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

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Two Gram-stain-negative, oxidase-negative, catalase-positive, aerobic and coccus-shaped bacterial strains, KSY3-6T and JSH6-18, were isolated from soil in South Korea. Strains KSY3-6T and JSH6-18 showed high resistance to gamma-ray and UVC irradiation. The 16S rRNA gene sequences of strains KSY3-6T and JSH6-18 showed a novel subline within the genus Deinococcus in the family Deinococcaceae. They shared 94.8–86.4 % nucleotide similarities with other species of the genus Deinococcus. Strain KSY3-6T exhibited high DNA–DNA hybridization values with JSH6-18 (77±0.8 %). The two strains showed typical chemotaxonomic characteristics of the genus Deinococcus, including the presence of menaquinone 8 (MK-8) as predominant respiratory quinone and C16 : 0, C17 : 0 cyclo and summed feature 3 (C16 : 1ω7c/C16 : 1ω6c) as major fatty acids. The G+C content of the DNA of strains KSY3-6T and JSH6-18 was 62.0 and 62.4 mol%, respectively. Polar lipids in strains KSY3-6T and JSH6-18 were mainly phosphoglycolipids. Based on their phenotypic and genotypic properties, strains KSY3-6T and JSH6-18 should be classified as representatives of a novel species in the genus Deinococcus, for which the name Deinococcus persicinus sp. nov. is proposed. The type strain is KSY3-6T (=KCTC 33787T=JCM 31313T). The reference strain is JSH6-18 (=KCTC 33788=JCM 31312).

The genus Deinococcus was originally established by Brooks & Murray (1981). Most members of the genus Deinococcus are resistant to gamma radiation and UV. Ionizing radiation such as gamma rays can cause cellular damage by producing reactive oxygen species (ROS). ROS cause diseases in human cells (Lee et al., 2014; Son et al., 2014). Radiation-resistant organisms have enzymes for nucleotide excision repair pathways to recover damaged DNA caused by irradiation (Kisker et al., 2013; Joo et al., 2015; Kim et al., 2015a, b). Members of the genera Deinococcus, Hymenobacter and Spirosoma have been reported to be resistant to UV and gamma radiation (Joo et al., 2015; Lee et al., 2015; Srinivasan et al., 2015).

During a course of study to isolate radiation-resistant bacteria from soil samples, two bacterial strains, KSY3-6T and JSH6-18, were isolated from soil samples collected near a water stream in Seoul (GPS: 37° 36’ 9.73” N 127° 1’ 59.73” E) and a flower garden in Nonsan-si, Korea (GPS: 36° 11’ 34.74” N 127° 5’ 18.65” E), respectively. Most radiation-resistant members of the genus Deinococcus were initially isolated from soil irradiated with UV or gamma rays (Oyaizu et al., 1987; Rainey et al., 2005). In our study, the soil samples were irradiated with gamma rays before isolating bacteria. The application of radiation eliminates most of the sensitive bacteria and supports the isolation of...
radiation-resistant bacteria of relatively low abundance (Ryan et al., 2008). After irradiation with 3 kGy using a cobalt-60 gamma irradiator (Advanced Radiation Technology Institute, Republic of Korea), 1 g dry soil sample was thoroughly immersed in 50 ml saline (0.85 % NaCl) solution, vortexed and serially diluted. These serially diluted soil samples (100 µl) were spread onto R2A agar (Difco) plates and incubated at 25 °C for 1 week. Ten colonies were purified by transferring them onto new agar plates and identified based on partial 16S rRNA gene sequencing using EzTaxon-e (http://eztaxon-e.ezbiocloud.net) (Kim et al., 2012); only two strains, KSY3-6T and JSH6-18, were representatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species. The isolates and reference strain strains KSY3-6 and JSH6-18 were repre-sentatives of a novel species.

