A report of 35 unrecorded bacterial species belonging to the classes *Alphaproteobacteria* and *Betaproteobacteria* in Korea

Hye Su Jung¹, Jung-Hoon Yoon², Kiseong Joh³, Chi-Nam Seong⁴, Won-Yong Kim⁵, Wan-Taek Im⁶, Myung-Kyum Kim⁷, Chang-Jun Cha⁸, Seung-Bum Kim⁹ and Che-Ok Jeon^{1,*}

¹Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

²Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea

³Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Yongin 02450, Republic of Korea

⁵Department of Microbiology, College of Medicine, Chung-Ang University, Seoul 06974, Republic

⁶Department of Biotechnology, Hankyong National University, Anseong 17546, Republic of Korea

⁷Department of Bio and Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 17987, Republic of Korea

⁸Department of Systems Biotechnology, Chung-Ang University, Anseong 17546, Republic of Korea ⁹Department of Microbiology, Chungnam National University, Daejeon 34134, Republic of Korea

*Correspondent: cojeon@cau.ac.kr

During a comprehensive investigation of indigenous prokaryotic species in Korea, 25 and 10 bacterial strains assigned to the classes *Alphaproteobacteria* and *Betaproteobacteria*, respectively, were isolated from diverse environmental habitats, including soil, mud, tidal field, sea water, sand, rusted iron, and leaf. Based on their high 16S rRNA gene sequence similarities (>98.7%) and the formation of robust phylogenetic clades with type species, each strain was assigned to an independent and predefined bacterial species. Since there were no published or official reports regarding these 35 isolates in Korea, they - 25 species of 14 families in the 5 orders of *Alphaproteobacteria* and 10 species of 3 families in the two orders of *Betaproteobacteria* - have been reported as unrecorded species in Korea. In addition, Gram reaction, colony and cell morphology, basic biochemical characteristic, isolation source, and strain ID of each species are also described in the species description sections.

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

© 2021 National Institute of Biological Resources DOI:10.12651/JSR.2021.10.1.012

INTRODUCTION

The Proteobacteria is the largest bacterial phylum of Gram-negative bacteria, including the classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, Zetaproteobacteria, Acidithiobacillia, and Oligoflexia (Williams et al., 2013; Nakai et al., 2014). At the time of writing, the taxonomic classification based on 16S rRNA gene sequences showed that the class Alphaproteobacteria includes 16 orders: Caulobacterales, Emcibacterales, Iodidimonadales, Kiloniellales, Kordiimonadales, Magnetococcales, Micropepsales, Minwuiales, Parvularculales, Pelagibacterales, Rhizobiales, Rhodobacterales, Rhodospirillales, Rickettsiales, Sneathiellales, and Sphingomonadales. Among them, Minwuiales and Micropepsales were recently established (Harbison et al., 2017; Sun et al., 2018). The class Betaproteobacteria includes nine orders: Burkholderiales, Ferritrophicales, Ferrovales, Gallionellales, Methylophilales, Neisseriales, Nitrosomonadales, Rhodocyclales, and Sulfuricellales. In 2019, we collected large number of environmental samples from diverse habitats in Korea and isolated various novel and unrecorded bacterial species. In this study, we describe 25 and 10 bacterial species belonging to the Alphaproteobacteria and Betaproteobacteria, respectively that were previously not reported in Korea.

⁴Department of Biology, Sunchon National University, Suncheon 57922, Republic of Korea

MATERIALS AND METHODS

A total of 35 bacterial strains assigned to the class *Alphaproteobacteria* and *Betaproteobacteria* were isolated from various environmental habitats, including soil, mud, tidal field, sea water, sand, rusted iron, and leaf (Table 1). All environmental samples were processed independently, serially diluted, spread onto diverse culture agar media including Mueller-Hinton agar (MH; BD, USA), R2A agar (BD, USA), marine agar 2216 (MA; BD, USA), and tryptic soy agar (TSA; BD, USA) and incubated at 25-37°C for 2–5 days (Table 1). The designated strain identifications (IDs), isolation sources, culture media, and incubation conditions of the isolates are described 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 the API 20NE kit (bioMérieux), according to the manufacturer's instruction.

