Deinococcus aquiradiocola sp. nov., isolated from a radioactive site in Japan

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A gamma- and UV-radiation-tolerant, pale-pink strain (TDMA-uv53^T) was isolated from a freshwater sample collected at Misasa (Tottori, Japan), after exposure of the water sample to UV radiation. The cells stained Gram-positive and were non-motile, rod-shaped and non-spore-forming. The DNA G + C content of the strain was 69.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain TDMA-uv53^T belongs to the genus *Deinococcus*, the highest sequence similarities being found with *Deinococcus claudionis* PO-04-19-125^T (96%), *D. altitudinis* ME-04-01-32^T (96%), *D. radiomollis* PO-04-20-132^T (95%), *D. deserti* VCD115^T (91.5%), *D. hopiensis* KR-140^T (91.0%) and *D. sonorensis* KR-87^T (91.0%). Major fatty acids were iso-15:0, 15:1 ω 6c, 15:0, 16:0 and summed feature 3 (iso-15:0 2-OH and/or 16:1 ω 7c). MK-8 was the predominant respiratory quinone. Phylogenetic distinctiveness and unique phenotypic characteristics differentiated strain TDMA-uv53^T from closely related *Deinococcus* species. The results of our polyphasic taxonomic analyses suggested that TDMA-uv53^T represents a novel *Deinococcus* species, for which the name *Deinococcus aquiradiocola* sp. nov. is proposed. The type strain is TDMA-uv53^T (=JCM 14370^T =NBRC 102118^T =CCUG 53612^T).

The family *Deinococcaceae* (Battista & Rainey, 2001a; Brooks & Murray, 1981; Rainey *et al.*, 1997) belongs to the phylum ‘*Deinococcus–Thermus*’ and currently comprises the single genus *Deinococcus* (Battista & Rainey, 2001a; Brooks & Murray, 1981; Rainey *et al.*, 1997). In the chapter on the genus *Deinococcus* in the latest edition of *Bergey’s Manual of Systematic Bacteriology* (Battista & Rainey, 2001b), the genus was reported to contain seven species. In the past few years, numerous further species of the genus have been described (de Groot *et al.*, 2005; Hirsch *et al.*, 2004; Lai *et al.*, 2006; Rainey *et al.*, 2005; Suresh *et al.*, 2004; Zhang *et al.*, 2007). Members of the genus have the distinctive feature of being the most radiation-resistant of vegetative cells. UV or gamma radiation has been used to isolate many species of the genus from soil (Christensen & Kristensen, 1981; de Groot *et al.*, 2005; Rainey *et al.*, 2005), room dust (Christensen &

Kristensen, 1981), foods (Anderson *et al.*, 1956; Davis *et al.*, 1963) and faeces (Kobatake *et al.*, 1973). Meanwhile, a few species have been isolated with no selective pressure in the isolation strategies, from a hot spring (Ferreira *et al.*, 1997), continental Antarctica (Hirsch *et al.*, 2004), an arsenic-contaminated aquifer (Suresh *et al.*, 2004) and the rhizosphere of a *Ficus* species (Lai *et al.*, 2006). Around the time of writing, another four psychrophilic, ionizing-radiation-sensitive *Deinococcus* species were described (Callegan *et al.*, 2008).

The Misasa spa region (Tottori, Japan) is known for the high natural radioactivity of its waters (²²⁶Ra, 0.60 Bq l^{−1}; ²²⁸Ra, 0.41 Bq l^{−1}) (Kametani & Matsumura, 1983). In such a radioactive environment, ionizing radiation damages living cells mainly by inducing oxidative stress; reactive oxygen species and free radicals induced by the partial reduction of oxygen attack biological macromolecules such as nucleic acids, proteins and lipids (Halliwell, 1996). Only ionizing radiation-resistant micro-organisms, which are adapted to this harsh environment, can grow and survive. Recently, carotenoid-producing bacteria were isolated from a water sample collected at Misasa. A comprehensive taxonomic characterization based on 16S rRNA gene sequences revealed a unique diversity of carotenoid producers that exist in this radioactive region

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Deinococcus aquiradiocola* TDMA-uv53^T is AB265180.

