Deinococcus peraridilitoris sp. nov., isolated from a coastal desert

Fred A. Rainey,¹ Margarida Ferreira,² M. Fernanda Nobre,³ Keren Ray,¹ Danielle Bagaley,¹ Ashlee M. Earl,¹ John R. Battista,¹ Benito Gómez-Silva,⁴ Christopher P. McKay⁵ and Milton S. da Costa²

Correspondence
Fred A. Rainey
frainey@lsu.edu

¹Department of Biological Sciences, 202 Life Sciences Building, Louisiana State University, Baton Rouge, LA 70803, USA

²Departamento de Bioquímica and Centro de Neurociências e Biologia Celular, Universidade de Coimbra, 3004-517 Coimbra, Portugal

³Departamento de Zoologia, Universidade de Coimbra, 3004-517 Coimbra, Portugal

⁴Instituto del Desierto y Unidad de Bioquímica, Facultad Ciencias de la Salud, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile

⁵Space Science Division, NASA Ames Research Center, Moffett Field, CA 94035, USA

Three ionizing-radiation-resistant bacterial strains (designated KR-196, KR-198 and KR-200^T) were isolated from a sample of arid soil collected from a coastal desert in Chile. The soil sample was irradiated before serial dilution plating was performed using one-tenth-strength plate count agar. Phylogenetic analysis of the 16S rRNA gene sequences showed these organisms to represent a novel species of the genus *Deinococcus*, having sequence similarities of 87.3–90.8% with respect to recognized *Deinococcus* species. Strains KR-196, KR-198 and KR-200^T were aerobic and showed optimum growth at 30 °C and pH 6.5–8.0. The major respiratory menaquinone was MK-8. The predominant fatty acids in these strains were 16:1 ω 7c, 16:0, 15:1 ω 6c, 17:0 and 18:0. The DNA G+C content of strain KR-200^T was 63.9 mol%. Strains KR-196, KR-198 and KR-200^T were found to be resistant to >10 kGy gamma radiation. On the basis of the phylogenetic, chemotaxonomic and phenotypic data, strain KR-200^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus peraridilitoris* sp. nov. is proposed. The type strain is KR-200^T (=LMG 22246^T = CIP 109416^T).

The genus *Deinococcus* comprises 24 species with validly published names and represents a distinct phylogenetic lineage that is related to the genera *Truepera*, *Thermus*, *Meiothermus*, *Oceanothermus* and *Vulcanithermus* (<http://www.bacterio.cict.fr/d/deinococcus.html>; Albuquerque *et al.*, 2005). All of the species of the genus *Deinococcus* tested have shown resistance to levels of ionizing radiation to which they would never be exposed in the natural environment. Of the 24 species with validly published names, 13 have been isolated from arid environments, i.e. desert soils or rocks (Hirsch *et al.*, 2004; de Groot *et al.*, 2005; Rainey *et al.*, 2005). A recent study of an arid soil collected from the Sonoran Desert yielded 60 strains of the genus *Deinococcus*, subsequently assigned to nine novel species (Rainey *et al.*,

2005). Two strains of the genus *Deinococcus* were isolated from Sahara Desert soil/sand and were subsequently described as *Deinococcus deserti* (de Groot *et al.*, 2005). In addition, three *Deinococcus* species were isolated from soils and rock surfaces in Antarctica (Hirsch *et al.*, 2004). In a study of the ionizing-radiation-resistant bacterial communities of arid soils, a sample of surface soil from the coastal desert north of Antofagasta in Chile was exposed to various doses of ionizing radiation and the surviving bacterial populations were isolated and identified. The strains described here survived the exposure of this arid soil to 9 and 11 kGy gamma radiation. Three of these strains were fully characterized and are proposed here as a novel species of the genus *Deinococcus*.

Strains KR-196, KR-198 and KR-200^T were isolated from a coastal desert soil sample collected north of Antofagasta in the area between the coast and the coastal range some 12 km from the Pacific Ocean south-west of Cerro Gordo. A surface sample of soil (i.e. taken from the upper 2 cm), designated AT97-3, was collected using a sterile scoop from

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KR-200^T and KR-198 are EF141348 and EF141349, respectively.

