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A report of 42 unrecorded bacterial species belonging to the *Alphaproteobacteria* in Korea

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As a subset study to discover indigenous prokaryotic species in Korea, a total of 42 bacterial strains assigned to the class *Alphaproteobacteria* were isolated from diverse environmental habitats including plant roots, ginseng soil, forest soil, marsh, mud flat, freshwater, and seawater. From the high 16S rRNA gene sequence similarity (>99.1%) and formation of a robust phylogenetic clade with the closest species, it was determined that each strain belonged to each independent and predefined bacterial species. There is no official report that these 42 species have been described in Korea; therefore 4 species of 1 genera in the order *Caulobacterales*, 18 species of 10 genera in the order *Rhizobiales*, 7 species of 5 genera in the order *Sphingomonadales* and 13 species of 11 genera in the order *Rhodobacterales* within the *Alphaproteobacteria* are reported for alphaproteobacterial species found in Korea. 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, *Alphaproteobacteria*, bacterial diversity, indigenous prokaryotic species in Korea, unrecorded species

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Introduction

The class *Alphaproteobacteria* that form one of the largest proteobacterial groups within bacteria include diverse phototrophs, chemolithotrophs, chemoorganotrophs, and aerobic photoheterotrophs and are abundant in various terrestrial and marine habitats (Giovannoni *et al*., 2005; Kersters *et al*., 2006). The taxonomic classification based on 16S rRNA gene sequences shows that the class *Alphaproteobacteria* are recognized as a class within the phylum *Proteobacteria* and they are subdivided into 8 main subgroups or orders (*Caulobacterales*, *Magnetococcales*, *Parvularculales*, *Rickettsiales*, *Rhizobiales*, *Rhodobacterales*, *Rhodospirillales*, and *Sphingomonadales*; Ferla *et al*., 2013).

Members of *Alphaproteobacteria* provide enormously valuable biological resources in various industries as well as valuable tools for taxonomic, biochemical and molecular biological studies. Some members of *Alphaproteobacteria* exhibit interactions with eukaryotic host organisms, which is of central importance from agricultural and medical perspectives (Skorpil and Broughton, 2005;

Fredricks, 2006). For example, symbiotic interactions of the *Rhizobiaceae* family members with the plant root nodules are responsible for most of the atmospheric nitrogen fixation, which is very important in agriculture (Kaneko *et al*., 2000; 2002; Fredricks, 2006). Many other *Alphaproteobacteria* such as *Brucella*, *Bartonella*, and *Rickettsiales* that constitute important pathogens in humans and animals have been adapted to intracellular life styles (Andersson *et al*., 2003; Alsmark *et al*., 2004; Fredricks *et al*., 2006; Moreno and Moriyon, 2006). Aerobic anoxygenic phototrophic bacteria, *Pelagibacter of Alphaproteobacteria*, are widely distributed as marine planktons that constitute more than 10% of the open ocean microbial population (Giovannoni *et al*., 2005). In addition, it has been reported that the *Alphaproteobacteria* also play a seminal role in the eukaryotic cell evolution (Gray *et al*., 1999; Andersson *et al*., 2003).

In 2013, we collected diverse environmental samples and isolated many novel and unrecorded bacterial species by the research program supported by NIBR in Korea. The present report focuses on the description of bacterial species belonging to the *Alphaproteobacteria* that were not previously reported in Korea. Here we report 42 unrecorded bacterial species belonging to *Alphaproteobacteria* in Korea, which consisted of 27 genera within four orders.

Materials and Methods

A total of 42 bacterial strains assigned to the class *Alphaproteobacteria* were isolated from various environmental habitats including plant root, ginseng soil, forest soil, marsh, mud flat, freshwater, and seawater (Table 1). Each environmental sample was processed separately, spread onto diverse culture agar media including R2A agar (BD, USA), marine agar 2216 (MA; BD, USA), tryptic soy agar (TSA; BD, USA) and nutrient agar (NA; BD, USA), and incubated at 25-30°C for 2-5 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 10-20% glycerol suspension at −80°C and as lyophilized ampoules.

Colony morphology of the strains was observed on agar plates with a magnifying glass after the cells were cultivated to their stationary phase. Cellular morphology and cell size were examined by either transmission electron microscopy or scanning electron microscopy. Gram staining was performed using a Gram-staining kit or the standard procedures. Biochemical characteristics were tested by 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 described elsewhere (Lee *et al*., 2014). The 16S rRNA gene sequences of the strains assigned to the *Alphaproteobacteria* were compared with those of the valid type strains using the EzTaxon-e server (Kim *et al*., 2012). For phylogenetic analyses, multiple alignments between the 16S rRNA gene sequences of the isolates and those of the valid type strains were carried out using the Clustal_X program (Thompson *et al*., 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) and the phylogenetic trees were constructed by using the neighbour-joining algorithm (Saitou and Nei, 1987) with bootstrap values based on 1,000 replications (Felsenstein, 2002).

Results and Discussion

The 42 strains were distributed into 4 orders of the *Alphaproteobacteria*; 4 strains for the *Caulobacterales*, 17 strains for the order *Rhizobiales*, 13 strains for the *Rhodobacterales*, and 7 strains for the *Sphingomonadales* (Table 1). These strains were Gram-staining-negative or positive, chemoheterotrophic, and rod-shaped bacteria (Fig. 1).

The strains in the order *Rhizobiales* (Fig. 2) belonged to 5 families 10 separate genera: *Bosea* (1 species), *Ensifer* (1 species), *Hoeflea* (1 species), *Hyphomicrobium* (1 species), *Kaistia* (1 species), *Methylobacterium* (3 species), *Mesorhizobium* (2 species), *Phyllobacterium* (2 species), *Rhizobium* (5 species), and *Shinella* (1 species). Seven strains were assigned to the family *Sphingomonadaceae* of the order *Sphingomonadales*. Two strains assigned to the family *Sphingomonadaceae* belonged to the genus *Sphingomonas*. Five strains assigned to the family *Erythrobacteraceae* belonged to the genera *Altererythrobacter*, *Erythrobacter*, *Erythromicrobium*, and *Porphyrobacter* (Fig. 3).

