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A report of 29 unrecorded bacterial species in Korea, belonging to the *Alphaproteobacteria*

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As a subset study to discover indigenous prokaryotic species in Korea, a total of 29 bacterial strains assigned to the classes *Alphaproteobacteria* were isolated from various environmental samples collected from plant root, 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 29 species included in *Alphaproteobacteria* is have been described in Korea; therefore 14 species of 9 genera in the order *Rhizobiales*, 7 species of 6 genera in the order *Sphingomonadales* and 4 species of 2 genera in the order *Caulobacterales* and 3 species in the order *Rhodobacterales* and 1 species in the order *Rhodospirillales* 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, unrecorded species

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Introduction

In 2012, we collected diverse environmental samples and isolated a great number of novel bacterial species and unrecorded bacterial species in Korea. The identified bacterial species belonged to the classes/phyla *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Deinococci*, and *Verrucomicrobia*. As a subset of this study, the present report focuses on the description of unrecorded species belonging to the *Alphaproteobacteria*.

Bacteria in the class *Alphaproteobacteria* have a wide variety of lifestyles and physiologies. They comprise most phototrophic genera, but also several genera metabolizing C1-compounds (e.g., *Methylobacterium* spp.), symbionts of plants (e.g., *Rhizobium* spp.), agriculturally valuable strains (e.g., *Agrobacterium* spp.) and animals, and a group of pathogens of humans and livestock, the *Rickettsiaceae*, and several highly abundant soil (e.g., the *Sphingobacteriaceae*) and marine groups (Williams *et al*., 2007; Matteo *et al*., 2013). The Class *Alphaproteobacteria* is divided into three subclasses *Caulobacteridae*, *Rickettsidae*, and *Magnetococcidae* (Matteo *et al*., 2013). The first, environmental big group is *Caulobac-*

teridae which is composed of the *Holosporales*, *Rhodospirillales*, *Sphingomonadales*, *Rhodobacterales*, *Caulobacterales*, *Kiloniellales*, *Kordiimonadales*, *Parvularculales* and *Sneathiellales*. The second, *Rickettsidae* is composed of the intracellular *Rickettsiales* and the free-living *Pelagibacterales*. The third, *Magnetococcidae*, which is composed by a large diversity of magnetotactic bacteria, but only one is described, *Magnetococcus marinus* (Bazylinski *et al*., 2012).

Genera *Azospirillum* and *Rhizobium* of *Alphaproteobacteria* are nitrogen fixers that are important in agriculture. Aerobic anoxygenic phototrophic bacteria are *Alphaproteobacteria*, widely distributed marine plankton that may constitute over 10% of the open ocean microbial community. Members of the genus *Nitrobacter* are nitrifying bacteria that oxidize nitrogen compounds to NO_3^- via a process called Nitrification which are important geochemical pathway of nitrogen cycle. Pathogens in this class include *Rickettsia*, which causes typhus and Rocky Mountain spotted fever; *Brucella*, which causes brucellosis; and *Ehrlichia*, which causes ehrlichiosis. In industry, *Acetobacter* and *Gluconobacter* are used to synthesize acetic acid, and *Agrobacterium* is used in genetic recombination in plants to transfer foreign DNA into plant genomes, so they also have many other biotechnological properties (Chilton *et al*., 1977).

Recently, comparative analyses of the sequenced genomes have also led to discovery of many conserved molecular signatures in widely distributed proteins and whole proteins (i.e., signature proteins) that are distinctive characteristics of either all *Alphaproteobacteria*, or their different main orders which provide novel means for the circumscription of these taxonomic groups and for identification/assignment of new species into these groups. These provide evidence that *Alphaproteobacteria* have branched off later than most other phyla and Classes of Bacteria with the exception of *Betaproteobacteria* and *Gammaproteobacteria* (Oren *et al*., 2014; Parte, 2014).

As a part of results obtained from the research program supported by NIBR, the present report focuses on the description of bacterial species belonging to the *Alphaproteobacteria* which have not been previously isolated in Korea. Here we report 29 unrecorded bacterial species in Korea belonging to 11 families of 5 orders in the *Alphaproteobacteria*.

Materials and methods

A total of 29 bacterial strains assigned to the classes *Alphaproteobacteria* were isolated from various environmental samples collected from plant root, ginseng soil, forest soil, marsh, mud flat, freshwater and seawater (Table 1). Each environmental sample was processed separately, spread onto diverse culture media including R2A, Marine Agar 2216, Tryptic Soy Agar and Nutrient Agar, 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 −80°C as well as lyophilized ampoules.

