Deinococcus wulumuqiensis sp. nov., and *Deinococcus xibeiensis* sp. nov., isolated from radiation-polluted soil

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The taxonomic positions of two gamma- and UV-ray-resistant strains isolated from radiation-polluted soil in north-west China were determined in a polyphasic study. The organisms, designated R12^T and R13^T, were Gram-stain-positive, non-spore-forming cocci, which contained MK-8 as the major respiratory quinone and $C_{16:1}\omega7c$ and $C_{16:0}$ as major fatty acids. The cell walls of strains R12^T and R13^T contained ornithine. Phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA hybridizations showed that strains R12^T and R13^T are members of novel species belonging to the genus *Deinococcus,* with *Deinococcus radiodurans* DSM 20539^T as the closest relative. The isolates R12^T and R13^T shared 97 and 97.1 % 16S rRNA gene similarity, respectively, and 29.5 and 33.3 % DNA–DNA relatedness, respectively, with *D. radiodurans* DSM 20539^T. The DNA G+C contents of isolates R12^T and R13^T were 66.7 and 63.8 %, respectively. On the basis of phenotypic tests and other results, two species, *Deinococcus wulumuqiensis* sp. nov. (type strain R12^T =CGMCC 1.8884^T =NBRC 105665^T) and *Deinococcus xibeiensis* sp. nov. (type strain R13^T =CGMCC 1.8885^T =NBRC 105666^T), are proposed.

The genus *Deinococcus* was proposed by Brooks & Murray (1981) to accommodate a group of bacteria of which the most prominent characteristic is extreme resistance to UV light, gamma radiation and desiccation (Mattimore & Battista, 1996; Callegan *et al.*, 2008; de Groot *et al.*, 2005). The type strains of species of the genus *Deinococcus* form a phylogenetically diverse group in a deeply branching lineage within the *Bacteria* (Asker *et al.*, 2008). Deinococci have been isolated from diverse sources, notably from arid environments such as desert soils and rocks (Hirsch *et al.*, 2004; de Groot *et al.*, 2005; Rainey *et al.*, 2005, 2007; Peng *et al.*, 2009). At the time of writing, the genus contains 39 validly described species, nearly all of which have been described in the past five years (http://www.bacterio.cict.fr/d/deinococcus.html).

The present study was designed to establish the taxonomic status of two radiation-resistant, tetrad-forming strains, designated R12^T and R13^T, which were isolated during a study on the bioremediation of radiation-contaminated soils in Xinjiang Province, China. The isolates were the subject of a polyphasic taxonomic study, which showed that they warranted recognition as novel species of the genus *Deinococcus*.

Strains R12^T and R13^T were isolated from a soil suspension after incubation at 30 °C for 14 days on a tryptone-glucose-yeast extract agar plate [TGY; 1 % (w/v) tryptone; 0.1 % (w/v) glucose; 0.5 % (w/v) yeast extract; 1.5 % (w/v) agar (Brim *et al.*, 2003)]. The soil sample, which was collected from radiation-contaminated soil in the Xinjiang Uigur Autonomous Region of north-west China, had been exposed to a ^{60}Co source at a dose rate of 0.167 kGy min^{-1} at room temperature (1 kGy=10^5 rads) until it had received

The GenBank accession numbers for the 16S rRNA gene sequences of strains R12^T and R13^T are EU025028 and FJ439568, respectively.

Fatty acid compositions of strains R12^T and R13^T and two-dimensional thin-layer chromatography of polar lipids of *D. radiodurans* R1^T, strain R12^T and strain R13^T are available with the online version of this paper.

a dose of 15 kGy gamma radiation. The isolates were maintained on TGY slants at 4 °C and as suspensions of cells in 20 % (v/v) glycerol at −20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared in shake flasks of TGY broth at 30 °C for 2 days, harvested by centrifugation and washed twice in distilled water; cells for the chemical studies were freeze-dried.

