

A report of 23 unrecorded bacterial species belonging to the class *Alphaproteobacteria*

Muhammad Zubair Siddiqi¹, Seung-Bum Kim², Jang-Cheon Cho³, Jung-Hoon Yoon⁴, Kiseong Joh⁵, Chi-Nam Seong⁶, Jin-Woo Bae⁷, Kwang-Yeop Jahng⁸, Che-Ok Jeon⁹ and Wan-Taek Im^{1,*}

¹Department of Biotechnology, Hankyong National University, Anseong 17579, Republic of Korea

²Department of Microbiology, Chungnam National University, Daejeon 34134, Republic of Korea

³Department of Biological Sciences, Inha University, Incheon 22212, Republic of Korea

⁴Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea

⁵Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Gyeonggi 17035, Republic of Korea

⁶Department of Biology, Sunchon National University, Suncheon 57922, Republic of Korea

⁷Department of Biology, Kyung Hee University, Seoul 02447, Republic of Korea

⁸Department of Life Sciences, Chonbuk National University, Jeonju-si 54896, Republic of Korea

⁹Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

*Correspondent: wandra@hknu.ac.kr

To study the biodiversity of bacterial species, here we report indigenous prokaryotic species of Korea. A total of 23 bacterial strains affiliated to the class *Alphaproteobacteria* were isolated from various environmental sources including seaweeds, seawater, fresh water, wetland/marsh, tidal sediment, plant roots, sewage and soil. Considering higher than 98.8% 16S rRNA gene sequence similarities and formation of a well-defined phylogenetic clade with named species, it was confirmed that each strain belonged to the predefined bacterial species of the class *Alphaproteobacteria*. There is no official report of these 23 species in Korea; 20 species of 16 genera (*Mameliella*, *Yangia*, *Paracoccus*, *Ruegeria*, *Loktanella*, *Phaeobacter*, *Dinoroseobacter*, *Tropicimonas*, *Lutimaribacter*, *Litoreibacter*, *Sulfitobacter*, *Roseivivax*, *Labrenzia*, *Hyphomonas*, *Maricaulis*, *Thalassospira*) in the order *Rhodobacterales* and 3 species of a single genus (*Brevundimonas*) in the order *Caulobacterales*. Gram-staining, cell morphology, basic biochemical characteristics, isolation sources, optimum temperature, growth media, and strain IDs are detailed in the species description as well as Table 1.

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

© 2021 National Institute of Biological Resources
DOI:10.12651/JSR.2021.10.3.191

INTRODUCTION

In 2016, many novel and unreported bacterial species were isolated from different environmental samples collected in Korea. Based on the 16S rRNA gene sequence analysis, the identified bacterial species belong to the class *Alphaproteobacteria*.

Therefore, the aim of this study is to describe the unrecorded species belonging to the class *Alphaproteobacteria*.

In 1987, Carl Woese (Woese, 1987) suggested that based on nucleotide sequences similarity of the bacterial genome, a large and diverse group of bacteria which were called purple bacteria should be classified as a separate phylum within domain Bacteria. Afterwards, this phylum

was established under the name *Proteobacteria*. The phylum *Proteobacteria* includes many bacterial strains that are pathogens and part of the normal human microbiota, and can be further classified into five classes: 1. *Alphaproteobacteria*, 2. *Betaproteobacteria*, 3. *Gammaproteobacteria*, 4. *Deltaproteobacteria*, and 5. *Epsilonproteobacteria*.

