

Description of 39 unrecorded bacterial species in Korea, belonging to the class *Alphaproteobacteria*

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During an investigation of the biodiversity of bacterial species in Korea, we discovered many indigenous prokaryotic species. A total of 39 bacterial strains in the class *Alphaproteobacteria* were isolated from various environmental samples collected from marine organisms, sea water, fresh water, tap water, mud flats, activated sludge, mineral water, tidal flats, soil and decayed plants. From the high 16S rRNA gene sequence similarity (>98.7%) and formation of robust phylogenetic clades with the most closely related species, it was determined that each strain belonged to each independent and predefined bacterial species. There is no official report that any of these 39 *Alphaproteobacteria* species have been described in Korea. Specifically, 18 species in 11 genera in the order *Sphingomonadales*, 11 species in 10 genera in the order *Rhizobiales*, two species in two genera in the order *Caulobacteriales*, six species in six genera in the order *Rhodobacterales* and two species in two genera in the order *Rhodospirillales* were found in Korea. Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs are described in the species description section.

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

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INTRODUCTION

In 2014, we isolated many novel and unrecorded bacterial species from various environmental samples collected in Korea. The identified bacterial species belonged to the classes/phyla *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Deinococci*, and *Verrucomicrobia*. The aim of this study was to describe unrecorded species belonging to the *Alphaproteobacteria*.

In the phylum Proteobacteria, members of the class *Alphaproteobacteria* are a highly diverse group of bac-

teria. and keep very little harmonies. Members of the *Alphaproteobacteria* are gram negative and some parasitic intracellular members lack peptidoglycan and thus are gram variable (Brenner *et al.*, 2005; Euzéby, 2011). Members of this class have stalked, stellate, and spiral morphology. With respect to this diversity of life histories and ecological functions, the class *Alphaproteobacteria* is divided into three subclasses, *Magnetococcidae*, *Rickettsiidae* and *Caulobacteridae*, in which only one (*Magnetococcus marinus*) has been described by Bazyliński *et al.* 2012. The diversity and evolution of cell cycle regulation of these subclasses are well studied and among them *Caulobacter crescentus* has recently be-

come a model organism for similar studies (Skerker and Laub, 2004; Bowers *et al.*, 2008). In *Caulobacter crescentus*, each cell divides asymmetrically and produces two functionally and morphologically different daughter cells, the replicating “stalked” cell type and the vegetative “swarmer” type. Developmental programs occur that adjust between cell types, controlled by a web of regulatory systems (Viollier and Shapiro, 2004).

The subdivision of *Alphaproteobacteria* is a heterogeneous group of bacteria and includes pathogens of plants (*Agrobacterium*) and animals (*Brucella*, *Rickettsia*), symbionts of plants (*Rhizobia*), photosynthetic bacteria (*Rhodobacter*) and several genera that metabolize C1-compounds (e.g. *Methylobacterium*) (Williams *et al.*, 2007; Matteo *et al.*, 2013). Currently, there are no studies that have assessed differences in biochemical or molecular features that may help distinguish these bacteria from other groups.

Members of the class *Alphaproteobacteria* exhibit a variety of metabolic strategies such as nitrogen fixation bacteria (*Azospirillum* and *Rhizobium*), photosynthetic bacteria, ammonia oxidation bacteria, methylotrophic bacteria, and nitrifying bacteria. Nitrogen fixing bacteria are especially important in maintaining agricultural soil fertility. Members of the genus *Nitrobacter* are nitrifying bacteria that oxidize nitrogen compounds to NO_3^- via a process called nitrification, which increases soil fertility and is also an important component in the geochemical pathway of the nitrogen cycle. *Alphaproteobacterial* genera such as *Rickettsia*, *Ehrlichia*, and *Brucella* are important pathogens in this class, and cause typhus and Rocky Mountain spotted fever, ehrlichiosis, and brucellosis, respectively. Similarly, *Acetobacter* and *Gluconobacter* are used to synthesize acetic acid in industry. *Agrobacterium* is used to transfer foreign DNA in plant genomes, and has many other biotechnological properties (Chilton *et al.*, 1977).

Of special interest in the *Alphaproteobacteria* is the protomitochondrion, which is the ancestral group for mitochondria, organelles in eukaryotic cells; however, there has been disagreement regarding this issue (Esser *et al.*, 2004; Wu *et al.*, 2004; Fitzpatrick *et al.*, 2006). Moreover, genomic studies have discovered conserved molecular markers in a wide variety of proteins including whole proteins (i.e. signature proteins) which are the unique characteristics of either all *Alphaproteobacteria*, or their different main orders. This evidence provided the assignment of new species into these groups, and suggest that the *Alphaproteobacteria* branched off later than most other phyla and classes of Bacteria with the exception of *Betaproteobacteria* and *Gammaproteobacteria* (Oren and Garrity, 2014; Parte, 2014).

As a part of the results obtained from this research program that was conducted and supported by NIBR

Korea, the present study focuses on the description of bacterial species belonging to the class *Alphaproteobacteria* that have not been previously isolated or reported in Korea. Here, we briefly describe 39 unrecorded bacterial species in the class *Alphaproteobacteria* belonging to 15 families in five orders.

MATERIALS AND METHODS

A total of 39 bacterial strains in the class *Alphaproteobacteria* were isolated from numerous environmental samples collected from decayed plants (timber), soil, activated sludge, marine organisms, mud flats, freshwater and seawater (Table 1). Each environmental sample was processed separately, spread onto diverse culture media including R2A, marine agar 2216, tryptic soy agar (TSA) and nutrient (NA) agar, and incubated at 15, 25 and 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 as well as lyophilized ampoules.

