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Description of 41 unrecorded bacterial species in Korea, isolated from freshwater in 2021

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Here we describe indigenous prokaryotic species in Korea, a total of 41 bacterial strains were isolated from freshwater from the Republic of Korea. From the high 16S rRNA gene sequence similarity (>98.7%) and formation of a robust phylogenetic clade with the closest species, it was determined that each strain belonged to predefined bacterial species. There is no official report that these 41 species belonged to 5 phyla, 10 classes, 18 orders, 23 families, and 29 genera, which were assigned to *Streptomyces*, *Mycolicibacterium*, *Smaragdicoccus*, *Nocardiopsis*, and *Nocardia* of the phylum *Actinobacteria*; *Runella*, *Flavobacterium*, *Algoriphagus*, *Sphingobacterium*, and *Aequorivita* of the phylum *Bacteroidota*; *Paenibacillus*, *Bacillus*, *Metabacillus*, and *Fredinandcohnia* of the phylum *Firmicutes*; *Sphingobium*, *Erythrobacter*, *Duganella*, *Methylopila*, *Novosphingobium*, *Azospirillum*, *Simplicispira*, *Corallococcus*, *Pseudomonas*, *Devosia*, *Pseudorhodoferax*, *Pseudomonas*, *Prolinoborus*, *Pectobacterium*, and *Aquabacterium* of the phylum *Proteobacteria*; *Proshecobacter* of the phylum *Verrucomicrobia*. Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs are also described in the species description section.

Keywords: 16S rRNA, bacterial diversity, freshwater, indigenous prokaryotic species in Korea, unrecorded species

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INTRODUCTION

Fresh water accounts for 2.5% of the water-based environment, and seawater accounts for 97.5%. Fresh water is slightly different from seawater containing 3.5% salt, and includes various types of ecosystems, including rivers and river soils, where water flows depending on the appeal and altitude difference where the flow is stopped. And it exists in the form of rivers, lakes, and groundwater, and accounts for only about 0.6% of the total water system present on earth except for glaciers, but it is an essential resource for human survival (Wetzel, 2001). In a freshwater environment, most dominant prokaryotes are not cultured except for some groups. Microorganisms are more common in downstream water than in upstream water, and there are many low-nutritive microorganisms in upstream water, and many high-nutritive microorganisms multiply in downstream water. In general, most groups of bacteria form biofilm, but fungi and birds are often found. Microorganisms living in poor and eutrophobic lakes are also different. In eutrophobic lakes, photosynthetic bacteria such as *Cyanobacteria* multiply in summer. So far, bacterial clusters in the freshwater environment have been known as about 20 phyla, and among them, *Actinomycetota*, *Bacteroidota*, *Pseudomonadota*, *Cyanobacteriota*, and *Verrucomicrobiota* were dominant (Newton *et al*., 2011). According to GOLD statistics, environmental bacteria account for about 10% of all genetic projects, and most of environmental ecological research is biased toward digestive organs, oceans, and soil, accounting for 9.2% of fresh water (Mukherjee *et al*., 2022). Like the marine and land environment, freshwater-derived prokaryotes basically serve as decomposers to convert organic matter into inorganic substances by transferring organic matter to higher nutritional levels through microfed networks, and are well-known for their function in the circulation of greenhouse gases. Therefore, research on the analysis of microbial diversity derived from freshwater, which has a close impact on human life, but relatively insignificant excavation research, can contribute to the role of freshwaterspecific prokaryotes and thus the expansion of domestic microbial species discovery research.

In 2021, we collected environmental samples from freshwater in Korea and isolated many novel and unrecorded bacterial species during a research program supported by NNIBR (Nakdonggang National Institute of Biological Resources) of Korea. The identified bacterial species belongs to the classes *Bacilli*, *Actinomycetia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Verrucomicrobiae*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia*. Here we shortly describe 41 unrecorded bacterial species in the 10 classes belonging to 18 orders.

MATERIALS AND METHODS

A total of 41 bacterial strains affiliated to the classse *Bacilli*, *Actinomycetia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Verrucomicrobiae*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia* were isolated from freshwater samples. Freshwater sample was processed separately, spread onto diverse culture media including R2A, 1/5 R2A, nutrient, 1/5 nutrient, and incubated at 20, 25 and 30°C for 5–10 days (Table 1). The designated strain IDs, sources, culture media, and incubation conditions are summarized in Table 1. All strains were purified as single colonies and stored as 20–25% (w/v) glycerol suspension −80°C as well as lyophilized ampoules.

The morphology was studied on agar plates for 2–5 days under their optimum temperature. Cell size and shape were examined either by transmission electron microscopy or scanning electron microscopy. Gram staining was performed using a Gram-staining kit of the standard procedures. Using API 20NE (bioMérieux) the biochemical characteristics were performed according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification and 16S rRNA gene sequencing were performed using the standard procedures described by Lane (1991). The 16S rRNA gene sequences of the strains assigned to the 41 bacterial strains were compared with the sequences held in GenBank by BLASTN and also analyzed using the EzBioCloud blast (https://www.ezbiocloud.net) (Yoon *et al.*, 2017). For phylogenetic analyses, multiple alignments were performed using the Clustal_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using the neighbor-joining (Saitou et al., 1987) methods with the MEGA X Program (Kumar *et al.*, 2018) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Table 1. The taxonomic affiliations of isolated strains belong to the class *Alphaproteobacteria*.

