Journal of Species Research 7(3):193-201, 2018

A report of 22 unrecorded bacterial species in Korea, isolated from the North Han River basin in 2017

Yochan Joung, Miri Park, Hye-Jin Jang, Ilsuk Jung and Jang-Cheon Cho*

Department of Biological Sciences, Inha University, Incheon 22212, Republic of Korea

**Correspondent: chojc@inha.ac.kr*

Culturable bacterial diversity was investigated using freshwater and sediment samples collected from the North Han River basin in 2017, as a part of the research program 'Survey of freshwater organisms and specimen collection'. Over a thousand bacterial strains were isolated from the samples and identified based on 16S rRNA gene sequences. Among the bacterial isolates, 22 strains showing higher than 98.7% sequence similarity with validly published bacterial species, but not reported in Korea, were classified as unrecorded species in Korea. The 22 bacterial strains were phylogenetically diverse and assigned to 6 classes, 11 orders, 15 families, and 21 different genera. At the generic level, the unreported species were affiliated with *Flavobacterium* of the class *Flavobacteria*, *Flexibacter* of the class *Cytophagia*, *Blastomonas*, *Brevundimonas*, *Elstera*, *Rhizobium*, *Roseomonas*, *Sphingomonas*, and *Xanthobacter* of the class *Alphaproteobacteria*, *Albidiferax*, *Cupriavidus*, *Curvibacter*, *Ferribacterium*, *Hydrogenophaga*, *Iodobacter*, *Limnohabitans*, *Polaromonas*, *Undibacterium*, and *Variovorax* of the class *Betaproteobacteria*, *Pseudomonas* of the class *Gammaproteobacteria*, and *Arcobacter* of the class *Epsilonproteobacteria*. The unreported bacterial species were further characterized by examining Gram reaction, colonial and cellular morphology, and biochemical properties. The detailed descriptions of 22 strains of the unreported bacterial species are also provided.

Keywords: North Han River, unrecorded bacterial species, freshwater, 16S rRNA

© 2018 National Institute of Biological Resources
DOI:10.12651/JSR.2018.7.3.193

Introduction

Freshwater in the form of lakes, rivers, and groundwater comprises only approximately 0.6% of the total water on Earth. Although the proportion of freshwater is far less than that of marine water, microorganisms present in freshwater environments are regarded as potential indicators of global climate change (Williamson *et al*., 2008). The bacterial community structure of freshwater environments have been considered to be similar to that of soil environments due to the close proximity of the two habitats (Rheinheimer, 1980). However, culture-independent 16S rRNA gene sequence analyses showed distinctly different bacterial community structures between the two habitats (Lozupone and Knight, 2007). Freshwater bacterial communities are characterized by high abundance of bacterial members of the phyla *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Verrucomicrobia* and the classes *Alphaproteobacteria* and *Betaproteobacteria* (Newton *et al*., 2011). Of these abundant freshwater bacteria, many major groups including the acI, acIV and acSTL clades of the *Actinobacteria* have not yet been cultured (Kara *et al*., 2013).

The Han River is one of the largest rivers in Korea and has a variety of natural environments such as wetlands and lakes as well as artificial environments in major cities and land dedicated to agricultural. Bacterial distribution in the Han River has been widely studied at upstream artificial reservoirs such as Lake Soyang and Lake Paldang as well as downstream Han River. Studies of bacterial distribution have revealed many new taxa affiliated with the *Proteobacteria* and *Cytophagacae* that are present in the Han River waters (Han *et al*., 1998). Recently, many bacterial strains belonging to the genera *Deinococcus*, *Exiguobacterium*, and *Hymenobacter* were isolated from the Han River and shown to be radiation-resistant (Lee *et al*., 2017). Culture-independent bacterial community structure analyses in the Lake Paldang of the Han River basin also revealed that uncultured groups of the *Proteobacteria* and *Actinobac-*

teria predominate in the ecosystem, showing seasonal fluctuations (Kim *et al*., 2016).

