Deinococcus ruber sp. nov., a radiation-resistant bacterium isolated from soil

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Abstract

Two gamma- and UVC-resistant bacterial strains, designated JSH3-1T and 9-2-2, were isolated from garden soil in South Korea. Cells were Gram-stain-positive, aerobic, non-motile and spherical. A polyphasic approach was used to study the taxonomic properties of strains JSH3-1T and 9-2-2. Phylogenetic analysis based on nearly full-length 16S rRNA gene sequences of strains JSH3-1T and 9-2-2 indicated highest similarity with Deinococcus radiomollis PO-04-20-132T (94.7 and 94.9 %, respectively); levels of sequence similarity with the type strains of other Deinococcus species were less than 94.0 %. Strains JSH3-1T and 9-2-2 shared relatively high 16S rRNA gene sequence similarity (98.7 %) and had a high DNA reassociation value of 81±0.5 %. Meanwhile, they showed low levels of DNA reassociation (<25 %) with other closely related species of the genus Deinococcus. The two strains showed chemotaxonomic features typical of the genus Deinococcus, with the presence of menaquinone 8 as the respiratory quinone. The predominant fatty acids were iso-C17:0, iso-C13:0 and anteiso-C15:0. The polar lipids comprised phosphoglycolipid, aminophospholipid, glycolipid and unknown aminolipids. The DNA G+C contents of strains JSH3-1T and 9-2-2 were 62.0 and 61.9 mol%, respectively. On the basis of their phenotypic and genotypic characteristics, and phylogenetic distinction, strains JSH3-1T (=KCTC 33790=JCM 31311T) and 9-2-2 (=KCTC 33789=JCM 31310) should be classified within a novel species of the genus Deinococcus, for which the name Deinococcus ruber sp. nov. is proposed.

The genus Deinococcus was first proposed by Brooks and Murray [1]. At the time of writing, the genus comprises more than 58 species (www.bacterio.net/deinococcus.html). Members of the genus Deinococcus are generally Gram-stain-positive (although some strains are Gram-stain-negative) [2], aerobic, coccus- or rod-shaped, non-spore-forming and mostly show resistance to gamma and UV radiation. They produce enzymes that can protect and repair damaged DNA [3] after irradiation. Ionizing radiation such as gamma radiation can cause cellular damage by producing reactive oxygen species and these can cause disruption to human cells [4]. Radiation-resistant organisms have enzymes for nucleotide excision repair pathways to recover damaged DNA caused by irradiation [5–8]. Radiation-resistant bacteria are able to maintain the damaged segments in a controlled area and can also repair many small fragments from an entire chromosome [9]. Several bacteria of comparable radio-resistance are now known, including some species of the genus Chroococcidiopsis (phylum Cyanobacteria) and species of Rubrobacter (phylum Actinobacteria); among the Archaea, the species Thermococcus giammatolerans shows comparable radio-resistance [10].

Two radiation-resistant bacteria, designated strains JSH3-1T and 9-2-2, were isolated from wet garden soil samples collected at Noeun-myeon, Chungju-si, Chungcheongbuk-do, and Gyorae-ri, Jocheon-eup, Jeju-si, South Korea, respectively (GPS: JSH3-1T, 37° 02’ 11.9” N 127° 41’ 20.9” E; 9-2-2, 37° 24’ 37.8” N 126° 36’ 40.5” E). Radiation-resistant species of the genus Deinococcus have been isolated from soil irradiated with gamma or UVC radiation [11, 12]. The soil samples were irradiated with gamma radiation prior to isolation. One gram of each soil sample was irradiated with 3 kGy using a cobalt-60 gamma irradiator (Advanced Radiation Technology Institute) and thoroughly immersed in 50

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Keywords: Deinococcus; gamma radiation resistance; UVC radiation resistance.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JSH3-1T and 9-2-2 are KU865686 and KU865684, respectively.
Five supplementary figures and two supplementary tables are available with the online Supplementary Material.
ml saline (0.85 %, w/v, NaCl), vortexed and serially diluted. The dilutions were spread onto R2A agar (Difco) and incubated at 25 °C for 7 days. Exposure of soil samples to ionizing radiation eliminates radiation-sensitive strains and enriches ionizing radiation-resistant organisms [11]. Colonies were purified by transferring them to new agar plates. They were then incubated under the conditions described above. The isolates were routinely cultured on R2A agar at 25 °C and preserved as a suspension in distilled water with 20 % (w/v) glycerol at −80 °C.

