

## A report of 31 unrecorded bacterial species belonging to the class *Alphaproteobacteria* in Korea

Kyung Hyun Kim<sup>1</sup>, Jung-Hoon Yoon<sup>2</sup>, Seung-Bum Kim<sup>3</sup>, Kwang-Yeop Jahng<sup>4</sup>, Jang-Cheon Cho<sup>5</sup>, Ki-seong Joh<sup>6</sup>, Chang-Jun Cha<sup>7</sup>, Chi-Nam Seong<sup>8</sup>, Jin-Woo Bae<sup>9</sup>, Wan-Taek Im<sup>10</sup> and Che Ok Jeon<sup>1,\*</sup>

<sup>1</sup>Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

<sup>2</sup>Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea

<sup>3</sup>Department of Microbiology, Chungnam National University, Daejeon 34134, Republic of Korea

<sup>4</sup>Department of Life Sciences, Chonbuk National University, Jeonju-si 54899, Republic of Korea

<sup>5</sup>Department of Biological Sciences, Inha University, Incheon 22212, Republic of Korea

<sup>6</sup>Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Geonggi 02450, Republic of Korea

<sup>7</sup>Department of Systems Biotechnology, Chung-Ang University, Anseong 17546, Republic of Korea

<sup>8</sup>Department of Biology, Sunchon National University, Suncheon 57922, Republic of Korea

<sup>9</sup>Department of Biology, Kyung Hee University, Seoul 02453, Republic of Korea

<sup>10</sup>Department of Biotechnology, Hankyong National University, Anseong 17546, Republic of Korea

\*Correspondent: cojeon@cau.ac.kr

During a comprehensive investigation of indigenous prokaryotic species in Korea, a total 31 bacterial strains assigned to the class *Alphaproteobacteria* were isolated from diverse environmental habitats including freshwater, seawater, brackish water, ginseng soil, plant roots, natural caves, and tidal flats. Based on their high 16S rRNA gene sequence similarities (>99.1%) and formation of robust phylogenetic clades with the closest type species, each strain was assigned to an independent and predefined bacterial species. Because there were no published or official reports regarding the isolation of these 31 species in Korea, this study identified three species in two genera in the order *Caulobacterales*, 12 species in 10 genera in the order *Rhodobacterales*, three species in two genera in the order *Rhizobiales*, two species in two genera in the order *Rhodospirillales* and 11 species in seven genera, all in the order *Sphingomonadales* within the *Alphaproteobacteria* are reported as new alphaproteobacterial species 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, taxonomy, unrecorded species

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### INTRODUCTION

The *Alphaproteobacteria* is a bacterial class within the phylum *Proteobacteria* that forms one of the largest proteobacterial groups and includes diverse phototrophs, chemolithotrophs, chemoorganotrophs, and aerobic photoheterotrophs that are abundant in various terrestrial and marine habitats. For example, the aerobic anoxygenic phototrophic bacteria *Pelagibacter* are widely distributed marine plankton that constitute over 10% of microbial populations in the open ocean (Giovannoni *et al.*, 2005). The taxonomic classification based on 16S rRNA

gene sequences shows that the class *Alphaproteobacteria* is divided into 3 subclasses: *Magnetococcidae*, *Rickettsiidae*, and *Caulobacteridae*, which includes eight primary subgroups or orders (*Caulobacterales*, *Magnetococcales*, *Parvularculales*, *Rickettsiales*, *Rhizobiales*, *Rhodobacterales*, *Rhodospirillales*, and *Sphingomonadales* (Ferla *et al.*, 2013; Matteo *et al.*, 2013).

Members of the *Alphaproteobacteria* provide enormously valuable biological resources for various industries and are commonly used in taxonomic, biochemical and molecular biological studies. Some members of *Alphaproteobacteria* exhibit intimate interactions with

eukaryotic host organisms, which are of central importance in the agricultural and medical fields (Skorpil and Broughton, 2005; Fredricks, 2006). For example, symbiotic interactions of the *Rhizobiaceae* family with plant roots are responsible for most atmospheric nitrogen fixation, an essential environmental service for agriculture (Kaneko *et al.*, 2000; 2002; Fredricks, 2006).

In 2015, we collected environmental samples in Korea from diverse habitats and isolated various novel and unrecorded bacterial species through a research program supported by NIBR of Korea. This report focuses on the description of bacterial species in the *Alphaproteobacteria* that were not previously reported in Korea. Here we report 31 unrecorded bacterial species in the *Alphaproteobacteria* isolated in Korea, which consisted of 23 genera in five orders.

## MATERIALS AND METHODS

A total of 31 bacterial strains assigned to the class *Alphaproteobacteria* were isolated from various environmental habitats, including freshwater, seawater, brackish water, ginseng soil, plant roots, natural caves, and tidal flats (Table 1). All environmental samples were processed independently, serially diluted, spread onto diverse culture agar media including R2A agar (BD, USA), marine agar 2216 (MA; BD, USA), Kuster's agar (KUA) and nutrient agar (NA; BD, USA), and incubated at 20–30°C for 1–11 days (Table 1). The designated strain IDs, isolation sources, culture media, and incubation conditions are summarized in Table 1. All strains were isolated as pure cultures and stored as 10–20% glycerol suspension at –80°C and as lyophilized ampoules.

