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A report of 28 unrecorded bacterial species in Korea, isolated from freshwater and sediment of the Han River watershed in 2020

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To obtain unrecorded freshwater bacterial species in Korea, water and sediment samples were collected from streams, lakes, and wetland of the Han River watershed in 2020. Approximately 800 bacterial strains were isolated on R2A agar after aerobic or anaerobic incubation, and identified using 16S rRNA gene sequences. A total of 28 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, eight classes, 13 orders, 19 families, and 25 genera. The unreported species were assigned to *Acetobacter*, *Alsobacter*, *Mesorhizobium*, *Prosthecomicrobium*, and *Microvirga* of the class *Alphaproteobacteria*; *Vogesella*, *Formosimonas*, *Aquincola*, *Massilia*, *Acidovorax*, and *Brachymonas* of the class *Betaproteobacteria*; *Pseudoxanthomonas*, *Thermomonas*, *Lysobacter*, *Enterobacter*, *Kosakonia*, and *Acinetobacter* of the class *Gammaproteobacteria*; *Sulfuricurvum* of the class *Epsilonproteobacteria*; *Mycolicibacterium*, *Agromyces*, *Phycicoccus*, and *Microbacterium* of the class *Actinobacteria*; *Paenibacillus* of the class *Bacilli*; *Clostridium* of the class *Clostridia*; and *Flavobacterium* of the class *Flavobacteriia*. The details of the unreported species, including Gram reaction, colony and cell morphology, biochemical characteristics, and phylogenetic position are also provided in the description of the strains.

Keywords: 16S rRNA, anaerobic bacteria, freshwater, Han River, sediment, unrecorded bacterial species

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Introduction

Whereas freshwater ecosystems cover only less than 1% of the Earth's surface, fresh waters are vulnerable to climate change and thus considered to be indicators for global warming. As prokaryotic microorganisms inhabiting freshwater ecosystems play an essential role in ecosystem functioning, it is important to cultivate key microbial taxa for the elucidation of their physiology, metabolism, and ecological niches. In 2006, National Institute of Biological Resources (NIBR) launched the research program of 'The Survey of Korean Indigenous Species,' and in 2016, the Nakdonggang National Institute of Biological Resources (NNIBR) also started a similar research grant named 'Survey of freshwater organisms and specimen collection (Prokaryotes)". Owing to these research programs, many unrecorded or new prokaryotic species have been discovered from diverse freshwater ecosystems on the Korean Peninsula (i.e, Jeon *et al*., 2017; NIBR, 2017; Joung *et al*., 2018; Joung *et al*., 2019).

The study reported herein is a part of the research program supported by NNIBR, focusing on the isolation of unrecorded bacterial species from diverse habitats of the Han River watershed. In 2020, we targeted to isolate previously unrecorded bacterial species in water or sediment samples collected from eight different Han River watershed stations (Hanpo, Anyang, Gyeongan, and Hadong streams; Chungju, Cheongpyong, and Soyang lakes; and Janghang wetland). Bacterial cultivation was conducted under aerobic conditions, except for samples collected from the Gyeongan stream and the Janghang wetland, which were conducted under anaerobic conditions.

Based on the 16S rRNA gene-based phylogenetic analyses herein, 28 bacterial strains assigned to the classes *Alpha-proteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Epsilonproteobacteria*, *Actinobacteria*, *Bacilli*, *Clostridia*, and *Flavobacteriia* were identified as new records for bacterial species in Korea, for which taxonomic information and phenotypic characteristics are reported.

