Deinococcus daejeonensis sp. nov., isolated from sludge in a sewage disposal plant

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A Gram-stain-positive, strictly aerobic, spherical, non-motile red-pigmented bacterial strain, designated MJ27^T, was isolated from a sludge sample of the Daejeon sewage disposal plant in South Korea. A polyphasic approach was used to study the taxonomic position of strain MJ27^T. Strain MJ27^T shared highest 16S rRNA gene sequence similarity with *Deinococcus grandis* DSM 3963^T (98.8%), *Deinococcus caeni* Ho-08^T (97.5%) and *Deinococcus aquaticus* PB314^T (96.6%); levels of sequence similarity with the type strains of other *Deinococcus* species were less than 96.0%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain MJ27^T belonged to the clade formed by members of the genus *Deinococcus* in the family *Deinococcaceae*. The G + C content of the genomic DNA of strain MJ27^T was 67.6 mol%. The chemotaxonomic characteristics of strain MJ27^T were typical of members of the genus *Deinococcus*, with MK-8 as the predominant respiratory quinone, C_{16:1ω7c}, C_{15:1ω6c}, C_{16:0} and C_{15:0} as major fatty acids (>12%), ornithine as the diamino acid in the cell-wall peptidoglycan and resistance to gamma radiation [D₁₀ (dose required to reduce the bacterial population by tenfold) >9 kGy]. The low levels of DNA–DNA relatedness reported here (5.3 ± 1.5–29.2 ± 2.3%) indicate that strain MJ27^T represents a species that is separate from its closest relatives in the genus *Deinococcus*. On the basis of phylogenetic inference, fatty acid profile and other phenotypic properties, strain MJ27^T is considered to represent a novel species of the genus *Deinococcus*, for which the name *Deinococcus daejeonensis* sp. nov. is proposed. The type strain is MJ27^T (=KCTC 13751^T=JCM 16918^T).

Concerns about cell damage caused by X-ray irradiation in hospitals or radioactive material emitted from nuclear power stations have led to studies on the effect of radiation on the human body (Kwon & Seo, 2010; Seong *et al.*, 2010). The mechanisms by which damaged cells recover from radiation are a major issue, so radiation-resistant bacteria are becoming increasingly important as sources of radiation-resistant enzymes and other molecules. The genus *Deinococcus* is notable for its radiation resistance and is a good model for recovery of particular biomolecules.

The genus *Deinococcus* was proposed by Brooks & Murray (1981) and, at the time of writing, comprised more than 45 recognized species. Species of the genus *Deinococcus* are aerobic and show high resistance to ionizing radiation, colony colour ranges from yellow to pink, orange and red, they are commonly Gram-stain-positive (some strains are Gram-stain-negative), they have L-ornithine in the cell-wall peptidoglycan and they lack teichoic acids.

During the course of collecting micro-organisms from sludge at the Daejeon sewage disposal plant, South Korea, a Gram-stain-positive, non-motile, aerobic, non-spore-forming strain, MJ27^T, was isolated. On the basis of 16S rRNA gene sequence analysis, strain MJ27^T was considered to belong to the genus *Deinococcus*. Strain MJ27^T was subjected to a polyphasic taxonomic investigation and the results indicated that it represents a novel species of the genus *Deinococcus*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MJ27^T is JF806527.

One supplementary figure is available with the online version of this paper.

Fig. 1. Representative survival plot for strain MJ27^T (▲) following exposure to gamma radiation. Survival rates of *D. radiodurans* R1 (●) and *E. coli* (x) are also shown. Each increment on the y-axis represents a tenfold reduction in viability.

For isolation of strain MJ27^T, 1 g soil sample was mixed with 50 ml saline (0.85 % NaCl), vortexed, serially diluted and 100 µl of each dilution was spread onto 0.1 × R2A agar (Difco) and incubated at 30 °C for 1 week. Single colonies were transferred onto new plates and the purified colonies were tentatively identified by analysis of partial 16S rRNA gene sequences. Isolate MJ27^T was routinely cultured on nutrient agar (NA; Difco) at 30 °C and preserved as a suspension in Luria–Bertani (LB; Difco) broth with 20 % glycerol (w/v) at –70 °C.