Four strains that were assigned to the family *caulobacteraceae* of the order *caulobacterales* belonged to the genus *Brevundimonas* (Fig. 4). The strains in the order *Rhodobacterales* (Fig. 5) were found to belong to 11 separate genera within one family: *Albidovulum* (1 species), *Albirhodobacter* (1 species), *Jannaschia* (2 species), *Loktanella* (1 species), *Nautella* (1 species), *Paracoccus* (1 species), *Planktomarina* (1 species), *Phaeobacter* (1 species), *Ruegeria* (2 species), *Sulfitobacter* (1 species), and *Yangia* (1 species). Here we report 42 unrecorded bacterial species in Korea belonging to 9 families of 4 orders in the *Alphaproteobacteria*.

Description of *Albidovulum inexpectatum* HME9313

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

Table 1. Summary of strains isolated belonging to the *Alphaproteobacteria* and their taxonomic affiliations.

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Caulobacterales	*Caulobacteraceae*	*Brevundimonas*	GDR2	NIBRBA0000114314	*Brevundimonas basaltis*	99.3	Seawater	R2A	25°C, 3d
		Brevundimonas	JJ9005	NIBRBA0000114156	*Brevundimonas subvibriodes*	99.9	Fresh water	R2A	25°C, 2d
		Brevundimonas	W4-2-3	NIBRBA0000114204	*Brevundimonas nasdae*	99.7	Mineral water	R2A	25°C, 2d
		Brevundimonas	W5-3-2	NIBRBA0000114205	*Brevundimonas bacteroides*	99.8	Mineral water	R2A	25°C, 2d
Rhodobacterales	*Rhodobacteraceae*	*Albidovulum*	HME9313	NIBRBA0000114402	*Albidovulum inexpectatum*	99.9	Seawater	MA	30°C, 2d
		Albirhodobacter	SC11	NIBRBA0000114383	*Albirhodobacter marinus*	99.9	Mud flat	MA	25°C, 2d
		Jannaschia	IMCC3052	NIBRBA0000114257	*Jannaschia rubra*	99.5	Seawater	MA	25°C, 10d
		Jannaschia	IMCC12052	NIBRBA0000114258	*Jannaschia cystaugens*	99.9	Seawater	MA	25°C, 5d
		Loktanella	HME9315	NIBRBA0000114403	*Loktanella vestfoldensis*	99.3	Seawater	MA	30°C, 2d
		Nautella	G8	NIBRBA0000114354	*Nautella italic*	99.6	Mud flat	MA	25°C, 2d
		Paracoccus	R8-4	NIBRBA0000114151	*Paracoccus zeaxanthinifaciens*	100.0	Seawater	R2A	25°C, 2d
		Phaeobacter	MBM3	NIBRBA0000114145	*Phaeobacter arcticus*	99.2	Seawater	MA	25°C, 2d
		Planktomarina	IMCC1933	NIBRBA0000114256	*Planktomarina temperata*	99.9	Seawater	MA	25°C, 10d
		Ruegeria	F4	NIBRBA0000114348	*Ruegeria atlantica*	98.8	Gut of haliotis discus hannai	MA	25°C, 3d
		Ruegeria	HME9308	NIBRBA0000114400	*Ruegeria intermedia*	99.3	Seawater	MA	30°C, 2d
		Sulfitobacter	16-4-1	NIBRBA0000114369	*Sulfitobacter mediterraneus*	99.7	Mud flat	MA	25°C, 2d
		Yangia	24-5	NIBRBA0000114368	*Yangia pacifica*	99.5	Mud flat	MA	25°C, 2d
Rhizobiales	*Bradyrhizobiaceae*	*Bosea*	SR5-12	NIBRBA0000114201	*Bosea thiooxidans*	99.2	Mug wort root	R2A	30°C, 3d
	Hyphomicrobiaceae	*Hyphomicrobium*	W2-3-3	NIBRBA0000114206	*Hyphomicrobium facile* subsp. *ureaphilum*	99.4	Mineral water	R2A	25°C, 2d
	Methylobacteriaceae	*Methylobacterium*	01SU1-P	NIBRBA0000114424	*Methylobacterium rhodesianum*	99.7	Freshwater	R2A	25°C, 5d
		Methylobacterium	2013 N5	NIBRBA0000114260	*Methylobacterium extorquens*	99.8	Freshwater	R2A	25°C, 2d
		Methylobacterium	JJ9004	NIBRBA0000114155	*Methylobacterium brachiatum*	100.0	Freshwater	R2A	25°C, 5d
	Phyllobacteriaceae	*Hoeflea*	CDR12	NIBRBA0000114310	*Hoeflea alexandrii*	99.6	Estuary water	R2A	25°C, 3d
		Phyllobacterium	Gsoil033	NIBRBA0000114220	*Phyllobacterium ifriqiyense*	99.9	Ginseng cultivating filed	R2A	25°C, 2d
		Phyllobacterium	WS140	NIBRBA0000114433	*Phyllobacterium myrsinacearum*	99.9	Freshwater	R2A	25°C, 3d
	Rhizobiaceae	*Rhizobium*	SR1-03	NIBRBA0000114193	*Rhizobium laguerreae*	99.7	False acasia root	R2A	30°C, 3d
		Ensifer	MS6Y-8-2	NIBRBA0000114282	*Ensifer adhaerens*	99.6	Ginseng soil	MA	30°C, 3d
		Kaistia	MMD3Y-10-1	NIBRBA0000114275	*Kaistia adipata*	99.9	Ginseng soil	MA	25°C, 3d
		Mesorhizobium	SR1-05	NIBRBA0000114194	*Mesorhizobium silamurunense*	99.9	False acasia root	R2A	25°C, 2d
		Mesorhizobium	BDM25	NIBRBA0000114304	*Mesorhizobium opportunistum*	99.8	Mud flat	MA	25°C, 3d
		Rhizobium	ST6-05	NIBRBA0000114185	*Rhizobium cellulosilyticum*	99.2	Canadian horseweed roo	TSA	30°C, 2d
		Rhizobium	2013 C32	NIBRBA0000114258	*Rhizobium rosettiformans*	99.9	Freshwater	MA	25°C, 2d
		Rhizobium	MK6Y-2-1	NIBRBA0000114272	*Rhizobium mesosinicum*	99.9	Ginseng soil	MA	30°C, 3d
		Rhizobium	MS6Y-8-4	NIBRBA0000114283	*Rhizobium grahamii*	99.3	Ginseng soil	MA	30°C, 3d
		Shinella	BS17	NIBRBA0000114214	*Shinella zoogloeoides*	99.4	Compost	R2A	25°C, 2d
Sphingomonadales	*Erythrobacteraceae*	*Erythrobacter*	CDM27	NIBRBA0000114309	*Erythrobacter citreus*	99.4	Estuary water	MA	30°C, 3d
		Altererythrobacter	HME9328	NIBRBA0000114406	*Altererythrobacter gangjinensis*	100.0	Seawater	MA	30°C, 2d
		Erythrobacter	IMCC19253	NIBRBA0000114259	*Erythrobacter citreus*	99.3	Freshwater	R2A	25°C, 5d
		Erythromicrobium	JJ9007	NIBRBA0000114158	*Erythromicrobium ramosum*	99.9	Freshwater	R2A	25°C, 2d
		Porphyrobacter	KEM112	NIBRBA0000114318	*Porphyrobacter sanguineus*	99.5	Tidal flat	R2A	25°C, 3-4d
	Sphingomonadaceae	*Sphingomonas*	JJ9002	NIBRBA0000114153	*Sphingomonas faeni*	99.7	Fresh water	R2A	25°C, 2d
		Sphingomonas	PMX-RY	NIBRBA0000114218	*Sphingomonas yabuuchiae*	99.4	Tap water	R2A	25°C, 2d

