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A report of 26 unrecorded bacterial species in Korea, isolated from urban streams of the Han River watershed in 2018

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Owing to a distinct environmental regime and anthropogenic effects, freshwater bacterial communities of urban streams are considered to be different from those of large freshwater lakes and rivers. To obtain unrecorded, freshwater bacterial species in Korea, water and sediment samples were collected from various urban streams of the Han River watershed in 2018. After plating the freshwater samples on R2A agar, approximately 1000 bacterial strains were isolated from the samples as single colonies and identified using 16S rRNA gene sequence analyses. A total of 26 strains, with >98.7% 16S rRNA gene sequence similarity with validly published bacterial species but not reported in Korea, were determined to be unrecorded bacterial species in Korea. The unrecorded bacterial strains were phylogenetically diverse and belonged to four phyla, six classes, 12 orders, 16 families, and 21 genera. At the generic level, the unreported species were assigned to *Nocardioides*, *Streptomyces*, *Microbacterium*, *Kitasatospora*, *Herbiconiux*, *Corynebacterium*, and *Microbacterium* of the class *Actinobacteria*; *Paenibacillus* and *Bacillus* of the class *Bacilli*; *Caulobacter*, *Methylobacterium*, *Novosphingobium*, and *Porphyrobacter* of the class *Alphaproteobacteria;* *Aquabacterium*, *Comamonas*, *Hydrogenophaga*, *Laribacter*, *Rivicola*, *Polynucleobacter*, and *Vogesella* of the class *Betaproteobacteria*; *Arcobacter* of the class *Epsilonproteobacteria*; and *Flavobacterium* of the class *Flavobacteriia*. The details of the 26 unreported species, including Gram reaction, colony and cell morphology, biochemical properties, and phylogenetic position are also provided in the strain descriptions.

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

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Introduction

Aquatic bacteria residing in lakes, rivers, streams, and groundwater play a crucial role in the terrestrial ecosystem for climate control, nutrient cycling, and energy conservation (Wetzel, 2001; Williamson *et al*., 2008). Although *in situ* biogeochemical and molecular microbial ecology studies have identified high microbial diversity in aquatic ecosystems, many predominant and key microbial taxa have yet to be cultivated (Newton *et al*., 2011). Recent cultivation efforts employing the dilution-to-extinction method have shown that major or key bacterial taxa could be cultivated under the culturing conditions mimicking natural environmental conditions (Kang *et al*., 2017; Kim *et al*., 2017; Neuenschwander *et al*., 2018).

Since 2016, the Nakdonggang National Institute of Biological Resources has supported microbial taxonomists and ecologists to describe new or unrecorded bacterial species of Korea, under the research program of "Survey of freshwater organisms and specimen collection (Prokaryotes)". With this research program, we previously described unrecorded freshwater bacterial species of Korea, isolated from Soyang and Chungju Lakes in 2016 (Jeon *et al*., 2017) and from the Han River in 2017 (Joung *et al*., 2018). The present study is a part of this research program, focusing on the isolation of unrecorded bacterial species of Korea from diverse urban streams.

The Han River consists of two major rivers, the North Han River and the South Han River, along with many minor streams. After traversing the mid-western part of the Korean Peninsula, the two rivers merge at Yangsuri and form the Han River. The Han River flows through Seoul,

the largest metropolitan city in Korea, and is connected to the Yellow Sea. Many streams, such as Wangsuk, Jungnag, Gongneung, and Gulpo, flow into metropolitan cities and constitute the Han River watershed. Influenced by human activity, freshwater bacterial communities of urban streams are distinct from those of large freshwater lakes, reservoirs, and rivers and thus may contain new bacterial taxa that have not been reported previously in Korea.

To investigate the diversity of bacterial colony-formers present in urban streams, diverse water or sediment samples were collected from six streams of the Han River watershed, Korea, in 2018. As a result of 16S rRNA gene-based phylogenetic analyses of isolated stains, 26 bacterial species were determined as unrecorded bacterial species in Korea. Herein we report the taxonomic information and phenotypic characteristics of the unrecorded bacterial species.

