Journal of Species Research 12(3):212-223, 2023

A report on 30 unrecorded bacteria species in Korea belonging to the classes *Betaproteobacteria* and *Gammaproteobacteria* in 2021

Yunjeong Lee[1], Jung-Hoon Yoon[2], Myung Kyum Kim[3], Kiseong Joh[4], Seung Bum Kim[5], Che-Ok Jeon[6], Chang-Jun Cha[7], Wan-Taek Im[8] and Wonyong Kim[1,*]

[1]*Department of Microbiology, Chung-Ang University College of Medicine, Seoul 06974, Republic of Korea*
[2]*Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea*
[3]*Department of Bio and Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 01797, Republic of Korea*
[4]*Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Yongin 02450, Republic of Korea*
[5]*Department of Microbiology and Molecular Biology, Chungnam National University, Daejeon 34134, Republic of Korea*
[6]*Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea*
[7]*Department of Systems Biotechnology, Chung-Ang University, Anseong 17546, Republic of Korea*
[8]*Department of Biotechnology, Hankyong National University, Anseong 17579, Republic of Korea*

**Correspondent: kimwy@cau.ac.kr*

A total of 30 bacterial strains were identified in the classes *Betaproteobacteria* and *Gammaproteobacteria* in the study of prokaryotic species in Korea. These strains were isolated from a variety of environmental sources, including soil, tidal flat, mud, wetland, pine cone, seaweed, sea sediment, and brackish water. Phylogenetic analysis showed that isolates were identified based on high 16S rRNA gene sequence similarities ($\geq$98.7%) with the predefined bacterial type species. In this study, we present data on previously unreported species from Korea, including 10 species from three families of one order in the class *Betaproteobacteria* and 20 species from 12 families of nine order in the class *Gammaproteobacteria*. Morphological, biochemical characteristics, isolation sources, and NIBR deposit numbers are provided in the description sections.

Keywords: 16S rRNA, *Betaproteobacteria*, *Gammaproteobacteria*, unrecorded species

DOI:10.12651/JSR.2023.12.3.212

Introduction

In 2021, 30 unrecorded bacteria species were isolated from diverse habitats in Korea, belonging to the classes *Betaproteobacteria* and *Gammaproteobacteria*. The phylum *Proteobacteria* is the largest bacterial group (Kersters *et al*., 2006), which has undergone a recent name change to *Pseudomonadoat* (Oren and Garrity, 2021). The members of the phylum *Proteobacteria* are Gram-staining-negative with a wide range of morphologies and isolated from diverse environments. The class *Betaproteobacteria* and *Gammaproteobacteria* are the largest groups within the phylum. The class *Betaproteobacteria* includes four validly published orders: *Burkholderiales*, *Neisseriales*, *Nitrosomonadales*, and *Rhodocyclales*. The class *Gammaproteobacteria* is divided into 17 validly published orders: *Acidiferrobacterales*, *Aeromonadales*, *Alteromonadales*, *Arenicellales*, *Cardiobacteriales*, *Cellvibrionales*, *Chromatiales*, *Enterobacterales*, *Immundisolibacterales*, *Legionellales*, *Lysobacterales*, *Methylococcales*, *Nevskiales*, *Oceanospirillales*, *Orbales*, *Pasteurellales*, and *Pseudomonadales*. In this study, we focus on describing 30 previously unreported strains from various environmental habitats.

Materials and Methods

A total of 30 bacterial strains belonging to the class *Betaproteobacteria* and *Gammaproteobacteria* were isolated from diverse environments, including soil, pine cone, tidal flat, wetland, mud, sea sediment, seaweed, and brackish water. All samples were serially diluted using a standard dilution plating technique. Each sample was

spread onto R2A agar (BD, USA) and marine agar 2216 (MA; BD, USA), and incubated at 4−30°C for 2−5 days. The strain IDs, isolation sources, media, and culture conditions are described in Tables 1 and 2. All strains were purified as pure cultures and stored using 25% glycerol at −80°C as lyophilized ampoules.

The colony of the strains was observed for appearance, size, shape, and texture on agar plates and the morphology of the strains was observed by using transmission electron micrograph (TEM; JEM 1010) using cells grown at optical temperatures in the exponential phase. Gram-staining was tested using a Gram-staining kit (bioMérieux) according to the manufacturer's instructions. Biochemical characteristics were assessed using API 20NE strips (bioMérieux) according to the manufacturer's instructions.

Genomic DNA was extracted using a genomic DNA extraction kit and the 16S rRNA gene was isolated and purified. Using the bacterial universal primers 27F and 1525R (Lane, 1991), the 16S rRNA gene sequence was amplified with PCR. The 16S rRNA gene sequences of the strains were compared to the type strains of validly published bacterial species from the EzBioCloud server (Kim *et al*., 2012). Multiple alignments were performed with Clustal X (Thompson *et al*., 1997). The phylogenetic tree was constructed using the neighbor-joining (NJ; Saitou and Nei, 1987), maximum likelihood (ML; Felsenstein, 1981), and maximum parsimony (MP; Fitch and Margoliash, 1967) methods in MEGA7 (Kumar *et al*., 2016). Evolutionary distances were calculated using the Jukes-Cantor model from the NJ (Saitou and Nei, 1987) and the Kimura two-parameter model from the ML (Kimura, 1980) with 1,000 random replications (Felsenstein, 1985).

Results and discussion

In the class of *Betaproteobacteria*, all strains were assigned to the order *Burkholderiales*. All strains were Gram-negative and rod-shaped (Fig. 1). Ten strains of *Betaproteobacteria* were distributed into three families: four strains in *Burkholderiaceae*, three strains in *Comamonadaceae*, and three strains in *Oxalobacteraceae* (Table 1). In the family *Burkholderiaceae*, two strains belonged to the genus *Caballeronia* and two strains belonged to the genus *Paraburkholderia*. Three strains of the family *Comamonadaceae* were assigned to the genera *Acidovorax*, *Aquincola*, and *Hydrogenophaga*. Moreover, three strains of the family *Oxalobacteraceae* were assigned to the genera *Duganella*, *Noviherbaspirillum*, and *Massilia*.