The 16S rRNA genes of strains JSH3-1T and 9-2-2 were amplified using primers 27F/1492R as described previously [13]. The purified PCR product was sequenced using the universal bacterial primers 785F and 907R, provided by Macrogen. The almost-complete 16S rRNA gene sequence was determined using the SeqMan software (DNASTAR). Using the BioEdit software [14], the 16S rRNA gene sequences of reference taxa obtained from GenBank were analysed. The EzTaxon-e server (http://www.zebiocloud.net/eztaxon; [15]) was used for calculation of pairwise 16S rRNA gene sequence similarities and identification of phylogenetic neighbours. Multiple alignments were conducted using the Muscle software [16]. Evolutionary distances were computed using the Kimura two-parameter model [17]. A phylogenetic dendrogram was reconstructed using the neighbour-joining [18] method in the MEGAS software [19] with bootstrap values based on 1000 replications [20]. On the basis of 16S rRNA gene sequence similarities, the most closely related species to strains JSH3-1T and 9-2-2 was *Deinococcus radiomollis* PO-04-20-132T (94.7 and 94.9 %, respectively) while other *Deinococcus* species shared less than 94.0 % similarity. In the neighbour-joining phylogenetic dendrogram (Fig. 1), strains JSH3-1T and 9-2-2 comprised a monophyletic group and formed a cluster including members of the genus *Deinococcus*, supported by a high bootstrap value of 99 %.

Gram staining was performed according to Doetsch [21]. Cell morphology and motility were examined by light microscopy (Neoscope NB-2000B). Oxidase activity was assayed based on the oxidation of 1 % (w/v) N,N,N′,N′-tetramethyl-p-phenylenediamine and catalase activity was determined using 3 % (v/v) hydrogen peroxide solution. Growth on tripticase soy agar (TSA), Luria–Bertani (LB) agar and nutrient agar (NA) media was also estimated. BIOLOG testing was performed according to the recommendations of the manufacturer (BIOLOG GEN III). Growth at 4, 10, 15, 20, 25, 30 and 37 °C was determined on R2A agar (Difco) after 1 week. Growth at pH 5, 6, 7, 8, 9 and 10 was determined in R2A broth (MBcell) at 25 °C. NaCl tolerance was assessed in R2A broth (MBcell) at 25 °C with added NaCl at concentrations of 0, 1, 2, 3, 4 and 5 % (w/v).

Ethanol (95 %) extracts of strains JSH3-1T and 9-2-2 exhibited absorbance peaks typical of carotenoid-containing organisms, but no flexirubin pigments were found (Fig. S1, available in the online Supplementary Material). Cells were aerotrophic, Gram-stain-positive, non-motile and coccus shaped (Fig. S2). The phenotypic properties of strains JSH3-1T and 9-2-2 are provided in the species description, and those that differentiated them from related *Deinococcus* species are listed in Table 1.

Cells were irradiated at 100 kCi (3.7 PBq) with a dose rate of 70 Gy min⁻¹ [3, 22, 23]. A UVC ultraviolet cross-linker (UVP, CX-2000) at 254 nm was used for irradiation [24, 25]. After irradiation, the cells were plated on R2A agar. *Deinococcus radiodurans* R1T (=DSM 20539T) and *Escherichia coli* K12 (=KCTC 1116) were used as positive and negative controls, respectively. The numbers of colony-forming units (c.f.u.) of strains were determined, and the survival rate was calculated and displayed graphically (Fig. S3). Strains JSH3-1T and 9-2-2 showed both gamma and UVC radiation resistance, which is in agreement with the distinctive features of the genus *Deinococcus* (Fig. S4).