A maximum-likelihood tree based on 16S rRNA gene sequences, an SEM of cells of strain TDMA-uv53^T, a 2D TLC of total polar lipids of strain TDMA-uv53^T and comparisons of fatty acid profiles and biochemical characteristics of strain TDMA-uv53^T and related *Deinococcus* species are available as supplementary material with the online version of this paper.

(Asker *et al.*, 2007a, d). In this article, we report the results of a polyphasic taxonomic study of a gamma- and UV-radiation-tolerant, aerobic, pale pink-pigmented bacterium, strain TDMA-uv53^T, also isolated from Misasa, which produces unknown carotenoids. The novel bacterium showed marked tolerance to gamma and UV radiation; the ratios of survival after exposure to gamma rays and UV light were higher than those exhibited by *Deinococcus radiodurans* NBRC 15346^T (positive control). On the basis of biochemical and chemical criteria and the results of 16S rRNA gene sequence analysis, we propose that the isolate represents a novel species within the genus *Deinococcus*.

Strain TDMA-uv53^T was isolated as described in our previous paper (Asker *et al.*, 2007a). Briefly, 0.1 ml of a tenfold dilution of a water sample collected on 1 September 2005 was spread on nutrient agar (NA; Difco). Before incubation, the agar plate was irradiated for 3 min using a UV lamp (GL-15; Toshiba) (53 µW cm⁻²). The plate was incubated aerobically at 40 °C for 2–5 days. After incubation, pink-coloured colonies were isolated. Unless otherwise specified, all characteristics described below were determined after growth of strain TDMA-uv53^T on NA for 48 h at 37 °C.

The phylogenetic position of the isolate was studied by standard analysis based on 16S rRNA gene sequences. Genomic DNA of TDMA-uv53^T was extracted using a bacterial genomic DNA purification kit (Edge BioSystems). The DNA fragment that contained nearly the complete 16S rRNA gene of TDMA-uv53^T (1410 bp) was amplified according to a method described previously (Asker *et al.*, 2007c). The 16S rRNA gene was sequenced directly by using a BigDye Terminator v. 3.1 cycle sequencing kit on an ABI 3100 automated DNA sequencer (Applied Biosystems).

A sequence similarity search in the GenBank/EMBL/DDBJ nucleotide sequence databases performed using the BLASTN program (<http://www.ncbi.nlm.nih.gov/BLAST/>) revealed that strain TDMA-uv53^T belonged to the family *Deinococcaceae*, and the highest degrees of sequence similarity were to *Deinococcus claudionis* PO-04-19-125^T (96 %), *D. altitudinis* ME-04-01-32^T (96 %), *D. radiomollis* PO-04-20-132^T (95 %), *D. deserti* VCD115^T (91.5 %), *D. hopiensis* KR-140^T (91.0 %), *D. sonorensis* KR-87^T (91.0 %), *D. radiophilus* DSM 20551^T (90.9 %), *D. proteolyticus* DSM 20540^T (90.3 %), *D. murrayi* ALT-1b^T (90.2 %), *D. alpinitundrae* ME-04-04-52^T (89 %), *D. geothermalis* AG-3a^T (88.3 %) and *D. apachensis* KR-36^T (88.1 %). Levels of sequence similarity between strain TDMA-uv53^T and representatives of the genus *Deinococcus* ranged from 87.0 to 96 %.

A neighbour-joining phylogenetic tree was constructed using the CLUSTAL W (Thompson *et al.*, 1994) and NJPlot (Perrière & Gouy, 1996) programs. The tree topology was estimated by a bootstrap analysis (Felsenstein, 1993) with 1000 resamplings of the dataset. Phylogenetic analysis of

the nearly full-length 16S rRNA gene sequence of strain TDMA-uv53^T indicated that it is a member of the genus *Deinococcus*, forming a coherent cluster with *D. claudionis* PO-04-19-125^T, *D. altitudinis* ME-04-01-32^T and *D. radiomollis* PO-04-20-132^T (Fig. 1). A tree based on the maximum-likelihood method showed essentially the same topology (see Supplementary Fig. S1 in IJSEM Online).