Colony morphology of the strains was observed by eye or a magnifying glass after the cells were cultivated to their stationary phase on their culture agar media. Cellular morphology and cell size were examined by using either transmission electron or scanning electron microscopy. Gram staining tests were performed using a Gram-staining kit according to the standard procedures. Biochemical characteristics were evaluated 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 type strains of validated bacterial species using the Ez-Taxon-e server (Kim *et al.*, 2012). For phylogenetic analyses, multiple alignments of the 16S rRNA gene sequences between the isolates and established bacterial species 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 using a neighbor-joining algorithm (Saitou and Nei, 1987) with bootstrap values based on 1,000 replications (Felsenstein, 2002).

## RESULTS AND DISCUSSION

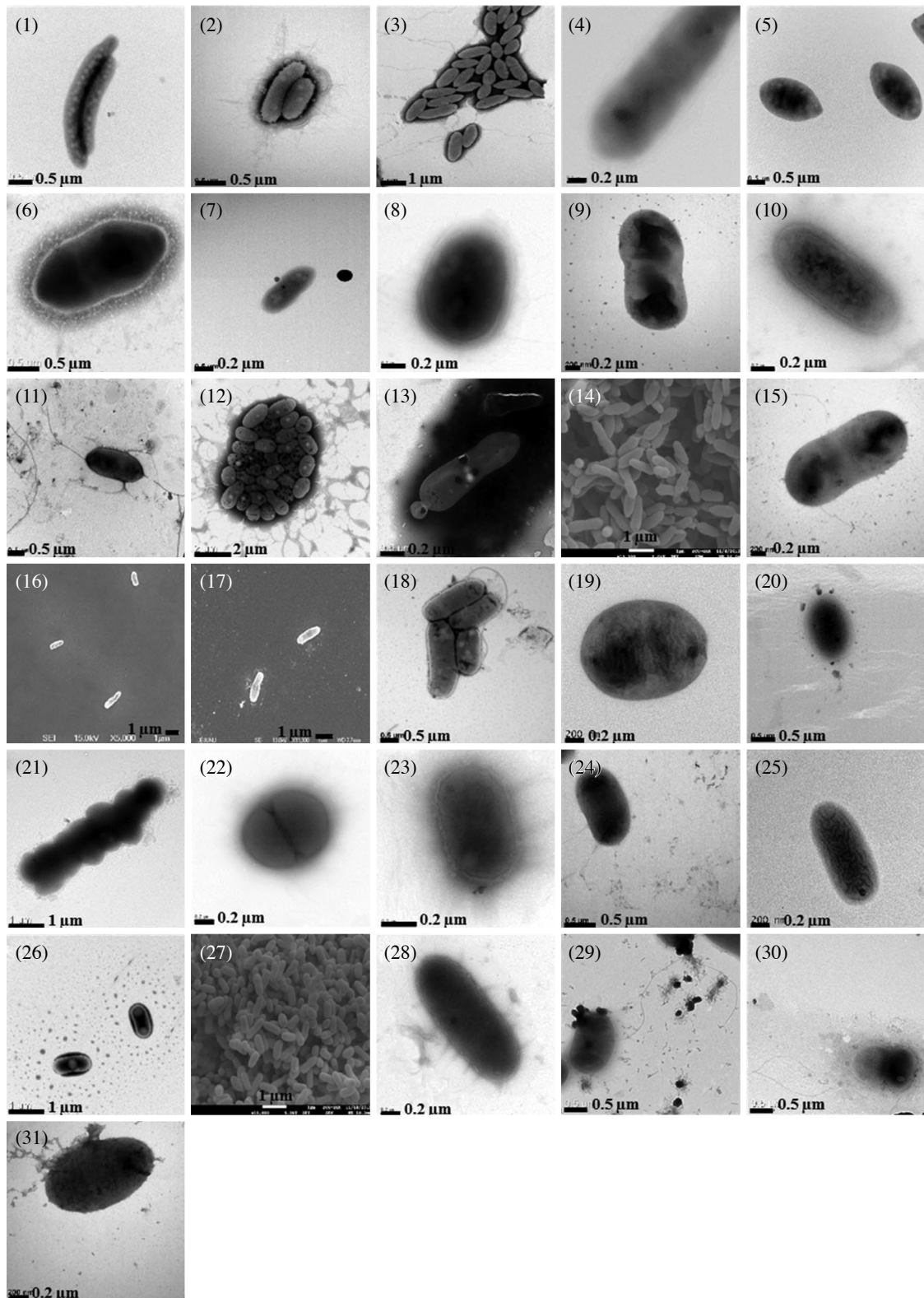
The 31 strains were distributed into five orders of the *Alphaproteobacteria*: three strains in the *Caulobacterales*, 12 strains in the *Rhodobacterales*, three strains in the *Rhizobiales*, two strains in the *Rhodospirillales*, and 11 strains in the *Sphingomonadales* (Table 1). These strains were gram-staining-negative, chemoheterotrophic, and rod-shaped bacteria (Fig. 1). Three strains that were assigned to the family *Caulobacteraceae* in the order *Caulobacterales* within the genera *Brevundimonas* and *Phenylobacterium* (Fig. 2). Two strains in the order *Rhizobiales* (Fig. 3) belonged to two genera of two separate families: *Mesorhizobium* (1 species) and *Rhizobium* (1 species). Twelve strains that were assigned to the family *Rhodobacteraceae* of the order *Rhodobacterales* belonged to the genera *Albirhodobacter*, *Cereibacter*, *Gemmobacter*, *Loktanella*, *Marinovum*, *Marivita*, *Rhodobacter*, *Roseivivax*, *Roseovarius*, and *Ruegeria* (Fig. 4). Two strains that were assigned to the family *Acetobacteraceae* in the order *Rhodospirillales* belonged to the genera *Acidisoma* and *Roseomonas* (Fig. 5). The strains in the order *Sphingomonadales* (Fig. 6) belonged to two families in seven genera: *Altererythrobacter* (1 species), *Citrimicrobium* (1 species), *Novosphingobium* (1 species), *Sandarakinorhabdus* (1 species), *Sphingobium* (2 species), *Sphingomonas* (4 species), and *Sphingopyxis* (1 species). Here we report 31 unrecorded bacterial species belonging to seven families of five orders in the *Alphaproteobacteria*, which were isolated in Korea.