Alphaproteobacteria is the first class of phylum *Proteobacteria*, with many important biological characteristics. Members of this class are oligotrophs and are able to live in low-nutrient environments such as sediments, deep under-surface, deep ocean, glacial ice and soil (Krom *et al.*, 1991; Pitta *et al.*, 2005; DeLong *et al.*, 2006; Thompson *et al.*, 2013). Affiliates of the class *Alphaproteobacteria* are gram-

stain-negative and some parasitic members lack peptidoglycan and are thus gram variable (Brenner *et al.*, 2005; Euzéby, 2011). Furthermore, the class *Alphaproteobacteria* is divided into three subdivisions: *Rickettsidae*, *Magnetococcidae*, and *Caulobacteridae* (Ferla *et al.*, 2013). Among these subclasses, the basal class/group *Magnetococcidae* consists of a large variety of magnetotactic bacteria, in which only [*Magnetococcus marinus*] is described by Bazylinski *et al.*, 2012. As such, these subdivisions of *Alphaproteobacteria* are comprised of diverse group of bacteria that are plant and animal pathogens, plant mutualists, photosynthetic, and also several genera metabolizing C1-compounds (*Methylobacterium*) (Williams *et al.*, 2007). However, until now, no scientist has studied the molecular or biochemical characteristics that can differentiate these bacteria from other groups.

Similarly, changes in the metabolic strategies are found in the class *Alphaproteobacteria*, including ammonia oxidation, nitrogen fixation, photosynthesis and methylotrophic. Different morphologies (stellate, stalked, and spiral) are also found within the members of class *Alphaproteobacteria*. Therefore, some developmental programs switch between cell types, and are controlled by a web of regulatory systems (Viollier and Shapiro, 2004).

A special interest has been taken in the class *Alphaproteobacteria* as the ancestral group for mitochondria. The most often cited subgroup of *Alphaproteobacteria* is *Rickettsiales*, from which mitochondria are inferred to have arisen from, which there is disagreement on this point (Esser *et al.*, 2004; Wu *et al.*, 2004; Fitzpatrick *et al.*, 2006). Moreover, genome sequencing has revealed a well-maintained molecular marker which is characteristics of either all *Alphaproteobacteria* or its main orders. This suggestion provides the assignment of new bacterial species into these groups, which conveys that *Alphaproteobacteria* has branched off later than most other classes, with the exception of *Beta-* and *Gammaproteobacteria* (Parte, 2014; Oren and Garrity, 2014).

In the current investigation, we briefly describe 23 unrecorded bacterial species in Korea in the class *Alphaproteobacteria* belonging to 4 families of 2 orders. This research program was conducted and supported by NIBR Korea.

MATERIALS AND METHODS

A total of 23 bacterial strains belong to the class *Alphaproteobacteria* were isolated from different environmental sources collected from plant roots, soil, tidal sediment, sea sources (including water, weeds, grasses) and freshwater (Table 1). Each environmental sample was processed separately, spread onto diverse culture media including R2A and marine agar 2216, and incubated at 20, 25, 30 and

35°C for 1–5 days (Table 1). The designated strain IDs, sources, culture media, and incubation conditions are summarized in Table 1. All strains were purified as single colonies and stored both as 10–20% glycerol suspension at –80°C and as lyophilized ampoules.

The colony morphology was studied on agar plates until the cell grew up to their stationary phase. Cell size and shape were examined either by transmission electron microscopy or scanning electron microscopy. Gram staining was performed using a Gram-staining kit (bioMérieux) or standard procedures. The biochemical characteristics were performed using API 20NE (bioMérieux) according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification and 16S rRNA gene sequencing were performed using the standard procedures described elsewhere. The 16S rRNA gene sequences of the strains assigned to *Alphaproteobacteria* were compared with the sequences held in GenBank by BLASTN and also analyzed using the EzBioCloud (<https://www.ezbiocloud.net>) (Kim *et al.*, 2017). For phylogenetic analyses, multiple alignments were performed using the Clustal_X program (Thompson *et al.*, 1997), with gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA6 (Tamura *et al.*, 2013) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

The 23 strains were distributed in 2 orders of *Alphaproteobacteria*, 20 strains in the order *Rhodobacterales* and 3 strains in the order *Caulobacterales* (Table 1). These strains were Gram-staining-negative, chemoheterotrophic, coccoid, rod and short-rod shaped bacteria, except for strain CSC-1 (23) (Fig. 1).

