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A report of 5 unrecorded bacterial species of the *Deinococcus* genus in Korea

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Five bacterial strains designated DY37, BS333, JJ521, BM1, and DG13-2 were assigned to the genus *Deinococcus* were isolated from forest soil samples collected from Deogyusan, Busan, Changwon, and Seoul of South Korea. The isolates were Gram-staining negative or positive, and pale pink- or red-pigmented, short-rod shaped. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strains DY37, BS333, JJ521, BM1, and DG13-2 were most closely related to *Deinococcus aquatilis* CCM 7524^T (with 99.0% similarity), *D. ficus* CC-FR2-10^T (100.0%), *D. grandis* KS 0485^T (99.2%), *D. roseus* TDMA-uv51^T (98.9%), and *D. yunweiensis* YIM007^T(100.0%), respectively. These 5 species have never been proposed in Korea; therefore 5 species of 1 genera in the family *Deinococcaceae* in the order *Deinococcales* within the class *Deinococci* are reported for proteobacterial species found in Korea.

Keywords: 16S rRNA gene, *Deinococcus*, *Deinococcaceae*, *Deinococcus-Thermus*, unrecorded species in Korea

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Introduction

The genus *Deinococcus* was first proposed by Brooks & Murray (1981) when the type species *Deinococcus radiodurans* was isolated from gamma-ray-irradiated food. At the time of writing, the genus *Deinococcus* comprises 53 species with validly names isolated from diverse environments such as air, a hot spring, continental Antarctica, desert soil, fish, and water (http://www.bacterio.cict.fr/d/deinococcus.html). Members of the genus *Deinococcus* are Gram-positive (Brooks & Murray, 1981; Srinivasan *et al*., 2012a; 2012b) or Gram-negative (Suresh *et al*., 2004; Zhang *et al*., 2007; Im *et al*., 2008; Chen *et al*., 2012), have L-ornithine as the di-amino acid in the cell-wall peptidoglycan, and have cell colors ranging from yellow to red. They are characterized by their extreme resistant to UV light, gamma radiation and desiccation (Mattimore *et al*., 1996; Hirsch *et al*., 2004; de Groot *et al*., 2005; Rainey *et al*., 2005; Callegan *et al*., 2008; Srinivasan *et al*., 2012a, 2012b). The ionizing radiation and deciccation induced DNA damage and it has been reported that the members of the genus *Deinococcus* showed resistance and had ability to repair the damaged DNA (Mattimore & Battista, 1996).

In 2014, we collected diverse local forest soil samples and isolated novel bacterial species and unrecorded bacterial species in Korea. The identified bacterial species belonged to the (class/phylum) *Deinococci*/*Deinococcus-Thermus*. As a subset of this study, the present report focuses on the description of unrecorded radiation-resistant species belonging to the genus *Deinococcus*. Here we report 5 unrecorded bacterial species in Korea belonging to familiy *Deinococcaceae* of order *Deinococcales* in the *Deinococcus-Thermus*.

Materials and Methods

A total of 5 bacterial strains assigned to the family *Deinococcaceae* were isolated from forest soil samples collected from Deogyusan, Busan, Changwon, and Seoul (Table 1). Each environmental sample was processed separately, spread onto R2A culture media and incubated at 25°C for 3 days (Table 1). The designated strain IDs, sources, culture media, and incubation conditions are

Table 1. 16S rRNA gene sequence similarity, Isolation source, medium, and conditions of unrecorded strains isolated belonging to the *Deinococcus*.

