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Report of 29 unrecorded bacterial species from the phylum *Proteobacteria*

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Our study aimed to discover indigenous prokaryotic species in Korea. A total of 29 bacterial species in the phylum *Proteobacteria* were isolated from freshwater and sediment of rivers and brackish zones in Korea. From the high 16S rRNA gene sequence similarity (≥98.8%) and formation of a robust phylogenetic clade with the closest species, it was determined that each strain belonged to an independent and predefined bacterial species. To our knowledge, there is no official report or publication that has previously described these 29 species in Korea. Specifically, we identified 10, 12, and seven species of eight, 12, and seven genera that belong to classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*, respectively; all are reported as previously unrecorded bacterial species in Korea. The Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs for each are also described.

Keywords: 16S rRNA gene, *Proteobacteria*, unrecorded species

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Introduction

Proteobacteria (Stackebrandt *et al*., 1988; Garrity *et al*., 2005) is the largest phylum of bacteria, containing over 500 genera. *Proteobacteria* constitutes the most diverse phylogenetic lineage with regard to phenotypic, physiological, and metabolic characteristics. Members of the phylum *Proteobacteria* have been observed in various environmental habitats ranging from aquatic and terrestrial environments to mammals (Costello *et al*., 2009; Joung *et al*., 2014; Pascault *et al*., 2014). Based on 16S rRNA gene sequences, the phylum *Proteobacteria* is further divided into six classes: *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, and *Zetaproteobacteria*. Previously, it was reported that the six classes of *Proteobacteria* showed significant differences in distribution based on environment: *Alphaproteobacteria* and *Betaproteobacteria* were enriched in the freshwater environment, *Gammaproteobacteria* and *Zetaproteobacteria* in the marine environment, *Gammaproteobacteria* and *Epsilonproteobacteria* in the intertidal zone, and *Deltaproteobacteria* were found in all environments (Emerson *et al*., 2007; Wang *et al*., 2012).

In 2015-16, we collected freshwater samples from the major rivers (Han, Nakdong, and Seomjin Rivers) in Korea and isolated myriads of novel and unrecorded bacterial species. Here, we report 29 previously unrecorded bacterial species in Korea from the phylum *Proteobacteria*, especially the classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*.

Materials and Methods

A total of 29 bacterial strains from the phylum *Proteobacteria* were isolated from environmental samples including freshwater, brackish water, and sediment. Each environmental sample was processed separately, spread onto various culture media, including R2A agar, marine agar 2216, and nutrient agar, and incubated at 15-25°C for 2-3 weeks. All the strains were purified as single colonies and stored in a 10-20% glycerol suspension at −80°C. The designated strain IDs, isolation sources, culture media, and incubation conditions are summarized in Table 1.

Bacterial DNA preparation, PCR amplification, and 16S rRNA gene sequencing were carried out as previ-

Table 1. Summary of strains belonging to the phylum *Proteobacteria* and their taxonomic affiliations.

Class	Family	Genus	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Alphaproteobacteria	*Acetobacteraceae*	*Belnapia*	AS5-06	NNIBRBA 60	*B. moabensis*	98.9	Sediment	R2A	30°C, 3d
	Bradyrhizobiaceae	*Tardiphaga*	BK16-1	NNIBRBA 43	*T. robiniae*	98.9	Fresh water	R2A	25°C, 3d
		Afipia	SH41	NNIBRBA 42	*A. massiliensis*	98.9	Fresh water	R2A	25°C, 3d
	Methylobacteriaceae	*Methylobacterium*	CS4-36	NNIBRBA 67	*M. bullatum*	100	Sediment	R2A	30°C, 3d
	Rhodobacteraceae	*Pseudorhodobacter*	BES-63	NNIBRBA 48	*P. ferrugineus*	99.4	Fresh water	MA	25°C, 3d
		Pseudooceanicola	ES2-15	NNIBRBA 64	*P. nanhaiensis*	99.4	Sediment	MA	25°C, 3d
	Sphingomonadaceae	*Sphingomonas*	SS1-08	NNIBRBA 3	*S. abaci*	99.6	Sediment	R2A	25°C, 3d
			SS1-17	NNIBRBA 5	*S. endophytica*	99.9	Sediment	R2A	25°C, 3d
			SS1-22	NNIBRBA 6	*S. yunnanensis*	99.4	Sediment	R2A	25°C, 3d
		Blastomonas	SH33	NNIBRBA 41	*B. aquatica*	98.8	Fresh water	R2A	25°C, 3d
Betaproteobacteria	*Burkholderiaceae*	*Polynucleobacter*	BK-182	NNIBRBA 29	*P. necessarius* subsp. *asymbioticus*	99.7	Fresh water	R2A	30°C, 3d
	Comamonadaceae	*Hydrogenophaga*	BK-176	NNIBRBA 27	*H. atypica*	98.8	Fresh water	R2A	25°C, 3d
		Malikia	SJ55	NNIBRBA 37	*M. granosa*	99.5	Brackish water	R2A	25°C, 3d
		Kinneretia	BK-77	NNIBRBA 22	*K. asaccharophila*	99.7	Fresh water	R2A	25°C, 3d
	Neisseriaceae	*Chitinibacter*	BK-219	NNIBRBA 33	*C. tainanensis*	99.9	Fresh water	NA	30°C, 4d
	Oxalobacteraceae	*Herminiimonas*	BK-438	NNIBRBA 46	*H. contaminans*	100	Fresh water	R2A	25°C, 3d
	Pseudomonadaceae	*Rhizobacter*	SS1-70	NNIBRBA 11	*R. dauci*	99.2	Sediment	R2A	25°C, 3d
	Rhodocyclaceae	*Dechloromonas*	BK-210	NNIBRBA 30	*D. hortensis*	99.1	Fresh water	R2A	30°C, 3d
		Thauera	ES1-46	NNIBRBA 63	*T. aminoaromatica*	99.8	Sediment	R2A	25°C, 3d
	*Sphaerotilus*_f	*Ideonella*	BK-22	NNIBRBA 19	*I. dechloratans*	99.5	Fresh water	R2A	25°C, 3d
		Inhella	BK-179	NNIBRBA 28	*I. inkyongensis*	99.2	Fresh water	R2A	15°C, 3d
		Piscinibacter	SS2-102	NNIBRBA 17	*P. aquaticus*	99.3	Sediment	R2A	25°C, 3d
Gammaproteobacteria	*Chromatiaceae*	*Rheinheimera*	BK-128	NNIBRBA 25	*R. aquatica*	99.1	Fresh water	R2A	35°C, 2d
	Nevskiaceae	*Nevskia*	BK-213	NNIBRBA 31	*N. ramosa*	98.7	Fresh water	R2A	25°C, 3d
	Pseudoalteromonadaceae	*Pseudoalteromonas*	BES3-108	NNIBRBA 49	*P. tunicata*	100	Sediment	R2A	25°C, 3d
	Pseudomonadaceae	*Pseudomonas*	KS1-13	NNIBRBA 56	*P. avellanae*	98.8	Sediment	R2A	25°C, 3d
	Xanthomonadaceae	*Lysobacter*	GS1-30	NNIBRBA 52	*L. brunescens*	99.8	Sediment	R2A	30°C, 3d
		Thermomonas	04KS1-07	NNIBRBA 57	*T. carbonis*	99.5	Sediment	R2A	30°C, 3d
		Arenimonas	CS4-45	NNIBRBA 68	*A. subflava*	99.9	Sediment	R2A	25°C, 3d

