Deinococcus swuensis sp. nov., a Gamma-Radiation-Resistant Bacterium Isolated from Soil[§]

Jae-Jin Lee¹, Hyun Ji Lee², Gi Seon Jang², Ja Myoung Yu², Ji Yoon Cha², Su Jeong Kim², Eun Bit Lee², and Myung Kyum Kim^{1*}

¹Department of Bio & Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 139-774, Republic of Korea ²Honor's Class, Seoul Women's University, Seoul 139-774, Republic of Korea

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Strain DY59^T, a Gram-positive non-motile bacterium, was isolated from soil in South Korea, and was characterized to determine its taxonomic position. Phylogenetic analysis based on the 16S rRNA gene sequence of strain DY59^T revealed that the strain DY59^T belonged to the family *Deinococcaceae* in the class Deinococci. The highest degree of sequence similarities of strain DY59^T were found with *Deinococcus radio*pugnans KACC 11999^T (99.0%), Deinococcus marmoris KACC 12218^T (97.9%), *Deinococcus saxicola* KACC 12240^T (97.0%), Deinococcus aerolatus KACC 12745^T (96.2%), and Deinococcus frigens KACC 12220^T (96.1%). Chemotaxonomic data revealed that the predominant fatty acids were iso-C_{15:0} (19.0%), C_{16:1} w7c (17.7%), C_{15:1} w6c (12.6%), iso-C_{17:0} (10.3%), and iso- $C_{17:1} \omega 9c$ (10.3%). A complex polar lipid profile consisted of a major unknown phosphoglycolipid. The predominant respiratory quinone is MK-8. The cell wall peptidoglycan contained D-alanine, L-glutamic acid, glycine, and L-ornithine (di-amino acid). 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 5 kGy. Based on the phylogenetic, chemotaxonomic, and phenotypic data, strain DY59^T (=KCTC 33033^T =JCM 18581^T) should be classified as a type strain of a novel species, for which the name Deinococcus swuensis sp. nov. is proposed.

Keywords: *Deinococcaceae*, *Deinococci*, *Deinococcus*, taxonomy

Introduction

The genus *Deinococcus* was first proposed by Brooks and Murray (1981) and *Deinococcus radiodurans* is the type species. At the time of writing, the genus *Deinococcus* comprises 50 species with validly published names (http://www. bacterio.cict.fr/d/deinococcus.html). Members of the genus Deinococcus are aerobic or facultatively aerobic, mostly Grampositive, have L-ornithine as di-amino acid in the cell wall peptidoglycan, and lack teichoic acids. Most Deinococcus species are characterized by their extreme resistance to gamma radiation, UV radiation, and desiccation (Hirsch et al., 2004; Rainey et al., 2005; Srinivasan et al., 2012a, 2012b). In the course of isolating gamma-radiation-resistant microorganism from soil, we isolated a strain (designated as DY59^T) from a soil sample collected from the mountain Deogyusan, Jeonbuk province, South Korea. Strain DY59^T showed gamma-radiation-resistance, were Gram-positive, and presented as pink colonies on Luria-Bertani (LB; Difco) agar. On the basis of 16S rRNA gene sequence analysis, strain DY59^T was considered to belong to the genus *Deino*coccus. Strain DY59^T was subjected to a polyphasic taxonomic investigation, and the results indicated that it represents a novel Deinococcus species.

Materials and Methods

Isolation of bacterial strain and culture conditions

Strain DY59^T was originally isolated from a soil sample (pH 6.6) collected from a mountain of Deogyusan (GPS; N 35° 51' 38" E 127° 44' 47"; altitude 1,500 m), Jeonbuk province, South Korea. The soil was exposed to 5 kGy gamma radiation using a cobal-60 gamma irradiator (point source; AECL, IR-79). One gram of the irradiated soil sample was immersed in 10 ml saline [0.85% (w/v) NaCl] and was vortexed. The resultant suspension was serially diluted and 100 µl of each dilution was spread on a Luria-Bertani (LB; Difco) agar and incubated at 30°C. Single colonies on the plate were purified by transferring onto new plate of LB agar, and were incubated for an additional 3 days at 30°C. This purified colony was identified by 16S rRNA gene sequence using the EzTaxon server 2.1 (http://www.eztaxon. org), and was preserved in a glycerol solution (20%, w/v) at -70°C.

