## A report of 27 unrecorded bacterial species within the class *Alphaproteobacteria* isolated from various sources of Korea in 2021

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In 2021, a total of 27 bacterial strains were isolated from soil, tree bark, moss, wetland, sea sediment, tidal flat, seawater and seaweed within Republic of Korea. Based on the analysis of 16S rRNA gene sequence (>98.7% sequence similarity), these isolates were assigned to the class *Alphaproteobacteria* as unrecorded species in Korea. The 27 strains were classified into the 10 families: *Maricaulaceae* of the order *Caulobacterales*; *Brucellaceae*, *Methylobacteriaceae*, *Nitrobacteraceae* and *Rhizobiaceae* of the order *Hyphomicrobiales*; *Micropepsaceae* of the order *Micropepsales*; *Rhodobacteraceae* of the order *Rhodobacterales*; *Azospirillaceae* of the order *Rhodospirillales*; and *Erythrobacteraceae* and *Sphingomonadales* of the order *Sphingomonadaceae*. There is no official report of these 27 species in Korea. Therefore, we report 27 isolates as unrecorded species, and described isolation sources, Gram-stain reactions, physiological and biochemical properties and morphologies of these strains.

Keywords: 16S rRNA gene, Alphaproteobacteria, bacterial diversity, phylum Pseudomonadota, unrecorded species

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## INTRODUCTION

The class *Alphaproteobacteria* within the phylum *Pseudomonadota* was proposed by Garrity in 2005 (Garrity *et al.*, 2005a), although the first representatives of the group were isolated in 1898 (Beijerinek, 1898). This group classification is currently being studied by polyphasic approach based on phenotypic and genomic data. In 2020, some members of the class *Alphaproteobacteria* were reclassified and emended by genome-scale phylogeny (Hördt *et al.*, 2020). The *Alphaproteobacteria* species were isolated from various environments, such as soil, freshwater, glacial ice, marine environments and lichens

(Hördt et al., 2020). At the time of writing, the 15 orders belonged to the class Alphaproteobacteria; Caulobacterales (Henrici and Johnson, 1935), Emcibacterales (Iino et al., 2016), Holosporales (Szokoli et al., 2016), Hyphomicrobiales (Douglas, 1957), Iodidimonadales (Iino et al., 2016), Kordiimonadales (Kwon et al., 2005), Magnetococcales (Bazylinski et al., 2013), Micropepsales (Harbison et al., 2017), Minwuiales (Sun et al., 2018), Rhodobacterales (Garrity et al., 2005b), Rhodospirillales (Pfenning and Trüper, 1971), Rhodothalassiales (Ramana et al., 2013), Rickettsiales (Gieszczykiewicz, 1939), Sneathiellales (Kurahashi et al., 2008) and Sphingomonadales (Yabuuchi and Kosako, 2005). The members of the class Alphaproteobacteria possess diverse metabolism. They are represented as Gram-stain negative bacteria. The majority of the class Alphaproteobacteria are aerobic, free living or chemoorganoheterotropic bacteria, but some of the species are facultative anaerobic, symbiotic or phototrophic bacteria. For example, the genus *Rhizobium* is known as endosymbiotic nitrogen-fixing bacteria associated with legumes. And some pathogens belong to the class Alphaproteobacteria, such as the genera Brucella and Roseomonas (Liu et al., 2019; Hördt et al., 2020).

In this article, we collected domestic terrestrial and marine environmental samples and obtained 27 unrecorded bacterial isolates in 2021. In addition, basic information about Gram reaction, cellular morphology and biochemical features of unrecorded species are described.

## **MATERIALS AND METHODS**

Unrecorded bacterial isolates were obtained primarily from terrestrial and marine environment sources. The terrestrial samples (soil, wetland, tree bark and moss) were collected from Daejeon, Goyang, Gurye, Jeju, Okcheon, Samcheok, Seogwipo, Seoul, Uijeongbu, Wando and Yongin. Marine environment samples (seawater, sea sediment, seaweed and tidal flat) were collected from Ganghwa, Geoje, Incheon, Jangheung, Sokcho and Taean. The collected samples were processed using a standard dilution-plating method on R2A, GYE and marine agars. These plates were cultured at 25°C, 30°C and 37°C for 2-5 days. The pure isolates were stored in cryogenic tubes with 10-20% glycerol stock at -80°C and by freeze-drying. The information of designated strain IDs, NIBR IDs, isolation sources, culture media and incubation conditions were described in Table 1. For bacterial identification of the unrecorded species, PCR-mediated amplification and sequencing of the 16s rRNA gene were performed as described Kim and Chun (Kim and Chun, 2014). The 16S rRNA gene sequences were determined using an Automatic Sequencer 3730XL (Applied Biosystems) with universal primers (27F and 1492R; (Lane, 1991)). A nearly complete 16S rRNA gene sequences (>1350 bp) were assembled using the BioEdit program (Hall, 1999) from the raw sequence data and submitted to the NCBI GenBank. The sequence similarity calculation and unrecorded species validation were performed using the EzBioCloud Database (Yoon et al., 2017); https://www.ezbiocloud.net/) and National Institute of Biological Resources database (https://species.nibr.go.kr/server/gatePage.do), respectively. The phylogenetic tree was constructed by using neighbor-joining method within MEGA7 software package with bootstrap resampling method with 1000 replicates (Felsenstein, 1985; Saitou and Nei, 1987; Kumar et al., 2016). Colony morphology was observed on agar plates

after cells were cultivated to their stationary phase. Cellular morphology was examined using transmission electron microscopy with negative staining. Gram reaction was carried out using a Gram-staining kit according to the standard procedures. API 20NE strips (bioMérieux) were inoculated according to the manufacturer's instructions. Oxidase activity determined using 1% N, N, N', N-tetramethyl-*p*-phenylenediamine dihydrochloride (Sigma).