Materials and Methods

Various freshwater and sediment samples were collected from streams (Hanpo, 37°04′42″N, 127°50′19″E; Anyang, 37°32′14″N, 126°53′15″E; Gyeongan, 37°22′27″N, 127°14′37″E; Hadong, 37°41′03″N, 126°38′57″E), large lakes (Chungju, 36°59′57″N, 127°59′49″E; Cheongpyong, 37°42′14″N, 127°26′56″E; Soyang, 37°56′49″N, 127°49′10″E), and wetland (Jangahang, 37°37′14″N, 126°42′27″E) of the Han River water systems in 2020. Using a spread plating technique, 100 μL of the freshwater samples were spread onto R2A agar (BD Diagnostics) and aerobically incubated at 20−25°C for 2−7 days. The sediment samples were collected at a depth of 30 cm using a stainless-steel soil sampler (30 cm long and 2 cm diameter), and immediately transported to the laboratory in an anaerobic jar (Mitsubishi Gas Chemical). After transport to the laboratory, the samples were placed in a vinyl anaerobic chamber (Coy Laboratory Products) filled with $N_2 : H_2 : CO_2$ (90 : 5 : 5). Using a homogenizer (IKA), a 1 g sub-sample was thoroughly mixed with 100 mL of sterile lake water. An aliquot (100 μL) of the homogenized sample was spread onto R2A agar and anaerobically incubated in an anaerobic jar at 20−25°C for 2−7 days. Bacterial strains were purified as single colonies after several serial dilution spreadings, 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.

For the determination of colony morphology, bacterial colonies were observed with a magnifying glass after reaching the stationary phase on r2A agar plates. Cellular morphology, including cell shape, presence of flagella, and cell size, were examined using a transmission electron microscope (CM200; Philips) after staining with 2% (w/v) uranyl acetate. Gram staining was performed using a Gram-staining kit (bioMérieux) and the KOH method. API 20NE galleries (bioMérieux) were employed for additional biochemical characterization. In API 20NE, aerobic and facultatively anaerobic bacteria were tested according to the manufacturer's instructions, while anaerobic bacteria were tested using phosphate buffered saline (PBS) solution harboring 1% L-cysteine.

For the determination of phylogenetic position of the isolated strains, bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures as previously described (Yang and Cho, 2008). Partial 16S rRNA gene sequences were obtained using the primers 518F and 800R. The resultant 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). A 16S rRNA gene sequence similarity of 98.7% was used as the cut-off value and applied for bacterial species demarcation (Chun *et al*., 2018). The bacterial isolates exhibiting ≥98.7% 16S rRNA gene sequence similarities with validly published species, but never reported in Korea were determined 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 implemented in MEGA X (Kumar *et al*., 2013). Using the unambiguously aligned 16S rRNA gene sequences, phylogenetic trees were reconstructed by employing neighbor-joining, maximum parsimony, and maximum likelihood methods. The robustness of the inferred phylogenetic trees was evaluated by bootstrap analyses based on 1000 random re-samplings (Felsenstein, 1985).

Results and Discussion

The 16S rRNA gene sequence analyses using approximately 800 bacterial strains revealed that many strains belonged to novel or previously unreported species from Korea. Of these, a total of 28 strains were identified as unrecorded bacterial species in Korea. The strain information, identification, taxonomic assignment from species to classes, and sequence accession numbers including NNIBR and GenBank are listed in Table 1. Phylogenetic assignment of the strains to established bacterial species based on 16S rRNA gene sequence similarity was confirmed by phylogenetic tree analysis (Fig. 1). All strains identified as unrecorded species each formed a robust clade with the type strains of corresponding species (Fig. 1).

The 28 unrecorded bacterial species were phylogenetically diverse, belonging to four phyla, eight classes, 13 orders, 19 families, and 25 genera (Table 1). At the generic level, the unreported species were assigned to *Acetobacter*, *Alsobacter*, *Mesorhizobium*, *Prosthecomicrobium*, and *Microvirga* of the class *Alphaproteobacteria*; *Vogesella*, *Formosimonas*, *Aquincola*, *Massilia*, *Acidovorax*, and *Brachymonas* of the class *Betaproteobacteria*; *Pseudoxanthomonas*, *Thermomonas*, *Lysobacter*, *Enterobacter*, *Kosakonia*, and *Acinetobacter* of the class *Gammaproteobacteria*; *Sulfuricurvum* of the class *Epsilonproteobacteria*; *Mycolicibacterium*, *Agromyces*, *Phycicoccus*, and

Table 1. Summary of the bacterial strains isolated from freshwater habitats and their taxonomic affiliations.