Fatty acid compositions for strains KR-200^T, KR-198, KR-196 and species of the genus *Deinococcus* are shown in a supplementary table available with the online version of this paper.

an area devoid of vegetation between the coastal range and the Pacific Ocean. The sample was transported and stored at ambient temperature until being processed. Aliquots (1 g) of dry soil were exposed to levels of radiation between 0 and 30 kGy at a dose of 2.57 kGy h⁻¹ at room temperature, using a Shephard model 484 ⁶⁰Co irradiator. After exposure, the samples were plated by serial dilution on a number of nutrient growth media, including one-tenth-strength plate count agar (1/10 PCA; Difco). The dilutions were made in liquid media of the same composition as the agar plates. Plates were incubated at 28 °C for up to 20 days. When plated on 1/10 PCA before exposure to ionizing radiation, the soil sample (AT97-3) was found to contain 1.3 × 10⁴ c.f.u. g⁻¹. A number of colonies were selected, purified and further characterized using a polyphasic approach. Strains were maintained in 1/10 plate count broth (Difco) containing 15 % (v/v) glycerol at -80 °C. Strains KR-196 and KR-198 were recovered from a sample exposed to 11 kGy gamma radiation and strain KR-200^T was recovered from a sample exposed to 9 kGy.

Almost-complete 16S rRNA gene sequences comprising 1461 nt were determined for strains KR-196, KR-198 and KR-200^T as described previously (Rainey *et al.*, 1996). The 16S rRNA gene sequences were aligned against

representative reference sequences for members of the genus *Deinococcus* and related taxa, using MEGA, version 3.1 (Kumar *et al.*, 2004). The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms and bootstrap analyses were generated using algorithms contained in the PHYLIP package (Felsenstein, 1993). Comparative sequence analysis showed the 16S rRNA gene sequences of strains KR-196, KR-198 and KR-200^T to share 100 % similarity, and phylogenetic analyses grouped them within the radiation of the species that currently comprise the genus *Deinococcus* (Fig. 1). The distinct phylogenetic lineage of strains KR-196, KR-198 and KR-200^T shows no well-supported relationship with any of the lineages containing previously described *Deinococcus* species. The levels of 16S rRNA gene sequence similarity between strains KR-196, KR-198 and KR-200^T and *Deinococcus* species were in the range 87.3–90.8 %. The highest level of 16S rRNA gene sequence similarity was found with respect to the type strain of *Deinococcus pimensis* (90.8 %), while similarity values in the range 90.0–90.6 % were found with respect to strains of *Deinococcus yavapaiensis*, *Deinococcus papagonensis*, *Deinococcus maricopensis* and *Deinococcus sonorensis*. With such low levels of 16S rRNA gene sequence similarity, the branching points in the phylogenetic tree are not well supported by bootstrap analyses (Fig. 1). The DNA

Fig. 1. 16S rRNA gene sequence-based phylogeny, showing the relationships of strains KR-196, KR-198 and KR-200^T and *Deinococcus* species. The dendrogram was constructed from distance matrices by using the neighbour-joining method. Numbers at branching points represent bootstrap percentages based on 1000 replicates. Bar, 2 inferred nucleotide substitutions per 100 nucleotides. *Truepera radiovictrix* RQ-24^T was used as the outgroup.

used to determine the G+C content was isolated as described by Cashion *et al.* (1977). The G+C content of the DNA was determined for strain KR-200^T by HPLC (Mesbah *et al.*, 1989) and was found to be 63.9 ± 0.4 mol%.

Cultures used for polar lipid analysis were grown on nutrient agar (Difco) and extraction of lipids was performed as described previously (Prado *et al.*, 1988; Donato *et al.*, 1990). Lipoquinones were extracted from freeze-dried cells and purified by TLC before being separated by HPLC (Gilson apparatus) (Tindall, 1989). Cultures for fatty acid analysis were grown on nutrient agar plates incubated in sealed plastic bags submerged in a water bath at 30 °C for 72 h. Fatty acid methyl esters were obtained from fresh wet biomass by saponification, methylation and extraction as described previously by Kuykendall *et al.* (1988) and separated, identified and quantified as described previously (Moreira *et al.*, 2000).

