Deinococcus depolymerans sp. nov., a gamma- and UV-radiation-resistant bacterium, isolated from a naturally radioactive site

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Four gamma- and UV-radiation-resistant bacterial strains, designated TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4, were isolated from a fresh-water sample collected at Misasa, Tottori, Japan. Cells of these strains were Gram-reaction-positive, non-motile, non-spore-forming, rod-shaped and formed red colonies. The genomic DNA G + C contents ranged from 70.5 to 70.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel isolates belong to the genus *Deinococcus*, the highest sequence similarities being with *Deinococcus aquaticus* PB314^T (98 %) and *Deinococcus caeni* Ho-08^T (97 %). The polar lipid profile of strain TDMA-24^T comprised three unidentified phosphoglycolipids, five unidentified glycolipids and seven unidentified polar lipids. MK-8 was the predominant respiratory quinone. Major fatty acids were iso-C_{15:0}, C_{15:1}ω6c, C_{15:0}, C_{16:0} and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c). On the basis of their phylogenetic positions and chemotaxonomic and phenotypic characteristics, the novel isolates represent a novel species of the genus *Deinococcus*, for which the name *Deinococcus depolymerans* sp. nov. is proposed. The type strain is TDMA-24^T (=JCM 14369^T =NBRC 102115^T =CCUG 53609^T).

The genus *Deinococcus* (Battista & Rainey, 2001; Brooks & Murray, 1981; Rainey *et al.*, 1997) belongs to the family *Deinococcaceae* (Battista & Rainey, 2001; Brooks & Murray, 1981; Rainey *et al.*, 1997) and comprises >40 species, 26 of which have been described as being highly resistant to radiation (Asker *et al.*, 2008, 2009; de Groot *et al.*, 2005; Hirsch *et al.*, 2004; Im *et al.*, 2008; Lai *et al.*, 2006; Rainey *et al.*, 2005; Suresh *et al.*, 2004; Zhang *et al.*, 2007). Four other species of the genus *Deinococcus* have been described as being sensitive to ionizing radiation (Callegan *et al.*, 2008).

Four gamma- and UV-radiation-resistant bacterial strains, designated TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4, which produce unknown carotenoids and

form red-pigmented colonies, were isolated from the Misasa spa, Tottori, Japan, a region known for its high levels of natural radioactivity (Asker *et al.*, 2007a, b). Based on 16S rRNA gene sequence similarities (Asker *et al.*, 2007b) these strains were phylogenetically identical (100 % sequence similarity). Here, we report the results of a polyphasic taxonomic study on the four novel strains. On the basis of their biochemical and chemical characteristics and the results of 16S rRNA gene sequence analysis, we propose that the isolates represent a novel species within the genus *Deinococcus*.

Strains TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4 were isolated as described by Asker *et al.* (2007b), except that incubations were carried out at 30 °C. Genomic DNA of the isolates was extracted using a bacterial genomic DNA purification kit (Edge BioSystems). DNA fragments containing the nearly complete 16S rRNA gene sequence were amplified according to the method described previously by Asker *et al.* (2007b). The 16S rRNA genes were sequenced directly by using a BigDye Terminator v3.1 cycle sequencing kit and an ABI 3100

Abbreviation: RAPD, random amplification of polymorphic DNA.

The GenBank/EMBL/DDBJ/accession numbers for the 16S rRNA gene sequences of strains TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4 are AB264134, AB479215, AB479216 and AB479217.

Four supplementary figures and one supplementary table are available with the online version of this paper.

automated DNA sequencer (Applied Biosystems). DNA G+C contents were determined by using HPLC (Mesbah & Whitman, 1989).

Sequence similarity searches in the GenBank database, performed using the BLASTN program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Gertz, 2005), revealed that strains TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4 belonged to the genus *Deinococcus* with the highest sequence similarities being found with *D. aquaticus* PB314^T (98.0%), *D. caeni* Ho-08^T (97.0%), *D. indicus* DSMZ 15307^T (96.6%), *D. grandis* DSM 3963^T (95.9%), *D. hohokamensis* KR-40^T (94.8%), *D. deserti* VCD115^T (94.4%), *D. navajonensis* KR-114^T (94.2%), *D. aquatilis* CCUG 53370^T (94.0%), *D. radiodurans* DSM 20539^T (94.0%) and *D. ficus* CC-FR2-10^T (92.9%). In addition, the four strains exhibited high sequence similarities (100%) to each other. A neighbour-joining phylogenetic tree was reconstructed using the CLUSTAL W (Thompson *et al.*, 1994) and NJPlot (Perrière & Gouy, 1996) programs. The tree topology was supported by bootstrap analysis (Felsenstein, 1993) with 1000 resamplings of the dataset. The four novel strains formed a coherent group and clustered together with *Deinococcus aquaticus* PB314^T and *Deinococcus caeni* Ho-08^T (Fig. 1). A similar topology was obtained in the tree reconstructed using the maximum-likelihood method (Supplementary Fig. S1, available in IJSEM Online).