Colony morphology of the strains was observed on agar plates with a magnifying glass after cells grew up to 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 the standard procedures described elsewhere. The 16S rRNA gene sequences of the strains assigned to the *Alphaproteobacteria* were compared with the sequences held in GenBank by BLASTN and also analyzed using the EzTaxon-e server (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). The phylogenetic trees were constructed by using the neighbor-joining (Saitou and Nei, 1987) and the maximum-parsimony (Fitch, 1971) methods with the MEGA5 Program (Tamura, *et al*., 2011) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Results and Discussion

The 29 strains were distributed in 5 orders of the *Alphaproteobacteria*; 14 strains for the order *Rhizobiales*, 7 strains for the *Sphingomonadales*, 4 strains for the *Caulobacterales*, 3 strains for the *Rhodobacterales*, 1 strain for the *Rhodospirillales* (Table 1). These strains were Gram-staining-negative, chemoheterotrophic, and rod-shaped bacteria except for strain MA11 showing coccoid-shaped (Fig. 1).

The strains in the order *Rhizobiales* (Fig. 2) found belonged to 6 families 9 separate Genus: *Rhizobium* (5 species), *Bradyrhizobium* (2 species), *Ochrobactrum* (1 species), *Phyllobacterium* (1 species), *Azorhizobium* (1 species), *Xanthobacter* (1 species), *Labrys* (1 species), *Besea* (1 species) and *Devosia* (1 species). 7 strains were assigned to the order *Sphingomonadales*: 5 strains for the family *Sphingomonadaceae* and 2 strains for the *Erythrobac-*

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 (Name of type strain)	Similarity (%)	Isolation source	Medium	Incubation conditions
Rhizobiales	*Rhizobiaceae*	*Rhizobium*	CT6-3	NIBRBA0000113857	*Rhizobium skierniewicense* Ch11^T	99.6	Mugwort root	TSA	30°C, 2d
		Rhizobium	CR5-1	NIBRBA0000113863	*Rhizobium nepotum* 39/7^T	99.9	Ochotensis root	R2A	30°C, 3d
		Rhizobium	WR-M3W	NIBRBA0000113999	*Rhizobium rosettiformans* W3^T	99.9	Soil	MA	25°C, 3d
		Rhizobium	RS3-4_B	NIBRBA0000113958	*Rhizobium giardinii* H152^T	99.9	Ginseng soil	R2A	30°C, 2d
		Rhizobium	CR1-2	NIBRBA0000113868	*Rhizobium lusitanum* P1-7^T	99.9	Bracken root	R2A	30°C, 5d
	Brucellaceae	*Ochrobactrum*	MU5-14	NIBRBA0000113955	*Ochrobactrum pituitosum* CCUG 50899^T	99.9	Ginseng soil	MA	30°C, 2d
	Phyllobacteriaceae	*Phyllobacterium*	CR4-2	NIBRBA0000113864	*Phyllobacterium myrsinacearum* IAM 13584^T	99.5	Isodon excisus (Maxim.) Kudo root	R2A	30°C, 3d
	Xanthobacteraceae	*Azorhizobium*	IK41	NIBRBA0000113909	*Azorhizobium doebereinerae* UFLA1-100^T	99.7	Freshwater	PCA	25°C, 2d
		Xanthobacter	IK20	NIBRBA0000113911	*Xanthobacter flavus* 301^T	99.1	Freshwater	1/10 R2A	25°C, 3d
		Labrys	Gsoil 106	NIBRBA0000113888	*Labrys neptuniae* Liujia-146^T	99.2	Ginseng soil	R2A	25°C, 2d
	Bradyrhizobiaceae	*Bosea*	IK38	NIBRBA0000113857	*Bosea massiliensis* 63287^T	99.3	Freshwater	NA	25°C, 3d
		Bradyrhizobium	UKS-12	NIBRBA0000113887	*Bradyrhizobium betae* PL7HG1^T	99.6	Marsh	R2A	25°C, 2d
		Bradyrhizobium	UKS-27	NIBRBA0000113884	*Bradyrhizobium paxllaeri* LMTR 21^T	99.5	Marsh	R2A	25°C, 2d
	Hyphomicrobiaceae	*Devosia*	mGW21	NIBRBA0000113966	*Devosia riboflavina* DSM 7230^T	99.3	Forest soil	R2A	37°C, 2d
Sphingomonadales	*Sphingomonadaceae*	*Novosphingobium*	CR6-9	NIBRBA0000113861	*Novosphingobium resinovorum* NCIMB 8767^T	99.6	Mugwort root	R2A	30°C, 3d
		Sphingopyxis	NUG4-1	NIBRBA0000113935	*Sphingopyxis chilensis* S37^T	99.2	Marsh	NA	30°C, 2d
		Sphingobium	CR2-3	NIBRBA0000113867	*Sphingobium yanoikuyae* GIFU 988^T	99.5	Vegetable root	R2A	30°C, 5d
		Sphingomonas	HME8658	NIBRBA0000114087	*Sphingomonas astaxanthini-faciens* TDMA-17^T	100	Water	R2A	37°C, 2d
	Erythrobacteraceae	*Porphyrobacter*	HME8673	NIBRBA0000114088	*Erythromicrobium ramosum* DSM 8510^T	99.9	Water	R2A	30°C, 2d
		Altererythrobacter	MMH1-3	NIBRBA0000113936	*Altererythrobacter dongtanensis* JM27^T	99.6	Mud flat	MA	30°C, 2d
	Sphingomonadaceae	*Novosphingobium*	KYW772	NIBRBA0000114110	*Novosphingobium indicum* H25^T	99.5	Seawater	MA	25°C, 3d

Table 1. Continued.