Materials and Methods

Various water and sediment samples were collected from wetland, urban streams of the Han River watershed, and Soyang Lake in 2018 (Fig. 1). Using a spread plating technique, 100 μL of the samples were spread onto R2A agar (BD Difco) and subsequently incubated at 20−25°C for 2−7 days. Bacterial strains were purified as single colonies after several serial dilution spreading and the pure cultures were preserved at −80°C in 20% (v/v) glycerol suspension, as well as lyophilized ampoules. The designation of the strains and source of isolation are presented in Table 1.

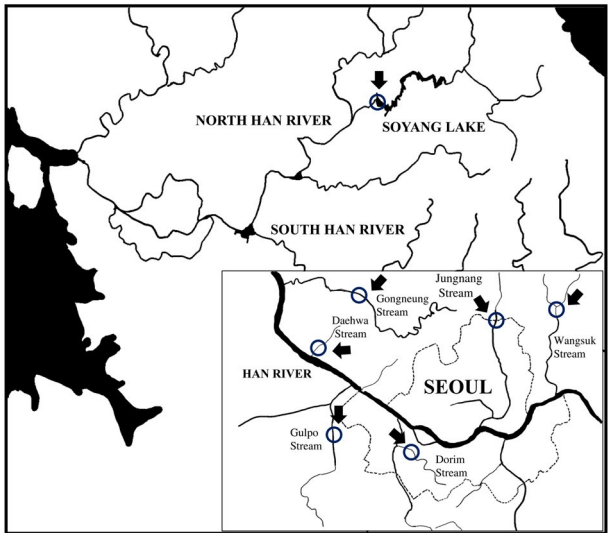

Fig. 1. Map of sampling stations. Open circles and arrows indicate the sampling stations where freshwater and sediment samples were collected in the Han River watershed.

Colony morphology of the bacterial strains was observed on agar plates with a magnifying glass after the cells grew to the stationary phase. Cellular morphology, presence of flagella, and cell size were examined by transmission electron microscopy (CM200; Philips). Gram staining was performed using a Gram-staining kit (bioMérieux) and the KOH method. Biochemical characteristics were determined using API 20NE galleries (bioMérieux) according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures as previously described (Yang and Cho, 2008). For the determination of 16S rRNA gene sequences, primers 27F, 518F, 800R and 1492R were used. The 16S rRNA gene sequences were initially compared with those of other bacterial strains with validly published names using the EzTaxon-e server (Kim *et al*., 2012). The cutoff value of 98.7% 16S rRNA gene sequence similarity was applied for bacterial species demarcation. Bacterial strains showing 98.7% or higher 16S rRNA gene sequence similarities with validly published species, but never reported in Korea were selected as unreported bacterial species. For phylogenetic analyses, multiple sequence alignments between the 16S rRNA gene sequences of the bacterial isolates and those of the closely related type strains were performed using ClustalW and manually checked with EzEditor (Jeon *et al*., 2014). Using the unambiguously aligned 16S rRNA gene sequences, phylogenetic trees were reconstructed by employing neighbor-joining (Saitou and Nei, 1987), maximum parsimony (Fitch, 1971), and maximum likelihood (Felsenstein, 1981) methods in MEGA 6.0 (Tamura *et al*., 2013). The robustness of the inferred phylogenetic trees was evaluated by bootstrap analyses based on 1000 random re-samplings (Felsenstein, 1985).

Results and Discussion

After analyses of the 16S rRNA gene sequences obtained from approximately 1000 bacterial strains, many strains belonging to novel and previously-unreported bacterial species were identified. A total of 26 strains were identified as unrecorded bacterial species in Korea. The strain information, identification, taxonomic assignment, and sequence accession numbers are listed in Table 1. The assignment of the strains to established bacterial species based on 16S rRNA gene sequence similarity was confirmed by phylogenetic inference (Fig. 2), where each bacterial isolate was the member of a robust clade with high bootstrap values with its most closely related species.