Class	Order	Family	Strain ID	NNIBR	Accession number	Closest species	16S rDNA similarity (%)*
Alphaproteobacteria	*Rhodospirillales*	*Acetobacteraceae*	IMCC33714	2020642BA7	MW261886	*Acetobacter peroxydans*	99.9
	Rhizobiales	*Alsobacteraceae*	IMCC34758	2020642BA9	MW261888	*Alsobacter soli*	98.8
	Rhizobiales	*Phyllobacteriaceae*	IMCC34759	2020642BA10	MW261889	*Mesorhizobium metallidurans*	99.7
	Rhizobiales	*Hyphomicrobiaceae*	IMCC34762	2020642BA13	MW261892	*Prosthecomicrobium hirschii*	99.6
	Rhizobiales	*Methylobacteriaceae*	IMCC34782	2020642BA19	MW261898	*Microvirga flavescens*	99.3
Betaproteobacteria	*Neisseriales*	*Chromobacteriaceae*	IMCC33705	2020642BA3	MW261882	*Vogesella fluminis*	99.0
	Burkholderiales	*Burkholderiaceae*	IMCC34764	2020642BA14	MW261893	*Formosimonas limnophila*	99.2
	Burkholderiales	Not assigned yet	IMCC34767	2020642BA17	MW261896	*Aquincola amnicola*	99.1
	Burkholderiales	*Oxalobacteraceae*	IMCC34768	2020642BA18	MW261897	*Massilia haematophila*	99.0
	Burkholderiales	*Comamonadaceae*	IMCC34789	2020642BA26	MW261905	*Acidovorax kalamii*	99.2
	Burkholderiales	*Comamonadaceae*	IMCC34787	2020642BA24	MW261903	*Brachymonas denitrificans*	99.9
Gammaproteobacteria	*Lysobacterales*	*Lysobacteraceae*	IMCC33701	2020642BA1	MW261880	*Pseudoxanthomonas icgebensis*	99.7
	Lysobacterales	*Lysobacteraceae*	IMCC34788	2020642BA25	MW261904	*Thermomonas brevis*	98.9
	Lysobacterales	*Lysobacteraceae*	IMCC34790	2020642BA27	MW261906	*Pseudoxanthomonas jiangsuensis*	99.6
	Lysobacterales	*Lysobacteraceae*	IMCC34769	2020642BA28	MW261906	*Lysobacter antibioticus*	100
	Enterobacterales	*Enterobacteriaceae*	IMCC34766	2020642BA16	MW261895	*Enterobacter soli*	99.8
	Enterobacterales	*Enterobacteriaceae*	IMCC34785	2020642BA22	MW261901	*Kosakonia oryzendophytica*	99.3
	Pseudomonadales	*Moraxellaceae*	IMCC34784	2020642BA21	MW261900	*Acinetobacter brisouii*	99.1
Epsilonproteobacteria	*Campylobacterales*	*Helicobacteraceae*	IMCC34783	2020642BA20	MW261899	*Sulfuricurvum kujiense*	99.7
Actinobacteria	*Mycobacteriales*	*Mycobacteriaceae*	IMCC33707	2020642BA4	MW261883	*Mycolicibacterium crocinum*	98.8
	Mycobacteriales	*Mycobacteriaceae*	IMCC33708	2020642BA5	MW261884	*Agromyces mediolanus*	100
	Micrococcales	*Intrasporangiaceae*	IMCC30636	2020642BA6	MW261885	*Phycicoccus duodecadis*	99.5
	Micrococcales	*Microbacteriaceae*	IMCC34786	2020642BA23	MW261902	*Microbacterium telephonicum*	99.1
Bacilli	*Caryophanales*	*Paenibacillaceae*	IMCC33715	2020642BA8	MW261887	*Paenibacillus humicus*	99.0
	Caryophanales	*Paenibacillaceae*	IMCC34765	2020642BA15	MW261894	*Paenibacillus hunanensis*	99.9
Clostridia	*Clostridiales*	*Clostridiaceae*	IMCC34760	2020642BA11	MW261890	*Clostridium neuense*	98.8
	Clostridiales	*Clostridiaceae*	IMCC34761	2020642BA12	MW261891	*Clostridium guangxiense*	99.0
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	IMCC33702	2020642BA2	MW261881	*Flavobacterium circumlabens*	99.7