To determine the survival rate after exposure to gamma radiation, cultures were grown at 30 °C in liquid nutrient-rich TGY medium (1 % tryptone, 0.1 % glucose, 0.5 % yeast extract) or on TGY solid medium (TGY medium plus 1.5 % agar). To measure ionizing radiation resistance, cultures grown to the early stationary phase (~10⁹ cells ml⁻¹) were divided into 1 ml aliquots without change of broth and were exposed on ice to a cobalt-60 gamma irradiator (point source; AECL, IR-79). The source strength was approximately 100 kCi (3.7 GBq) at a dose rate of 70 Gy min⁻¹; actual doses were within 2 % of the target dose. Irradiated cells were diluted, plated in triplicate on TGY agar plates and incubated for 2 days before survival rates were scored. *Deinococcus radiodurans* R1 and *Escherichia coli* cells were used as positive and negative control strains for the experiment. Strain MJ27^T showed good resistance to 3 kGy gamma radiation (90 % survival for *D. radiodurans* and MJ27^T); at 9 kGy, strain MJ27^T showed 20 % survival compared with 50 % for *D. radiodurans* R1 (Fig. 1). Radiation resistance is one of the main characteristics of members of the genus *Deinococcus*. Most radiation-resistant *Deinococcus* species were isolated by selection using UV or gamma radiation (Rainey *et al.*, 2005; Oyaizu *et al.*, 1987); however, strain MJ27^T was isolated without any radiation selection method, but showed resistance to gamma radiation.

The genomic DNA of strain MJ27^T was extracted by using a commercial genomic DNA extraction kit (Solgent);

PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). The nearly complete sequence of the 16S rRNA gene (1464 nt) was compiled with SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank and were edited by using the program BioEdit (Hall, 1999). Multiple alignments were performed with the program CLUSTAL X (Thompson *et al.*, 1997). Evolutionary distances were calculated with the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in MEGA3 (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985). In the neighbour-joining tree based on 16S rRNA gene sequences, strain MJ27^T belonged to the clade formed by members of the genus *Deinococcus* in the family *Deinococcaceae* (Fig. 2).

On the basis of levels of 16S rRNA gene sequence similarity, the closest relatives of strain MJ27^T were *Deinococcus grandis* DSM 3963^T (98.8 %), *Deinococcus caeni* Ho-08^T (97.5 %), *Deinococcus aquaticus* PB314^T (96.6 %) and *Deinococcus indicus* Wt/1a^T (95.6 %). Levels of sequence similarity to other *Deinococcus* species were ≤95 %. Consequently, the above type strains of *Deinococcus* species were obtained and grown under the same conditions as for strain MJ27^T and used as reference strains for phenotypic and chemotaxonomic experiments.

Cell morphology and motility were observed with an Olympus light microscope (1000 × magnification), by using cells grown for 24 h in nutrient broth at 30 °C. For electron microscopy, cells were gently resuspended in one drop of deionized H₂O and samples were placed on carbon- and Formvar-coated nickel grids for 30 s. Grids were floated on one drop of 0.1 % (w/v) aqueous uranyl acetate, blotted dry and then viewed with a Carl Zeiss LEO912AB electron microscope at 100 kV under standard operating conditions. The Gram reaction was performed by using the non-staining method, as described by Buck (1982). Catalase activity was assessed by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was assessed by using 1 % (w/v) tetramethyl *p*-phenylenediamine (Cappuccino & Sherman, 2002). Anaerobic growth was examined in serum bottles with sodium thioglycolate (1 g l⁻¹) added to nutrient broth and the upper air layer was replaced with nitrogen. Physiological characteristics were determined with API 20NE, API ID 32 GN and API ZYM galleries (bioMérieux) according to the manufacturer's instructions. Growth at 4, 10, 20, 25, 28, 30, 37, 40, 42 and 45 °C and at pH 3.0–12.0 (0.5 pH unit intervals) was assessed in LB broth for 5 days. The pH of the medium was adjusted by using diluted NaOH and HCl. Salt tolerance was tested in R2A broth supplemented with 0–10 % (w/v) NaCl incubated for 5 days at 30 °C. Growth on trypticase soy agar (TSA; Difco), R2A agar and LB agar was also evaluated at 30 °C. The phenotypic characteristics of strain MJ27^T are given in the species description below and those that differentiated strain