The colony morphology was studied on agar plates until the cells grew to their stationary phase. Cell size and shape were examined either by transmission electron microscopy or scanning electron microscopy. Gram staining was performed using a Gram-staining kit or the standard procedures. The biochemical characteristics were performed using APIU 20NE (bioMérieux) according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification and 16S rRNA gene sequencing were performed using the standard procedures described elsewhere. The 16S rRNA gene sequences of the strains assigned to the *Alphaproteobacteria* were compared with sequences in GenBank by BLASTN and also analyzed using the EzBioCloud blast (<http://eztaxon-e.ezbiocloud.net/>) (Kim *et al.*, 2012). 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). Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) and the maximum-parsimony (Fitch, 1971) methods with the MEGA6 Program (Tamura *et al.*, 2013) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

The 39 strains were distributed in five orders of the *Alphaproteobacteria*; 18 strains in the order *Sphingo-*

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

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions		
Erythrobacteraceae	<i>Altererythrobacter</i>	EgM1111	NIBRBA0000114939	<i>Altererythrobacter nambicola</i> KYW48 ^T	97.1	Gut of <i>Fulvia mutica</i>	MA	25°C, 3d			
	<i>Croceicoccus</i>	IMCC12376	NIBRBA0000114862	<i>Croceicoccus marinus</i> E4A9 ^T	100	Sea water	MA	15°C, 3d			
	<i>Porphyrobacter</i>	63DPR2	NIBRBA0000114799	<i>Porphyrobacter sanguineus</i> IAMI12620 ^T	99.3	Fresh water	R2A	25°C, 3d			
Sphingomonadales	Sphingomonadaceae	<i>Blastomonas</i>	FW-2	NIBRBA0000114840	<i>Blastomonas nataoria</i> DSM 3183 ^T	100	Activated sludge	R2A	30°C, 2d		
		<i>Sandaracinorhabdus</i>	WW59	NIBRBA0000115025	<i>Sandaracinorhabdus limnophila</i> DSM 17366 ^T	99.0	Fresh water	R2A	25°C, 3d		
		<i>Sphingomicrobium</i>	HME9618	NIBRBA0000114989	<i>Sphingomicrobium astaxanthinifaciens</i> CC-AMO ^T	99.5	Sea water	MA	25°C, 3d		
		<i>Sphingomonas</i>	WS23	NIBRBA0000115019	<i>Sphingomonas faeni</i> MA-olki ^T	99.6	Fresh water	R2A	25°C, 3d		
		<i>Sphingomonas</i>	01SU6	NIBRBA0000115026	<i>Sphingomonas huanaensis</i> JSM 083058 ^T	99.6	Fresh water	R2A	25°C, 3d		
		<i>Sphingomonas</i>	SDN0101	NIBRBA0000114952	<i>Sphingomonas mucosissima</i> CPI73-2 ^T	98.5	Fresh water	NA	25°C, 3d		
		<i>Sphingomonas</i>	WM24	NIBRBA0000115018	<i>Sphingomonas echinoides</i> ATCC 14820 ^T	99.8	Fresh water	R2A	25°C, 3d		
		<i>Sphingorhabdus</i>	M41	NIBRBA0000114971	<i>Sphingorhabdus flavinaris</i> SW-151 ^T	99.3	Tidal flat	MA	25°C, 2d		
		<i>Blastomonas</i>	01SU7-P	NIBRBA0000115021	<i>Blastomonas nataoria</i> DSM 3183 ^T	99.4	Fresh water	R2A	25°C, 3d		
		<i>Novosphingobium</i>	RI-11	NIBRBA0000114813	<i>Novosphingobium barchaimii</i> LL02 ^T	99.4	Soil	R2A	30°C, 3d		
		<i>Novosphingobium</i>	LR-3	NIBRBA0000114805	<i>Novosphingobium capsulatum</i> GIFU11526 ^T	99.0	Fresh water pond	TSA	30°C, 3d		
		<i>Sphingomonas</i>	MT2F 6	NIBRBA0000114783	<i>Sphingomonas humi</i> PB323 ^T	99.5	Timber	R2A	25°C, 2d		
		<i>Sphingopyxis</i>	W5-3-3	NIBRBA0000114818	<i>Sphingopyxis alaskensis</i> RB2256 ^T	100	Mineral water	R2A	25°C, 2d		
		<i>Sphingopyxis</i>	7C-17	NIBRBA0000114838	<i>Sphingopyxis italic</i> SCI3E-S71 ^T	100	Activated sludge	R2A	30°C, 2d		
<i>Sphingopyxis</i>	KTCe-5	NIBRBA0000114842	<i>Sphingopyxis ummariensis</i> UI2 ^T	99.8	Activated sludge	R2A	30°C, 2d				
Methylobacteriaceae	<i>Methylobacterium</i>	LB-3	NIBRBA0000114803	<i>Methylobacterium tardum</i> RB677 ^T	99.9	Fresh water pond	TSA	30°C, 3d			
	<i>Methylobacterium</i>	PMX-R	NIBRBA0000114821	<i>Methylobacterium aquaticum</i> GR16 ^T	99.9	Tap water	R2A	25°C, 2d			
Bradyrhizobiaceae	<i>Bosea</i>	WM92	NIBRBA0000115022	<i>Bosea enae</i> 34614 ^T	99.5	Fresh water	R2A	25°C, 3d			
	<i>Rhodopsseudomonas</i>	MW2F51	NIBRBA0000114773	<i>Rhodopsseudomonas penitheaenatexigens</i> JA575 ^T	99.7	Fresh water	R2A	25°C, 2d			
Rhizobiales	Rhizobiaceae	<i>Phreatobacter</i>	61DPR27	NIBRBA0000114795	<i>Phreatobacter oligotrophus</i> PI 21 ^T	98.8	Fresh water	R2A	25-30°C, 5d		
		<i>Pseudoxanthobacter</i>	M49	NIBRBA0000114972	<i>Amorphus sueciae</i> YC6899 ^T	99.3	Tidal flat	MA	25°C, 2d		
		<i>Stappia</i>	HME9619	NIBRBA0000114990	<i>Stappia taiwanensis</i> CC-SPIO-10 ^T	99.8	Sea water	MA	25°C, 3d		
		<i>Martella</i>	BSW2	NIBRBA0000114955	<i>Martella radialis</i> BM5-7 ^T	98.7	Tidal flat	MA	25°C, 2d		
		<i>Aurantimonas</i>	IMCC12390	NIBRBA0000114863	<i>Aurantimonas litoralis</i> HTCC 2156 ^T	100	Sea water	MA	15°C, 3d		
		<i>Phyllobacteriaceae</i>	SDM0103	NIBRBA0000114945	<i>Mesorhizobium tamadyense</i> Ala-3 ^T	98.4	Gut of <i>Todarodes pacificus</i>	MA	15°C, 3d		
		<i>Brucecellaceae</i>	BS16	NIBRBA0000114833	<i>Ochrobactrum gallinifaciens</i> Iso196 ^T	98.9	Mushroom compost waste	R2A	30°C, 2d		
		<i>Caulobacter</i>	LR-4	NIBRBA0000114806	<i>Caulobacter segnis</i> ATCC21756 ^T	99.6	Freshwater pond	TSA	30°C, 3d		
		<i>Phenylobacterium</i>	IMCC12425	NIBRBA0000114866	<i>Phenylobacterium fulsum</i> AC-49 ^T	99.7	Sea water	MA	15°C, 3d		
		Rhodobacterales	Rhodobacteraceae	<i>Paracoccus</i>	BMI5	NIBRBA0000114936	<i>Paracoccus zhejiangensis</i> J6 ^T	96.9	Gut of <i>Fulvia mutica</i>	MA	25°C, 3d
				<i>Ruegeria</i>	EgM3207	NIBRBA0000114938	<i>Ruegeria lacuscaerulensis</i> 2111-1157 ^T	99.8	Gut of <i>Tulvia mutica</i>	MA	25°C, 3d
				<i>Sedimentitalea</i>	KHS03	NIBRBA0000114943	<i>Leisingera nanhaiensis</i> DSM 24252 ^T	97.4	Gut of <i>Todarodes pacificus</i>	MA	25°C, 3d
				<i>Planctotalea</i>	KHS07	NIBRBA0000114944	<i>Planctotalea frisia</i> SH6-1 ^T	98.2	Gut of <i>Todarodes pacificus</i>	MA	25°C, 3d
				<i>Sulfitobacter</i>	SDM0205	NIBRBA0000114948	<i>Sulfitobacter donghicola</i> DSW-25 ^T	99.8	Gut of <i>Todarodes pacificus</i>	MA	25°C, 3d
Rhodospirillales	Thalassobaculum_f Acetobacteraceae	<i>Altitrosovarius</i>	HME9615	NIBRBA0000114988	<i>Altitrosovarius crassostreae</i> CV919-312 ^T	99.7	Sea water	MA	25°C, 3d		
		<i>Oceanibaculum</i>	IMCC12392	NIBRBA0000114864	<i>Oceanibaculum indicum</i> P24 ^T	100	Sea water	MA	15°C, 3d		
		<i>Roseomonas</i>	WW106	NIBRBA0000115024	<i>Roseomonas gilardii</i> subsp. <i>gilardii</i> ATCC 49956 ^T	100	Fresh water	R2A	25°C, 3d		

