Deinococcus humi sp. nov., isolated from soil

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A Gram-staining-positive, strictly aerobic, spherical, non-motile, red-pigmented bacterium, designated strain MK03T, was isolated from a soil sample collected in South Korea. The taxonomic position of the novel strain was investigated using a polyphasic approach. In phylogenetic analyses based on 16S rRNA gene sequences, strain MK03T was placed in a clade formed by members of the genus *Deinococcus* in the family *Deinococcaceae* and appeared to be most closely related to *Deinococcus aerolatus* 5516T-9T (97.4 % sequence similarity), *Deinococcus marmoris* AA-63T (97.2 %), *Deinococcus radiopugnans* ATCC 19172T (97.2 %) and *Deinococcus saxicola* AA-1444T (96.9 %). The genomic DNA G+C content of the novel strain was 64.5 mol%. The chemotaxonomic characteristics of strain MK03T were typical of members of the genus *Deinococcus*: MK-8 was identified as the predominant respiratory quinine, the major fatty acids were C16:1ω7c, C15:1ω6c, C16:0 and C15:0, ornithine was found to be the diamino acid in the cell-wall peptidoglycan and the novel strain showed resistance to gamma radiation, with a D10 value (i.e. the dose required to reduce the bacterial population by 10-fold) in excess of 9 kGy. In hybridization experiments, only low DNA–DNA relatedness values (11.6–34.5 %) were recorded between the novel strain and its closest relatives in the genus *Deinococcus*. Based on the phylogenetic, chemotaxonomic, phenotypic and DNA–DNA relatedness data, strain MK03T represents a novel species of the genus *Deinococcus*, for which the name *Deinococcus humi* sp. nov. is proposed. The type strain is MK03T (=KCTC 13619T =JCM 17915T).

At the time of writing, the genus *Deinococcus*, which was first established by Brooks & Murray (1981), contained 49 species. The current members of the genus *Deinococcus* are aerobic, generally Gram-staining-positive (some strains are Gram-staining-negative) and have L-ornithine in their cell-wall peptidoglycan but no detectable teichoic acids. As some members of the genus *Deinococcus* also show remarkable resistance to radiation, they may prove useful as sources of radiation-resistant enzymes and other biomolecules (Kwon & Seo, 2010; Seong et al., 2010) and for cleaning up waste sites that contain radioactive contaminants as well as other hazardous materials (Servinsky & Julin, 2007; Cox & Battista, 2005).

In the course of isolating micro-organisms from soil samples collected on the campus of Seoul Women’s University, a Gram-staining-positive, non-motile, aerobic, non-spore-forming bacterium, designated strain MK03T, was isolated. The results of preliminary 16S rRNA gene sequence analysis indicated that strain MK03T should be placed in the genus *Deinococcus*. The exact taxonomic position of the novel strain was subsequently determined using a polyphasic approach.