Strain DY59^T was deposited in Korean Collection for Type Cultures (KCTC 33033^T), as well as the Japan Collection of Microorganisms (JCM18581^T). The reference type strains for comparisons, *D. radiopugnans* KACC 11999^T, *D. marmoris* KACC 12218^T, *D. saxicola* KACC 12240^T, *D. aerolatus* KACC 12745^T, and *D. frigens* KACC 12220^T, were obtained from the Korean Agricultural Culture Collection (KACC). *D. marmoris* KACC 12218^T was grown in the presence of 1 ml vitamin solution (containing biotin 4 mg/L, folic acid 4 mg/L, pyridoxamine-HCl 20 mg/L, riboflavin

^{*}For correspondence. E-mail: biotech@swu.ac.kr; Tel.: +82-2-970-5667; Fax: +82-2-970-5974

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10 mg/L, thiamine-HCl $2H_2O$ 10 mg/L, nicotinamide 10 mg/L, Ca-pantothenate 10 mg/L, vitamin B_{12} 0.2 mg/L, and p-aminobenzoic acid 10 mg/L) on R2A agar (Difco).

All the strains (except KACC 12218¹) were maintained and cultivated aerobically on R2A (Difco) media at pH 7.0 aerobically, unless otherwise stated.

16S rRNA sequencing and phylogenetic analysis

The 16S rRNA gene of strain DY59^T was amplified using the 9F and 1492R universal bacterial primer set (Weisburg et al., 1991). The purified PCR product was sequenced using the 9F, 518F, 800R and 1492R universal bacterial primer set by Genotech (Korea). The full sequence of the 16S rRNA gene was compiled with SeqMan software (DNASTAR Inc., USA) and was then compared with corresponding sequences of other taxa, using the EzTaxon-e server (Kim et al., 2012). The 16S rRNA sequences of related taxa were obtained from GenBank and were edited with the BioEdit program (Hall, 1999). Multiple alignments were performed with the ClustalX program (Thompson et al., 1997). Pairwise distances for the neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) were calculated according to the Kimura two-parameter model (Kimura, 1983), and a phylogenetic tree was constructed using the MEGA 5 program (Tamura et al., 2011). A bootstrap analysis with 1,000 replicates was also conducted to obtain confidence levels for the branches (Felsenstein, 1985). The min-mini heuristic method with a search factor of one was applied in a maximum-parsimony (MP) analysis using the MEGA 5 Program.

Phenotypic and biochemical characteristics

Gram-staining was performed as described by Gerhardt et al. (1994). Cell morphology and motility were examined by light microscopy (Nikon E600), transmission electron microscopy (Call Zeiss LEO912AB), and SIM test (Brown, 2008), after the cells had grown for 2 days at 30°C on R2A agar. Oxidase activity was evaluated by the oxidation of 1% (w/v) tetramethyl-p-phenylene diamine. Catalase activity was determined by measuring bubble production after applying 3% (v/v) hydrogen peroxide solution. Growth on different media was also assessed on trypticase soy agar (TSA), nutrient agar (NA), and TGY (1% tryptone, 0.1% glucose, 0.5% yeast extract) agar. The API 20NE, API ID32GN, API 50CHB, and API ZYM microtest systems were employed according to the recommendations of the manufacturer (bioMérieux, France) to study carbon source utilization and enzyme activities of the strains. Growth at different temperatures (4, 15, 20, 25, 30, 37, and 42°C) was assessed on R2A agar for 2 days. Growth at various pH levels (4, 5, 6, 7, 8, 9, 10, and 11) was assessed in R2A broth at 30°C. The pH of the medium was maintained using three buffers (final concentration of 50 mM): acetate buffer (for pH 4.0-5.5); phosphate buffer (for pH 6.0-8.0); and Tris buffer (for pH 8.5-11.0). NaCl tolerance was tested at 30°C on R2A broth (MBcell) that had been supplemented with 0-10% (w/v) NaCl.

Growth under anaerobic conditions was tested in GasPak jars (BBL) at 30°C.