Genomic DNA, extracted as described by Earl *et al.* (2002), was used as template for PCR-mediated amplification and sequencing following standard procedures (Rainey *et al.*, 1997). The resultant almost complete sequences of isolates R12^T and R13^T (1459 and 1422 nt, respectively) were compared with 16S rRNA gene sequences from GenBank by using the BLAST program (http://www.ncb.nlm.nih.gov/BLAST) to determine their approximate phylogenetic position. The two gene sequences were then aligned with corresponding sequences of closely related species of the genus *Deinococcus* by using CLUSTAL W software (Thompson *et al.*, 1994). Pairwise evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980) and phylogenetic trees were generated by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Goodman & Pechère, 1977) tree-making algorithms from the MEGA 4.0 program (Kumar *et al.*, 2004); this software was also used to generate bootstrap confidence values based on 1000 resamplings. The G+C content of the genomic DNA of the strains was determined by using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* K-12 as control.

It can be seen from Fig. 1 that the two isolates and the type strain of *Deinococcus radiodurans* (DSM 20539^T) formed a well delineated subclade in the 16S rRNA gene tree, a taxon that was supported by all of the tree-making algorithms and by a 100 % bootstrap value. Isolates R12^T and R13^T shared 16S rRNA gene similarities with the *D. radiodurans* strains of 97 and 97.1 %, respectively, values which corresponded to 7 and 8 nt differences. The isolates also shared a high 16S rRNA gene similarity (99 %), thereby necessitating DNA–DNA relatedness studies. The DNA G+C contents of isolates R12^T and R13^T were 66.7 and 63.8 %, respectively.

The levels of DNA–DNA relatedness between the three members of the *D. radiodurans* 16S rRNA gene subclade were determined by using the renaturation method (De Ley *et al.*, 1970) and a Perkin Elmer Lambda 35UV/VIS spectrophotometer fitted with a PTP-1 peltier temperature controller. Genomic DNA preparations were sheared by ultrasonication to give a mean fragment size (300–700 bp) and the resultant samples adjusted to give an OD_{260} of 2.0 in 2 × SSC buffer (1 × SSC is 0.15 M sodium chloride and 0.015 M sodium citrate). Denaturation was carried out at 99 °C for 10 min and hybridization at 76 °C. Isolates R12^T and R13^T shared a DNA–DNA relatedness value of 58.5 % and corresponding values of 29.5 and 33.3 % with *D. radiodurans* DSM 20539^T, respectively. These results indicate that the three strains belong to distinct genomic species as the relatedness values are well below the 70 % cut-off point recommended for the circumscription of bacterial species (Wayne *et al.*, 1987).

Isolates R12^T and R13^T were examined for a range of diagnostic chemotaxonomic markers to determine whether or not they belonged to the genus *Deinococcus*. To this end, standard procedures were used to extract and detect the diamino acid in peptidoglycan preparations (Schleifer & Kandler, 1972) and major polar lipids in whole cells (Tindall, 1990), including those of *D. radiodurans* DSM 20539^T. Similarly, menaquinones extracted after Minnikin *et al.* (1984) were separated by HPLC (Kroppenstedt, 1982). Cellular fatty acids were extracted, methylated, separated and identified by using the Sherlock Microbial Identification System (MIDI) (Sasser, 1990).

The isolates contained L-ornithine as the diamino acid of the peptidoglycan, and MK-8 as the predominant menaquinone. The major cellular fatty acids of isolates R12^T and R13^T were hexadecanoic acid ($C_{16:1}\omega7c$: 46.4 and 54.3 %,