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain MJ27^T and the type strains of recognized *Deinococcus* species. Numbers at branch points are bootstrap values (percentages of 1000 replications); only values >50 % are shown. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. The sequence from *Thermus aquaticus* DSM 625^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

MJ27^T from the type strains of closely related species are given in Table 1.

Isoprenoid quinones were extracted from bacterial cells of strain MJ27^T with chloroform/methanol (2:1, v/v), evaporated under vacuum and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane/quinone solution was then purified by using silica Sep-Pak Vac cartridges (Waters) and was subsequently analysed by HPLC, as described by Hiraishi *et al.* (1996). Strain MJ27^T contained menaquinone MK-8 as the major respiratory quinone. The peptidoglycan was analysed as described by Schleifer & Kandler (1972) by using TLC on cellulose

sheets instead of paper chromatography. Strain MJ27^T had ornithine and glycine as major amino acids in the peptidoglycan structure, in line with data for members of the genus *Deinococcus*. Cell-wall sugars contained rhamnose and ribose, as commonly observed in *Deinococcus* species.

For fatty acid methyl ester analysis, strain MJ27^T was grown on TSA and harvested at the early stationary growth phase at 30 °C. Two loopfuls of bacterial mass were collected and subjected to saponification, methylation and extraction according to the methods of Kuykendall *et al.* (1988). The fatty acid methyl ester mixtures were separated by using the Sherlock Microbial Identification System

Table 1. Differential characteristics of strain MJ27^T and the type strains of closely related *Deinococcus* species

Strains: 1, MJ27^T; 2, *D. aquaticus* KCTC 12552^T; 3, *D. caeni* KCTC 12553^T; 4, *D. grandis* KACC 11979^T; 5, *D. radiodurans* KACC 12248^T. All data are from this study and were determined under the same experimental conditions. All strains are non-motile and do not produce acid from glucose. w, Weakly positive reaction.

Characteristic	1	2	3	4	5
Gram staining	+	–	–	–	+
Morphology	Coccus	Rod	Rod	Rod	Coccus
Colony colour	Red	Red	Pink	Pink	Red
Growth at/with (LB):					
15 °C	+	–	–	–	+
42 °C	–	–	–	+	+
pH 6	+	–	–	–	+
pH 8.5	+	+	+	–	–
2 % NaCl	+	+	+	–	+
4 % NaCl	+	–	–	–	+
Nitrate reduction	–	–	–	+	–
Enzyme activity					
Oxidase	–	+	–	–	+
Alkaline phosphatase	+	+	+	+	+
Arginine dihydrolase	+	–	–	–	–
α-Chymotrypsin	+	+	–	–	+
Esterase (C4)	+	+	w	+	w
β-Galactosidase (ONPG)	+	+	w	+	w
β-Glucosidase	+	+	–	+	–
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	–
Valine arylamidase	+	w	w	–	–
Gelatin hydrolysis	+	–	–	–	+
Starch hydrolysis	+	+	+	+	w
Assimilation of:					
3-Hydroxybenzoate	w	w	–	w	+
4-Hydroxybenzoate	+	+	w	+	+
2-Ketogluconate (α)	–	w	–	+	+
5-Ketogluconate	+	+	w	+	+
Acetate	–	–	–	w	+
Caprate	w	w	–	+	+
Citrate	w	w	–	+	+
DL-Lactate	+	+	–	w	+
Propionate	–	–	+	–	–
L-Fucose	–	+	–	+	+
D-Glucose	+	+	–	+	+
Melibiose	+	w	–	w	+
L-Rhamnose	+	w	+	–	+
Sucrose	+	+	–	+	–
myo-Inositol	+	+	–	+	+
D-Mannitol	+	+	–	+	+
L-Alanine	w	w	+	–	–
L-Serine	–	+	–	+	+
N-Acetyl-D-glucosamine	–	+	+	–	+
Salicin	+	+	–	w	–
Glycogen	–	–	+	–	–
DNA G + C content (mol%)	67.6	68.3	68.4	68.4	67.0