ously described (Chun & Goodfellow, 1995). The 16S rRNA gene sequences of the related taxa were obtained from the EzTaxon-e server (Kim *et al*., 2012). The 16S rRNA gene sequences were aligned in the SILVA aligner version 1.2.1.11 (http://www.arb-silva.de/aligner/; Pruesse *et al*., 2012). Phylogenetic trees were reconstructed by neighbor-joining (NJ) (Saitou & Nei, 1987), maximum-parsimony (MP) (Fitch, 1971), and maximum-likelihood (ML) (Felsenstein, 1981) algorithms using the MEGA software version 6 (Tamura *et al*., 2013). For the neighbor-joining analysis, the evolutionary distances between sequences were calculated using the Jukes-Cantor distance (Jukes & Cantor, 1969), and the tree topology was evaluated with 1,000 replicates of bootstrap resampling (Felsenstein, 1985).

The colony morphology of the strains was observed on agar plates with a magnifying glass after cells grew to stationary phase. Cellular morphology and cell size were examined by transmission electron microscopy (Fig. 1). Gram staining was performed using a Gram Staining Kit (Sigma). Biochemical characteristics were tested using the API 20 NE galleries (bioMérieux) according to the manufacturer's instructions.

Results and Discussion

Based on 16S rRNA gene sequence comparisons and phylogenetic analyses, a total 29 strains were identified as previously unrecorded bacterial species in Korea. These strains were gram-negative and rod-shaped bacteria, except for strains AS5-06 and ES1-46 which had either a coccoid or oval shape (Fig. 1). The 29 unrecorded bacterial species belonged to three classes, 10 orders, 17 families, and 27 genera. They were assigned to 10 species of *Alphaproteobacteria*, 12 species of *Betaproteobacteria*, and seven species of *Gammaproteobacteria*. Fig. 2 shows the phylogenetic assignments of 10 species within the class *Alphaproteobacteria*; these strains were identified as the species *Sphingomonas abaci* (Busse *et al*., 2005), *S. endophytica* (Huang *et al*., 2012), *S. yunnanensis* (Zhang *et al*., 2005), and *Blastomonas aquatica* (Xiao *et al*., 2015) of the order *Sphingomonadales*; *Tardiphaga robiniae* (De Mayer *et al*., 2012), *Afipia massiliensis* (La Scola *et al*., 2002), and *Methylobacterium bullatum* (Hoppe *et al*., 2011) of the order *Rhizobiales*; *Pseudorhodobacter ferrugineus* (Uchino *et al*., 2002) and *Pseudooceanicola nanhaiensis* (Lai *et al*., 2015) of the order *Rhodobacterales*; and *Belnapia moabensis* (Reddy *et al*., 2006) of the order *Rhodospirillales*. In the class *Betaproteobacteria*, 12 strains were assigned to the three orders *Burkholderiales*, *Rhodocyclales*, and *Neisseriales* (Fig. 3). The strains were identified as the species *Ideonella dechloratans* (Malmqvist *et al*., 1994), *Kinneretia asaccharophila* (Gomila *et al*., 2010), *Hydrogenophaga atypica* (Kämpfer *et al*., 2005), *Inhella inkyongensis* (Chen *et al*., 2012), *Polynucleobacter necessarius* subsp. *asymbioticus* (Hahn *et al*., 2016), *Rhizobacter dauci* (Goto & Kuwata, 1988), *Piscinibacter aquaticus* (Stackebrandt *et al*., 2009), *Malikia granosa* (Spring *et al*., 2005), and *Herminiimonas contaminans* (Kämpfer *et al*., 2013) of the order *Burkholderiales*; *Dechloromonas hortensis* (Wolterink *et al*., 2005) and *Thauera aminoaromatica* (Mechichi *et al*., 2002) of the order *Rhodocyclales*; and *Chitinibacter tainanensis* (Chern *et al*., 2004) of the order *Neisseriales*. Fig. 4 shows the phylogenetic assignments of seven species of the orders *Xanthomonadales*, *Alteromonadales*, *Nevskiales*, and *Pseudomonadales* within the class *Gammaproteobacteria*. *Lysobacter brunescens* (Christensen & Cook, 1978), *Thermomonas carbonis* (Wang *et al*., 2014), and *Arenimonas subflava* (Makk *et al*., 2015) of the order *Xanthomonadales*; *Rheinheimera aquatica* (Chen *et al*., 2010) and *Pseudoalteromonas tunicata* (Holmström *et al*., 1998) of the order *Alteromonadales*; *Nevskia ramosa* (Famintzin, 1892) of the order *Nevskiales*; and *Pseudomonas avellanae* (Janse *et al*., 1996) of the order *Pseudomonadales* were identified.