To investigate culturable bacterial diversity present in the North Han River and collect multiple specimen of previously unreported bacterial resources as a part of the research program 'Survey of freshwater organisms and specimen collection', diverse samples were collected from wetland, stream, and river environments in the North Han River basin, Korea, in 2017. The 16S rRNA gene-based phylogenetic analyses of bacterial strains collected revealed that 22 bacterial species were unrecorded bacterial species in Korea. Here, we report the phylogenetic information and phenotypic characteristics of these unrecorded bacterial species in order to expand the inventory record of bacterial species found in Korea.

Materials and Methods

Freshwater environmental samples for bacterial isolation were collected from a wetland in Lake Cheongpyeong (37°43′29.3 N, 127°31′26.1 E) on April 6, 2017 and a river surface (37°43′18.2 N, 127°35′34.9 E) and a sediment surface of the Hongcheon River (37°42′10.5 N, 127°35′48.4 E) on June 30, 2017. Using a spread plating technique, the samples from freshwater environments were spread onto R2A agar (BD Difco, USA) and the plates were incubated at 20-25°C for 3-7 days. Bacterial strains were isolated from colonies grown on R2A agar plates, purified as pure cultures, and preserved at −80°C in 20% (v/v) glycerol suspension as well as lyophilized ampoules. The designation of strains, source of isolation, culture medium, and incubation conditions are summarized in Table 1.

Bacterial DNA extraction, PCR amplification with universal primers 27F and 1492R, and 16S rRNA gene sequencing were performed using standard procedures as described previously (Yang and Cho, 2008). For 16S rRNA gene sequencing, primers 518F and 800R were used. The 16S rRNA gene sequences were compared with other bacterial strains with validly published names using the EzTaxon-e server (Kim *et al*., 2012). The cutoff value of 98.7% sequence similarity was applied for bacterial identification. Bacterial strains showing 98.7% or higher sequence similarity with validly published species never reported in Korea were selected as unreported bacterial species. For phylogenetic analyses, multiple sequence alignments between the 16S rRNA gene sequences of the isolates and those of the reference type strains of the type species were carried out using ClustalW and manually checked with EzEditor (Jeon *et al*., 2014). Based on the sequences aligned, phylogenetic trees were generated by using neighbor-joining (Saitou and Nei, 1987), maximum parsimony (Fitch, 1971), and maximum likelihood (Felsenstein, 1981) methods that are programmed in MEGA 6.0 software (Tamura *et al*., 2013). The robustness of the phylogenetic trees was confirmed by bootstrap analyses based on 1,000 random replications (Felsenstein, 1985).

Colony morphology of strains of the unrecorded bacterial species was observed on agar plates with a magnifying glass after the cells were cultivated to their stationary phase. Cellular morphology and cell size were examined by transmission electron microscopy. Gram staining was performed using a Gram-staining kit (bioMérieux). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions.

Results and Discussion

From the present study on cultural bacterial diversity of the North Han River basin, many strains belonging to novel species and established species were isolated. Based on 16S rRNA gene sequence analyses of approximately 1,400 bacterial strains isolated, 22 strains were identified as unrecorded bacterial species that exhibit higher than 98.7% sequence similarity to known bacterial species and have never been reported in Korea. The strain information and identification results of these species are summarized in Table 1. The delineation of the species based on sequence similarity was confirmed by the phylogenetic trees (Fig. 1), which showed that each bacterial strain formed a robust clade with high bootstrap values with the most closely related species. The tree topology of neighbor-joining, maximum-parsimony, and maximum-likelihood methods was basically identical at least at species-level.