Strain ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
DY37	*Deinococcus aquatilis*	99.0	forest soil of Deogyusan	R2A	25°C, 3d
BS333	*Deinococcus ficus*	100.0	forest soil of Busan	R2A	25°C, 3d
JJ521	*Deinococcus grandis*	99.2	forest soil of Changwon	R2A	25°C, 3d
BM1	*Deinococcus roseus*	98.9	forest soil of Seoul	R2A	25°C, 3d
DG13-2	*Deinococcus yunweiensis*	100.0	forest soil of Seoul	R2A	25°C, 3d

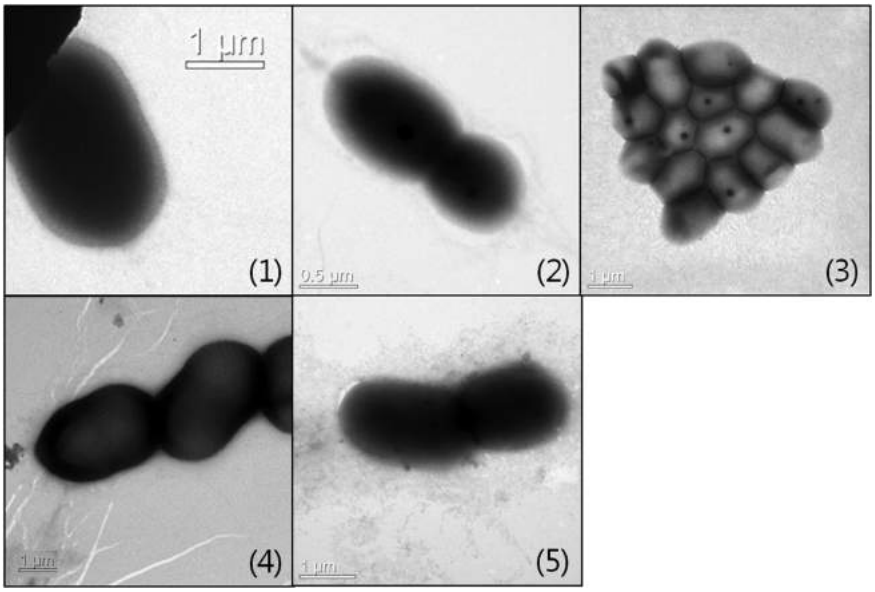

Fig. 1. Transmission electron micrographs of the strains isolated in this study. Strains: 1, DY37; 2, BS333; 3, JJ521; 4, BM1; 5, DG13-2.

summarized in Table 1. All strains were purified as single colonies and stored as 10-20% glycerol suspension at −80°C as well as lyophilized ampoules.

Colony morphology and cell size of the strains was observed on R2A agar plates after cells grown for 3 days at 25°C by using transmission electron microscopy (LIBRA 120, Carl Zeiss). Transmission electron micrograph of the strains are shown in Fig. 1. Gram reaction was performed according to the classic Gram procedure described by Doetsch (1981). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions and the results are presented in Table 1 and in the strains description.

Genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent) and the 16S rRNA gene was amplified by PCR with 9F and 1492R universal bacterial primers (Weisburg *et al*., 1991). The nearly complete sequence of the 16S rRNA gene was compiled with SeqMan software (DNASTAR). The 16S rRNA gene sequences of the 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 alignments were performed with the CLUSTAL_X program (Thompson *et al*., 1997). The evolutionary distances were calculated using the two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) and maximum-likelihood methods in MEGA5 program (Tamura, 2011) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Results and Discussion

Based on the comparative 16S rRNA gene sequence analyses and phylogeny, 5 strains, designated DY37, BS333, JJ521, BM1, and DG13-2, were assigned to the class *Deinococci*. They were all Gram-staining-negative or -positive and short-rod shaped bacteria (Fig. 1). Morphology and physiological characteristics are shown in the species description section.

Strains DY37, BS333, JJ521, BM1, and DG13-2 were most closely related to *Deinococcus aquatilis* CCM