Genomic DNA was extracted using cetyltrimethylammono- nium bromide (CTAB)/NaCl solution (Marmur, 1961). The 16S rRNA gene was amplified and sequenced at Macrogen, Seoul, Korea, using a 3730XL automated DNA sequencing system (Applied Biosystems) with 9F, 985F, 907R and 1492R universal bacterial primers. The 16S rRNA gene sequences of related taxa were obtained from EzTaxon-e (http://eztaxon-e.ezbiocloud.net) (Kim et al., 2012) and edited using the BioEdit program (Hall, 1999). Multiple sequence alignments were performed using the MUSCLE program (Edgar, 2004). The Kimura two-parameter model (Kimura, 1983) was used to calculate the evolutionary distances. A neighbour-joining phylogenetic tree (Saitou & Nei, 1987) was reconstructed using the MEGAG program (Tamura et al., 2011) with bootstrap values based on 1000 replications (Felsenstein, 1985). Based on 16S rRNA gene sequence similarities, the closest relative of the two strains was Deinococcus alpinitundrae ME-04-04-52T (94.7 and 94.8 %, respectively). The two strains shared ≤94.6 % sequence similarities with other species of the genus Deino-coccus. In the neighbour-joining phylogenetic tree (Fig. 1), strains KSY3-6T and JSH6-18 formed a monophyletic group and joined the cluster of members of the genus Deinococcus with a high bootstrap value (99 %).

The closely related type strain Deinococcus alpinitundrae LMG 24283T was obtained from the Belgian Co-ordinated Collections of Micro-organisms (LMG, Brussels, Belgian). It was grown under the same conditions.

Gram-staining was performed according to the method described by Doetsch (1981). Cell morphology was examined under a light microscope (NEOscope Binocular NB-2000B; BioActs). Catalase activity was determined by mea-suring bubble production after applying 3 % (v/v) hydrogen peroxide solution to bacteria. Oxidase activity was deter-mined using 1 % (w/v) tetramethyl-p-phenylene diamine, which turns oxidase-positive bacteria purple. R2A medium was used throughout unless otherwise stated. Growth on nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco) plates was assessed at 25 °C. Growth at different tem-peratures (4, 10, 15, 20, 25, 30, 37 and 42 °C) was assessed for 1 week. Growth at various pH levels (pH 5, 6, 7, 8, 9 and 10) was assessed at 25 °C. The pH of medium was main-tained using the following three buffers (final concentration of 50 mM): acetate buffer (for pH 4.0–5.0); phosphate buffer (for pH 6.0–8.0) and tris buffer (for pH 9.0–10.0). The NaCl tolerance was tested in R2A broth (MBcell) at 25 °C supplemented with 0–10 % (w/v) NaCl (1 % intervals). Degradation of DNA [using DNase agar (Difco)], Tween 40, Tween 20, starch, skimmed milk and triple sugar iron [using triple sugar iron agar (Difco)] was also investigated (Atlas, 1993). API 20NE, API ZYM (bioMérieux) and Biolog Gen III microplate systems were used to determine the utilization and fermentation of different carbon sources and enzymic activities, according to manufacturers’ recommendations (Wragg et al., 2014).

Cells of strains KSY3-6T and JSH6-18 were aerobic, Gram-stain-negative, non-spor-forming and coccus-shaped. The phenotypic characteristics of strains KSY3-6T and JSH6-18 and the most closely related species of the genus Deinococcus are summarized in Table 1.

After being exposed to gamma and UV radiation, survival rates of strains KSY3-6T, JSH6-18 and D. alpinitundrae LMG 24283T (only UV test) were calculated with ~10⁷ c.f.u. ml⁻¹ cells in TGY broth (Difco). These cells were irradiated with a cobalt-60-based gamma irradiator. The irradiation strength was approximately 100 kCi (3.7 PBq) at a dose rate of 70 Gy min⁻¹ (Im et al., 2008; Srinivasan et al., 2015; Lee et al., 2015). For ultraviolet radiation, a UVC ultraviolet crosslinker (CX-2000; UVP) at 254 nm was used with different doses (Im et al., 2013; Selvam et al., 2013). In the UV and gamma radi-a-tion experiment, Deinococcus radiodurans R1T (=DSM 20539T) was used as a positive control and Escherichia coli K12 (=KCTC 1116) was used as a negative control (Kämpfer et al., 2008). The c.f.u. were counted, and the survival rate was cal-cu-lated as described by Tamaoka & Komagata (1984). The two strains (KSY3-6T and JSH6-18) showed low levels of resistance to gamma-ray radiation compared with D. radiodurans R1T (see Fig. S1, available in the online Supplementary Material). The D. radiodurans R1T exhibited a D₅₀ value of 9 kGy but the newly isolated strains showed a D₅₀ value of ≤3 kGy. Similarly, the closely related strain D. alpinitundrae LMG 24283T showed a D₅₀ value of 4kGy (Ryan et al., 2008). The newly isolated strains and D. alpinitundrae LMG 24283T showed slightly lower resistance to UVC radiation compared with D. radiodurans R1T (see Fig. S2) (Ryan et al., 2008).