### Description of *Acidisoma tundrae* TW2K3

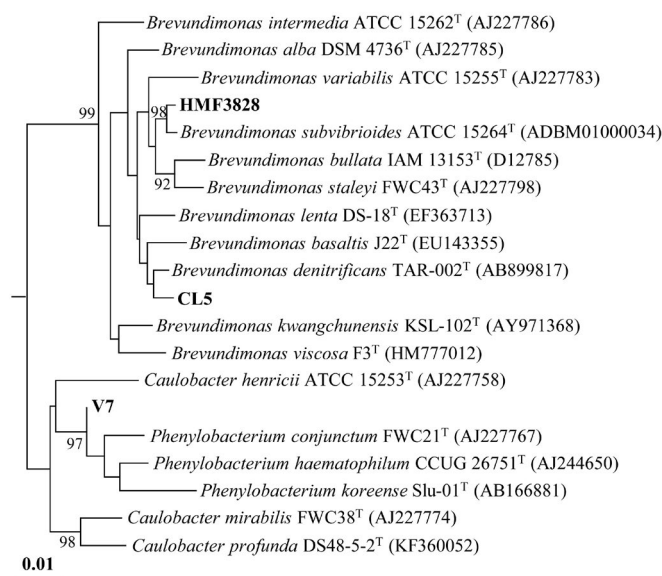
Cells are gram-staining-negative, non-flagellated, and short rod-shaped. Colonies are punctiform, flat, entire, and white-colored after 5 days of incubation at 30°C on Kuster's agar. Positive for nitrate reduction, glucose fermentation, and urease activity. Negative for indole production, arginine dihydrolase, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain TW2K3 (=NIBRBAC000498081) was isolated from abandoned mine soil sample, Boeungun, Korea.