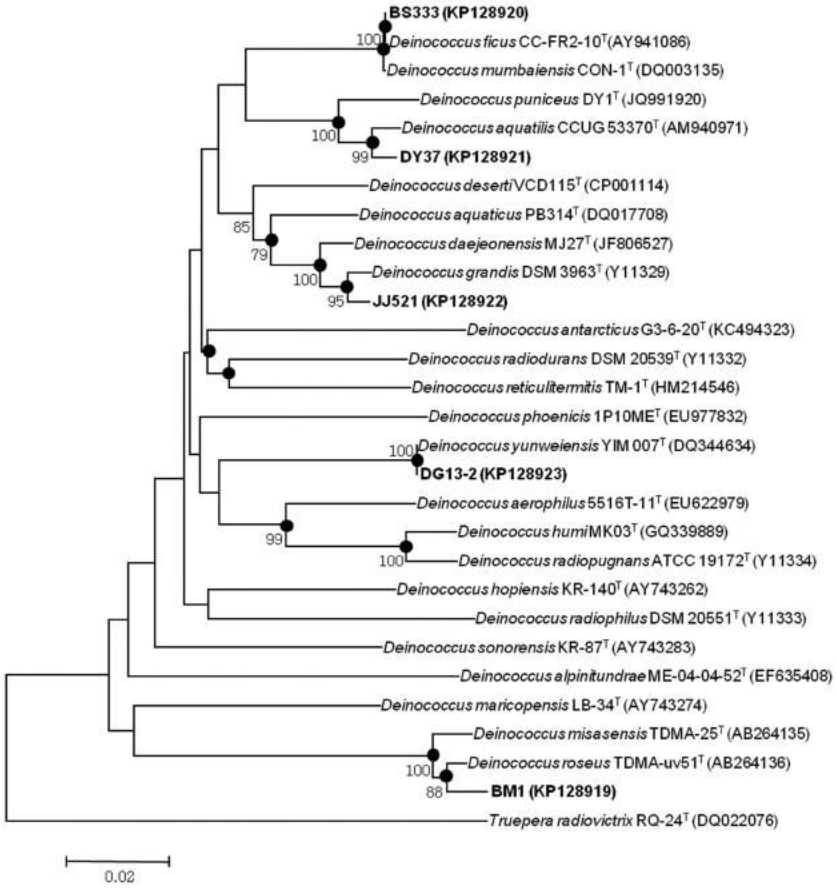

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between the strains isolated in this study and their relatives of the genus *Deinococcus*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining and below nodes for the maximum-likelihood methods. Filled circles indicate the nodes recovered by the two treeing methods. Bar: 0.02 substitutions per nucleotide position.

7524^T (AM940971; 99.0% 16S rRNA gene sequence similarity), *Deinococcus ficus* CC-FR2-10^T (AY941086; 100%), *Deinococcus grandis* KS 0485^T (Y11329; 99.2 %), *Deinococcus roseus* TDMA-uv51^T (AB264136; 98.9 %), and *Deinococcus yunweiensis* YIM 007^T (DQ344634; 100.0%), respectively (Table 1). As expected from high 16S rRNA gene sequence similarities of the 5 strains with their closest relatives, each strain formed a robust phylogenetic clade with the most closely related species (Fig. 2). From the high 16S rRNA gene sequence similarity and robust formation of phylogenetic clade, it is concluded that strains DY37, BS333, JJ521, BM1, and DG13-2 are members of the species *Deinococcus aquatilis* (Kämpfer *et al*., 2008), *Deinococcus ficus* (Lai *et al*., 2006), *Deinococcus grandis* (Oyaizu *et al*., 1987), *Deinococcus roseus* (Asker *et al*., 2008), and *Deinococcus yunweiensis* (Zhang *et al*., 2007), respectively.

There is no official report that these 5 species in the genus *Deinococcus* have been isolated in Korea and hence the strains DY37, BS333, JJ521, BM1, and DG13-2 are proposed to be unreported strains of *Deinococcus aquatilis*, *D. ficus*, *D. grandis*, *D. roseus*, and *D. yunweiensis* for *Deinococcial* species found in Korea.

Description of *Deinococcus aquatilis* DY37

Cells are Gram-staining-positive, non-flagellated, and short rod-shaped. Colonies are circular and pale pinkish-colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease. D-glucose is utilized. Does not utilize *N*-acetyl-glucosamine, adipic acid, L-arabinose, capric acid, D-maltose, malic acid, D-mannitol, D-mannose, phenylacetic acid, potassium gluconate, and trisodium citrate. Strain DY37 (=NIBRBA0000115047) has been isolated from a soil sample, mountain of Deogyusan, Jeonbuk province, South Korea.