## **RESULTS AND DISCUSSION**

In 2021, 27 unrecorded species of Alphaproteobacteria group were isolated from the terrestrial and marine environments. They were distributed in 21 genera of ten families within six orders according to sequence comparison and phylogenetic analysis based on the 16S rRNA gene. Seventeen soil bacteria were assigned to the families Brucellaceae, Methylobacteriaceae, Nitrobacteraceae, Rhizobiaceae, Micropepsaceae, Azospirillaceae, Erythrobacteraceae and Sphingomonadaceae of classes Hyphomicrobiales, Micropepsales, Rhodospirillales and Sphingomonadales: ten species in the seven genera Ochrobactrum, Methylobacterium, Microvirga, Rhodoplanes, Rhodopseuomonas, Agrobacterium and Rhizobium of the four families Brucellaceae, Methylobacteriaceae, Nitrobacteraceae and Rhizobiaceae within the order Hyphomicrobiales. One species in genus Rhizomicrobium of the family Micropepsaceae within the order Micropepsales, one species in the genus Azospirillum of the family Azospirillaceae within the order Rhodospirillales, five species in three genera Novosphingobium, Sphingobium and Sphingomonas of two families Erythrobacteraceae and Sphingomonadaceae within the order Sphinogomonadales. Strains HMF5357, BT721, HMF4628, MMS21-SJ4-13, BT288, ATS65, NA30, HMF6701, NS12-3, HMF5594, P4, MMS21-AI2-12, MMS21-M39, BT751, BT752, BT753 and HMG3570 were most closely related to Ochrobactrum teleogrylli (99.1% 16S rRNA gene sequence similarity), Methylobacterium indicum (98.8%), Methylobacterium pseudosasicola (99.8%), Methylobacterium radiotolerans (99.9%), Microvirga tunisiensis (99.3%), Rhodoplanes tepidicaeni (98.8%), Rhodopseudomonas thermotolerans (99.7%), Agrobacterium pusense (99.9%), Rhizobium aquaticum (100%), *Rhizobium paranaense* (100%), *Rhizomicrobium palustre* (100%), Azospirillum soli (98.9%), Novosphingobium pokkalii (99.2%), Sphingobium quisquiliarum (99.4%), Sphingomonas alpine (99.7%), Sphingomonas gei (99.2%) and Sphingomonas montanisoli (99.1%), respectively. Ten marine bacterial isolates were assigned to the families Maricaulaceae and Rhodobacteraceae of the classes Caulobacterales and Rhodobacterales, respectively: one species in the genus Hyphobacterium of the family Mari-