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

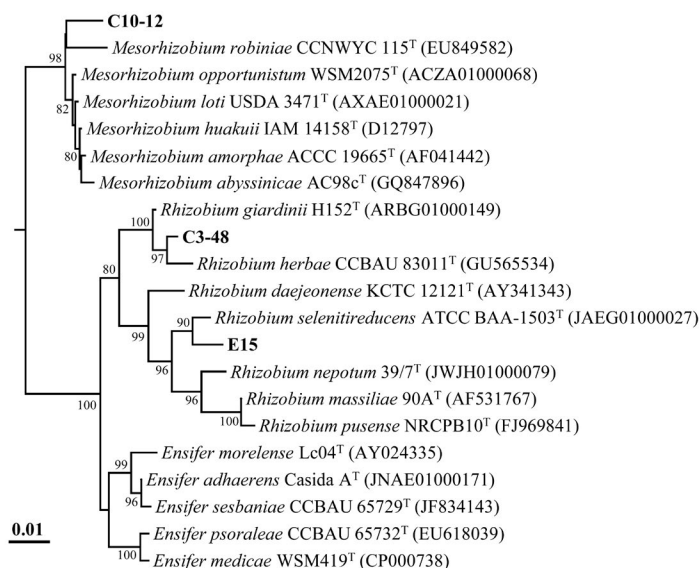
| Order                   | Family                    | Genus                     | Strain ID                              | NIBR ID          | Most closely related species           | Similarity (%)                       | Isolation source | Medium       | Incubation conditions |           |
|-------------------------|---------------------------|---------------------------|--|------------------|--|--------------------------------------|------------------|--------------|-----------------------|-----------|
| <i>Caulobacterales</i>  | <i>Caulobacteraceae</i>   | <i>Brevundimonas</i>      | HMF3828                                | NIBRBAC000497899 | <i>Brevundimonas subvibrioides</i>     | 99.6                                 | Freshwater       | R2A          | 30°C, 4d              |           |
|                         |                           | <i>Brevundimonas</i>      | CL5                                    | NIBRBAC000498062 | <i>Brevundimonas basaltis</i>          | 99.3                                 | Tidal flat       | R2A          | 30°C, 2d              |           |
|                         |                           | <i>Phenyllobacterium</i>  | V7                                     | NIBRBAC000498073 | <i>Phenyllobacterium haematophilum</i> | 99.6                                 | Tidal flat       | R2A          | 30°C, 2d              |           |
| <i>Rhodobacterales</i>  | <i>Rhodobacteraceae</i>   | <i>Albirhodobacter</i>    | IMCC25608                              | NIBRBAC000497998 | <i>Albirhodobacter marinus</i>         | 99.6                                 | Brackish water   | NA           | 20°C, 7d              |           |
|                         |                           | <i>Cereibacter</i>        | NU4                                    | NIBRBAC000498050 | <i>Cereibacter changensis</i>          | 100                                  | Freshwater       | R2A          | 25°C, 2d              |           |
|                         |                           | <i>Gemmobacter</i>        | SIL02                                  | NIBRBAC000498103 | <i>Gemmobacter caeni</i>               | 99.8                                 | Freshwater       | R2A          | 30°C, 2d              |           |
|                         |                           | <i>Gemmobacter</i>        | RDH1                                   | NIBRBAC000498075 | <i>Gemmobacter fontiphilus</i>         | 99.6                                 | Freshwater pond  | R2A          | 30°C, 2d              |           |
|                         |                           | <i>Loktanella</i>         | IMCC25609                              | NIBRBAC000497999 | <i>Loktanella vesfoldensis</i>         | 99.6                                 | Brackish water   | NA           | 20°C, 10d             |           |
|                         |                           | <i>Marinovum</i>          | LPB0094                                | NIBRBAC000497976 | <i>Marinovum algicola</i>              | 98.9                                 | Seawater         | MA           | 26°C, 2d              |           |
|                         |                           | <i>Marivita</i>           | IMCC25610                              | NIBRBAC000498000 | <i>Marivita lactus</i>                 | 100                                  | Brackish water   | MA           | 20°C, 5d              |           |
|                         |                           | <i>Rhodobacter</i>        | JAF4                                   | NIBRBAC000498043 | <i>Rhodobacter viridis</i>             | 99.5                                 | Freshwater       | R2A          | 25°C, 2d              |           |
|                         |                           | <i>Rosevivax</i>          | HMF2668                                | NIBRBAC000497897 | <i>Rosevivax halodurans</i>            | 99.4                                 | Salt pond        | MA           | 30°C, 3d              |           |
|                         |                           | <i>Roseovarius</i>        | HMF3938                                | NIBRBAC000497906 | <i>Roseovarius nanhaiticus</i>         | 99.9                                 | Seawater         | MA           | 30°C, 3d              |           |
| <i>Rhodospirillales</i> | <i>Acetobacteraceae</i>   | <i>Ruegeria</i>           | KYW1034                                | NIBRBAC000497926 | <i>Ruegeria scottomollitcae</i>        | 100                                  | Seawater         | MA           | 25°C, 3d              |           |
|                         |                           | <i>Ruegeria</i>           | LPB0098                                | NIBRBAC000497978 | <i>Ruegeria mobilis</i>                | 99.9                                 | Seawater         | MA           | 26°C, 1d              |           |
| <i>Rhizobiales</i>      | <i>Phyllobacteriaceae</i> | <i>Mesorhizobium</i>      | C10-12                                 | NIBRBAC000498034 | <i>Mesorhizobium opportunistum</i>     | 99.5                                 | Natural cave     | R2A          | 30°C, 6d              |           |
|                         |                           | <i>Rhizobium</i>          | C3-48                                  | NIBRBAC000498027 | <i>Rhizobium herbae</i>                | 99.7                                 | Natural cave     | R2A          | 30°C, 11d             |           |
|                         |                           | <i>Rhizobium</i>          | E15                                    | NIBRBAC000498064 | <i>Rhizobium selenitireducens</i>      | 99.0                                 | Tidal flat       | R2A          | 30°C, 2d              |           |
| <i>Rhodospirillales</i> | <i>Acetobacteraceae</i>   | <i>Acidisoma</i>          | TW2K3                                  | NIBRBAC000498081 | <i>Acidisoma tundrae</i>               | 99.3                                 | Mine soil        | KUA          | 30°C, 3d              |           |
|                         |                           | <i>Roseomonas</i>         | G11                                    | NIBRBAC000498068 | <i>Roseomonas lacus</i>                | 99.9                                 | Tidal flat       | R2A          | 30°C, 2d              |           |
| <i>Sphingomonadales</i> | <i>Erythrobacteraceae</i> | <i>Altererythrobacter</i> | MW26                                   | NIBRBAC000497864 | <i>Altererythrobacter xiamenensis</i>  | 99.2                                 | Tidal flat       | MA           | 30°C, 2d              |           |
|                         |                           | <i>Citrimicrobium</i>     | IMCC25619                              | NIBRBAC000498009 | <i>Citrimicrobium luteum</i>           | 99.7                                 | Freshwater       | MA           | 20°C, 15d             |           |
|                         | <i>Sphingomonadales</i>   | <i>Sphingomonadaceae</i>  | <i>Sandarakinorhabdus</i>              | IMCC25606        | NIBRBAC000497996                       | <i>Sandarakinorhabdus limnophila</i> | 99.1             | Freshwater   | 1/10 R2A              | 20°C, 7d  |
|                         |                           |                           | <i>Sphingobium</i>                     | LPB0124          | NIBRBAC000497990                       | <i>Sphingobium lactosutens</i>       | 99.0             | Seawater     | MA                    | 26°C, 2d  |
|                         |                           |                           | <i>Sphingobium</i>                     | NR 4-05          | NIBRBAC000498089                       | <i>Sphingobium vulgare</i>           | 99.6             | Plant root   | R2A                   | 30°C, 3d  |
|                         |                           |                           | <i>Sphingomonas</i>                    | HMF4018          | NIBRBAC000497909                       | <i>Sphingomonas jaspisi</i>          | 99.1             | Freshwater   | R2A                   | 30°C, 3d  |
|                         |                           |                           | <i>Sphingomonas</i>                    | JJ13             | NIBRBAC000497933                       | <i>Sphingomonas rosea</i>            | 99.8             | Freshwater   | R2A                   | 25°C, 5d  |
|                         |                           |                           | <i>Sphingomonas</i>                    | IMCC25620        | NIBRBAC000498010                       | <i>Sphingomonas echinooides</i>      | 99.7             | Freshwater   | 1/3 R2A               | 20°C, 10d |
|                         |                           |                           | <i>Sphingomonas</i>                    | BR5-29-1         | NIBRBAC000498124                       | <i>Sphingomonas pani</i>             | 99.2             | Ginseng soil | R2A                   | 30°C, 1d  |
|                         |                           |                           | <i>Sphingopyxis</i>                    | S6-5             | NIBRBAC000497886                       | <i>Sphingopyxis bauzanensis</i>      | 98.9             | Tidal flat   | MA                    | 30°C, 2d  |
| <i>Novosphingobium</i>  | LPB0117                   | NIBRBAC000497988          | <i>Novosphingobium lidamiclasticum</i> | 98.8             | Seawater                               | MA                                   | 26°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, HMF3828; 2, CL5; 3, V7; 4, IMCC25608; 5, NU4; 6, SIL02; 7, RDH1; 8, IMCC25609; 9, LPB0094; 10, IMCC25610; 11, JAF4; 12, HMF2668; 13, HMF3938; 14, KYW1034; 15, LPB0098; 16, C10-12; 17, C3-48; 18, E15; 19, TW2K3; 20, G11; 21, MW26; 22, IMCC25619; 23, IMCC25606; 24, LPB0124; 25, NR 4-05; 26, HMF4018; 27, JJ13; 28, IMCC25620; 29, BR5-29-1; 30, S6-5; 31, LPB0117.