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species (Name of type strain)	Similarity (%)	Isolation source	Medium	Incubation conditions
Caulobacterales	*Caulobacteraceae*	*Brevundimonas*	HME8471	NIBRBA0000114082	*Brevundimonas variabilis* ATCC 15255^T	100	Water	R2A	30°C, 2d
		Brevundimonas	WR-M10	NIBRBA0000113996	*Brevundimonas nasdae* GTC 1043^T	99.9	Soil	MA	25°C, 3d
		Caulobacter	WR-R2Y	NIBRBA0000114001	*Caulobacter fusiformis* ATCC 15257^T	99.8	Soil	R2A	25°C, 3d
		Caulobacter	IK06	NIBRBA0000113855	*Caulobacter vibrioides* CB51^T	99.4	Freshwater	R2A	25°C, 3d
Rhodobacterales	*Rhodobacteraceae*	*Paracoccus*	MA11	NIBRBA0000113969	*Paracoccus marcusii* DSM 11574^T	99.7	Forest soil	MA	25°C, 2d
		Nereida	ES05-2S-4-MA	NIBRBA0000113916	*Nereida ignava* 2SM4^T	99.8	Seawater	MA	25°C, 3d
		Litoreibacter	HD48	NIBRBA0000113992	*Litoreibacter albidus* KMM 3851^T	100	Mud flat	MA	25°C, 3d
Rhodospirillales	*Acetobacteraceae*	*Roseomonas*	HME8528	NIBRBA0000114073	*Roseomonas stagni* HS-69^T	99.8	Water	R2A	30°C, 2d

teraceae (Fig. 3, Table 1). 2 of strains assigned to the family *Sphingomonadaceae* belong to the genus *Novosphingobium* and others belonged to the genus *Sphingopyxis*, *Sphingobium* and *Sphingomonas*. 2 strains for the *Erythrobacteraceae* belong to genus *Porphyrobacter* and *Altererythrobacter*.

Fig. 4 shows phylogenetic assignment of 8 strains of the orders *Caulobacterales*, *Rhodobacterales* and *Rhodospirillales*. 2 strains belong to *Brevundimonas* and 2 strains belong to *Caulobacter* of family *Caulobacter*aceae. 3 strains belong to *Paracoccus*, *Nereida* and *Litoreibacter* of family *Rhodobacteraceae*. 1 strain belongs to *Roseomonas* of family *Acetobacteraceae*.

Here we report 29 unrecorded bacterial species in Korea belonging to 11 families of 5 orders in the *Alphaproteobacteria*.

Description of *Rhizobium skierniewicense* CT6-3

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and ivory-colored after 2 days of incubation on TSA at 30°C. Positive for nitrate reduction, esculin hydrolysis, urease, gelatinase, β-galactosidase in API 20NE but negative for indole production, glucose fermentation and arginine dihydrolase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain CT6-3 (=NIBRBA0000113857) has been isolated from mugwort root, Wonju, Korea.

Description of *Rhizobium nepotum* CR5-1

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are round, umbonate, and pale yellow colored after 3 days on R2A at 30 °C. Negatives for nitrate reduction, Indole production, glucose fermentation, gelatin hydrolysis and arginine dihydrolase in API 20NE, but positive for urease, esculin hydrolysis, and β-galactosidase. Does not utilize capric acid, adipic acid and phenylacetic acid. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, trisodium citrate and malic acid are utilized. Strain *Rhizobium nepotum* CR5-1 (=NIBRBA0000113863) has been isolated from ochotensis root, Wonju, Korea.

Description of *Rhizobium rosettiformans* WR-M3W

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, smooth, glistening, and pale yellow colored after 3 days on MA at 25°C. Positive for glucose fermentation, urease, esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate production, indole production, arginine