Class	Order	Family	Genus	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions (°C, days)
Bacilli	*Bacillales*	*Bacillaceae*	*Bacillus*	MMS21-DH2R7	NNIBR2021642BA1	*Bacillus cytotoxicus*	98.91	Freshwater	MA	30°C, 2d
	Bacillales	*Bacillaceae*	*Fredinandcohnia*	MMS21-DH2MA11	NNIBR2021642BA49	*Fredinandcohnia onubensis*	99.18	Freshwater	MA	30°C, 3d
	Bacillales	*Bacillaceae*	*Metabacillus*	MMS21-DH3MA7	NNIBR2021642BA3	*Metabacillus mangrovi*	99.93	Freshwater	MA	30°C, 2d
	Bacillales	*Paenibacillaceae*	*Paenibacillus*	AS127	NNIBR2021642BA40	*Paenibacillus ihuae*	99.35	Freshwater	R2A	25°C, 3d
	Bacillales	*Paenibacillaceae*	*Paenibacillus*	AS51	NNIBR2021642BA18	*Paenibacillus massiliensis* subsp. *massiliensis*	99.73	Freshwater	R2A	25°C, 3d
	Bacillales	*Paenibacillaceae*	*Paenibacillus*	K11	NNIBR2021642BA23	*Paenibacillus ottowii*	99.12	Freshwater	R2A	25°C, 3d
Actinomycetia	*Corynebacteriales*	*Mycobacteriaceae*	*Mycolicibacterium*	CG42	NNIBR2021642BA32	*Mycolicibacterium aichiense*	98.74	Freshwater	R2A	25°C, 3d
	Corynebacteriales	*Nocardiaceae*	*Nocardia*	MMS21-JYBA42	NNIBR2021642BA8	*Nocardia africana*	99.29	Freshwater	R2A	30°C, 3d
	Streptosporangiales	*Nocardiopsaceae*	*Nocardiopsis*	K69	NNIBR2021642BA35	*Nocardiopsis dassonvillei* subsp. *dassonvillei*	99.93	Freshwater	R2A	25°C, 3d
	Corynebacteriales	*Nocardiaceae*	*Smaragdicoccus*	K21	NNIBR2021642BA34	*Smaragdicoccus niigatensis*	100	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	WG8	NNIBR2021642BA26	*Streptomyces bryophytorum*	98.75	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	AS52	NNIBR2021642BA19	*Streptomyces bullii*	98.96	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	CG18	NNIBR2021642BA20	*Streptomyces cellostaticus*	99.17	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS21-OR1	NNIBR2021642BA9	*Streptomyces shenzhenensis*	99.31	Freshwater	R2A	30°C, 3d

Table 1. Continued.

Class	Order	Family	Genus	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions (°C, days)
Actinomycetia	*Streptomycetales*	*Streptomycetaceae*	*Streptomyces*	MMS21-DH1MA22	NNIBR2021642BA48	*Streptomyces speibonae*	98.97	Freshwater	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS21-305	NNIBR2021642BA46	*Streptomyces violarus*	99.59	Freshwater	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS21-MKJW7	NNIBR2021642BA45	*Streptomyces wuyuanensis*	100	Freshwater	R2A	30°C, 3d
Alphaproteobacteria	*Rhodospirillales*	*Azospirillaceae*	*Azospirillum*	AS37	NNIBR2021642BA30	*Azospirillum oryzae*	99.41	Freshwater	R2A	25°C, 3d
	Hyphomicrobiales	*Devosiaceae*	*Devosia*	AS105	NNIBR2021642BA38	*Devosia elaeis*	99.93	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Erythrobacteraceae*	*Erythrobacter*	K14	NNIBR2021642BA24	*Erythrobacter tepidarius*	99.64	Freshwater	R2A	25°C, 3d
	Hyphomicrobiales	*Methylocystaceae*	*Methylopila*	CG59	NNIBR2021642BA28	*Methylopila oligotropha*	99.64	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Novosphingobium*	WG87	NNIBR2021642BA29	*Novosphingobium arabidopsis*	99	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Novosphingobium*	AS117	NNIBR2021642BA39	*Novosphingobium sediminis*	99.78	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Sphingobium*	K3	NNIBR2021642BA21	*Sphingobium sufflavum*	98.85	Freshwater	R2A	25°C, 3d
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	*Aquabacterium*	MMS21-SJ6-8	NNIBR2021642BA47	*Aquabacterium lacunae*	99.59	Freshwater	MA	30°C, 3d
	Burkholderiales	*Oxalobacteraceae*	*Duganella*	CG34	NNIBR2021642BA27	*Duganella rivi*	99.24	Freshwater	R2A	25°C, 3d
	Neisseriales	*Neisseriaceae*	*Prolinoborus*	MMS21-Sa21	NNIBR2021642BA7	*Prolinoborus fasciculus*	98.83	Freshwater	R2A	30°C, 3d
	Burkholderiales	*Comamonadaceae*	*Pseudorhodoferax*	AS150	NNIBR2021642BA42	*Pseudorhodoferax aquiterrae*	99.72	Freshwater	R2A	25°C, 3d
	Burkholderiales	*Comamonadaceae*	*Simplicispira*	CG15	NNIBR2021642BA31	*Simplicispira lacusdiani*	99.31	Freshwater	R2A	25°C, 3d
Gammaproteobacteria	*Enterobacterales*	*Pectobacteriaceae*	*Pectobacterium*	MMS21-Er7	NNIBR2021642BA10	*Pectobacterium aroidearum*	99.52	Freshwater	R2A	30°C, 3d
	Enterobacterales	*Pectobacteriaceae*	*Pseudomonas*	AS210	NNIBR2021642BA44	*Pseudomonas batumici*	99.04	Freshwater	R2A	25°C, 3d
	Enterobacterales	*Pectobacteriaceae*	*Pseudomonas*	AS199	NNIBR2021642BA43	*Pseudomonas huaxiensis*	99.86	Freshwater	R2A	25°C, 3d
	Enterobacterales	*Pectobacteriaceae*	*Pseudomonas*	CG31	NNIBR2021642BA36	*Pseudomonas neuropathica*	98.86	Freshwater	R2A	25°C, 3d
Deltaproteobacteria	*Myxococcales*	*Myxococcaceae*	*Corallococcus*	CG50	NNIBR2021642BA33	*Corallococcus interemptor*	100	Freshwater	R2A	25°C, 3d
Verrucomicrobiae	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Prosthecobacter*	CG69	NNIBR2021642BA37	*Prosthecobacter dejongeii*	99.39	Freshwater	R2A	25°C, 3d
Shpingobacteriia	*Sphingobacteriales*	*Sphingobacteriaceae*	*Sphingobacterium*	MMS21-DH2R2	NNIBR2021642BA2	*Sphingobacterium canadense*	98.97	Freshwater	R2A	30°C, 2d
Cytophagia	*Cytophagales*	*Cyclobacteriaceae*	*Algoriphagus*	AS131	NNIBR2021642BA41	*Algoriphagus aquaeductus*	98.92	Freshwater	R2A	25°C, 3d
	Cytophagales	*Spirosomaceae*	*Runella*	K7	NNIBR2021642BA22	*Runella aurantiaca*	98.74	Freshwater	R2A	25°C, 3d
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Aequorivita*	MMS21-MC1	NNIBR2021642BA11	*Aequorivita sinensis*	99.9	Freshwater	MA	30°C, 2d
	Flavobacteriales	*Flavobacteriaceae*	*Flavobacterium*	MMS21-SJ5-12	NNIBR2021642BA51	*Flavobacterium difficile*	97.77	Freshwater	R2A	30°C, 3d
	Flavobacteriales	*Flavobacteriaceae*	*Flavobacterium*	WG6	NNIBR2021642BA25	*Flavobacterium squillarum*	99.86	Freshwater	R2A	25°C, 3d