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 Alphaproteobacteria and Betaproteobacteria were compared with those of the type strains of validated bacterial species by using the nucleotide similarity search program in the EzBioCloud server (http://www.ezbiocloud.net/identify) (Yoon et al. 2017). For phylogenetic analyses, multiple alignments of the 16S rRNA gene sequences between the isolates and established bacterial species were carried out using the ClustalX program (Thompson et al., 1997). Evolutionary distances were calculated using the Kimura two-parameter model and the phylogenetic trees were constructed using the MEGA7 program (Kumar et al., 2016) based on the neighbor-joining algorithm with 1,000 replications.

RESULTS AND DISCUSSION

Twenty-five strains of Alphaproteobacteria were distributed into five orders: one strain in Caulobacterales, 10 strains in Rhizobiales, five strains in Rhodobacterales, four strains in Rhodospirillales, and five strains in Sphingomonadales (Table 1). Ten strains of Betaproteobacteria were distributed into two orders: nine strains in Burk-

Fable 1. Summary	of strains isolated t	belonging to the Alpha	ı <i>proteobacteria</i> and	Betaproteoba	cteia and their taxonor	nic affiliations.				
Class/phylum	Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
	Caulobacterales	Caulobacteraceae	Brevundimonas	19D2A5	NIBRBAC000503268	Brevundimonas faecalis	99.4	soil	НМ	30°C, 4d
		Brucellaceae	Ochrobactrum	19D1V26	NIBRBAC000503266	Ochrobactrum thiophenivorans	9.66	soil	НМ	30°C, 4d
		$Devosia_f$	Devosia	BSSL-BR9	NIBRBAC000503333	Devosia naphthalenivorans	98.9	pnu	R2A	25°C, 5d
		Methylobacteriaceae	Microvirga	MMS19-R33	NIBRBAC000503369	Microvirga flocculans	9.66	soil	R2A	30°C, 3d
Al nhanrate ohacteria		Nitrobacteraceae	Bradyrhizobium	MMS19-T29	NIBRBAC000503385	Bradyrhizobium liaoningense	100	soil	R2A	30°C, 3d
n an	Rhizobiales	Phyllobacteriaceae	Mesorhizobium Phyllobacterium Pseudaminobacter	G187 BT55 19D1A35	NIBRBAC000503410 NIBRBAC000502989 NIBRBAC000503255	Mesorhizobium plurifarium Phyllobacterium zundukense Pseudaminobacter arsenicus	99.2 100 99.2	soil soil soil	R2A R2A MH	25°C, 3d 25°C, 3d 30°C, 4d
		Rhizobiaceae	Rhizobium Rhizobium	HMF6710 BT341	NIBRBAC000503112 NIBRBAC000502997	Rhizobium straminoryzae Rhizobium cauense	98.9 99.2	tidal field soil	R2A R2A	30°C, 3d 25°C, 3d
		Rhodobiaceae	Parvibaculum	CAU 1597	NIBRBAC000503247	Parvibaculum lavamentivorans	9.66	sand	MA	30°C, 3-4d