The 26 unrecorded bacterial species were phylogenetically diverse, belonging to four phyla, six classes, 12 orders, 16 families, and 21 genera. The unrecorded species

Table 1. Summary of isolated strains from the freshwater 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		
Actinobacteria	*Corynebacteriales*	*Corynebacteriaceae*	IMCC34927	NNIBR2018143BA579	MK138649	*Corynebacterium efficiens*	BA000035	99.71	Water
	Propionibacteriales	*Nocardioidaceae*	IMCC34902	NNIBR2018143BA555	MK138625	*Nocardioides cavernae*	KX815990	99.51	Sediment
	Micrococcales	*Microbacteriaceae*	IMCC34917	NNIBR2018143BA570	MK138640	*Herbiconiux flava*	AB583921	100	Water
			IMCC34941	NNIBR2018143BA593	MK226317	*Microbacterium aureliae*	KF793922	98.75	Sediment
			IMCC34912	NNIBR2018143BA565	MK138635	*Microbacterium keratanolyticum*	AB004717	99.86	Sediment
	Streptomycetales	*Streptomycetaceae*	IMCC34913	NNIBR2018143BA566	MK138636	*Kitasatospora aburaviensis*	AY999779	98.92	Sediment
			IMCC34906	NNIBR2018143BA559	MK138629	*Streptomyces canus*	KQ948708	99.71	Water
Bacilli	*Bacillales*	*Bacillaceae*	IMCC34903	NNIBR2018143BA556	MK138626	*Bacillus zhangzhouensis*	JOTP01000061	99.73	Sediment
		Paenibacillaceae	IMCC34901	NNIBR2018143BA554	MK138624	*Paenibacillus mucilaginosus*	FJ039528	99.59	Water
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	IMCC34935	NNIBR2018143BA603	MK256313	*Flavobacterium branchiarum*	HE612097	98.83	Water
			IMCC34944	NNIBR2018143BA595	MK226319	*Flavobacterium nitratireducens*	FR827898	100	Water
Alphaproteobacteria	*Caulobacterales*	*Caulobacteraceae*	IMCC34905	NNIBR2018143BA558	MK138628	*Caulobacter rhizosphaerae*	KX792139	99.41	Water
	Rhizobiales	*Methylobacteriaceae*	IMCC34930	NNIBR2018143BA582	MK138652	*Methylobacterium adhaesivum*	AM040156	99.48	Water
	Sphingomonadales	*Erythrobacteraceae*	IMCC34934	NNIBR2018143BA602	MK256312	*Porphyrobacter colymbi*	MUYK01000003	99.49	Water
		Sphingomonadaceae	IMCC34933	NNIBR2018143BA601	MK217371	*Novosphingobium fuchskuhlense*	KQ954244	99.71	Water
Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	IMCC34928	NNIBR2018143BA580	MK138650	*Polynucleobacter cosmopolitanus*	NJGG01000001	99.93	Water
			IMCC34925	NNIBR2018143BA577	MK138647	*Polynucleobacter sinensis*	LOJJ01000001	100	Water
			IMCC34948	NNIBR2018143BA599	MK226323	*Polynucleobacter victoriensis*	FYEX01000001	99.57	Water
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	IMCC34942	NNIBR2018143BA594	MK226318	*Aquabacterium commune*	AF035054	98.92	Water
			IMCC34946	NNIBR2018143BA597	MK226321	*Comamonas thiooxydans*	BBVD01000034	99.86	Water
			IMCC34914	NNIBR2018143BA567	MK138637	*Hydrogenophaga flava*	BCTF01000049	98.71	Water
Betaproteobacteria	*Neisseriales*	*Chromobacteriaceae*	IMCC34909	NNIBR2018143BA562	MK138632	*Laribacter hongkongensis*	AUHR01000037	100	Water
			IMCC34910	NNIBR2018143BA563	MK138633	*Vogesella amnigena*	LK056648	99.04	Water
		Neisseriaceae	IMCC34916	NNIBR2018143BA569	MK138639	*Rivicola pingtungensis*	JN104394	99.78	Water
Epsilonproteobacteria	*Campylobacterales*	*Campylobacteraceae*	IMCC34908	NNIBR2018143BA561	MK138631	*Arcobacter aquimarinus*	HG932574	99.49	Water
			IMCC34926	NNIBR2018143BA578	MK138648	*Arcobacter suis*	FJ573216	99.42	Water