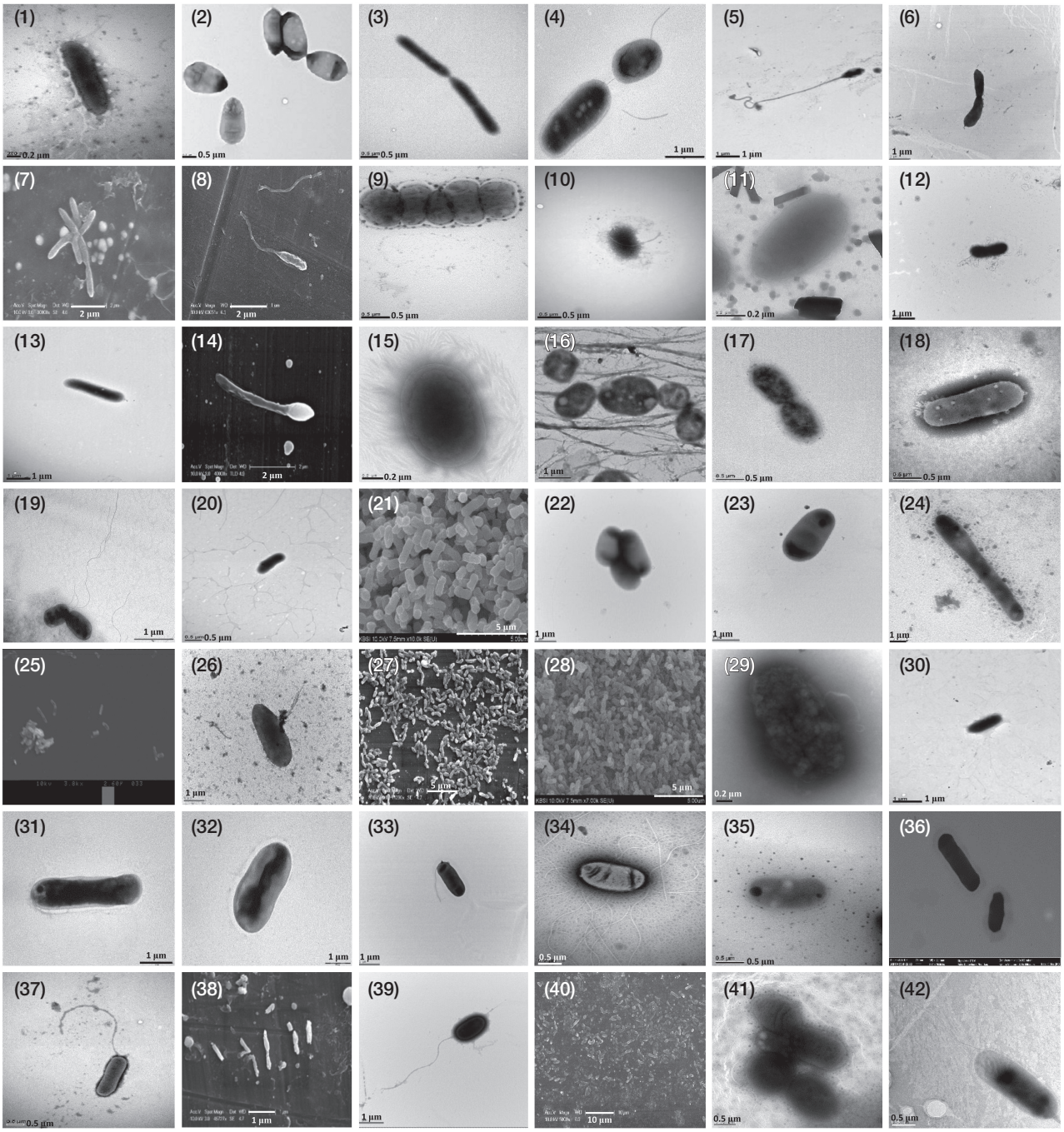

Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strains: 1, HME9313; 2, SC11; 3, HME9328; 4, SR5-12; 5, GDR2; 6, JJ9005; 7, W4-2-3; 8, W5-3-2; 9, MS6Y-8-2; 10, CDM27; 11, IMCC19253; 12, JJ9007; 13, CDR12; 14, W2-3-3; 15, IMCC3052; 16, IMCC12052; 17, MMD3Y-10-1; 18, HME9315; 19, SR1-05; 20, BDM25; 21, 01SU1-P; 22, 2013N5; 23, JJ9004; 24, G8; 25, R8-4; 26, MBM3; 27, Gsoil033; 28, WS140; 29, IMCC1933; 30, KEM112; 31, SR1-03; 32, ST6-05; 33, 2013C32; 34, MK6Y-2-1; 35, MS6Y-8-4; 36, F4; 37, HME9308; 38, BS17; 39, JJ9002; 40, PMX-RY; 41,16-4-1; 42, 24-5.

rod-shaped. Colonies are circular, convex, entire, and cream colored after 2 days on MA at 30°C. Positive for esculin hydrolysis and β-galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, gelatinase, and urease activities. D-Glucose, L-arabinose, D-mannitol, D-maltose, adipic acid and malic acid are utilized. Does not utilize D-mannose, potassium gluconate, *N*-acetyl-