The pink pigments were extracted and analysed using HPLC-MS (LCMS-2010EV; Shimadzu) according to a method described previously (Asker *et al.*, 2007b, e). The menaquinone content was determined by an HPLC method (Collins, 1994) using an extract of *D. radiodurans* NBRC 15346^T as a reference for menaquinone-8 (MK-8). Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990). The fatty acid methyl esters of TDMA-uv53^T were extracted and analysed according to the standard protocol of the Sherlock Microbial Identification System (version 5.0; MIDI). Purified peptidoglycan was prepared and analysed by the method of Schleifer & Kandler (1972). DNA G + C content was determined by an HPLC method (Mesbah & Whitman, 1989).

The pink pigments of strain TDMA-uv53^T were unknown carotenoid compounds (UV-visible λ_{max} 479 and 510 nm). The peptidoglycan of strain TDMA-uv53^T contained L-ornithine and the respiratory quinone was MK-8. The polar lipid profile of the strain was dominated by two unknown glycopospholipids (GPL1, 2) and two unknown aminophospholipids (APL1, 2). One unknown phospholipid (PL), two unknown glycolipids (GL1, 2) and four unidentified lipids (L1–4) were also detected (Supplementary Fig. S2). It differed from *D. claudionis* PO-04-19-125^T, the closest relative, by the presence of GL1, GPL1, APL2, PL and L1–4. It also differed from *D. altitudinis* ME-04-01-32^T by the absence of aminoglycopospholipid and the presence of the unknown phospholipid and a second unknown glycopospholipid (GPL1). It differed from *D. radiomollis* PO-04-20-132^T by the presence of unknown glycolipid GL1 and unknown glycopospholipid GPL1 and the absence of two additional unknown aminophospholipids and an additional unknown phospholipid.

The predominant cellular fatty acids were iso-15:0 (16.7 %), 15:1 ω 6c (15.0 %), 15:0 (10.5 %), 16:0 (10.5 %) and summed feature 3 (iso-15:0 2-OH and/or 16:1 ω 7c) (25.0 %). These fatty acids represented 77.7 % of the total fatty acids of TDMA-uv53^T. The complete fatty acid profile of TDMA-uv53^T is given in Supplementary Table S1. This profile is in excellent agreement with those of representative of the genus *Deinococcus* (Supplementary Table S1; de Groot *et al.*, 2005; Ferreira *et al.*, 1997; Rainey *et al.*, 2005) and supports its assignment to the genus. However, the profile of strain TDMA-uv53^T differed both qualitatively and quantitatively from those of other members of genus *Deinococcus* with regard to certain fatty acids (Supplementary Table S1). The DNA G + C content

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of strain TDMA-uv53^T and representatives of related species of the genus *Deinococcus*. The tree was constructed by the neighbour-joining method (Saitou & Nei, 1987). Bar, 2 substitutions per 100 nucleotide positions. Bootstrap values, expressed as percentages of 1000 replicates, above 50% are given at branching points. GenBank accession numbers are shown in parentheses.

of TDMA-uv53^T was 69.1 mol% (Table 1), in agreement with the values of 60–71.5 mol% reported for the genus (Battista & Rainey, 2001; de Groot *et al.*, 2005; Ferreira *et al.*, 1997; Rainey *et al.*, 2005).

Gram staining was performed according to the method described by Smibert & Krieg (1994) and bacterial cells were observed under a Zeiss Axioskop 2 microscope. Scanning electron microscope observation was performed using a model VE-8800 instrument (Keyence). To determine the optimal growth temperature, the strain was cultivated on NA at 4, 10, 20, 30, 37, 40 and 45 °C. NaCl tolerance was studied using nutrient broth supplemented with different concentrations of NaCl. Growth at various pH values (pH 3.0–10.0) was evaluated in NB adjusted with HCl or NaOH. UV- and gamma-irradiation tolerance of strain TDMA-uv53^T was tested according to the method described by Rainey *et al.* (2005) and Suresh *et al.* (2004). *D. radiodurans* NBRC 15346^T and *Escherichia coli* C600 were used as positive and negative controls, respectively. Briefly, to determine survival after exposure to UV irradiation, strains were grown in NB to exponential phase. Cells were harvested by centrifugation, washed with 0.067 M potassium phosphate buffer (pH 7.0) and then diluted serially. Aliquots (0.1 ml) of these dilutions were spread on NA plates. The inoculated plates without their lids were exposed to UV light to the desired dose and subsequently incubated at 30 °C (*D. radiodurans* NBRC