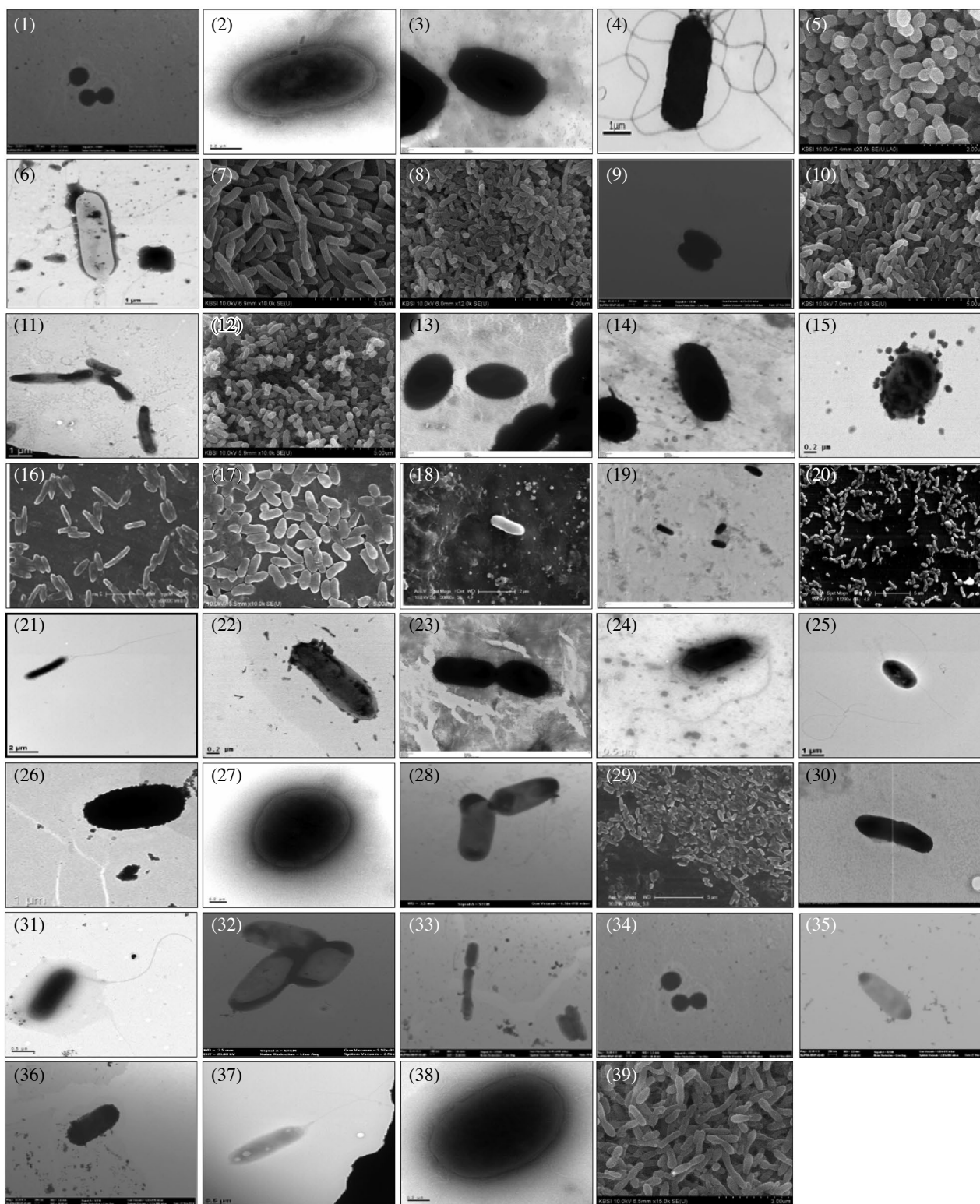


Fig. 1. Transmission and scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, EgM1111; 2, IMCC12376; 3, 63DPR2; 4, FW-2; 5, WW59; 6, HME9618; 7, WS23; 8, 01SU6; 9, SDN0101; 10, WM24; 11, M41; 12, 01SU7-P; 13, R1-11; 14, LR-3; 15, MT2F 6; 16, W5-3-3; 17, 7C-17; 18, KTCe-5; 19, LB-3; 20, PMX-R; 21, WM92; 22, MW2F51; 23, 61DPR27; 24, M49; 25, HME9619; 26, BSW2; 27, IMCC12390; 28, SDM0103; 29, BS16; 30, LR-4; 31, IMCC12425; 32, BM15; 33, EgM3207; 34, KHS03; 35, KHS07; 36, SDM0205; 37, HME9615; 38, IMCC12392; 39, WW106.