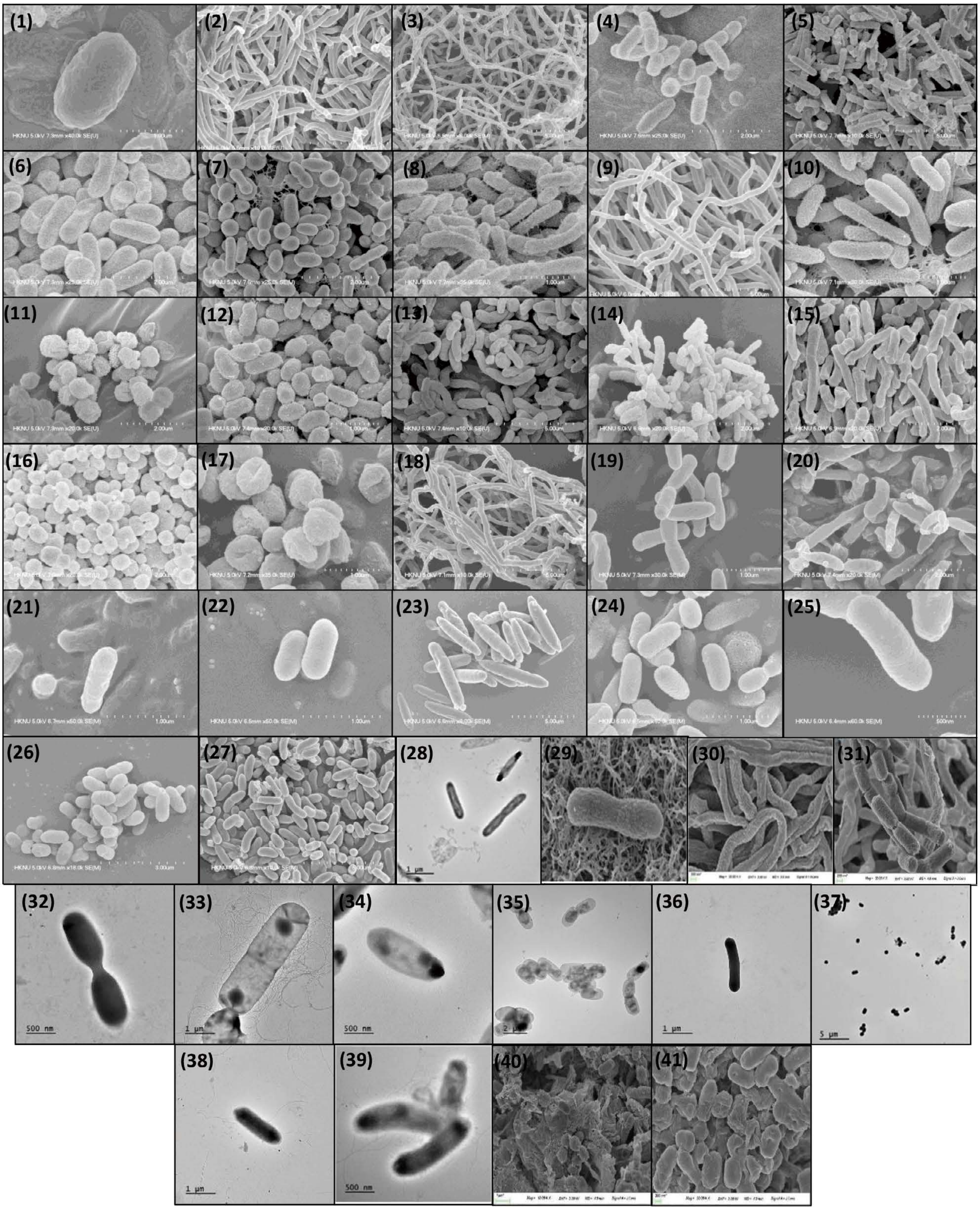

Fig. 1. Transmission and scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, AS51; 2, AS52; 3, CG18; 4, K3; 5, K7; 6, K11; 7, K14; 8, WG6; 9, WG8; 10, CG34; 11, CG59; 12, WG87; 13, AS37; 14, CG15; 15, CG42; 16, CG50; 17, K21; 18, K69; 19, CG31; 20, CG69; 21, AS105; 22, AS117; 23, AS127; 24, AS131; 25, AS150; 26, AS199; 27, AS210; 28, MMS21-SJ5-12; 29, MMS21-305; 30, MMS21-MKJW7; 31, MMS21-DH1MA22; 32, MMS21-Sa21; 33, MMS21-DH2R7; 34, MMS21-DH2R2; 35, MMS21-DH3MA7; 36, MMS21-DH2MA11; 37, MMS21-MC1; 38, MMS21-Er7; 39, MMS21-SJ6-8; 40, MMS21-OR1; 41, MMS21-JYBA42.

RESULTS AND DISCUSSION

The 41 strains were distributed in 10 classes of the *Bacilli*, *Actinomycetia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Verrucomicrobiae*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia*; 6 strains for the class *Bacilli*, 11 strains for the *Actinomycetia*, 7 strains for the *Alphaproteobacteria*, 5 strains for the *Betaproteobacteria*, 4 strains for the *Gammaproteobacteria*, 1 strain for the *Deltaproteobacteria*, 1 strain for the *Verrucomicrobiae*, 1 strain for the *Shpingobacteriia*, 2 strains for the *Cytophagia* and 3 strains for the *Flavobacteriia* (Table 1). These strains were Gram-staining-negative or positive, chemoheterotrophic, rod, short-rod and coccoid shaped showing in Fig. 1.

The strains in the phylum *Actinobacteria* (Fig. 2) were found to belong to 1 class 5 separate genera: *Streptomyces* (7 species), *Mycolicibacterium* (1 species), *Smaragdicoccus* (1 species), *Nocardiopsis* (1 species), *Nocardia* (1 species).

Fig. 3 shows phylogenetic assignment of 17 strains of the phylum *Proteobacteria* belong to 4 classes 14 separate genera: *Aquabacterium* (1 species), *Azospirillum* (2 species), *Corallococcus* (1 species), *Devosia* (1 species), *Duganella* (1 species), *Erythrobacter* (1 species), *Methylopila* (1 species), *Novosphingobium* (2 species), *Pectobacterium* (1 species), *Prolinoborus* (1 species), *Pseudomonas* (3 species), *Pseudorhodoferax* (1 species), *Simplicispira* (1 species) and *Sphingobium* (1 species).