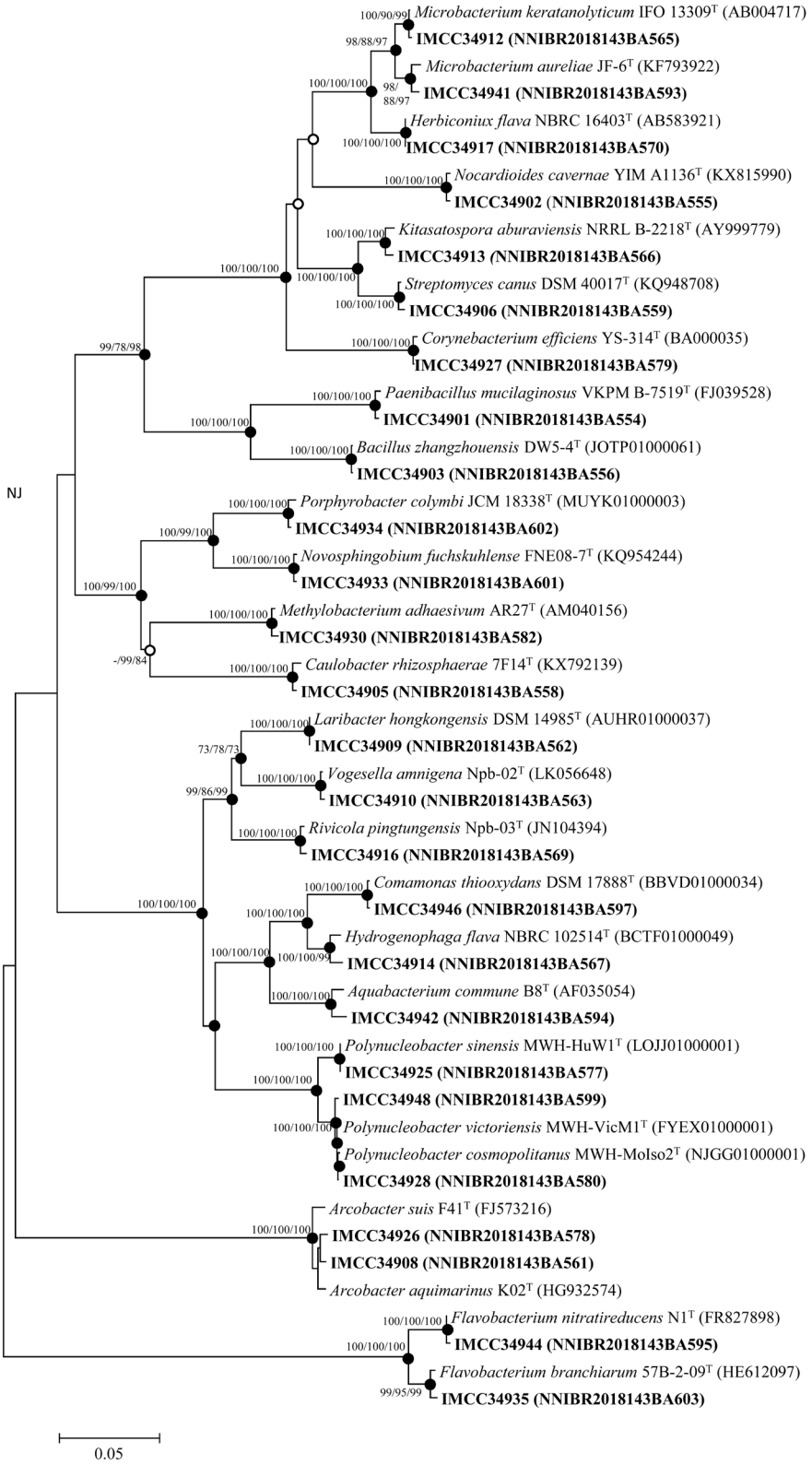

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest bacterial species. Bootstrap values over 70% are shown at nodes for neighbor-joining, maximum parsimony, and maximum likelihood methods, respectively. Filled circles indicate that the corresponding node was also recovered in the trees reconstructed with both the maximum parsimony and maximum likelihood algorithms, while open circles indicate that the corresponding node was recovered in the tree generated with only one of these algorithms. Scale bar = 0.05 substitutions per nucleotide position.