Class/phylum	Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
		Hyphomonadaceae	Euryhalocaulis	CAU 1595	NIBRBAC000503245	Euryhalocaulis caribicus	99.3	sand	МА	30°C, 2-3d
	Rhodobacterales	Rhodobacteraceae	Nioella Paracoccus Paracoccus Salipiger	YSTF-M5 KYW1791 KYW1736 CAU 1521	NIBRBAC000503332 NIBRBAC000503285 NIBRBAC000503286 NIBRBAC000503227	Nioella sediminis Paracoccus halotolerans Paracoccus rhizosphaerae Salipiger profiundus	9.99 5.99 5.99 7.99	mud sea water sea water sand	MA MA MA MA	25°C, 3d 25°C, 3d 25°C, 3d 30°C, 2d
Alphaproteobacteria	Rhodospirillales	Acetobacteraceae Rhodospirtilaceae	Roseomonas Roseomonas Roseomonas Indioceanicola	HMF5336 R36 19D1F4 KYW1819	NIBRBAC000503107 NIBRBAC000503419 NIBRBAC000503271 NIBRBAC000503292	Roseomonas aquatica Roseomonas wenyumeiae Roseomonas mucosa Indioceanicola profundi	98.8 99.9 100 8.99.8	rusted iron soil soil sea water	R2A R2A MH MA	30°C, 3d 25°C, 3d 30°C, 4d 25°C, 5d
		Erythrobacteraceae	Erythrobacter	KYW1376	NIBRBAC000503287	Erythrobacter zhengü	6.66	sea water	MA	25°C, 5d
	Sphingomonadales	Sphingomonadaceae	Sphingobium Sphingomonas Sphingomonas Sphingomonas	FS72 G119 BT373 HMF6093	NIBRBAC000503299 NIBRBAC000503407 NIBRBAC000503006 NIBRBAC000503109	Sphingobium mellinum Sphingomonas changbaiensis Sphingomonas mali Sphingomonas prati	8.89 9.89 9.99	soil soil soil sea water	R2A R2A R2A R2A R2A	25°C, 3d 25°C, 3d 25°C, 3d 30°C, 3d
Betaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia Burkholderia Caballeronia Paraburkholderia Paraburkholderia	BT358 BT359 BG9 HMF5328 BT367 BT372	NIBRBAC000502988 NIBRBAC000502999 NIBRBAC000503390 NIBRBAC000503106 NIBRBAC000503004 NIBRBAC000503005	Burkholderia contaminans Burkholderia territorii Caballeronia glathei Paraburkholderia caledonica Paraburkholderia sabiae Paraburkholderia sabiae	99.8 99.9 99.9 99.3 99.3	soil soil soil soil soil	R2A MA TSA R2A R2A R2A R2A	25°C, 3d 37°C, 3d 30°C, 3d 30°C, 3d 25°C, 3d 25°C, 3d
		Comamonadaceae	Azohydromonas Pseudacidovorax Ramlibacter	BT39 HMF4787 BT69	NIBRBAC000502985 NIBRBAC000503105 NIBRBAC000502987	Azohydromonas australica Pseudacidovorax intermedius Ramlibacter henchirensis	99.2 99.8 99.4	soil leaf soil	R2A R2A R2A	25°C, 2d 30°C, 3d 25°C, 3d
	Rhodocyclales	Zoogloeaceae	Thauera	CAU 1556	NIBRBAC000503231	Thauera chlorobenzoica	66	sand	MA	30°C, 2d

Table 1. Continued.



Fig. 1. Transmission electron micrographs or scanning electron micrographs showing the cell morphologies of the strains isolated in this study. The strains were cultured at their optimal growth conditions. Strains: 1, YSTF-M5; 2, BSSL-BR9; 3, HMF5336; 4, HMF6093; 5, HMF6710; 6, KYW1791; 7, KYW1736; 8, KYW1376; 9, KYW1819; 10, FS72; 11, CAU 1521; 12, CAU 1595; 13, CAU 1597; 14, G119; 15, G187; 16, R36; 17, BT55; 18, BT341; 19, BT373; 20, 19D1A35; 21, 19D1V26; 22, 19D2A5; 23, 19D1F4; 24, MMS19-R33; 25, MMS19-T29; 26, HMF4787; 27, HMF5328; 28, CAU 1556; 29, BT39; 30, BT69; 31, BT358; 32, BT359; 33, BT367; 34, BT372; 35, BG9.

holderiales and one strain in *Rhodocyclales*. All strains were Gram-staining-negative and chemoheterotrophic, and 27 strains were rod shaped and eight strains were cocci shaped (Fig. 1).