Fig. 4 shows phylogenetic assignment of 13 strains of

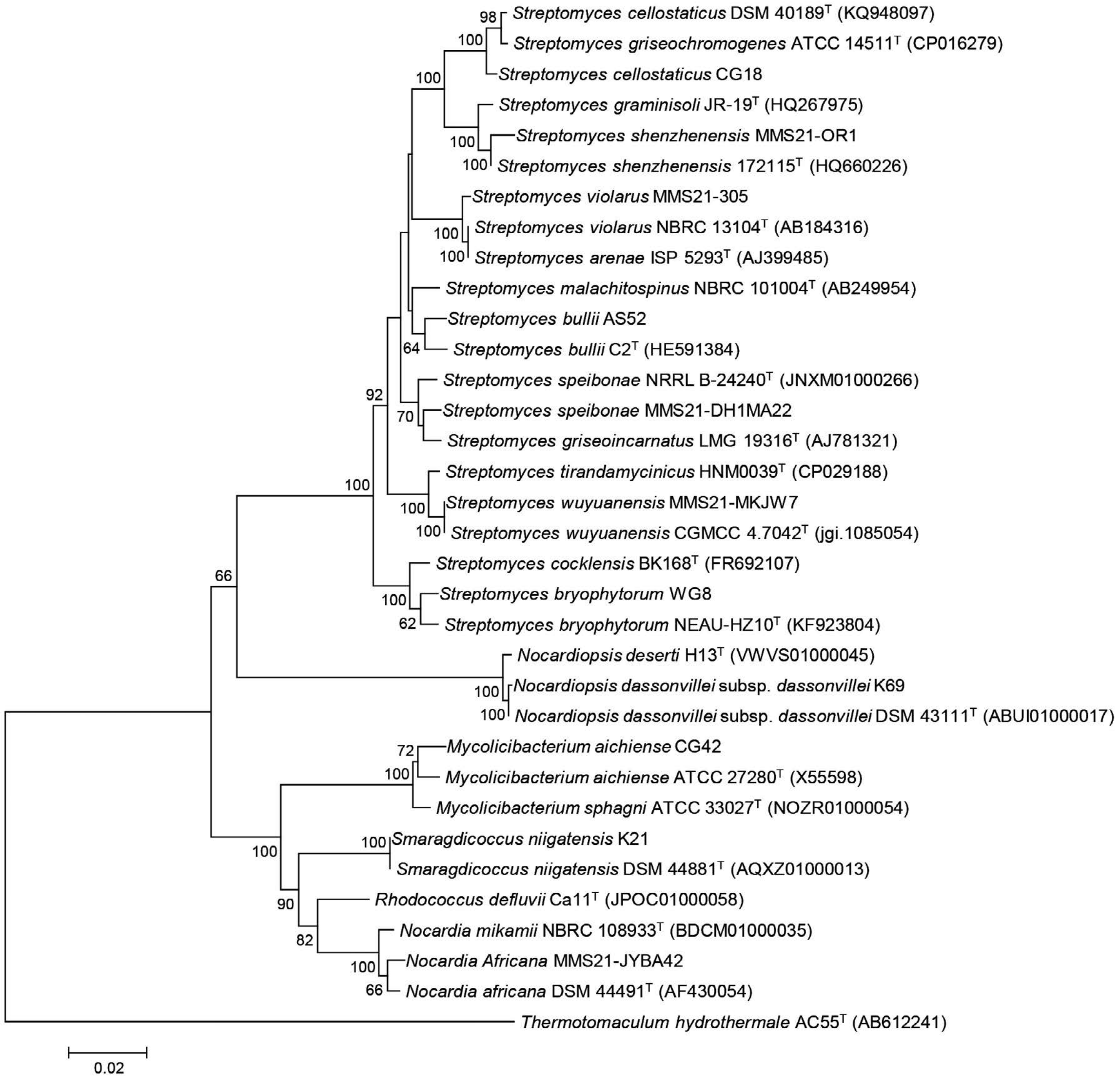

Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Actinobacteria*. Bootstrap values (>60%) are shown in the neighbor-joining method. Bar, 0.02 substitutions per nucleotide position.