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

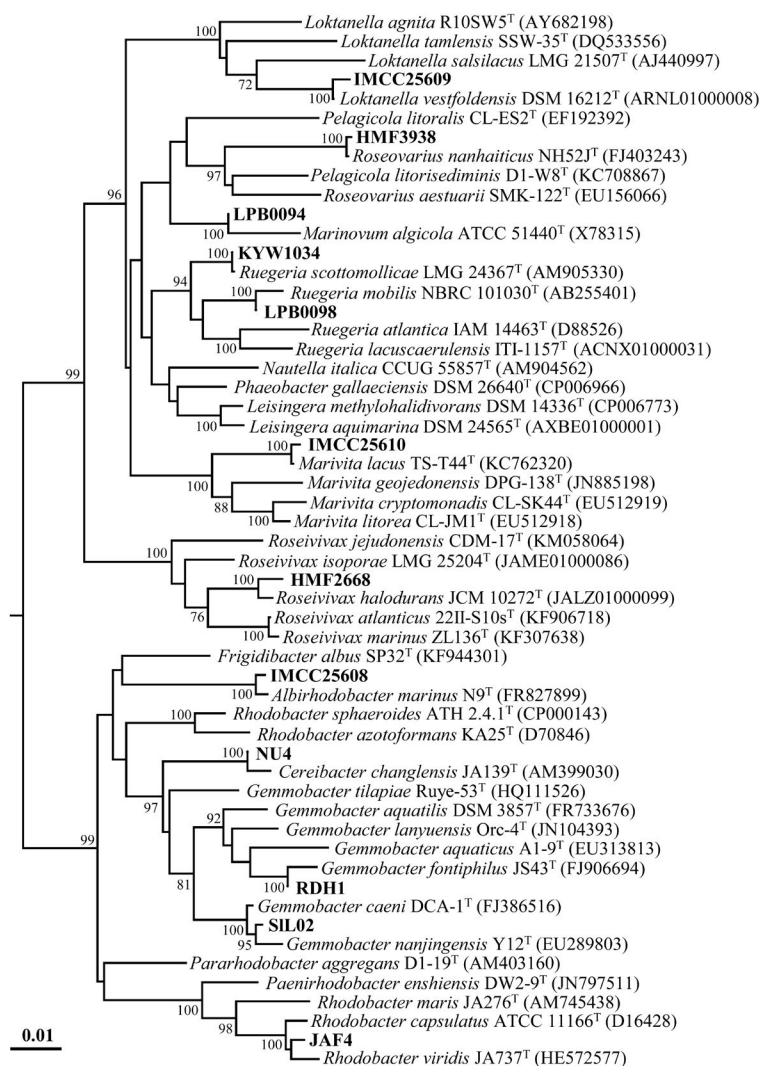


**Fig. 3.** Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationship between the strains isolated in this study and their relatives in the order *Rhizobiales*, class *Alphaproteobacteria*. *Escherichia coli* ATCC 11775<sup>T</sup> (X80725) was used as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

### Description of *Albirhodobacter marinus* IMCC25608

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire and white-colored after 7 days of incubation at 20°C on NA. Positive for nitrate reduction and urease activity. D-Glucose, L-arabinose, D-mannose, D-mannitol, potassium gluco-

nate, and malic acid are utilized. Negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25608 (= NIBRBAC000497998) was isolated from a brackish water sample, Incheon, Korea.