In conclusion, we have described 29 previously unrecorded bacterial species from the phylum *Proteobacteria*, which were isolated from freshwater environments in Korea.

Description of *Sphingomonas abaci* SS1-08

Cells are gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, shiny, leathery, dry and yellow-colored after 3 days on R2A agar at 25°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannose, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Weakly utilize *N*-acetyl-glucosamine. Dose not utilize D-mannitol, capric acid, adipic acid and phenylacetic acid. The strain SS1-08 (=NNIBRBA 3) was isolated from sediment of the Nakdong River, Gyeongcheon-island, Sangju, Korea.

Description of *Sphingomonas endophytica* SS1-17

Cells are gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, opaque and yellow-colored after 3 days on R2A agar at 25°C. Positive for esculin hydrolysis and β-galactosidase, weakly positive for gelatinase, but negative for oxidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose and malic

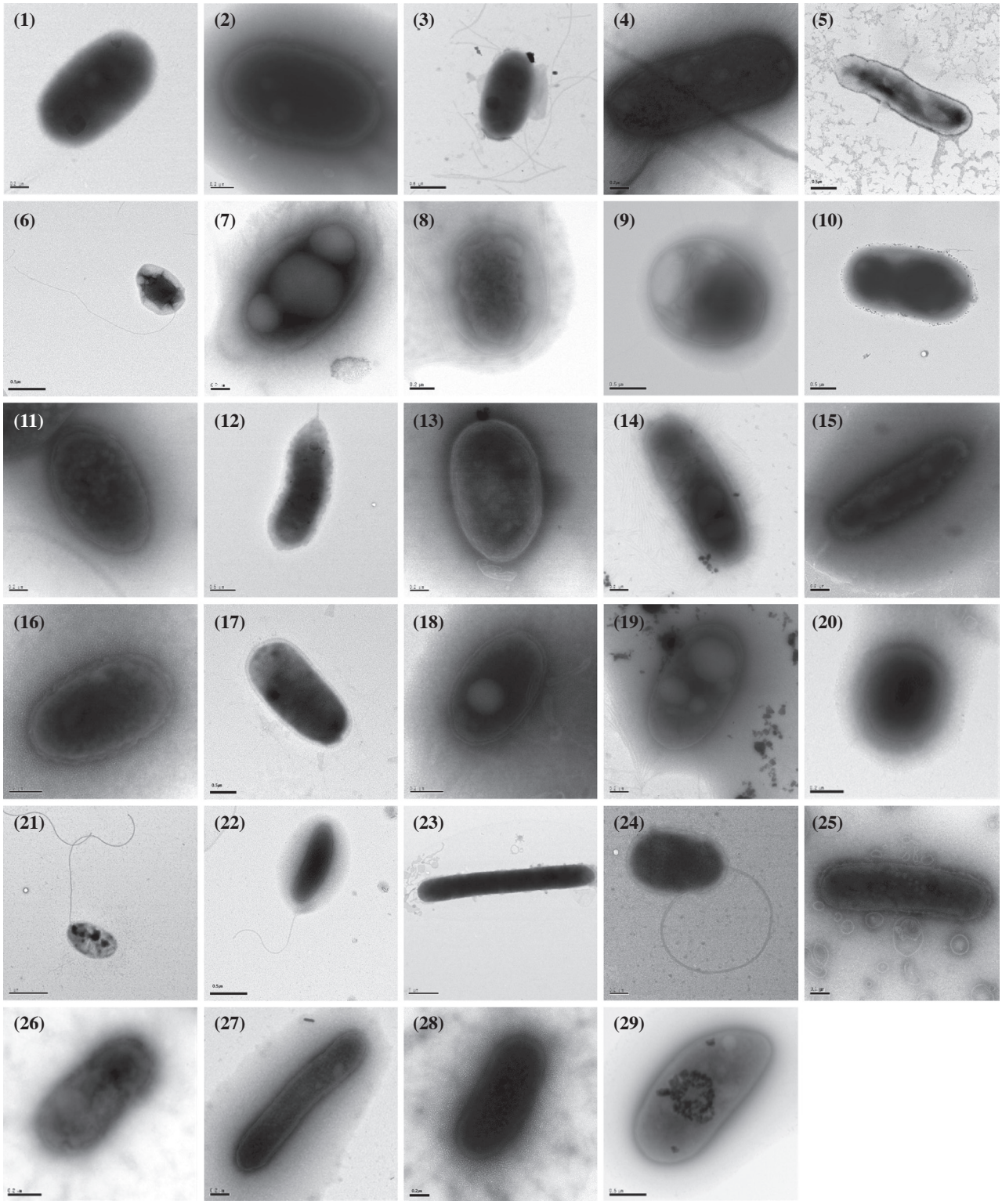

Fig. 1. Transmission electron micrographs of the strains isolated in this study. Strains: 1, S1-08; 2, SS1-17; 3, SS1-22; 4, SH33; 5, BK16-1; 6, SH41; 7, BES-63; 8, ES2-15; 9, AS5-06; 10, CS4-36; 11, BK-22; 12, BK-77; 13, BK-179; 14, SS1-70; 15, SS2-102; 16, BK-176; 17, SJ55; 18, BK-182; 19, BK-210; 20, ES1-46; 21, BK-219; 22, BK-438; 23, BK-128; 24, BK-213; 25, GS1-30; 26, 04KS1-07; 27, CS4-45; 28, BES3-108; 29, KS1-13.

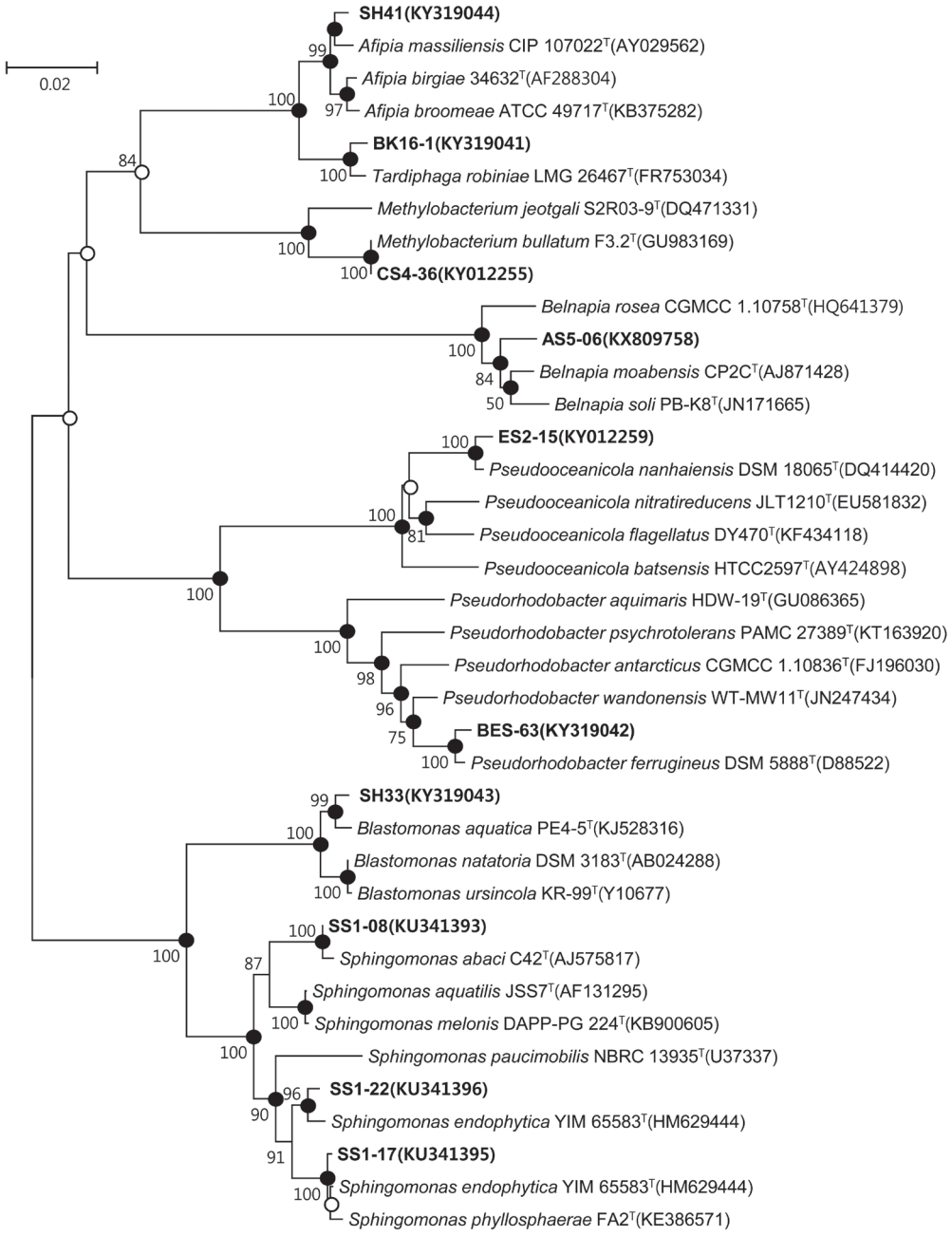

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between 10 unrecorded bacterial species and other representatives of the class *Alphaproteobacteria*. Bootstrap values (>70%) based on 1000 resamplings are shown at branching points. Filled circles indicate that the corresponding nodes were recovered by all treeing methods. Open circles indicate that the corresponding nodes were recovered by the neighbor-joining and maximum-likelihood methods. *Escherichia coli* NCTC9001^T (LN831047) was used as an outgroup (not shown). Bar, 0.02 substitutions per nucleotide position.