Twenty strains were classified into nine orders in the class *Gammaproteobacteria*: two strains in *Aeromonadales*, three strains in *Alteromonadales*, one strain in *Cellvibrionales*, one strain in *Enterobacterales*, one strain in *Lysobacterales*, one strain in *Moraxellales*, two strains in *Oceanospirillales*, seven strains in *Pseudomonadales*, and two strains in *Vibrionales* (Table 2). The cell morphology of the isolates is presented in Fig. 1. At the family level, 20 strains were distributed into 12 families: *Aeromonadaceae* (two strains) in the order *Aeromonadales*; *Colwelliaceae* (one strain), *Ferrimonadaceae* (one strain), and *Pseudoalteromonadaceae* (one strain) in the order *Alteromonadales*; *Microbulbiferaceae* (one strain) in the order *Cellvibrionales*; *Pectobacteriaceae* (one strain) in the order *Enterobacterales*; *Lysobacteraceae* (one strain) in the order *Lysobacterales*; *Moraxellaceae* (one strain) in the order *Moraxellales*; *Oceanospirillaceae* (two strains) in the order *Oceanospirillales*; *Marinobacteraceae* (one strain) and *Pseudomonadaceae* (six strains) in the order *Pseudomonadales*; and *Vibrionaceae* (two strains) in the order *Vibrionales*. The phylogenetic relationship between the isolates and those of the most closely related strains is provided in Figs. 2 and 3. The detailed descriptions of 30 unrecorded species are given below.

Description of *Paraburkholderia madseniana* HMG1432

Cells are Gram-stain-negative, non-flagellated, and rod shaped. After 3 days at 30°C on R2A medium, colonies are circular, convex, smooth, and white colored. The result from the API 20NE tests show positive for esculin hydrolysis, β-galactosidase, arginine dihydrolase, urease, and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, malic acid, capric acid, and phenylacetic acid are utilized; but not D-maltose, adipic acid, and trisodium citrate. Strain HMG1432 (=NIBRBAC000508756) was isolated from soil in Hwahyeon-myeon (37°52′53.5″N, 127°18′17.4″E), Pocheon-si, Gyeonggi-do, Korea. The 16S rRNA gene sequence of strain HMG1432 have been deposited in GenBank under accession numbers OL347558.

Description of *Caballeronia telluris* HMG1944

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 30°C on R2A medium, colonies are circular, convex, smooth, and white colored. The result from the API 20NE tests show positive for arginine dihydrolase, urease, and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, β-galactosidase, esculin hydrolysis and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, malic acid, adipic acid, and phenylacetic acid are utilized; but not D-maltose, capric acid, and trisodium citrate. Strain HMG1944 (=NIBRBAC000508757) was isolated from soil in Cheoin-gu (37°20′23.4″N, 127°16′16.0″E),