The 22 unrecorded bacterial species were assigned to the classes *Flavobacteria* (1 strain) and *Cytophagia* (1 strain) of the phylum *Bacteroidetes* and the classes *Alphaproteobacteria* (8 strains), *Betaproteobacteria* (10 strains), *Gammaproteobacteria* (1 strain), and *Epsilonproteobacteria* (1 strain) of the phylum *Proteobacteria*. At the generic and family level, the strains belonged to 21 genera of 15 families: *Flavobacterium* of *Flavobacteriaceae*, *Flexibacter* of *Cytophagaceae*, *Brevundimonas* of *Caulobacteraceae*, *Rhizobium* of *Rhizobiaceae*, *Xanthobacter* of *Xanthobacter*aceae, *Roseomonas* of *Acetobacteraceae*, *Elstera* of *Rhodospirillaceae*, *Blastomonas* and *Sphingomonas* of *Sphingomonadaceae*, *Cupriavidus* of *Burkholderiaceae*, *Albidiferax*, *Curvibacter*, *Hydrogenophaga*, *Limnohabitans*, *Polaromonas*, *Variovorax* of *Comamonadaceae*, *Undibacterium* of *Oxalobacteraceae*, *Iodobacter* of *Chromobacteriaceae*, *Ferribacterium* of *Rhodocyclaceae*, *Pseudomonas* of *Pseudomonadaceae*, and *Arcobacter* of *Campylobacteraceae*.

Table 1. Summary of isolated strains from the North Han River basin and their taxonomic affiliations.

Class	Order	Family	Strain ID	NNIBR ID	Accession number	Most closely related species		Similarity (%)	Isolation source
						Closest type strain	Accession number		
Flavobacteria	*Flavobacteriales*	*Flavobacteriaceae*	IMCC34522	NNIBRBA134	MG456789	*Flavobacterium collinsii* 983-08^T	HE612088	99.0	River sediment
Cytophagia	*Cytophagales*	*Cytophagaceae*	IMCC34590	NNIBRBA137	MG456792	*Flexibacter aurantiacus* DSM 6792^T	M62792	99.5	River water
Alphaproteobacteria	*Caulobacterales*	*Caulobacteraceae*	IMCC34630	NNIBRBA141	MG456796	*Brevundimonas alba* DSM 4736^T	AJ227785	99.0	River water
			IMCC34661	NNIBRBA147	MG456802	*Brevundimonas intermedia* ATCC 15262^T	AJ227786	99.6	River water
	Rhizobiales	*Rhizobiaceae*	IMCC34666	NNIBRBA148	MG456803	*Rhizobium capsica* CC-SKC2^T	HQ113369	99.2	River water
		Xanthobacteraceae	IMCC34578	NNIBRBA136	MG456791	*Xanthobacter autotrophicus* DSM 432^T	X94201	98.9	River water
	Rhodospirillales	*Acetobacteraceae*	IMCC34610	NNIBRBA140	MG456795	*Roseomonas tokyonensis* K-20^T	AB297501	98.9	River water
		Rhodospirillaceae	IMCC34605	NNIBRBA138	MG456793	*Elstera litoralis* Dia-1^T	EU678309	99.3	Wetland water
	Sphingomonadales	*Sphingomonadaceae*	IMCC34726	NNIBRBA153	MG45680	*Blastomonas natatorial* DSM 3183^T	AB024288	99.9	Wetland water
			IMCC34658	NNIBRBA145	MG456800	*Sphingomonas yantingensis* 1007^T	JX566547	99.7	Wetland water
Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	IMCC34655	NNIBRBA143	MG456798	*Cupriavidus pinatubonensis* 1245^T	AB121221	99.1	River sediment
		Comamonadaceae	IMCC34711	NNIBRBA151	MG456806	*Albidiferax ferrireducens* T118^T	CP000267	98.9	River sediment
			IMCC34728	NNIBRBA154	MG456809	*Curvibacter fontanus* AQ9^T	AB120963	99.0	Wetland water
			IMCC34689	NNIBRBA150	MG456805	*Hydrogenophaga intermedia* S1^T	AF019037	99.4	River sediment
			IMCC34713	NNIBRBA152	MG45680	*Limnohabitans australis* MWH-BRAZ-DAM2D^T	FM178226	99.2	River sediment
			IMCC34644	NNIBRBA142	MG456797	*Polaromonas aquatica* CCUG 39402^T	AM039830	98.9	River water
			IMCC34656	NNIBRBA144	MG456799	*Variovorax guangxiensis* GXGD002^T	JF495126	98.9	Wetland water
		Oxalobacteraceae	IMCC34688	NNIBRBA149	MG456804	*Undibacterium squillarum* CMJ-15^T	HE648174	99.7	River sediment
	Neisseriales	*Chromobacteriaceae*	IMCC34510	NNIBRBA133	MG456788	*Iodobacter limnosediminis* E1^T	HM031078	99.8	River sediment
	Rhodocyclales	*Rhodocyclaceae*	IMCC34659	NNIBRBA146	MG456801	*Ferribacterium limneticum* cda-1^T	Y17060	98.9	Wetland water
Gammaproteobacteria	*Pseudomonadales*	*Pseudomonadaceae*	IMCC34607	NNIBRBA139	MG456794	*Pseudomonas abietaniphila* ATCC 700689^T	AJ011504	99.7	Wetland water
Epsilonproteobacteria	*Campylobacterales*	*Campylobacteraceae*	IMCC34525	NNIBRBA135	MG456790	*Arcobacter venerupis* F67-11^T	HE565359	99.6	River sediment