The strains in the order *Rhodobacterales* (Fig. 2) belong to 3 families and 16 genera: *Mameliella* (1 species), *Yangia* (1 species), *Paracoccus* (2 species), *Tropicimonas* (1 species), *Lutimaribacter* (1 species), *Litoreibacter* (1 species), *Sulfitobacter* (1 species), *Roseivivax* (1 species), *Hyphomonas* (1 species), and *Thalassospira* (2 species) (Fig. 2, Table 1).

Figure 3 shows phylogenetic assignment of 10 strains of the order *Rhodobacterales* and 3 strains belong to *Brevundimonas* of the family *Caulobacteraceae*.

Here we report 23 unrecorded bacterial species in Korea belonging to 4 families of 2 orders in the *Alphaproteobacteria*.

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

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
<i>Caulobacterales</i>	<i>Caulobacteraceae</i>	<i>Brevundimonas</i>	YHD2	NIBRBAC000498430	<i>Brevundimonas bullata</i> IAM 13153 ^T	99.0	Sewage treatment plant	R2A	30°C, 3d
		<i>Brevundimonas</i>	HMF4573	NIBRBAC000498442	<i>Brevundimonas variabilis</i> ATCC 15255 ^T	100.0	Wetland or marsh	R2A	25°C, 3d
		<i>Brevundimonas</i>	HMF4667	NIBRBAC000498446	<i>Brevundimonas staleyii</i> FWC43 ^T	99.0	Wetland or marsh	R2A	25°C, 3d
<i>Rhodobacteriales</i>		<i>Mameliella</i>	FIL 61	NIBRBAC000498407	<i>Mameliella phaeodactyli</i> KD53 ^T	100.0	Tidal	MA	30°C, 2d
		<i>Yangia</i>	T4-2	NIBRBAC000498427	<i>Yangia pacifica</i> DSM 26894 ^T	99.7	Seashore	MA	30°C, 2d
		<i>Paracoccus</i>	YH6C	NIBRBAC000498429	<i>Paracoccus aminovorans</i> DSM 8537 ^T	99.4	Sewage treatment plant	R2A	30°C, 3d
		<i>Ruegeria</i>	SF30	NIBRBAC000498476	<i>Ruegeria atlantica</i> CECT 4292 ^T	100.0	Gulfweed	MA	25°C, 3d
		<i>Loktanella</i>	ZOD2-5	NIBRBAC000498479	<i>Loktanella rosea</i> DSM 29591 ^T	99.4	Seagrass	MA	25°C, 3d
		<i>Phaeobacter</i>	EC2	NIBRBAC000498481	<i>Phaeobacter inhibens</i> DSM 16374 ^T	100.0	Seaweed	MA	25°C, 3d
		<i>Dinoroseobacter</i>	GLB36	NIBRBAC000498483	<i>Dinoroseobacter shibae</i> DFL 12 ^T	100.0	Scallop	MA	25°C, 5d
		<i>Tropicimonas</i>	CAU 1140	NIBRBAC000498503	<i>Tropicimonas sediminiticola</i> DSM 29339 ^T	99.5	Reclaimed soil	MA	30°C, 5d
		<i>Lutimaribacter</i>	CAU 1340	NIBRBAC000498508	<i>Lutimaribacter saemankamensis</i> DSM 28010 ^T	99.1	Sea soil	MA	35°C, 5d
		<i>Litoreibacter</i>	LPB0157	NIBRBAC000498528	<i>Litoreibacter albidus</i> DSM 26922 ^T	100.0	Seashore	MA	35°C, 1d
		<i>Sulfitobacter</i>	LPB0162	NIBRBAC000498532	<i>Sulfitobacter mediterraneus</i> KCTC 32188 ^T	99.9	Seashore	MA	25°C, 1d
		<i>Roseivivax</i>	IMCC25645	NIBRBAC000498544	<i>Roseivivax pacificus</i> 22DY03 ^T	99.8	Plant roots	MA	20°C, 3d
		<i>Paracoccus</i>	JHR-13	NIBRBAC000498633	<i>Paracoccus seriniphilus</i> MBT-A4 ^T	99.9	Sea water	R2A	25°C, 3d
<i>Paracoccus</i>	CSC-1	NIBRBAC000498638	<i>Paracoccus yeei</i> ATCC BAA-599 ^T	99.6	Fresh water	R2A	25°C, 3d		
<i>Labrenzia</i>	SFD13	NIBRBAC000498470	<i>Labrenzia alba</i> CECT 5094 ^T	98.8	Gulf weed	R2A	25°C, 5d		
<i>Labrenzia</i>	EC2D15	NIBRBAC000498485	<i>Labrenzia aggregata</i> IAM 12614 ^T	98.9	Sea weed	MA	25°C, 3d		
<i>Hyphomonadaceae</i>	<i>Hyphomonas</i>	IMCC25644	NIBRBAC000498543	<i>Hyphomonas jannaschiana</i> VP2 ^T	99.9	Plant roots	MA	20°C, 3d	
	<i>Maricaultis</i>	HMF6043	NIBRBAC000498449	<i>Maricaultis maris</i> MCS10 ^T	99.2	Sea water	MA	25°C, 3d	
<i>Rhodospirillaceae</i>	<i>Thalassospira</i>	IMCC25636	NIBRBAC000498535	<i>Thalassospira profundimaris</i> WP0211 ^T	99.9	Plant roots	MA	20°C, 3d	
	<i>Thalassospira</i>	IMCC25646	NIBRBAC000498545	<i>Thalassospira australica</i> NP3b2 ^T	99.6	Fresh water	MA	20°C, 3d	