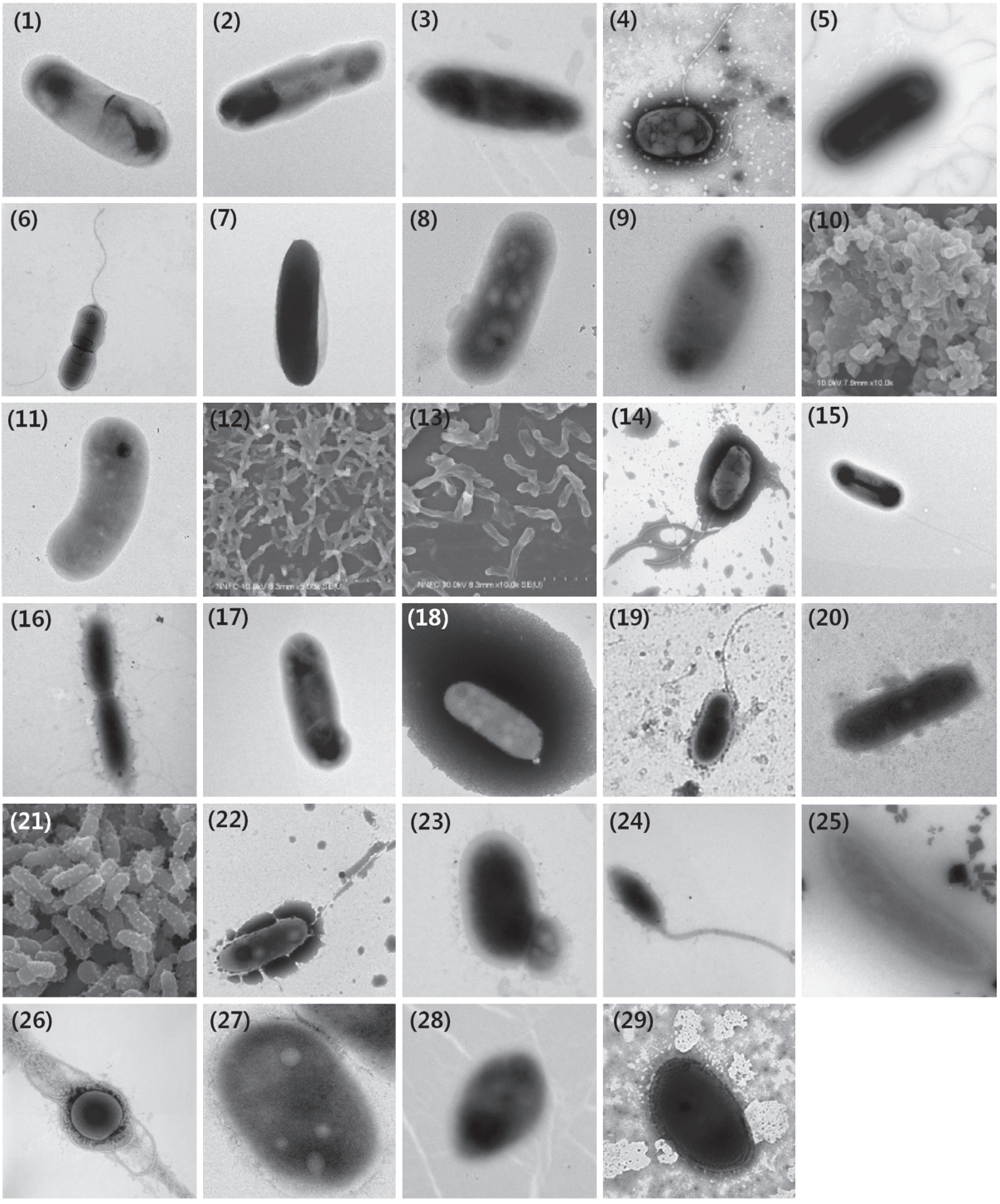

Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1. CT6-3; 2. CR5-1; 3. WR-M3W; 4. RS3-4_B; 5. CR1-2; 6. MU5-14; 7. CR4-2; 8. IK41; 9. IK20; 10. Gsoil 106; 11. IK38; 12. UKS-12; 13. UKS-27; 14. mGW21; 15. CR6-9; 16. NUG4-1; 17. CR2-3; 18. HME8658; 19. HME8673; 20. MMH1-3; 21. KYW772; 22. HME8471; 23. WR-M10; 24. WR-R2Y; 25. IK06; 26. MA11; 27. ES05-2S-4-MA; 28. HD48; 29. HME8528.

dihydrolase and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate are utilized. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain WR-M3W (=NIBRBA0000113999) has been isolated from a soil sample, Wando, Korea.

Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains isolated in this study and their relatives of the order *Rhizobiales* in the class *Alphaproteobacteria*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining and below nodes for the maximum-likelihood methods. Filled circles indicate the nodes recovered by the two treeing methods. Bar, 0.02 substitutions per nucleotide position.

Description of *Rhizobium giardinii* RS3-4_B

Cells are Gram-staining-negative, flagellated, and short-rod shaped. Colonies are circular, entire, smooth, and white colored after 2 days on R2A at 30°C. Positive for urease, esculin hydrolysis, glucose fermentation and