15346^T) or 37 °C (TDMA-uv53^T and *E. coli* C600). Relative survival was determined by comparison with unirradiated cultures. To determine survival after exposure to gamma radiation, cells were prepared as above and then irradiated at room temperature using a ¹³⁷Cs source (dose rate 13 Gy min⁻¹). After exposure to 2.35, 5, 10 or 16 kGy, suspensions were diluted and plated on NA plates. All plates were then incubated at 30 or 37 °C as appropriate, and colonies were counted after 4 or 5 days. Survival was expressed as a percentage of the number of colonies that were formed in the absence of irradiation.

Anaerobic growth was assessed on NA incubated in a GasPak anaerobic system (BBL). The following tests were performed as described in the indicated references: casein hydrolysis (O'Brien & Colwell, 1987), catalase activity, spore formation and hydrolysis of Tween 80, DNA and starch (Smibert & Krieg, 1994) and hydrolysis of chitin, CM-cellulose (high viscosity; Sigma) and agar (Barrow & Feltham, 1993). Oxidase activity was tested by using commercial cytochrome oxidase test strips (BioChemika). Other enzyme activities, growth on carbohydrates, acid production from carbohydrates, nitrate reduction and the production of H₂S, indole and acetoin were examined using the commercial systems API 20E, API 20NE and API 50CH (bioMérieux) according to the manufacturer's instructions. A set of related type strains including *Deinococcus pimensis* NRRL B-23994^T, *D. yavapaiensis*

Table 1. Differential characteristics of strain TDMA-uv53^T and related type strains

Strains: 1, TDMA-uv53^T; 2, *D. claudionis* PO-04-19-125^T; 3, *D. altitudinis* ME-04-01-32^T; 4, *D. radiomollis* PO-04-20-132^T (data in columns 2–4 from Callegan *et al.*, 2008); 5, *D. sonorensis* KR-87^T (data from Rainey *et al.*, 2005). ND, No data available.

Characteristic	1	2	3	4	5
Pigmentation*	PP	P	P	P	P
Cell morphology†	R	SP/SR	R	R	SP/SR
Oxidase	+	–	–	–	+
Hydrolysis of:					
Casein	+	–	–	–	–
Gelatin	+	–	–	–	+
Starch	+	–	–	–	–
Optimum growth temperature (°C)	37	10	10	10	30
Growth at:					
37 °C	+	–	–	–	+
5 °C	–	+	+	+	–
pH 10	–	+	–	+	ND
Assimilation of:					
Glucose	+	+	–	+	+
Mannose	+	+	–	+	+
Glucosamine	–	+	–	+	+
DNA G + C content (mol%)	69.1	63.1	65.9	63.2	70.3

*PP, Pale pink; P, pink.

†R, Rods; SP, spherical; SR, short rods.

NRRL B-23960^T, *D. proteolyticus* DSM 20540^T, *D. radiophilus* DSM 20551^T, *D. geothermalis* DSM 11300^T, *D. grandis* DSM 3963^T, *D. indicus* DSM 15307^T, *D. murrayi* DSM 11303^T, *D. deserti* DSM 17065^T and *D. radiodurans* NBRC 15346^T were used as reference strains. They were received as gifts from the Agricultural Research Service Culture Collection (Peoria, IL, USA) or purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) and the National Institute of Technology and Evaluation, Biological Resource Center (NBRC; Tokyo, Japan).

Strain TDMA-uv53^T was tolerant of UV irradiation, showing 78 % survival after exposure to UV light at a dose of 64 J m^{–2}. At this dose, *D. radiodurans* NBRC 15346^T exhibited 50 % survival and *E. coli* C600 exhibited 0 % survival. Strain TDMA-uv53^T and *D. radiodurans* NBRC 15346^T could grow at the highest dose, 640 J m^{–2}. In addition, strain TDMA-uv53^T was tolerant of gamma irradiation, showing 95 % survival after exposure to a dose of 2.3 kGy. At this dose, *D. radiodurans* NBRC 15346^T exhibited 76 % survival and *E. coli* C600 exhibited 0 % survival. At 16.0 kGy gamma radiation, growth of *D. radiodurans* NBRC 15346^T and strain TDMA-uv53^T was still observed. Other phenotypic properties of strain TDMA-uv53^T are given in the species description, and those characteristics that differentiate strain TDMA-uv53^T

from related members of the genus *Deinococcus* are listed in Table 1 and Supplementary Table S2.