The fatty acid profile of strains JSH3-1T and 9-2-2 was evaluated using cells grown on R2A agar for 2 days at 25 °C. Two loopfuls of third and fourth quadrant cells were harvested from plates when colonies began to appear and subjected to saponification, methylation and extraction using the techniques of Kuykendall et al. [26]. The fatty acids were extracted, and examined by GC (6890; Hewlett Packard) according to the Sherlock system MIDI 6.0. The cellular fatty acid methyl esters were identified using the TSBA 6 database of the Sherlock Aerobic Bacterial Identification System [27]. The fatty acid profiles of strains JSH3-1T and 9-2-2, together with those of closely related *Deinococcus* species, are shown in Table S1. The major fatty acids were iso-C₁₇:₀, iso-C₁₃:₀ and anteiso-C₁₃:₀ which are the predominant fatty acids of members of the genus *Deinococcus* [26]. The fatty acid profiles of strains JSH3-1T and 9-2-2 were similar, but there were several qualitative and quantitative differences with that of the closely related species *Deinococcus radiomollis* LMG 24019T.

Cells of the two novel strains were grown on R2A agar, collected by centrifugation and lyophilization, and subjected to respiratory menaquinone and polar lipid analysis. Iso- and anteiso menaquinones were extracted from lyophilized cells, purified using a Sep-pak kit (cartridge) and separated by HPLC as described by Collins and Jones [28]. Following HPLC separation, the two strains showed menaquinone 8 (MK-8) as the major respiratory menaquinone, which is a common characteristic of members of the genus *Deinococcus*. Polar lipids were extracted and analysed by two-dimensional TLC [29]. Total lipids were stained with 5 % ethanolic molybdophosphoric acid, nynhidrin and α-naphthol. Spots were identified using specific reagents. The results of two-dimensional TLC revealed that strain JSH3-1T contained major amounts of an unknown phosphoglycolipid and moderate to minor amounts of unknown glycolipids, unknown aminophospholipids and unknown aminolipids (Fig. S5).
Genomic DNA was isolated as described above and enzymatically depolymerized into nucleosides. The nucleosides were examined using HPLC and the DNA G+C contents were determined as described previously [30]. DNA–DNA reassociation experiments were performed fluorometrically, according to the method described by Ezaki et al. [31], using DNA probes labelled with photobiotin and 96 micro-dilution wells. Levels of DNA reassociation were analysed reciprocally using five replications per sample. The highest and lowest values of the five results were excluded; the remaining three values were utilized in the determination of reassociation values. The DNA reassociation values were the means of these three values. Strain JSH3-1$^T$ exhibited a relatively high level of DNA reassociation with strain 9-2-2 (81±0.9 %), and showed similar phenotypic characters; thus, these strains probably belong to the same species (DNA–DNA reassociation values are shown in Table S2).

The reported DNA G+C content range of the genus *Deinococcus* is 61.4–70.2 mol% [32]. The DNA G+C contents of strains JSH3-1$^T$ and 9-2-2 were 62.0 and 61.9 mol%, respectively.

The chemotaxonomic characteristics of strains JSH3-1$^T$ and 9-2-2 showed features typical of the genus *Deinococcus*, with the presence of MK-8 as the major respiratory menaquinone, and iso-C$_{17:0}$ iso-C$_{13:0}$ and anteiso-C$_{13:0}$ as major fatty acids. The major polar lipid was an unknown phosphoglycolipid. Strains JSH3-1$^T$ and 9-2-2 could be distinguished from *D. radiomollis* LMG 24019$^T$ by differences in morphological and phenotypic characteristics. Cells of *D. radiomollis* LMG 24019$^T$ were rod shaped and utilized fructose, mannose, L-rhamnose, L-arabinose, sucrose, xylose and galactose whereas the two new isolates were coccus shaped and unable to utilize these carbon sources. This polyphasic analysis thus suggests that strains JSH3-1$^T$ and 9-2-2 represent a novel species within the genus *Deinococcus*, for which the name *Deinococcus ruber* sp. nov. is proposed.