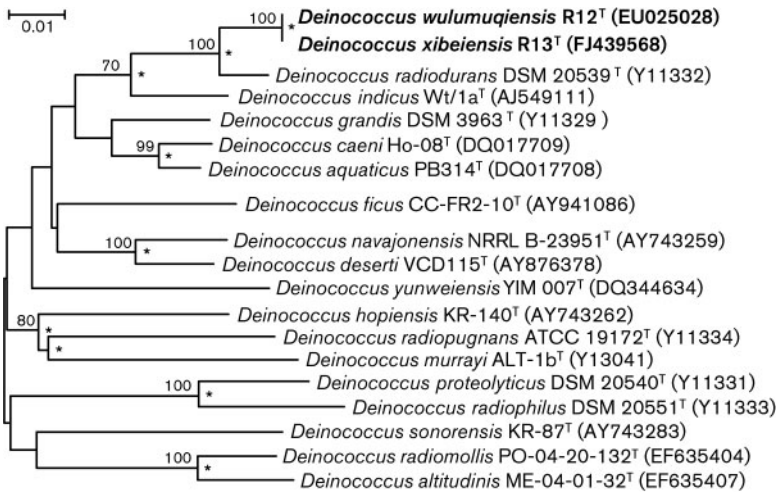

Fig. 1. Phylogenetic tree based on a distance matrix analysis of almost complete 16S rRNA gene sequences showing relationships between isolates R12^T and R13^T and phylogenetically close members of the genus *Deinococcus*. Branches of the tree with asterisks were also found using the neighbour-joining and maximum-parsimony tree-making algorithms. Numbers at nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >70 % are shown. Bar, 1 % sequence divergence.

respectively) and hexadecanoic acid ($C_{16:0}$: 14.5 and 21.0 %, respectively). Small qualitative and quantitative differences were found in the fatty acid composition of the minor components of the two isolates, notably a higher proportion of $C_{17:1}\omega 8c$ in isolate R12^T compared with isolate R13^T (6.7 against 2.7 %), which is also predominant in *D. radiodurans* DSM 20539^T (Rainey *et al.*, 2005).

Based on their staining behaviour, the polar lipid patterns of *D. radiodurans* DSM 20539^T and the two novel isolates consisted of various unknown glycolipids, phosphoglycolipids and polar lipids (Supplementary Figure S1, available in IJSEM Online). In all three strains, the patterns were dominated by unknown phosphoglycolipid PGL2. This is in line with previous results for species of the genus *Deinococcus* (Thompson *et al.*, 1980; Embley *et al.*, 1987; Ferreira *et al.*, 1997; de Groot *et al.*, 2005; Weon *et al.*, 2007; Callegan *et al.*, 2008; Im *et al.*, 2008). The organisms also contained phosphoglycolipid PGL1, glycolipids GL2 and GL3, and an unknown polar lipid, a pattern typical of other species of the genus *Deinococcus* (Lai *et al.*, 2006; Im *et al.*, 2008; Kämpfer *et al.*, 2008). However, the strains also showed differences in their polar lipid profiles which may be of diagnostic value. The two isolates lack a fifth glycolipid (GL5) compared with *D. radiodurans* DSM 20539^T. Isolate R13^T contained a first glycolipid and, like *D. radiodurans* DSM 20539^T, contained a characteristic aminophospholipid. All of the strains contained a spot composed of a red pigment.

The isolates were examined for phenotypic properties known to be of value in *Deinococcus* systematics. Gram staining was carried out by using a standard procedure, and cellular morphology and motility sought using light microscopy following growth on TGY agar for 3 days at 30 °C. Catalase activity was determined by assessing bubble production following the addition of 3 % (v/v) H_2O_2 to colonies, and oxidase activity was determined by using 1 % (v/v) tetramethyl-*p*-phenylenediamine. The ability of the strains to grow under different temperature and pH conditions and in the presence of NaCl was carried out using TGY medium. Single carbon source tests were carried out after Ferreira *et al.* (1997), and starch degradation was assessed using TGY agar as the basal medium; Lugol's iodine was added after plates had been incubated at 30 °C for 3 days. Susceptibility to erythromycin and rifampicin at 15 mg ml^{-1} was determined by using TGY agar plates that had been incubated at 30 °C for 3 days.

The novel isolates were Gram-stain-positive, non-motile, tetrad-forming cocci, which formed circular, shiny, reddish-orange colonies on TGY agar. Catalase and oxidase reactions were positive. Both strains grew at 10 to 55 °C and at pH 5.0 to 12.0, but differed in their optimal growth temperatures; isolate R12^T grew optimally at 37 °C and isolate R13^T at 30 °C. It can be seen from Table 1 that the isolates can be readily distinguished from one another and from the type strain of *D. radiodurans* by using a combination of phenotypic properties.