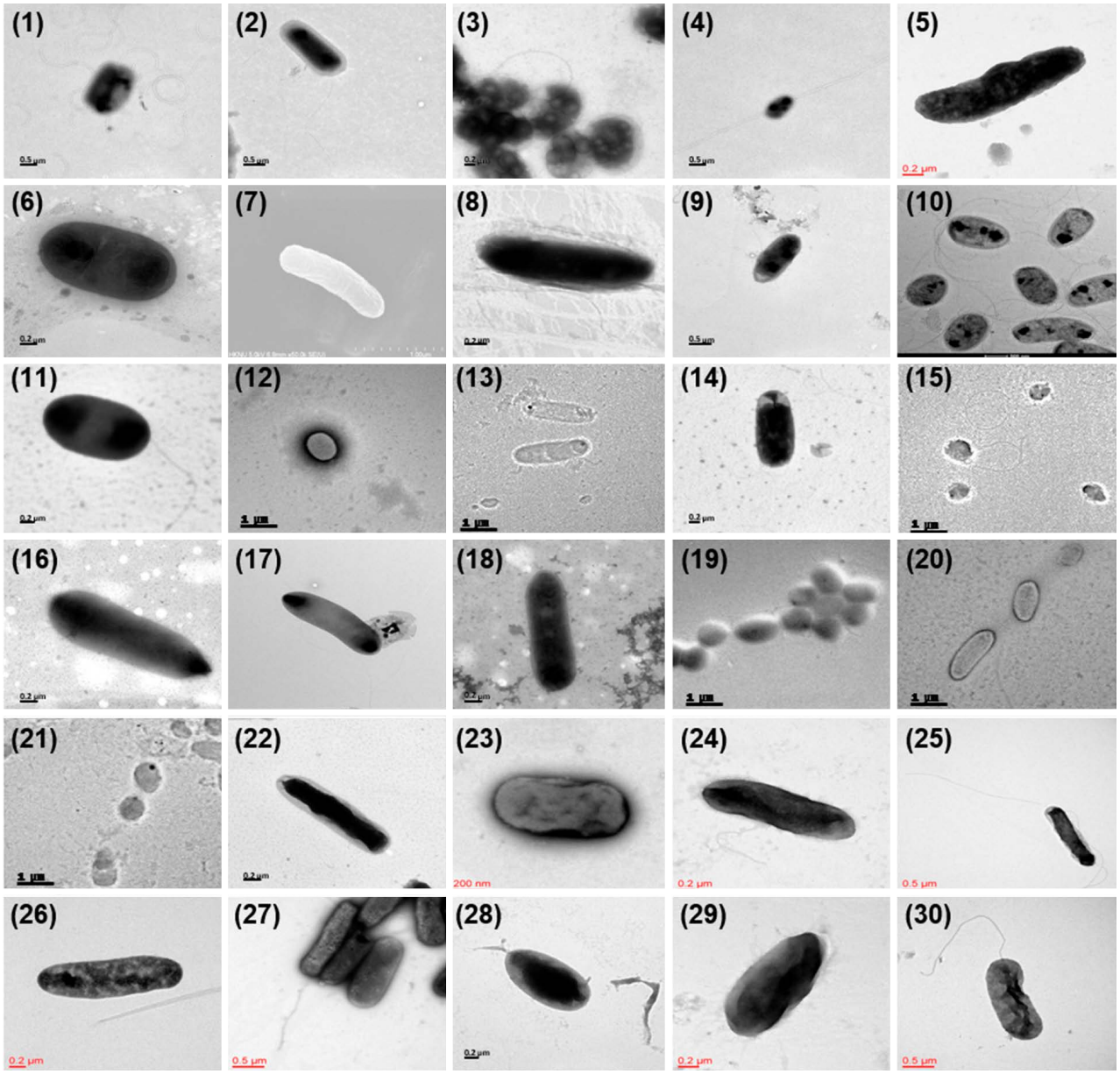

Fig. 1. Transmission electron micrographs of cells of the strains belonging to the *Betaproteobacteria* and *Gammaproteobacteria*. The strains were cultured at their optimal growth conditions. Strain: 1, HMG1432; 2, HMG1944; 3, HMG2081; 4, HMG5310; 5, SATF-R7; 6, ATS74; 7, PS9; 8, 18JY6-4; 9, BT33; 10, MMS21-YDR2; 11, CAU 1658; 12, 16_SB_L4; 13, G1-2; 14, CAU 1657; 15, 15G1-13; 16, CAU 1650; 17, MMS21-M35; 18, CAU 1655; 19, 17_H6_M9; 20, GJ51-11; 21, G5-10; 22, CAU 1666; 23, SATF-R3; 24, AMTF-M1; 25, AMTF-M15; 26, AMTF-R2; 27, DSTF-M2; 28, BT758; 29, JHKTF-M108; 30, DSTF-M15.

Yongin-si, Gyeonggi-do, Korea. The 16S rRNA gene sequence of strain HMG1944 have been deposited in GenBank under accession numbers OL347559.

Description of *Caballeronia calidae* HMG2081

Cells are Gram-stain-negative, rod shaped, and flagellated. After 3 days at 30°C on R2A medium, colonies are circular, convex, smooth, and cream colored. The result from the API 20NE tests show positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, and cytochrome oxidase; but negative for indole production, esculin hydrolysis, gelatinase, and β-galactosidase. D-Mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are utilized; but not D-maltose. Strain HMG2081 (=NIBRBAC000508758) was isolated from soil in Cheoin-gu

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

Order	Family	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Culture conditions
Burkholderiales	*Burkholderiaceae*	HMG1432	NIBRBAC000508756	*Paraburkholderia madseniana*	100	Soil	R2A	30°C, 3 days
		HMG1944	NIBRBAC000508757	*Caballeronia telluris*	99.04	Soil	R2A	30°C, 3 days
		HMG2081	NIBRBAC000508758	*Caballeronia calidae*	99.17	Soil	R2A	30°C, 3 days
		HMG3510	NIBRBAC000508767	*Paraburkholderia xenovorans*	99.72	Pine cone	R2A	30°C, 3 days
	Comamonadaceae	SATF-R7	NIBRBAC000508910	*Hydrogenophaga aquatica*	99.24	Tidal flat	R2A	25°C, 5 days
		ATS74	NIBRBAC000508882	*Aquincola tertiaricarbonis*	100	Soil	R2A	25°C, 3 days
		PS9	NIBRBAC000508781	*Acidovorax cattleyae*	99.1	Wetland	R2A	30°C, 3 days
	Oxalobacteraceae	18JY6-4	NIBRBAC000508863	*Massilia horti*	98.82	Soil	R2A	25°C, 3 days
		BT33	NIBRBAC000508889	*Noviherbaspirillum aerium*	98.89	Soil	R2A	25°C, 3 days
		MMS21-YDR2	NIBRBAC000508737	*Duganella levis*	98.97	Soil	R2A	30°C, 3 days

(37°20′19.4″N, 127°15′58.0″E), Yongin-si, Gyeonggi-do, Korea. The 16S rRNA gene sequence of strain HMG2081 have been deposited in GenBank under accession numbers OL347560.

Description of *Paraburkholderia xenovorans* HMG3510

Cells are Gram-stain-negative, rod shaped, and flagellated. After 3 days at 30°C on R2A medium, colonies are circular, convex, smooth, and cream colored. The result from the API 20NE tests show positive for arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, and gelatinase. D-Mannitol, D-glucose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are utilized; but not L-arabinose and D-maltose. Strain HMG3510 (=NIBRBAC000508767) was isolated from pine cone in Chodang-dong (37°47′54.0″N, 128°54′29.6″E), Gangneung-si, Gangwon-do, Korea. The 16S rRNA gene sequence of strain HMG3510 have been deposited in GenBank under accession numbers OL347569.

Description of *Hydrogenophaga aquatica* SATF-R7

Cells are Gram-stain-negative, ovoid shaped, and non-flagellated. After 5 days at 25°C on R2A medium, colonies are circular, slightly convex, glistening, and grayish-yellow colored. The result from the API 20NE tests show positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase; but negative for indole production, glucose fermentation, cytochrome oxidase, and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, malic acid, and trisodium citrate are utilized; but not *N*-acetyl-glucosamine, potassium gluconate, adipic acid, capric acid, and phenylacetic acid. Strain SATF-R7 (=NIBRBAC000508910) was isolated from tidal flat in Sinan-gun (34°43′14.0″N, 125°56′12.4″E), Jeollanam-do, Korea. The 16S rRNA gene sequence of strain SATF-R7 have been deposited in GenBank under accession numbers OR398947.

Description of *Aquincola tertiaricarbonis* ATS74

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 25°C on R2A medium, colonies are circular, convex, glistening, and white colored. The result from the API 20NE tests show positive for urease and esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, cytochrome oxidase, β-galactosidase, and gelatinase. D-Mannitol, D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic

Table 2. The taxonomic affiliations of isolated strains belonging to the class *Gammaproteobacteria*.

