# **Report on 14 unrecorded bacterial species in Korea that belong to the phyla** *Bacteroidetes* and *Deinococcus-Thermus*

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As a result of the research project 'Survey and excavation of Korean indigenous species' to secure unrecorded species in Korea, a total of 14 unreported bacterial strains assigned to the phyla *Deinococcus*-*Thermus* and *Bacteroidetes* were isolated from various environmental habitats all around Korea. On the basis of 16S rRNA gene sequence similarity and affiliation to the clade with the closest species, it was concluded that the isolates represent deep groups of the phyla *Deinococcus*-*Thermus* and *Bacteroidetes*. There have been no records about these 14 unreported species in Korea; therefore 2 species of 2 genera in the class *Deinococci* within the phylum *Deinococcus*-*Thermus*, and 11 species of 9 genera in the class *Flavobacteriia* and one species in one genus in the class *Sphigobacteriia* within the phylum *Bacteroidetes* are described as unreported species found in Korea. Gram staining reaction, morphological and other biochemical characteristics are described in the species description section.

Keywords: 16S rRNA, bacterial diversity, Bacteroidetes, Deinococcus-Thermus, unrecorded species

© 2015 National Institute of Biological Resources DOI:10.12651/JSR.2015.4.2.137

# **INTRODUCTION**

The number of prokaryotes on earth is estimated to be around  $5 \times 10^{30}$  cells, and these cells make up over half of the biomass on earth (Whitman *et al.*, 1998). As a large fraction of the biomass, prokaryotes dominate the flux of energy and biologically important chemical elements. Although the prokaryotes constitute a major part of living organism phylogeny, many prokaryotes in nature have not yet been described. Therefore, National Institute Biological Resources has supported the project 'Survey and excavation of Korean indigenous species' in an attempt to secure new and unrecorded species in Korea. The phylum *Deinococcus-Thermus* is a small group of bacteria, which is resistant to extreme environmental hazard condition (Griffiths and Gupta, 2007). The phylum contains one class, *Deinococci*. The class *Deinococci* includes 2 orders, 3 families, and 10 described genera. The order *Deinococcales* have become famous for their several species that are resistant to radiation and their ability to eat nuclear waste and other toxic materials, and can survive on the vacuum of space and extremes of heat and cold (Rainey *et al.*, 1997). The order *Thermales* includes several genera resistant to heat.

The phylum *Bacteroidetes* is a phenotypically diverse group of Gram-stain-negative rods that do not form endospores (Ludwig *et al.*, 2010). The phylum contains 4 classes, *Bacteroidia*, *Cytophagia*, *Flavobacteriia* and *Sphingobacteriia*. The class *Bacteroidia* presently contains one order, *Bacteroidales*, the well-studied *Bacteroidetes* (Krieg *et al.*, 2010). The class *Cytophagia* is comprised of Gram-stain-negative bacteria with chemo-organotrophic characteristics. The class *Flavobacteriia* consists of Gram-stain-negative, non-spore forming rods or filaments devoid of gas vesicles and intracellular granules of poly- $\beta$ -hydroxybutyrate, which usually multiply by binary fission. The class *Sphingobacteriia* contains high concentration of sphingophospholipids as cellular lipid components.

As a result of the project in 2012, we report 2 unrecorded bacterial species belonging to *Deinococcus-Thermus* and 12 species belonging to *Bacteroidetes* in this communication.