Strain MK03T was originally isolated from soil collected from a mountain within the campus of Seoul Women’s University in Seoul, South Korea. A subsample (1 g) was immersed in 50 ml saline [0.85 % (w/v) NaCl] and vortexed. The resultant suspension was serially diluted and 100 μl of each dilution was spread on a plate of 10-fold-diluted 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. One isolate, designated MK03T, was selected for further study and routinely cultured on nutrient agar (NA; Difco) at 30 °C. This novel strain was also preserved as a suspension in Luria–Bertani (LB) broth (Difco) with 20 % (w/v) glycerol at −70 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MK03T is GQ339889. A supplementary figure is available with the online version of this paper.
The genomic DNA of strain MK03<sup>T</sup> was extracted using a commercial genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified by PCR, and the PCR product was purified and sequenced, by the methods of Kim et al. (2005). The almost-complete sequence of the 16S rRNA gene (1469 nt) was compiled with SeqMan (DNASTAR) and then compared with corresponding sequences of other taxa by use of the EzTaxon server (Kim et al., 2012). The 16S rRNA gene sequences of the taxa that were found to be related were obtained from Deinococcus frigens AA-692<sup>T</sup> (AJ585981), Deinococcus saxicola AA-1444<sup>T</sup> (AJ585984), Deinococcus marmoros AA-63<sup>T</sup> (AJ585986), Deinococcus radiopugnans ATCC 19172<sup>T</sup> (Y11334), Deinococcus aerolatus 5516T-9<sup>T</sup> (EU622978), Deinococcus humi MK03<sup>T</sup> (GQ339889), Deinococcus aerophilus 5516T-11<sup>T</sup> (EU622979), Deinococcus yunweiensis YIM 007<sup>T</sup> (DQ344634), Deinococcus murrayi ALT-1b<sup>T</sup> (Y13041), Deinococcus geothermalis DSM 11300<sup>T</sup> (CP000359), Deinococcus aerius TR0125<sup>T</sup> (AB087287), Deinococcus radiomollis PO-04-20-132<sup>T</sup> (EF635404), Deinococcus alpinitundrae ME-04-04-52<sup>T</sup> (EF635408), Deinococcus roseus TMDA-u511<sup>T</sup> (AB264136), Deinococcus misasensis TMDA-25<sup>T</sup> (AB264135), Deinococcus peraridilitoris KR-200<sup>T</sup> (EF141348), Deinococcus papagonensis KR-241<sup>T</sup> (AY743280), and Deinococcus yavapaiensis KR-236<sup>T</sup> (AY743279), respectively. Truepera radiovictrix RQ-24<sup>T</sup> (DQ022076) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain MK03<sup>T</sup> and members of the genus Deinococcus and related genera. Percentage bootstrap values >50% (based on 1000 replications) are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the maximum-parsimony tree. Thermus aquaticus DSM 625<sup>T</sup> (L09663) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
GenBank and edited using the BioEdit program (Hall, 1999). Multiple alignments were then made with CLUSTAL_X (Thompson et al., 1997) and evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed in MEGA3 (Kumar et al., 2004) using the neighbour-joining algorithm (Saitou & Nei, 1987; Fig. 1) and the maximum-parsimony algorithm (Fitch, 1971). The topologies of the trees were evaluated by bootstrap analysis with 1000 replications (Felsenstein, 1985). This phylogenetic analysis based on 16S rRNA gene sequences indicated that the closest relatives of strain MK03T were Deinococcus aerolatus 5516T-9^{T} (97.4 % sequence similarity), Deinococcus marmoris AA-63^{T} (97.2 %), Deinococcus radiopugnans ATCC 19172^{T} (97.2 %), Deinococcus saxicola AA-1444^{T} (96.9 %) and Deinococcus frigens AA-692^{T} (96.0 %). Consequently, for use as reference strains in the phenotypic and chemotaxonomic investigations, type strains of D. aerolatus, D. marmoris, D. radiopugnans, D. saxicola and D. frigens were obtained from the Korean Agricultural Culture Collection (KACC) and grown under the same conditions as strain MK03T.

To determine the percentage of cells that survived exposure to fixed doses of gamma radiation, cultures were grown at 30 °C in liquid TGY medium containing 1 % (w/v) tryptone, 0.1 % (w/v) glucose and 0.5 % (w/v) yeast extract. These cultures were grown to the early stationary phase (when the medium contained about 10^{9} cells ml^{-1}), divided into 1 ml aliquots, and then exposed, on ice, to an IR-79 cobalt-60 point source (AECL). The source strength was approximately 100 kCi at a dose rate of 70 Gy min^{-1}, and the actual doses were within 2 % of the target doses. Irradiated cells were diluted, plated in triplicate on plates of TGY agar and incubated at 30 °C for 2 days before the surviving cells were scored. For these irradiation experiments, Deinococcus radiodurans R-1 and E. coli K2 cells were used as positive and negative controls, respectively.

After exposure to 3 and 9 kGy gamma radiation, the novel strain showed 75 % and 14 % survival, respectively, whereas the positive control (D. radiodurans R-1) showed 85 % and 50 % survival, respectively (Fig. 2). The novel strain’s D_{10} value (i.e. the dose required to reduce the bacterial population by 10-fold) is therefore in excess of 9 kGy. Radiation resistance is one of the main characteristics of the genus Deinococcus and most radiation-resistant members of this genus were initially isolated using UV or gamma radiation (Rainey et al., 2005; Oyaizu et al., 1987). Although strain MK03^{T} was isolated without using radiation for selection, it was still found to be radiation-resistant (see also Yang et al., 2009, 2010; Yoo et al., 2010; Srinivasan et al., 2012).