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strains JSH3-1$^T$ and 9-2-2 and other members of the genus *Deinococcus*. Bootstrap values of >50 % (percentages of 1000 replications) are shown at branch points. Accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position.
**Table 1.** Differential phenotypic characteristics between strains JSH3-1T and 9-2-2 and closely related species

<table>
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<td>Rod</td>
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<tr>
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<td>DNA G+C content (mol%)</td>
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<td>61.9</td>
<td>63.2</td>
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**DESCRIPTION OF DEINOCOCCUS RUBER SP. NOV.**

*Deinococcus ruber* (ru’ber, L. masc. adj. ruber red, referring to the colour of colonies).

Cells are Gram-stain-positive, non-motile, coccus-shaped and 2.0–2.5 µm in diameter. Colonies on R2A agar are circular, smooth and pale reddish. Growth occurs at 4–37 °C; the optimum temperature for growth is 25 °C. Oxidase- and catalase-positive. The pH range for growth is pH 7.0–8.0, with an optimum of pH 7.0. Utilizes D-fructose 6-phosphate and glucuronamide in Biolog GEN III microplates. The following substrates are not utilized: acetic acid, acetoacetic acid, N-acetyl-D-galactosamine, N-acetyl-D-mannosamine, N-acetyl-neuraminic acid, N-acetyl-D-glucosamine, L-alanine, γ-amino butyric acid, D-arabitol, D-arginine, D-aspartic acid, L-aspartic acid, bromosuccinic acid, cellobiose, citric acid, dextrin, formic acid, D-fructose, D-fucose, L-fucose, L-galactonic acid lactone, D-galactose, D-galacturonic acid, gelatin, gentiobiose, D-gluconic acid, α-D-glucose, D-glucose 6-phosphate, D-glucuronic acid, L-glutamic acid, glycerol, L-histidine, α-hydroxybutyrilic acid, β-hydroxy-DL-butyric acid, p-hydroxy-phenylacetic acid, inosine, α-ketobutyric acid, α-ketoglutarlic acid, L-lactic acid, D-lactic acid methyl ester, α-D-lactose, D-malic acid, L-malic acid, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, 3-methyl glucose, methyl pyruvate, mucc acid, myo-inositol, pectin, propionic acid, glycol L-proline, L-pyroglutamic acid, quinic acid, raffinose, L-rhamnose, D-saccharic acid, D-salicin, D-serine, L-serine, D-sorbitol, stachyose, sucrose, trehalose, D-turanose and Tween 40. In sensitivity tests, the tetrazolium redox dye was not reduced with minocycin or tetrazolium violet; the dye was reduced in the presence of 1 % sodium lactate, 1, 4 and 8 % NaCl, aztreonam, fusidic acid, guanidine HCl, lincomycin, lithium chloride, nalidixic acid, Niaproof 4, potassium tellurite, rifamycin SV, D-serine, sodium bromate, sodium butyrate, tetrazolium blue, treloandymycin and vancomycin. MK-8 was the major respiratory quinone. The fatty acid profile included major amounts of iso-C17:0, iso-C15:0 and anteiso-C15:0. The polar lipid profile comprised major amounts of unknown an phosphoglycolipid. The G +C content of the genomic DNA was 61.9–62.0 mol%.

The type strain, JSH3-1T (=KCTC 33790T=JCM 31311T), and strain 9-2-2 (=KCTC 33789=JCM 31310) were isolated from wet soil samples collected in Noeun-myeon, Chungju-si, Chungcheongbuk-do, and Gyorae-ri, Jocheon-eup, Jeju-si, South Korea, respectively.

**Funding information**

This work was supported by a research grant from Seoul Women’s University (2016) and a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 201501113).

**Conflicts of interest**

The authors declare that there are no conflicts of interest

**References**