In the class of Alphaproteobacteria, one strain was assigned to the genus Brevundimonas of the family Caulobacteraceae in the order Caulobacterales and 10 strains in the order Rhizobiales belonged to seven different families: Brucellaceae (one strain), Devosia_f (one strain), Methylobacteriaceae (one strain), Nitrobacteraceae (one strain), Phyllobacteriaceae (three strains), Rhizobiaceae (two strains), and Rhodobacteraceae (one strain). Five strains in the order Rhodobacterales belonged to two separate families: Hyphomonadaceae (one strain) and Rhodobacteraceae (four strains), while four strains in the order Rhodospirillales were separated into two families: Acetobacteraceae (three strains) and Rhodospirillaceae (one strain), and five strains in the order Sphingomonadales were separated into two families: Erythrobacteraceae (one strain) and Sphingomonadaceae (four strains) (Fig. 2).

Ten strains were distributed into two orders, *Burkholderiales* and *Rhodocyclales* of the class *Betaproteobacteria*. Nine strains belonged to two families in the order of *Burkholderiales: Burkholderiaceae* (six strains) and *Comamonadaceae* (three strains). One strain belonged to the genus *Thauera* of the family *Zoogloeaceae* of the order *Rhodocyclales* (Fig. 3).

In conclusion, in this study we report 35 unrecorded bacterial species belonging to two proteobacterial classes, *Alphaproteobacteria* and *Betaproteobacteria*, which were isolated in Korea.

Description of Nioella sediminis YSTF-M5

Cells are Gram-staining-negative, flagellated and rod shaped. Colonies are circular, slightly convex, glistening, and yellowish gray colored after 3 days of incubation at 25°C on MA. Positive for nitrate reduction, esculin hydrolysis, and β -galactosidase activity. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease activity, 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 YSTF-M5 (=NIBRBAC000503332) was isolated from a mud sample, Incheon, Korea.

Description of Devosia naphthalenivorans BSSL-BR9

Cells are Gram-staining-negative, flagellated and rod shaped. Colonies are circular, convex, glistening, and yellowish white colored after 5 days of incubation at 25°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and arginine dihydrolase, urease, and β -galactosidase activities. Negative for indole production, glucose fermenta-

tion, and gelatinase activity. 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 BSSL-BR9 (= NIBRBAC000503333) was isolated from a mud sample, Boryeong, Korea.

Description of Roseomonas aquatica HMF5336

Cells are Gram-staining-negative, non-flagellated and coccus shaped. Colonies are circular, convex, smooth, wet and pale red colored after 3 days of incubation at 30°C on R2A. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and gelatinase, arginine dihydrolase, urease, and β -galactosidase activities. D-Mannitol, *N*-acetylglucosamine, and potassium gluconate are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain HMF5336 (=NIBRBAC000503107) was isolated from a rusted iron sample, Yongin, Korea.

Description of Sphingomonas prati HMF6093

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, smooth, and yellow colored after 3 days of incubation at 30°C on R2A. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose and potassium gluconate are utilized. Does not utilize capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain HMF 6093 (=NIBRBAC000503109) was isolated from a sea water sample, Jeju, Korea.

Description of Rhizobium straminoryzae HMF6710

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, smooth and cream colored after 3 days of incubation at 30°C on R2A or MA. Positive for esculin hydrolysis and urease and β -galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate and malic acid are utilized. Does not utilize capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain HMF6710 (= NIBRBAC000503112) was isolated from a tidal field sample, Wando, Korea.

Description of Paracoccus halotolerans KYW1791

Cells are Gram-staining-negative, non-flagellated, and coccus shaped. Colonies are circular, convex, smooth,



Fig. 2. A 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 orders *Caulobacterales*, *Rhizobiales*, *Rhodobacterales*, *Rhodobacterales*, *and Sphingomonadales* of the class of *Alphaproteobacteria*. Bootstrap values (>70%) are shown above nodes. Scale bar: 0.02 changes per nucleotide.

opaque, and pale orange colored after 3 days of incubation at 25°C on MA. Positive for nitrate reduction, esculin hydrolysis, and β -galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, and urease and gelatinase activities. D-Glucose, D-mannose, D-mannitol, D-maltose, potassium gluconate, malic acid, trisodium citrate are utilized. Does not utilize L-arabinose, *N*-acetylglucosamine, capric acid, adipic acid, and phenylacetic acid. Strain KYW1791 (=NIBRBAC000503285) was isolated from a sea water sample, Gwangyang, Korea.