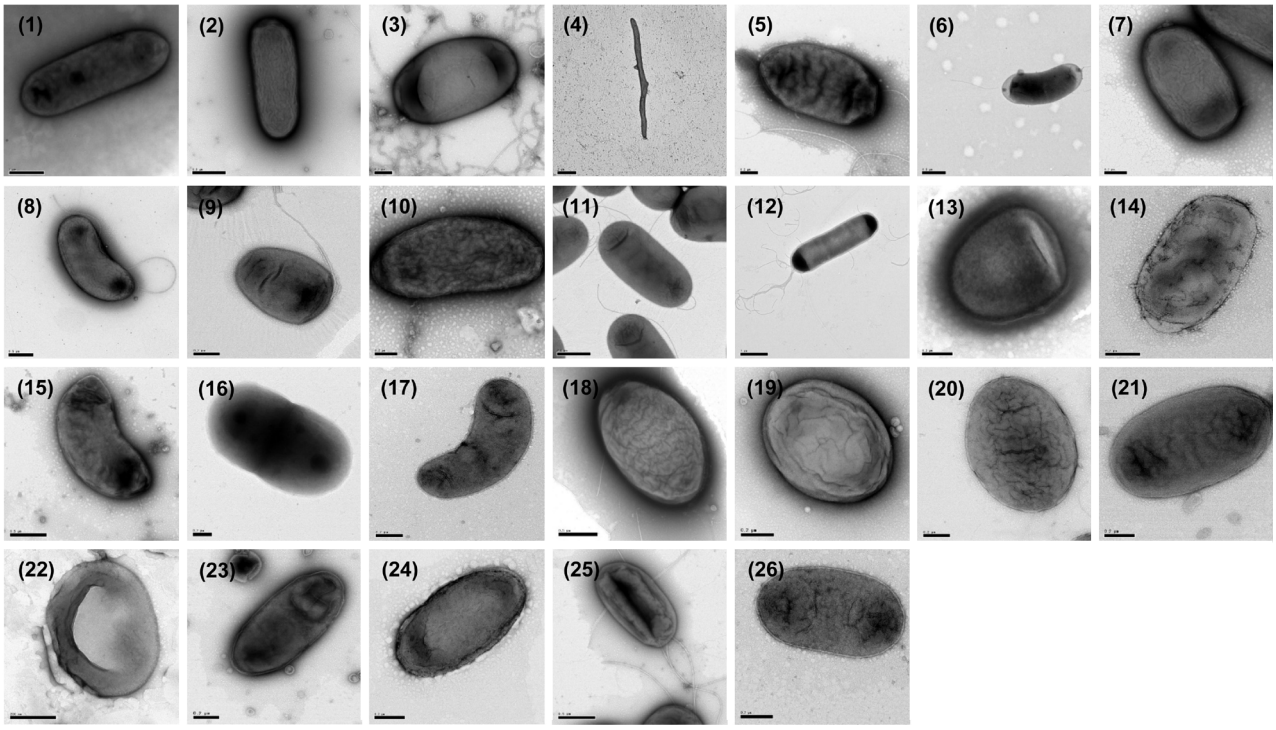

Fig. 3. Transmission electron micrographs of cells of the strains isolated in this study. Strains: 1, IMCC34901 (1.0 μm); 2, IMCC34902 (0.5 μm); 3, IMCC34903 (0.2 μm); 4, IMCC34905 (2.0 μm); 5, IMCC34906 (0.2 μm); 6, IMCC34908 (0.5 μm); 7, IMCC34909 (0.7 μm); 8, IMCC34910 (0.5 μm); 9, IMCC34912 (0.7 μm); 10, IMCC34913 (0.2 μm); 11, IMCC34914 (0.5 μm); 12, IMCC34916 (1.0 μm); 13, IMCC34917 (0.2 μm); 14, IMCC34925 (0.7 μm); 15, IMCC34926 (0.5 μm); 16, IMCC34927 (0.7 μm); 17, IMCC34928 (0.7 μm); 18, IMCC34930 (0.5 μm); 19, IMCC34933 (0.2 μm); 20, IMCC34934 (0.2 μm); 21, IMCC34935 (0.2 μm); 22, IMCC34941 (0.2 μm); 23, IMCC34942 (0.2 μm); 24, IMCC34944 (0.2 μm); 25, IMCC34946 (0.5 μm); 26, IMCC34948 (0.2 μm). Sizes of scale bars are indicated in parenthesis after the strain ID.

were assigned to seven species of the class *Actinobacteria*, two species of the class *Bacilli*, two species of the class *Flavobacteriia*, four species of the class *Alphaproteobacteria*, nine species of the class *Betaproteobacteria*, and two species of the class *Epsilonproteobacteria* (Table 1). At the generic level, these unreported species belonged to the genera *Corynebacterium*, *Nocardioides*, *Herbiconiux*, *Microbacterium*, *Kitasatospora*, and *Streptomyces* of the class *Actinobacteria*; *Bacillus* and *Paenibacillus* of the class *Bacilli*; *Flavobacterium* of the class *Flavobacteriia*; *Caulobacter*, *Methylobacterium*, *Porphyrobacter*, and *Novosphingobium* of the class *Alphaproteobacteria*; *Polynucleobacter*, *Aquabacterium*, *Comamonas*, *Hydrogenophaga*, *Laribacter*, *Vogesella*, and *Rivicola* of the class *Betaproteobacteria*; and *Arcobacter* of the class *Epsilonproteobacteria*.