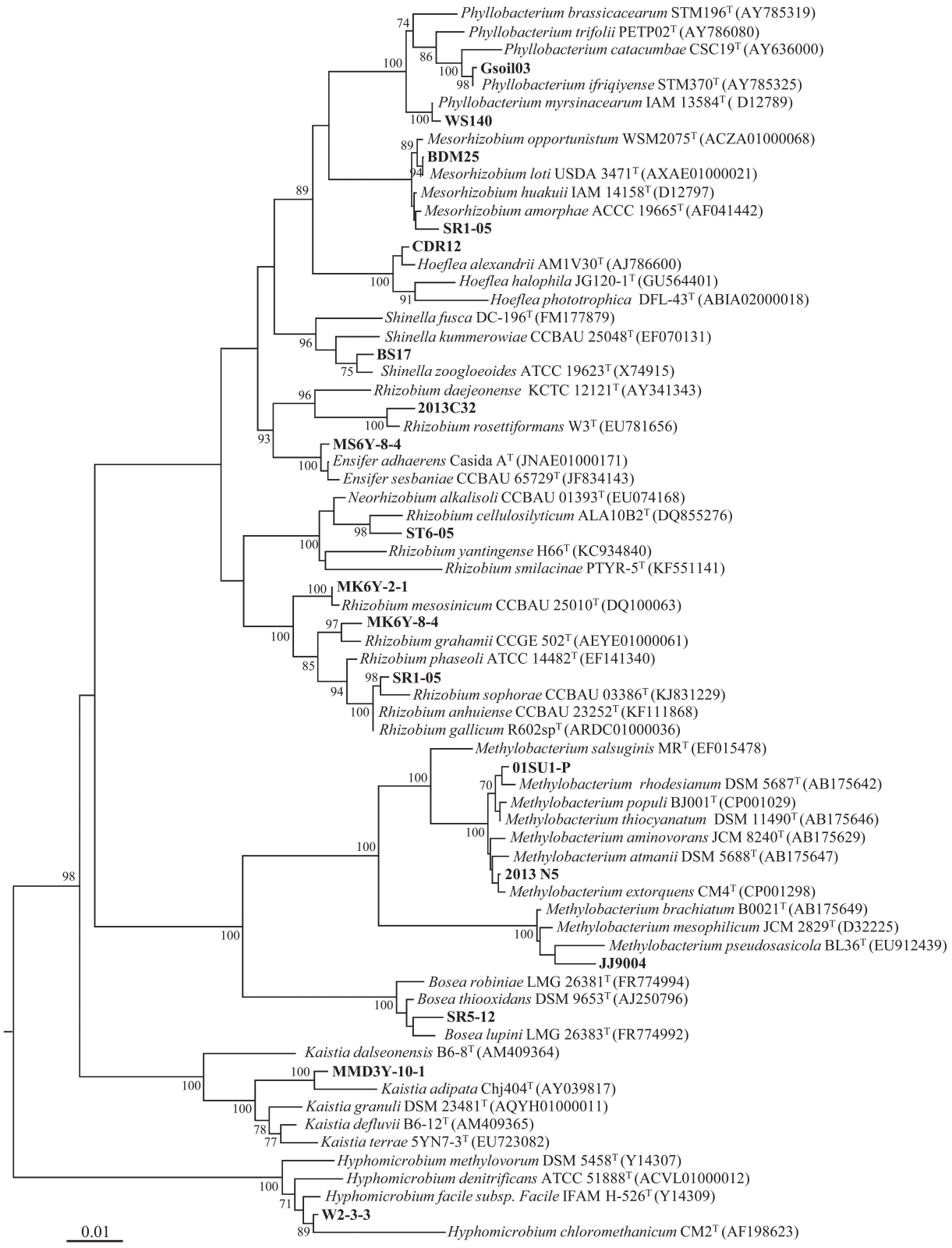

Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships between the strains isolated in this study and their relatives of the order *Rhizobiales* in the class *Alphaproteobacteria*. *Escherichia coli* ATCC 11775^T (X80725) was used as an outgroup (not shown). Bootstrap values (>70%) are shown above nodes for the neighbor-joining. Scale bar: 0.01 changes per nucleotide.

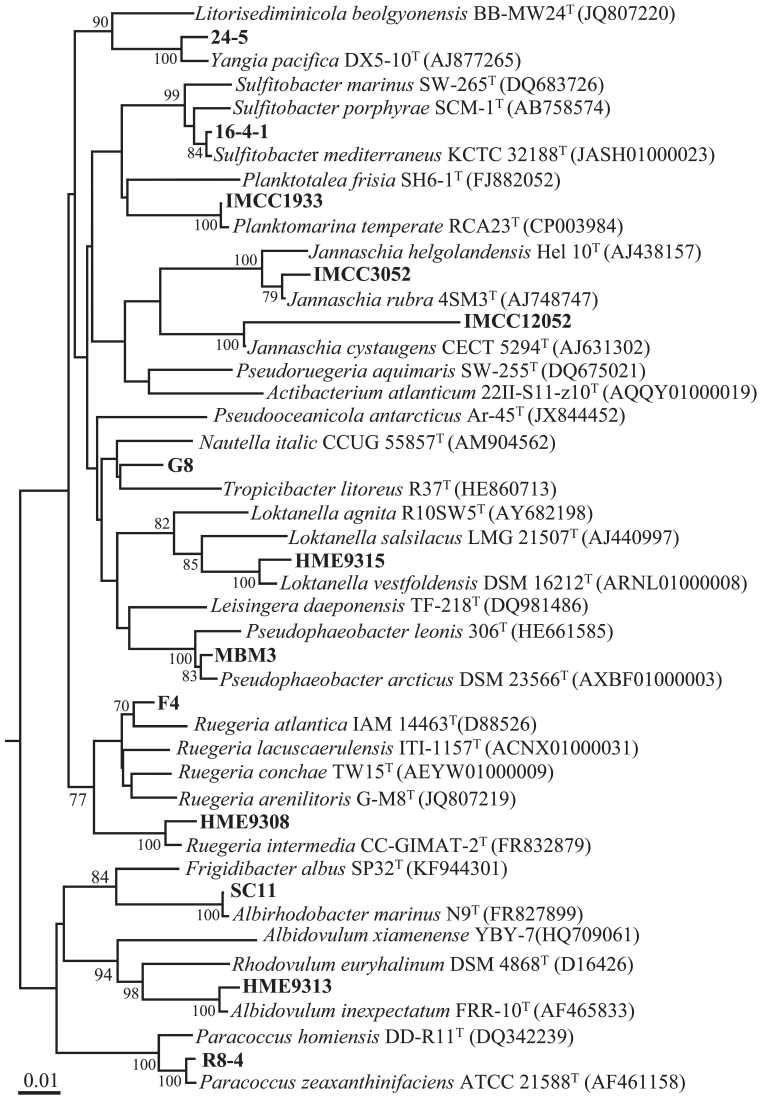

Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships between the strains isolated in this study and their relatives of the order *Rhodobacterales* in the class *Alphaproteobacteria*. *Escherichia coli* ATCC 11775^T (X80725) was used as an outgroup (not shown). Bootstrap values (>70%) are shown above nodes for the neighbor-joining. Scale bar: 0.01 changes per nucleotide.

glucosamine, phenylacetic acid, trisodium citrate, and capric acid. Strain HME9313 (=NIBRBA0000114402) was isolated from a sea water sample, Shinan-gun, Korea.