Description of Paracoccus rhizosphaerae KYW1736

Cells are Gram-staining-negative, non-flagellated, and coccus shaped. Colonies are circular, smooth, convex, opaque, and pale orange colored after 3 days of incubation at 25°C on MA. Positive for nitrate reduction, esculin hydrolysis, and β -galactosidase activity. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. D-Glucose, L-arabinose, D-mannitol, D-maltose, potassium gluconate and malic acid



Fig. 3. A 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 orders *Burkholderiales* and *Rhodocyclales* of the class of *Betaproteobacteria*. Bootstrap values (>70%) are shown above nodes. Scale bar: 0.01 changes per nucleotide.

are utilized. Does not utilize D-mannose, N-acetylglucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain KYW1736 (=NIBRBAC00050 3286) was isolated from a sea water sample, Gwangyang, Korea.

Description of Erythrobacter zhengii KYW1376

Cells are Gram-staining-negative, non-flagellated, and short rod or coccus shaped. Colonies are circular, smooth, convex, opaque, and orange-yellow colored after 5 days of incubation at 25°C on MA. Positive for indole production and esculin hydrolysis. Negative for nitrate reduction, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and β -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 KYW1376 (=NIBRBAC000503287) was isolated from a sea water sample, Gwangyang, Korea.

Description of Indioceanicola profundi KYW1819

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, smooth, convex, and pale pink colored after 5 days of incubation at 25°C on MA. Positive for indole production. Negative for nitrate reduction, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, gelatinase, and β -galactosidase activities. Malic acid is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain KYW1819 (=NIBRBAC000503292) was isolated from a sea water sample, Gwangyang, Korea.

Description of Sphingobium mellinum FS72

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, smooth, and yellow colored after 3 days of incubation at 25°C on R2A. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and β -galactosidase activities. D-Glucose is utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain FS72 (=NIBRBAC000503299) was isolated from a soil sample, Suncheon, Korea.

Description of Salipiger profundus CAU 1521

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, mucoid, rough, opaque, and cream colored after 2 days of incubation at 30° C on MA. Positive for esculin hydrolysis and urease and β -galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase and gelatinase 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 CAU 1521 (=NIBRBAC000503227) was isolated from a sand sample, Busan, Korea.

Description of Euryhalocaulis caribicus CAU 1595

Cells are Gram-staining-negative, flagellated, and short rod shaped. Colonies are circular, convex, shiny, opaque, and cream colored after 2–3 days of incubation at 30°C on MA. Positive for nitrate reduction, esculin hydrolysis, and gelatinase and urease activities. Negative for indole production, glucose fermentation, and arginine dihydrolase and β -galactosidase activities. D-Glucose and L-arabinose are utilized. Does not utilize D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CAU 1595 (=NIBRBAC00050 3245) was isolated from a sand sample, Incheon, Korea.

Description of *Parvibaculum lavamentivorans* CAU 1597

Cells are Gram-staining-negative, flagellated, and rod shaped. Colonies are circular, convex, smooth, opaque, and cream colored after 3–4 days of incubation at 30°C on MA. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. Does not utilize 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 CAU 1597 (= NIBRBAC000503247) was isolated from a sand sample, Jeju, Korea.

Description of Sphingomonas changbaiensis G119

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, entire, and light-yellow colored after 3 days of incubation at 25°C on R2A. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, gelatinase, and β -galactosidase activities. D-Glucose, L-arabinose, D-manose, D-maltose, and phenylacetic acid are utilized. Does not utilize D-mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, and trisodium citrate. Strain G119 (= NIBRBAC 000503407) was isolated from a soil sample, Yeongju, Korea.