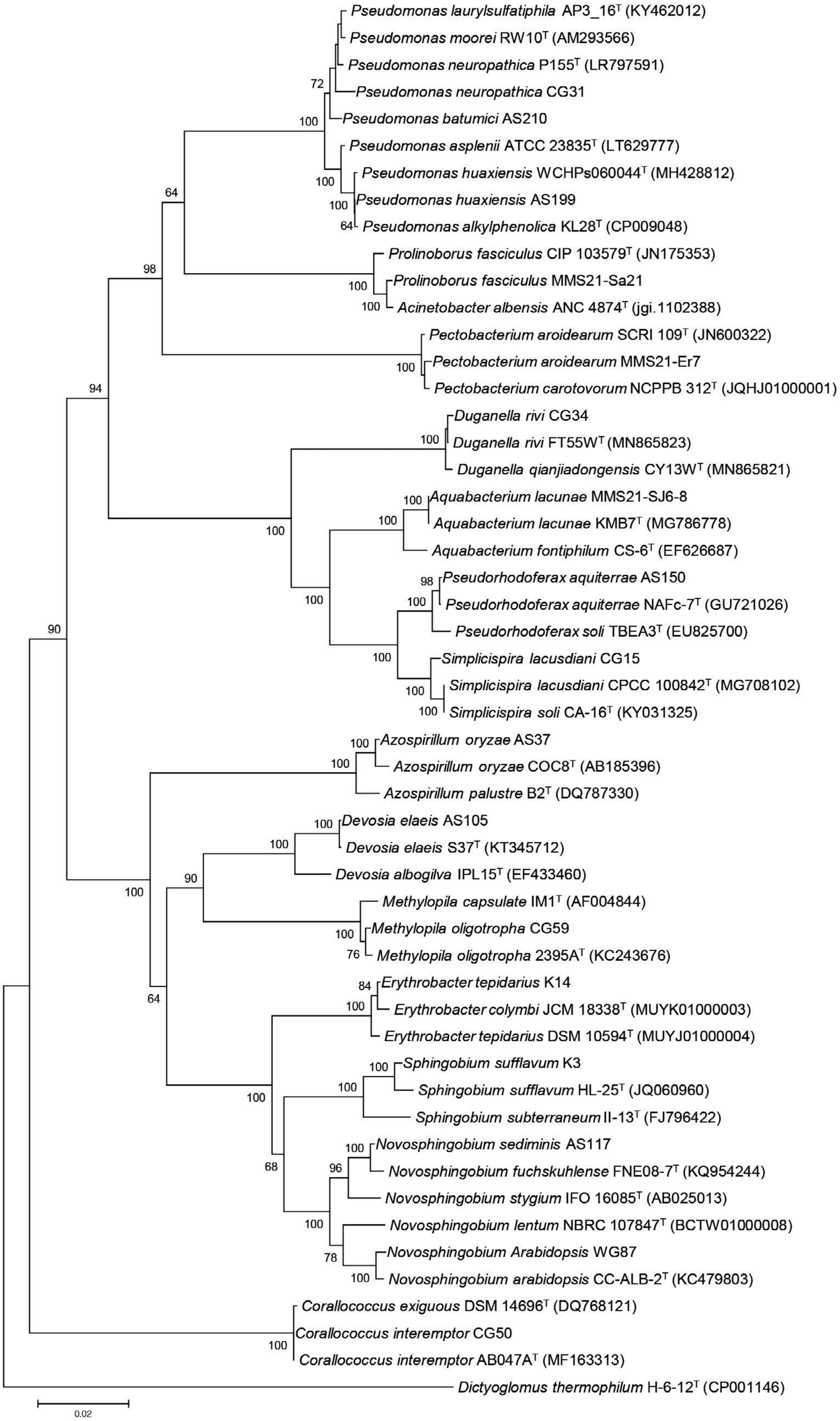

Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Proterobacteria*. Bootstrap values are greater than 60% are shown the neighbor-joining tree method. Bar, 0.02 substitutions per nucleotide position.