Table 1. Phenotypic properties separating isolates R12^T and R13^T from one another and from the type strain of *D. radiodurans*

Strains: 1, isolate R12^T; 2, isolate R13^T; 3, *D. radiodurans* DSM 20539^T. +, Positive; −, negative; ND, no data available; W, weakly positive result or growth. Data from this study.

Characteristic	1	2	3
Temperature range for growth (°C)	10–55	10–55	ND
Optimum temperature (°C)	37	30	30
pH range for growth	5–12	5–12	ND
Optimum pH	7–8	7	ND
Growth on sole carbon sources			
Sucrose	−	+	−
Sorbitol	−	+	−
Arabinose	−	+	−
Maltose	W	+	W
Fucose	−	+	−
D-Mannose	−	+	+
Melezitose	+	+	−
D-Xylose	−	+	−
Rhamnose	−	+	−
Sodium citrate	−	+	−
Sodium oxalate	−	+	+
Starch degradation	+	+	−
Susceptibility to antibiotics (15 μg ml^{-1})			
Rifampicin	+	−	+
Erythromycin	−	−	+

Survival rates of the novel isolates in response to gamma and UV radiation were compared with those of *D. radiodurans* DSM 20539^T and *E. coli* DH5α via an established procedure (Ferreira *et al.*, 1997). Strains were grown in modified TGY broth to exponential growth phase, at which point biomass was washed with sodium chloride (0.85 %, w/v), centrifuged at 4 °C and resuspended in saline (0.85 %, w/v) to give a concentration of 1×10^7–10^8 c.f.u. ml^{-1}. Each suspension was divided into 2 ml aliquots and exposed to a ^{60}Co source at a dose rate of 0.167 kGy min^{-1} at room temperature; the gamma radiation doses were from zero to 20.0 kGy in steps of 2.0 kGy. Treated samples were plated onto TGY agar plates and incubated at 30 °C. Similarly, preparations were exposed to a 254 nm UV lamp for the desired dose and subsequently incubated at 30 °C. The dose was monitored by using a VLX-30 radiometer. It can be seen from Fig. 2 that the isolates and positive control, unlike the negative control, showed little decrease in per cent survival when treated with gamma radiation at 3kGy; the *Deinococcus* strains also showed resistance to >10kGy. In the case of UV radiation, the *Deinococcus* strains grew at the highest dosage of 746 J m^{-2}; in contrast, the lethal dosage for the *E. coli* strain was 30 J m^{-2}.

It is evident from the genotypic and phenotypic data that each of the isolates represents a novel species of the genus

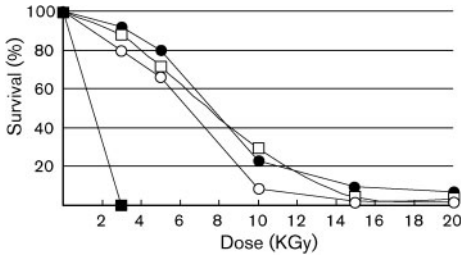

Fig. 2. Survival curves for isolates R12^T, R13^T, *Deinococcus radiodurans* DSM 20539^T (DR) and *Escherichia coli* DH5α following exposure to gamma radiation. ○, R13^T; ●, DR; □, R12^T; ■, *E. coli*.

Deinococcus. It is proposed that isolates R12^T and R13^T be given the names *Deinococcus wulumuqiensis* sp. nov., and *Deinococcus xibeiensis* sp. nov., respectively.

Description of *Deinococcus wulumuqiensis* sp. nov.

Deinococcus wulumuqiensis sp. nov. (wu.lu.mu.qi.en′sis. N.L. masc. adj. *wulumuqi* referring to Urumchi, the Chinese phoneticization for Urumchi city, where the type strain was isolated).