acid are utilized. Dose not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. The strain SS1-17 (=NNIBRBA 5) was isolated from sediment of the Nakdong River, Gyeongcheon-island, Sangju, Korea.

Description of *Sphingomonas yunnanensis* SS1-22

Cells are gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, opaque and yellow-colored after 3 days on R2A agar at 25°C. Positive for oxidase, esculin hydrolysis, gelatinase and β-galactosidase, but negative for nitrate reduction, indole

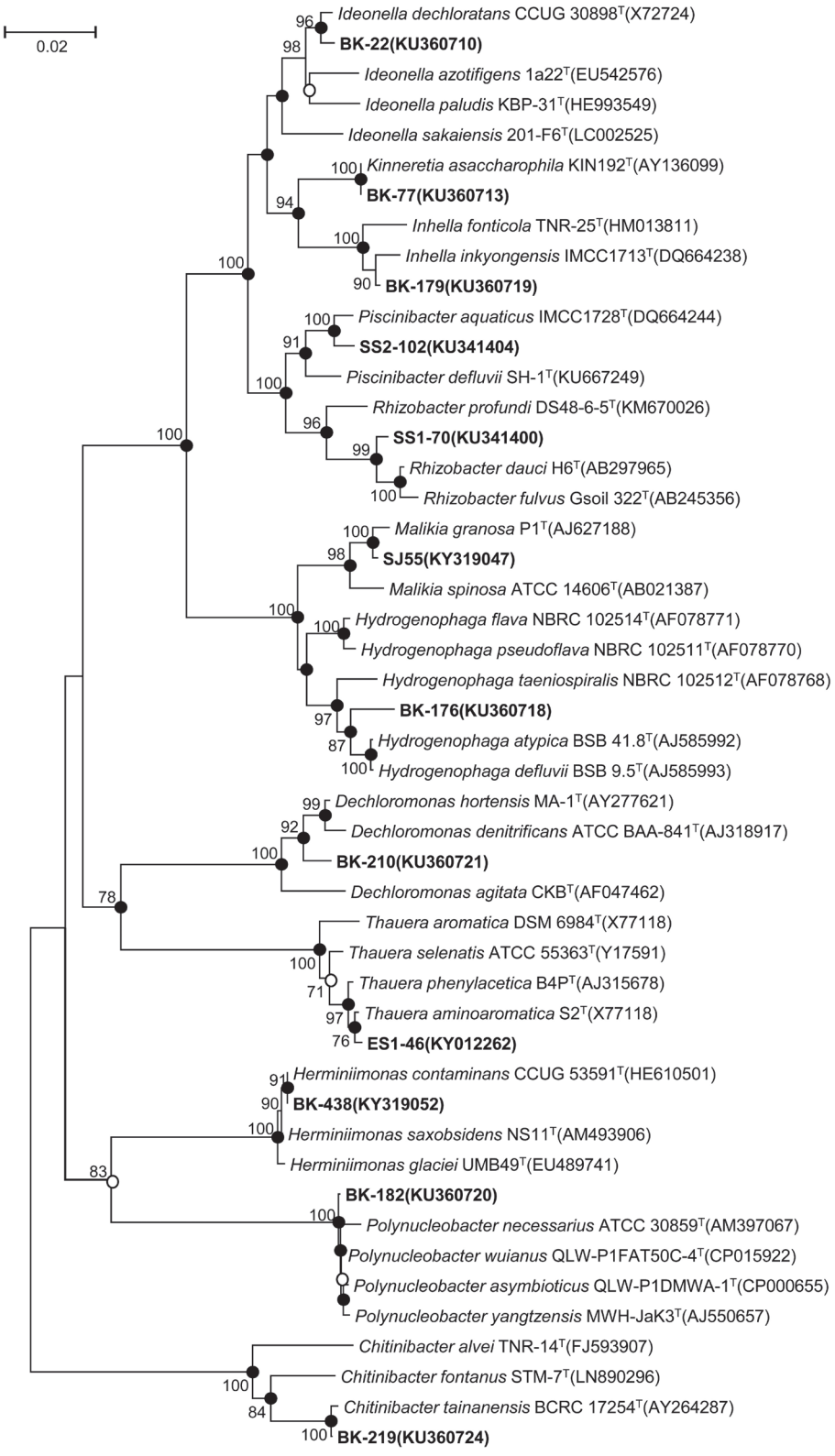

Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between 12 unrecorded bacterial species and other representatives of the class *Betaproteobacteria*. Bootstrap values (>70%) based on 1000 resamplings are shown at branching points. Filled circles indicate that the corresponding nodes were recovered by all treeing methods. Open circles indicate that the corresponding nodes were recovered by the neighbour-joining and maximum-likelihood methods. *Escherichia coli* NCTC9001^T (LN831047) was used as an outgroup (not shown). Bar, 0.02 substitutions per nucleotide position.