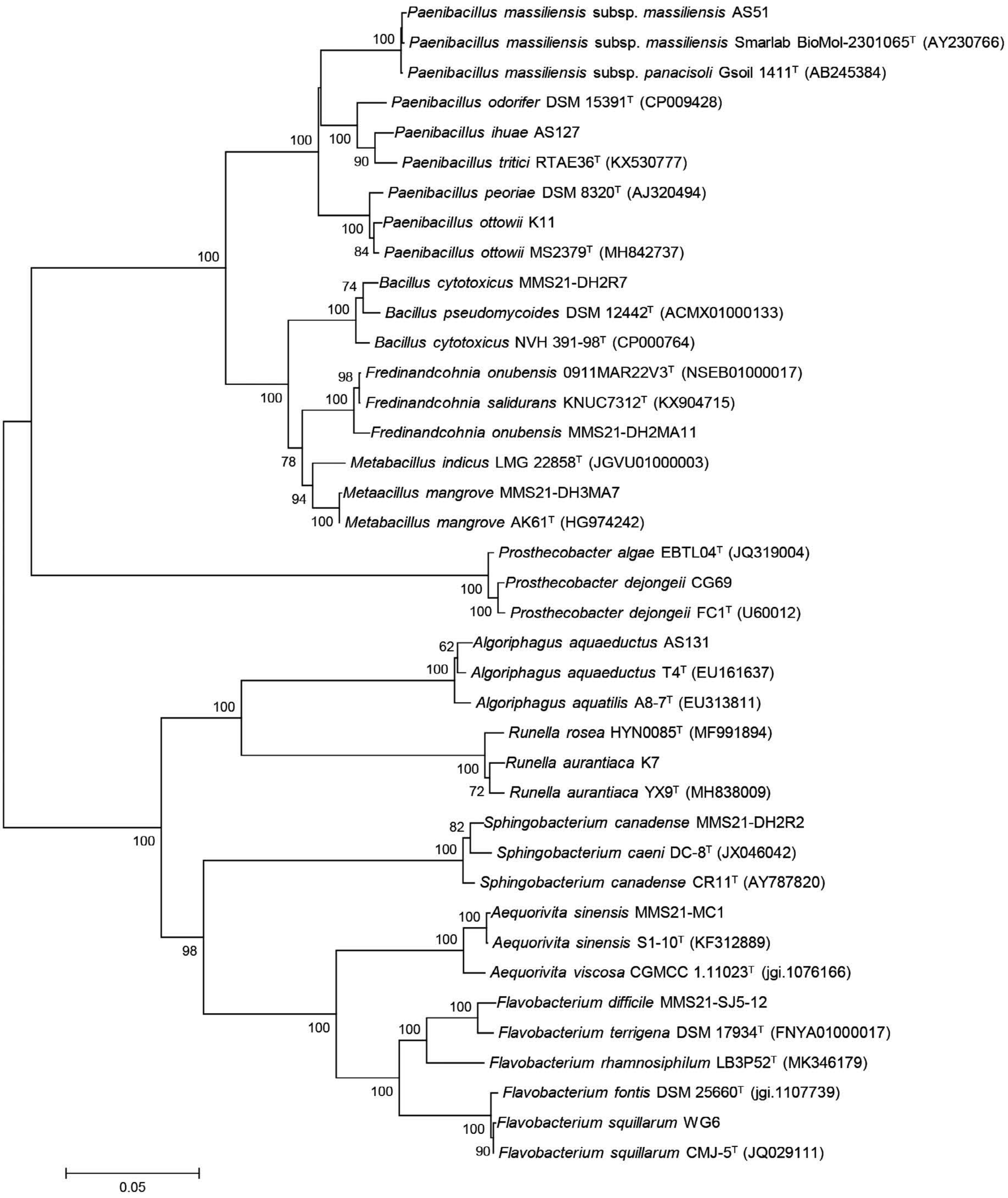

Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Firmicutes*, *Bacteroidota* and *Verrucomicrobia*. Bootstrap values ($>60\%$) are shown in the neighbor-joining tree. Bar, 0.05 substitutions per nucleotide position.

the phyla *Firmicutes*, *Bacteroidota* and *Verrucomicrobia* belong to 5 classes 10 separate genera: *Aequorivita* (1 species), *Algoriphagus* (1 species), *Bacillus* (1 species), *Flavobacterium* (2 species), *Fredinandcohnia* (1 species), *Metabacillus* (1 species), *Paenibacillus* (3 species), *Prosthecobacter* (1 species), *Runella* (1 species) and *Sphingobacterium* (1 species).

Here we report 41 unrecorded bacterial species in Korea belonging to 10 classes of 5 phyla.