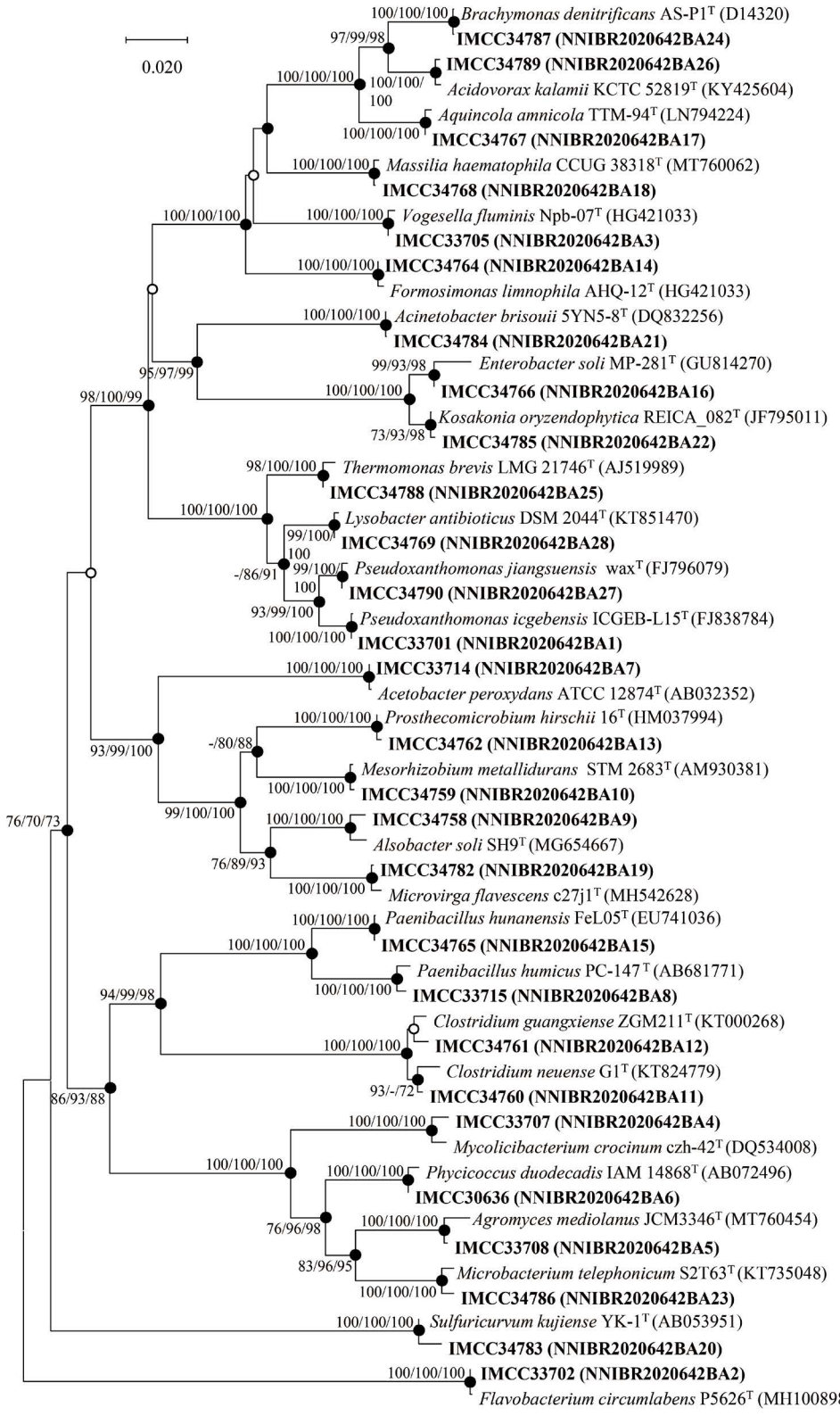

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest bacterial species. Bootstrap values over 70% are shown at nodes for neighbor-joining, maximum parsimony, and maximum likelihood methods, respectively. 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 = 0.02 substitutions per nucleotide position.