The 26 unrecorded bacterial species were Gram-staining-negative, flagellated or non-flagellated, short- or straight-rod or coccoid-shaped bacteria (Fig. 3). Detailed morphological and physiological characteristics of the unrecorded bacterial species determined in this study are given in the following strain descriptions.

Description of *Paenibacillus mucilaginosus* IMCC34901

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for indole production, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and oxidase 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 IMCC34901 (=NNIBR2018143BA554) was isolated from a freshwater sample, Daehwa Stream, Ilsan, Gyeonggi-do, Korea.

Description of *Nocardioides cavernae* IMCC34902

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Positive for nitrate reduction, urease, esculin hydrolysis, and β-galactosidase, but negative for oxidase, indole pro-

duction, glucose fermentation, arginine dihydrolase, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, and malic acid are utilized as sole carbon sources, but not capric acid, adipic acid, trisodium citrate, phenylacetic acid, and potassium gluconate. Strain IMCC34902 (=NNIBR2018143BA555) was isolated from a sediment sample, Daehwa Stream, Ilsan, Gyeonggi-do, Korea.

Description of *Bacillus zhangzhouensis* IMCC34903

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease 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 IMCC34903 (=NNIBR2018143BA556) was isolated from a sediment sample, Daehwa Stream, Ilsan, Gyeonggi-do, Korea.

Description of *Caulobacter rhizosphaerae* IMCC34905

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Maltose is utilized as a sole carbon source, 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 IMCC34905 (=NNIBR2018143BA558) was isolated from a freshwater sample, Gongneung Stream, Paju, Gyeonggi-do, Korea.