Description of *Paenibacillus massiliensis* subsp. *massiliensis* AS51

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilizes D-glucose, L-arabinose, D-mannose and D-mannitol. Does not utilize *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS51 (=NNIBR2021642BA18) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Streptomyces bullii* AS52

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilizes D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize D-mannose, D-mannitol, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain AS52 (=NNIBR2021642BA19) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Streptomyces cellostaticus* CG18

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and white → gray color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, urease, esculin hydrolysis, β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase. Utilizes D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and malic acid. Does not utilize D-mannitol, capric acid, trisodium citrate and phenylacetic acid. Strain CG18 (=NNIBR2021642BA20) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Sphingobium sufflavum* K3

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose and malic acid. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain K3 (=NNIBR2021642BA21) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Sphingobium sufflavum* K7

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and pink color after 3 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine and D-maltose. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain K7 (=NNIBR2021642BA22) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Paenibacillus ottowii* K11

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, gelatinase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase. Utilizes D-glucose, D-mannose, D-mannitol and D-maltose. Does not utilize L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain K11 (=NNIBR2021642BA23) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Erythrobacter tepidarius* K14

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and orange color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose, D-mannose and phenylacetic acid. Does not utilize L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. Strain K14 (=NNIBR2021642BA24) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Flavobacterium squillarum* WG6

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, positive for gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain WG6 (=NNIBR2021642BA25) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Streptomyces bryophytorum* WG8

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and ivory color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate and adipic acid. Does not utilize L-arabinose, D-mannitol, D-maltose, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain WG8 (=NNIBR2021642BA26) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Duganella rivi* CG34

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for urease, esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase and β-galactosidase. Utilizes D-glucose and L-arabinose. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain CG34 (=NNIBR2021642BA27) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Methylopila oligotropha* CG59

Cells are Gram-staining-negative, non-flagellated and oval-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes potassium gluconate and malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain CG59 (=NNIBR2021642BA28) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Novosphingobium arabidopsis* WG87

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, positive for urease, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase. Utilizes D-glucose, L-arabinose, D-mannose and potassium gluconate. Does not utilize D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain WG87 (=NNIBR2021642BA29) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Azospirillum oryzae* AS37

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, but negative for indole production, glucose fermentation, gelatinase. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Does not utilize D-mannose and D-maltose. Strain AS37 (=NNIBR2021642BA30) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Simplicispira lacusdiani* CG15

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, β-galactosidase. Utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, adipic acid and malic acid. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Strain CG15 (=NNIBR2021642 BA31) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Mycolicibacterium aichiense* CG42

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, adipic acid, malic acid and trisodium citrate. Does not utilize *N*-acetyl-glucosamine, D-maltose, capric acid and phenylacetic acid. Strain CG42 (=NNIBR2021642BA32) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Corallococcus interemptor* CG50

Cells are Gram-staining-negative, non-flagellated and coccoid-shaped. Colonies are swarming and dark orange color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, indole production, esculin hydrolysis, gelatinase, but negative for glucose fermentation, arginine dihydrolase, urease, β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain CG50 (=NNIBR2021642BA33) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Smaragdicoccus niigatensis* K21

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular and dark green color after 3 days on R2A at 25°C. In API 20NE, positive for urease, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase. Utilize *N*-acetylglucosamine and malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain K21 (=NNIBR2021642 BA34) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Nocardiopsis dassonvillei* subsp. *dassonvillei* K69

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and white color after 3 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, β-galactosidase. Utilize *N*-acetylglucosamine. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain K69 (=NNIBR2021642BA35) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudomonas neuropathica* CG31

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are irregular raised undulate and cream color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase and esculin hydrolysis, but negative for indole production, glucose fermentation, urease, gelatinase, β-galactosidase. Utilize capric acid and malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain CG31 (=NNIBR2021642BA36) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Prosthecobacter dejongeii* CG69

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and cream color after 3 days on R2A at 25°C. In API 20NE, positive for urease, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase. Utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain CG69 (=NNIBR2021642BA37) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Devosia elaeis* AS105

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and cream color after 3 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, weak positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine and D-maltose. Does not utilize potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS105 (=NNIBR 2021642BA38) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Novosphingobium sediminis* AS117

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and adipic acid. Does not utilize *N*-acetylglucosamine, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS117 (=NNIBR2021642BA 39) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Paenibacillus ihuae* AS127

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and yellow color after

3 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, gelatinase β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and malic acid. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain AS127 (= NNIBR2021642BA40) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Algoriphagus aquaeductus* AS131

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and red color after 3 days on R2A at 25°C. In API 20NE, positive for gelatinase and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis. Utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose and phenylacetic acid. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. Strain AS131 (= NNIBR2021642BA41) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudorhodoferax aquiterrae* AS150

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction and urease, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase. Utilize L-arabinose, D-mannitol, D-maltose, potassium gluconate, adipic acid and malic acid. Does not utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, capric acid, trisodium citrate and phenylacetic acid. Strain AS150 (= NNIBR2021642BA 42) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudomonas huaxiensis* AS199

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and red color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilize D-glucose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose and adipic acid. Strain AS199 (= NNIBR2021642BA43) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudomonas batumici* AS210

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and cream color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid and trisodium citrate. Does not utilize *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain AS210 (= NNIBR2021642BA44) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Flavobacterium difficile* MMS21-SJ5-12

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-SJ5-12 (= NNIBR2021642BA51) has been isolated from a freshwater sample, Okcheon-gun, Korea.