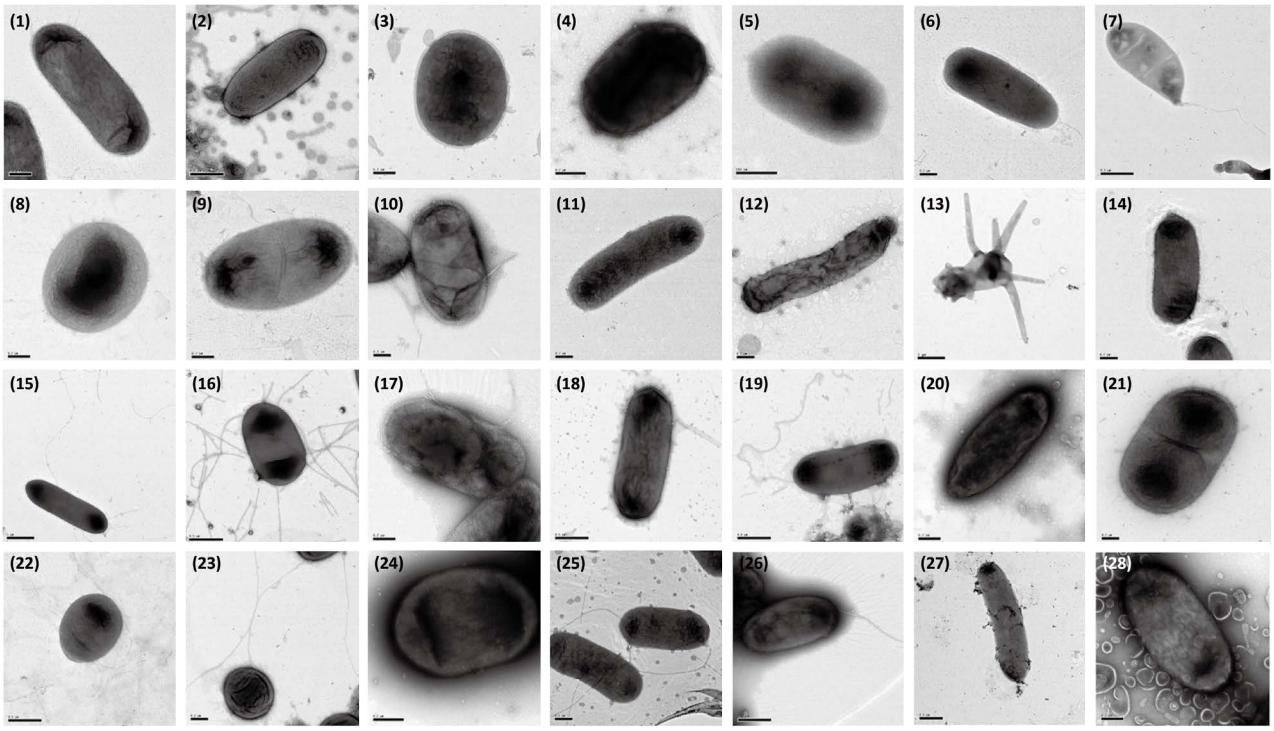

Fig. 2. Transmission electron micrographs of cells of the strains isolated in this study. Strains: 1, IMCC33701 (0.2 μm); 2, IMCC33702 (0.5 μm); 3, IMCC33703 (0.2 μm); 4, IMCC33707 (0.2 μm); 5, IMCC33708 (200 nm); 6, IMCC30636 (0.2 μm); 7, IMCC33714 (0.5 μm); 8, IMCC33715 (0.2 μm); 9, IMCC34758 (0.2 μm); 10, IMCC34759 (0.2 μm); 11, IMCC34760 (0.5 μm); 12, IMCC34761 (0.2 μm); 13, IMCC34762 (1.0 μm); 14, IMCC34764 (0.2 μm); 15, IMCC34765 (1.0 μm); 16, IMCC34766 (0.5 μm); 17, IMCC34767 (0.2 μm); 18, IMCC34768 (0.5 μm); 19, IMCC34782 (0.5 μm); 20, IMCC34783 (0.2 μm); 21, IMCC34782 (0.5 μm); 22, IMCC34785 (0.5 μm); 23, IMCC34786 (0.2 μm); 24, IMCC34787 (0.2 μm); 25, IMCC34788 (0.2 μm); 26, IMCC34789 (0.5 μm); 27, IMCC34790 (0.5 μm); 28, IMCC34769 (0.2 μm). Scale bars are indicated in parenthesis after strain ID.

Microbacterium of the class *Actinobacteria*; *Paenibacillus* of the class *Bacilli*; *Clostridium* of the class *Clostridia*; and *Flavobacterium* of the class *Flavobacteriia*.

The 28 unrecorded bacterial species identified in this study were Gram-staining-negative or positive, flagellated or non-flagellated, short- or straight-rod or coccoid-shaped bacteria (Fig. 2). Detailed morphological, physiological, and biochemical characteristics of the unrecorded bacterial species are given in the following strain descriptions.

Description of *Pseudoxanthomonas icgebensis* IMCC33701

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and yellow-colored after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase, urease, and esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, β-galactosidase, and cytochrome 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 IMCC33701 (=NNIBR2020642BA1) was isolated from a freshwater sample, Hanpo Stream, Chungju, Chungcheongbuk-do, Korea.

Description of *Flavobacterium circumlabens* IMCC33702

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are irregular, convex, undulate, and yellow-colored after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, and cytochrome 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 weakly utilized as sole carbon sources. Strain IMCC33702 (=NNIBR2020642BA2) was isolated from a freshwater sample, Chungju Lake, Chungju, Chungcheongbuk-do, Korea.

Description of *Vogesella fluminis* IMCC33705