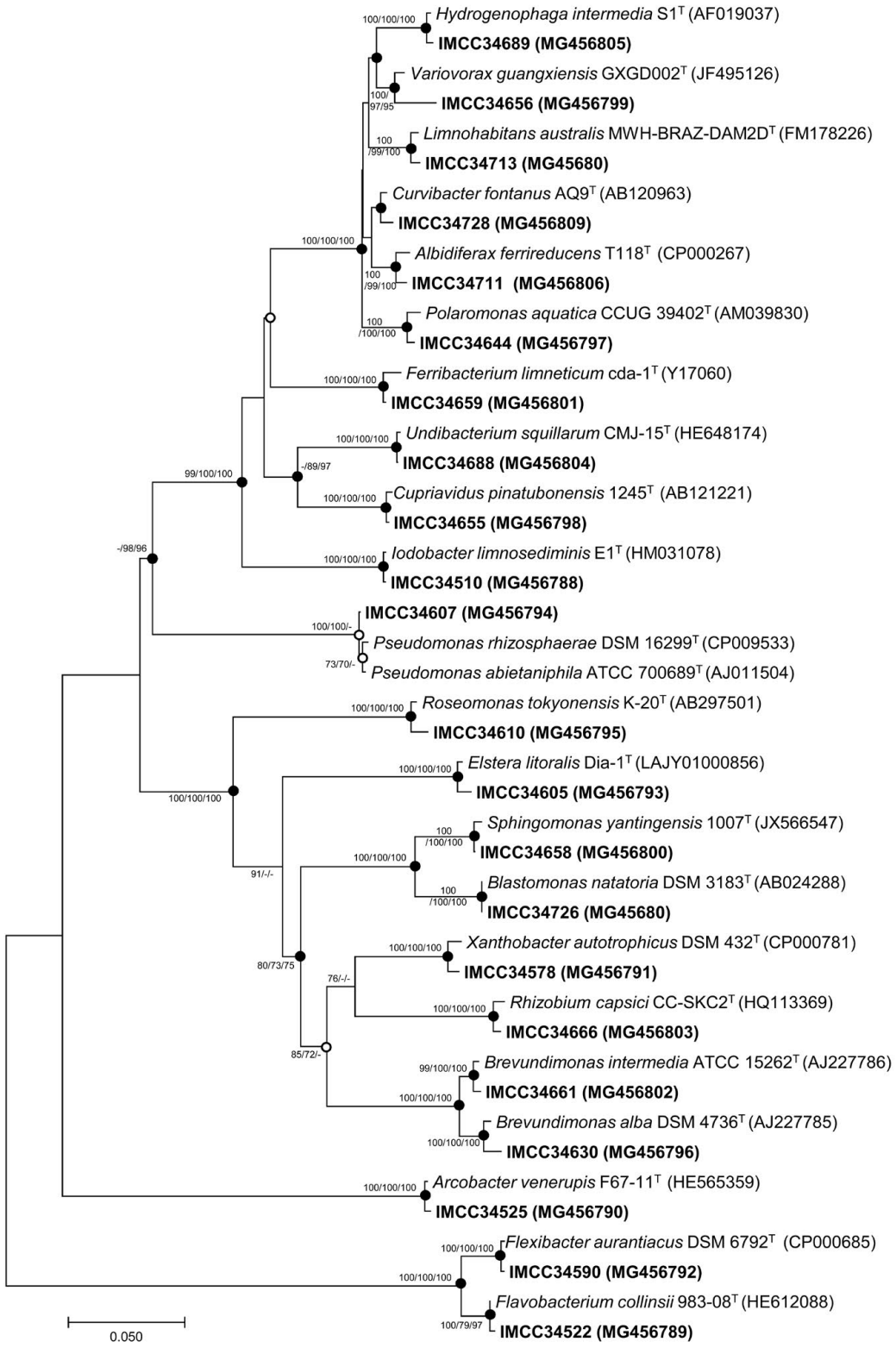

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest relatives. Bootstrap values (expressed as percentages of 1000 replications) over 70% are shown at nodes for neighbor-joining, maximum parsimony, and maximum likelihood methods, respectively. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms, while open circles indicate that the corresponding nodes were also recovered in the tree generated with one of these algorithms. Bar, 0.05 substitutions per nucleotide position.