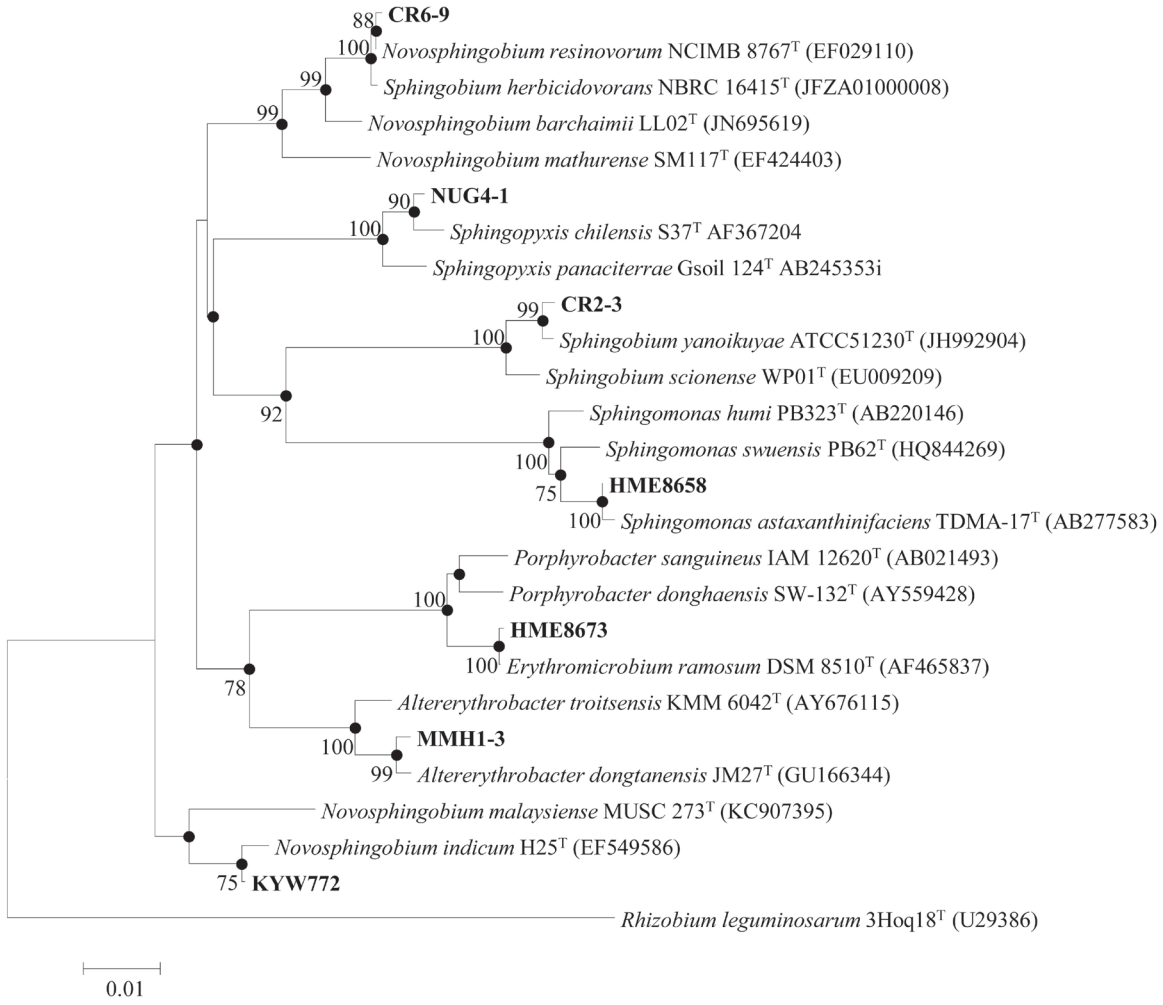

Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains isolated in this study and their relatives of the order *Sphingomonadales* in the class *Alphaproteobacteria*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining and below nodes for the maximum-likelihood methods. Filled circles indicate the nodes recovered by the two treeing methods. Bar, 0.01 substitutions per nucleotide position.

β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase and gelatinase. Does not utilize L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid but utilize D-glucose and D-mannose. Strain RS3-4_B (=NIBRBA0000113958) has been isolated from a ginseng soil, Anseong, Korea.

Description of *Rhizobium lusitanum* CR1-2

Cells are Gram-staining-negative, flagellated, pigmented, and rod shaped. Colonies are round, smooth, drop like, and pale white colored after 5 days on R2A at 30 °C. Positive for urea, esculin hydrolysis, and β-galactosidase and weakly positive for arginine dihydrolase in API 20NE, but negative for nitrate reduction, indole production, gelatinase and glucose fermentation. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, D-mannitol, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize, capric acid, adipic acid, and phenylacetic acid. Strain CR1-2 (=NIBRBA0000113868) has been isolated from bracken root, Wonju, Korea.