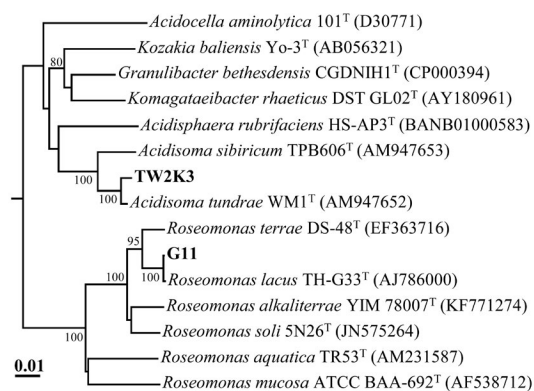
**Fig. 4.** Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationships between the strains isolated in this study and their relatives in the order Rhodobacterales, class Alphaproteobacteria. *Escherichia coli* ATCC 11775<sup>T</sup> (X80725) was used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

#### Description of *Altererythrobacter xiamenensis* MW26

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire and pale yellow-colored after 2 days of incubation at 30°C on MA. Positive for nitrate reduction, esculin hydrolysis, gelatinase, and  $\beta$ -galactosidase activities. Capric acid is utilized. Negative for indole production, glucose fermentation, and arginine dihydrolase and urease 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, and phenylacetic acid. Strain MW26 (= NIBRBAC 000497864) was isolated from a tidal flat sample, Taean-gun, Korea.

#### Description of *Brevundimonas basaltis* CL5

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, convex, entire and white-colored after 2 days of incubation at 30°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and gelatinase and  $\beta$ -galactosidase activities. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, and trisodium citrate are utilized. Negative for indole production, glucose fermentation, and arginine dihydrolase and urease activities. Does not utilize L-arabinose, capric acid, and phenylacetic acid. Strain CL5 (= NIBRBAC 000498062) was isolated from a tidal flat sample, Incheon, Korea.



**Fig. 5.** 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 *Rhodospirillales*, class *Alphaproteobacteria*. *Escherichia coli* ATCC 11775<sup>T</sup> (X80725) was used as an outgroup (not shown). Bootstrap values (>70%) are shown above nodes. Scale bar: 0.01 changes per nucleotide.

#### Description of *Brevundimonas subvibrioides* HMF3828

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and yellow-colored after 4 days of incubation at 30°C on R2A. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. 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 HMF3828 (=NIBRBAC000497899) was isolated from a fresh water sample, Yong-in, Korea.

#### Description of *Cereibacter changlensis* NU4

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex and white-colored after 2 days of incubation at 25°C on R2A. D-Glucose, L-arabinose, D-mannose, and *N*-acetyl-glucosamine are utilized. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain NU4 (=NIBRBAC000498050) was isolated from a fresh water sample, Jeonju, Korea.

#### Description of *Citrimicrobium luteum* IMCC25619

Cells are gram-staining-negative, non-flagellated, and coccoid-shaped. Colonies are circular, raised, entire, and red yellow-colored after 15 days of incubation at 20°C

on MA. Positive for  $\beta$ -galactosidase activity. D-Mannitol, potassium gluconate, and malic acid are utilized. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25619 (=NIBRBAC000498009) was isolated from a fresh water sample, Incheon, Korea.

#### Description of *Gemmobacter caeni* SIL02

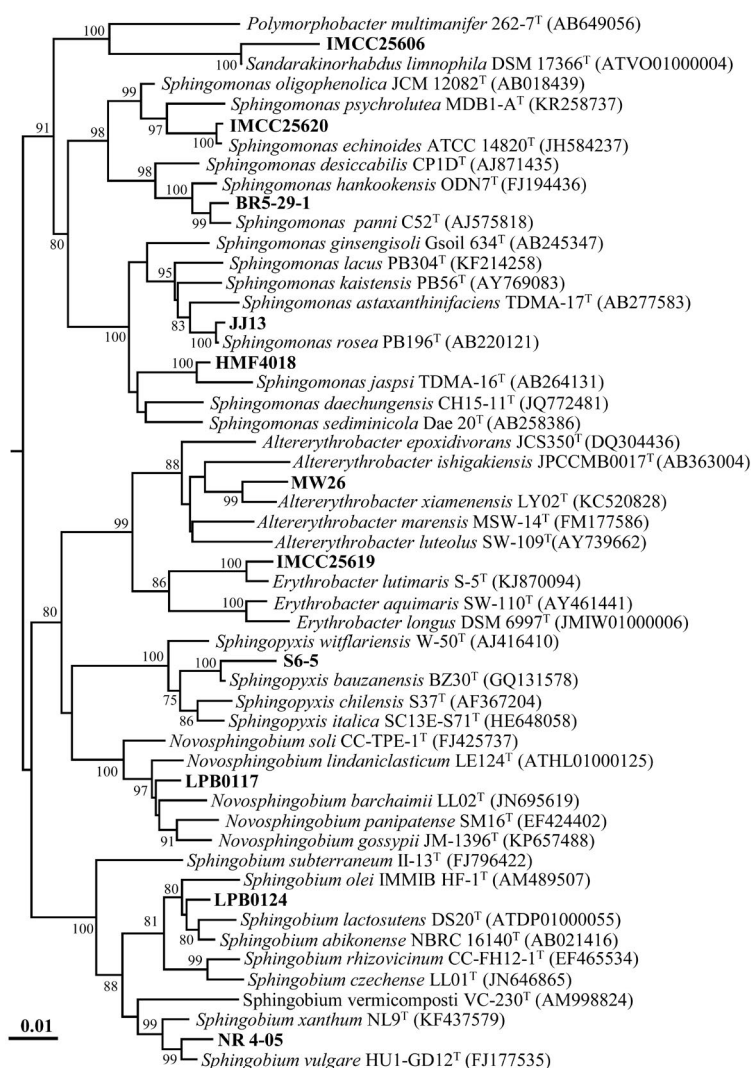
Cells are gram-staining-negative, non-flagellated, and round, circular, and convex-shaped. Colonies are circular, round, entire, and light yellow-colored after 2 days of incubation at 30°C on R2A. Positive for glucose fermentation, esculin hydrolysis, and urease and  $\beta$ -galactosidase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Negative for nitrate reduction, indole production, and arginine dihydrolase and gelatinase activities. Does not utilize *N*-acetyl-glucosamine and capric acid. Strain SIL02 (=NIBRBAC000498103) was isolated from an activated sludge sample, Daejeon, Korea.