Description of *Albirhodobacter marinus* SC11

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, and entire yellow colored after 2 days on MA at 25°C. Positive for indole production and urea and esculin hydrolysis. D-Glucose, L-arabinose, D-mannose, and D-mannitol are utilized. Negative for nitrate reduction, glucose fermentation, and arginine dihydrolase, gelatinase, and β-galactosidase activities. Does not utilize potassium gluconate, adipic acid, malic acid, *N*-acetyl-glucosamine, D-maltose, phenylacetic acid, trisodium citrate, and capric acid. Strain SC11 (=NIBRBA0000114383) was isolated from a tidal flat sample, Taean, Korea.

Description of *Altererythrobacter gangjinensis* HME9328

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and ochre colored after 2 days on MA at 30°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, gelatinase, and urease

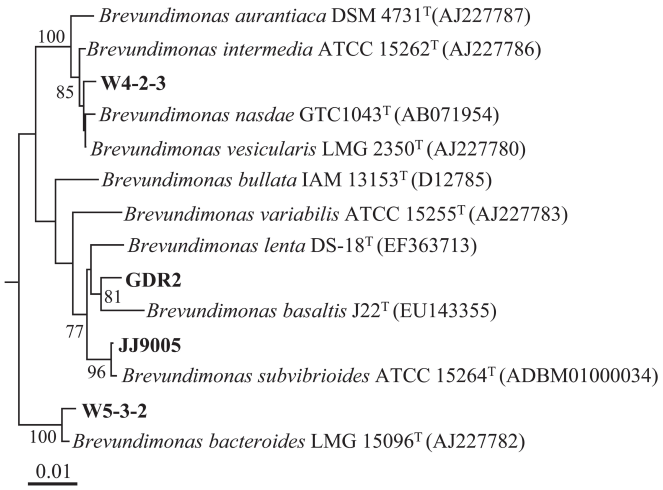

Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships between the strains isolated in this study and their relatives of the order *Caulobacterales* in the class *Alphaproteobacteria*. *Escherichia coli* ATCC 11775^T (X80725) was used as an outgroup (not shown). Bootstrap values (>70%) are shown above nodes for the neighbor-joining. Scale bar: 0.01 changes per nucleotide.

activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-maltose, adipic acid, D-mannitol, *N*-acetyl-glucosamine, malic acid, potassium gluconate, phenylacetic acid, trisodium citrate, and capric acid. Strain HME9328 (=NIBRBA0000114406) was isolated from a sea water sample, Shinan-gun, Korea.

Description of *Bosea thiooxidans* SR5-12

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, entire, convex, and white colored after 3 days on R2A agar at 30°C. Positive for nitrate reduction and urease activity in API 20NE, but negative for indole production, esculin hydrolysis, glucose fermentation, and arginine dihydrolase, β-galactosidase, and gelatinase activities. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, trisodium citrate, and phenylacetic acid, but utilizes L-arabinose, potassium gluconate, adipic acid, malic acid, and capric acid. Strain SR5-12 (=NIBRBA 0000114201) was isolated from a mug wort root, Daejeon, Korea.

Description of *Brevundimonas bacteroides* W5-3-2

Cells are Gram-staining-negative and rod-shaped. Colonies are circular, raised, and entire yellow colored after 2 Days on R2A agar at 25°C. Positive for nitrate reduction in API 20NE, but negative for urea and esculin hydrolysis, indole production, glucose fermentation, and β-galactosidase, arginine dihydrolase, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, D-mannitol, potassium gluconate, malic acid, trisodium citrate, capric acid, adipic acid, and phenylacetic acid. Strain W5-3-2 (=NIBRBA0000114205) was isolated from mineral water, Daejeon, Korea.

Description of *Brevundimonas basaltis* GDR2

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, smooth, convex, glistening, and cream colored after 3 days on R2A agar at 25°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, urease, glucose fermentation, indole production, and arginine dihydrolase and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, phenylacetic acid, and capric acid. Strain GDR2 (=NIBRBA0000114314) was isolated from a sea water, Pohang, Korea.

Description of *Brevundimonas nasdae* W4-2-3

Cells are Gram-staining-negative and rod-shaped. Colonies are circular, raised, and entire light yellow colored after 2 days on R2A agar at 25°C. Positive for nitrate reduction, esculin hydrolysis, and arginine dihydrolase and urease and activities in API 20NE, but negative for indole production, glucose fermentation, and gelatinase and β-galactosidase activities. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, malic acid, trisodium citrate, D-mannitol, capric acid, adipic acid, and phenylacetic acid, but utilizes D-glucose and D-maltose. Strain W4-2-3 (=NIBRBA

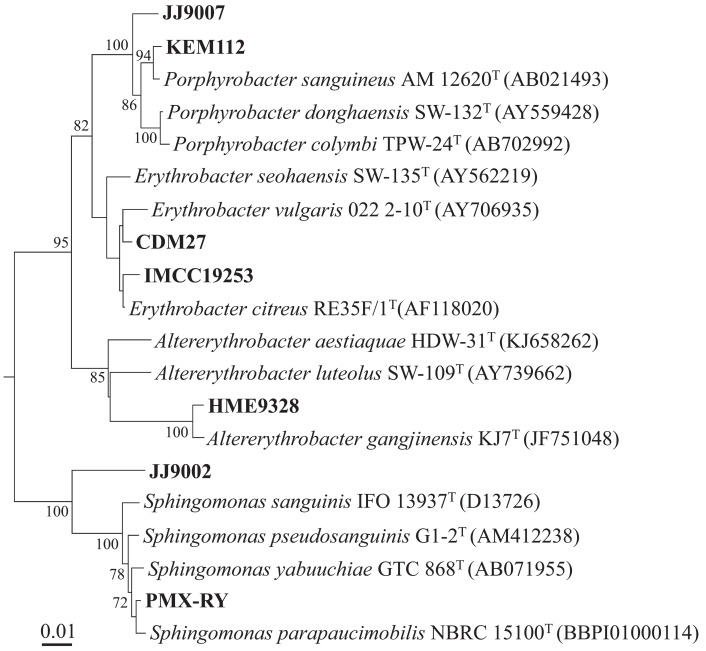

Fig. 5. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationship between the strains isolated in this study and their relatives of the order *Sphingomonadales* in the class *Alphaproteobacteria*. *Escherichia coli* ATCC 11775^T (X80725) was used as an outgroup (not shown). Bootstrap values (>70%) are shown above nodes for the neighbor-joining. Scale bar: 0.01 changes per nucleotide.