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation condition
Caulobacterales	Maricaulaceae	Hyphobacterium	CAU 1667	NIBRBAC000508846	Hyphobacterium vulgare	99.4	Sea sediment	MA	30°C, 3–5d
Hyphomicrobiales	Brucellaceae	Ochrobactrum	HMF5357	NIBRBAC000508753	Ochrobactrum teleogrylli	99.1	Soil	R2A	30°C, 3d
	Methylobacteriaceae	Methylobacterium	BT721 HMF4628 MMS21-SJ4-13	NIBRBAC000508870 NIBRBAC000508752 NIBRBAC000508742	Methylobacterium indicum Methylobacterium pseudosasicola Methylobacterium radiotolerans	8.89 8.99 9.99	Soil Costata birch bark Soil	R2A R2A R2A	25°C, 3d 30°C, 3d 30°C, 3d
		Microvirga	BT288	NIBRBAC000508867	Microvirga tunisiensis	99.3	Soil	R2A	25°C, 3d
	Nitrobacteraceae	Rhodoplanes Rhodopseudomonas	ATS65 NA30	NIBRBAC000508881 NIBRBAC000508780	Rhodoplanes tepidicaeni Rhodopseudomonas thermotolerans	98.8 99.7	Soil Wetland	R2A R2A	25°C, 3d 30°C, 3d
	Rhizobiaceae	Agrobacterium Rhizobium	HMF6701 NS12-3 HMF5594	NIBRBAC000508755 NIBRBAC000508931 NIBRBAC000508754	Agrobacterium pusense Rhizobium aquaticum Rhizobium paranaense	9.99 100.0 100.0	Soil Soil Soil	MA R2A R2A	30°C, 3d 30°C, 4d 30°C, 3d
Micropepsales	Micropepsaceae	Rhizomicrobium	P4	NIBRBAC000508935	Rhizomicrobium palustre	100.0	Soil	R2A	30°C, 4d
Rhodobacterales	Rhodobacteraceae	Celeribacter	15MA-21	NIBRBAC000508955	Celeribacter halophilus	100.0	Seaweed	МА	10-45°C, 2d
		Marivita Nioella	CAU 1659 SCSW-6	NIBRBAC000508839 NIBRBAC000508908	Marivita roseacus Nioella ostreopsis	99.4 100.0	Sea sediment Seawater	MA MA	30°C, 3–5d 25°C, 3d
		Pararhodobacter	CAU 1660	NIBRBAC000508840	Pararhodobacter marinus	98.8	Sea sediment	МА	30°C, 3-5d
		Pseudooceanicola	CAU 1661	NIBRBAC000508842	Pseudooceanicola lipolyticus	6.66	Sea sediment	MA	30°C, 3-5d
		Kuegeria Seohaeicola	14MAJJD-6 CAU 1648	NIBRBAC000508830 NIBRBAC000508830	Kuegeria projundi Seohaeicola nanhaiensis	8.89 7.99	l idal flat Seawater	MA	25°C, 3d 30°C, 3d
			CAU 1651	NIBRBAC000508833	Seohaeicola zhoushanensis	5.66	Seawater	GYE	30°C, 3-5d
		Tritonibacter	AMTF-M12	NIBRBAC000508906	Tritonibacter mobilis subsp. pelagius	99.4	Tidal flat	MA	25°C, 5d
Rhodospirillales	Azospirillaceae	Azospirillum	MMS21-AI2-12	NIBRBAC000508731	Azospirillum soli	98.9	Soil	R2A	30°C, 2d
Sphingomonadales	Erythrobacteraceae	Novosphingobium	MMS21-M39	NIBRBAC000508740	Novosphingobium pokkalii	99.2	Soil	R2A	30°C, 3d
	Sphingomonadaceae	Sphingobium	BT751	NIBRBAC000508874	Sphingobium quisquiliarum	99.4	Soil	R2A	25°C, 3d
		Sphingomonas	BT752	NIBRBAC000508875	Sphingomonas alpina	<i>T.</i> 66	Soil	R2A	25°C, 3d
			BT753	NIBRBAC000508876	Sphingomonas gei	99.2	Soil	R2A P24	25°C, 3d
			U/ CSUMH	NIBKBACUUUU 80	sphingomonas montanisoti	1.66	Moss	KZA	30°C, 30

Table 1. Taxonomic affiliation and isolation information of the isolates belonging to the class Alphaproteobacteria.

caulaceae. Nine species in the genera Celeribacter, Marivita, Nioella, Pararhodobacter, Pseudooceanicola, Ruegeria, Seohaeicola and Tritonibacter of the family Rhodobacteracea within the Rhodobacterales. Strain CAU 1667 was related to Hyphobacterium vulgare (99.4% 16S rRNA gene sequence similarity). Strains 15MA-21, CAU 1659, SCSW-6, CAU 1660, CAU 1661, 14MAJJD-6, CAU 1648, CAU 1651 and AMTF-M12 had the highest similarities to Celeribacter halophilus (100%), Marivita roseacus (99.4%), Nioella ostreopsis (100%), Pararhodobacter marinus (98.8%), Pseudooceanicola lipolyticus (99.9%), Ruegeria profundi (98.8%), Seohaeicola nanhaiensis (99.7%), Seohaeicola zhoushanensis (99.5%) and Tritonibacter mobilis subsp. pelagius (99.4%) (Table 1). All strains were Gram-staining-negative and rod shaped (Fig. 1). Additionally, the phylogenetic positions of unrecorded bacteria were presented in Figs. 2 and 3. The detailed morphological, physiological and biochemical characteristics are shown description part. Herein, 27 bacterial isolates were collected from various sources of Korea areas are reported as unrecorded species of the class Alphaproteobacteria.

### Description of Hyphobacterium vulgare CAU 1667

Cells are Gram-stain-negative, aerobic, flagellated and rod shaped. Colonies grown on marine agar are cream, circular, entire, convex, smooth and opaque. Oxidase activity is positive. In the API 20NE test, cells are positive for nitrate reduction, esculin hydrolysis, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malate and trisodium citrate; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase,  $\beta$ -galactosidase, and assimilation of capric acid and phenylacetic acid. Strain CAU 1667 (= NIBRBAC000508846) was isolated from sea sediment collected from Inchon, Republic of Korea (37°26'01.9"N 126°24'53.1"E).

#### Description of Ochrobactrum teleogrylli HMF5357

Cells are Gram-stain-negative, aerobic, flagellated and rod shaped. Colonies grown on R2A are white, circular, convex and smooth. Oxidase activity is positive. In the API 20NE test, cells are positive for nitrate reduction, glucose fermentation, esculin hydrolysis, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malate and trisodium citrate; but negative for indole production, arginine dihydrolase, urease, gelatinase,  $\beta$ -galactosidase, and assimilation of adipic acid and phenylacetic acid. Strain HMF5357 (=NI-BRBAC000508753) was isolated from a soil sample collected from Yongin, Republic of Korea (37°20'22.9"N

## 127°16′15.3″E).

#### **Description of Methylobacterium indicum BT721**

Cells are Gram-stain-negative, aerobic, flagellated and rod shaped. Colonies grown on R2A are pink, circular, raised and opaque. Oxidase activity is negative. In the API 20NE test, cells are positive for indole production, esculin hydrolysis, gelatinase,  $\beta$ -galactosidase, and assimilation of potassium gluconate and capric acid; but negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain BT721 (=NIBRBAC000508870) was isolated from a soil sample collected from Samcheok, Republic of Korea (37°13'02.5"N 129°03'52.9"E).