Order	Family	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Culture conditions
Aeromonadales	*Aeromonadaceae*	CAU 1658	NIBRBAC000508838	*Oceanimonas doudoroffii*	98.91	Sea sediment	MA	30°C, 3−5 days
		16_SB_L4	NIBRBAC000508789	*Aeromonas tecta*	99.79	Sediment soil	R2A	30°C, 2−3 days
Alteromonadales	*Colwelliaceae*	G1-2	NIBRBAC000508957	*Colwellia agarivorans*	100	Seaweed	MA	4−30 °C, 2 days
	Ferrimonadaceae	CAU 1657	NIBRBAC000508837	*Ferrimonas balearica*	98.92	Sea sediment	MA	30°C, 2−3 days
	Pseudoalteromonadaceae	15G1-13	NIBRBAC000508959	*Psychrosphaera aquimarina*	100	Seaweed	MA	4−30°C, 2 days
Cellvibrionales	*Microbulbiferaceae*	CAU 1650	NIBRBAC000508832	*Microbulbifer arenaceous*	98.92	Mud	MA	30°C, 3−5 days
Enterobacterales	*Pectobacteriaceae*	MMS21-M35	NIBRBAC000508739	*Dickeya fangzhongdai*	99.93	Soil	R2A	30°C, 3 days
Lysobacterales	*Lysobacteraceae*	CAU 1655	NIBRBAC000508835	*Luteimonas vadosa*	99.73	Sea sediment	MA	30°C, 3−5 days
Moraxellales	*Moraxellaceae*	17_H6_M9	NIBRBAC000508788	*Acinetobacter modestus*	100	Brackish water	R2A	30°C, 2−3 days
Oceanospirillales	*Oceanospirillaceae*	GJ51-11	NIBRBAC000508953	*Amphritea opalescens*	99.29	Seaweed	MA	15−30°C, 3 days
		G5-10	NIBRBAC000508956	*Marinomonas gallaica*	99.18	Seaweed	MA	10−30°C, 2 days
Pseudomonadales	*Marinobacteraceae*	CAU 1666	NIBRBAC000508849	*Marinobacter similis*	99.66	Sea sediment	MA	30°C, 3−5 days
	Pseudomonadaceae	SATF-R3	NIBRBAC000508909	*Pseudomonas xionganensis*	100	Tidal flat	R2A	25°C, 3 days
		AMTF-M1	NIBRBAC000508911	*Pseudomonas fulva*	99.73	Tidal flat	MA	25°C, 5 days
		AMTF-M15	NIBRBAC000508912	*Pseudomonas khazarica*	99.93	Tidal flat	MA	25°C, 5 days
		AMTF-R2	NIBRBAC000508913	*Pseudomonas straminea*	99.73	Tidal flat	R2A	25°C, 3 days
		DSTF-M2	NIBRBAC000508914	*Pseudomonas toyotomiensis*	99.59	Tidal flat	MA	25°C, 3 days
		BT758	NIBRBAC000508878	*Pseudomonas donghuensis*	100	Soil	R2A	25°C, 3 days
Vibrionales	*Vibrionaceae*	JHKTF-M108	NIBRBAC000508904	*Vibrio diabolicus*	99.01	Tidal flat	MA	25°C, 3 days
		DSTF-M15	NIBRBAC000508907	*Vibrio galatheae*	98.98	Tidal flat	MA	25°C, 3 days

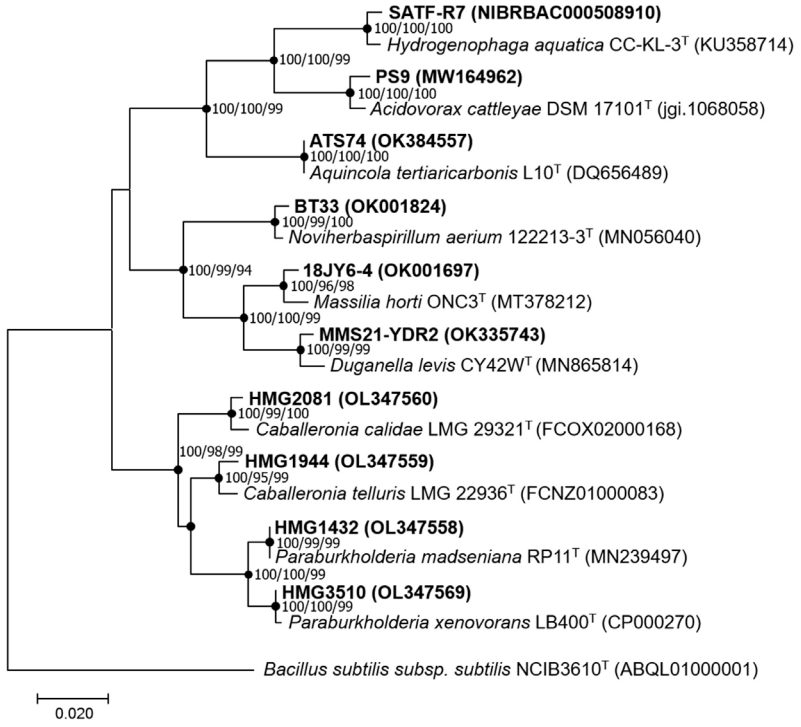

Fig. 2. Neighbor-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between the strains identified in this study and their relatives of the order *Burkholderiales* in the class *Betaproteobacteira*. Black dots indicate that the corresponding nodes were also recovered in the trees generated using the maximum likelihood (ML) and maximum parsimony (MP) algorithms. Bootstrap values (>70%) based on 1,000 replications are indicated at branch points (NJ/ML/MP). The scale bar represents a 0.02 nucleotide sequence divergence.

acid, trisodium citrate, and phenylacetic acid are utilized; but not L-arabinose, capric acid, and adipic acid. Strain ATS74 (=NIBRBAC000508882) was isolated from soil in Gangdong-gu (37°34′01.4″N, 127°08′59.3″E), Seoul, Korea. The 16S rRNA gene sequence of strain ATS74 have been deposited in GenBank under accession numbers OK384557.