The predominant fatty acids of strains KR-196, KR-198 and KR-200^T were 16:1 ω 7c (31–36 % of the total), 16:0 (12–17 %), 15:1 ω 6c (4.9–5.1 %), 17:0 (4.5–6.1 %) and 18:0 (5.8–7.2 %). The combination of 16:1 ω 7c, 16:0, 17:0, 17:1 ω 8c and 18:0 allows these strains to be distinguished from recognized species of the genus *Deinococcus* (see Supplementary Table S1 available in IJSEM Online). The major respiratory quinone in strains KR-196, KR-198 and KR-200^T is MK-8, as in all recognized *Deinococcus* strains. Several phosphoglycolipids and glycolipids, including the major phosphoglycolipid which co-migrated with that found in *Deinococcus radiodurans*, are prominent components of these strains.

Cell morphology and motility were examined by phase-contrast microscopy after cultivation of the strains on agar plates. On 1/10 PCA, colonies were light pink in colour. The cells, which stained Gram-positive, were spherical or short rods (2.0×1.5 – 2.0 μ m), non-motile and lacked spores. The temperature range for growth was determined on 1/10 PCA plates incubated for 10 days at temperatures between 5 and 50 °C. Growth occurred between 10 and 37 °C but not at 5 or 42 °C, and the optimum growth temperature was 30 °C. The pH range for growth was determined at 30 °C on buffered 1/10 PCA plates (range, pH 5.5–9.5); media buffered between pH 5.5 and pH 9.5 were prepared as described previously (Ferreira *et al.*, 1997). Strains KR-196, KR-198 and KR-200^T grew at pH 5–8.5. Growth of strains KR-198 and KR-200^T was observed at pH 9.0 but not at pH 9.5. The optimum pH for growth was in the range 6.5–8.0.

Catalase, cytochrome oxidase and the hydrolysis of starch, casein and gelatin were determined as described by Smibert & Krieg (1981). All strains were cytochrome oxidase-positive and catalase-positive. It was observed that the cytochrome oxidase reaction was negative in older cultures. Variation in the hydrolysis of starch, casein and gelatin was found among the three strains (Table 1). The assimilation of various sole carbon sources was examined using a defined medium solidified with deionized water-washed agar, as

described previously (Rainey *et al.*, 2005). Growth was examined visually on plates incubated at 30 °C for up to 7 days. Negative-control plates did not include carbon sources. Positive-control cultures were grown on both nutrient agar and solidified Degryse medium 162 (Degryse *et al.*, 1978). The strains utilized a number of substrates for growth. Strains KR-196, KR-198 and KR-200^T gave identical results for all sole carbon sources tested. Results from phenotypic characterization are given in Table 1 and the species description below.

The survival of isolates after exposure to various doses of gamma radiation was determined by exposing aliquots of strains KR-196, KR-198 and KR-200^T to gamma radiation from a cobalt-60 source at a dose rate of 2.57 kGy h⁻¹ (Rainey *et al.*, 2005). Strains KR-196, KR-198 and KR-200^T were found to be resistant to >10 kGy gamma radiation.

Although the 16S rRNA gene sequences of strains KR-196, KR-198 and KR-200^T share only 89.9 % similarity with that of the type strain of *D. radiodurans* (the type species of the genus *Deinococcus*), the phylogenetic placement within the radiation of the genus (as currently defined) as well as the combination of chemotaxonomic and phenotypic characteristics provide evidence justifying the inclusion of these strains within the genus *Deinococcus*. On the basis of the distinct phylogenetic position, the presence of the combination of fatty acids 16:1 ω 7c, 16:0, 17:0, 17:1 ω 8c and 18:0 and the phenotypic characteristics, strains KR-196, KR-198 and KR-200^T represent a novel species of the genus *Deinococcus*, for which the name *Deinococcus peraridilitoris* sp. nov. is proposed.

Description of *Deinococcus peraridilitoris* sp. nov. Rainey and da Costa

Deinococcus peraridilitoris (pe.ra.ri.di.li'to.ris. L. adj. *peraridus* very dry, very arid; L. gen. n. *litoris* of the seashore, of a coast; N.L. gen. n. *peraridilitoris* of a very dry coast).

