Deinococcus aerius sp. nov., isolated from the high atmosphere

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An orange-pigmented, non-motile, coccoid bacterial strain, designated TR0125^T, was isolated from dust samples collected in the high atmosphere above Japan. Phylogenetic analysis based on 16S rRNA gene sequences showed that the strain was within the radiation of *Deinococcus* species. The major peptidoglycan amino acids were D-glutamic acid, glycine, D-alanine, L-alanine and ornithine. The predominant fatty acids were iso-C_{17:0}, iso-C_{17:1ω9c} and iso-C_{15:0}. Strong resistance to desiccation, UV-C and gamma radiation and high DNA G+C content also supported the affiliation of strain TR0125^T to the genus *Deinococcus*. Strain TR0125^T showed the highest 16S rRNA gene sequence similarity value (95.7%) to the type strain of *Deinococcus apachensis*, and phylogenetic analysis showed that it was further separated from *D. apachensis* than from *Deinococcus geothermalis*, indicating that strain TR0125^T was not a member of these two *Deinococcus* species. In addition, phenotypic differences were found between strain TR0125^T and the type strains of these two *Deinococcus* species. Therefore, a novel species of the genus *Deinococcus*, *Deinococcus aerius* sp. nov. (type strain, TR0125^T=JCM 11750^T=DSM 21212^T), is proposed to accommodate this isolate.

The genus *Deinococcus* was created in 1981 to accommodate radiation-resistant cocci exemplified by '*Micrococcus radiodurans*' (Brooks & Murray, 1981). Although one of the species, *Deinococcus erythromyxa*, has been transferred to the genus *Kocuria* (Rainey *et al.*, 1997), the number of species in the genus *Deinococcus* has increased to 30 at the time of writing (Callegan *et al.*, 2008). Airborne bacteria of the genus *Deinococcus* were first isolated as a contaminant from a hospital (*Deinococcus radiodurans* strain SARK) (Murray & Robinow, 1958). Another *Deinococcus*-like micro-organism was later isolated from indoor air dust (Christensen & Kristensen, 1981). Recently, *Deinococcus cellulosilyticus* was isolated from an air sample from an island (Weon *et al.*, 2007). Here we report the characterization of a novel *Deinococcus* strain, designated TR0125^T, isolated from the high atmosphere.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TR0125^T is AB087288.

Tables showing the differential characteristics and fatty acid compositions of strain TR0125^T and the type strains of closely related *Deinococcus* species are available as supplementary material with the online version of this paper.

Air dust at various high altitudes above the main island of Japan was collected on membrane filters (type HA: diameter 47 mm, pore size 0.45 µm; Millipore) using an ADS1 air sampler (Japan Machinery Co.) loaded on an aircraft (Gulfstream-2; Diamond Air Service). After sampling, the membrane filters were placed on mTGE agar composed of Difco mTGE broth (1^l: beef extract, 6.0 g; tryptone, 10.0 g; glucose, 2.0 g) plus Difco Bacto agar (15 g l⁻¹), and incubated at 30 °C for 2 weeks. Strain TR0125^T was isolated from a sample collected at an altitude of 0.8–5.8 km.

Unless stated otherwise, bacterial cells used for biochemical and molecular studies were obtained by cultivation in mTGE broth. Growth temperatures were determined by spreading exponentially grown cells on mTGE agar, followed by incubation at 4, 10, 15 and 47 °C and at specific temperatures from 20 to 45 °C for 2 weeks, and checking the development of colonies on the plates. Gram staining, and cell morphology and motility were examined under a microscope (Olympus BX-FLA). For scanning electron microscopy, bacterial cells were placed on a plastic sheet coated with poly-L-lysine and fixed with 2%

glutaraldehyde. The preparations were then dehydrated in ethanol, critical-point dried, coated with metallic gold and examined in a low vacuum JSM-5800LV microscope (JEOL) operated at 15 kV.

Gamma-radiation resistance of strain TR0125^T was determined as described by Satoh *et al.* (2006). *D. radiodurans* ATCC 13939^T was used as a control strain. Strains TR0125^T and ATCC 13939^T were grown in mTGE broth at 30 °C to early stationary phase. Cells were harvested by centrifugation at 5000 g, 4 °C for 5 min, washed twice with 10 mM sodium phosphate buffer (pH 7.0) and resuspended in the same buffer. Aliquots (100 µl) of the cell suspension were dispensed into test tubes and irradiated at room temperature for 5.5 h with ⁶⁰Co gamma-rays at dose rates of 0.37–1.45 kGy h⁻¹ that were regulated by changing the distance of the samples from the gamma-ray source. Serial dilutions of the irradiated suspension and a non-irradiated control were plated on mTGE agar in triplicate. UV survival experiments were carried out according to the methods described by Miller (1992) and Arrage *et al.* (1993). *D. radiodurans* JCM 6273 and ATCC 13939^T were used as control strains. Percentage survival was calculated as colony counts of irradiated cells divided by those of unirradiated control cells, multiplied by 100. The D₁₀ value (radiation dose that reduced the number of cells to 10 % of the original number) was calculated from the survival curve. Desiccation resistance of strain TR0125^T and the control strains ATCC 13939^T and *Escherichia coli* MG1665 wild-type was determined according to the method of Callegan *et al.* (2008).