# **MATERIALS AND METHODS**

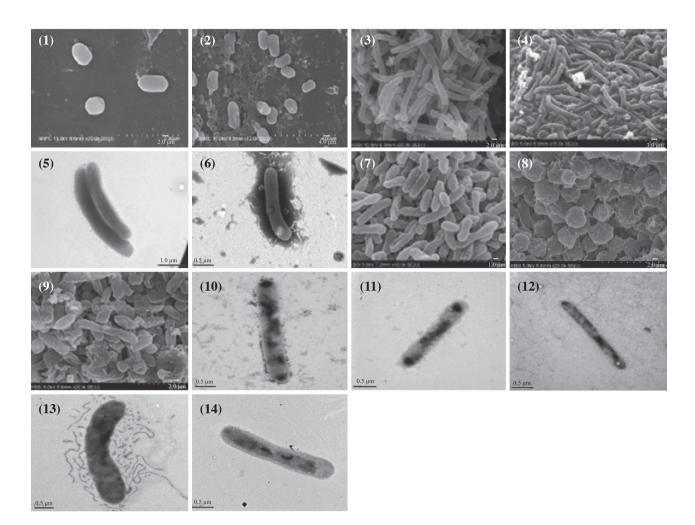
A total of 14 bacterial strains assigned to the phyla *Deinococcus-Thermus* and *Bacteroidetes* were isolated from diverse environmental samples collected from sediment, plant, seawater, freshwater and tidal flat (Table 1). Each environmental sample was serially diluted separately and plated on diverse culture media including R2A (BD Difco), Marine Agar 2216 (MA; BD Difco) and Tryptic Soy Agar (TSA; BD Difco), and incubated at 25-37°C for 2-4 days (Table 1). The designated strain IDs, sources, culture media, and incubation conditions are summarized in Table 1. All strains were purified as single colonies and preserved as 20% glycerol suspension at -70°C and also lyophilized.

The phylogenetic position of the strains assigned to the Deinococcus-Thermus and Bacteroidetes was investigated by 16S rRNA gene analysis. The gene was amplified and sequenced using universal bacterial primers 27F and 1492R. The sequence was aligned with closely related 16S rRNA gene sequences using the EzTaxon-e server (Kim et al., 2012b). Phylogenetic trees were reconstructed by neighbor-joining (Saitou and Nei, 1987) and maximum-likelihood (Fitch, 1971) algorithms using the software package MEGA5 (Tamura et al., 2011), after generating multiple sequence alignments with the program CLUSTAL\_X version 2.1 (Thompson et al., 1997). For the neighbor-joining analysis, the evolutionary distances between sequences were calculated using Kimura's two-parameter model (Kimura, 1980), and the tree topology was evaluated with 1,000 replicates of bootstrap resampling.

Optical microscopy was used to characterize the morphological properties of the strains on stationary phase. Cellular morphology and cell size were examined by either transmission electron microscopy or scanning ele-

Phylum	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Deinococcus-Thermus Deinococcaceae	Deinococcaceae	Deinococcus Deinococcus	DaeR-4 BE4-4	NIBRBA0000113891 NIBRBA0000113903	D. aquiradiocola TDMA-uv53 <sup>T</sup> D. humi MK03 <sup>T</sup>	99.3 100.0	Sediment Plant	R2A R2A	25°C, 2d 25°C, 2d
Bacteroidetes	Flavobacteriaceae	Cellulophaga Flavobacterium Flavobacterium Gramella Hyunsoonleella Lutimonas Maribacter Nonlabens Zobellia Zunongwangia	KA18 KYW884 PN22 HME 8473 KYW842 KYW842 KYW573 HD33 WSW-MO1 WSW-MO2 WSW-MO2 IMCC1073	NIBRB A0000114107 NIBRB A0000114126 NIBRB A0000113873 NIBRB A0000113873 NIBRB A0000114090 NIBRB A0000114109 NIBRB A0000114109 NIBRB A0000114006 NIBRB A0000114006 NIBRB A0000114006 NIBRB A0000114005 NIBRB A0000114005	C. fucicola NN015840 <sup>T</sup> F. alnfettiae 10Alg 130 <sup>T</sup> F. compostarboris 15C3 <sup>T</sup> F. limicola ST-82 <sup>T</sup> G. echinicola ST-82 <sup>T</sup> H. jejuensis CNU004 <sup>T</sup> L. saemankumensis RSS3-C1 <sup>T</sup> M. stanieri KMM 6046 <sup>T</sup> N. ulvanivorans PLR <sup>T</sup> Z. amurskyensis KMM 3526 <sup>T</sup> Z. profunda SM-A87 <sup>T</sup>	99.6 100.0 100.0 99.2 99.9 99.9 99.9 99.9 99.9	Seawater Seawater Fresh water Fresh water Seawater Seawater Tidal flat Seawater Seawater Fresh water Fresh water	MA MA TSA MA MA MA MA MA MA MA	25°C, 3d 25°C, 2d 25°C, 2d 30°C, 2d 25°C, 2d 25°C, 3d 25°C, 3d 25°C, 3d 25°C, 3d 25°C, 3d 25°C, 3d
	Sphingobacteriaceae Pedobacter	Pedobacter	HME8545	NIBRBA0000114077	NIBRBA0000114077 P. glucosidilyticus 1-2 <sup>T</sup>	99.3	Fresh water	R2A	37°C, 3d