Chemotaxonomic and genomic analyses

Cells were allowed to grow on R2A agar for 3 days at 20°C to perform the fatty acid methyl ester analysis, and then two loops of well-grown cells were harvested. Fatty acid methyl esters were prepared, separated, and identified with the Sherlock Microbial Identification System (Sherlock version 6.01; data base TSBA6; MIDI, Inc., USA) (Sasser, 1990). The amino acid composition of the cell-wall peptidoglycan was determined, using TLC after hydrolysis with 6 M HCl at 100°C for 18 h, as described by Komagata and Suzuki (1987). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified via TLC, and subsequently analyzed by HPLC, as described previously (Collins and Jones, 1981; Shin et al., 1996). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and were identified using two-dimensional thin-layer chromatography (TLC) followed by spraying with the appropriate detection reagents (Minnikin et al., 1984; Komagata and Suzuki, 1987). The mobile phase for TLC development was chloroform/methanol/water (65:25:4, v/v/v), and the second mobile phase was chloroform/methanol/acetic acid/ water (80:12:15:4, v/v/v/v). The total lipid profile was detected by spraying with molybdophosphoric acid solution (Sigma-Aldrich, USA) followed by heating at 150°C; aminolipids were detected by spraying with 0.2% (w/v) ninhydrin solution, followed by heating at 105°C for 10 min; glycolipids were detected with 0.5% 1-naphthol in methanol/ water (1:1, v/v) and sulfuric acid/ethanol (1:1, v/v), followed by heating at 120°C for 5-10 min; phospholipids were detected by spraying with Zinzadze reagent; and phosphatidylcholine was detected by spraying with Dragendorff reagent (Sigma-Aldrich).

To determine G+C content, genomic DNA was extracted and purified with the Genomic-tip system 100/G (QIAGEN, Japan). The DNA was enzymatically degraded into nucleosides and was analyzed using reverse-phase high performance liquid chromatography (HPLC), as previously described (Tamaoka and Komagata, 1984; Mesbah *et al.*, 1989). DNA-DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labeled DNA probes and micro-dilution wells. Hybridization was performed with five replications per sample. The highest and lowest values obtained for each sample were excluded, and the remaining three values were utilized to calculate hybridization values. DNA relatedness was expressed as the means of these three values.

Gamma-radiation-resistant analyses

To determine the survival rate after exposure to gamma radiation, cultures were grown at 30°C in liquid nutrient rich medium TGY broth. To measure ionizing radiation resistance, cultures grown to the early stationary phase ($\sim 10^9$ cells/ml) were divided into 1 ml aliquots, without change of broth, and were exposed, on ice, to a cobal-60 gamma irradiator (point source; AECL, IR-79). 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 dose. Irradiated cells were diluted, plated in triplicate on TGY agar plates and incubated for 2 days, after which survivors were scored. *D*.



Fig. 1. Cell morphology of strain DY59^T, as determined by transmission electron microscopy after growth on R2A agar for 2 days at 30°C. Bar, 0.5 μ m.

radiodurans $R1^{T}$ (=DSM 20539^T) (Brooks and Murray, 1981; White *et al.*, 1999) and *E. coli* K12 (=KCTC 1116) cells (Kämpfer *et al.*, 2008) were used as positive and negative control strains for the radiation resistance analysis (Im *et*

al., 2008; Lim *et al.*, 2006, 2012; Srinivasan *et al.*, 2012a, 2012b).

Results and Discussion

Morphological and phenotypic characteristics

Strain DY59^T was pink colored when routinely cultured on R2A agar at 30°C. Cells are Gram-positive, strictly aerobic, non-motile, and coccus-shaped (Fig. 1). They grew at temperatures of 15–37°C. Optimal growth occurred at 30°C. Strain DY59^T grew at pH values of 6–10. Other physiological characteristics of strain DY59^T are summarized in the species description. Differential characteristics between strain DY59^T and the most closely related type strains are shown in Table 1.

Phylogenetic analysis

The 16S rRNA gene sequence of strain DY59^T was a continuous stretch of 1360 nucleotides. Strain DY59^T belongs to the class *Deinococci*, order *Deinococcales*, and family *Deinococcaceae*. The highest degree of sequence similarity of strain DY59^T was found with *Deinococcus* species, *D. radiopugnans* KACC 11999^T (99.0%) (Brooks and Murray, 1981), *D. marmoris* KACC 12218^T (97.9%) (Hirsch *et al.*, 2004), *D. saxicola*

Table 1. Differential characteristics of strain DY59^T and closely related species

Strains: 1, DY59^T; 2, D. radiopugnans KACC 11999^T; 3, D. marmoris KACC 12218^T; 4, D. saxicola KACC 12240^T; 5. D. aerolatus KACC 12745^T; 6. D. frigens KACC 12220^T.