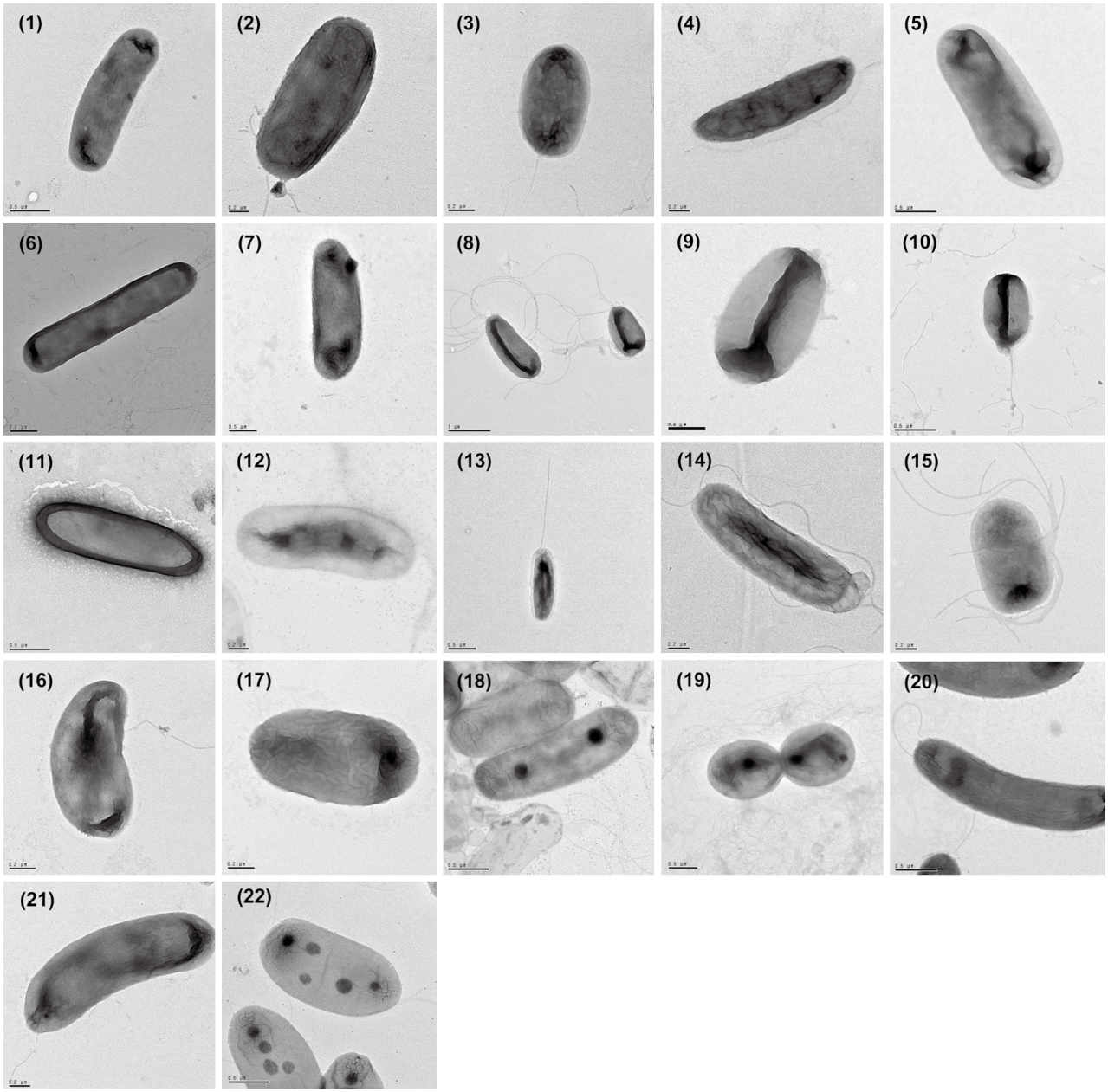

Fig. 2. Transmission electron micrographs of cells of the strains isolated in the study. Strains: 1, IMCC34522 (0.5 μm); 2, IMCC34590 (0.2 μm); 3, IMCC34630 (0.2 μm); 4, IMCC34661 (0.2 μm); 5, IMCC34666 (0.5 μm); 6, IMCC34578 (0.5 μm); 7, IMCC34610 (0.5 μm); 8, IMCC34605 (1.0 μm); 9, IMCC34726 (0.2 μm); 10, IMCC34658 (0.5 μm); 11, IMCC34655 (0.5 μm); 12, IMCC34711 (0.2 μm); 13, IMCC34728 (0.5 μm); 14, IMCC34689 (0.2 μm); 15, IMCC34713 (0.2 μm); 16, IMCC34644 (0.2 μm); 17, IMCC34656 (0.2 μm); 18, IMCC34688 (0.5 μm); 19, IMCC34510 (0.5 μm); 20, IMCC34659 (0.5 μm); 21, IMCC34607 (0.2 μm); 22, IMCC34525 (0.5 μm). Scale bars are indicated in parenthesis after strain ID.

The 22 unrecorded bacterial species were Gram-staining-negative, flagellated or non-flagellated, rod- or coccoid-shaped bacteria (Fig. 2). Detailed morphological and physiological characteristics of the isolates are given in the strain descriptions. Based on the results from the present study, the 22 bacterial isolates were classified as members of *Flavobacterium collinsii*, *Flexibacter aurantiacus*, *Brevundimonas alba*, *Brevundimonas intermedia*, *Rhizobium capsica*, *Xanthobacter autotrophicus*, *Roseomonas tokyonensis*, *Elstera litoralis*, *Blastomonas natatorial*, *Sphingomonas yantingensis*, *Cupriavidus pinatubonensis*, *Albidiferax ferrireducens*, *Curvibacter fontanus*, *Hydrogenophaga intermedia*, *Limnohabitans australis*, *Polaromonas aquatica*, *Variovorax guangxiensis*,

Undibacterium squillarum, *Iodobacter limnosediminis*, *Ferribacterium limneticum*, *Pseudomonas abietaniphila*, and *Arcobacter venerupis*, which have not been officially reported in Korea (Kang and Yoon, 2015). Therefore, here we describe the characteristics of these unrecorded bacterial species.

Description of *Flavobacterium collinsii* IMCC34522

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are irregular, undulate, and raised after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid are utilized as sole carbon sources, but not capric acid and adipic acid. Strain IMCC34522 (=NNIBRBA134) was isolated from river sediment, the North Han River basin, Korea.

Description of *Flexibacter aurantiacus* IMCC34590

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, urease, and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and β-galactosidase in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34590 (=NNIBRBA137) was isolated from river water, the North Han River basin, Korea.

Description of *Brevundimonas alba* IMCC34630

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34630 (=NNIBRBA141) was isolated from river water, the North Han River basin, Korea.