Description of Mesorhizobium plurifarium G187

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex and cream colored after 3 days of incubation at 25°C on R2A. Positive for urease activity, esculin hydrolysis and β -galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, and malic acid are utilized. Does not utilize D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain G187 (=NIBRBAC000503410) was isolated from a soil sample, Yeongju, Korea.

Description of Roseomonas wenyumeiae R36

Cells are Gram-staining-negative, flagellated, and coccus or ovoid shaped. Colonies are circular, convex, smooth, and light orange colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and gelatinase, arginine dihydrolase, and urease activities. Negative for indole production, glucose fermentation, and β -galactosidase activity. D-Glucose, D-mannose, D-mannitol, D-maltose, and trisodium citrate are utilized. Does not utilize L-arabinose, *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain R36 (=NIBRBAC000503419) was isolated from a soil sample, Yeongju, Korea.

Description of Phyllobacterium zundukense BT55

Cells are Gram-staining-negative, non-flagellated, and short rod shaped. Colonies are circular, smooth, and white colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction. Negative for indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, gelatinase, and β -galactosidase activities. D-Glucose, D-maltose and malic acid are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain BT55 (=NIBRBAC000502989) was isolated from a soil sample, Pyeongchang, Korea.

Description of Rhizobium cauense BT341

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, smooth, and white colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and urease and β -galactosidase activities. Negative for indole production, glucose fermentation, and arginine dihydrolase and gelatinase activities. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, and malic acid are utilized. Does not utilize potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain BT341 (=NIBRBAC000502997) was isolated from a soil sample, Jeju, Korea.

Description of Sphingomonas mali BT373

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, smooth, and yellow colored after 3 days of incubation at 25°C on R2A agar. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. D-Glucose and D-maltose are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain BT373 (= NIBRBAC000503006) was isolated from a soil sample, Cheongdo, Korea.

Description of Pseudaminobacter arsenicus 19D1A35

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, glistening and brown colored after 4 days of incubation at 30°C on MH. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease, gelatinase and β -galactosidase activities. D-Glucose, D-mannose, D-mannitol, *N*-acetylglucosamine, adipic acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Does not utilize L-arabinose, D-maltose, potassium gluconate, and capric acid. Strain 19D1A35 (=NIBRBAC000503255) was isolated from a soil sample, Jeongseon, Korea.

Description of *Ochrobactrum thiophenivorans* 19D1V26

Cells are Gram-staining-negative, flagellated, and rod shaped. Colonies are circular, raised, umbonate, and cream colored after 4 days of incubation at 30°C on MH. Positive for nitrate reduction, esculin hydrolysis, and urease activity. Negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase and β -galactosidase activities. D-Glucose, L-arabinose, D-mannose, N-acetylglucosamine and malic acid are utilized. Does not utilize D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain 19D1V26 (=NIBRBAC000503266) was isolated from a soil sample, Jeongseon, Korea.

Description of Brevundimonas faecalis 19D2A5

Cells are Gram-staining-negative, flagellated, and rod shaped. Colonies are circular, convex, glistening, and light yellow colored after 4 days of incubation at 30°C on MH. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and β -galactosidase activities. Capric acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol,

N-acetylglucosamine, D-maltose, potassium gluconate, adipic acid, trisodium citrate, and phenylacetic acid. Strain 19D2A5 (=NIBRBAC000503268) was isolated from a soil sample, Jeongseon, Korea.

Description of Roseomonas mucosa 19D1F4

Cells are Gram-staining-negative, non-flagellated, and coccus shaped. Colonies are circular, convex, glistening, and pink colored after 4 days of incubation at 30°C on MH. Positive for urease activity and esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, gelatinase, and β -galactosidase activities. D-Glucose, L-arabinose, D-mannitol, potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Does not utilize D-mannose, *N*-acetylglucosamine, D-maltose, and capric acid. Strain 19D1F4 (= NIBRBAC000503271) was isolated from a soil sample, Jeongseon, Korea.