The fatty acid profiles of the two strains were analysed using cells grown for 48 h at 25 °C. Two loopfuls of bacterial mass were collected, and fatty acids were extracted using the methods of Kuykendall et al. (1988). The Microbial Identification software package for the Hewlett Packard 6890 cap-il-lary GLC of the Sherlock system version 6.0 and the Sherlock Aerobic Bacterial Database (TSBA6) (MIDI; Sasser, 1990) were used to identify fatty acids. The major fatty acids of the two strains were C₁₆ : 0, C₁₇ : 0 cyclo and summed feature 3 (C₁₆ : 0 _ω7c/C₁₆ : 0 _ω6c), the dominant fatty acids in the members of the genus Deinococcus. The fatty acid profiles of strains KSY3-6T and JSH6-18 were
similar to those of the most closely related type strain. However, some quantitative and qualitative differences were found (see Table S1).

For isoprenoid quinone and polar lipid analysis, cells of the two strains were grown, collected by centrifugation and freeze-dried. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified with a Sep-pak kit (cartridge; Waters), and subsequently analysed by HPLC as described by Collins & Jones (1981) and Shin et al. (1996).

Polar lipids were extracted and examined by two-dimensional TLC (Minnikin et al., 1984). Total lipids were revealed by staining with 5% ethanolic molybdophosphoric acid. Different spots were identified by using specific reagents. The two strains contained menaquinone 8 (MK-8) as the major respiratory quinone, which is common in the members of the genus *Deinococcus*. KSY3-6<sup>T</sup> and JSH6-18 contained major amounts of an unknown phosphoglycolipid. Moderate to minor amounts of unknown glycolipids, unknown aminophospholipids, unknown aminolipids, and unknown polar lipids were also present (see Fig. S3).

Genomic DNA was extracted and enzymically degraded into nucleosides. The nucleosides were then analysed by HPLC. The DNA G+C content was measured as described by Tamaoka & Komagata (1984). DNA–DNA hybridization experiments were performed fluorometrically according to the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and 96-well micro-dilution plates. Hybridization was performed in the presence of 50% (v/v) formamide with ionic strength of 0.3 M NaCl. The hybridization was carried out reciprocally with five replications per sample. The highest and lowest hybridization rates obtained for each sample were excluded. The remaining three values were used to calculate hybridization values. DNA relatedness values were expressed as means of these values. The DNA G+C content of genomic DNA for the most closely related type strain, *D. alpinitundrae* ME-04-04-52<sup>T</sup>, was 62.6 mol% (Ryan et al., 2008). The DNA G+C contents of strains KSY3-6<sup>T</sup> and JSH6-18 were 62.0 and 62.4 mol%, respectively. Strain KSY3-6<sup>T</sup> exhibited a relatively high level of DNA relatedness with JSH6-18 (77±0.8%), and the two novel strains showed a low level of DNA relatedness to *D. alpinitundrae* LMG 24283<sup>T</sup>. The detailed DNA–DNA hybridization values are shown in Table S2.

The chemotaxonomic characteristics of strains KSY3-6<sup>T</sup> and JSH6-18 showed typical features of the genus *Deinococcus*, including the major fatty acids summed C<sub>16:0</sub>, C<sub>17:0</sub> cyclo

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of KSY3-6<sup>T</sup> and JSH6-18. Bootstrap values of >50% (percentages of 1000 replications) are shown at branching points. The sequences used for this comparative study are included in parentheses. Bar, 0.02 substitutions per nucleotide position. *Thermus aquaticus DSM 625<sup>T</sup>* (L09663) was used as an out-group.](http://js.microbiologyresearch.org)
and summed feature 3 (composed of C₁₆ : 1ω7c/C₁₆ : 1ω6c), the presence of the predominant respiratory quinone MK-8 and phosphoglycolipid as the major polar lipid. Strains KSY3-6⁴ and JSH6-18 can be distinguished from other phylogenetically related members of the genus Deinococcus based on the production of alkaline phosphatase and assimilation of melibiose. Results of the polyphasic analysis showed that strains KSY3-6⁴ and JSH6-18 represent a novel species within the genus Deinococcus, for which the name Deinococcus persicinus sp. nov. is proposed.