## Description of *Methylobacterium pseudosasicola* HMF4628

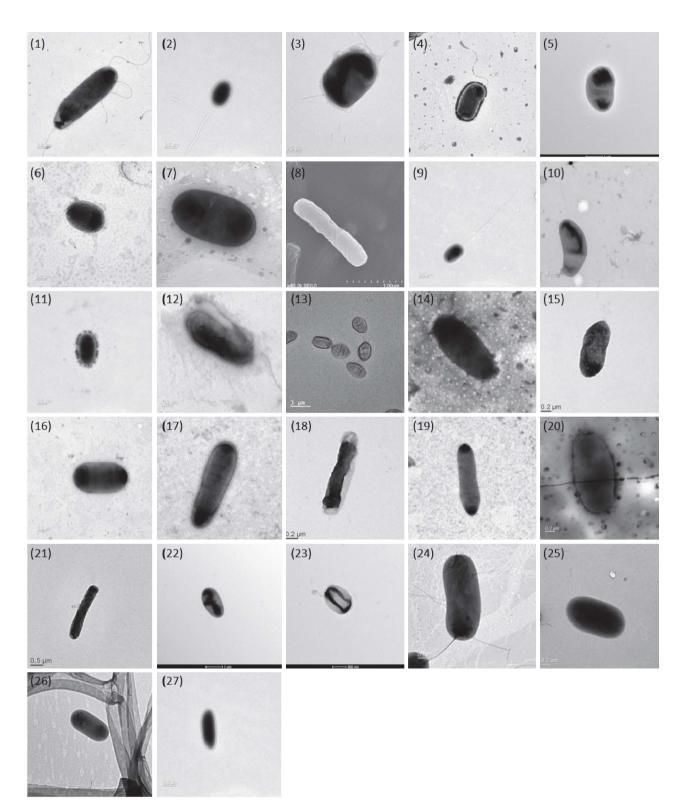
Cells are Gram-stain-negative, aerobic, flagellated and rod shaped. Colonies grown on R2A are pink, circular, convex and smooth. Oxidase activity is positive. In the API 20NE test, cells are positive for arginine dihydrolase, urease, and assimilation of D-glucose, L-arabinose, potassium gluconate, adipic acid; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatinase,  $\beta$ -galactosidase, and assimilation of D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, capric acid, malate, trisodium citrate and phenylacetic acid. Strain HMF4628 (=NIBRBAC000508752) was isolated from a costata birch bark sample collected from Gurye, Republic of Korea (35°17'46.6″N 127°31'14.6″E).

## Description of *Methylobacterium radiotolerans* MMS21-SJ4-13

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A are brown, circular, convex and erose. Oxidase activity is positive. In the API 20NE test, cells are positive for nitrate reduction, esculin hydrolysis, arginine dihydrolase and urease; but negative for indole production, glucose fermentation, gelatinase,  $\beta$ -galactosidase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain MMS21-SJ4-13 (=NIBRBAC000508742) was isolated from a soil sample collected from Okcheon, Republic of Korea (36°21'40.6"N 127°36'02.0"E).

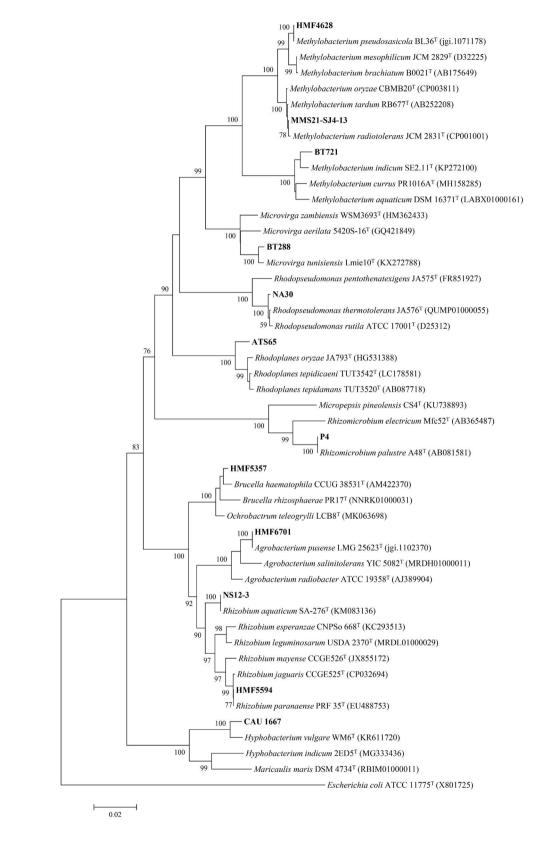
#### **Description of Microvirga tunisiensis BT288**

Cells are Gram-stain-negative, aerobic, non-flagellated and oval-shaped. Colonies grown on R2A agar are