#### Description of *Gemmobacter fontiphilus* RDH1

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are convex, round, smooth, entire, and green-colored after 2 days of incubation at 30°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and  $\beta$ -galactosidase activity. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, adipic acid, and malic acid are utilized. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. Does not utilize potassium gluconate, capric acid, trisodium citrate, and phenylacetic acid. Strain RDH1 (=NIBRBAC000498075) was isolated from a fresh water pond sample, Seoul, Korea.

#### Description of *Loktanella vestfoldensis* IMCC25609

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire, and white-colored after 10 days of incubation at 20°C on NA. Positive for esculin hydrolysis and urease, gelatinase, and  $\beta$ -galactosidase activities. D-Mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase activity. Does not utilize D-glucose, L-arabinose, D-mannose, and D-maltose. Strain IMCC25609 (=NIBRBAC000497999) was



**Fig. 6.** 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 *Sphingomonadales*, class *Alphaproteobacteria*. *Escherichia coli* ATCC 11775<sup>T</sup> (X80725) was used as an outgroup (not shown). Bootstrap values (>70%) are shown above nodes. Scale bar: 0.01 changes per nucleotide.

isolated from a brackish water sample, Incheon, Korea.

#### Description of *Marinovum algicola* LPB0094

Cells are gram-staining-negative, non-flagellated, and coccoid-shaped. Colonies are ovoid and beige-colored after 2 days of incubation at 26°C on MA. Positive for  $\beta$ -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, and gelatinase activities. 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 LPB0094 (= NIBRBAC000497976) was isolated from a sea water sample, Busan, Korea.

#### Description of *Marivita lacus* IMCC25610

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire, and white yellow-colored after 5 days of incubation at 20°C on MA. Positive for esculin hydrolysis and  $\beta$ -galactosidase activity. D-Mannitol, D-maltose, potassium gluconate, and capric acid are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25610 (= NIBRBAC 000498000) was isolated from a Brackish water sample, Incheon, Korea.



**Description of *Mesorhizobium opportunistum* C10-12**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and cream-colored after 6 days of incubation at 30°C on R2A. Positive for esculin hydrolysis. D-Mannitol, *N*-acetylglucosamine, and D-maltose are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain C10-12 (=NIBRBAC000498034) was isolated from a natural cave sample, Jeju island, Korea.

**Description of *Novosphingobium lidaniclasticum* LPB0117**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular and light yellow-colored after 2 days on MA at 26°C. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. L-Arabinose, D-maltose, and malic acid are utilized. Does not utilize D-glucose, D-mannose, D-mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain LPB0117 (=NIBRBAC000497988) was isolated from sea water sample, Busan, Korea.

**Description of *Phenylobacterium haematophilum* V7**

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are convex, entire, and colorless after 2 days of incubation at 30°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and  $\beta$ -galactosidase activity. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. D-Glucose, L-arabinose, *N*-acetylglucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid are utilized. Does not utilize D-mannose, D-mannitol, capric acid, trisodium citrate, and phenylacetic acid. Strain V7 (=NIBRBAC000498073) was isolated from a tidal flat, Incheon, Korea.

**Description of *Rhizobium herbae* C3-48**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and cream-colored after 11 days of incubation at 30°C on R2A. Positive for urease and  $\beta$ -galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannitol, and *N*-acetylglucosamine are

utilized. Does not utilize D-mannose, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain C3-48 (=NIBRBAC000498027) was isolated from a natural cave, Jeju island, Korea.

**Description of *Rhizobium selenitireducens* E15**

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, entire, and cream-colored after 2 days on R2A at 30°C. Positive for nitrate reduction, urease, esculin hydrolysis, and  $\beta$ -galactosidase activity. Negative for indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, adipic acid, and malic acid are utilized. Does not utilize *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, trisodium citrate, and phenylacetic acid. Strain E15 (=NIBRBAC000498064) was isolated from a tidal flat sample, Incheon, Korea.