Cells are Gram-stain-negative, facultatively anaerobic, non-flagellated, and coccus-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on R2A at 20°C. Positive for indole production; but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, and cytochrome 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 IMCC33705 (=NNIBR2020642BA3) was isolated from a sediment sample, Janghang Wetland, Ilsan, Gyeonggi-do, Korea.

Description of *Mycolicibacterium crocinum* IMCC33707

Cells are Gram-stain-positive, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and yellow-colored after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase; but negative for nitrate reduction, indole production, glucose fermentation, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase in API 20NE. L-Arabinose is utilized as a sole carbon source, but not D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC 33707 (=NNIBR2020642BA4) was isolated from a sediment sample, Janghang Wetland, Ilsan, Gyeonggi-do, Korea.

Description of *Agromyces mediolanus* IMCC33708

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and yellow-colored after incubation for 3 days on R2A at 20°C. Positive for glucose fermentation, arginine dihydrolase, urease, and β-galactosidase; but negative for nitrate reduction, indole production, esculin hydrolysis, gelatin hydrolysis, and cytochrome oxidase in API 20NE. L-Arabinose, D-mannitol, and *N*-acetyl-glucosamine are utilized as sole carbon sources, but not D-glucose, D-mannose, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC33708 (=NNIBR2020642BA5) was isolated from a freshwater sample, Soyang Lake, Chuncheon, Gangwon-do, Korea.

Description of *Phycicoccus duodecadis* IMCC30636

Cells are Gram-stain-positive, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, and cytochrome oxidase in API 20NE. D-Mannose is utilized as a sole carbon source, but not D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC 30636 (=NNIBR2020642BA6) was isolated from a freshwater sample, Soyang Lake, Chuncheon, Gangwon-do, Korea.

Description of *Acetobacter peroxydans* IMCC33714

Cells are Gram-stain-negative, aerobic, flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for nitrate reduction and gelatin hydrolysis; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, and cytochrome 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 IMCC33714 (=NNIBR2020642BA7) was isolated from a sediment sample, Janghang Wetland, Ilsan, Gyeonggi-do, Korea.