All strains are Gram-positive, non-motile, oxidase-positive, and catalase-positive. They produced acid phosphatase and alkaline phosphatase. They did not produce α -fucosidase, α -galactosidase, Lipase (C14), α -mannosidase, or β -glucuronidase. Maximum NaCl tolerance for strain DY59^T and *D. radiopugnans* KACC 11999^T was determined through this study, and data for other strains were obtained from references (Hirsch *et al.*, 2004; Yoo *et al.*, 2010). Gram staining, motility, cell size, colony color, oxygen requirement, pH range, and G+C content data for other type strains were obtained from references (Brooks and Murray, 1981; Hirsch *et al.*, 2004; Yoo *et al.*, 2010). +, positive; -, negative; w, weak positive.

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Characteristic	1	2	3	4	5	6
Cell size (µm)	1.4-1.4	1.0-2.0	1.3-4.5	2.0-4.0	1.2-1.7	0.9-2.0
Colony color	Pink	Orange to red	Pink to red	Pink to red	Pink	Beige pink to orange
Oxygen requirement	Strictly aerobic	Aerobic to facultatively anaerobic	Aerobic	Aerobic	Aerobic	Aerobic to facultatively anaerobic
Nitrate reduction	+	+	-	-	-	+
Temperature range	15-37	15-37	15-25	15-25	15-37	15-25
pH range	6-10	6-10	7-8	4-9	7-9	4-9
Max. NaCl tolerance (w/v %)	3	8	2	1	8	10
Enzyme activity						
N-Acetyl- β -glucosaminidase	w	+	-	-	-	-
a-Chymotrypsin	+	+	+	+	-	-
Cystine arylamidase	w	-	-	-	+	-
Esterase (C4)	-	-	-	-	w	W
Esterase (C8)	w	-	-	-	w	W
β -Galactosidase (ONPG)	w	w	+	+	+	W
α-Glucosidase	+	W	-	-	w	W
β -Glucosidase	-	-	-	+	-	-
Leucine arylamidase	+	W	+	+	+	+
Naphtol-AS-BI-phosphohydrolase	-	-	-	+	-	+
Trypsin	+	w	w	-	-	-
Valine arylamidase	-	-	-	-	+	W
Growth on TSA	+	+	W	-	-	-
Growth on LB	+	+	w	w	-	-
G+C content	66.5	70.0	62.8	59.4	61.0	65.5



Fig. 2. A phylogenetic tree based on the 16S rRNA gene sequences of strain DY59^T and representatives of related taxa. The neighbor-joining (NJ) method was used. Bar represents 0.02 substitutions per nucleotide position. Numbers at the nodes indicate the bootstrap values (greater than 50%) calculated on the basis of either NJ or NJ/MP tree algorithms, and expressed as a percentage of 1,000 replicates.

KACC 12240^{T} (97.0%) (Hirsch *et al.*, 2004), *D. aerolatus* KACC 12745^{T} (96.2%) (Yoo *et al.*, 2010), and *D. frigens* KACC 12220^{T} (96.1%) (Hirsch *et al.*, 2004). In the phylogenetic tree (Fig. 2), strain DY59^T clearly belonged to the genus *Deinococcus* linage (neighbor-joining and maximum-parsimony trees), as evidenced by the high bootstrap value of 100%.

Chemotaxonomic and genomic analyses

The predominant cellular fatty acids of strain DY59^T were iso-C_{15:0} (19.0%), C_{16:1} ω7c (17.7%), C_{15:1} ω6c (12.6%), iso- $C_{17:0}$ (10.3%), and iso- $C_{17:1}$ $\omega 9c$ (10.3%). Minor fatty acids of strain DY59^T were $C_{16:0}$ (5.8%), summed feature 1 ($C_{13:0}$ $3OH/C_{15:1}$ i H) (4.3%), $C_{17:1} \omega 8c$ (3.4%), $C_{17:0}$ (2.7%), iso- $C_{13:0}$ (2.5%), anteiso B-C_{17:1} (2.5%), C_{15:1} $\omega 8c$ (1.8%), C_{14:0} (1.6%), $C_{17:1} \ \omega 6c$ (1.4%), and iso F-C_{15:1} (1.1%). Strain DY59¹ has larger amounts of iso-C_{15:0} (19.0%), iso-C_{17:0} (10.3%), summed feature 1 (13:0 3OH/15:1 i H; 4.3%), and iso-C_{13:0} (2.5%), whereas other closely related Deinococcus species have smaller amounts of corresponding fatty acids, and 17:1 anteiso B (2.5%) was present in strain DY59^T but was absent in other closely related species. Strain DY59^T has low amounts of $C_{16:1} \omega 7c$ (17.7%). Strain DY59^T could be separated from the other closely related species based on qualitative and quantitative differences in their fatty acids compositions (Table 2). Strain DY59^T contained D-alanine, L-glutamic acid, glycine, and L-ornithine (di-amino acid) in cell wall peptidoglycan. L-Ornithine is the commonly found di-amino acid in the cell walls of Deinococcus species (Brooks and Murray, 1981; Im et al., 2008; Wang et al., 2010). MK-8

