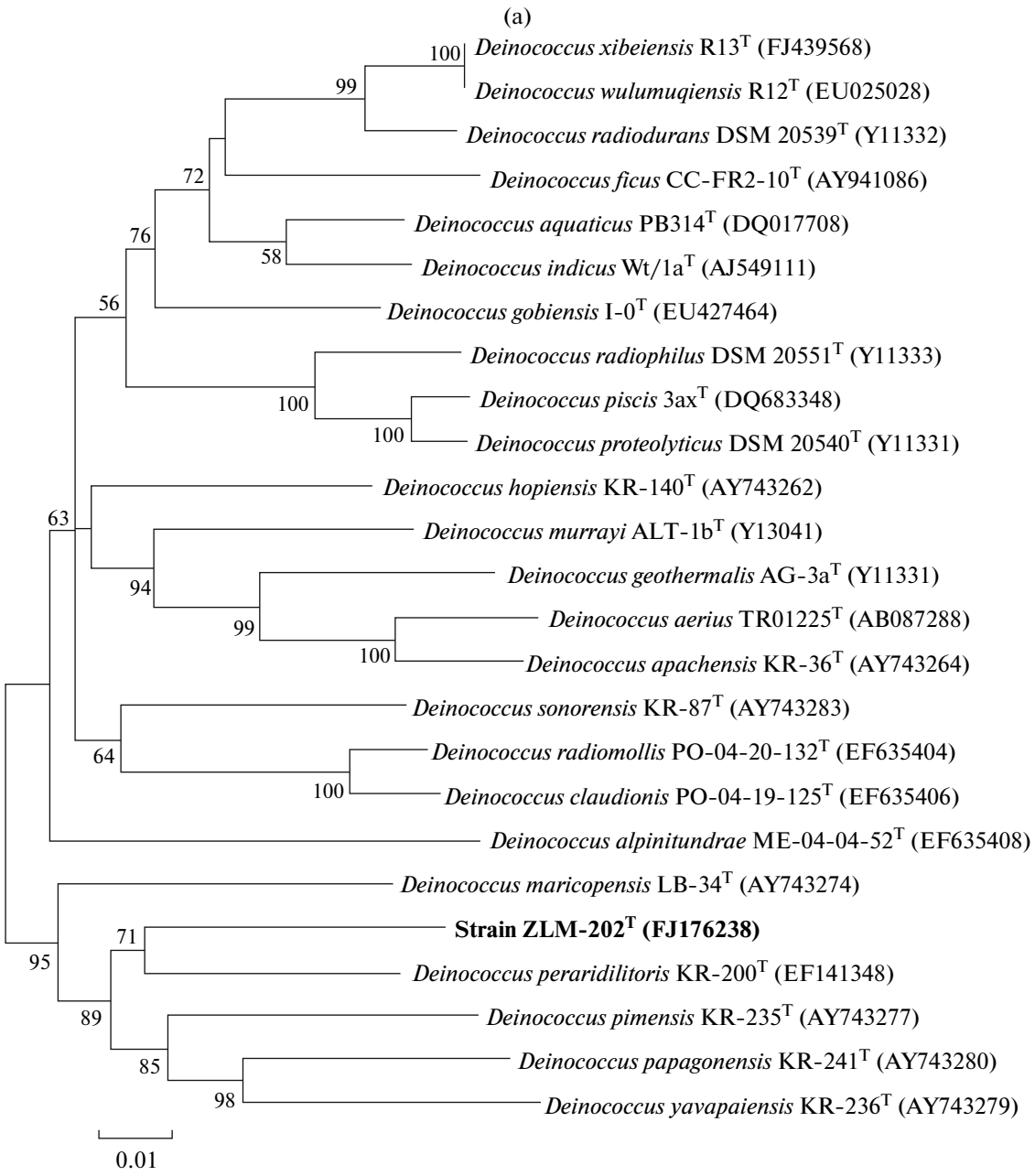

Fig. 2. a – Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing relationships between strain ZLM-202^T and phylogenetically close members of the genus *Deinococcus*. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position. b – Phylogenetic tree constructed under the maximum-parsimony criterion performed by using the software package MEGA with the following settings. For maximum-parsimony tree search options: the heuristic search (Close-Neighbour-Interchange). For scoring changes: the MEGA standard method (in which all nucleotide changes are weighted equally). Tree length is given by the sum of minimum numbers of substitutions. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 20 substitutions.

ilarity to *D. peraridilitoris* KR-200^T (92.2%); this value is well below the threshold for demarcating bacterial species [21]. 16S rRNA gene sequence similarities between strain ZLM-202^T and *D. maricopensis* LB-34^T, *D. papagonensis* KR-241^T, *D. pimensis* KR-235^T and *D. yavapaiensis* KR-236^T were 91.3, 91.0, 91.0 and 90.0%, respectively. No other recognized bacterial species showed more than 90% 16S rRNA gene sequence similarity to the new isolate. These results also suggest that strain ZLM-202^T represents a novel species within the genus *Deinococcus*.