MA, marine agar; TSA, tryptic soy agar



**Fig. 1.** Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1. DaeR-4; 2. BE4-4; 3. KA18; 4. KYW884; 5. PN22; 6. HME8473; 7. KYW842; 8. KA3; 9. KYW573; 10. HD33; 11. WSW-MO1; 12. WSW-MO2; 13. IMCC1073; 14. HME8545.

ctron microscopy in the stationary growth phase at each temperature. Gram staining was performed using a Gram stain kit (BD Difco) or the standard procedures. Various physiological and biochemical tests were performed using API 20NE galleries (bioMérieux) accordance with the manufacturer's instructions.

# **RESULTS AND DISCUSSION**

#### Strains assigned to the Deinococcus-Thermus

Based on the comparative 16S rRNA gene sequence analyses and phylogeny, 2 strains, designated DaeR-4 and BE4-4, were assigned to the *Deinococcus-Thermus*. They were all Gram-staining-negative, chemoheterotrophic and rod-shaped bacteria (Fig. 1). Colony size, morphology and physiological characteristics are shown in the species description section.

Strains DaeR-4 and Be4-4 were most closely related to *Deinococcus aquiradiocola* TDMA-uv53<sup>T</sup> (AB265180; 99.3% 16S rRNA gene sequence similarity) and D. humi MK03<sup>T</sup> (GQ339889; 100.0%), respectively (Table 1). As expected from high 16S rRNA gene sequence similarities of the 2 strains with their closest relatives, each strain formed a robust phylogenetic clade with the most closely related species (Fig. 2). From the robust formation of phylogenetic clade with the high 16S rRNA gene sequence similarity and morphological and physiological characteristics, it is concluded that strains DaeR-4 and BE4-4 are members of the species D. aquiradiocola (Asker et al., 2009) and D. humi (Srinivasan et al., 2012), respectively. There has been no official report that these species have been isolated in Korea; therefore, D. aquiradiocola and D. humi of the family Deinococcaceae are reported for Deinococcus-Thermus species found in Korea.

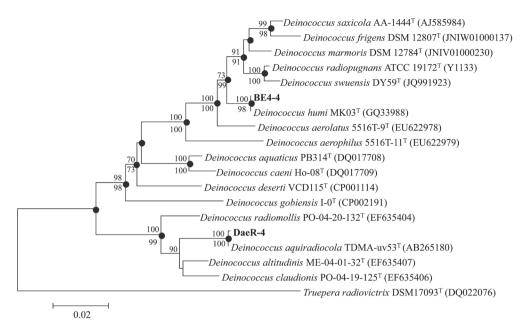


Fig. 2. Neighbor-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships between the strains isolated in this study and their relatives of the class *Deinococcus-Thermus*. Numbers at nodes are levels of bootstrap support for branch points, based on 1,000 resampling; values are shown only if greater than 70%. Filled circles at nodes indicate that the corresponding nodes were also recovered using maximum-likelihood algorithm. Bar, 2% sequence divergence.

#### Strains assigned to the Bacteroidetes

On the basis of 16S rRNA gene sequence comparisons and phylogenetic analyses, a total of 12 strains were assigned to the class *Bacteroidetes*. The 12 strains were distributed in 2 orders of the *Bacteroidetes*; 11 strains for the order *Flavobacteriales* and one strain for the *Sphingobacteriales* (Table 1). These strains were Gram-stainingnegative, chemoheterotrophic and rod-shaped bacteria except for strain KA3 showing coccoid-shaped (Fig. 1). Colony size, morphology and physiological characteristics are also shown in the species description section.