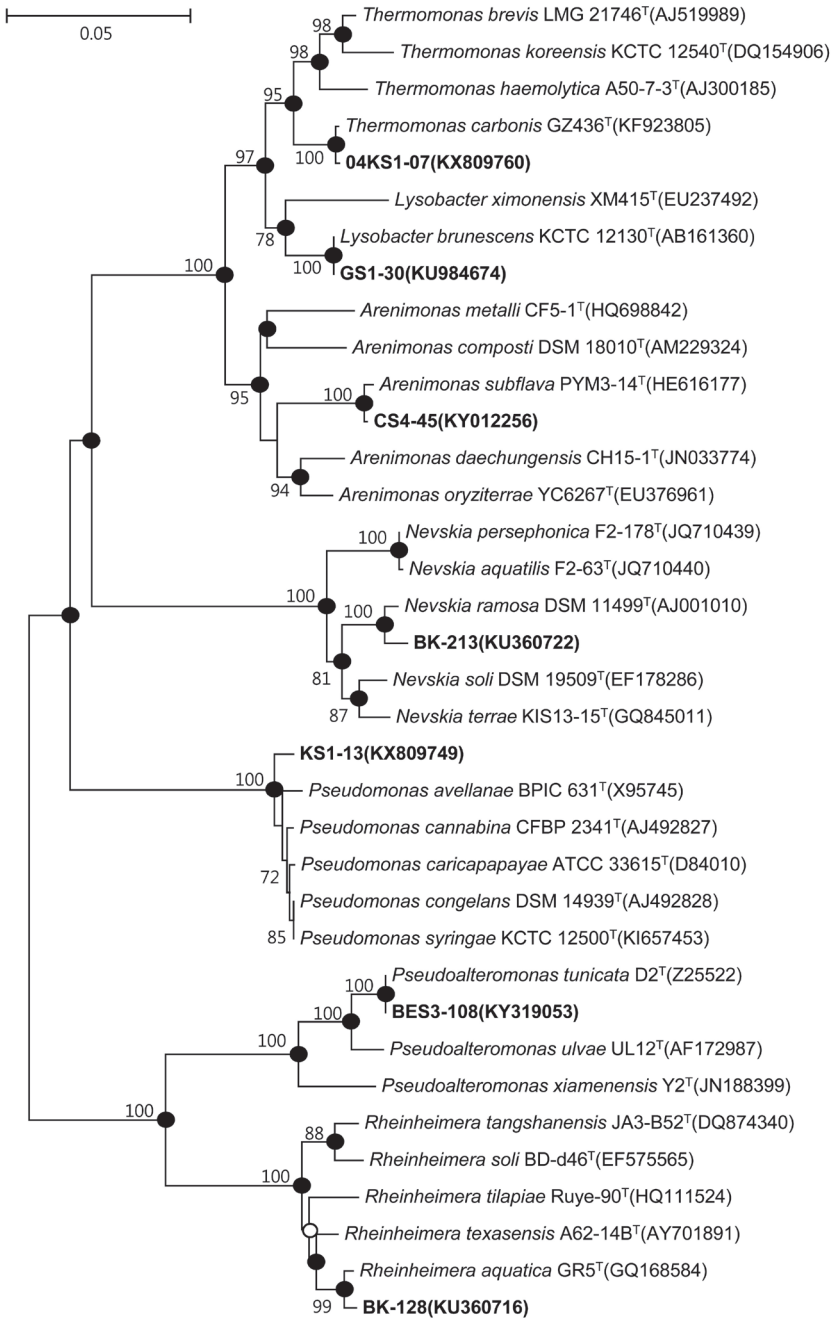

Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between 7 unrecorded bacterial species and other representatives of the class *Gammaproteobacteria*. Bootstrap values (>70%) based on 1000 resamplings are shown at branching points. Filled circles indicate that the corresponding nodes were recovered by all treeing methods. Open circles indicate that the corresponding nodes were recovered by the neighbor-joining and maximum-likelihood methods. *Flavobacterium aquatile* LMG 4008^T (AM230485) was used as an outgroup (not shown). Bar, 0.05 substitutions per nucleotide position.

production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid are utilized. Dose not utilize D-mannitol, capric acid, adipic acid, trisodium citrate and phenylacetic acid. The strain SS1-22 (=NNIBRBA 6) was isolated from a sediment of the Nakdong River, Gyeongcheon-island, Sangju, Korea.

Description of *Tardiphaga robiniae* BK16-1

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

rod-shaped. Colonies are circular, convex with entire edge and white-colored after 3 days on R2A agar at 25 °C. Positive for oxidase, urease and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase 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. The strain BK16-1 (=NNIBRBA 43) was isolated from freshwater of the Geomyeongso (origin of Han River), Taebaek, Korea.

Description of *Pseudorhodobacter ferrugineus* BES-63

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, convex with entire edge and yellow-colored after 3 days on MA at 25°C. Positive for oxidase, esculin hydrolysis and β-galactosidase but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain BES-63 (=NNIBRBA 48) was isolated from freshwater, Eulsuk-island at the end of the Nakdong River, Busan, Korea.