Description of *Streptomyces canus* IMCC34906

Cells are Gram-stain-positive, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and β-galactosidase, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, adipic acid, and malic acid are utilized as sole carbon sources, but not potassium gluconate, capric acid, trisodium citrate, and phenylacetic acid. Strain IMCC34906 (=NNIBR2018143BA559) was isolated from a freshwater sample, Gongneung Stream, Paju, Gyeonggi-do, Korea.

Description of *Arcobacter aquimarinus* IMCC34908

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for nitrate reduction and esculin hydrolysis, but negative for oxidase, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, 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 IMCC34908 (=NNIBR2018143BA561) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Laribacter hongkongensis* IMCC34909

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase and esculin hydrolysis, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, urease, and β-galactosidase in API 20NE. Capric acid, adipic acid, and malic acid are utilized as sole carbon sources, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, trisodium citrate, and phenylacetic acid. Strain IMCC34909 (=NNIBR2018143BA562) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Vogesella amnigena* IMCC34910

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase and nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, esculin hydrolysis, and β-galactosidase in API 20NE. D-Glucose is utilized as a sole carbon source, but not L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, *N*-acetyl-glucosamine, and D-maltose. Strain IMCC34910 (=NNIBR2018143BA563) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Microbacterium keratanolyticum* IMCC34912

Cells are Gram-stain-positive, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and en-

tire after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and β-galactosidase, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Glucose, D-mannose, and *N*-acetyl-glucosamine are utilized as sole carbon sources, but not L-arabinose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34912 (=NNIBR2018143BA565) was isolated from a sediment sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Kitasatospora aburaviensis* IMCC34913

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and undulate after incubation for 3 days on R2A at 20°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase, but negative for oxidase, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease 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 IMCC34913 (=NNIBR2018143BA566) was isolated from a sediment sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Hydrogenophaga flava* IMCC34914

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, nitrate reduction, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, and malic acid are utilized as sole carbon sources, but not D-mannitol, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34914 (=NNIBR2018143BA567) was isolated from a freshwater sample, Jungnang Stream, Dobong, Seoul, Korea.

Description of *Rivicola pingtungensis* IMCC34916

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, nitrate reduction and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, and esculin hydrolysis 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 IMCC34916 (=NNIBR2018143BA569) was isolated from a freshwater sample, Jungnang Stream, Dobong, Seoul, Korea.

Description of *Herbiconiux flava* IMCC34917

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and short rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and β-galactosidase, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, and malic acid are utilized as sole carbon sources, but not *N*-acetyl-glucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34917 (=NNIBR2018143BA570) was isolated from a freshwater sample, Wangsuk Stream, Namyangju, Gyeonggi-do, Korea.

Description of *Polynucleobacter sinensis* IMCC34925

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, esculin 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 IMCC34925 (=NNIBR2018143BA577) was isolated from a freshwater sample, Daehwa Stream, Ilsan, Gyeonggi-do, Korea.

Description of *Arcobacter suis* IMCC34926

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, 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 IMCC34926 (=NNIBR2018143BA578) was isolated from a freshwater sample, Soyang Lake, Chuncheon, Gangwon-do, Korea.

Description of *Corynebacterium efficiens* IMCC34927

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex,

and entire after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and β-galactosidase, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Glucose, L-arabinose, trisodium citrate and phenylacetic acid are utilized as sole carbon sources, but not D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, and malic acid. Strain IMCC34927 (=NNIBR2018143BA579) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Polynucleobacter cosmopolitanus* IMCC34928

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, esculin 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 IMCC34928 (=NNIBR2018143BA580) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Methylobacterium adhaesivum* IMCC34930

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, urease, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, potassium gluconate, malic acid, and trisodium citrate are utilized as sole carbon sources, but not D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, phenylacetic acid, and adipic acid. Strain IMCC34930 (=NNIBR2018143BA582) was isolated from a freshwater sample, Jungnang Stream, Dobong, Seoul, Korea.