Fig. 3 shows phylogenetic assignment of 12 strains into 12 species of the orders *Flavobacteriales* and *Sphingobacteriales*. These strains belonged to *Cellulophaga fucicola* (Johansen *et al.*, 1999), *Flavobacterium ahnfeltiae* (Nedashkovskaya *et al.*, 2014), *F. compostarboris* (Kim *et al.*, 2012a), *F. limicola* (Tamaki *et al.*, 2003), *Gramella echinicola* (Nedashkovskaya *et al.*, 2005), *Hyunsoonleella jejuensis* (Yoon *et al.*, 2010), *Lutimonas saemankumensis* (Kim *et al.*, 2014), *Maribacter stanieri* (Nedashkovskaya *et al.*, 2010), *Nonlabens ulvanivorans* (Kopel *et al.*, 2014), *Zobellia amurskyensis* (Nedashkovskaya *et al.*, 2004) and *Zunongwangia profunda* (Qin *et al.*, 2010) of the family *Flavobacteriaceae* and *Pedobacter glucosidilyticus* (Luo *et al.*, 2010) of the family *Sphingobacteriaceae*.

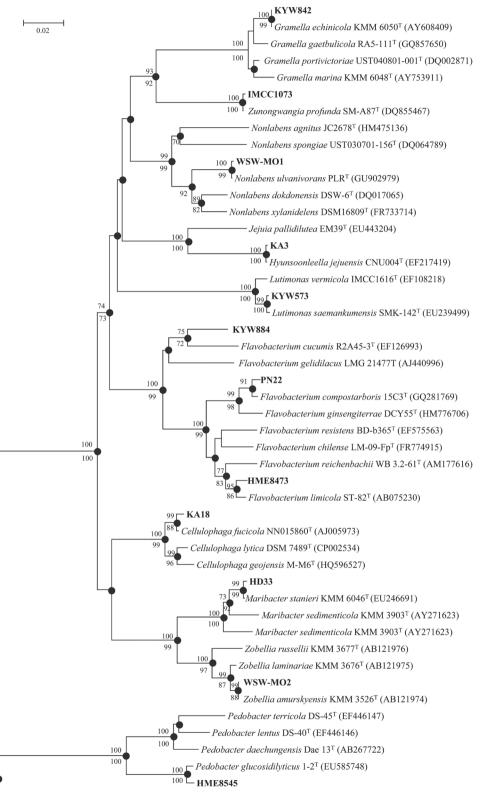
There is no official report that these 12 species have been isolated in Korea; therefore 11 species in 9 genera of one family in the order *Flavobacteriales* and one species in the order *Sphingobacteriales* are reported for *Bacteroidetes* species found in Korea.

#### Description of Deinococcus aquiradiocola DaeR-4

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are circular, raised, entire and yellow-colored after 2 days of incubation on R2A at 25°C. Positive for  $\beta$ -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol and D-maltose are utilized. Does not utilize *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain DaeR-4 (=NIBRBA0000113 891) has been isolated from a sediment sample of Daechung stream, Daejeon, Korea.

#### Description of Deinococcus humi BE4-4

Cells are Gram-staining-negative, non-flagellated, nonpigmented and short rod-shaped. Colonies are circular, raised, entire and orange-colored after 2 days of incubation on R2A at 25°C. Positive for  $\beta$ -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. Does not utilize D-



- Bacteroides fragilis ATCC25285<sup>T</sup> (X83935)

**Fig. 3.** Neighbor-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships between the strains isolated in this study and their relatives of the class *Bacteroidetes*. Numbers at nodes are levels of bootstrap support for branch points, based on 1,000 resampling; values are shown only if greater than 70%. Filled circles at nodes indicate that the corresponding nodes were also recovered using maximum-likelihood algorithm. Bar, 5% sequence divergence.

glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain BE4-4 (=NIBRBA0000113903) has been isolated from a plant sample, Daejeon, Korea.

#### Description of Cellulophaga fucicola KA18

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are opaque, complex, hair-lock like, convex and yellow-colored after 3 days of incubation on MA at 25°C. Positive for  $\beta$ -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KA18 (=NIBRBA 0000114107) has been isolated from a seawater sample, Gwangyang Bay, Korea.