Description of Microvirga flocculans MMS19-R33

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, raised, entire, and palecream colored after 3 days of incubation at 30°C on R2A. Positive for nitrate reduction and esculin hydrolysis. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and β -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 MMS19-R33 (=NIBRBAC000503369) was isolated from a soil sample, Gurye, Korea.

Description of *Bradyrhizobium liaoningense* MMS19-T29

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, entire, and cream colored after 3 days of incubation at 30°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and β -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*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MMS19-T29 (= NIBRBAC000503385) was isolated from a soil sample, Gurye, Korea.

Description of *Pseudacidovorax intermedius* HMF4787

Cells are Gram-staining-negative, non-flagellated, and short rod shaped. Colonies are circular, convex, smooth,

slimy, and light-yellow colored after 3 days of incubation at 30°C on R2A. Positive for urease activity. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, gelatinase, and β -galactosidase activities. D-Glucose, potassium gluconate, and malic acid are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain HMF4787 (=NIBRBAC00050 3105) was isolated from a leaf sample, Yongin, Korea.

Description of *Paraburkholderia caledonica* HMF5328

Cells are Gram-staining-negative, flagellated, and rod shaped. Colonies are circular, convex, smooth, and lightyellow colored after 3 days of incubation at 30°C on R2A. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase, urease, and gelatinase activities. D-Glucose, L-arabinose, D-mannitol, *N*-acetylglucosamine, and potassium gluconate are utilized. Does not utilize D-mannose, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain HMF5328 (= NIBRBAC000503106) was isolated from a soil sample, Gangneung, Korea.

Description of Thauera chlorobenzoica CAU 1556

Cells are Gram-staining-negative, flagellated, and rod shaped. Colonies are circular, convex, shiny, opaque, and cream colored after 2 days of incubation at 30°C on MA. Positive for nitrate reduction and esculin hydrolysis. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease, gelatinase, and β -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 CAU 1556 (=NIBRBAC000503231) was isolated from a sand sample, Jeju, Korea.

Description of Azohydromonas australica BT39

Cells are Gram-staining-negative, non-flagellated, and coccus shaped. Colonies are circular, smooth, and white colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction. Negative for indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, gelatinase, and β -galactosidase activities. D-Glucose is utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain BT39 (=NIBRBAC000502985) was isolated from a soil sample, Pyeongchang, Korea.

Description of Ramlibacter henchirensis BT69

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, smooth, and white colored after 3 days of incubation at 25°C on R2A. Positive for gelatinase activity. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, and β -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 BT69 (=NIBRBAC000502987) was isolated from a soil sample, Pyeongchang, Korea.

Description of Burkholderia contaminans BT358

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, smooth, and white colored after 3 days of incubation at 25°C on R2A. Positive for arginine dihydrolase, urease, and gelatinase activities. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and β -galactosidase activity. D-Glucose, L-arabinose, potassium gluconate, malic acid, and trisodium citrate and are utilized. Does not utilize D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, capric acid, adipic acid, and phenylacetic acid. Strain BT358 (=NIBRBAC000502988) was isolated from a soil sample, Jeju, Korea.

Description of Burkholderia territorii BT359

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, convex, smooth, and yellow colored after 3 days of incubation at 37°C on MA. Positive for esculin hydrolysis and arginine dihydrolase, urease, gelatinase, and β -galactosidase activities. Negative for nitrate reduction, indole production, and glucose fermentation. 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 are utilized. Strain BT359 (= NIBRBAC 000502999) was isolated from a soil sample, Jeju, Korea.