0000114204) was isolated from mineral water, Daejeon, Korea.

Description of *Brevundimonas subvibriodes* JJ9005

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, and orange-colored after 2 days on R2A agar at 25°C. Positive for hydrolysis of esculin. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, β-galactosidase, and gelatinase activities in API 20NE. D-Glucose, D-mannose, and D-mannitol are utilized. Does not utilize L-arabinose, malic acid, trisodium citrate, *N*-acetyl-glucosamine, capric acid, potassium gluconate, adipic acid, D-maltose, and phenylacetic acid. Strain JJ9005 (=NIBRBA0000114156) was isolated from a freshwater sample, Wanju-gun, Korea.

Description of *Ensifer adhaerens* MS6Y-8-2

Cells are Gram-staining-positive, non-flagellated, and rod-shaped. Colonies are circular, entire, smooth, raised, and white colored after 3 days of incubation on MA at 30°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, and urease and β-galactosidase activities. Negative for indole production, glucose fermentation, and gelatinase and arginine dihydrolase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, trisodium citrate, malic acid, and phenylacetic acid are utilized. Does not utilize capric acid. Strain MS6Y-8-2 (=NIBRBA0000114282) was isolated from a ginseng soil sample, Anseong, Korea.

Description of *Erythrobacter citreus* CDM27

Cells are Gram-staining-negative, flagellated, and coccoid- or oval-shaped. Colonies are circular, convex, smooth, glistening, and yellow colored after 3 days on MA at 30°C. Positive for esculin hydrolysis and gelatinase activity, but negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and β-galactosidase activities in API 20NE. Does not utilize D-glucose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-arabinose, D-mannose, D-mannitol, and D-maltose. Strain CDM27 (=NIBRBA0000114309) was isolated from an estuary water, Jeju island, Korea.

Description of *Erythrobacter citreus* IMCC19253

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, entire, convex, smooth, and yellow colored after 5 days on R2A agar at 25°C. Positive for esculin hydrolysis and urease and arginine dihydrolase activities. Negative for nitrate re-

duction, indole production, glucose fermentation, and β-galactosidase and gelatinase activities. D-Glucose, D-mannitol, adipic acid, trisodium citrate, and potassium gluconate are utilized. Does not utilize L-arabinose, *N*-acetyl-glucosamine, D-maltose D-mannose, capric acid, malic acid, and phenylacetic acid. Strain IMCC19253 (=NIBRBA0000114259) was isolated from a freshwater sample, Chuncheon, Korea.

Description of *Erythromicrobium ramosum* JJ9007

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, and orange colored after 2 days on R2A agar at 25°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activity in API 20NE, but negative for indole production, glucose fermentation, and gelatinase, arginine dihydrolase, and ureasea activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, trisodium citrate, and phenylacetic acid. Adipic acid and malic acid are utilized. Strain JJ9007 (=NIBRBA0000114158) was isolated from a freshwater sample, Seocheon, Korea.

Description of *Hoeflea alexandrii* CDR12

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, glistening, and cream colored after 3-4 days on R2A agar at 37°C. Positive for nitrate reduction, urease, β-galactosidase and esculin hydrolysis. Negative for glucose fermentation, indole production, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, and trisodium citrate are utilized. Does not utilize phenylacetic acid and capric acid. Strain CDR12 (=NIBRBA 0000114310) was isolated from an estuary water, Jeju island, Korea.

Description of *Hyphomicrobium facile* W2-3-3

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, and entire yellow colored after 2 days on R2A agar at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, gelatinase, urease, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, *N*-acetyl-glucosamine, capric acid, adipic acid, and potassium gluconate. Strain W2-3-3 (=NIBRBA0000114206) was isolated from mineral water, Daejeon, Korea.

Description of *Jannaschia cystaugens* IMCC12052

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, and cream colored after 5 days on MA at 25°C. Negative for nitrate reduction, glucose fermentation, indole production, esculin hydrolysis, and arginine dihydrolase, β-galactosidase, urease, and gelatinase activities in API 20NE. Does not utilize D-glucose, L-arabinose, D-maltose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid, trisodium citrate, phenylacetic acid, capric acid, and adipic acid. Strain IMCC 12052 (=NIBRBA0000114258) was isolated from seawater, Incheon, Korea.

Description of *Jannaschia rubra* IMCC3052

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are irregular, smooth, shiny, and dark red colored after 10 days on MA at 25°C. Diffusible pigments are produced on MA. Positive for nitrate reduction, esculin hydrolysis, and urease and β-galactosidase activities, but negative for indole production, glucose fermentation, arginine dihydrolase and gelatinase in API 20NE. D-Glucose, D-mannose and malic acid are utilized. Does not utilize L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, trisodium citrate, adipic acid, and phenylacetic acid. Strain IMCC3052 (=NIBRBA0000114257) was isolated from a seawater sample, Incheon, Korea.

Description of *Kaistia adipata* MMD3Y-10-1

Cells are Gram-staining-negative, non-flagellated, and oval-shaped. Colonies are circular, entire, smooth, raised, and white colored after 3 days on MA at 30°C. Positive for nitrate reduction, esculin hydrolysis, and urease and β-galactosidase activities in API 20NE, but negative for indole production, gelatinase activity, and glucose fermentation. *N*-Acetyl-glucosamine and D-maltose are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, trisodium citrate, phenylacetic acid, adipic acid, and malic acid. Strain MMD3Y-10-1 (=NIBRBA0000114275) was isolated from a ginseng soil sample, Anseong, Korea.