The high 16S rRNA gene sequence similarities (100%) between the four strains suggested that they belong to the same species. To confirm this, standard DNA–DNA hybridizations were carried out on these strains. DNA–DNA hybridizations were also performed with strain TDMA-24^T and type strains of the closest related phylogenetic members of the genus *Deinococcus*, *D. aquaticus* PB314^T and *D. caeni* Ho-08^T. Genomic DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by using chromatography on hydroxyapatite (Cashion *et al.*, 1977). DNA–DNA hybridization was

carried out following the method of De Ley *et al.* (1970) with modifications (Huß *et al.*, 1983) using a Cary 100 Bio UV/VIS- spectrophotometer equipped with a Peltier-thermostated 6×6 multi-cell changer and a temperature controller with an *in-situ* temperature probe (Varian). The four novel strains showed high levels of DNA–DNA relatedness (98–100%) to each other. DNA–DNA hybridizations between strain TDMA-24^T and *D. aquaticus* NBRC 101311^T and *D. caeni* NBRC 101312^T showed relatedness values of 42.5–44.7% and 48.9–58.9%, respectively. Based on the recommended criteria for the delineation of bacterial species (Wayne *et al.*, 1987), the four novel strains clearly represented the same species, distinct from other members of the genus *Deinococcus*. In addition, random amplification of polymorphic DNA (RAPD) was performed to provide further evidence of the similarities between the four strains. RAPD was performed by using four primers, Primer 1 (5'-GGTGC GGGA-3'), Primer 3 (5'-GTAGACCCGT-3'), Primer 5 (5'-AACGCGCAAC-3') and Primer 6 (5'-CCCGTCAGCA-3') (Pharmacia Biotech), and ~50 ng extracted DNA as template. PCR amplification was carried out in reaction volumes of 50 µl using a thermal cycler (MyCycler BIO RAD) programmed for 45 cycles of 95 °C for 1 min, 36 °C for 1 min and 72 °C for 2 min. Amplified PCR products (8 µl) were electrophoresed in a 2% agarose gel in TBE buffer with 1 kb DNA ladder as a molecular size marker. Agarose gels were stained with ethidium bromide and visualized under UV light using a Gel Doc 2000 system (Bio-Rad). The RAPD patterns were identical for the four novel strains (Supplementary Fig. S2), indicating that the four strains were not only members of a single species but were also clonally related. The DNA G+C contents of the four strains ranged from 70.5 to 70.6 mol% (Table 1), which is within the range of values characteristic of the genus *Deinococcus* (60–71.5%) (Battista & Rainey, 2001; de Groot *et al.*, 2005; Ferreira *et al.*, 1997; Rainey *et al.*, 2005).

Gram staining was performed according to the method of Smibert & Krieg (1994) and bacterial cells were observed

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of strains TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4 and closely related members of the genus *Deinococcus*. The tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Bar, 0.01 substitutions per nucleotide position. Bootstrap values >50%, expressed as a percentage of 1000 replicates, are given at the branching points. GenBank accession numbers are shown in parentheses.

Table 1. Differential characteristics of strain TDMA-24^T and related species of the genus *Deinococcus*

Taxa: 1, strain TDMA-24^T (strains TDMA-24-2, TDMA-24-3 and TDMA-24-4 show identical results); 2, *D. aquaticus* PB314^T (Im *et al.*, 2008); 3, *D. caeni* Ho-08^T (Im *et al.*, 2008); 4, *D. indicus* (Asker *et al.*, 2008; Rainey *et al.*, 2005); 5, *D. radiodurans* (Asker *et al.*, 2008; Battista & Rainey, 2001); 6, *D. aquatilis* CCUG 53370^T (Kämpfer *et al.*, 2008); 7, *D. grandis* (Asker *et al.*, 2008; Battista & Rainey, 2001). Sugar utilization in *D. aquaticus* and *D. caeni* were tested by using API 50 CH test strips. R, Red; P, pink; P-R, pink-red; r, rod-shaped; sp, spherical; –, negative; +, positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Pigmentation	R	R	P	R	R	P	P-R
Cell morphology	r	r	r	r	sp	r	r
Gram reaction	+	–	–	+	+	+	–
Growth temperature range (°C)	18–40	20–37	20–37	20–37	20–45	15–36	30–40
Tolerance to NaCl (% w/v)	0–2	0–3	0–3	0–1	ND	ND	0–1
Oxidase	+	+	–	+	+	+	+
β-Galactosidase	+	+	+	+	–	ND	+
Nitrate reduction	–	–	–	+	+	ND	+
Arginine dihydrolase	–	–	–	+	+	ND	+
Hydrolysis of:							
Aesculin	+	+	–	+	–	ND	+
Starch	+	+	+	+	+	ND	+
Casein	+	ND	ND	+	–	ND	–
Gelatin	+	–	–	+	–	ND	+
Tween 80	+	ND	ND	–	–	ND	–
DNA	+	–	–	–	+	ND	+
Assimilation of:							
Glucose	+	+	+	–	+	+	+
L-Arabinose	–	–	–	+	–	–	–
D-Mannose	+	+	–	+	+	–	–
Mannitol	–	+	–	–	+	–	+
N-Acetylglucosamine	+	+	+	+	+	+	–
Gluconate	+	+	+	+	+	–	–
Malate	+	+	–	+	+	–	+
Melibiose	–	+	–	+	ND	–	+
D-Ribose	–	–	–	–	+	–	–
Salicin	–	+	–	+	ND	–	–
Sorbitol	–	+	–	+	ND	–	+
DNA G + C content (mol%)	70.5–70.6	68.3	68.4	65.8	67.0	ND	69