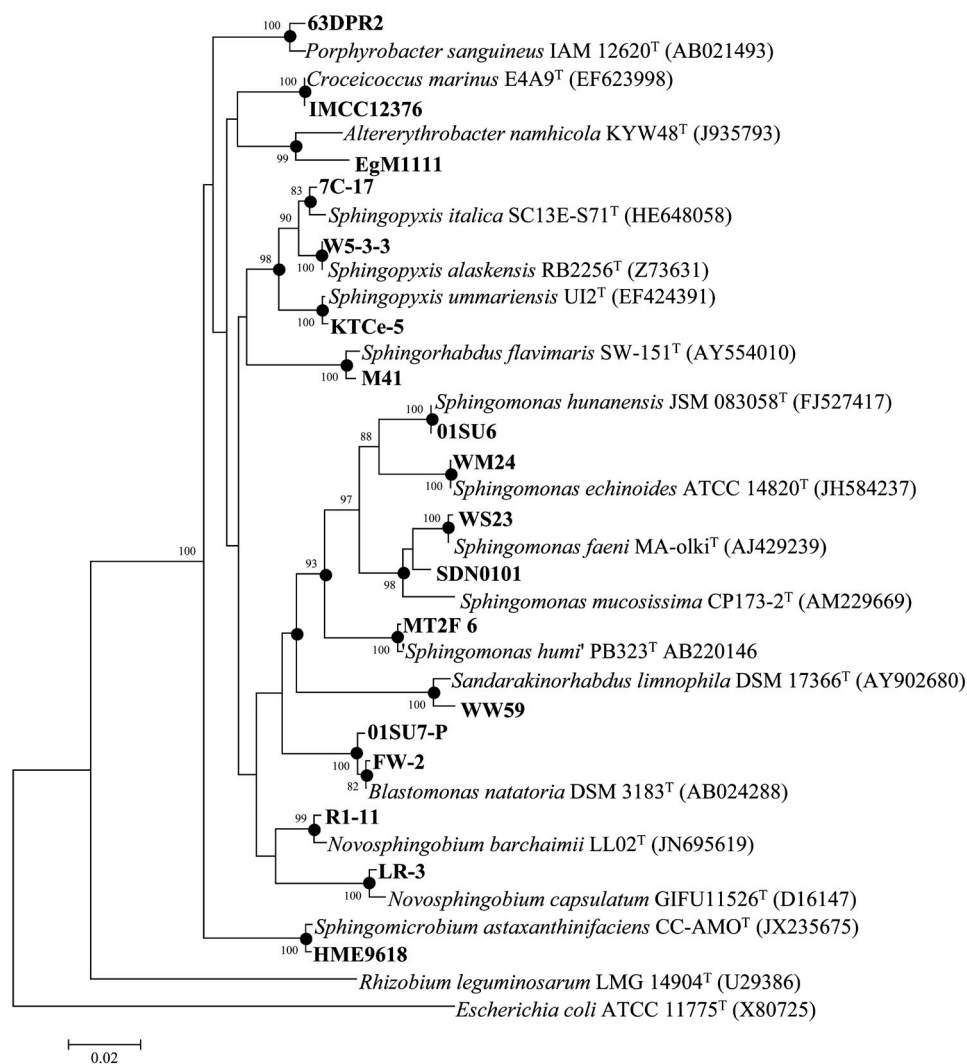


Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Sphingomonadales* in the class *Alphaproteobacteria*. Bootstrap values (>70%) are shown. Filled circles indicate the nodes recovered by the maximum-likelihood & maximum-parsimony tree algorithms. Bar, 0.02 substitutions per nucleotide position.

monadales, 11 strains in the *Rhizobiales*, two strains in the *Caulobacteriales*, six strains in the *Rhodobacterales*, and two strains in the *Rhodospirillales* (Table 1). These strains were Gram-staining-negative, chemoheterotrophic, rod and short-rod shaped bacteria except for strain EgM1111 (1) (Fig. 1).

The strains in the order *Sphingomonadales* (Fig. 2) belonged to two families and 11 genera: *Sphingomonas* (5 species), *Altererythrobacter* (1 species), *Croceicoccus* (1 species), *Porphyrobacter* (1 species), *Blastomonas* (2 species), *Sandarakinorhabdus* (1 species), *Sphingomicrobium* (1 species), *Sphingorhabdus* (1 species), *Novosphingobium* (2 species) and *Sphingopyxis* (3 species). 11 strains were assigned to the order *Rhizobiales*: two strains in the family

Methylobacteriaceae, two strains in the *Bradyrhizobiaceae* and seven strains were assigned to families *Phreatobacter_f*, *Pseudoxanthobacter_f*, *Stapia_f*, *Marteletella_f*, *Aurantimonadaceae*, *Phyllobacteriaceae* and *Brucellaceae*, respectively (Fig. 3, Table 1).

Fig. 4 shows phylogenetic assignments of 10 strains of the orders *Caulobacteriales*, *Rhodobacterales* and *Rhodospirillales*. Two strains belonged to *Caulobacter* in the family *Caulobacteraceae*. Six strains belonged to *Paracoccus*, *Ruegeria*, *Sedimentitalea*, *Planktotalea*, *Sulfitobacter* and *Aliiroseovarius* in the family *Rhodobacteraceae*. Two strains belonged to the family *Rhodospirillales*.