Description of *Loktanella vestfoldensis* HME9315

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and beige colored after 2 days on MA at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, gelatinase, and urease activities. Positive for esculin hydrolysis and β-galactosidase activity. D-Mannitol and *N*-acetyl-glucosamine are utilized. Does not utilize D-glucose, L-arabinose, D-

mannose, D-maltose, adipic acid, malic acid, potassium gluconate, phenylacetic acid, trisodium citrate, and capric acid. Strain HME9315 (=NIBRBA0000114403) was isolated from a sea water sample, Shinan-gun, Korea.

Description of *Mesorhizobium opportunistum* BDM25

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, smooth, convex, glistening, and cream colored after 3 days on MA at 25°C. Positive for nitrate reduction, esculin hydrolysis, and gelatinase and β-galactosidase activities in API 20NE, but negative for glucose fermentation, indole production, and arginine dihydrolase and urease activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, trisodium citrate, malic acid, and phenylacetic acid are utilized. Does not utilize capric acid and adipic acid. Strain BDM25 (=NIBRBA0000114304) was isolated from a tidal flat sample, Wando, Korea.

Description of *Mesorhizobium silamurunense* SR1-05

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, convex, and pale yellow colored after 3 days on R2A agar at 30°C. Positive for nitrate production, esculin hydrolysis, and urease activity in API 20NE, but negative for glucose fermentation, indole production, and arginine dihydrolase, β-galactosidase, and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid are utilized. Does not utilize potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain SR1-05 (=NIBRBA0000114194) was isolated from false acasia root, Daejeon, Korea.

Description of *Methylobacterium brachiatum* JJ9004

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex after 2 days on R2A agar at 25°C. Positive for urease in API 20NE, but negative for nitrate reduction, glucose fermentation, indole production, esculin hydrolysis, and β-galactosidase, arginine dihydrolase, and gelatinase activities. L-Arabinose, potassium gluconate, adipic acid, and D-maltose are utilized. Does not utilize D-glucose, D-mannose, D-mannitol, malic acid, trisodium citrate, *N*-acetyl-glucosamine, capric acid, and phenylacetic acid. Strain JJ9004 (=NIBRBA0000114155) was isolated from a freshwater sample, Wanju-gun, Korea.

Description of *Methylobacterium extorquens* N5

Cells are Gram-staining-negative, non-flagellated, pigmented, and asporogenous rod shaped. Colonies are round and pink colored after 2 days on R2A agar at 25°C. Positive for glucose fermentation and arginine dihydrolase and urease activities in API 20NE, but negative for nitrate reduction, indole production, esculin hydrolysis, and gelatinase and β-galactosidase activities. Malic acid is utilized. Does not utilize capric acid, trisodium citrate, phenylacetic acid, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and adipic acid. Strain N5 (=NIBRBA0000114170) was isolated from a freshwater sample, Jinan, Korea.

Description of *Methylobacterium rhodesianum* 01SU1-P

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are opaque, round, smooth, and pink colored after 5 days on MA at 25°C. Positive for urease. Negative for nitrate reduction, esculin hydrolysis, indole production, glucose fermentation, and β-galactosidase, arginine dihydrolase, and gelatinase activities. Malic acid is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-maltose, adipic acid, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, phenylacetic acid, trisodium citrate, and capric acid. Strain 01SU1-P (=NIBRBA0000 114424) was isolated from a fresh water sample, Changnyeong-gun, Korea.

Description of *Nautella italica* G8

Cells are Gram-staining-negative and rod-shaped. Colonies are circular, rasised, entire, convex, and pale yellow colored after 2 days on MA at 25°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. Does not utilize potassium gluconate, adipic acid, malic acid D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, phenylacetic acid, trisodium citrate, and capric acid. Strain G8 (=NIBRBA0000114354) was isolated from a tidal flat sample, Taean, Korea.

Description of *Paracoccus zeaxanthinifaciens* R8-4

Cells are Gram-staining-negative, non-flagellated, pigmented, and rod-shaped. Colonies are circular, raised, and entire yellow colored after 2 days on R2A agar at 25°C. Positive for esculin hydrolysis and urease and β-galactosidase activities, while negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities. Does not utilize D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. D-Mannose, D-mannitol, potas-

sium gluconate, and malic acid are utilized. Strain R8-4 (=NIBRBA0000114151) was isolated from a Sea water sample, Yeongdeok-gun, Korea.

Description of *Phaeobacter arcticus* MBM3

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire and yellow colored after 2 days of incubation on MA at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and urease, arginine dihydrolase, gelatinase, and β-galactosidase activities. Dose not assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, D-maltose, potassium gluconate, trisodium citrate, capric acid, phenylacetic acid, and adipic acid in API 20NE. Strain MBM3 (=NIBRBA0000114145) was isolated from a Sea water sample, Busan, Korea.

Description of *Phyllobacterium ifriqiyense* Gsoil 033

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, and entire white colored after 2 days on R2A agar at 25°C. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, and β-galactosidase, arginine dihydrolase, urease, and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and malic acid are utilized. Does not utilize capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain Gsoil 033 (=NIBRBA0000114220) was isolated from a ginseng cultivating filed sample, Pocheon, Korea.

Description of *Phyllobacterium myrsinacearum* WS140

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are opaque, round, smooth, convex, and white colored after 3 days on MA at 25°C. Positive for glucose fermentation. Negative for nitrate reduction, esculin hydrolysis, indole production, and urease, β-galactosidase, arginine dihydrolase, and gelatinase activities. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid, and trisodium citrate are utilized. Does not utilize L-arabinose, adipic acid, potassium gluconate, phenylacetic acid, and capric acid. Strain WS140 (=NIBRBA0000114433) was isolated from a fresh water sample, Changnyeong-gun, Korea.

Description of *Planktomarina temperata* IMCC1933

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are small, circular, convex, and light beige colored after 10 days on MA at 25°C. Positive for esculin hydrolysis and β-galactosidase activity in API 20NE. Does not utilize D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, phenylacetic acid, L-arabinose, capric acid, and adipic acid. Strain IMCC 1933 (=NIBRBA0000114256) was isolated from a seawater sample, Goseong-gun, Korea.

Description of *Porphyrobacter sanguineus* KEM112

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, smooth, convex, glistening, and orange colored after 3-4 days on R2A agar at 25°C. Positive for nitrate reduction, β-galactosidase, and esculin hydrolysis. Negative for glucose fermentation, indole production, and urease, arginine dihydrolase, and gelatinase activities. Potassium gluconate, adipic acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, phenylacetic acid, trisodium citrate, and capric acid. Strain KEM112 (=NIBRBA0000114318) was isolated from a tidal flat, Pohang, Korea.