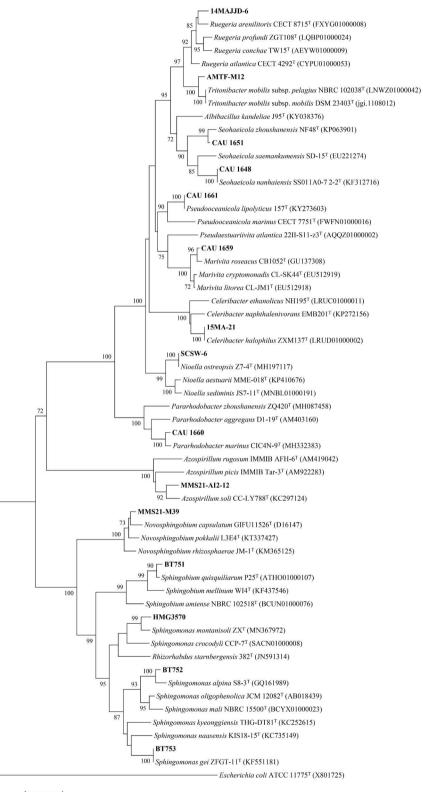


**Fig. 1.** Transmission electron micrographs of cells. Strains: 1, CAU 1667; 2, HMF5357; 3, BT721; 4, HMF4628; 5, MMS21-SJ4-13; 6, BT288; 7, ATS65; 8, NA30; 9, HMF6701; 10, NS12-3; 11, HMF5594; 12, P4; 13, 15MA-21; 14, CAU 1659; 15, SCSW-6; 16, CAU 1660; 17, CAU 1661; 18, 14MAJJD-6; 19, CAU 1648; 20, CAU 1651; 21, AMTF-M12; 22, MMS21-AI2-12; 23, MMS21-M39; 24, BT751; 25, BT752; 26, BT753; 27, HMG3570.

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**Fig. 2.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between the isolates and their relatives of the order *Caulobacterales*, *Hyphomicrobiales* and *Micropepsales*. Bootstrap values (>70%) are shown. *Escherichia coli* ATCC 11775<sup>T</sup> (X80725) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.



0.02

**Fig. 3.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between the isolates and their relatives of the order *Rhodobacterales*, *Rhodospirillales* and *Sphingomonadales*. Bootstrap values (>70%) are shown. *Escherichia coli* ATCC 11775<sup>T</sup> (X80725) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

light brown and circular. Oxidase activity is positive. In the API 20NE test, cells are positive for esculin hydrolysis; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase,  $\beta$ -galactosidase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain BT288 (=NIBRBAC000508867) was isolated from a soil sample collected from Uijeongbu, Republic of Korea (37°44′23.0″N 127°01′28.0″E).

#### Description of Rhodoplanes tepidicaeni ATS65

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A are white, circular, convex and glistening. Oxidase activity is negative. In the API 20NE test, cells are positive for nitrate reduction, esculin hydrolysis, urease, and assimilation of  $\beta$ -galactosidase and adipic acid; but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malate, trisodium citrate and phenylacetic acid. Strain ATS65 (=NIBRBAC000508881) was isolated from a soil sample collected from Seoul, Republic of Korea (37°34'01.4"N 127°08'59.3"E).

## Description of *Rhodopseudomonas thermotolerans* NA30

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A are white, round, entire and flat. Oxidase activity is negative. In the API 20NE test, cells are positive for urease and assimilation of D-glucose, L-arabinose, adipic acid, malate and phenylacetic acid; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, gelatinase,  $\beta$ -galactosidase, and assimilation of D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid and trisodium citrate. Strain NA30 (=NIBRBAC000508780) was isolated from a wetland sample collected from Seoul, Republic of Korea (37°34'02.2"N 127°08'59.1"E).

#### **Description of Agrobacterium pusense HMF6701**

Cells are Gram-stain-negative, facultative anaerobic, flagellated and rod shaped. Colonies grown on marine agar are white, circular, convex and smooth. Oxidase activity is positive. In the API 20NE test, cells are positive for nitrate reduction, esculin hydrolysis, arginine dihydrolase, urease,  $\beta$ -galactosidase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malate and trisodium citrate; but negative for indole production, glucose fermentation, gelatinase, and assimilation of capric acid, adipic acid and phenylacetic acid. Strain HMF6701 (=NIBRBAC000508755) was isolated from a soil sample collected from Wando, Republic of Korea  $(34^{\circ}19'36.9''N 126^{\circ}49'06.4''E)$ .

### Description of Rhizobium aquaticum NS12-3

Cells are Gram-stain-negative, aerobic, flagellated and rod shaped. Colonies grown on R2A agar are white, circular, convex and smooth. Oxidase activity is negative. In the API 20NE test, cells are positive for urease,  $\beta$ -galactosidase, and assimilation of D-glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and malate; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, gelatinase, and assimilation of L-arabinose, D-mannose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain NS12-3 (=NIBRBAC000508931) was isolated from a soil sample collected from Goyang, Republic of Korea (37°40'27.9″N 126°48'20.5″E).

#### Description of Rhizobium paranaense HMF5594

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A agar are white, circular, convex and smooth. Oxidase activity is positive. In the API 20NE test, cells are positive for nitrate reduction, esculin hydrolysis, urease,  $\beta$ -galactosidase, and assimilation of *N*-acetyl-glucosamine; but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain HMF5594 (= NIBRBAC000508754) was isolated from a soil sample collected from Yongin, Republic of Korea (37°20'18.3"N 127°16'01.3"E).