Here, we report 39 unrecorded bacterial species in

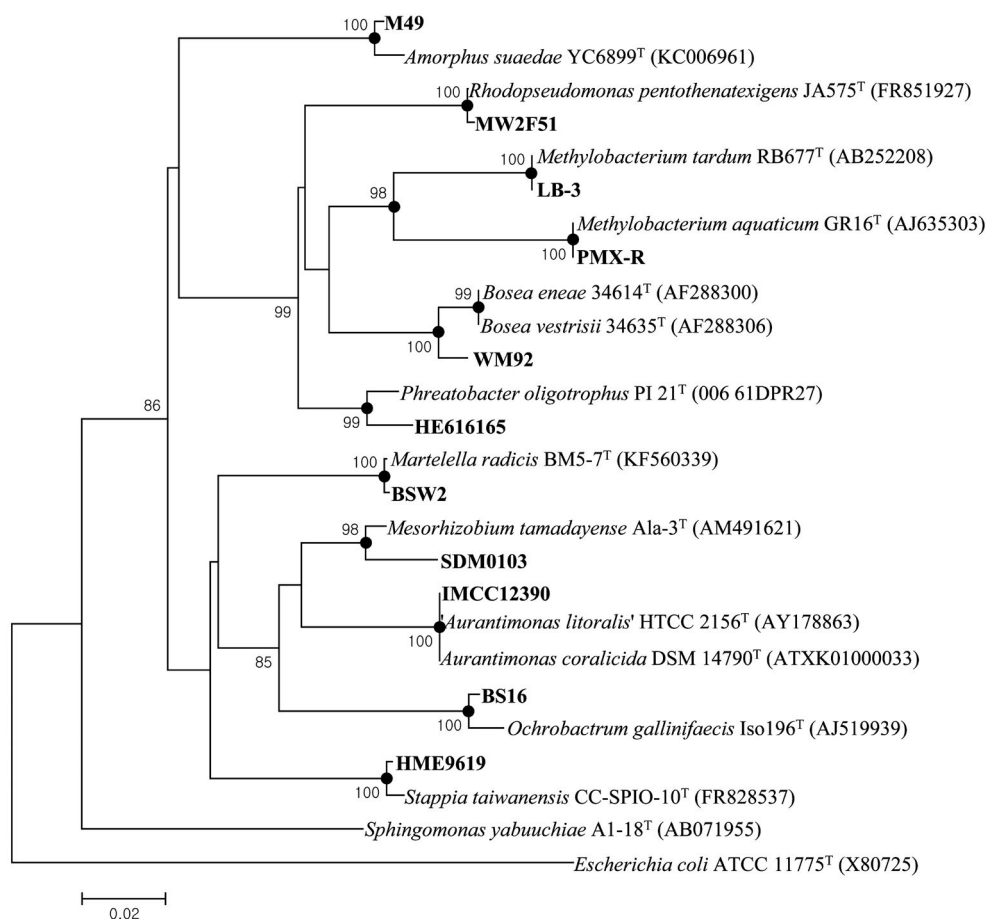


Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Rhizobiales* in the class *Alphaproteobacteria*. Bootstrap values greater than 70% are shown. Filled circles indicate the nodes recovered by the maximum-likelihood & maximum-parsimony tree methods. Bar, 0.02 substitutions per nucleotide position.

Korea belonging to 15 families in five orders of the *Alphaproteobacteria*.

Description of *Altererythro bacter namhicola* EgM1111

Cells are Gram-staining-negative, non-flagellated, diffusible pigmented and cocci-shaped. Colonies are circular, convex and orange-yellow colored after 3 days of incubation on MA at 25°C. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, urease, gelatinase, β -galactosidase, indole production, glucose fermentation and arginine dihydrolase. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain EgM1111 (= NIBRBA0000114939) has been isolated from gut of *Fulvia mutica*, Wando, Korea.

Description of *Croceicoccus marinus* IMCC12376

Cells are Gram-staining-negative, non-flagellated, diffusible-pigmented, and rod-shaped. Colonies are circular, convex, smooth and yellow colored after 3 days of incubation on MA at 15°C. Strain IMCC12376 positive for nitrate reduction, hydrolysis of esculin, β -galactosidase and negative for arginine dihydrolase, urease, Indole production, glucose fermentation and gelatin. Utilizes D-glucose, L-arabinose, N-acetyl-glucosamine and malic acid but does not utilize capric acid, adipic acid, phenylacetic acid, D-mannose, D-mannitol, D-maltose, potassium gluconate and trisodium citrate. Strain IMCC 12376 (= NIBRBA0000114862) has been isolated from sea water, Sokcho, Korea.

Description of *Porphyrobacter sanguineus* 63DPR2

Cells are Gram-staining-negative, non-flagellated, diffusible-pigmented and rod-shaped. Colonies are punc-

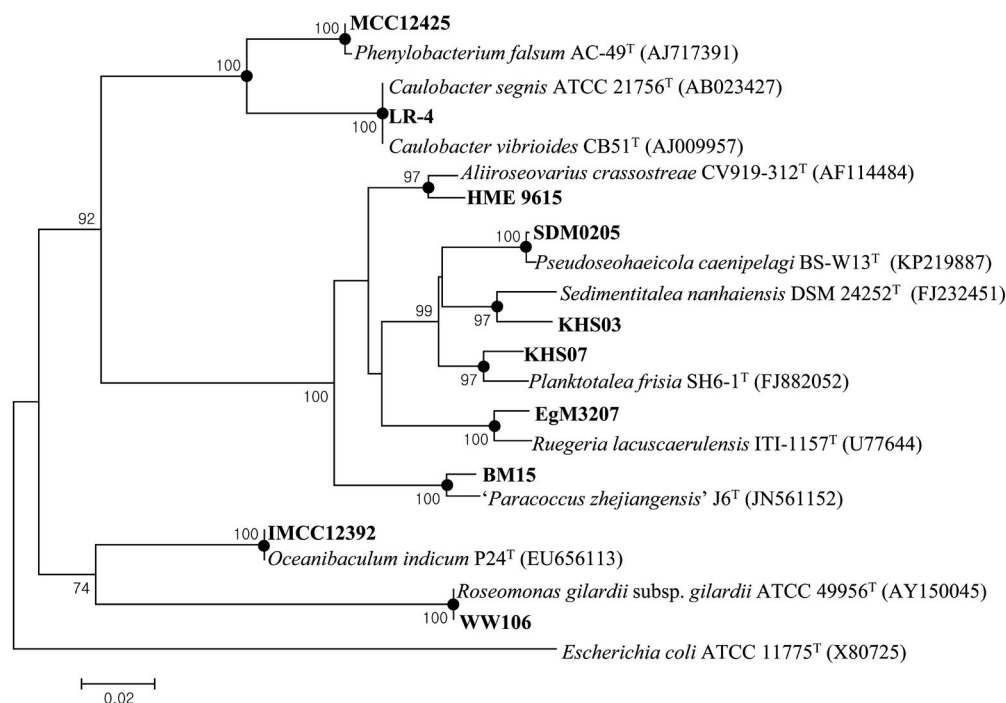


Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Rhodobacterales*, *Caulobacterales* and *Rhodospirillales* in the class *Alphaproteobacteria*. Bootstrap values > 70% are shown. Filled circles indicate the nodes recovered by the maximum-likelihood & maximum-parsimony tree methods. Bar, 0.02 substitutions per nucleotide position.

tiform, smooth, and red orange colored after 3 days of incubation on R2A at 25°C. In API 20NE, positive for esculin hydrolysis but negative for nitrate production, glucose fermentation, urease, indole β -galactosidase production, arginine dihydrolase and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, capric acid, adipic acid and phenylacetic acid. Strain 63DPR2 (= NIBRBA 0000114799) has been isolated from fresh water sample, Daejeon, Korea.