Description of *Ochrobactrum pituitosum* MU5-14

Cells are Gram-staining-negative, flagellated, pigmented, and rod-shaped. Colonies are punctiform, entire, smooth and light yellow colored after 2 days on MA at 30°C. Positive for nitrate reduction and urea in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase, and β-galactosidase. Does not utilize D-mannitol, D-maltose, capric acid,

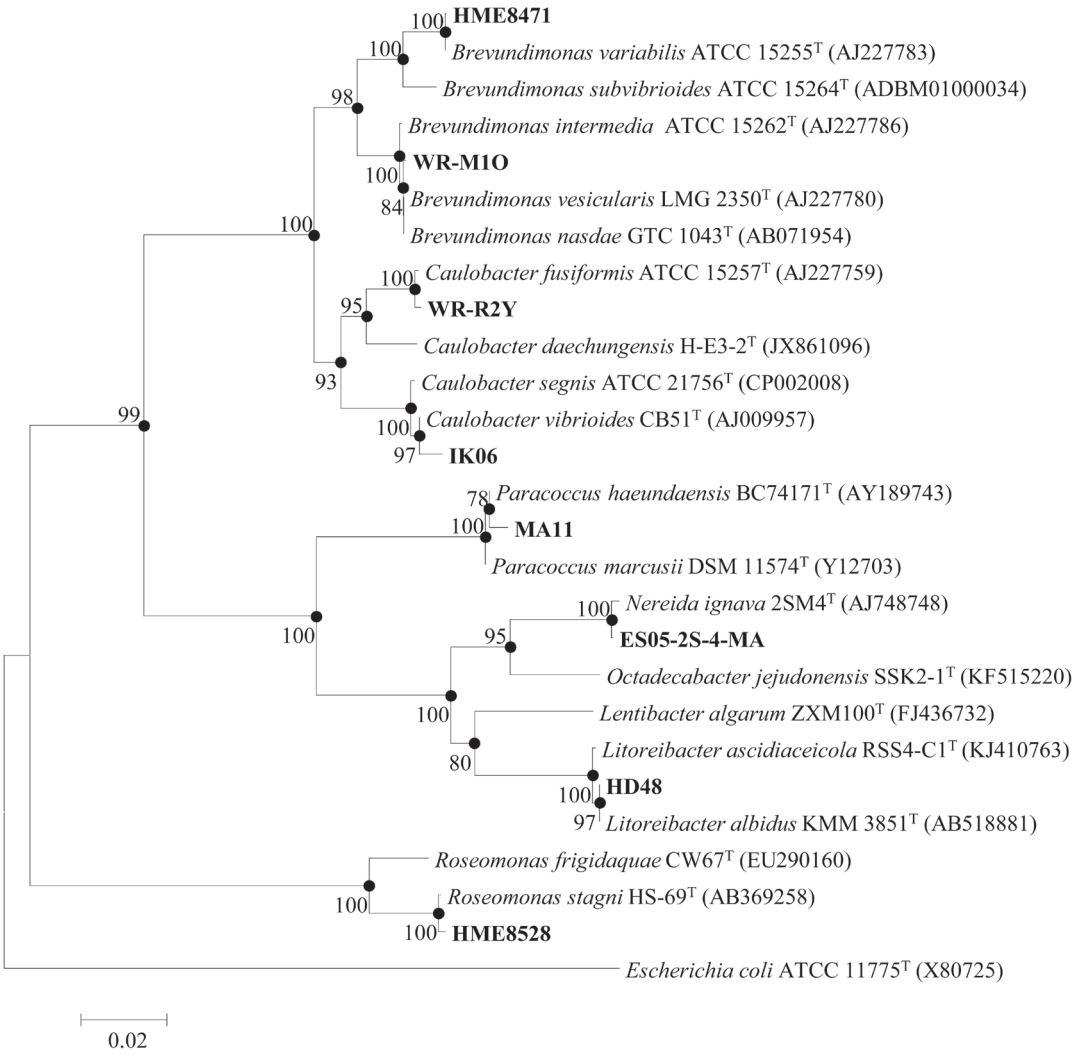

Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains isolated in this study and their relatives of the order *Caulobacterales*, *Rhodobacterales* and *Rhodospirillales* in the class *Alphaproteobacteria*. Bootstrap values (>70%) are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

adipic acid and phenylacetic acid but utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, malic acid, trisodium citrate. Strain MU5-14 (=NIBRBA0000113955) has been isolated from ginseng soil, Anseong, Korea.

Description of *Phyllobacterium myrsinacearum* CR4-2

Cells are Gram-staining-negative, non-flagellated, pigmented, and rod-shaped. Colonies are round, smooth, convex, and gray colored after 3 days on R2A at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and gelatinase but positive for urea, esculin hydrolysis and β-galactosidase. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, *N*-acetyl-glucosamine are utilized. Does not utilize, capric acid, adipic acid and potassium gluconate. Strain CR4-2 (=NIBRBA0000113864) has been isolated from Isodon excisus (Maxim) Kudo root, Wonju, Korea.

Description of *Azorhizobium doebereinerae* IK41

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, raised, entire, and yellow colored after 2 days of incubation on PCA at 25°C. Positive for nitrate reduction, arginine dihydrolase, urease, D-glucose, L-arabinose, D-mannitol, D-mannose, potassium gluconate, malic acid and trisodium citrate in API 20NE, but negative for indole production, glucose fermentation, esculin hydrolysis, gelatinase, β-galactosidase, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid and phenylacetic acid. Strain IK41 (=NIBRBA

0000113909) has been isolated from a freshwater sample, Incheon, Korea.