Description of *Brevundimonas intermedia* IMCC34661

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and raised after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis in API 20NE. D-Glucose, D-mannose, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized as sole carbon sources, but not L-arabinose, D-mannitol, *N*-acetyl-glucosamine, and capric acid. Strain IMCC34661 (=NNIBRBA147) was isolated from river water, the North Han River basin, Korea.

Description of *Rhizobium capsici* IMCC34666

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and raised after incubation for 3 days on R2A at 25°C. Positive for arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate are utilized as sole carbon sources, but not capric acid, adipic acid, and phenylacetic acid. Strain IMCC34666 (=NNIBRBA148) was isolated from river water, the North Han River basin, Korea.

Description of *Xanthobacter autotrophicus* IMCC34578

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, arginine dihydrolase, and urease, but negative for indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34578 (=NNIBRBA136) was isolated from river water, the North Han River basin, Korea.

Description of *Roseomonas tokyonensis* IMCC34610

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and cocci-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for urease and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fer-

mentation, arginine dihydrolase, gelatin hydrolysis, and β-galactosidase in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34610 (=NNIBRBA140) was isolated from river water, the North Han River basin, Korea.

Description of *Elstera litoralis* IMCC34605

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, L-arabinose, and D-mannitol are utilized as sole carbon sources, but not D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34605 (=NNIBRBA138) was isolated from a wetland water sample, the North Han River basin, Korea.

Description of *Blastomonas natatoria* IMCC34726

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Maltose is utilized as sole carbon sources, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34726 (=NNIBRBA153) was isolated from a wetland water sample, the North Han River basin, Korea.

Description of *Sphingomonas yantingensis* IMCC34658

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and raised after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, and malic acid are utilized as sole carbon sources, but not D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34658 (=NNIBRBA145) was isolated from a wetland water sample, the North Han River basin, Korea.

Description of *Cupriavidus pinatubonensis* IMCC34655

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and raised after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction and potassium gluconate, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. Capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized as sole carbon sources, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and D-maltose. Strain IMCC34655 (=NNIBRBA143) was isolated from river sediment, the North Han River basin, Korea.

Description of *Albidiferax ferrireducens* IMCC34711

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34711 (=NNIBRBA151) was isolated from river sediment, the North Han River basin, Korea.

Description of *Curvibacter fontanus* IMCC34728

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for arginine dihydrolase and urease, but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34728 (=NNIBRBA154) was isolated from a wetland water sample, the North Han River basin, Korea.

Description of *Hydrogenophaga intermedia* IMCC34689

Cells are Gram-stain-negative, flagellated, non-pig-

mented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, arginine dihydrolase, and urease, but negative for indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. Potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized as sole carbon sources, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, and capric acid. Strain IMCC34689 (=NNIBRBA150) was isolated from river sediment, the North Han River basin, Korea.

Description of *Limnohabitans australis* IMCC34713

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid, and trisodium citrate are utilized as sole carbon sources, but not potassium gluconate, capric acid, adipic acid, and phenylacetic acid. Strain IMCC34713 (=NNIBRBA152) was isolated from river sediment, the North Han River basin, Korea.

Description of *Polaromonas aquatica* IMCC34644

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, D-mannose, and D-maltose are utilized as sole carbon sources, but not L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34644 (=NNIBRBA142) was isolated from a river water sample, the North Han River basin, Korea.

Description of *Variovorax guangxiensis* IMCC34656

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, D-mannitol, potassium gluconate, malic acid, and phenylacetic acid are utilized as sole carbon sources, but not L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, and trisodium citrate. Strain IMCC34656 (=NNIBRBA144) was isolated from a wetland water sample, the North Han River basin, Korea.

Description of *Undibacterium squillarum* IMCC34688

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Maltose is utilized as sole carbon sources, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34688 (=NNIBRBA149) was isolated from river sediment sample, the North Han River basin, Korea.