Description of *Paenibacillus humicus* IMCC33715

Cells are Gram-stain-positive, aerobic, non-flagellated, and coccus-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase, urease, and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. Capric acid is utilized as a sole carbon source, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC 33715 (=NNIBR2020642BA8) was isolated from a sediment sample, Janghang Wetland, Ilsan, Gyeonggi-do, Korea.

Description of *Alsobacter soli* IMCC34758

Cells are Gram-stain-negative, facultative anaerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-

galactosidase, and cytochrome oxidase in API 20NE. Phenylacetic acid is utilized as a sole carbon source, but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, and trisodium citrate. Strain IMCC34758 (=NNIBR2020642BA9) was isolated from a sediment sample, Janghang Wetland, Ilsan, Gyeonggi-do, Korea.

Description of *Mesorhizobium metallidurans* IMCC34759

Cells are Gram-stain-negative, facultative anaerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase in API 20NE. 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, capric acid, trisodium citrate, and phenylacetic acid. Strain IMCC34759 (=NNIBR2020642BA10) was isolated from a sediment sample, Janghang, Ilsan, Gyeonggi-do, Korea.

Description of *Clostridium neuense* IMCC34760

Cells are Gram-stain-positive, anaerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and gelatin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, and cytochrome 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 IMCC34760 (=NNIBR2020642BA11) was isolated from a sediment sample, Janghang Wetland, Ilsan, Gyeonggi-do, Korea.

Description of *Clostridium guangxiense* IMCC34761

Cells are Gram-stain-positive, anaerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and gelatin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, and cytochrome oxidase in API 20NE. L-Arabinose and trisodium citrate are utilized as sole carbon sources, but not D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain IMCC34761 (=NNIBR2020642BA12) was isolated from a sediment sample, Janghang Wetland, Ilsan, Gyeonggi-do, Korea.

Description of *Prosthecomicrobium hirschii* IMCC34762

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, 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 IMCC34762 (=NNIBR2020642BA13) was isolated from a freshwater sample, Soyang Lake, Chuncheon, Gangwon-do, Korea.

Description of *Formosimonas limnophila* IMCC34764

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, D-mannose, D-mannitol, and adipic acid are utilized as sole carbon sources, but not L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC 34764 (=NNIBR2020642BA14) was isolated from a freshwater sample, Soyang Lake, Chuncheon, Gangwon-do, Korea.

Description of *Paenibacillus hunanensis* IMCC34765

Cells are Gram-stain-positive, aerobic, flagellated, and rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and cytochrome oxidase in API 20NE. D-Mannose and *N*-acetyl-glucosamine are utilized as sole carbon sources, but not D-glucose, L-arabinose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34 765 (=NNIBR2020642BA15) was isolated from a freshwater sample, Anyang Stream, Yangcheon, Seoul, Korea.

Description of *Enterobacter soli* IMCC34766

Cells are Gram-stain-negative, aerobic, flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for glucose fermentation, esculin hydrolysis, and β-galactosidase; but negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatin hydrolysis, and cytochrome 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 IMCC34766 (=NNIBR2020642BA16) was isolated from a freshwater sample, Anyang Stream, Yangcheon, Seoul, Korea.

Description of *Aquincola amnicola* IMCC34767

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and beige-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase, and cytochrome 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 IMCC34767 (=NNIBR2020642BA17) was isolated from a freshwater sample, Anyang Stream, Yangcheon, Seoul, Korea.

Description of *Massilia haematophila* IMCC34768

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and yellow-colored after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, and cytochrome oxidase in API 20NE. D-Mannose and D-mannitol are utilized as sole carbon sources, but not D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34768 (=NNIBR2020642BA18) was isolated from a freshwater sample, Anyang Stream, Yangcheon, Seoul, Korea.