Description of *Streptomyces violarus* MMS21-305

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous, raised, undulate and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, urease, glucose fermentation, gelatinase and β-galactosidase, but negative for esculin hydrolysis, indole production and arginine dihydrolase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose and adipic acid. Does not utilize capric acid and phenylacetic acid. Strain MMS21-305 (= NNIBR2021642BA46) has been isolated from a freshwater sample, Gimhae-si, Korea.

Description of *Streptomyces wuyuanensis* MMS21-MKJW7

Cells are Gram-staining-positive and rod-shaped. Colonies are filamentous, raised, undulate and brown color after 3 days on R2A at 30°C. In API 20NE, positive for glucose fermentation, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease and esculin hydrolysis. Utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize L-arabinose, adipic acid, D-mannitol, capric acid, trisodium citrate and phenylacetic acid. Strain MMS21-MKJW7 (=NNIBR 2021642BA45) has been isolated from a freshwater sample, Gimhae-si, Korea.

Description of *Streptomyces speibonae* MMS21-DH1MA22

Cells are Gram-staining-positive and rod-shaped. Colonies are circular, convex, entire and gray color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction and glucose fermentation, but negative for indole production, esculin hydrolysis, β-galactosidase, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-DH1MA22 (=NNIBR2021642BA 48) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Prolinoborus fasciculus* MMS21-Sa21

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction and urease, but negative for esculin hydrolysis, β-galactosidase, indole production, glucose fermentation, arginine dihydrolase and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-Sa21 (=NNIBR2021 642BA7) has been isolated from a freshwater sample, Gangneung-si, Korea.

Description of *Bacillus cytotoxicus* MMS21-DH2R7

Cells are Gram-staining-positive, flagellate and rod-shaped. Colonies are rhizoid, raised, undulate and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for arginine dihydrolase, urease, glucose fermentation, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production and esculin hydrolysis. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol and potassium gluconate. Does not utilize capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-DH2R7 (=NNIBR2021642BA1) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Sphingobacterium canadense* MMS21-DH2R2

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow color after 2 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and β-galactosidase, but negative for indole production and gelatinase. Utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine and D-maltose. Does not utilize D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, adipic acid and phenylacetic acid. Strain MMS21-DH2R2 (=NNIBR2021642BA2) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Metabacillus mangrovi* MMS21-DH3MA7

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for arginine dihydrolase, esculin hydrolysis, urease, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production and glucose fermentation. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate. Does not utilize capric acid, malic acid, trisodium citrate, adipic acid and phenylacetic acid. Strain MMS21-DH3MA7 (=NNIBR2021642BA3) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Fredinandcohnia onubensis* MMS21-DH2MA11

Cells are Gram-staining-positive, flagellate and rod-shaped. Colonies are irregular, flat, entire and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, gelatinase and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. Utilize L-arabinose, potassium gluconate, *N*-acetyl-glucosamine and D-maltose. Does not utilize D-glucose, D-mannose, D-mannitol, capric acid, malic acid, trisodium citrate, adipic acid and phenylacetic acid. Strain MMS21-DH2MA11 (=NNIBR2021642BA49) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Aequorivita sinensis* MMS21-MC1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are beige color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for urease and esculin hydrolysis, but negative for nitrate reduction, indole production, β-galactosidase, glucose fermentation, arginine dihydrolase and gelatinase. Utilize D-mannose, D-mannitol and trisodium citrate. Does not utilize D-glucose, L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-MC1 (=NNIBR2021642BA11) has been isolated from a freshwater sample, Asan-si, Korea.

Description of *Pectobacterium aroidearum* MMS21-Er7

Cells are Gram-staining-negative, flagellate and rod-shaped. Colonies are circular, flat, undulate and white color after 3 days on R2A at 30°C. In API 20NE, positive for glucose fermentation, but negative for esculin hydrolysis, β-galactosidase, nitrate reduction, indole production, arginine dihydrolase, urease and gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and D-maltose. Does not utilize, adipic acid, phenylacetic acid, potassium gluconate, capric acid, malic acid and trisodium citrate. Strain MMS21-Er7 (=NNIBR2021642BA10) has been isolated from a freshwater sample, Daejeon, Korea.

Description of *Aquabacterium lacunae* MMS21-SJ6-8

Cells are Gram-staining-negative, flagellate and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and β-galactosidase. Utilize D-glucose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-SJ6-8 (=NNIBR2021642BA47) has been isolated from a freshwater sample, Okcheon-gun, Korea.

Description of *Streptomyces shenzhenensis* MMS21-OR1

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and brown color after 3 days on R2A at 30°C. In API 20NE, positive for urease and esculin hydrolysis, but negative for nitrate reduction, β-galactosidase, indole production, glucose fermentation, arginine dihydrolase, and gelatinase. Utilize D-mannose, D-mannitol and trisodium citrate. Does not utilize *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, D-glucose, L-arabinose, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-OR1 (=NNIBR2021642BA9) has been isolated from a freshwater sample, Cheongju-si, Korea.

Description of *Nocardia africana* MMS21-JYBA42

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are punctiform, convex, entire and peach color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, but negative for esculin hydrolysis, β-galactosidase, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-JYBA42 (=NNIBR2021642BA8) has been isolated from a freshwater sample, Gangneung-si, Korea.

CONFLICTS OF INTEREST

The author of this paper has no affiliation with any interests and is solely responsible for the paper.

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