#### Table 1. Different phenotypic characteristics of Deinococcus persicinus sp. nov. and the most closely related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 20</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Production of acid from glucose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D₁₀</td>
<td>2.9</td>
<td>1.5</td>
<td>4*</td>
</tr>
<tr>
<td>Gamma (kJy)</td>
<td>300</td>
<td>330</td>
<td>690*</td>
</tr>
</tbody>
</table>

Enzyme activity

- N-Acetyl-β-glucosaminidase: + + +
- Arginine dihydrolase: + − −
- Alkaline phosphatase: + + −
- Esterase (C4): + − +
- α-Fucosidase: + + +
- α-Galactosidase: + − −
- β-Galactosidase (ONPG): + − −
- β-Glucuronidase: + − −
- Lipase (C14): + − −
- Naphthol-AS-BI-phosphohydrolase: + − +
- Protease (gelatin hydrolysis): + − +
- Assimilation of l-arabinose: + − +
- Growth at (R2A): 20° C: + − − *
- pH 5: − − +*  
- pH 10: − − +*
- DNA G+C content: 62.0 62.4 62.6*

*Data from Ryan et al. (2008).

Cells are Gram-stain-negative, non-motile, non-spore-forming, aerobic and coccus-shaped with a diameter of 1.5–2.0 μm. Colonies are circular, smooth and pink in colour. Growth occurs at 30 °C. The optimum temperature for growth is 25 °C. The pH range for growth is pH 6.0–8.0, with optimum growth at pH 7.0. Growth occurs on tryptic soy agar, Luria–Bertani agar and R2A agar. Oxidase-negative and catalase-positive. Can hydrolyse Tween 40, DNA, skimmed milk, and starch, but not triple sugar iron. Cells show positive enzyme activities for esterase (C8), leucine arylamidase and valine arylamidase, but show negative enzyme activities for acid phosphatase, cystine arylamidase, α-chymotrypsin, β-galactosidase (PNPG), α-glucosidase (starch hydrolysis), β-glucosidase (aesculin hydrolysis), β-glucosidase and α-mannosidase. Enzyme activities of N-acetyl-β-glucosamine, alkaline phosphatase, esterase (C4), α-fucosidase, β-galactosidase (ONPG), β-glucuronidase, lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin and protease (gelatin hydrolysis) are variable by strain (API ZYM and API 20NE). Cells show negative reactions for the assimilation of N-acetyl-D-glucosamine, adipate, l-arabinose, caprate, citrate, gluconate, D-glucose, L-malate, maltose, D-mannitol, D-mannose and phenylacetate (API 20NE). In Biolog GEN III microplates, D-glucose 6-phosphate and glucuronamide are utilized as the sole carbon source. In sensitivity tests, tetrалuminium redox dye is not reduced in the presence of 1 % NaCl, 1 % sodium lactate, 4 % NaCl, 8 % NaCl, aztreonam, guanidine hydrochloride, lincomycin, lithium chloride, nalidixic acid, pH 4, pH 5, pH 6, potassium tellurite, rifamycin SV, D-serine, sodium bromate, troleandomycin or vancomycin. The predominant respiratory quinone is menaquinone 8 (MK-8). The fatty acids profile includes major amounts of C₁₆ : 1ω7c/C₁₆ : 1ω6c, C₁₈ : 1ω7c/C₁₈ : 1ω6c, and C₁₈ : 1ω7c/C₁₈ : 1ω6c (starch hydrolysis). The polar lipids profile includes major amounts of phosphoglycolipid. The type strain, KSY3-6⁴ (=KCTC 33787T=JCM 31313T), and strain JSH6-18 (=KCTC 33788=JCM 31312) were isolated from a soil sample collected near a water stream in Seoul (GPS: 37° 36’ 9.73” N 127° 1’ 59.73” E) and a flower garden in Nonsan-si, Korea (GPS: 36° 11’ 34.74” N 127° 5’ 18.65” E), respectively, after exposure to gamma-ray radiation in the laboratory. The DNA G+C content of the type strain is 62.0 mol%.

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