is the predominant respiratory quinone of strain DY59^T, like other *Deinococcus* species (Brooks and Murray, 1981; Hirsch *et al.*, 2004; Yoo *et al.*, 2010). TLC results of polar lipid analysis suggested that strain DY59^T contains various unknown phosphoglycolipids (L_1), unknown glycolipids (GL_{1-7}), unknown phosphoglycolipids (PGL_{1-7}), unknown phospholipids (PL_{1-3}), and unknown aminolipids (AL_{1-2}) (Supplementary data Fig. S1). The polar lipid profile of strain DY59^T was dominated by an unknown phosphoglycolipid (PGL_5), which is common in members of the genus *Deinococcus* (Brooks and Murray, 1981; Ferreira *et al.*, 1997; Lai *et al.*, 2006; Im *et al.*, 2008; Kämpfer *et al.*, 2008; Srinivasan *et al.*, 2012a, 2012b),

The G+C content of genomic DNA from strain DY59^T was 66.5 mol%. Strain DY59^T exhibited low DNA-DNA relatedness with the closely related *D. radiopugnans* KACC 11999^T (33.8±2.5%; reciprocal analysis, 28.3±4.9%), *D. marmoris* KACC 12218^T (21.8±5.1%; reciprocal analysis, 19.0± 2.6%), *D. saxicola* KACC 12240^T (26.7±1.5%; reciprocal analysis, 23.2±0.7%), *D. aerolatus* KACC 12745^T (23.5±1.4%), and *D. frigens* KACC 12220^T (18.0±2.3%) (Supplementary data Table S1). DNA-DNA hybridization levels between strain DY59^T and other type strains were determined to be less than 70%, which is the threshold for delineating a genomic species (Wayne *et al.*, 1987; Stackebrandt and Goebel, 1994).

Gamma-radiation-resistant analyses

Radiation resistance is one of the main characteristics of the genus *Deinococcus* (Oyaizu *et al.*, 1987; Rainey *et al.*, 2005). Strain DY59^T has 21% survival resistance at 5 kGy gamma

Table 2. Cellular fatty acid profiles of strain DY59^T and closely related species

Strains: 1, DY59^T; 2, D. radiopugnans KACC 11999^T; 3, D. marmoris KACC 12218^T; 4, D. saxicola KACC 12240^T; 5. D. aerolatus KACC 12745^T; 6. D. frigens KACC 12220^T.

All strains were grown on R2A agar at 20°C for 3 days (except D. marmoris KACC 12218 ^T grown with vitamin solution, as mentioned in material and
method). The position of the double bond was located by counting from the methyl (ω) end of the carbon chain for unsaturated fatty acids. ^T Summed fea-
ture contained fatty acids that could not be separated by GLC with the Microbial Identification System, not detected; tr, trace (less than 1.0%)