Description of *Acidovorax cattleyae* PS9

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 30°C on R2A medium, colonies are round, entire, raised, and white colored. The result from the API 20NE tests show positive for cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, potassium gluconate, malic acid, adipic acid, and trisodium citrate are utilized; but not *N*-acetyl-glucosamine, D-maltose, capric acid, and phenylacetic acid. Strain PS9 (=NIBRBAC000508781) was isolated from wetland in Godeok-dong (37°34′02.2″N, 127°08′59.1″E), Gangdong-gu, Seoul, Korea. The 16S rRNA gene sequence of strain PS9 have been deposited in GenBank under accession numbers MW164962.

Description of *Massilia horti* 18JY6-4

Cells are Gram-stain-negative, oval shaped, and flagellated. After 3 days at 25°C on R2A medium, colonies are round, flat, and white colored. The result from the API 20NE tests show positive for esculin hydrolysis, cytochrome oxidase, β-galactosidase, and gelatinase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. D-Mannitol, malic acid, and phenylacetic acid are utilized; but not D-mannose, D-maltose, D-glucose, L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, capric acid, and trisodium citrate. Strain 18JY6-4 (=NIBRBAC000508863) was isolated from soil in Jungwon-gu (37°28′17.4″N, 127°10′38.3″E), Seongnam-si, Gyeonggi-do, Korea. The GeneBank accession number of the 16S rRNA gene sequence of strain is. The 16S rRNA gene sequence of strain 18JY6-4 have been deposited in GenBank under accession numbers OK001697.

Description of *Noviherbaspirillum aerium* BT33

Cells are Gram-stain-negative, oval shaped, and non-

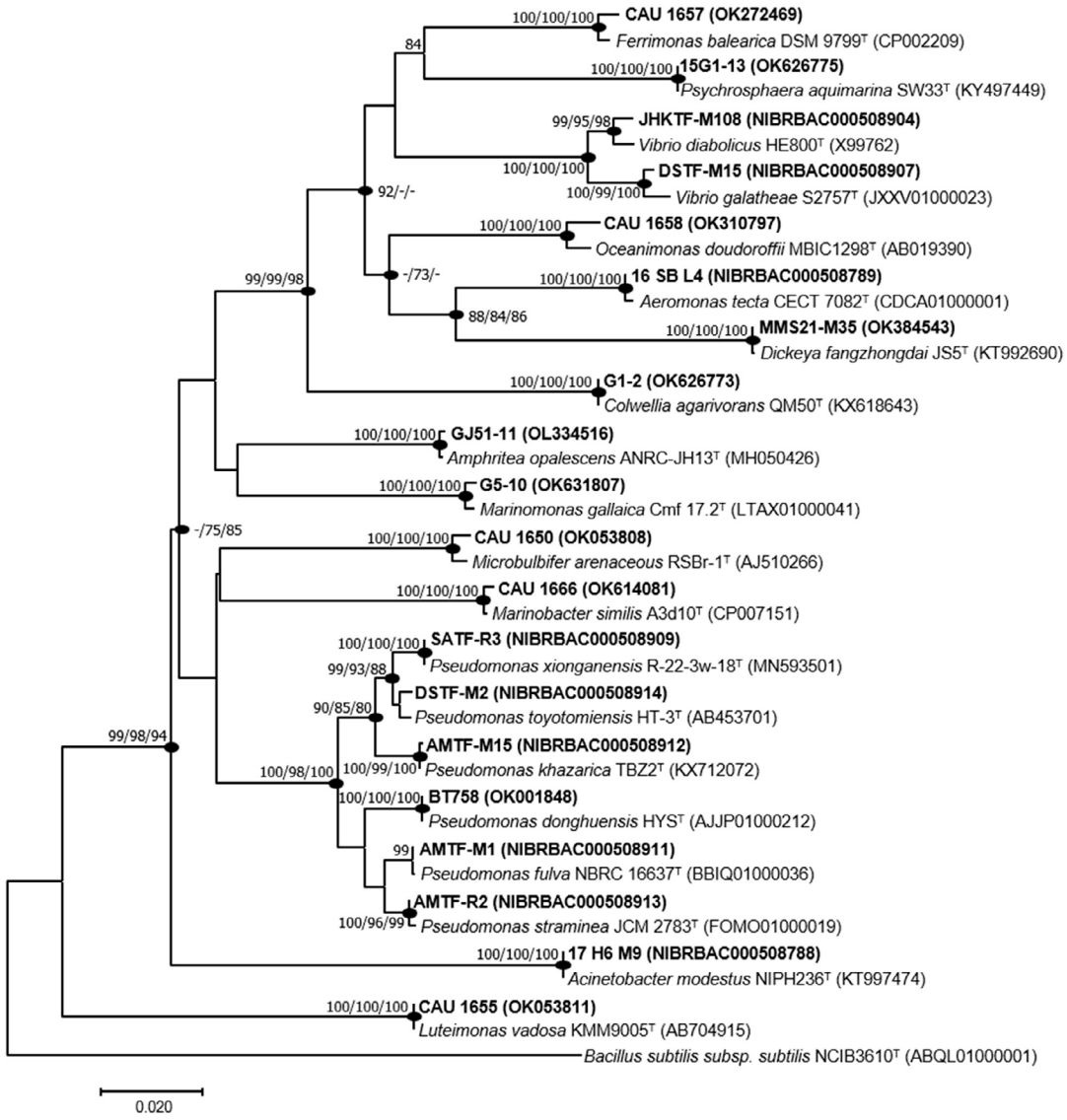

Fig. 3. Neighbor-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between the strains identified in this study and their relatives of the orders *Aeromonadales*, *Alteromonadales*, *Cellvibrionales*, *Enterobacterales*, *Lysobacterales*, *Moraxellales*, *Oceanospirillales*, *Pseudomonadales*, and *Vibrionales* in the class *Gammaproteobacteria*. Black dots indicate that the corresponding nodes were also recovered in the trees generated using the maximum likelihood (ML) and maximum parsimony (MP) algorithms. Bootstrap values (>70%) based on 1,000 replications are indicated at branch points (NJ/ML/MP). The scale bar represents a 0.02 nucleotide sequence divergence.

flagellated. After 3 days at 25°C on R2A medium, colonies are semi-transparent, circular, smooth, and orange colored. The result from the API 20NE tests show positive for esculin hydrolysis and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, D-maltose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are not utilized. Strain BT33 (=NIBRBAC000508889) was isolated from soil in Ganeung-dong (37°44′15.0″N, 127°01′02.2″E), Uijeongbu-si, Gyeonggi-do, Korea. The GeneBank accession number of the 16S rRNA gene sequence of strain is. The 16S rRNA gene sequence of strain BT33 have been deposited in GenBank under accession numbers OK001824.