Peptidoglycan was prepared and the amino acids were analysed by using the methods described by Komagata & Suzuki (1987). The fatty acid composition was analysed by NCIMB, Japan. Bacterial cultures were grown at 28 °C for 3 days. Methyl ester mixtures were prepared by using saponification, methylation and extraction (Kuykendall *et al.*, 1988), and separated by using a gas chromatograph according to the operation manual (version 6) of the Sherlock Microbial Identification System (version 3.00B; MIDI). Identification and quantification of the methyl esters were conducted by using TSBA (version 4.0) of MIS Standard Libraries (MIDI). Menaquinones were extracted and determined according to Tindall (1989) and Shimada *et al.* (2001). Menaquinone-8 from strain ATCC 13939^T and a commercial menaquinone-7 standard (Wako) were used as controls. Catalase, oxidase, β-galactosidase and hydrolysis and nitrate reduction tests were conducted according to the methods described by Barrow & Feltham (1993). Single-carbon-source assimilation studies were performed by using the methods described in the paper characterizing *Deinococcus apachensis* (Rainey *et al.*, 2005). Growth was examined visually on agar plates incubated at 30 °C for 2 weeks. Plates without a carbon source were used as negative controls. mTGE plates were used as positive controls.

The DNA G+C content was determined by using the method of Mesbah *et al.* (1989) with minor modifications.

DNA was isolated using a Blood & Cell Culture DNA kit (Qiagen). *E. coli* W3110 wild-type and *D. radiodurans* ATCC 13939^T were used as controls. For complete hydrolysis of DNA, 25 µl of a solution containing 2–5 µg DNA in a 1.5 ml microfuge tube was heated in boiling water for 5 min and then rapidly cooled on ice, then 25 µl of 2 mM ZnSO₄, 40 mM sodium acetate buffer (pH 5.3), 20 µl sterilized ultrapure water and 5 µl P1 nuclease (20 U ml⁻¹) were added. After incubation for 2 h at 37 °C, 2.5 µl 0.1 M glycine buffer (pH 10.4) and 6 µl bovine intestinal mucosa alkaline phosphatase (0.35 U ml⁻¹) were added. The sample was then incubated overnight at 37 °C and stored at 4 °C until analysis. A 10 µl sample was applied to an HPLC (Shimadzu LC-10Avp) equipped with a Capcell Pak C18 UG120 S-5 µm column (Shiseido) and a UV detector at 270 nm, and analysed at a flow rate of 1.0 ml min⁻¹ at 40 °C. The solvent used was 20 mM KH₂PO₄ : HPLC-grade acetonitrile (70 : 1, by vol.).

Genomic DNA was extracted and purified using a FastDNA kit (BIO101). A 16S rRNA gene fragment (corresponding to *E. coli* nucleotides 10–1500) was amplified by PCR with primers EU 10F (5'-AGAGTTTGATCCTGGCTCAG-3') and EU 1500R (5'-GGTTACCTTGTTACGACTT-3') (Takami *et al.*, 1999). The PCR products were cloned into pCR2.1-TOPO (Invitrogen). The sequences were determined using an ABI Big Dye Terminator Cycle Sequencing FS Ready Reaction kit on an ABI PRISM 377 DNA Sequencing system (Applied Biosystems). The 16S rRNA gene sequences of closely related species were searched using the BLAST algorithm (BLASTN). The partial 16S rRNA gene sequences were aligned for phylogenetic analysis using CLUSTAL_X 1.83 software with the sequences of type strains of recognized *Deinococcus* species. The phylogenetic tree was constructed by using the maximum-likelihood method with TREEFINDER software (Jobb *et al.*, 2004) under the GTR+I+G model selected by using the program MODELTEST. Bootstrap analysis (1000 pseudore samplings) was performed to obtain the bootstrap probability of each node. 16S rRNA gene sequence similarity values between strain TR0125^T and the type strains of two closely related species (*D. geothermalis* and *D. apachensis*) were calculated using BLAST 2 sequences (Tatusova & Madden, 1999) on the NCBI website.