under an Axioskop 2 microscope (Carl Zeiss). Scanning electron microscopy was performed using a model VE-8800 microscope (Keyence). Physiological characterization, including determination of growth at optimum pH, temperature and salt concentration, was performed according to the methods described by Asker *et al.* (2008). Growth was assessed under anaerobic conditions on nutrient agar (NA) in a GasPak anaerobic system (BBL). Tests for the following properties were performed as previously described: casein hydrolysis (O'Brien & Colwell, 1988); catalase activity, spore formation and hydrolysis of Tween 80, DNA and starch (Smibert & Krieg, 1994); and hydrolysis of chitin, carboxymethyl cellulose (high viscosity; Sigma) and agar (Barrow & Feltham, 1993). Oxidase activity was tested by using commercial cytochrome oxidase test strips (BioChemika). Other enzymic activities as well as growth and acid production from carbohydrates, nitrate reduction and the production of hydrogen sulfide, indole and acetoin were examined using

commercial API 20 E, API 20 NE and API 50 CH systems (bioMérieux) according to the manufacturer's instructions. The resistance of strain TDMA-24^T to UV- and gamma-rays was tested according to the method described by Asker *et al.* (2007b, 2009).

Strains TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4 exhibited identical morphological and physiological traits, which are listed in the species description and Table 1 and shown in Supplementary Fig. S3. Characteristics that differentiated the novel strains from related members of the genus *Deinococcus* are listed in Table 1.

Since the four strains are clonally related, it is not surprising that their phenotypes do not differ. They did, however, differ from *Deinococcus aquaticus* NBRC 101311^T and *D. caeni* NBRC 101312^T by being Gram-reaction-positive, able to hydrolyse gelatin and DNA and less halotolerant. In addition, the four novel strains differed

from *D. aquaticus* NBRC 101311^T by being unable to assimilate mannitol, melibiose, ribose, salicin and sorbitol and differed from *D. indicus* DSMZ 15307^T by being able to produce oxidase and β -galactosidase and hydrolyse Tween 80 and DNA and by being unable to assimilate L-arabinose or reduce nitrate. The four novel strains differed from *D. radiodurans* NBRC 15346^T by being able to produce β -galactosidase, hydrolyse aesculin, casein, gelatin and Tween 80 and assimilate N-acetylglucosamine and by being unable to assimilate L-arabinose and mannitol or reduce nitrate. Characteristics that distinguished the four novel strains from other closely related species of the genus *Deinococcus* are detailed in Table 1.

Strain TDMA-24^T was tolerant of UV irradiation, showing an 83 % survival rate after exposure to UV light at a dose of 64 J m⁻². At this dose, *Deinococcus radiodurans* NBRC 15346^T exhibited a 50 % survival rate and *Escherichia coli* C600 exhibited a 0 % survival rate. Strains TDMA-24^T and *D. radiodurans* NBRC 15346^T grew after being subjected to a higher dose of 640 J m⁻². In addition strain TDMA-24^T was resistant against gamma-rays, showing a 100 % survival rate after exposure to a dose of 2.3 kGy. At this dose, *Deinococcus radiodurans* NBRC 15346^T exhibited a 76 % survival rate and *E. coli* C600 exhibited a 0 % survival rate. At 16.0 kGy gamma radiation, growth of *D. radiodurans* NBRC 15346^T and strain TDMA-24^T was still observed.

Pigments were extracted and analysed using HPLC–MS (LCMS-2010EV, Shimadzu) according to the method described previously (Asker *et al.*, 2007b). The menaquinone content was determined by HPLC (Collins, 1994) using an extract of *D. radiodurans* NBRC 15346 as a reference for MK-8. The fatty acid methyl esters of strain TDMA-24^T were extracted and analysed according to the standard protocol of the Sherlock Microbial Identification System (Version 5.0; MIDI). Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990). Purified peptidoglycan was prepared and analysed by the method of Schleifer & Kandler (1972).