Description of *Duganella levis* MMS21-YDR2

Cells are Gram-stain-negative, rod shaped, and flagellated. After 3 days at 30°C on R2A medium, colonies are circular, convex, entire, and white colored. The result

from the API 20NE tests show positive for glucose fermentation, urease, esculin hydrolysis, and cytochrome oxidase; but negative for nitrate reduction, indole production, arginine dihydrolase, gelatinase, and β-galactosidase. D-Glucose, L-arabinose, and D-maltose are utilized; but not D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid. Strain MMS21-YDR2 (=NIBRBAC000508737) was isolated from soil in Taepyeong-dong (36°19′08.1″N, 127°23′04.1″E), Jung-gu, Daejeon, Korea. The 16S rRNA gene sequence of strain MMS21-YDR2 have been deposited in GenBank under accession numbers OK335743.

Description of *Oceanimonas doudoroffii* CAU 1658

Cells are Gram-stain-negative, rod shaped, and flagellated. After 3−5 days at 30°C on MA medium, colonies are circular, convex, entire, smooth, opaque and cream. The result from the API 20NE tests show positive for arginine dihydrolase, urease, esculin hydrolysis, and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, β-galactosidase, and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, adipic acid, trisodium citrate, and phenylacetic acid are utilized; but not capric acid. Strain CAU 1658 (=NIBRBAC000508838) was isolated from sea sediment in Masiran Beach (37°26′01.9″N, 126°24′53.1″E), Jung-gu, Incheon, Korea. The 16S rRNA gene sequence of strain CAU 1658 have been deposited in GenBank under accession numbers MW020537.

Description of *Aeromonas tecta* 16_SB_L4

Cells are Gram-stain-negative, coccibacilli shaped, and non-flagellated. After 2−3 days at 30°C on R2A medium, colonies are circular, convex, smooth, and white. The result from the API 20NE tests show positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, cytochrome oxidase, β-galactosidase, and gelatinase; but negative for indole production. D-Mannitol, D-maltose, D-glucose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, and trisodium citrate are utilized; but not L-arabinose, adipic acid, and phenylacetic acid. Strain 16_SB_L4 (=NIBRBAC000508789) was isolated from sediment soil in Daesin-myeon (37°40′27.27″N, 127°54′23.87″E), Yeoju-gun, Gyeonggi-do, Korea. The 16S rRNA gene sequence of strain 16_SB_L4 have been deposited in GenBank under accession numbers OR367260.

Description of *Colwellia agarivorans* G1-2

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 2 days at 4−30°C on MA medium, colonies are circular, and yellow colored. The result from the API 20NE tests show positive for nitrate reduction, esculin hydrolysis, cytochrome oxidase, β-galactosidase, and gelatinase; but negative for indole production, glucose fermentation, arginine dihydrolase, and urease. D-Mannose, D-Mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are not utilized. Strain G1-2 (=NIBRBAC000508957) was isolated from seaweed in Jugwang-myeon (38°21′23.8″N, 128°30′47.3″E), Goseong-gun, Gangwon-do, Korea. The 16S rRNA gene sequence of strain G1-2 have been deposited in GenBank under accession numbers OK626773.

Description of *Ferrimonas balearica* CAU 1657

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 2−3 days at 30°C on MA medium, colonies are circular, entire, convex, smooth, opaque, and orange pink colored. The result from the API 20NE tests show positive for esculin hydrolysis, gelatinase, and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase. D-Maltose, D-glucose, *N*-acetyl-glucosamine, and malic acid are utilized; but not L-arabinose, D-mannose, D-mannitol, potassium gluconate, adipic acid, capric acid, trisodium citrate, and phenylacetic acid. Strain CAU 1657 (=NIBRBAC000508837) was isolated from sea sediment in Masiran Beach (37°26′01.9″N, 126°24′53.1″E), Jung-gu, Incheon, Korea. The 16S rRNA gene sequence of strain CAU 1657 have been deposited in GenBank under accession numbers OK272469.

Description of *Psychrosphaera aquimarina* 15G1-13

Cells are Gram-stain-negative, rod shaped, and flagellated. After 2 days at 4−30°C on MA medium, colonies are circular, and white colored. The result from the API 20NE tests show positive for esculin hydrolysis, β-galactosidase, and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are not utilized. Strain 15G1-13 (=NIBRBAC000508959) was isolated from seaweed in Jugwang-myeon (38°21′23.8″N, 128°30′47.3″E), Goseong-gun, Gangwon-do, Korea. The 16S rRNA gene sequence of strain 15G1-13 have been deposited in GenBank under accession numbers OK626775.

Description of *Microbulbifer arenaceous* CAU 1650

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3−5 days at 30°C on MA medium, colonies are circular, entire, flat, smooth, translucent and cream colored. The result from the API 20NE tests show positive for esculin hydrolysis and cytochrome oxidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are not utilized. Strain CAU 1650 (=NIBRBAC000508832) was isolated from mud in Minmeoru Beach (37°38′57.9″N, 126°20′07.4″E), Ganghwa-gun, Incheon, Korea. The 16S rRNA gene sequence of strain CAU 1650 have been deposited in GenBank under accession numbers OK053808.