Cells of strain TR0125^T were non-spore-forming, non-motile, spherical and 1.0–1.5 µm in diameter, and occurred as single cells, or in pairs, tetrads or clusters (Fig. 1). Gram-staining yielded a positive reaction. Colonies on mTGE agar were circular, convex, shiny and orange. The edges of the colonies formed were wavy and smooth. Strain TR0125^T grew at 15–45 °C. The optimum growth temperature range was 25–30 °C. No growth occurred at 4, 10 or 47 °C. Strain TR0125^T was catalase-, oxidase-, β-galactosidase- and alkaline phosphatase-positive and hydrolysed aesculin. The strain did not reduce nitrate to nitrite. Tests for urease, arginine dihydrolase, ornithine decarboxylase, β-glucuronidase, arginine arylamidase and pyrrolidone

Fig. 1. Scanning electron micrograph showing the morphology of cells of strain TR0125^T. Bar, 1 μm.

arylamidase were negative. Strain TR0125^T was unable to utilize the following carbon sources: glucose, fructose, mannose, rhamnose, ribose, xylose, lactose, trehalose, melibiose, lactate, malate, aspartate, alanine, asparagine, glutamine, glycine, galactose, cellobiose, sorbose or acetate. Arabinose, glucosamine, ornithine, glutamate, maltose, sucrose, proline, raffinose, cysteine, lysine and methionine were utilized. Strain TR0125^T exhibited higher UV-resistance, evaluated by D_{10} values (strain TR0125^T, 1200 J m⁻²), than *D. radiodurans* strains (JCM 6273, 860 J m⁻²; ATCC 13939^T, 870 J m⁻²). The strain exhibited lower resistance to gamma radiation than *D. radiodurans* ATCC 13939^T (D_{10} values for strains TR0125^T and ATCC 13939^T were 4.9 and 6.7 kGy, respectively). Desiccation resistance of strain TR0125^T was also lower than that of strain ATCC 13939^T, but was much higher than that of *E. coli*. Surviving fractions after desiccation for 6 weeks were 32 %, 24 % and 0 for strains ATCC 13939^T, TR0125^T and

Fig. 2. Phylogenetic tree showing the relationship of strain TR0125^T to closely related species. *Thermus ruber* ATCC 35948^T and *Thermus thermophilus* HB27 were used as an outgroup. Bootstrap probabilities of each node greater than 50 % are shown. Bar, 0.1 substitutions per site.

E. coli, respectively. Compared with *D. radiodurans* ATCC 13939^T, strain TR0125^T formed rigid cell pellets and desiccated cell aggregates that were harder to evenly resuspend during experiments.

The predominant fatty acids detected were branched-chain, iso-C_{17:0} (23.5 %), iso-C_{17:1ω9c} (16.3 %) and iso-C_{15:0} (15.7 %). The major peptidoglycan amino acids were D-glutamic acid, glycine, D-alanine, L-alanine and ornithine (molar ratio of 1:2:1:1:1). The major menaquinone was MK-8. The DNA G+C content was 70.5 mol%. The BLAST search indicated that strain TR0125^T was most closely related to members of the genus *Deinococcus*. Strain TR0125^T showed 95.7 and 92.3 % 16S rRNA gene sequence similarities to the type strains of *D. apachensis* and *D. geothermalis*, respectively.

The 16S rRNA gene-based phylogenetic analysis showed that strain TR0125^T fell in the radiation of the *Deinococcus* cluster (Fig. 2). In addition, its cell morphology, high DNA G+C content, desiccation tolerance, gamma- and UV-resistance were also consistent with properties reported of recognized *Deinococcus* species. Among the hitherto recognized *Deinococcus* species, strain TR0125^T was relatively closer to the type strains of *D. apachensis* and *D. geothermalis*. The pairwise similarity values of the 16S rRNA gene sequences among the three strains were not more than 97 %. In the phylogenetic tree, strain TR0125^T was further separated from *D. apachensis* than from *D. geothermalis*, indicating that strain TR0125^T was not a member of these two *Deinococcus* species. In addition, there were phenotypic differences between strain TR0125^T and the two strains as shown in Supplementary Tables S1 and S2 (available in IJSEM Online). Therefore, strain TR0125^T is considered to represent a novel species of the genus *Deinococcus*, for which the name *Deinococcus aerius* sp. nov. is proposed.

Description of *Deinococcus aerius* sp. nov.

Deinococcus aerius (a.e'ri.us. L. masc. adj. *aerius* belonging to the air, referring to the isolation site of the type strain).

Colonies are circular, convex, orange-pigmented (non-fluorescent) and shiny with smooth edges. Cells are spherical, 1.0–1.5 µm in diameter, aerobic, non-spore-forming and non-motile. Optimum temperature range for growth in mTGE broth is 25–30 °C. Cells typically grow singly, and in pairs, tetrads and clusters and are resistant to desiccation, and gamma and UV radiation. Major fatty acids are iso-C_{17:0}, iso-C_{17:1ω9c} and iso-C_{15:0}. Major peptidoglycan amino acids are D-glutamic acid, glycine, D-alanine, L-alanine and ornithine. Catalase- and oxidase-positive. Positive for alkaline phosphatase and β-galactosidase activities and hydrolysis of aesculin, but negative for reduction of nitrate to nitrite. L-Arabinose, glucosamine, ornithine, glutamate, maltose, sucrose, proline, raffinose, cysteine, lysine and methionine are utilized as carbon sources. The DNA G+C content of the type strain is 70.5 mol%.

The type strain, TR0125^T (=JCM 11750^T=DSM 21212^T), was isolated from an air sample collected from the high atmosphere, Japan.

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