Description of *Xanthobacter flavus* IK20

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire and yellow colored after 3 days on 1/10 R2A at 25°C. Positive for nitrate reduction, arginine dihydrolase and urease in API 20NE, but negative for indole production, glucose fermentation, esculin hydrolysis, gelatinase and β-galactosidase. D-Glucose, L-arabinose, D-mannose, malic acid, trisodium citrate and potassium gluconate is utilized. Does not utilize *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid and phenylacetic acid. Strain IK20 (=NIBRBA0000113911) has been isolated from a freshwater sample, Incheon, Korea.

Description of *Labrys neptuniae* Gsoil 106

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire and yellow colored after 2 days 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, 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 106 (=NIBRBA0000113888) has been isolated from a ginseng soil sample, Pocheon, Korea.

Description of *Bosea massiliensis* IK38

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are rhizoid, convex and light brown colored after 3 days of incubation on NA medium at 25°C. Positive for nitrate reduction, Indole production, urea and glucose fermentation but negative for arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. Show positive assimilates for D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, D-maltose, potassium gluconate, trisodium citrate and adipic acid in API 20NE, but negative for capric acid and phenyl acetic acid. Strain IK38 (=NIBRBA0000113857) has been isolated from a freshwater sample, Incheon, Korea.

Description of *Bradyrhizobium japonicum* UKS-12

Cells are Gram-staining-negative, non-flagellated, pigmented, and rod-shaped. Colonies are circular, raised, entire yellow colored after 2 days on R2A at 25°C. Positive for nitrate reduction while negative for glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, indole production and 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 UKS-12 (=NIBRBA0000113887) has been isolated from a Marsh sample, Gochang-gun, Korea.

Description of *Bradyrhizobium lablabi* UKS-27

Cells are Gram-staining-negative, non-flagellated, diffusible-pigmented, and rod-shaped. Colonies are circular, raised, entire, and yellow colored after 2 days on R2A at 25°C. Negative for nitrate reduction, arginine dihydrolase, esculin hydrolysis, β-galactosidase, indole production, glucose fermentation, urease, and gelatinase. D-Glucose, D-mannose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate, D-maltose, adipic acid and phenylacetic acid are not utilized. Strain UKS-27 (=NIBRBA 0000113884) has been isolated from a marsh sample, Gochang-gun, Korea.

Description of *Devosia riboflavin* mGW21

Cells are Gram-staining-negative, flagellated, diffusible pigmented, and rod-shaped. Colonies are circular after 5 days on minimal medium at 25°C. Positive for glucose fermentation, esculin hydrolysis, urea and β-galactosidase in API 20NE, but negative for nitrate reduction, arginine dihydrolase, gelatinase and indole production. D-Glucose, L-arabinose, D-mannose, D-mannitol, malic acid, trisodium citrate, *N*-acetyl-glucosamine and D-maltose are utilized. Does not utilize potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain mGW21 (=NIBRBA0000113966) has been isolated from a forest soil sample, Gwanaksan, Seoul, Korea.

Description of *Novosphingobium resinovorum* CR6-9

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are round, raised, smooth and pale yellow-colored after 3 days on R2A at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase in API 20NE. Positive for β-galactosidase and hydrolyze esculin. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid and phenylacetic acid, but utilize trisodium citrate, D-maltose, malic acid and D-glucose. Strain CR6-9 (=NIBRBA0000113861) has been isolated from a mugwort root sample, Wonju, Korea.

Description of *Sphingopyxis chilensis* NUG4-1

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

shaped. Colonies are circular, entire smooth, and yellow colored after 2 days on NA at 30°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 capric acid, but utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain NUG4-1 (=NIBRBA0000113935) has been isolated from a Marsh sample, Taean, Korea.

Description of *Sphingobium yanoikuyae* CR2-3

Cells are Gram-staining-negative, non-flagellated, pigmented, and rod-shaped. Colonies are round, smooth, convex, and orange colored after 5 days incubated on R2A at 30°C. Weakly positive for nitrate reduction and positive for esculin hydrolysis and β-galactosidase in API 20NE. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. Does not utilize D-mannose, D-mannitol, capric acid, adipic acid and phenylacetic acid. Utilized D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate. Strain CR2-3 (=NIBRBA0000113867) has been isolated from a vegetable root sample, Wonju, Korea.

Description of *Sphingomonas astaxanthinifaciens* HME8658

Cells are Gram-staining-negative, non-flagellated, pigmented, and short rod shaped. Colonies are circular, convex, entire and red colored after 2 days on R2A at 37°C. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, urease, glucose fermentation, arginine dihydrolase, gelatinase, and β-galactosidase. Does not utilize capric acid, malic acid, trisodium citrate, phenylacetic acid, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and adipic acid. Strain HME8658 (=NIBRBA0000114087) has been isolated from a Water sample, Lake Juam, Korea.