The red pigments of strains TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4 were unknown carotenoid compounds, which had identical spectra (UV-Vis $\lambda_{\max}$ 477 and 505 nm). The four strains contained peptidoglycan type A3 β (L-Orn–Gly–Gly). The four strains contained a quinone system composed predominantly of MK-8. This is in agreement with the menaquinone systems of other species of the genus *Deinococcus*, which all contain menaquinone MK-8 as the major quinone (Embley *et al.*, 1987; Ferreira *et al.*, 1997; Kämpfer *et al.*, 2008; Lai *et al.*, 2006; Suresh *et al.*, 2004).

The predominant cellular fatty acids of strain TDMA-24^T were iso-C_{15:0} (17.5 %), C_{15:1} ω 6c (16.0 %), C_{15:0} (11.2 %), C_{16:0} (9.7 %) and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c) (23.9 %). The fatty acid profiles of strain TDMA-24^T and closely related members of the genus *Deinococcus* were in excellent agreement (Supplementary Table S1; Callegan *et al.*, 2008; de Groot *et al.*, 2005;

Ferreira *et al.*, 1997; Rainey *et al.*, 2005); however, strain TDMA-24^T differed from members of the genus *Deinococcus* both qualitatively and quantitatively with regard to certain fatty acids (Supplementary Table S1). The polar lipid profile of strain TDMA-24^T was dominated by an unknown phosphoglycolipid (PGL2) and also contained various other unknown phosphoglycolipids, glycolipids and polar lipids (Supplementary Fig. S4). These results are in agreement with previous results reported for species of the genus *Deinococcus* (Ferreira *et al.*, 1997; de Groot *et al.*, 2005; Lai *et al.*, 2006; Kämpfer *et al.*, 2008); however, strain TDMA-24^T showed a unique combination of unknown polar lipids, which might distinguish them from other members of the genus *Deinococcus*. For instance, strain TDMA-24^T contained unidentified phosphoglycolipid PGL3, seven unidentified lipids and five unidentified glycolipids, whereas *D. aquaticus* contained no PGL3, only four unidentified lipids and six unidentified glycolipids (Im *et al.*, 2008).

Based on their phylogenetic positions, DNA–DNA relatedness, DNA G+C contents, fatty acid compositions and physiological and morphological characteristics, strains TDMA-24^T, TDMA-24-2, TDMA-24-3 and TDMA-24-4 represent a novel species of the genus *Deinococcus*, for which the name *Deinococcus depolymerans* sp. nov. is proposed, with the type strain TDMA-24^T.

Description of *Deinococcus depolymerans* sp. nov.

Deinococcus depolymerans (de.po.ly'me.rans. N.L. v. *depolymere* depolymerize; N.L. part. adj. *depolymere* depolymerizing).

Cells are strictly aerobic, non-motile, non-spore-forming and Gram-reaction-positive rods (0.4–0.6 $\times$ 2–1.8 μ m; sometimes elongated up to 3.2 μ m), which grow singly or in pairs. Forms red, circular colonies (2–4 mm in diameter) within 2 days of incubation at 30 °C on NA. Carotenoids are produced. Growth occurs at 18–40 °C (optimum 30 °C), at pH 5–11 (optimum pH 7) and in 0–2 % (w/v) NaCl (optimum 0.5 %). Aesculin, casein, gelatin, starch, DNA and Tween 80 are hydrolysed but agar, cellulose and chitin are not. Indole and hydrogen sulfide are not produced. Nitrate is not reduced. Catalase- and oxidase-positive. β -Galactosidase-positive. Tryptophan deaminase, lysine decarboxylase, ornithine decarboxylase and urease activities are negative. Glucose, mannose, N-acetylglucosamine, maltose, gluconate and malate are assimilated but arabinose, mannitol, capric acid, adipic acid, trisodium citrate and phenylacetic acid are not. In the API 50 CH system, acid is produced from glycerol, galactose, glucose, aesculin, cellobiose, maltose, sucrose and turanose. The polar lipids profile consists of three unidentified phosphoglycolipids, five unidentified glycolipids and seven unidentified lipids. MK-8 is the predominant menaquinone and the major fatty acids are iso-C_{15:0}, C_{15:1} ω 6c, C_{15:0}, C_{16:0} and summed feature 3 (iso-C_{15:0}

2-OH and/or C_{16:1ω7c}). The DNA G+C contents are 70.5–70.6 mol%.

The type strain, TDMA-24^T (=JCM 14369^T =NBRC 102115^T =CCUG 53609^T), was isolated from a fresh water sample collected at Misasa, Tottori, Japan.

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