Description of *Iodobacter limnosediminis* IMCC34510

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and short-rod-shaped. Colonies are irregular, undulate, and flat after incubation for 3 days on R2A at 25°C. Positive for nitrate reduction, glucose fermentation, and arginine dihydrolase, but negative for indole production, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, and potassium gluconate are utilized as sole carbon sources, but not D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34510 (=NNIBRBA133) was isolated from river sediment, the North Han River basin, Korea.

Description of *Ferribacterium limneticum* IMCC34659

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Positive for arginine dihydrolase and urease, but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34659 (=NNIBRBA146) was isolated from a wetland water sample, the North Han River basin, Korea.

Description of *Pseudomonas abietaniphila* IMCC34607

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and convex after incubation for 3 days on R2A at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, and *N*-acetyl-glucosamine in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, and trisodium citrate are utilized as sole carbon sources, but not D-maltose, adipic acid, and phenylacetic acid. Strain IMCC34607 (=NNIBRBA139) was isolated from a wetland water sample, the North Han River basin, Korea.

Description of *Arcobacter venerupis* IMCC34525

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, and raised after incubation for 3 days on R2A at 25°C. Positive for arginine dihydrolase and urease, but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. 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 are not utilized as sole carbon sources. Strain IMCC34525 (=NNIBRBA135) was isolated from river sediment, the North Han River basin, Korea.

Acknowledgements

This study was supported by the research grant "Survey of freshwater organisms and specimen collection (Prokaryotes)" from Nakdonggang National Institute of Biological Resources of the Ministry of Environment in Korea.

References

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368-376.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406-416.

Han, S.K., I.G. Lee and T.Y. Ahn. 1998. Bacterial diversity of the Han River as determined by 16S rRNA gene analysis. Kor J Microbiol 34:194-199.

Jeon, Y.S., K. Lee, S.C. Park, B.S. Kim, Y.J. Cho, S.M. Ha and J. Chun. 2014. EzEditor: a versatile sequence alignment editor for both rRNA- and protein-coding genes. Int J Syst Evol Microbiol 64:689-691.

Kang, M.-S. and J.-H. Yoon. 2015. National List of Species of Korea [Prokaryotes]: National Institute of Biological Resources.

Kara, E.L., P.C. Hanson, Y.H. Hu, L. Winslow and M.D. McMahon. 2013. A decade of seasonal dynamics and co-occurrences within freshwater bacterioplankton communities from eutrophic Lake Mendota, WI, USA. ISME J 7:680-684.

Kim, H., D. Kaown, C. Kim and S. Lee. 2016. Analysis of bacterial diversity in water from the Han River water source protection area via a pyrosequencing Assay. Kor J Environ Health Sci 42:274-279.

Kim, O.S., Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H. Yi, S. Won and J. Chun. 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. Rare variant alleles in the light of the neutral theory. Mol Biol Evol 1:84-93.

Lozupone, C.A. and R. Knight. 2007. Global patterns in bacterial diversity. Proc Natl Acad Sci USA 104:11436-11440.

Lee, J.-J., E.S. Joo, D.H. Lee, H.-Y. Jung and M.K. Kim. 2016. Phylogenetic diversity and UV resistance analysis of radiation-resistant bacteria isolated from the water in Han River. Kor J Microbiol 52:65-73.

Newton, R.J., S.E. Jones, A. Eiler, K.D. McMahon and S. Bertilsson. 2011. A guide to the natural history of freshwater lake bacteria. Microbiol Mol Biol Rev 75:1449.

Rheinheimer, G. 1980. Aquatic microbiology. Wiley, New York.

Saitou, N. and M. Nei. 1987. The neighbor-joining method-a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725-2729.

Wetzel, R.G. 2001. Limnology: lake and river ecosystems. Gulf Professional Publishing.

Williamson, C.E., W. Dodds, T.K. Kratz and M.A. Palmer. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Front Ecol Environ 6:247-254.

Yang, S.-J. and J.-C. Cho. 2008. *Gaetbulibacter marinus* sp. nov., isolated from coastal seawater, and emended description of the genus *Gaetbulibacter*. Int J Syst Evol Microbiol 58:315-318.

Submitted: March 9, 2018
Revised: July 13, 2018
Accepted: July 25, 2018