#### Description of Rhizomicrobium palustre P4

Cells are Gram-stain-negative, aerobic, flagellated and rod shaped. Colonies grown on R2A agar are white, circular, opaque and smooth. Oxidase activity is negative. In the API 20NE test, cells are positive for  $\beta$ -galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, gelatinase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain P4 (=NIBRBAC000508935) was isolated from a soil sample collected from Goyang, Republic of Korea (37°40'27.9″N 126°48'20.5″E).

#### **Description of Celeribacter halophilus 15MA-21**

Cells are Gram-stain-negative, aerobic, non-flagellated and short rod shaped. Colonies grown on marine agar are light white, circular and convex. Oxidase activity is positive. In the API 20NE test, cells are positive for esculin hydrolysis, arginine dihydrolase, urease,  $\beta$ -galactosidase, and assimilation of D-glucose, D-mannitol, capric acid, adipic acid and trisodium citrate; but negative for nitrate reduction, indole production, glucose fermentation, gelatinase, and assimilation of L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malate and phenylacetic acid. Strain 15MA-21 (=NI-BRBAC000508955) was isolated from a seaweed sample collected from Goseong, Republic of Korea (38°30'13.1"N 128°25'37.6"E).

#### Description of Marivita roseacus CAU 1659

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on marine agar are cream, circular, entire, convex, smooth and opaque. Oxidase activity is positive. In the API 20NE test, cells are positive for nitrate reduction, esculin hydrolysis,  $\beta$ -galactosidase; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain CAU 1659 (= NI-BRBAC000508839) was isolated from a sea sediment sample collected from Incheon, Republic of Korea (37°26'01.9"N 126°24'53.1"E).

#### **Description of Nioella ostreopsis SCSW-6**

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on marine agar are yellowish white, circular, convex and glistening. Oxidase activity is negative. In API 20NE test, cells are positive for nitrate reduction, esculin hydrolysis, gelatinase,  $\beta$ -galactosidase, and assimilation of potassium gluconate and adipic acid; but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, malate, trisodium citrate and phenylacetic acid. Strain SCSW-6 (=NIBRBAC000508908) was isolated from a seawater sample collected from Sokcho, Republic of Korea (38°13'42"N 128°35'13"E).

#### Description of Pararhodobacter marinus CAU 1660

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on marine agar are cream, circular, entire, convex, smooth and opaque. Oxidase activity is positive. In the API 20NE test, cells are positive for esculin hydrolysis, urease, gelatinase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase,  $\beta$ -galactosidase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain CAU 1660 (=NIBRBAC000508840) was isolated from a sea sediment sample collected from Geoje, Republic of Korea (34°81'29.6"N 128°70'70.5"E).

## Description of *Pseudooceanicola lipolyticus* CAU 1661

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on marine agar are cream, circular, entire, convex, smooth and opaque. Oxidase activity is positive. In the API 20NE test, cells are positive for urease, esculin hydrolysis, and assimilation of *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malate and phenylacetic acid; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase,  $\beta$ -galactosidase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, capric acid and trisodium citrate. Strain CAU 1661 (=NIBRBAC000508842) was isolated from a sea sediment sample collected from Geoje, Republic of Korea (34°81'29.6″N 128°70'70.5″E).

#### Description of Ruegeria profundi 14MAJJD-6

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown marine agar are pale yellow, circular, slightly convex and glistening. Oxidase activity is negative. In the API 20NE test, cells are positive for nitrate reduction and  $\beta$ -galactosidase; but negative for indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, gelatinase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain 14MAJJD-6 (=NIBRBAC000508915) was isolated from a tidal flat sample collected from Jangheung, Republic of Korea (34°36′49.2″N 127°00′41.7″E).

#### Description of Seohaeicola nanhaiensis CAU 1648

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on marine agar are cream, punctiform entire, convex, smooth and translucent. Oxidase activity is positive. In the API 20NE test, cells are positive for esculin hydrolysis and assimilation of D-glucose, L-arabinose, D-mannitol, potassium gluconate, adipic acid, malate and phenylacetic acid; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase,  $\beta$ -galactosidase, and assimilation of D-mannose, N-acetyl-glucosamine, D-maltose, capric acid and trisodium citrate. Strain CAU 1648 (=NIBRBAC000508830) was isolated from a seawater sample collected from Ganghwa, Republic of Korea (37°42'01.1″N 126°22'44.6″E).

#### Description of Seohaeicola zhoushanensis CAU 1651

Cells are Gram-stain-negative, facultative anaerobic, non-flagellated and rod shaped. Colonies grown on GYE agar are cream, circular, entire, convex, smooth and translucent. Oxidase activity is positive. In the API 20NE test, cells are positive for urease, esculin hydrolysis, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, adipic acid, malate, trisodium citrate and phenylacetic acid; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase,  $\beta$ -galactosidase, and assimilation of *N*-acetyl-glucosamine and capric acid. Strain CAU 1651 (=NIBRBAC000508833) was isolated from a seawater sample collected from Ganghwa, Republic of Korea (37°38'59.6"N 126°20'02.8"E).