Aerobic, Gram-stain-positive, non-spore-forming, non-motile, tetrad-forming cocci. Reddish-orange, circular, opaque colonies (approx. 1.8–3.8 mm in diameter) are formed after incubation on TGY medium for 14 days at 37 °C. The optimal growth pH and temperature are pH 7.0–8.0 and 37 °C, respectively. L-Cystine, D-fructose, melezitose, L-leucine, L-proline, L-threonine, L-tryptophan, L-tyrosine and lactate can be utilized as sole carbon sources. Arabinose, galactose, fucose, dextrin, rhamnose, histidine, mannitose, sorbitol, cellobiose, D-xylose, raffinose, citrate, oxalate, succinate and benzoate cannot be utilized as sole carbon sources. Growth occurs in the presence of 1 % (w/v) NaCl. The major cellular fatty acids are $C_{16:1}\omega7c$, $C_{16:0}$, $C_{17:1}\omega8c$, $C_{16:1}\omega9c$, $C_{17:1}\omega6c$ with small amounts of iso-$C_{16:0}$, $C_{15:1}\omega6c$, $C_{15:0}$, $C_{17:0}$, iso-$C_{16:0}$ H, iso-$C_{17:1}\omega9c$, $C_{15:1}\omega8c$, $C_{18:1}\omega7c$, $C_{14:0}$, $C_{16:1}\omega5c$, $C_{18:1}\omega9c$ and $C_{18:0}$ (Supplementary Table S1). Gamma radiation resistant to >10 kGy, UV resistant to >700 J m^{-2}. The 16S rRNA gene similarity and DNA–DNA relatedness of the type strain with *Deinococcus radiodurans* DSM 20539^T are 97 and 29.5 %, respectively. The DNA G+C content of the type strain is 66.7 mol%.

The type strain, isolate R12^T (=CGMCC 1.8884^T =NBRC 105665^T), was recovered from radiation-contaminated soil collected in Xinjiang Province, China.

Description of *Deinococcus xibeiensis* sp. nov.

Deinococcus xibeiensis sp. nov. [xi.bei.en′sis. N.L. masc. adj. *xibeiensis* referring to Xibei, the Chinese phoneticization for north-west China, where the soil sample was collected (i.e. Xinjiang Province)].

Aerobic, Gram-stain-positive, non-spore-forming, non-motile tetrad-forming cocci. Pinkish-red, circular, opaque colonies (approx. 3.2–4.5 mm in diameter) are formed after incubation on TGY medium for 14 days at 30 °C. The optimal growth pH and temperature are pH 7.0 and 30 °C. Growth occurs in the presence of 1 % (w/v) NaCl. Sucrose, sorbitol, maltose, galactose, fucose, mannitose, melezitose, xylose, arabinose, rhamnose, dextrin, mannitol, L-cystine, D-fructose, melezitose, L-leucine, L-proline, L-threonine, L-tryptophan, L-tyrosine, tartrate, oxalate and lactate can be utilized as sole carbon sources. Succinate, benzoate, histidine, raffinose and cellobiose cannot be utilized as sole carbon sources. The main cellular fatty acids are $C_{16:1}\omega7c$, $C_{16:0}$, $C_{16:1}\omega9c$ with small amounts of $C_{17:1}\omega8c$, $C_{15:0}$, $C_{18:1}\omega7c$, $C_{17:1}\omega6c$, $C_{15:1}\omega6c$, $C_{17:0}$, iso-$C_{16:0}$, iso-$C_{17:1}\omega9c$, $C_{14:0}$, iso-$C_{16:0}$ H, $C_{16:1}\omega5c$, $C_{15:1}\omega8c$, $C_{18:1}\omega9c$, $C_{14:0}$ 2-OH, $C_{18:0}$ and $C_{10:0}$ (Supplementary Table S1). Gamma radiation resistant to >10 kGy, UV resistant to >700 J m^{-2}. The 16S rRNA gene similarity and DNA–DNA relatedness of the type strain with *Deinococcus radiodurans* DSM 20539^T are 97.1 and 33.3 %, respectively. The DNA G+C content of the type strain is 63.8 mol%.

The type strain, isolate R13^T (=CGMCC 1.8885^T =NRBC 105666^T), was recovered from radiation-contaminated soil collected from Xinjiang Province, China.

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