Description of *Dickeya fangzhongdai* MMS21-M35

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 30°C on R2A medium, colonies are irregular, raised, entire, translucent, and cream colored. The result from the API 20NE tests show negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, and gelatinase. D-Mannitol is utilized; but not D-mannose, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid. Strain MMS21-M35 (=NIBRBAC000508739) was isolated from soil in Doan-dong (36°19′08.5″N, 127°20′19.9″E), Seo-gu, Daejeon, Korea. The 16S rRNA gene sequence of strain MMS21-M35 have been deposited in GenBank under accession numbers OK384543.

Description of *Luteimonas vadose* CAU 1655

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3−5 days at 30°C on MA medium, colonies are circular, entire, convex, smooth, translucent, and yellow colored. The result from the API 20NE tests show positive for esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, cytochrome oxidase, β-galactosidase, and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are not utilized. Strain CAU 1655 (=NIBRBAC000508835) was isolated from sea sediment in Minmeoru Beach (37°38′58.4″N, 126°20′07.0″E), Ganghwa-gun, Incheon, Korea. The 16S rRNA gene sequence of strain CAU 1655 have been deposited in GenBank under accession numbers OK053811.

Description of *Acinetobacter modestus* 17_H6_M9

Cells are Gram-stain-negative, coccus shaped, and non-flagellated. After 2−3 days at 30°C on R2A medium, colonies are circular, convex, smooth, and white colored. The result from the API 20NE tests show positive for esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, cytochrome oxidase, β-galactosidase, and gelatinase. Capric acid and malic acid are utilized; but not D-mannose, D-mannitol, D-maltose, D-glucose, L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, trisodium citrate, and phenylacetic acid. Strain 17_H6_M9 (=NIBRBAC000508788) was isolated from brackish water in Gaehwa-dong (37°60′04.8″N, 126°80′92.4″E), Gangseo-gu, Seoul, Korea. The 16S rRNA gene sequence of strain 17_H6_M9 have been deposited in GenBank under accession numbers OR367259.

Description of *Amphritea opalescens* GJ51-11

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 15−30°C on MA medium, colonies are circular and cream colored. The result from the API 20NE tests show positive for nitrate reduction and cytochrome oxidase; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, and gelatinase. Trisodium citrate is utilized; but not D-mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, capric acid, and phenylacetic acid. Strain GJ51-11 (=NIBRBAC000508953) was isolated from seaweed in Gwangjin Beach (37°57′17.6″N, 128°46′21.2″E), Yangyang-gun, Gangwon-do, Korea. The 16S rRNA gene sequence of strain GJ51-11 have been deposited in GenBank under accession numbers OL334516.

Description of *Marinomonas gallaica* G5-10

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 2 days at 10−30°C on MA medium, colonies are circular and white colored. The result from the API 20NE tests show positive for esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, cytochrome oxidase, β-galactosidase, and gelatinase. D-Mannitol, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid are utilized; but not D-mannose, D-glucose, L-arabinose, *N*-acetyl-glucosamine, capric acid, and adipic acid. Strain G5-10 (=NIBRBAC000508956) was isolated from seaweed in Jugwang-myeon (38°21′23.8″N, 128°30′47.3″E), Goseong-gun, Gangwon-do, Korea. The GeneBank accession number of the 16S rRNA gene sequence of strain

is. The 16S rRNA gene sequence of strain G5-10 have been deposited in GenBank under accession numbers OK631807.

Description of *Marinobacter similis* CAU 1666

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3−5 days at 30°C on MA medium, colonies are circular, entire, convex, smooth, translucent and cream colored. The result from the API 20NE tests show positive for nitrate reduction, esculin hydrolysis, and cytochrome oxidase; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase, and gelatinase. D-Mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, capric acid, trisodium citrate, and phenylacetic acid are not utilized. Strain CAU 1666 (=NIBRBAC000508849) was isolated from sea sediment in Ayajin Beach (38°16′32.0″N, 128°33′12.0″E), Goseong-gun, Gangwon-do, Korea. The 16S rRNA gene sequence of strain CAU 1666 have been deposited in GenBank under accession numbers OK614081.

Description of *Pseudomonas xionganensis* SATF-R3

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 25°C on R2A medium, colonies are circular, convex, glistening and pale yellow colored. The result from the API 20NE tests show positive for nitrate reduction; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, cytochrome oxidase, β-galactosidase, and gelatinase. Capric acid, malic acid, and trisodium citrate are utilized; but not D-mannose, D-mannitol, D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and phenylacetic acid. Strain SATF-R3 (=NIBRBAC000508909) was isolated from tidal flat in Bigeum-myeon (34°43′14.0″N, 125°56′12.4″E), Sinan-gun, Jeollanam-do, Korea. The 16S rRNA gene sequence of strain SATF-R3 have been deposited in GenBank under accession numbers OR363648.

Description of *Pseudomonas fulva* AMTF-M1

Cells are Gram-stain-negative, rod shaped, and flagellated. After 5 days at 25°C on MA medium, colonies are circular, convex, glistening and vivid yellow colored. The result from the API 20NE tests show positive for arginine dihydrolase, urease, and gelatinase; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, β-galactosidase, and cytochrome oxidase. D-Mannose, D-mannitol, potassium gluconate, capric acid, malic acid, and trisodium citrate are utilized; but not D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, adipic acid, and phenylacetic acid. Strain AMTF-M1 (=NIBRBAC000508911) was isolated from tidal flat in Anmyeondo Island (36°33′58.6″N, 126°21′57.1″E), Taean-gun, Chungcheongnam-do, Korea. The 16S rRNA gene sequence of strain AMTF-M1 have been deposited in GenBank under accession numbers OR363691.