Fatty acids	1	2	3	4	5	6
Saturated						
13:0 iso	2.5	tr	2.0	tr	tr	tr
14:0	1.6	2.1	3.7	tr	tr	-
14:0 2OH	-	tr	-	2.3	tr	tr
15:0 iso	19.0	12.0	3.5	1.3	6.8	2.6
16:0	5.8	6.6	33.4	8.5	8.3	6.3
16:0 iso	tr	tr	-	2.7	-	5.0
16:0 N alcohol	-	-	1.2	-	-	-
17:0	2.7	2.1	1.3	1.2	2.4	3.0
17:0 iso	10.3	5.8	3.1	2.2	4.6	2.5
18:0	tr	-	1.0	tr	tr	-
Unsaturated						
15:1 iso F	1.1	1.6	-	-	1.0	tr
15:1 <i>ω6c</i>	12.6	12.1	2.3	4.9	10.8	10.8
15:1 <i>\u03b8c</i>	1.8	5.4	tr	1.2	4.6	2.3
16:1 iso H	tr	-	-	3.6	-	5.2
16:1 <i>w</i> 5 <i>c</i>	tr	-	tr	1.0	tr	tr
16:1 <i>ω</i> 7 <i>c</i>	17.7	24.3	41.9	48.9	31.8	37.3
16:1 <i>ω9c</i>	-	-	-	-	2.1	-
17:1 <i>ω6c</i>	1.4	1.3	-	3.8	1.6	4.2
17:1 <i>\u03b8c</i>	3.4	6.9	tr	4.7	9.0	9.8
17:1 iso <i>ω9c</i>	10.3	16.0	1.2	7.8	14.3	7.2
17:1 anteiso B	2.5	tr	-	-	-	-
17:1 anteiso <i>ω9c</i>	-	-	-	0.5	-	-
18:1 iso H	-	-	-	1.1	-	tr
18:1 <i>w</i> 7 <i>c</i>	-	-	-	3.2	-	tr
Summed Feature 1 (13:0 3OH / 15:1 i H)	4.3	1.1	-	-	-	-

radiation (85% survival for positive control *D. radiodurans* $R1^{T}$) and 1% survival rate at 10 kGy gamma radiation (70% survival rate for *D. radiodurans* $R1^{T}$) (Supplementary data Fig. S2). The negative control strain of *E. coli* has less than 0.001% survival resistance to 2 kGy gamma radiation.

Taxonomic conclusion

The results of chemotaxonomic analysis of strain DY59^T clearly showed typical features of the genus Deinococcus, with the presence of predominant respiratory quinone as MK-8; the cell wall di-amino acid as L-ornithine; resistance to gamma radiation ($D_{10}>5$ kGy), and the proportions of major fatty acids, such as $C_{16:1} \omega 7c$ and $C_{15:1} \omega 6c$. Strain DY59^T can be distinguished from the closely related species D. radiopugnans based on the significantly higher amounts of iso-C_{17:0} and anteiso B-C_{17:1} fatty acids; inability to grow without oxygen, and inability to tolerate NaCl concentrations greater than 3% (w/v). Strain DY59¹ can also be distinguished from other closely related members of the genus Deinococcus due to its containing higher amounts of iso-C_{15:0} and iso-C_{17:0} fatty acids; the presence of anteiso B-C_{17:1}; and the production of α -glucosidase and trypsin. Based on the phylogenetic, chemotaxonomic, and phenotypic data, we conclude that strain DY59^T is a representative novel species, for which the name *Deinococcus swuensis* sp. nov. is proposed.

Description of Deinococcus swuensis sp. nov.

Deinococcus swuensis (swu.en'sis. N.L. masc. adj. swuensis of or belonging to SWU, Seoul Women's University, where taxonomic study was performed for the new organisms).

Strain DY59^T is pink colored, 1.4 μ m wide, and 1.4 μ m long, Gram-positive, strictly aerobic, non-motile, and coccusshaped when grown on R2A agar (Difco) at 30°C for 2 days. Growth occurs on TSA, LB, NA, TGY, and R2A. Growth occurred at temperatures of 15-37°C, with optimum growth occurring at 30°C. Strain DY59^T grew well at pH values of 6–10, and could tolerate up to 3% NaCl (w/v). Nitrate was reduced to nitrite (API 20NE). Oxidase-positive and catalase-positive. Acid production from glucose and indole production is negative (API 20NE). Growth is observed with glycogen, L-histidine, D-maltose, D-mannitol, D-mannose, and L-rhamnose. Growth is not observed with acetate, Nacetyl-D-glucosamine, adipate, L-alanine, L-arabinose, caprate, citrate, L-fucose, gluconate, D-glucose, 3-hydroxybenzoate, 4-hydroxybenzoate, D,L-3-hydroxybutyrate, itaconate, 2-ketogluconate (a), 5-ketogluconate, D,L-lactate, L-malate, malonate, D-melibiose, myo-inositol, phenyl acetate, L-