Table 2. Characteristics of strains isolated belonging to the *Deinococcus* and their taxonomic affiliations.
Strains: 1, DY37; 2, BS333; 3, JJ521; 4, BM1; 5, DG13-2.

Characteristics	1	2	3	4	5
Nitrate reduction					
Nitrate reduction to NO_2	−	+	−	+	−
Nitrate reduction to N_2	−		−		−
Production of Indole	−	−	−	−	−
Production of acid from glucose	−	−	−	−	−
Enzyme activity of :					
Arginine dihydrolase	−	−	−	−	−
Urease	−	−	−	+	−
β-Glucosidase (esculin hydrolysis)	+	+	+	+	+
Protease (gelatin hydrolysis)	−	−	−	−	−
β-Galactosidase (PNPG)	+	+	+	+	+
Assimilation of :					−
D-Glucose	w	+	+	−	−
L-Arabinose	−	−	−	−	−
D-Mannose	−	−	+	−	−
D-Mannitol	−	−	+	−	−
N-Acetyl-D-glucosamine	−	−	w	−	−
D-Maltose	−	+	+	−	−
Gluconate	−	−	w	−	−
Caprate	−	−	−	−	−
Adipate	−	w	w	−	−
L-Malate	−	−	+	−	−
Citrate	−	−	−	−	−
Phenyl acetate	−	−	−	−	−

Description of *Deinococcus ficus* BS333

Cells are Gram-staining-positive, non-flagellated, and short rod-shaped. Colonies are circular and pale pinkish-colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis, β-galactosidase, and nitrate reduction in API 20NE, but negative for arginine dihydrolase, gelatinase, glucose fermentation, indole production, and urease. Adipic acid, D-glucose, and D-maltose are utilized. Does not utilize acetyl-glucosamine, L-arabinose, capric acid, *N*-malic acid, D-mannitol, D-mannose, phenylacetic acid, potassium gluconate, and trisodium citrate. Strain BS333 (=NIBRBA0000115048) has been isolated from a forest soil sample, Busan, South Korea.

Description of *Deinococcus grandis* JJ521

Cells are Gram-staining-negative, non-flagellated, and short rod-shaped. Colonies are circular and pinkish-colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE, but negative for arginine dihydrolase, gelatinase, glucose fermentation, indole production, nitrate reduction, and urease. *N*-acetyl-glucosamine, adipic acid, D-glucose, malic acid, D-maltose, D-mannitol, D-mannose, and potassium gluconate are utilized. Does not utilize L-arabinose, capric acid, phenylacetic acid, and trisodium citrate. Strain JJ521 (=NIBRBA0000115049) has been isolated from a forest soil sample, Changwon, South Korea.

Description of *Deinococcus roseus* BM1

Cells are Gram-staining-positive, non-flagellated, and short rod-shaped. Colonies are circular and pinkish-colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis, nitrate reduction, urease, and β-galactosidase in API 20NE, but negative for arginine dihydrolase, gelatinase, glucose fermentation, and indole production. Does not utilize *N*-acetyl-glucosamine, adipic acid, L-arabinose, capric acid, phenylacetic acid, D-glucose, malic acid, D-maltose, D-mannitol, D-mannose, potassium gluconate, and trisodium citrate. Strain BM1 (=NIBRBA0000115050) has been isolated from a forest soil sample, Seoul, Korea.

Description of *Deinococcus yunweiensis* DG13-2

Cells are Gram-staining-negative, non-flagellated, and short rod-shaped. Colonies are circular and red colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE, but negative for arginine dihydrolase, indole production, gelatinase, glucose fermentation, nitrate reduction, and urease. Does not utilize *N*-acetyl-glucosamine, adipic acid, L-arabinose, capric acid, D-glucose, malic acid, D-maltose, D-mannitol, D-mannose, phenylacetic acid, potassium gluconate, and trisodium citrate. Strain DG13-2 (=NIBRBA0000115051) has been isolated from a forest soil sample in Namsan, Seoul, Korea.

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