Description of *Pseudomonas khazarica* AMTF-M15

Cells are Gram-stain-negative, rod shaped, and flagellated. After 5 days at 25°C on MA medium, colonies are circular, slightly convex, glistening and yellowish white colored. The result from the API 20NE tests show positive for arginine dihydrolase and urease; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatinase, β-galactosidase, and cytochrome oxidase. D-Glucose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, and trisodium citrate are utilized; but not L-arabinose, *N*-acetyl-glucosamine, D-maltose, adipic acid, and phenylacetic acid. Strain AMTF-M15 (=NIBRBAC000508912) was isolated from tidal flat in Anmyeondo Island (36°33′58.6″N, 126°21′57.1″E), Taean-gun, Chungcheongnam-do, Korea. The 16S rRNA gene sequence of strain AMTF-M15 have been deposited in GenBank under accession numbers OR363693.

Description of *Pseudomonas straminea* AMTF-R2

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 25°C on R2A medium, colonies are circular, glistening and vivid yellow colored. The result from the API 20NE tests show positive for arginine dihydrolase, urease, and gelatinase; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, β-galactosidase, and cytochrome oxidase. D-Mannose, D-mannitol, D-glucose, L-arabinose, potassium gluconate, capric acid, malic acid, and trisodium citrate are utilized; but not *N*-acetyl-glucosamine, D-maltose, adipic acid, and phenylacetic acid. Strain AMTF-R2 (=NIBRBAC000508913) was isolated from tidal flat in Anmyeondo Island (36°33′58.6″N, 126°21′57.1″E), Taean-gun, Chungcheongnam-do, Korea. The 16S rRNA gene sequence of strain AMTF-R2 have been deposited in GenBank under accession numbers OR363695.

Description of *Pseudomonas toyotomiensis* DSTF-M2

Cells are Gram-stain-negative, rod shaped, and flagellated. After 3 days at 25°C on MA medium, colonies are circular, convex, glistening and moderate yellow colored. The result from the API 20NE tests show positive for nitrate reduction, arginine dihydrolase, and urease; but neg-

ative for indole production, glucose fermentation, esculin hydrolysis, cytochrome oxidase, β-galactosidase, and gelatinase. D-Glucose, D-maltose, potassium gluconate, capric acid, malic acid, and trisodium citrate are utilized; but not L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, adipic acid, and phenylacetic acid. Strain DSTF-M2 (=NIBRBAC000508914) was isolated from tidal flat in Nam-myeon (36°39′31.5″N, 126°17′49.3″E), Taean-gun, Chungcheongnam-do, Korea. The 16S rRNA gene sequence of strain DSTF-M15 have been deposited in GenBank under accession numbers OR363647.

Description of *Pseudomonas donghuensis* BT758

Cells are Gram-stain-negative, rod shaped, and flagellated. After 3 days at 25°C on R2A medium, colonies are circular, convex, glistening and white colored. The result from the API 20NE tests show positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatinase; but negative for indole production, β-galactosidase, and cytochrome oxidase. D-Glucose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, and trisodium citrate are utilized; but not D-mannitol, D-maltose, L-arabinose, D-mannose, adipic acid, and phenylacetic acid. Strain BT758 (=NIBRBAC000508878) was isolated from soil in Hangyeong-myeon (33°19′19″N, 126°15′55″E), Jeju-si, Jeju-do, Korea. The 16S rRNA gene sequence of strain BT758 have been deposited in GenBank under accession numbers OK001848.

Description of *Vibrio diabolicus* JHKTF-M108

Cells are Gram-stain-negative, rod shaped, and non-flagellated. After 3 days at 25°C on MA medium, colonies are circular, slightly convex, glistening and brownish-pink colored. The result from the API 20NE tests show positive for nitrate reduction, esculin hydrolysis, gelatinase, and β-galactosidase; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and cytochrome oxidase. L-Arabinose and D-mannitol are utilized; but not D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain JHKTF-M108 (=NIBRBAC000508904) was isolated from tidal flat in Janghang-eup (36°01′44.6″N, 126°39′56.8″E), Seocheon-gun, Chungcheongnam-do, Korea. The 16S rRNA gene sequence of strain JHKTF-M108 have been deposited in GenBank under accession numbers OR363643.

Description of *Vibrio galatheae* DSTF-M15

Cells are Gram-stain-negative, ovoid (or rod) shaped, and flagellated. After 3 days at 25°C on MA medium, colonies are circular, slightly convex, glistening and yellowish white colored. The result from the API 20NE tests show positive for esculin hydrolysis and β-galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, cytochrome oxidase, and gelatinase. Potassium gluconate and adipic acid are utilized; but not D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DSTF-M15 (=NIBRBAC000508907) was isolated from tidal flat in Nam-myeon (36°39′31.5″N, 126°17′49.3″E), Taean-gun, Chungcheongnam-do, Korea.

Acknowledgements

This work was supported by the project on survey of indigenous species of Korea of the National Institute of Biological Resources (NIBR) under the Ministry of Environment (MOE).

References

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal Molecular Evolution 17(6):368-376.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4):783-791.

Fitch, W.M. and E. Margoliash. 1967. Construction of phylogenetic trees. Science 155(3760):279-284.

Kersters, K., P. de Vos, M. Gillis, J. Swings, P. Vandamme and E. Stackebrandt. 2006. Introduction to the proteobacteria. The Prokaryotes, Springer, New York. pp. 3-37.

Kim, O.S., Y.J. Cho, K. Lee, S.H. Yoon, M. Kim, H. Na, S.C. Park, Y.S. Jeon, J.H. Lee, H. Yi, S. Won and J. Chun 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. International Journal of Systematic and Evolutionary Microbiology 62(Pt3):716-721.

Kimura, M.A. 1980. Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 16(2):111-120.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology Evolution 33(7):1870-1874.

Lane, D.J. 1991. 16S/23S RNA sequencing. In: E. Stackebrandt and M. Goodfellow (eds). Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons Ltd., London. pp. 115-175.

Oren, A. and G.M. Garrity. 2021. Valid publication of the names of forty-two phyla of prokaryotes. Int J Syst Evol Microbiol. 71:005056.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology Evolution 4(4):406-425.

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(24):4876-4882.

Submitted: November 15, 2022
Accepted: April 17, 2023