Description of *Blastomonas natatoria* FW-2

Cells are Gram-reaction-negative, flagellated, pigmented and rod shaped. Colonies are grown on R2A agar plates for 2 days are yellow, circular, raised, entire, with regular margins, and 2-3.5 mm in diameter. Positive for gelatinase in API 20NE, but negative for nitrate reduction, urease, esculin hydrolysis, glucose fermentation and β -galactosidase indole production and arginine dihydrolase. Does not utilize D-glucose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and phenyl acetic acid but utilizes L-arabinose, D-maltose, trisodium citrate and adipic acid. Strain FW-2 (= NIBRBA0000114840) has been isolated from

activated sludge, Daejeon, Korea.

Description of *Sandarakinorhabdus limnophila* WW59

Cells are Gram-staining-negative, non-spore-forming, non-flagellated, and pigmented rods. Colonies are circular, smooth, drop-like, and dark orange colored at 25°C on R2A agar medium after 3 days of incubation. In API 20NE, positive for gelatinase and β -galactosidase but negative for nitrate reduction, urea, arginine dihydrolase, esculin, indole production and glucose fermentation. 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 WW59 (= NIBR BA0000115025) has been isolated from fresh water, Changnyeong, Korea.

Description of *Sphingomicrobium astaxanthinifaciens* HME9618

Cells are Gram-staining-negative, non-spore-forming, flagellated, pigmented and rod-shaped. Colonies are circular, convex, entire and orange colored after 3 days of incubation on Marine agar medium at 25°C. In API 20NE, positive esculin hydrolysis but negative for ni-

trate reduction, urea indole production, glucose fermentation, arginine dihydrolase, gelatinase and β -galactosidase. Does not utilize D-glucose, L-arabinose, capric acid, and phenyl-acetic acid but D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and trisodium citrate are utilized. Strain HME9618 (= NIBRBA0000114989) has been isolated from sea water, Gangneung, Korea.

Description of *Sphingomonas faeni* WS23

Cells are Gram-staining-negative, non-flagellated, pigmented, and rod-shaped. Colonies are circular, entire, convex, smooth and orange colored after 3 days of incubation on R2A agar medium at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urea and gelatinase but positive for esculin hydrolysis and β -galactosidase. D-Glucose, L-arabinose, D-mannose, citric acid, *N*-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate are utilized. D-Mannitol, potassium gluconate, capric acid, adipic acid and phenyl-acetic acid are not utilized. Strain WS23 (= NIBRBA0000115019) has been isolated from fresh water, Changnyeong, Korea.

Description of *Sphingomonas hunanensis* 01SU6

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, smooth, convex and yellow colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and assimilation of D-maltose in API 20NE but negative for nitrate reduction, arginine dihydrolase, urease, indole production, glucose fermentation, gelatinase, β -galactosidase, D-glucose, L-arabinose, D-mannitol, D-mannose, potassium gluconate, malic acid, trisodium citrate, *N*-acetyl-glucosamine, capric acid, adipic acid and phenylacetic acid. Strain 01SU6 (= NIBRBA0000115026) has been isolated from a fresh water sample, Changnyeong, Korea.

Description of *Sphingomonas mucosissima* SDN0101

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular and yellow colored after 3 days on NA at 25°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE but negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase and glucose fermentation. D-Glucose, D-mannose, D-maltose, D-mannitol and trisodium citrate are utilized. Does not utilize L-arabinose *N*-acetyl-glucosamine, citrate, malic acid, potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid. Strain SDN0101 (= NIBRBA0000114952) has been isolated from a fresh water sample, Taean,

Korea.

Description of *Sphingomonas echinoides* WM24

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, entire, convex, smooth and yellow colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine and D-maltose are utilized. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain WM24 (= NIBRBA0000115018) has been isolated from fresh water sample, Changnyeong, Korea.

Description of *Sphingorhabdus flavimaris* M41

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are rhizoid, convex and white colored after 2 days of incubation on MA medium at 25°C. Positive for nitrate reduction and esculin hydrolysis but negative for Indole production, urea, glucose fermentation, arginine dihydrolase, gelatinase and β -galactosidase. Positive for assimilation for D-glucose and negative for capric acid, *N*-acetyl-glucosamine, L-arabinose, D-mannose, D-mannitol, D-maltose, D-maltose, potassium gluconate, trisodium citrate, adipic acid and phenyl acetic acid. Strain M41 (= NIBRBA 0000114971) has been isolated from a tidal flat sample, Taean, Korea.

Description of *Blastomonas natoria* 01SU7-P

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, smooth, convex and yellow colored after 3 days of incubation on R2A at 25°C. In API 20NE, only positive D-glucose utilization while negative for nitrate reduction glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, indole production, β -galactosidase and assimilation of 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 01SU7-P (= NIBRBA0000115021) has been isolated from a fresh water sample, Changnyeong, Korea.

Description of *Novosphingobium barchaimii* R1-11

Cells are Gram-staining-negative, non-flagellated, diffusible-pigmented, and rod-shaped. Colonies are circular, convex, smooth, and yellow colored after 3 days on R2A at 30°C. Negative for nitrate reduction,

indole production, arginine dihydrolase, urease, glucose fermentation, and gelatinase but positive for esculin hydrolysis and β -galactosidase in API 20NE. Utilizes D-glucose, D-mannose, L-arabinose and *N*-acetyl-glucosamine but D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, D-maltose, adipic acid and phenylacetic acid are not utilized. Strain R1-11 (= NIBRBA0000114813) has been isolated from a soil sample, Daejeon, Korea.

Description of *Novosphingobium capsulatum* LR-3

Cells are Gram-staining-negative, non-flagellated, diffusible pigmented and rod-shaped. Colonies are irregular, smooth, white margin after 3 days of incubation on TSA at 30°C. In API 20NE, positive for esculin hydrolysis but negative for nitrate reduction, arginine dihydrolase, gelatinase, glucose fermentation, urea, β -galactosidase and indole production. Utilizes D-glucose, D-mannose, D-maltose and D-mannitol but malic acid, trisodium citrate, *N*-acetyl-glucosamine L-arabinose, potassium gluconate, capric acid, adipic acid and phenylacetic acid are not utilized. Strain LR-3 (= NIBRBA0000114805) has been isolated from a fresh water pond, Daejeon, Korea.