proline, propionate, D-ribose, salicin, L-serine, D-sorbitol, suberate, D-sucrose, or n-valerate. Acid is produced with D-arabitol, arbutin, D-cellobiose, esculin, D-fructose, D-galactose, glycerol, glycogen, D-lactose, mannitol, D-mannose, D-melobiose, α -methyl-D-glucoside, ribose, salicin, sorbitol, starch, D-sucrose, D-trehalose, turanose, and D-xylose. Acid is not produced with N-acetyl-glucosamine, D-adonitol (ribitol), amygdalin, D-arabinose, L-arabinose, L-arabitol, dulicitol (galactitol), erythritol, D-fucose, L-fucose, gentiobiose, gluconate, inositol, inulin, 2-ketogluconate, 5-ketogluconate D-lyxose, maltose, melezitose, α -methyl-D-mannoside, β -methyl-D-xyloside, D-raffinose, L-rhamnose, Lsorbose, D-tagatose, xylitol, or L-xylose. In tests with the API Zym system, enzyme production is positive for N-acetyl- β -glucosaminidase, acid phosphatase, alkaline phosphatase, α -chymotrypsin, cysteine arylamidase, esterase (C8), β -galactosidase (OPNG), α -glucosidase, leucine arylamidase, and trypsin. Enzyme production is negative for esterase (C4), α -fucosidase, α -galactosidase, β -glucosidase, β -glucuronidase, lipase (C14), α -mannosidase, naphtol-AS-BI-phosphohydrolase, or valine arylamidase. The predominant cellular fatty acids of strain BS6^T are iso- $C_{15:0}$ (19.0%), $C_{16:1} \omega 7c$ (17.7%), C_{15:1} *ω*6*c* (12.6%), iso-C_{17:0} (10.3%) and iso-C_{17:1} *ω*9*c* (10.3%). The cell-wall peptidoglycan contains D-alanine, L-glutamic acid, glycine, and L-ornithine (di-amino acid). MK-8 is the predominant quinone. The major polar lipid is a phosphoglycolipid (PGL₅) (unidentified), which is common in species of the genus Deinococcus. The G+C content was 66.5 mol%. The type strain, DY59^T (=KCTC 33033^{T} =JCM 18581^{T}) was isolated from a soil sample collected from the mountain Deogyusan (GPS; N 35° 51' 38" E 127° 44' 47"; altitude 1,500 m), Jeonbuk province, South Korea.

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References

- Brooks, B.W. and Murray, R.G.E. 1981. Nomenclature for "Micrococcus radiodurans" and other radiation-resistant cocci: Deinococcaceae fam. nov. and Deinococcus gen. nov., including five species. Int. J. Syst. Bacteriol. 19, 353–360.
- **Brown, A.E.** 2008. Benson's microbiological applications: laboratory manual in general microbiology, complete version, 10th Edition. McGraw-Hill.
- **Collins, M.D. and Jones, D.** 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol. Rev.* **45**, 316–354.
- Ezaki, T., Hashimoto, Y., and Yabuuchi, E. 1989. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* **39**, 224–229.

- Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Ferreira, A.C., Nobre, M.F., Rainey, F.A., Silva, T.M.T., Wait, R., Burghardt, J., Chung, A.P., and Costa, M.S.D. 1997. Deinococcus geothermalis sp. nov. and Deinococcus murrayi sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. Int. J. Syst. Bacteriol. 47, 939–947.
- Gerhardt, P., Murray, R.G.E., Wood, W.A., and Krieg, N.R. 1994. Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, D.C., USA.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.
- Hirsch, P., Gallikowski, C.A., Siebert, J., Peissl, K., Kroppenstedt, R., Schumann, P., Stackebrandt, E., and Anderson, R. 2004. Deinococcus frigens sp. nov., Deinococcus saxicola sp. nov., and Deinococcus marmoris sp. nov., low temperature and draughttolerating, UV-resistant bacteria from continental Antarctica. Syst. Appl. Microbiol. 27, 636–645.
- Im, W.-T., Jung, H.-M., Ten, L.N., Kim, M.K., Bora, N., Goodfellow, M., Lim, S., Jung, J., and Lee, S.-T. 2008. *Deinococcus aquaticus* sp. nov., isolated from fresh water, and *Deinococcus caeni* sp. nov., isolated from activated sludge. *Int. J. Syst. Evol. Microbiol.* 58, 2348–2353.
- Kämpfer, P., Lodders, N., Huber, B., Falsen, E., and Busse, H.-J. 2008. Deinococcus aquatilis sp. nov., isolated from water. Int. J. Syst. Evol. Microbiol. 58, 2803–2806.
- Kim, O.-S., Cho, Y.-J., Lee, K., Yoon, S.-H., Kim, M., Na, H., Park, S.-C., Jeon, Y.S., Lee, J.-H., Yi, H., Won, S., and Chun, J. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* 62, 716–721.
- Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge: Cambridge University Press.
- Komagata, K. and Šuzuki, K. 1987. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19**, 161–207.
- Lai, W.-A., Kämpfer, P., Arun, A.B., Shen, F.-T., Huber, B., Rekha, P.D., and Young, C.-C. 2006. Deinococcus ficus sp. nov., isolated from the rhizosphere of Ficus religiosa L. Int. J. Syst. Evol. Microbiol. 56, 787–791.
- Lim, S., Song, D., Joe, M., and Kim, D. 2012. Development of a qualitative dose indicator for gamma radiation using lyophilized *Deinococcus*. J. Microbiol. Biotechnol. 22, 1296–1300.
- Lim, S., Yoon, H., Ryu, S., Jung, J., Lee, M., and Kim, D. 2006. A comparative evaluation of radiation-induced DNA damage using real-time PCR: influence of base composition. *Radiat. Res.* 165, 430–437.
- Mesbah, M., Premachandran, U., and Whitman, W.B. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* 39, 159–167.
- Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J. Microbiol. Methods* **2**, 233–241.
- Oyaizu, H., Stackebrandt, E., Schleifer, K.H., Ludwig, W., Pohla, H., Ito, H., Hirata, A., Oyaizu, Y., and Komagata, K. 1987. A radiation-resistant rod-shaped bacterium, *Deinobacter grandis* gen. nov., sp. nov., with peptidoglycan containing ornithine. *Int. J. Syst. Bacteriol.* **37**, 62–67.
- Rainey, F.A., Ray, K., Ferreira, M., Gatz, B.Z., Nobre, M.F., Bagaley, D., Rash, B.A., Park, M.-J., Earl, A.M., Shank, N.C., and et al. 2005. Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran desert soil and description of nine new species of the genus *Deinococcus* obtained from a single soil sample. *Appl. Environ. Microbiol.* 71, 5225–5235.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new

method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

- Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, DE, USA.
- Shin, Y.K., Lee, J.S., Chun, C.O., Kim, H.J., and Park, Y.H. 1996. Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. J. Microbiol. Biotechnol. 6, 68–69.
- Srinivasan, S., Kim, M.K., Lim, S., Joe, M., and Lee, M. 2012a. Deinococcus daejeonensis sp. nov., isolated from sludge in a sewage disposal plant. Int. J. Syst. Evol. Microbiol. 62, 1265–1270.
- Srinivasan, S., Lee, J.J., Lim, S., Joe, M., and Kim, M.K. 2012b. Deinococcus humi sp. nov., isolated from soil. Int. J. Syst. Evol. Microbiol. 62, 2844–2850.
- Stackebrandt, E. and Goebel, B.M. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* 44, 846–849.
- Tamaoka, J. and Komagata, K. 1984. Determination of DNA base composition by reversed phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* 25, 125–128.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* 28, 2731–2739.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and

Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **24**, 4876–4882.

- Wang, W., Mao, J., Zhang, Z., Tang, Q., Xie, Y., Zhu, J., Zhang, L., Liu, Z., Shi, Y., and Goodfellow, M. 2010. Deinococcus wulumuqiensis sp. nov., and Deinococcus xibeiensis sp. nov., isolated from radiation-polluted soil. Int. J. Syst. Evol. Microbiol. 60, 2006–2010.
- Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., and *et al.* 1987. International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37, 463–464.
- Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703.
- White, O., Eisen, J.A., Heidelberg, J.F., Hickey, E.K., Peterson, J.D., Dodson, R.J., Haft, D.H., Gwinn, M.L., Nelson, W.C., Richardson, D.L., and et al. 1999. Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1. Science 286, 1571–1577.
- Yoo, S.-H., Weon, H.-Y., Kim, S.-J., Kim, Y.-S., Kim, B.-Y., and Kwon, S.-W. 2010. Deinococcus aerolatus sp. nov. and Deinococcus aerophilus sp. nov., isolated from air samples. Int. J. Syst. Evol. Microbiol. 60, 1191–1195.