(TSBA, version 6.0; MIDI), analysed by GC (Hewlett Packard 6890) and identified via the Microbial Identification software package (Sasser, 1990). Strain MJ27^T contained major amounts of C_{16:0} (28.6 %), C_{16:1ω7c} (27.5 %), C_{15:1ω6c} (13.8 %) and C_{15:0} (12.6 %), a moderate amount of C_{17:0} (5.6 %) and minor amounts of iso-C_{16:0} (2.6 %), C_{16:1ω5c} (1.8 %), C_{14:0} (1.7 %), iso-C_{17:0} (1.5 %) and C_{15:0} (1.2 %). The major fatty acids of strain MJ27^T are common to most recognized *Deinococcus* species (see Table 2), but there were some quantitative and qualitative differences between strain MJ27^T and the reference type strains.

To determine the polar lipid profile of strain MJ27^T, total cellular polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1977). Total lipids were observed after staining with 5 % ethanolic molybdophosphoric acid and spots were detected by using 0.2 % ninhydrin reagent (for aminolipids), α-naphthol reagent (for glycolipids) and Zinzadze reagent (for phospholipids). Strain MJ27^T contained various unknown phosphoglycolipids (PGL_{1–2}), unknown glycolipids (GL_{1–7}) and unknown polar lipids (Fig. S1, available in IJSEM Online). The predominant polar lipid was unknown phosphoglycolipid PGL₂, which is common to members of the genus *Deinococcus* (Ferreira *et al.*, 1997; Weon *et al.*, 2007; Im *et al.*, 2008).

Table 2. Fatty acid profiles (%) of strain MJ27^T and the type strains of closely related *Deinococcus* species

Strains: 1, MJ27^T; 2, *D. aquaticus* KCTC 12552^T; 3, *D. caeni* KCTC 12553^T; 4, *D. grandis* KACC 11979^T; 5, *D. radiodurans* KACC 12248^T. All strains were incubated on TSA for 2 days at 30 °C. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. The *cis* isomers are indicated by the suffix *c*. Fatty acids amounting to <1 % of the total fatty acids in all strains are not shown. Tr, trace (<1 %); ND, not detected.

Fatty acid	1	2	3	4	5
Saturated					
C _{14:0}	1.7	3.4	2.4	ND	ND
iso-C _{14:0}	Tr	Tr	ND	ND	ND
C _{15:0}	12.6	8.4	ND	15.6	3.9
iso-C _{15:0}	1.2	2.3	ND	6.8	ND
C _{16:0}	28.6	32.9	37.8	7.5	16.8
iso-C _{16:0}	2.6	1.2	2.8	4.8	ND
C _{17:0}	5.6	3.8	1.8	8.3	8.4
iso-C _{17:0}	1.5	6.8	Tr	2.3	Tr
anteiso-C _{17:0}	ND	ND	ND	8.6	ND
Unsaturated					
C _{15:1ω6c}	13.8	ND	2.4	28.6	7.8
C _{16:1ω5c}	1.8	Tr	2.9	2.8	6.8
C _{16:1ω7c}	27.5	38.5	48.9	13.8	42.8
C _{16:1ω9c}	ND	ND	ND	ND	4.8
C _{18:1ω7c}	ND	ND	ND	ND	4.3
C _{18:1ω9c}	ND	ND	ND	ND	2.8

For determination of the DNA G+C content, genomic DNA was extracted as described above and enzymically degraded into nucleosides. The nucleosides were analysed by HPLC (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). The DNA G+C content of strain MJ27^T was 67.6 mol%.