Description of *Novosphingobium fuchskuhlense* IMCC34933

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and coccus-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 28°C. Positive for oxidase and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, 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 IMCC34933 (=NNIBR2018143BA601) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Porphyrobacter colymbi* IMCC34934

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for 3 days on R2A at 28°C. Positive for oxidase and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, and esculin hydrolysis in API 20NE. D-Maltose and malic acid 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, trisodium citrate, and phenylacetic acid. Strain IMCC34934 (=NNIBR2018143BA602) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Flavobacterium branchiarum* IMCC34935

Cells are Gram-stain-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are irregular, convex, undulate after incubation for 3 days on R2A at 28°C. Positive for oxidase, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, and malic acid are utilized as sole carbon sources, but not L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34935 (=NNIBR2018143BA603) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Microbacterium aureliae* IMCC34941

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, nitrate reduction, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease in API 20NE. D-Glucose, D-mannitol, and D-maltose are utilized as sole carbon sources, but not L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassi-

um gluconate, capric acid , adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34941 (=NNIBR2018143BA593) was isolated from a sediment sample, Daehwa Stream, Ilsan, Gyeonggi-do, Korea.

Description of *Aquabacterium commune* IMCC34942

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, nitrate reduction, and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, 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 IMCC34942 (=NNIBR2018143BA594) was isolated from a freshwater sample, Daehwa Stream, Ilsan, Gyeonggi-do, Korea.

Description of *Flavobacterium nitratireducens* IMCC34944

Cells are Gram-stain-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Positive for oxidase, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and urease 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 IMCC34944 (=NNIBR2018143BA595) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

Description of *Comamonas thiooxydans* IMCC34946

Cells are Gram-stain-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Positive for nitrate reduction and esculin hydrolysis, but negative for oxidase, indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, urease, and β-galactosidase in API 20NE. Potassium gluconate is utilized as a sole carbon source, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized as sole carbon sources. Strain IMCC34946 (=NNIBR2018143BA597) was isolated from a freshwater sample, Gongneung Stream, Paju, Gyeonggi-do, Korea.

Description of *Polynucleobacter victoriensis* IMCC34948

Cells are Gram-stain-positive, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, round, and entire after incubation for 3 days on R2A at 20°C. Positive for nitrate reduction, gelatin hydrolysis, and esculin hydrolysis, but negative for oxidase, indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase in API 20NE. D-Glucose and D-mannitol are utilized as sole carbon sources, but not L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34948 (=NNIBR2018143BA599) was isolated from a freshwater sample, Gulpo Stream, Bucheon, Gyeonggi-do, Korea.

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References

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

Jeon, H.T., Y. Joung, S. Kim, Y. Lim and J.-C. Cho. 2017. A report on 17 unrecorded bacterial species in Korea isolated from Lakes Soyang and Chungju in 2016. J. Species Res. 6:163-170.

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.

Joung, Y., M. Park, H.-J. Jang, I. Jung and J.-C. Cho. 2018. A report of 22 unrecorded bacterial species in Korea, isolated from the North Han River basin in 2017. J. Species Res. 7:193-201.

Kang, I., S. Kim, M.R. Islam and J.-C. Cho. 2017. The first complete genome sequences of the acI lineage, the most abundant freshwater *Actinobacteria*, obtained by whole-genome-amplification of dilution-to-extinction cultures. Sci. Rep. 7:42252.

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.

Kim, S., I. Kang and J.-C. Cho. 2017. Genomic analysis of a freshwater actinobacterium, "*Candidatus* Limnosphaera

aquatica" strain IMCC26207, isolated from Lake Soyang. J. Microbiol. Biotechnol. 27:825-833.

Neuenschwander, S.M., R. Ghai, J. Pernthaler and M.M. Salcher. 2018. Microdiversification in genome-streamlined ubiquitous freshwater *Actinobacteria*. Isme J. 12:185-198.

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. 75:14-49.

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.

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