**Description of *Rhodobacter viridis* JAF4**

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, convex, and brown-colored after 2 days of incubation at 25°C on R2A. Positive for glucose fermentation, esculin hydrolysis, and  $\beta$ -galactosidase activity. Negative for nitrate reduction, indole production, and arginine dihydrolase, urease, and gelatinase activities. D-Maltose and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain JAF4 (=NIBRBAC000498043) was isolated from a fresh water sample, JeonJu, Korea.

**Description of *Roseivivax halodurans* HMF2668**

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, convex, entire, and pink-colored after 3 days of incubation at 30°C on MA. Positive for esculin hydrolysis and  $\beta$ -galactosidase activity. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, and phenylacetic acid are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. Does not utilize capric acid and trisodium citrate. Strain HMF2668 (=NIBRBAC000497897) was isolated from a salt pond sample, Shinan-gun, Korea.

**Description of *Roseomonas lacus* G11**

Cells are gram-staining-negative, non-flagellated, and coccoid-shaped. Colonies are circular, raised, entire, and cream-colored after 2 days of incubation at 30°C on R2A.

Positive for nitrate reduction and urease activity. Negative for indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, gelatinase, and  $\beta$ -galactosidase activities. Potassium gluconate, capric acid, adipic acid, malic acid, and trisodium citrate are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, and phenylacetic acid. Strain G11 (=NIBRBAC000498068) was isolated from a tidal flat sample, Incheon, Korea.

#### **Description of *Roseovarius nanhaiticus* HMF3938**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and beige-colored after 3 days of incubation at 30°C on MA. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. 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 HMF3938 (=NIBRBAC000497906) was isolated from a sea water sample, Pohang, Korea.

#### **Description of *Ruegeria mobilis* LPB0098**

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular and brown-colored after 1 day of incubation at 26°C on MA. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. 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 LPB0098 (=NIBRBAC000497978) was isolated from a sea water sample, Busan, Korea.

#### **Description of *Ruegeria scottomollicae* KYW1034**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, opaque, and light yellow-colored after 3 days of incubation at 25°C on MA. Positive for esculin hydrolysis and  $\beta$ -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. 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 KYW1034 (=NIBRBAC000497926) was isolated from a sea water sample, Gwangyang, Korea.

#### **Description of *Sandarakinorhabdus limnophila* IMCC25606**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire, and white-colored after 7 days of incubation at 20°C on 1/10 diluted R2A. Positive for gelatinase and  $\beta$ -galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase and urease activities. 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 IMCC25606 (=NIBRBAC000497996) was isolated from a freshwater sample, Chuncheon, Korea.

#### **Description of *Sphingobium lactosutens* LPB0124**

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular and yellow-colored after 2 days of incubation at 26°C on MA. Positive for esculin hydrolysis. D-Glucose, L-arabinose, and D-maltose are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, Potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain LPB0124 (=NIBRBAC000497990) was isolated from a seawater sample, Busan, Korea.

#### **Description of *Sphingobium vulgare* NR 4-05**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, smooth, glistening, and yellow-colored after 3 days of incubation on R2A at 30°C. Positive for nitrate reduction, esculin hydrolysis, and  $\beta$ -galactosidase activity. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. 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 NR 4-05 (=NIBRBAC000498089) was isolated from a plant root sample, Jeongeup, Korea.

#### **Description of *Sphingomonas jaspisi* HMF4018**

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, entire, and yellow-colored after 3 days of incubation at 30°C on R2A agar. Positive for nitrate reduction and esculin hydrolysis. D-Glucose, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenyl-

acetic acid are utilized. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and capric acid. Strain HMF4018 (=NIBRBAC 000497909) was isolated from a freshwater sample, Gyeongju, Korea.

#### Description of *Sphingomonas rosea* JJ13

Cells are gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, smooth, convex, and orange red-colored after 5 days of incubation at 25°C on R2A agar. Positive for esculin hydrolysis and urease activity. D-Glucose and D-maltose are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain JJ13 (=NIBRBAC 000497933) was isolated from a freshwater sample, Changwon, Korea.

#### Description of *Sphingomonas echinoides* IMCC25620

Cells are gram-staining-negative, non-flagellated, and coccoid-shaped. Colonies are circular, raised, entire, and yellow-colored after 10 days of incubation at 20°C on 1/3 diluted R2A agar. Positive for esculin hydrolysis. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize D-mannitol, capric acid, trisodium citrate, and phenylacetic acid. Strain IMCC25620 (=NIBRBAC 000498010) was isolated from a freshwater sample, Incheon, Korea.

#### Description of *Sphingomonas panni* BR5-29-1

Cells are gram-staining-negative, flagellated, and round shaped rods. Colonies are irregular circular, transparent, convex, and dark yellow-colored after 1 day of incubation at 30°C on R2A. Positive for glucose fermentation and esculin hydrolysis. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, and malic acid are utilized. Negative for nitrate reduction, indole production, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain BR5-29-1 (=NIBRBAC000498124) was isolated from a ginseng soil sample, Anseong, Korea.

#### Description of *Sphingopyxis bauzanensis* S6-5

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, raised, entire, and pale yellow-colored after 2 days of incubation at 30°C on MA. Positive for esculin hydrolysis. D-Glucose, potassium gluconate, adipic acid, and malic acid are utilized. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and  $\beta$ -galactosidase activities. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, trisodium citrate, and phenylacetic acid. Strain S6-5 (=NIBRBAC000497886) was isolated from a tidal flat sample, Taean-gun, Korea.

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