Description of *Blastomonas aquatica* SH33

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, convex with entire edge and yellow-colored after 3 days on R2A agar at 25°C. Positive for oxidase, esculin hydrolysis and gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease 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. The strain SH33 (=NNIBRBA 41) was isolated from freshwater of the Sohan-stream, Samcheok, Korea.

Description of *Afipia massiliensis* SH41

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, convex with entire edge and white-colored after 3 days on R2A agar at 25°C. Positive for oxidase, arginine dihydrolase and urease, but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatinase and β-galactosidase. 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. The strain SH41 (=NNIBRBA 42) was isolated from freshwater of the Sohan-stream, Samcheok, Korea.

Description of *Belnapia moabensis* AS5-06

Cells are gram-staining-negative, non-flagellated, coccus-shaped. Colonies are convex, round, rough and red-pink colored after 3 days on R2A agar at 30°C. Positive for oxidase and urease, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. L-Arabinose, potassium gluconate and adipic acid are utilized. Does not utilize D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, malic acid, trisodium citrate and phenylacetic acid. The strain AS5-06 (=NNIBRBA 60) was isolated from sediment of the Seobu-dock, Andong, Korea.

Description of *Methylobacterium bullatum* CS4-36

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, convex, smooth and red-pink colored after 3 days on R2A agar at 30°C. Positive for oxidase, nitrate reduction, urease and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase and β-galactosidase. Potassium gluconate, adipic acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, trisodium citrate and phenylacetic acid. The strain CS4-36 (=NNIBRBA 67) was isolated from sediment of the Changnyeong-Haman weir, Changnyeong, Korea.

Description of *Pseudooceanicola nanhaiensis* ES2-15

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, convex, smooth and faint yellow-colored after 3 days on MA at 25°C. Positive for oxidase, arginine dihydrolase and urease, but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and activity of β-galactosidase and gelatinase. D-Glucose, L-arabinose, D-mannitol, malic acid and adipic acid are utilized. Does not utilize D-mannose, D-maltose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. The strain ES2-15 (=NNIBRBA 64) was isolated from sediment of the Eulsuk-island, Busan, Korea.

Description of *Ideonella dechloratans* BK-22

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are convex entire margins, mucoid

and cream-colored after 2 days on R2A agar at 30°C. Positive for oxidase, urease and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase and β-galactosidase. 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 are not utilized. The strain BK-22 (=NNIBRBA 19) was isolated from freshwater of the Nakdong River, Sangju, Korea.

Description of *Kinneretia asaccharophila* BK-77

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are translucent, irregular, margins and cream-colored after 3 days on R2A agar at 25°C. Positive for oxidase, nitrate reduction, esculin hydrolysis and gelatinase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and β-galactosidase. 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 are not utilized. The strain BK-77 (=NNIBRBA 22) was isolated from freshwater of the Nakdong River, Sangju, Korea.

Description of *Hydrogenophaga atypica* BK-176

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, regular and cream-colored after 3 days on R2A agar at 25°C. Positive for oxidase, nitrate reduction, urease and esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase and β-galactosidase. 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 are not utilized. The strain BK-176 (=NNIBRBA 27) was isolated from freshwater of the Nakdong River, Sangju, Korea.

Description of *Inhella inkyongensis* BK-179

Cells are gram-staining-negative non-flagellated and shot rod-shaped. Colonies are circular, convex, smooth and cream-colored after 3 days on R2A agar at 15°C. Positive for oxidase, esculin hydrolysis, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. 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 are not utilized. The strain BK-179 (=NNIBRBA 28) was isolated from freshwater of the Nakdong River, Sangju, Korea.

Description of *Polynucleobacter necessarius* subsp. *Asymbioticus* BK-182

Cells are gram-staining-negative non-flagellated and shot rod-shaped. Colonies are circular, convex, smooth and cream-colored after 3 days on R2A at 30°C. Positive for oxidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. 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 are not utilized. The strain BK-182 (=NNIBRBA 29) was isolated from freshwater of the Nakdong River, Sangju, Korea.

Description of *Dechloromonas hortensis* BK-210

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, regular and yellow-colored after 3 days on R2A agar at 30°C. Positive for oxidase and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and β-galactosidase. 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 are not utilized. The strain BK-210 (=NNIBRBA 30) was isolated from freshwater of the Nakdong River, Sangju, Korea.

Description of *Chitinibacter tainanensis* BK-219

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, entire, convex and milky white-colored after 4 days on NA at 30°C. Positive for oxidase, glucose fermentation, esculin hydrolysis and gelatinase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease and β-galactosidase. D-Mannose is utilized. Dose not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain BK-219 (=NNIBRBA 33) was isolated from freshwater of the Nakdong River, Sangju, Korea.

Description of *Rhizobacter dauci* SS1-70

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, tough and lemon-colored after 3 days on R2A agar at 25°C. Positive for oxidase, nitrate reduction and β-galactosidase, weakly positive for urease, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Mlatose is utilized. Dose not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-

glucosamine, potassium gluconate, capric acid, adipic acid, D-malic acid, trisodium citrate and phenylacetic acid. The strain SS1-70 (=NNIBRBA 11) was isolated from riverside sediment of the Nakdong River, Gyeongcheon-island, Sangju, Korea.