Description of *Erythromicrobium ramosum* HME 8673

Cells are Gram-staining-negative, flagellated, pigmented, and rod-shaped. Colonies are circular, convex, entire, and orange colored after 2 days on R2A at 30°C. Positive for β-galactosidase, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis, indole production, urease and gelatinase 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 HME8673 (=NIBRBA0000114088) has been isolated from a water sample of Lake Soyang, Korea.

Description of *Altererythrobacter dongtanensis* MMH1-3

Cells are Gram-staining-negative, non-flagellated, diffusible pigmented, and rod-shaped. Colonies are punctiform, entire, smooth, yellow colored after 2 days on MA at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and β-galactosidase in API 20NE but positive for esculin hydrolysis. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Utilize adipic acid. Strain MMH1-3 (=NIBRBA0000113936) has been isolated from a mud flat sample, Taean, Korea.

Description of *Novosphingobium indicum* KYW772

Cells are Gram-staining-negative, flagellated, pigmented, and rod-shaped. Colonies are opaque, round, smooth, convex, and light yellow colored after 3 days on MA at 25°C. Negative for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis, β-galactosidase, indole production, urease and gelatinase in API 20NE. D-maltose is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid, trisodium citrate, phenylacetic acid, capric acid and adipic acid. Strain KYW772 (=NIBRBA0000114110) has been isolated from seawater, Gwangyang-si, Korea.

Description of *Brevundimonas variabilis* HME8471

Cells are Gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, convex, entire and orange colored after 2 days on R2A at 30°C. Positive for esculin hydrolysis and β-galactosidase. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate are utilized. Does not utilize D-mannose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HME8471 (=NIBRBA0000114082) has been isolated from a water sample, Gyeongancheon, Korea.

Description of *Brevundimonas nasdae* WR-M10

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, glistening and orange yellow colored after 3 days on MA at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and

β-galactosidase while positive for esculin hydrolysis in API 20NE. D-Glucose, D-maltose and malic acid are utilized. Weakly utilize the capric acid. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain WR-M10 (=NIBRBA 0000113996) has been isolated from a soil sample, Wando, Korea.

Description of *Caulobacter fusiformis* WR-R2Y

Cells are Gram-staining-negative, flagellated, pigmented and oval-shaped. Colonies are circular, convex, glistening and yellow colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase in API 20NE, but negative for nitrate reduction, urease, indole production and glucose fermentation. Adipic acid and Malic acid are utilized. 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. Strain WR-R2Y (=NIBRBA0000114001) has been isolated from a soil sample, Wando, Korea.

Description of *Caulobacter vibrioides* IK06

Cells are Gram-staining-negative, non-flagellated, diffusible-pigmented, and rod-shaped. Colonies are circular, convex, and colorless (white) after 3 days of incubation on R2A at 25°C. In API 20NE, positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, urease and β-galactosidase. Negative for, indole production, arginine dihydrolase, D-glucose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IK06 (=NIBRBA0000113855) has been isolated from a freshwater sample, Incheon, Korea.

Description of *Paracoccus marcusii* MA11

Cells are Gram-staining-negative, flagellated, diffusible pigmented, and coccus shaped. Colonies are circular and orange-colored after 2 days on MA at 25°C. Positive for glucose fermentation, urease, esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase and gelatinase. Does not utilize Capric acid, malic acid, trisodium citrate, D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and phenylacetic acid. Strain MA11 (=NIBRBA0000113969) has been isolated from a forest soil sample, Gwanaksan, Seoul, Korea.

Description of *Nereida ignava* ES05-2S-4-MA

Cells are Gram-staining-negative, flagellated, non-pigmented and coccus. Colonies are circular, convex and beige colored after 3 days on MA at 25°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, indole production, urease and gelatinase. 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 ES05-2S-4-MA (=NIBRBA0000113916) has been isolated from a seawater sample, Uljin, east sea, Korea.

Description of *Litoreibacter albidus* HD48

Cells are Gram-staining-negative, non-flagellated, pigmented and oval or rod shaped. Colonies are circular, convex, smooth, and light yellow colored after 3 days on MA at 25°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, gelatinase, arginine dihydrolase, urease and glucose fermentation in API 20NE. 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 are not utilize. Strain HD48 (=NIBRBA0000113992) has been isolated from a mud flat sample, Taean, Korea.

Description of *Roseomonas stagni* HME8528

Cells are Gram-staining-negative, non-flagellated, pigmented and Rod-shaped. Colonies are circular, convex, entire and yellow colored after 2 days on R2A at 30°C. Negative for nitrate reduction, glucose fermentation, urease indole production, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. D-Glucose, D-mannose, adipic acid and malic acid are utilized. Does not utilize, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid and trisodium citrate. Strain HME8528 (=NIBRBA 0000114073) has been isolated from a water sample collected from Gyeongancheon, Korea.

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