Description of Paraburkholderia hiiakae BT367

Cells are Gram-staining-negative, flagellated, and rod shaped. Colonies are circular, smooth, and white colored after 3 days of incubation at 25°C on R2A. Positive for arginine dihydrolase, urease, and β -galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, and gelatinase activity. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic

acid, malic acid, and phenylacetic acid are utilized. Does not utilize D-maltose and trisodium citrate. Strain BT367 (=NIBRBAC000503004) was isolated from a soil sample, Cheongdo, Korea.

Description of Paraburkholderia sabiae BT372

Cells are Gram-staining-negative, non-flagellated, and rod shaped. Colonies are circular, smooth, and white colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction and β -galactosidase activity. Negative for indole production, glucose fermentation, esculin hydrolysis, and arginine dihydrolase, urease, and gelatinase activities. D-Glucose, D-mannose, D-mannitol, *N*-acetylglucosamine, potassium gluconate, capric acid, malic acid, and phenylacetic acid are utilized. Does not utilize L-arabinose, D-maltose, adipic acid, and trisodium citrate. Strain BT372 (= NIBRBAC000503005) was isolated from a soil sample, Cheongdo, Korea.

Description of Caballeronia glathei BG9

Cells are Gram-staining-negative, non-flagellated, and coccus shaped. Colonies are punctiform, convex, entire, and cream colored after 3 days of incubation at 30°C on TSA. Positive for glucose fermentation and arginine dihydrolase, urease, and β -galactosidase activities. Negative for nitrate reduction, indole production, esculin hydrolysis, and gelatinase activity. D-Glucose, L-arabinose, D-mannose, N-acetylglucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Does not utilize D-mannitol, D-maltose, and adipic acid. Strain BG9 (=NIBRBAC000503390) was isolated from a soil sample, Gurye, Korea.

REFERENCES

Harbison, A.B., L.E. Price, M.D. Flythe and S.L. Bräuer. 2017. *Micropepsis pineolensis* gen. nov., sp. nov., a mildly acidophilic *Alphaproteobacterium* isolated from a poor fen, and proposal of *Micropepsaceae* fam. nov. within *Micropepsales* ord. nov. International Journal of Systematic and Evolutionary Microbiology 67:839-844.

- Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.
- Lee, H.J., S.E. Jeong, M.S. Cho, S.H. Kim, S.S. Lee, B.H. Lee and C.O. Jeon. 2014. *Flavihumibacter solisilvae* sp. nov., isolated from forest soil. International Journal of Systematic and Evolutionary Microbiology 64:2897-2901.
- Nakai, R., M. Nishijima, N. Tazato, Y. Handa, F. Karray, S. Sayadi, H. Isoda and T. Naganuma. 2014. Oligoflexus tunisiensis gen. nov., sp. nov., a Gram-negative, aerobic, filamentous bacterium of a novel proteobacterial lineage, and description of Oligoflexaceae fam. nov., Oligoflexales ord. nov. and Oligoflexia classis nov. International Journal of Systematic and Evolutionary Microbiology 64:3353-3359.
- Sun, C., L. Xu, X.Y. Yu, Z. Zhao, Y.H. Wu, A. Oren, C.S. Wang and X.W. Xu. 2018. *Minwuia thermotolerans* gen. nov., sp. nov., a marine bacterium forming a deep branch in the *Alphaproteobacteria*, and proposal of *Minwuiaceae* fam. nov. and *Minwuiales* ord. nov. International Journal of Systematic and Evolutionary Microbiology 68:3856-3862.
- Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876-4882.
- Williams, K.P. and D.P. Kelly. 2013. Proposal for a new class within the phylum *Proteobacteria*, *Acidithiobacillia* classis nov., with the type order *Acidithiobacillales*, and emended description of the class *Gammaproteobacteria*. International Journal of Systematic and Evolutionary Microbiology 63:2901-2906.
- Yoon, S.H., S.M. Ha, S. Kwon, J. Lim, Y. Kim, H. Seo and J. Chun. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. International Journal of Systematic and Evolutionary Microbiology 67:1613-1617.

Submitted: September 27, 2020 Revised: January 22, 2021 Accepted: January 22, 2021