Description of *Piscinibacter aquaticus* SS2-102

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, smooth with entire margins and beige-milkish colored after 3 days on R2A agar at 25°C. Positive for oxidase, nitrate reduction, urease, gelatinase and β-galactosidase, weakly positive for esculin hydrolysis, but negative for indole production, glucose fermentation and arginine dihydrolase. D-Glucose, D-mannitol and D-maltose are utilized. Dose not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain SS2-102 (=NNIBRBA 17) was isolated from riverside sediment of the Nakdong River, Gyeongcheon-island, Sangju, Korea.

Description of *Malikia granosa* SJ55

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, convex with entire edge and white-colored after 3 days on R2A agar at 25 °C. Positive for oxidase, nitrate reduction, urease, esculin hydrolysis, gelatinase and β-galactosidase, but negative for indole production, glucose fermentation and arginine dihydrolase. D-Glucose, D-mannose and D-mannitol are utilized. Dose not utilize L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain SJ55 (=NNIBRBA 37) was isolated from brackish water of the Seomjin River, Gwangyang, Korea.

Description of *Herminiimonas contaminans* BK-438

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, convex with entire edge and white-colored after 3 days on R2A agar at 25 °C. Positive for oxidase, nitrate reduction, arginine dihydrolase, urease and esculin hydrolysis, but negative for indole production, glucose fermentation, gelatinase and β-galactosidase. Malic acid is utilized. Dose not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. The strain BK438 (=NNIBRBA 46) was isolated from freshwater of the Nakdong River, Changnyeong, Korea.

Description of *Thauera aminoaromatica* ES1-46

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are circular, convex, smooth and light-yellow colored after 3 days on R2A agar at 25°C. Positive for oxidase, glucose fermentation, urease, esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose and potassium gluconate are utilized. Dose not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain ES1-46 (=NNIBRBA 63) was isolated from brackish marsh sediment of Eulsuk-island at the end of the Nakdong River, Busan, Korea.

Description of *Rheinheimera aquatica* BK-128

Cells are gram-staining-negative non-flagellated and rod-shaped. Colonies are convex with entire margins and greenish-yellow colored after 2 days on R2A agar at 35°C. Positive for oxidase, nitrate reduction, esculin hydrolysis, gelatinase and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. 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 are not utilized. The strain BK-127 (=NNIBRBA 25) was isolated from freshwater of the Nakdong River, Gyeongcheon-island, Sangju, Korea.

Description of *Nevskia ramosa* BK-213

Cells are gram-staining-negative non-Flagellated and shot rod-shaped. Colonies are circular, regular and yellow-colored after 3 days on R2A agar at 25°C. Positive for oxidase, esculin hydrolysis and gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and β-galactosidase. 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 are not utilized. The strain BK-213 (=NNIBRBA 31) was isolated from freshwater of the Nakdong River, Gyeongcheon-island, Sangju, Korea.

Description of *Lysobacter brunescens* GS1-30

Cells are gram-staining negative non-flagellated and rod-shaped. Colonies are circular with entire edge and yellow-colored after 3 days on R2A agar at 30°C. Positive for oxidase, esculin hydrolysis, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. 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 are not utilized. The strain GS1-30 (=NNIBRBA 52) was isolated from riverside sediment of the Nakdong River, Gumi, Korea.

Description of *Pseudoalteromonas tunicata* BES3-108

Cell are gram-staining negative non-flagellated and rod-shaped. Colonies are circular, convex, opaque with entire edge and yellow-colored after 3 days on R2A agar at 25°C. Positive for oxidase, nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase and β-galactosidase, but negative for indole production, arginine dihydrolase and urease. D-Glucose, D-mannose, *N*-acetyl-glucosamine and D-maltose are utilized. L-Arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized. The strain BES3-108 (=NNIBRBA 49) was isolated from brackish marsh sediment of the Eulsuk-island at the end of the Nakdong River, Busan, Korea.

Description of *Thermomonas carbonis* 04KS1-07

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, convex, smooth and ivory-colored after 3 days on R2A agar at 30°C. Positive for oxidase, esculin hydrolysis, gelatinase and β-galactosidas, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. D-Mannitol is utilized. Weakly utilize D-maltose. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain 04KS1-07 (=NNIBRBA 57) was isolated from freshwater sediment of the Geomryeongso (origin of Han River), Taebaek, Korea.

Description of *Arenimonas subflava* CS4-45

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, convex, smooth and yellowish-white colored after 3 days on R2A agar at 25°C. Positive for oxidase, and gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis 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. The strain CS4-45 (=NNIBRBA 68) was isolated from sediment of the Nakdong River, Changnyeong, Korea.

Description of *Pseudomonas avellanae* KS1-13

Cells are gram-staining-negative, non-flagellated, rod-shaped. Colonies are circular, glistering, mucoid and cream-colored after 3 days on R2A agar at 25°C. Positive for oxidase, nitrate reduction and arginine dihydrolase, weakly positive for esculin hydrolysis, but negative for, indole production, glucose fermentation, urease, gelatinase and β-galactosidase. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and trisodium citrate are utilized. Does not utilize L-arabinose, D-maltose, adipic acid and phenylacetic acid. The strain KS1-13 (=NNIBRBA 56) was isolated from sediment of the Eulsuk-island, Busan, Korea.

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