Strain TDMA-uv53^T differed from *D. claudionis* PO-04-19-125^T, *D. altitudinis* ME-04-01-32^T and *D. radiomollis* PO-04-20-132^T by (i) having a different fatty acid profile and a higher DNA G + C content, (ii) being mesophilic, (iii) being less psychrotolerant, (v) being able to produce oxidase, (iv) being able to hydrolyse casein, gelatin and starch and (vi) being highly tolerant of gamma and UV radiation. Strain TDMA-uv53^T differed from *D. radiophilus* DSM 20551^T, *D. proteolyticus* DSM 20540^T and *D. deserti* DSM 17065^T by (i) having a different fatty acid profile and a higher DNA G + C content, (ii) being pale-pink in colour, (iii) being more mesophilic, (v) being able to produce oxidase, (iv) being able to hydrolyse Tween 80 and (vi) being able to assimilate L-arabinose. Furthermore, TDMA-uv53^T differed from *D. radiophilus* DSM 20551^T and *D. proteolyticus* DSM 20540^T by having rod-shaped cells and its ability to assimilate D-mannose and maltose. Additionally, strain TDMA-uv53^T was distinguished from other closely related species by traits detailed in Table 1 and Supplementary Table S2.

Based on the data from a polyphasic study of strain TDMA-uv53^T, including its phylogenetic position, fatty acid composition, DNA G + C content and physiological and morphological characteristics, it is proposed that strain TDMA-uv53^T represents a novel species of the genus *Deinococcus*, for which we propose the name *Deinococcus aquiradiocola* sp. nov.

Description of *Deinococcus aquiradiocola* sp. nov.

Deinococcus aquiradiocola (a'qui.ra'di.o.co'la. L. n. *aqua* water; L. n. *radius* a beam or ray; N.L. pref. *radio*-pertaining to radiation; L. suffix *-cola* dweller; N.L. n. *aquiradiocola* radioactive-water dweller).

Cells are strictly aerobic, non-motile, non-spore-forming and Gram-positive-staining rods (0.4–0.6 × 1.5–1.8 µm; sometimes elongated up to 3 µm) that grow singly, in pairs or short chains (Supplementary Fig. S3). On nutrient agar, it forms pale-pink, circular, compact colonies (1–2 mm in diameter) within 3 days at 37 °C. Carotenoids are produced. Growth occurs between 20 and 40 °C (optimum 37 °C) and between pH 5.0 and 9.0 (optimum pH 7.0). Optimal growth occurs in the presence of 0.25 % (w/v) NaCl; growth occurs at 0–1 % NaCl (w/v). No growth at >1.0 % (w/v) NaCl. Aesculin, casein, gelatin, starch and Tween 80 are hydrolysed, while agar, DNA, CM-cellulose and chitin are not. Indole and H₂S are not produced. Nitrate is not reduced. Catalase- and oxidase-positive. β-Galactosidase-positive. Tests for tryptophan deaminase, lysine decarboxylase, ornithine decarboxylase and urease activities are negative. Glucose, arabinose, mannose, mannitol and maltose are assimilated, but N-acetylglucosamine, gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid are not. Results

from the API 50 CH test system show that no acids are produced from any of the carbohydrates tested. The polar lipid profile contains two unknown glycopospholipids, two unknown aminophospholipids, one unknown phospholipid, one unknown glycolipid and four unidentified lipids. MK-8 is the predominant menaquinone, and the major fatty acids are iso-15:0, 15:1 ω 6c, 15:0, 16:0 and summed feature 3 (iso-15:0 2-OH and/or 16:1 ω 7c). The DNA G+C content of the type strain is 69.1 mol%.

The type strain is TDMA-uv53^T (=JCM 14370^T =NBRC 102118^T =CCUG 53612^T), which was isolated from a freshwater sample collected at Misasa (Tottori, Japan).

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