Cell survival rate. Survival rates after exposure to various doses of gamma radiation and UV light were

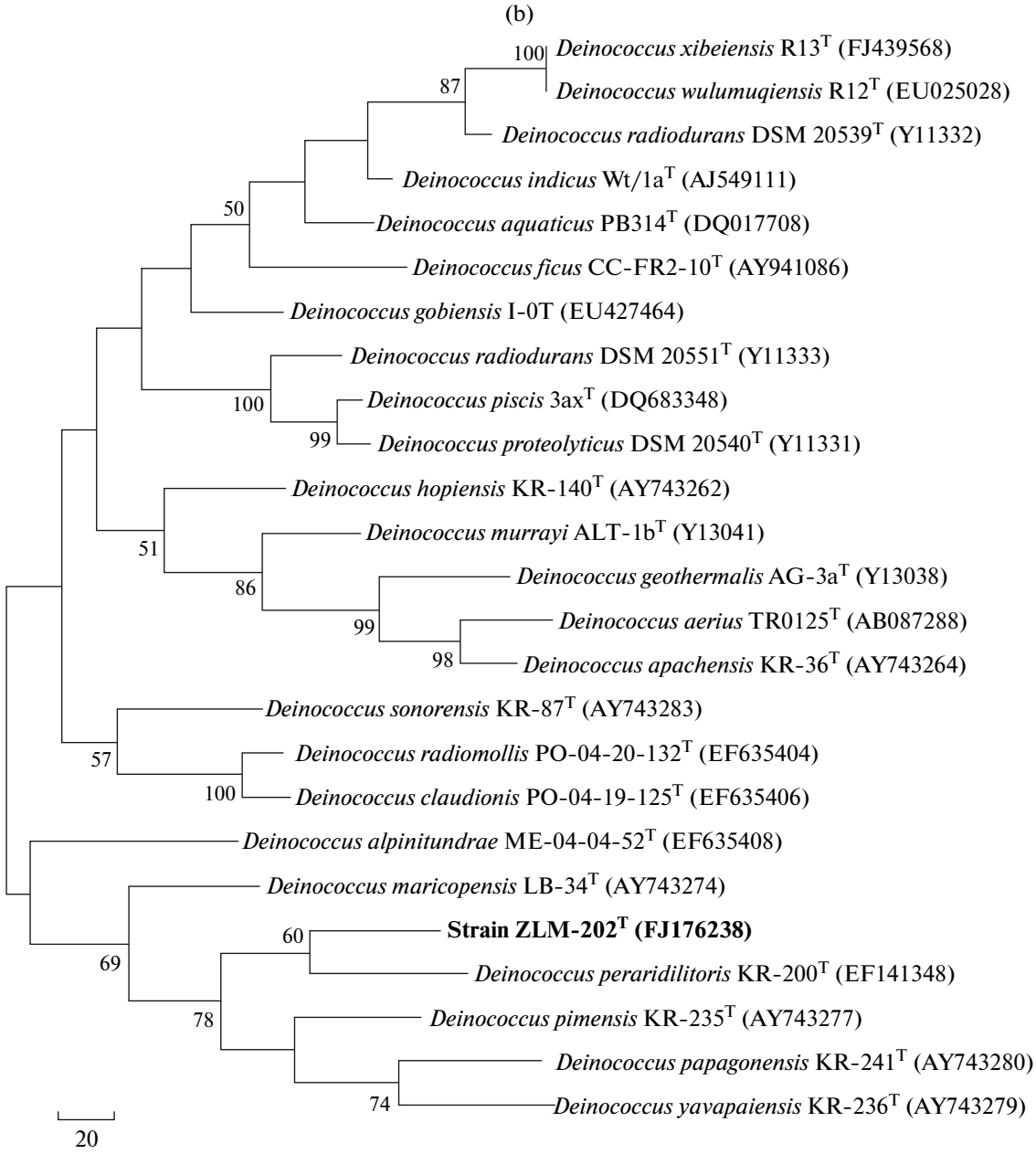

Fig. 2. Contd.

analysed for strain ZLM-202^T, *D. radiodurans* R1^T and *Escherichia coli* DH5α (Fig. 3). The gamma radiation (Fig. 3a) and UV light survival curves (Fig. 3b) of *Escherichia coli* DH5α dropped most sharply, while the two Deinococcus strains were significantly resistant to gamma radiation and UV light. Compared with *D. radiodurans* R1^T, strain ZLM-202^T showed somewhat lower resistance to gamma radiation and UV light. When cultures were exposed to 10 kGy gamma radiation, 3% and 12% survival were observed for strain ZLM-202^T and *D. radiodurans* R1^T, respectively. As for UV radiation tolerance, 6% and 15% survival were observed for ZLM-202^T and *D. radiodurans* R1^T, respectively, when they were exposure to UV dose as high as 600 J m^{-2}.