#### Description of Flavobacterium ahnfeltiae KYW884

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are opaque, round, smooth, convex and yellow-colored after 2 days of incubation on MA at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KYW884 (=NIBRBA0000114126) has been isolated from a seawater sample, Gwangyang Bay, Korea.

#### Description of Flavobacterium compostarboris PN22

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and yellow-colored after 2 days of incubation on TSA at 25°C. Positive for nitrate reduction, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine and D-maltose are utilized. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PN22 (=NIBRBA0000113873) has been isolated from a freshwater sample, Daejeon, Korea.

#### Description of Flavobacterium limicola HME8432

Cells are Gram-staining-negative, non-flagellated, non-

pigmented and rod-shaped. Colonies are circular, convex, entire and yellow-colored after 2 days on R2A at 30°C. Positive for esculin hydrolysis and  $\beta$ -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Mannose, D-maltose and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain HME8473 (=NIBRBA0000114090) has been isolated from a freshwater sample, Gyoungan stream, Korea.

#### Description of Gramella echinicola KYW842

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are opaque, round, smooth, convex and yellow-colored after 2 days on MA at 25°C. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KYW842 (=NIBRBA0000114112) has been isolated from a seawater sample, Gwangyang Bay, Korea.

#### Description of Hyunsoonleella jejuensis KA3

Cells are Gram-staining-negative, non-flagellated, nonpigmented and coccoid-shaped. Colonies are opaque, round, smooth, convex and yellow-colored after 3 days on MA at 25°C. Positive for esculin hydrolysis and  $\beta$ galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KA3 (=NIBRBA0000114106) has been isolated from a seawater sample, Gwangyang Bay, Korea.

## Description of Lutimonas saemankumensis KYW573

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are opaque, round, smooth, convex and yellow-colored after 4 days on MA at 25°C. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KYW573 (=NIBRBA0000114109) has been isolated from a seawater sample, Gwangyang Bay, Korea.

#### Description of Maribacter stanieri HD33

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are circular, convex, smooth and yellow-colored after 3 days on MA at 25°C. Positive for nitrate reduction, esculin hydrolysis and  $\beta$ -galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HD33 (=NIBRBA0000113990) has been isolated from a tidal flat sample, Taean, Korea.

#### Description of Nonlabens ulvanivorans WSW-MO1

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are circular, convex, glistening and orange-colored after 3 days on MA at 25°C. Positive for nitrate reduction and gelatinase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and  $\beta$ -galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain WSW-MO1 (=NIBRBA0000114006) has been isolated from a seawater sample, Wando, Korea.

#### Description of Zobellia amurskyensis WSW-MO2

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are circular, convex, glistening and orange-colored after 3 days on MA at 25°C. Positive for nitrate reduction, esculin hydrolysis and  $\beta$ -galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain WSW-MO2 (=NIBRBA0000114007) has been isolated from a seawater sample, Wando, Korea.

### Description of Zunongwangia profunda IMCC1073

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow-colored after 2 days on MA at 25°C. Positive for glucose fermentation and  $\beta$ -galactosidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. Malic acid and trisodium citrate are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain IMCC1073 (=NIBRBA0000113913) has been isolated from a freshwater sample, Lake Inkyoung, Korea.

#### **Description of Pedobacter glucosidilyticus HME8545**

Cells are Gram-staining-negative, non-flagellated, nonpigmented and rod-shaped. Colonies are circular, convex, entire and red-colored after 3 days on R2A at 37°C. Positive for esculin hydrolysis and  $\beta$ -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, D-mannose, N-acetylglucosamine and D-maltose are utilized. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HME8545 (= NIBRBA0000114007) has been isolated from a freshwater sample, Gyoungan stream, Korea.

# ACKNOWLEDGEMENTS

This study was supported by the research grant 'The Survey of Korean Indigenous Species' from the National Institute of Biological Resources of the Ministry of Environment in Korea.

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Submitted: May 22, 2015 Revised: June 29, 2015 Accepted: July 27, 2015