# Description of *Tritonibacter mobilis* subsp. *pelagius* AMTF-M12

Cells are Gram-stain-negative, aerobic, non-flagellated, and ovoid and rod shaped. Colonies grown on marine agar are dark yellow, circular, convex and glistening. Oxidase activity is negative. In the API 20NE test, cells are positive for esculin hydrolysis, gelatinase and  $\beta$ -galactosidase; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain AMTF-M12 (=NI-BRBAC000508906) was isolated from a tidal flat sample collected from Taean, Republic of Korea (36°33'58.6"N 126°21'57.1"E).

## Description of Azospirillum soli MMS21-AI2-12

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A are white, circular, convex and entire. Oxidase activity is positive. In the API 20NE test, cells are positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase,  $\beta$ -galactosidase, and assimilation of D-glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, malate, trisodium citrate and phenylacetic acid; but negative for indole production, arginine dihydrolase, urease, and assimilation of D-mannose, capric acid and adipic acid. Strain MMS21-AI2-12 (= NI-BRBAC000508731) was isolated from a soil sample collected from Okcheon, Republic of Korea (36°19′24.3″N 127°43′42.4″E).

## Description of *Novosphingobium pokkalii* MMS21-M39

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A are pale yellow, circular, convex and entire. Oxidase activity is positive. In the API 20NE test, cells are positive for glucose fermentation, arginine dihydrolase and esculin hydrolysis; but negative for nitrate reduction, indole production, urease, gelatinase,  $\beta$ -galactosidase, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain MMS21-M39 (=NIBRBAC000508740) was isolated from a soil sample collected from Daejeon, Republic of Korea (36°19'08.5"N 127°20'19.9"E).

#### Description of Sphingobium quisquiliarum BT751

Cells are Gram-stain-negative, aerobic, flagellated and rod shaped. Colonies grown on R2A agar are yellow, smooth, circular, convex and opaque. Oxidase activity is positive. In the API 20NE test, cells are positive for gelatinase and assimilation of D-glucose, L-arabinose and D-mannose; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease,  $\beta$ -galactosidase, and assimilation of D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain BT751 (=NI-BRBAC000508874) was isolated from a soil sample collected from Seogwipo, Republic of Korea (33°14′19″N 126°17′16″E).

## **Description of Sphingomonas alpina BT752**

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A agar are yellow, circular, convex, glistening, round and entire margins. Oxidase activity is positive. In the API 20NE test, cells are positive for gelatinase activity and assimilation of L-arabinose, potassium gluconate and malate; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease,  $\beta$ -galactosidase, and assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain BT752 (= NIBRBAC000508875) was isolated from a soil sample collected from Jeju, Republic of Korea (33°19′19″N 126°15′55″E).

#### Description of Sphingomonas gei BT753

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A agar are bright yellow, circular, convex, glistening and smooth. Oxidase activity is positive. In the API 20NE test, cells are positive for gelatinase,  $\beta$ -galactosidase, and assimilation of D-glucose, D-mannitol, D-maltose and potassium gluconate; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, and assimilation of L-arabinose, D-mannose, *N*-acetyl-glucosamine, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain BT753 (=NIBRBAC000508876) was isolated from a soil sample collected from Jeju, Republic of Korea (33°26′05″N 126°41′20″E).

#### Description of Sphingomonas montanisoli HMG3570

Cells are Gram-stain-negative, aerobic, non-flagellated and rod shaped. Colonies grown on R2A are cream, circular, convex and smooth. Oxidase activity is positive. In the API 20NE test, cells are positive for assimilation of D-glucose; but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, gelatinase,  $\beta$ -galactosidase, and assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. Strain HMG3570 (=NIBRBAC000508768) was isolated from a moss sample collected from Yongin, Republic of Korea (37°20'20.1"N 127°15'55.5"E).

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## REFERENCES

Bazylinski, D.A., T.J. Williams, C.T. Lefevre, R.J. Berg, C.L. Zhang, S.S. Bowser, A.J. Dean and T.J. Beveridge. 2013. *Magnetococcus marinus* gen. nov., sp. nov., a marine, magnetotactic bacterium that represents a novel lineage (*Magnetococcaceae* fam. nov., *Magnetococcales* ord. nov.) at the base of the *Alphaproteobacteria*. International Journal of Systematic and Evolutionary Microbiology 63(3):801-808.

Beijerinck, M.W. 1898. Ueber die arten der essigbakterien.

Cent. für Bakteriol. Parasitenkd. und Infekt. 4:209-216.