Cells are spherical or short rod-shaped (2.0×1.5 – 2.0 μ m). Gram stain is positive. Cells are non-motile and spores are not observed. Colonies on 1/10 PCA medium are pigmented light pink. Growth occurs between 10 and 37 °C but not at 5 or 42 °C. Optimum temperature for growth is 30 °C. Known strains grow at pH 5–8.5; growth of strains KR-198 and KR-200^T occurs at pH 9.0. Optimum pH for growth is 6.5–8.0. All strains are cytochrome oxidase-positive and catalase-positive. The predominant fatty acids are 16:1 ω 7c (31–36 %), 16:0 (12–17 %), 15:1 ω 6c (4.9–5.1 %) and 17:0 (4.5–6.1 %). Strains utilize L-arabinose, cellobiose, galactose, D-glucose, maltose, sucrose, trehalose, ribose, glucosamine, glutamate, asparagine and proline. The following substrates are not utilized: fructose, L-rhamnose, melibiose, lactose, glycerol, raffinose, sorbose, xylose, acetate, citrate, α -ketoglutarate, lactate, malate, aspartate, cysteine, alanine, glutamine, glycine, lysine, methionine and ornithine. Starch, casein and gelatin are degraded by some strains. DNA of the

Table 1. Differential characteristics of strains KR-200^T, KR-198, KR-196 and related members of the genus *Deinococcus*

Strains: 1, KR-200^T; 2, KR-198; 3, KR-196; 4, *D. pimensis* KR-235^T; 5, *D. maricopensis* LB-34^T; 6, *D. yavapaiensis* KR-236^T; 7, *D. papagonensis* KR-241^T; 8, *D. sonorensis* KR-87^T; 9, *D. radiodurans* R1^T. Data for reference strains were taken from Rainey *et al.* (2005). All strains were catalase-positive. Glucose, galactose, maltose, sucrose, cellobiose, glucosamine and proline were utilized by all of the strains. None of the strains utilized sorbose, raffinose, acetate, citrate, cysteine, lysine or methionine. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9
Pigmentation	Light pink	Light pink	Light pink	Pink	Pink	Pink	Slightly pink	Pink	Pink–red
Cell morphology	Spherical/short rods	Spherical/short rods	Spherical/short rods	Spherical	Rods	Rods	Rods	Spherical/short rods	Spherical
Optimum growth temperature (°C)	30	30	30	30	40	30	30	30	30
Cytochrome oxidase	+	+	+	–/+	+	+	+	+	+
Utilization of:									
Fructose	–	–	–	+	–/+	+	+	+	+
L-Rhamnose	–	–	–	+	–	+	+	+	–
L-Arabinose	+	+	+	+	+	–	+	+	–
Ribose	+	+	+	+	–	–	–	–	+
Xylose	+	+	+	w	–	–	+	+	–
Lactose	–	–	–	+	–	–	+/–	+	–
Glycerol	–	–	–	w	+	–	–	+	+
α-Ketoglutarate	–	–	–	–	–	–	–	+	–
Lactate	–	–	–	–	–	+	–	–	+
Malate	–	–	–	–	+	–	+/–	–	+
Ornithine	–	–	–	w	+	–	+	+	–
Aspartate	–	–	–	–	+	–	–	–	+
Glutamate	+	+	+	w	+	–	+	–	+
Alanine	–	–	–	w	–	–	+	–	–
Asparagine	w	w	w	w	+	–	–	–	–
Glutamine	–	–	–	w	+	w	+	w	+
Glycine	–	–	–	–	–	–	–	–	w
Degradation of:									
Starch	+	–	+	+	+	+	–	–	ND
Casein	–	–	+	+	+	+	–	–	ND
Gelatin	–	–	+	+	+	+	+	+	ND
DNA G + C content (mol%)	63.9	ND	ND	71.5	71.1	66.1	69.0	70.3	67.0

type strain has a G + C content of 63.9 mol%. Known strains are resistant to > 10 kGy gamma radiation.

The type strain, KR-200^T (=LMG 22246^T=CIP 109416^T), and strains KR-196 and KR-198 were isolated from a coastal desert soil sample exposed to 9 and 11 kGy gamma radiation. Strain KR-198 (=LMG 22247=CIP 109417) is a reference strain.

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