Description of *Microvirga flavescens* IMCC34782

Cells are Gram-stain-negative, facultative anaerobic, flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase, and cytochrome 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 IMCC34782 (=NNIBR2020642BA19) was isolated from a sediment sample, Gyeongan Stream, Gwangju, Gyeonggi-do, Korea.

Description of *Sulfuricurvum kujiense* IMCC34783

Cells are Gram-stain-negative, anaerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, 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 IMCC 34783 (=NNIBR2020642BA20) was isolated from a sediment sample, Gyeongan Stream, Gwangju, Gyeonggi-do, Korea.

Description of *Acinetobacter brisouii* IMCC34784

Cells are Gram-stain-negative, facultative anaerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and cytochrome oxidase in API 20NE. L-Arabinose, D-mannose, and D-mannitol are utilized as sole carbon sources, but not D-glucose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34784 (=NNIBR2020642 BA21) was isolated from a sediment sample, Gyeongan Stream, Gwangju, Gyeonggi-do, Korea.

Description of *Kosakonia oryzendophytica* IMCC34785

Cells are Gram-stain-negative, facultative anaerobic, non-flagellated, and coccus-shaped. Colonies are irregular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase,

urease, gelatin hydrolysis, and cytochrome 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 IMCC34785 (=NNIBR2020642BA22) was isolated from a sediment sample, Gyeongan Stream, Gwangju, Gyeonggi-do, Korea.

Description of *Microbacterium telephonicum* IMCC34786

Cells are Gram-stain-negative, facultative anaerobic, flagellated, and coccus-shaped. Colonies are irregular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, and cytochrome oxidase in API 20NE. Potassium gluconate is utilized as a sole carbon source, but 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. Strain IMCC34786 (=NNIBR2020642BA23) was isolated from a sediment sample, Gyeongan Stream, Gwangju, Gyeonggi-do, Korea.

Description of *Brachymonas denitrificans* IMCC34787

Cells are Gram-stain-negative, facultative anaerobic, non-flagellated, and rod-shaped. Colonies are irregular, convex, entire, and beige-colored after incubation for 3 days on R2A at 20°C. Positive for β-galactosidase and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatin hydrolysis in API 20NE. D-Mannose is utilized as a sole carbon source, but not 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 IMCC34787 (=NNIBR2020642BA24) was isolated from a sediment sample, Gyeongan Stream, Gwangju, Gyeonggi-do, Korea.

Description of *Thermomonas brevis* IMCC34788

Cells are Gram-stain-negative, facultative anaerobic, flagellated, and rod-shaped. Colonies are irregular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and gelatin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, and cytochrome oxidase in API 20NE. D-Mannose is utilized as a sole carbon source, but not D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34788 (=NNIBR2020642BA25) was isolated from a freshwater sample, Hadong Stream, Kimpo, Gyeonggi-do, Korea.

Description of *Acidovorax kalamii* IMCC34789

Cells are Gram-stain-negative, aerobic, flagellated, and rod-shaped. Colonies are irregular, convex, entire, and white-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and malic acid are utilized as sole carbon sources, but not D-mannitol, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC34789 (=NNIBR2020642BA26) was isolated from a freshwater sample, Anyang Stream, Yangcheon, Seoul, Korea.

Description of *Pseudoxanthomonas jiangsuensis* IMCC34790

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are irregular, convex, entire, and yellow-colored after incubation for 3 days on R2A at 20°C. Positive for esculin hydrolysis and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and cytochrome oxidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, malic acid, trisodium citrate, and phenylacetic acid are utilized as sole carbon sources, but not potassium gluconate and adipic acid. Strain IMCC34790 (=NNIBR2020642BA27) was isolated from a freshwater sample, Cheongpyong Lake, Gapyong, Gyeonggi-do, Korea.

Description of *Lysobacter antibioticus* IMCC34769

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod-shaped. Colonies are circular, convex, undulate, and pink to brown-colored after incubation for 3 days on R2A at 20°C Positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and cytochrome 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 IMCC34769 (=NNIBR2020642BA28) was isolated from a freshwater sample, Anyang Stream, Yangcheon, Seoul, Korea.

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