Description of *Sphingomonas humi* MT2F 6

Cells are Gram-staining-negative, non-motile, non-spore-forming, non-flagellated and pigmented rods. Colonies grown on R2A agar medium for 2 days are circular, raised, entire and pink-colored. In API 20NE, negative for β -galactosidase, esculin, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase, while positive for nitrate reduction. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid and phenylacetic acid, trisodium citrate, D-maltose, malic acid and D-glucose. Strain MT2F 6 (= NIBRBA0000114783) has been isolated from a timber sample, Wando, Korea.

Description of *Sphingopyxis alaskensis* W5-3-3

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire and yellow colored after 2 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE, but negative for nitrate reduction, indole production, gelatinase, glucose fermentation, arginine dihydrolase and urease. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid but utilizes D-glucose, adipic acid, D-maltose and malic acid. Strain W5-3-3 (= NIBRBA0000114818) has been isolated from mineral water, Daejeon, Korea.

Description of *Sphingopyxis italica* 7C-17

Cells are Gram-staining-negative, non-flagellated, pigmented, and rod-shaped. Colonies are circular, raised, entire, and yellow colored after 2 days incubated on R2A at 30°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE. Negative for indole production, nitrate reduction, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose and malic acid. Strain 7C-17 (= NIBRBA0000114838) has been isolated from activated sludge, Daejeon, Korea.

Description of *Sphingopyxis ummariensis* KTCe-5

Cells are Gram-staining-negative, non-flagellated, pigmented and rod shaped. Colonies are circular, raised, entire and yellow colored after 2 days on R2A at 30°C. Positive for arginine dihydrolase, urease and esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, gelatinase, and β -galactosidase. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate, malic acid and phenylacetic acid but utilizes D-glucose, D-maltose and adipic acid. Strain KTCe-5 (= NIBRBA0000114842) has been isolated from activated sludge, Daejeon, Korea.

Description of *Methylobacterium tardum* LB-3

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are punctiform, sticky and pink colored after 3 days of incubation on TSA at 30°C. Strain LB-3 negative for nitrate reduction, β -galactosidase, glucose fermentation, arginine dihydrolase, esculin hydrolysis, indole production, urease and gelatinase in API 20NE. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, phenylacetic acid, capric acid and adipic acid but utilizes D-glucose and L-arabinose. Strain LB-3 (= NIBRBA0000114803) has been isolated from fresh water pond, Daejeon, Korea.

Description of *Methylobacterium aquaticum* PMX-R

Cells are Gram-staining-negative, non-flagellated, diffusible pigmented and rod-shaped. Colonies are wrinkled, raised, entire, red pin colored after 2 days on R2A at 25°C. In API 20NE negative for nitrate reduction, indole production, glucose fermentation, gelatinase, esculin hydrolysis and β -galactosidase but positive for arginine dihydrolase and urease. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid

and phenylacetic acid. Does not utilize adipic acid and trisodium citrate. Strain PMX-R (= NIBRBA0000114821) has been isolated from tap water, Daejeon, Korea.

Description of *Bosea enea* WM92

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies grown on R2A agar medium are circular, smooth, drop-like and white colored after 3 days of incubation at 25°C. Negative for glucose fermentation, arginine dihydrolase, esculin hydrolysis, β -galactosidase, indole production and gelatinase but positive for nitrate reduction and urease in API 20NE. D-glucose, L-arabinose, D-mannose, adipic acid and malic acid are utilized. Does not utilize D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid trisodium citrate and phenylacetic acid. Strain WM92 (= NIBRBA0000115022) has been isolated from fresh water, Changnyeong, Korea.

Description of *Rhodopseudomonas pentothentaxigens* MW2F51

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white colored after 2 days on R2A at 25°C. Positive for nitrate reduction and urease, while negative for esculin hydrolysis, β -galactosidase, indole production, glucose fermentation, arginine dihydrolase and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate, D-mannose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MW2F51 (= NIBRBA0000114773) has been isolated from fresh water, Iksan, Korea.

Description of *Phreatobacter oligotrophus* 61DPR27

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are punctiform, transparent and white colored after 5 days of incubation on MA at 25-30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, β -galactosidase and esculin hydrolysis in API 20NE. Does not utilize D-glucose, D-maltose, malic acid, capric acid, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain 61DPR27 (= NIBRBA0000114795) has been isolated from fresh water, Daejeon, Korea.

Description of *Amorphus suaedae* M49

Cells are Gram-staining-negative, flagellated, diffusible pigmented and rod-shaped. Colonies are circular, raised, entire and white colored after 2 days on MA at

25°C. Positive for esculin hydrolysis and *N*-acetyl-glucosamine in API 20NE but negative for nitrate reduction, gelatinase, urease, indole production, β -galactosidase and glucose fermentation. Does not utilize adipic acid, malic acid, D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Strain M49 (= NIBRBA0000114972) has been isolated from tidal flat, Wando, Korea.

Description of *Stappia taiwanensis* HME9619

Cells are Gram-staining-negative, non-flagellated, diffusible-pigmented and rod-shaped. Colonies are circular, convex, entire and beige colored after 3 days of incubation on MA at 25°C. In API 20NE, positive for β -galactosidase and malic acid, while negative for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, urease, indole production, arginine dihydrolase and assimilation of D-glucose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, *N*-acetyl-glucosamine and phenylacetic acid. Strain HME9619 (= NIBRBA0000114990) has been isolated from sea water, Wando, Korea.

Description of *Martellella radices* BSW2

Cells are Gram-staining-negative, non-flagellated, diffusible pigmented, and rod shaped. Colonies are circular, raised, entire and ivory-colored after 2 days on MA at 25°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis and β -galactosidase in API 20NE, but negative for urease, indole production, arginine dihydrolase and gelatinase. D-glucose, L-arabinose, D-mannitol and phenylacetic acid are utilized. Capric acid, malic acid, trisodium citrate, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and adipic acid are not assimilated. Strain BSW2 (= NIBRBA0000114955) has been isolated from tidal flat, Taean, Korea.

Description of *Aurantimonas litoralis* IMCC12390

Cells are Gram-staining-negative, non-flagellated, pigmented and short-rod. Colonies are circular, convex, smooth and yellow colored after 3 days on MA at 15°C. Positive for nitrate reduction and urease in API 20NE, but negative for esculin hydrolysis, β -galactosidase, glucose fermentation, arginine dihydrolase, indole production, and gelatinase. Does not utilize D-Mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, trisodium citrate, phenylacetic acid, and capric acid. D-glucose, D-mannose, L-arabinose, adipic acid and malic acid are utilized. Strain IMCC12390 (= NIBRBA0000114863) has been isolated from a sea water sample, Wando,

Korea.