Cell morphology and motility were observed under a light microscope (Olympus), using cells grown for 24 h in nutrient broth (Difco) at 30 °C. For electron microscopy, cells were gently resuspended in a drop of deionized water and then the suspensions were placed on carbon- and Formvar-coated nickel grids for 30 s. Each grid was then floated on a drop of 0.1 % (w/v) aqueous uranyl acetate, blotted dry and viewed in a transmission electron microscope (LEO912AB; Carl Zeiss) at 100 kV, under standard operating conditions. The Gram reaction was performed by using the non-staining method described by Buck (1982). Catalase activity was assessed by bubble production in 3 % (v/v) H_{2}O_{2}, and oxidase activity was assessed using 1 % (w/v) tetramethyl p-phenylenediamine (Cappuccino & Sherman, 2002). Anaerobic growth was examined in serum bottles, using nutrient broth supplemented with sodium thioglycolate (1 g l^{-1}) and a nitrogen atmosphere. Physiological characteristics were determined by using the API 20NE, API ID 32 GN and API ZYM systems (bioMe`rieux) according to the manufacturer’s instructions. Growth at 4, 10, 20, 25, 28, 30, 37, 40, 42 and 45 °C and at pH 5.0–10.0 (at intervals of 0.5 pH unit) was assessed in LB broth for 5 days. The pH of the medium was maintained using 50 mM acetate buffer (pH 5.0–5.5), 50 mM phosphate buffer (pH 6.0–8.0) or 50 mM Tris buffer (pH 8.5–10.0). Salt tolerance was tested by incubation for 5 days at 30 °C in R2A broth (MBcell) that had been supplemented with 0–10 % (w/v) NaCl. Growth on tryptase soy agar (TSA; Difco), R2A agar and LB agar was also evaluated at 30 °C. The phenotypic characteristics of strain MK03^{T} are given in the species description. Those that can be used to differentiate the novel strain from its close relatives in the genus Deinococcus are also listed in Table 1. Strain MK03^{T} can be distinguished from closely related members of the genus Deinococcus by its arginine dihydrolase, x-fucosidase and lipase (C14) activities and its assimilation of l-arabinose.

Isoprenoid quinones were extracted from cells of strain MK03^{T} with chloroform/methanol (2:1, v/v), evaporated under vacuum and re-extracted in n-hexane/water (1:1, v/v). The resultant n-hexane/quinone solution was purified using silica cartridges (Sep-Pak Vac; Waters) and then
analysed by HPLC, as described previously (Hiraishi et al., 1996). The major respiratory quinone of strain MK03<sup>T</sup> was identified as MK-8. The cell-wall peptidoglycan was analysed as described by Schleifer & Kandler (1972) except that cellulose sheets were used for the TLC instead of paper. The results of this analysis were similar to those recorded for members of the genus *Deinococcus*, with ornithine and glycine identified as the major amino acids.

### Table 1. Characteristics that distinguish strain MK03<sup>T</sup> from the type strains of closely related members of the genus *Deinococcus*

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Table 2. Fatty acid contents (%) of strain MK03T and type strains of closely related members of the genus Deinococcus

| Strains: 1, MK03T; 2, D. aerolatus KACC 12745T; 3, D. marmoris KACC 12218T; 4, D. radiopugnans KACC 11999T; 5, D. saxicola KACC 12240T; 6, D. frigens KACC 12220T; 7, D. radiodurans KACC 12248T. All data are from this study and were produced using cells that had been incubated on TSA for 2 days at 30 °C. Fatty acids amounting to <1% of the total in all of the strains are not shown. TR, Trace (<1%); --, not detected.

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in the peptidoglycan and rhamnose and ribose identified as the major cell-wall sugars. For fatty acid methyl ester (FAME) analysis, strain MK03T was grown on TSA at 30 °C and harvested at early stationary growth phase. Two loopfuls of bacterial mass were collected and subjected to saponification, methylation and extraction using the methods of Kuykendall et al. (1988). The FAME mixtures were separated using the standard protocol of version 6.08 of the Sherlock Microbial Identification System (MIDI), analysed by GC (6890; Hewlett Packard) and identified using the TSBA6 database (Sasser, 1990). The major fatty acids of strain MK03T were identified as C16:0 (23.6%), C16:1ω7c (24.5%), C15:1ω6c (14.6%) and C15:0 (13.5%). The fatty acid profile of strain MK03T was similar to those of the novel strain’s closest relatives in the genus Deinococcus but showed some qualitative and quantitative differences (Table 2). Strain MK03T, for example, contained much less iso-C14:0 than any of the closely related type strains with which it was compared (Table 2).