DNA–DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), by using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was carried out reciprocally with five replications per sample. The highest and lowest values obtained for each sample were excluded; the remaining three values were utilized in the calculation of levels of relatedness, and values are quoted as means ($\pm$ SD) of these three values. Strain MJ27^T exhibited relatively low levels of DNA–DNA relatedness with *D. aquaticus* KCTC 12552^T (5.3 ± 1.5 %), *D. caeni* KCTC 12553^T (19.2 ± 2.8 %) and *D. grandis* KACC 11979^T (29.2 ± 2.3 %). These values indicated that strain MJ27^T is not related to members of the genus *Deinococcus* at the species level (Wayne *et al.*, 1987).

Data from the present polyphasic study clearly showed that strain MJ27^T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus daejeonensis* sp. nov. is proposed.

Description of *Deinococcus daejeonensis* sp. nov.

Deinococcus daejeonensis (da.e.je.on.en'sis. N.L. masc. adj. *daejeonensis* of or pertaining to Daejeon, a city in South Korea, from where the type strain was isolated).

Cells are Gram-stain-positive, strictly aerobic, non-motile, non-spore-forming cocci. After 3 days of incubation at 30 °C on R2A agar, colonies are 0.5–0.7 mm in diameter, red, smooth, convex and circular with regular edges. Good growth occurs at 15–37 °C (optimum, 30 °C; weak growth at 40 °C). Growth occurs at pH 6.0–8.5 (optimum, pH 7.0–7.5). Growth occurs with 0–4 % (w/v) NaCl, but not with 5 % NaCl. Growth occurs on TSA, LB agar, NA and R2A agar. Positive for catalase and negative for oxidase. Nitrate is not reduced. Cells are highly resistant to gamma radiation [D₁₀ (dose required to reduce the bacterial population by tenfold) >9 kGy]. In API ZYM and API 20NE strips, positive for acid phosphatase, alkaline phosphatase, arginine dihydrolase, α -chymotrypsin, esterase, esterase lipase, β -galactosidase, gelatinase, α -glucosidase, β -glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase activities. Negative for *N*-acetyl- β -glucosaminidase, cystine arylamidase, α -fucosidase, α -galactosidase, β -glucuronidase, lipase, α -mannosidase, trypsin and urease activities. In API ID 32 GN strips, assimilates D-glucose, 4-hydroxybenzoic acid, DL-lactate, D-mannitol, melibiose, myo-inositol, potassium 5-ketogluconate, L-rhamnose, salicin and sucrose, weakly assimilates L-alanine, caprate, citrate and 3-hydroxybenzoic acid, but does not assimilate *N*-acetylglucosamine, adipate, L-arabinose, L-fucose, gluconate,

glycogen, L-histidine, 3-hydroxybutyric acid, itaconate, 2-ketogluconate, L-malate, D-maltose, malonate, D-mannose, L-proline, phenylacetate, propionate, D-ribose, L-serine, sodium acetate, D-sorbitol, suberate or n-valerate. The predominant respiratory quinone is MK-8. The major fatty acids (>12 %) are C_{16:1} ω 7c, C_{15:1} ω 6c, C_{16:0} and C_{15:0}. The polar lipid profile is complex, containing various unknown phosphoglycolipids (including one major phosphoglycolipid), glycolipids and phospholipids. Ornithine and glycine are present in the peptidoglycan. The cell wall contains rhamnose and ribose.

The type strain, MJ27^T (=KCTC 13751^T=JCM 16918^T), was isolated from sludge at the Daejeon sewage disposal plant in South Korea. The genomic DNA G+C content of the type strain is 67.6 mol%.

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