Description of *Mesorhizobium tamadayense* SDM0103

Cells are Gram-staining-negative, non-flagellated, pigmented and rod shaped. Colonies are circular and light yellow-beige colored after 3 days on MA at 15°C. In API 20NE, positive for assimilation of D-glucose, D-mannose and D-mannitol but negative for esculin hydrolysis, β -galactosidase, nitrate reduction, indole production, gelatinase, arginine dihydrolase, urease, glucose fermentation and assimilation of *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-arabinose and D-maltose. Strain SDM0103 (=NIBRBA0000114945) has been isolated from gut of *Todarodes pacificus*, Korea.

Description of *Ochrobactrum gallinifaecis* BS16

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, raised, entire and white colored after 2 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and potassium gluconate. Similarly, negative for glucose fermentation, urease, indole production, arginine dihydrolase, esculin hydrolysis, gelatinase, β -galactosidase and assimilation of adipic acid, malic acid, D-maltose, capric acid and trisodium citrate. Strain BS16 (=NIBRBA0000114833) has been isolated from a mushroom compost waste collected from Yesan, Korea.

Description of *Caulobacter segnis* LR-4

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, convex, entire and white colored after 3 days on TSA at 30°C. In API 20NE, positive for esculin hydrolysis and β -galactosidase. Negative for nitrate reduction, glucose fermentation, urease, indole production, arginine dihydrolase, gelatinase and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, D-maltose, capric acid and trisodium citrate. Strain LR-4 (=NIBRBA0000114806) has been isolated from fresh water pond, Daejeon, Korea.

Description of *Phenylobacterium falsum* IMCC12425

Cells are Gram-staining-negative, flagellated, pigmented and rod-shaped. Colonies are circular, convex, entire and white colored after 3 days on MA at 15°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis and β -galactosidase but negative for glucose fermentation, urease, indole production, arginine dihydrolase and gelatinase. Does not utilize D-glucose, L-arab-

inose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, D-maltose, capric acid, phenylacetic acid and trisodium citrate. Strain IMCC12425 (=NIBRBA0000114866) has been isolated from sea water, Wando, Korea.

Description of *Paracoccus zhejiangensis* BM15

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, convex, entire and orange colored after 3 days of incubation on MA at 25°C. Positive for nitrate reduction, indole production, β -galactosidase and esculin hydrolysis but negative for arginine dihydrolase, urease, glucose fermentation and gelatinase in API 20NE. Utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine and malic acid. D-mannitol, L-arabinose, potassium gluconate, and D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid are not utilized. Strain BM15 (=NIBRBA0000114936) has been isolated from gut of *Fulvia mutica*, Korea.

Description of *Ruegeria lacuscaerulensis* EgM3207

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular and beige colored after 3 days of incubation on MA at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, β -galactosidase and negative for arginine dihydrolase, urease, indole production glucose fermentation, gelatinase and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain EgM3207 (=NIBRBA0000114938) has been isolated from gut of *Tulvia mutica*, Korea.

Description of *Leisingera nanhaiensis* KHS03

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular and beige colored after 3 days on MA at 25°C. In API 20NE, positive for nitrate reduction and negative for esculin hydrolysis, β -galactosidase arginine dihydrolase, urease, indole production glucose fermentation, gelatinase and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain KHS03 (=NIBRBA0000114943) has been isolated from gut of *Todarodes pacificus*, Korea.

Description of *Planktotalea frisia* KHS07

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

yellow-beige colored after 3 days of incubation on MA at 25°C. In API 20NE, negative for nitrate reduction, esculin hydrolysis, β -galactosidase arginine dihydrolase, urease, indole production, glucose fermentation and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain KHS07 (=NIBRBA0000114944) has been isolated from gut of *Todarodes pacificus*, Korea.

Description of *Sulfitobacter donghicola* SDM0205

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular and yellow-beige colored after 3 days of incubation on MA at 25°C. Positive for nitrate reduction, urease and gelatinase In API 20NE. Negative for esculin hydrolysis, β -galactosidase, arginine dihydrolase, indole production and glucose fermentation. Utilizes D-glucose, D-mannose, D-maltose and potassium gluconate. L-Arabinose, D-mannitol, *N*-acetyl-glucosamine, malic acid, capric acid, adipic acid, trisodium citrate and phenylacetic acid are not utilized. Strain SDM0205 (=NIBRBA0000114948) has been isolated from gut of *Todarodes pacificus*, Korea.

Description of *Aliiroseovarius crassostreae* HME9615

Cells are Gram-staining-negative, flagellated, pigmented and rod-shaped. Colonies are circular, convex, entire and grey colored after 3 days on MA at 25°C. Negative for nitrate reduction, urease, gelatinase, esculin hydrolysis, β -galactosidase, arginine dihydrolase, indole production and glucose fermentation in API 20NE. Does not assimilate D-glucose, D-mannose, D-maltose, potassium gluconate, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, malic acid, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain HME9615 (=NIBRBA0000114988) has been isolated from sea water, Wando, Korea.

Description of *Oceanibaculum indicum* IMCC12392

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, raised, entire and yellow colored after 3 days on MA at 15°C. Positive for nitrate reduction and β -galactosidase and negative for esculin hydrolysis, arginine dihydrolase, indole production, urease and glucose fermentation in API 20NE. Utilizes D-glucose, D-mannose, D-maltose, potassium gluconate, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, malic acid and adipic acid. Capric acid, trisodium citrate and phenylacetic acid are not utilized. Strain IMCC12392 (=NIBRBA0000114864) has been

isolated from sea water, Wando, Korea.

Description of *Roseomonas gilardii* subsp. *gilardii* WW106

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are circular, raised, entire and pink colored after 3 days on R2A at 25°C. Positive for urease while negative for nitrate reduction and β -galactosidase, esculin hydrolysis, arginine dihydrolase, indole production and glucose fermentation in API 20NE. Utilizes D-glucose, L-arabinose, malic acid and trisodium citrate. Does not utilize D-mannose, D-maltose, potassium gluconate, D-mannitol, *N*-acetyl-glucosamine, adipic acid, capric acid and phenylacetic acid. Strain WW106 (=NIBRBA0000115024) has been isolated from fresh water, Changnyeong, Korea.

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