Description of *Rhizobium cellulosilyticum* ST6-05

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, glistening, moist, and beige-colored after 2 days of incubation on TSA at 30°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, and urease and β-galactosidase activities in API 20NE, but negative for indole production and gelatinase and arginine dihydrolase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and malic acid are utilized. Does not utilize capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain ST6-05 (=NIBRBA0000114185) was isolated from Canadian horseweed root, Daejeon, Korea.

Description of *Rhizobium grahamii* MS6Y-8-4

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, entire, smooth, raised, and white-colored after 3 days on MA at 30°C. Positive for esculin hydrolysis and urease and β-galactosidase activities in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, trisodium citrate, malic acid, and phenylacetic acid are utilized. Does not utilize capric acid. Strain MS6Y-8-4 (=NIBRBA 0000114283) was isolated from a ginseng soil sample, Anseong, Korea.

Description of *Rhizobium laguerreae* SR1-03

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, convex, and white colored after 3 days on R2A agar at 30°C. Positive for nitrate reduction, esculin hydrolysis, and urease and β-galactosidase activities, but negatives for indole production, glucose fermentation, gelatin hydrolysis, and arginine dihydrolase activity in API 20NE. Does not utilize capric acid, potassium gluconate, trisodium citrate, adipic acid, malic acid, and phenylacetic acid. *N*-Acetyl-glucosamine, D-glucose, L-arabinose, D-mannose, D-mannitol, and D-maltose are utilized. Strain *Rhizobium nepotum* SR1-03 (=NIBRBA0000114193) was isolated from false acasia root, Daejeon, Korea.

Description of *Rhizobium mesosinicum* MK6Y-2-1

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, entire, smooth, raised, and white colored after 3 days on MA at 30°C. Negative for indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities, while positive for nitrate reduction, esculin hydrolysis, and urease and β-galactosidase activities in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, and *N*-acetyl-glucosamine are utilized. Does not utilize potassium gluconate, adipic acid, capric acid, trisodium citrate, malic acid, and phenylacetic acid. Strain MK6Y-2-1 (=NIBRBA0000114272) was isolated from a ginseng soil sample, Anseong, Korea.

Description of *Rhizobium rosettiformans* C32

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are round, convex, and cream colored after 2 days of incubation on MA at 25°C. Positive for glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, and β-galactosidase activities in API 20NE. Negative for nitrate reduction, indole production and gelatinase activity. D-Glucose, D-mannitol, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, malic acid, and trisodium citrate are utilized. Does not utilize potassium gluconate, capric acid, adipic acid, and phenylacetic acid. Strain C32 (=NIBRBA0000114168) was isolated from a seawater sample, Yeongdeok-gun, Korea.

Description of *Ruegeria atlantica* F4

Cells are Gram-staining-negative and rod-shaped. Colonies are convex and creamy yellow colored after 3 days on MA at 25°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase and arginine dihydrolase activities. Negative for glucose fermentation, indole production, and urease and gelatinase activities. Does not utilize potassium gluconate, adipic acid, malic acid, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, phenylacetic acid, trisodium citrate, and capric acid. Strain F4 (=NIBRBA0000114348) was isolated from a gut of haliotis discus hannai, Korea.

Description of *Ruegeria intermedia* HME9308

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, convex, entire, and cream colored after 2 days on MA at 30°C. Positive for indole production, β-galactosidase, and esculin hydrolysis. Negative for nitrate reduction, glucose fermentation, and arginine dihydrolase, gelatinase, and urease activities. D-Glucose, D-mannitol, adipic acid, and malic acid are utilized. Does not utilize L-arabinose, D-mannose, potassium gluconate, *N*-acetyl-glucosamine, D-maltose, phenylacetic acid, trisodium citrate, and capric acid. Strain HME9308 (=NIBRBA0000114400) was isolated from a sea water sample, Shinan-gun, Korea.

Description of *Shinella zoogloeoides* BS17

Cells are Gram-staining-negative, rod-shaped. Colonies are circular, raised, and entire white colored after 2 days of incubation on R2A agar at 25°C. Positive for acid production from D-glucose, L-arabinose, D-mannitol, D-mannose, *N*-acetyl-glucosamine, and D-maltose, esculin hydrolysis, and urease activity in API 20NE. Negative for nitrate reduction, indole production, glucose fermentation, activities of arginine dihydrolase, gelatinase, and β-galactosidase, and utilization of potassium gluconate, malic acid, capric acid, trisodium citrate, adipic acid, and phenylacetic acid. Strain BS17 (=NIBRBA 0000114214) was isolated from a compost sample, Yesan, Korea.

Description of *Sphingomonas faeni* JJ9002

Cells are Gram-staining- negative, flagellated, and rod-shaped. Colonies are circular, convex, and yellow colored after 2 days on R2A agar at 25°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. D-Glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid, and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannose, potassium gluconate, capric acid, adipic acid, and phenylacetic acid. Strain JJ9002 (=NIBRBA0000114153) was isolated from a freshwater sample, Wanju-gun, Korea.

Description of *Sphingomonas yabuuchiae* PMX-RY

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised,

and entire yellow colored after 2 days on R2A agar at 25°C. Positive for esculin hydrolysis and urease and β-galactosidase activities in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannose, trisodium citrate, *N*-acetyl-glucosamine, and potassium gluconate are utilized. Does not utilize D-maltose, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain PMX-RY (=NIBRBA0000114218) was isolated from a Tap water, Daejeon, Korea.

Description of *Sulfitobacter mediterraneus* 16-4-1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and pale yellow colored after 2 days on MA at 25°C. Positive for nitrate reduction and β-galactosidase. Negative for glucose fermentation, indole production, esculin hydrolysis, and arginine dihydrolase, gelatinase, and urease activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, malic acid, *N*-acetyl-glucosamine, phenylacetic acid, capric acid, adipic acid, and trisodium citrate. Strain 16-4-1 (=NIBRBA0000114369) was isolated from a tidal flat sample, Taean, Korea.

Description of *Yangia pacifica* 24-5

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and pale yellow colored after 2 days on MA at 25°C. Positive for nitrate reduction, urease and esculin hydrolysis. Negative for glucose fermentation, indole production, and β-galactosidase, arginine dihydrolase, and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, and malic acid are utilized. Does not utilize *N*-acetyl-glucosamine, phenylacetic acid, capric acid, adipic acid, and trisodium citrate. Strain 24-5 (=NIBRBA0000114368) was isolated from a tidal flat sample, Taean, Korea.

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