- Douglas, H.C. 1957. Order III. *Hyphomicrobiales* Douglas, ordo nov. In: R.S. Breed, E.G.D. Murray and N.R. Smith (eds), Bergey's Manual of Determinative Bacteriology, 7th edition, The Williams and Wilkins Co., Baltimore, pp. 276-280.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4):783.
- Garrity, G.M., J.A. Bell and T. Lilburn. 2005a. Class I. Alphaproteobacteria class. nov. In: D.J. Brenner, N.R. Krieg, J.T. Staley and G.M. Garrity (eds), Bergey's Manual of Systematic Bacteriology, 2nd edition, vol. 2 (The Proteobacteria), part C (The Alpha-, Beta-, Delta-, and Epsilonproteobacteria), Springer, New York, p. 1.
- Garrity, G.M., J.A. Bell and T. Lilburn. 2005b. Order III. *Rhodobacterales* ord. nov. In: D.J. Brenner, N.R. Krieg, J.T. Staley and G.M. Garrity. (eds), Bergey's Manual of Systematic Bacteriology, 2nd edition, vol. 2 (The *Proteobacteria*), part C (The Alpha-, Beta-, Delta-, and *Epsilonproteobacteria*), Springer, New York, p. 161.
- Gieszczykiewicz, M. 1939. Bulletin de l'Academie Polonaise des Sciences. Serie des Sciences Biologiques 1:9-27.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series Information 41:95-98.
- Harbison, A.B., L.E. Price, M.D. Flythe and S.L. Brauer. 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(4):839-844.
- Henrici, A.T. and D.E. Johnson. 1935. Studies of freshwater bacteria: II. Stalked bacteria, a new order of Schizomycetes. Journal of Bacteriology 30(1):61-93.
- Hördt, A., M.G. López, J.P. Meier-Kolthoff, M. Schleuning, L. Weinhold, B.J. Tindall, S. Gronow, N.C. Kyrpides, T. Woyke and M. Göker. 2020. Analysis of 1,000+ typestrain genomes substantially improves taxonomic classification of *Alphaproteobacteria*. Frontiers in Microbiology 11:468.
- Iino, T., M. Ohkuma, Y. Kamagata and S. Amachi. 2016. *Iodidimonas muriae* gen. nov., sp. nov., an aerobic iodide-oxidizing bacterium isolated from brine of a natural gas and iodine recovery facility, and proposals of *Iodidimonadaceae* fam. nov., *Iodidimonadales* ord. nov., *Emcibacteraceae* fam. nov. and *Emcibacterales* ord. nov. International Journal of Systematic and Evolutionary Microbiology 66(12):5016-5022.
- Kim, M. and J. Chun. 2014. 16S rRNA gene-based identification of bacteria and archaea using the EzTaxon server. In: Methods in microbiology, Academic Press, pp. 61-74.
- Kumar, S., G. Stecher and K. Tamura 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7):1870-

1874.

- Kurahashi, M., Y. Fukunaga, S. Harayama and A. Yokota. 2008. Sneathiella glossodoripedis sp. nov., a marine alphaproteobacterium isolated from the nudibranch Glossodoris cincta, and proposal of Sneathiellales ord. nov. and Sneathiellaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology 58(3):548-552.
- Kwon, K.K., H. Lee, S.H. Yang and S. Kim. 2005. Kordiimonas gwangyangensis gen. nov., sp. nov., a marine bacterium isolated from marine sediments that forms a distinct phyletic lineage (Kordiimonadales ord. nov.) in the 'Alphaproteobacteria'. International Journal of Systematic and Evolutionary Microbiology 55(5):2033-2037.
- Lane, D.J. 1991. 16S/23S rRNA sequencing. In: E. Stackebrandt and M. Goodfellow (eds.), Nucleic acid techniques in bacterial systematics, John Wiley and Sons, New York, pp. 125-175.
- Liu, Q., S. Kim, J. Yoon, K. Joh, C. Seong, C. Jeon, W. Kim, M.K. Kim and W. Im. 2019. Description of 42 unrecorded bacterial species in Korea, belonging to the class *Alphaproteobacteria*. Journal of Species Research 8(4):351-364.
- Pfenning, N. and H.G. Trüper. 1971. Higher taxa of the phototrophic bacteria. International Journal of Systematic and Evolutionary Microbiology 21(1):17-18.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4(4):406-425.
- Sun, C., L. Xu, X. Yu, Z. Zhao, Y. Wu, A. Oren, C. Wang and X. 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(12):3856-3862.

- Szokoli, F., M. Castelli, E. Sabaneyeva, M. Schrallhammer, S. Krenek, T.G. Doak, T.U. Berendonk and G. Petroni. 2016. Disentangling the taxonomy of *Rickettsiales* and description of two novel symbionts ("*Candidatus* Bealeia paramacronuclearis" and "*Candidatus* Fokinia cryptica") sharing the cytoplasm of the ciliate protist *Paramecium biaurelia*. Applied and Environmental Microbiology 82(24):7236-7247.
- Ramana, V.V., S.K. Chakravarthy, E.V.V. Ramaprasad, V. Thiel, J.F. Imhoff, C. Sasikala and C.V. Ramana. 2013. Emended description of the genus *Rhodothalassium* Imhoff *et al.*, 1998 and proposal of *Rhodothalassiaceae* fam. nov. and *Rhodothalassiales* ord. nov. Systematic and Applied Microbiology 36(1):28-32.
- Yabuuchi, E. and Y. Kosako. 2005. Order IV. Sphingomonadales ord. nov. In: D.J. Brenner, N.R. Krieg, J.T. Staley and G.M. Garrity (eds.), Bergey's Manual of Systematic Bacteriology, 2nd edition, vol. 2 (The Proteobacteria), part C (The Alpha-, Beta-, Delta-, and Epsilonproteobacteria), Springer, New York, pp. 230-233.
- 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 gene sequences and whole-genome assemblies. International Journal of Systematic and Evolutionary Microbiology 67(5):1613-1617.

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