Cellular polar lipids were extracted and examined by two-dimensional TLC (Minnikin et al., 1977). They were revealed by staining the TLC plates with 5% molybdophosphoric acid in ethanol (total lipids), 0.2% ninhydrin reagent (aminolipids), α-naphthol reagent (glycolipids) and Zinzadze reagent (phosphorus-containing lipids). Strain MK03T was found to contain three unidentified phosphoglycolipids (PGL1–PGL3), five unidentified glycolipids and another unidentified polar lipid (Fig. S1, available in IJSEM Online). The predominant polar lipid in strain MK03T, as in several current members of the genus Deinococcus (Ferreira et al., 1997; Weon et al., 2007; Im et al., 2008), was PGL2.

For the determination of its G+C content, genomic DNA was degraded into nucleosides with nuclease P1 (Sigma) and alkaline phosphatase (Sigma). The nucleosides were then analysed using HPLC, as described previously (Tamaoka & Komagata, 1984; Mesbah et al., 1989). The genomic DNA G+C content of strain MK03T was 64.5 mol%.

DNA–DNA hybridization experiments were performed fluorometrically, according to the method developed by Ezaki et al. (1989), using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was carried out reciprocally, with five replications. After the highest and lowest values obtained for each sample were excluded, the mean of the remaining three values was calculated and recorded as the DNA–DNA relatedness value. Strain MK03T showed relatively low levels of DNA–DNA relatedness with D. aerolatus KACC 12745T (34.5±1.5%), D. marmoris KACC 12218T (25.4±1.8%), D. radiopugnans KACC 11999T (24.3±2.1%), D. saxicola KACC 12240T (18.4±3.4%) and D. frigens KACC 12220T (11.6±2.4%), indicating that it did not belong to any of these closely related species in the genus Deinococcus (Wayne et al., 1987).

Based on the phenotypic, chemotaxonomic, phylogenetic and DNA–DNA relatedness data, strain MK03T represents a novel species within the genus Deinococcus, for which the name Deinococcus humi sp. nov. is proposed.

Description of Deinococcus humi sp. nov.

Deinococcus humi (hu’mi. L. gen. n. humi of/from soil, the source of the type strain).

Cells are Gram-reaction-positive, strictly aerobic, non-motile, non-spore-forming cocci. After 3 days of incubation at 30 °C on R2A agar, colonies are 0.7–1.2 mm in diameter, red, smooth, convex and circular, with regular edges. Grows well at 20–37 °C (optimum 30 °C) and weakly at 40 °C. Grows at pH 5.5–9.0 (optimum between pH 7.0 and pH 7.5) and with 0–3% (w/v) NaCl but not with 4%. Growth occurs on TSA, NA and LB and R2A agars. Catalase-positive and oxidase-negative. Nitrate is reduced to nitrite but nitrogen gas is not produced. Cells are highly resistant to gamma radiation (with a D10 of >9 kGy). Positive, on API ZYM and API 20NE strips, for arginine dihydrolase, esterase (C4), α-fucosidase, gelatinase, α-glucosidase, β-glucosidase, leucine arylamidase,
lipase, and valine arylamidase activities and weakly positive for acid phosphatase, esterase lipase (C8), β-galactosidase (ONPG) and naphthol-AS-Bl-phosphohydrolase activities but negative for N-acetyl-β-glucosamidase, x-chymotrypsin, cystine arylamidase, α-galactosidase, β-galactosidase (PNPG), β-glucuronidase, x-mannosidase, trypsin and urease activities. Positive, on API ID32GN strip, for the assimilation of N-acetyl-D-glucosamine, L-arabinose, L-alanine, caprate, citrate, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 5-ketogluconate, DL-lactate and melibiose and weakly positive for the assimilation of D-mannitol, myo-inositol and sucrose but negative for the assimilation of adipate, L-fucose, gluconate, D-glucose, glycogen, L-histidine, 3-hydroxybutyric acid, itaconate, 2-ketogluconate, L-malate, malonate, malonate, L-mannose, L-proline, phenylacetate, propionate, L-rhamnose, D-ribose, salicin, L-serine, sodium acetate, D-sorbitol, suberate and n-valerate. The predominant respiratory quinone is MK-8. The major fatty acids are C16:1(ω7c), C15:1(ω6c), C16:0, and C15:0. The polar lipid profile is complex, containing various unidentified phosphoglycolipids, glycolipids and phospholipids. Ornithine and glycine are present in the cell-wall peptidoglycan. The cell wall also contains rhamnose and ribose.

The type strain, MK03T (=KCTC 13619T =JCM 17915T), was isolated from a soil sample collected from the campus of Seoul Women’s University. The genomic DNA G+C content of the type strain is 64.5 mol%.

Acknowledgements

This work was supported by a special research grant from Seoul Women’s University (2012).

References