Taxonomic conclusion. Although the 16S rRNA gene sequence of strain ZLM-202^T shares only 88.4% similarity with that of *D. radiodurans* R1^T (the type species of the genus *Deinococcus*), the phylogenetic placement within the radiation of the genus as well as the combination of chemotaxonomic and phenotypic characteristics provide evidence justifying the inclusion of this strain within the genus *Deinococcus*. On the basis of the distinct phylogenetic position, polar lipid profile and the phenotypic characteristics, strain ZLM-202^T

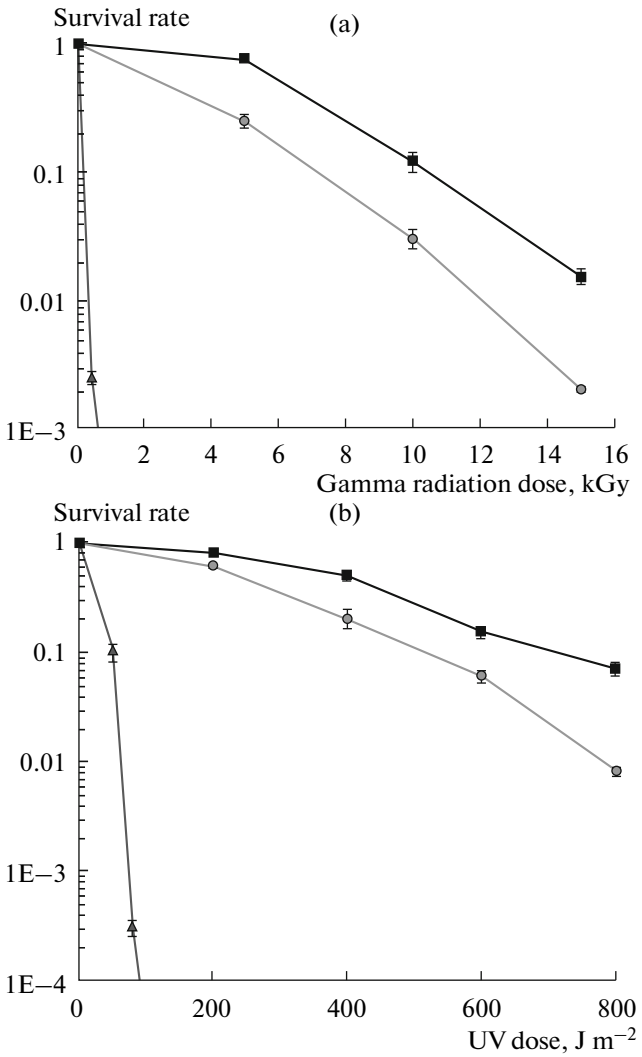

Fig. 3. Representative survival curves for strain ZLM-202^T (circles), *D. radiodurans* R1^T (squares) and *E. coli* DH5α (triangles) following exposure to gamma radiation (a) and UV light (b). Values are means ± standard deviations of duplicate experiments.

represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus soli* sp. nov. is proposed.

Description of *Deinococcus soli* sp. nov.

Deinococcus soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain)

Cells are 1.3–1.5 × 1.6–3.2 μm in size. Colonies on R2A agar for 10 days are opaque, flat, circular with entire edges, faintly pink-pigmented and 3–4 mm in diameter. Growth occurs at 10–45°C (optimum 30–37°C), 0–2% (w/v) NaCl (optimum 0%) and pH 6.0–10.0 (optimum pH 7.0). Oxidase- and catalase-positive. Negative for nitrate reduction, H_2S production, citrate utilization, indole production and urease. Hydrolyses aesculin, gelatin, starch and casein. Does not hydrolyse DNA or chitin. Utilizes L-rhamnose, ribose, sucrose, maltose, suberic acid, lactic acid, alanine, glycogen, D-glucose, D-melibiose, L-arabinose, 3-hydroxybutyric acid and proline. Does not utilize N-acetyl-D-glucosamine, inositol, itaconic acid, sodium malonate, sodium acetate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, D-mannitol, salicin, L-fucose, D-sorbitol, propionic acid, capric acid, valeric acid, trisodium citrate, L-histidine, potassium 2-ketogluconate or 4-hydroxybenzoic acid. According to the API ZYM gallery (bioMérieux), produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-galactosidase, but not lipase (C14), valine arylamidase, cystine arylamidase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. The predominant menaquinone is MK-8. The major fatty acids are 16:1 ω7*c* (33.3%), 16:0 (11.4%), 15:1 ω6*c* (4.9%), 15:0 *iso* (4.9%) and 16:1 ω5*c* (4.8%). The polar lipid profile contains two unknown glycolipids, two unknown phosphoglycolipids, six unknown phospholipids and five unknown aminophospholipids. The peptidoglycan contains L-ornithine. The DNA G + C content of the type strain is 65.5 mol. %. Tolerates high doses of gamma (>10 kGy) and UV radiation (>600 J m^{-2}). Susceptible to chloramphenicol, erythromycin, gentamicin, penicillin G, tetracycline, vancomycin, kanamycin and streptomycin.

Type strain ZLM-202^T has been deposited in the China Center for Type Culture Collection (CCTCC) and the Korean Collection for Type Cultures (KCTC). The DDBJ Nucleotide Sequence Database accession number is FJ176238.

Type strain ZLM-202^T (=CCTCC AB 208223^T = KCTC 13419^T) was isolated from soil exposed to 10 kGy gamma radiation, which was collected from an arid area in Xinjiang Province, north-west China.

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