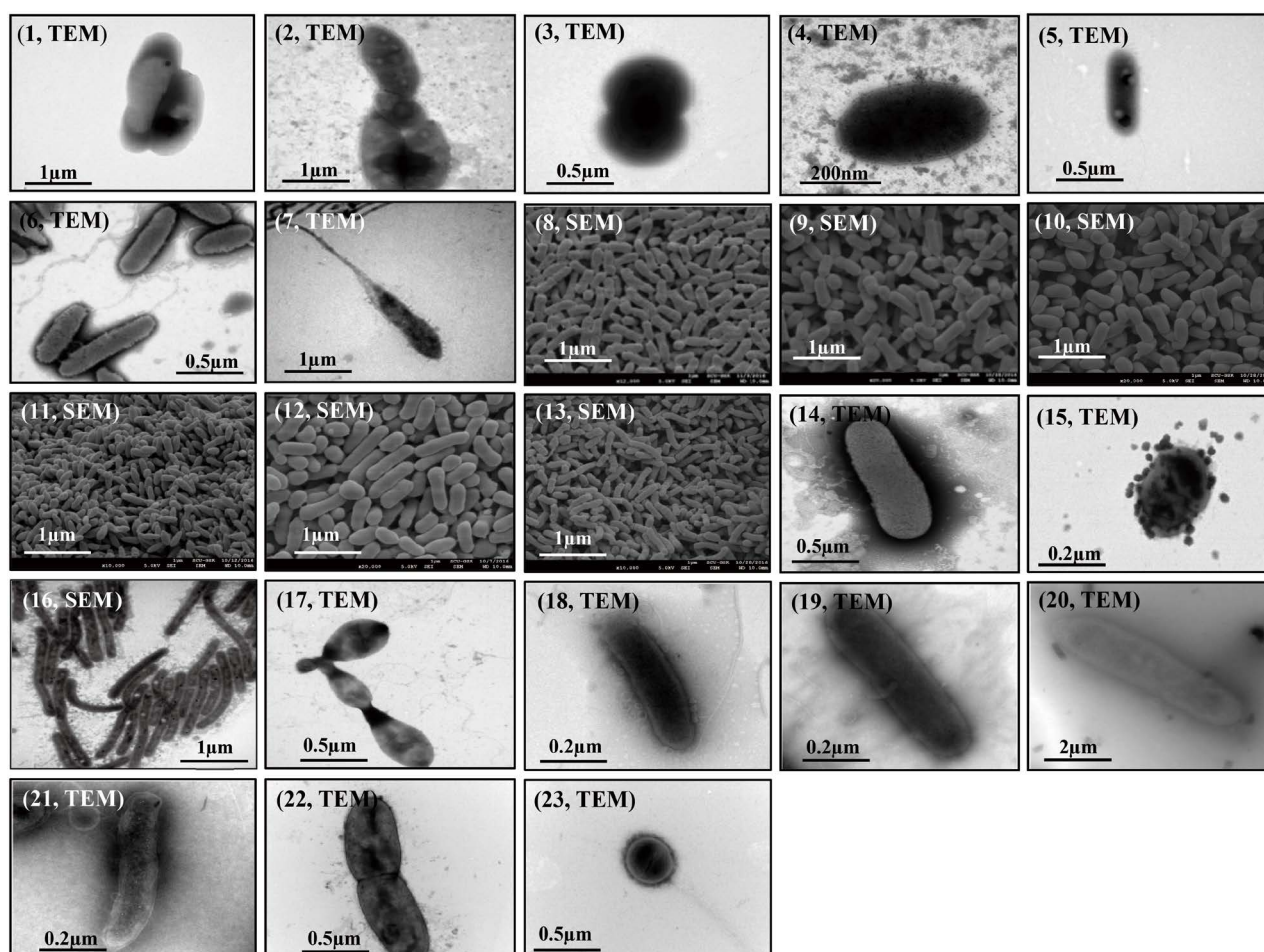


Fig. 1. Transmission and scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, *Mameliella phaeodactyli* FIL 61 (TEM); 2, *Yangia pacifica* T4-2 (TEM); 3, *Paracoccus aminovorans* YH6C (TEM); 4, *Brevundimonas bullata* YHD2 (TEM); 5, *Brevundimonas variabilis* HMF4573 (TEM); 6, *Brevundimonas staleyi* HMF4667 (TEM); 7, *Maricaulis maris* HMF6043 (TEM); 8, *Labrenzia alba* SFD13 (SEM); 9, *Ruegeria atlantica* SF30 (SEM); 10, *Loktanella rosea* ZOD2-5 (SEM); 11, *Phaeobacter inhibens* EC2 (SEM); 12, *Dinoroseobacter shibae* GLB36 (SEM); 13, *Labrenzia aggregata* EC2D15 (SEM); 14, *Tropicimonas sediminicola* CAU 1140 (TEM); 15, *Lutimaribacter saemankumensis* CAU 1340 (TEM); 16, *Litoreibacter albidus* LPB0157 (SEM); 17, *Sulfitobacter mediterraneus* LPB0162 (TEM); 18, *Thalassospira profundimaris* IMCC25636 (TEM); 19, *Hyphomonas jannaschiana* IMCC25644 (TEM); 20, *Roseivivax pacificus* IMCC25645 (TEM); 21, *Thalassospira tepidiphila* IMCC25646 (TEM); 22, *Paracoccus seriniphilus* JHR-13 (TEM); 23, *Paracoccus yeei* CSC-1 (TEM).

Description of *Mameliella phaeodactyli* FIL 61

Cells are Gram-staining-negative, non-flagellated, diffusible pigmented and short rod. Colonies are circular, convex and ivory colored after 2 days of incubation on MA at 30°C. Positive for nitrate reduction, urease and esculin hydrolysis in API 20NE; but negative for gelatinase, β -galactosidase, indole production, glucose fermentation and arginine dihydrolase. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain FIL 61 (= NIBRBAC000498407) was isolated from tidal water, Chungcheongnam-do, Korea.

Description of *Yangia pacifica* T4-2

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, raised, entire and pale-yellow colored after 2 days of incubation on MA at 30°C. Strain T4-2 is positive for hydrolysis of esculin, gelatin, glucose fermentation, and β -galactosidase; and negative for nitrate reduction, arginine dihydrolase, urease and indole production. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, N-acetyl-glucosamine, trisodium citrate and malic acid; but does not utilize capric acid, adipic acid, phenylacetic acid and potassium gluconate. Strain T4-2 (= NIBRBAC000498427) was isolated from seashore sand, Chungcheongnam-do, Korea.

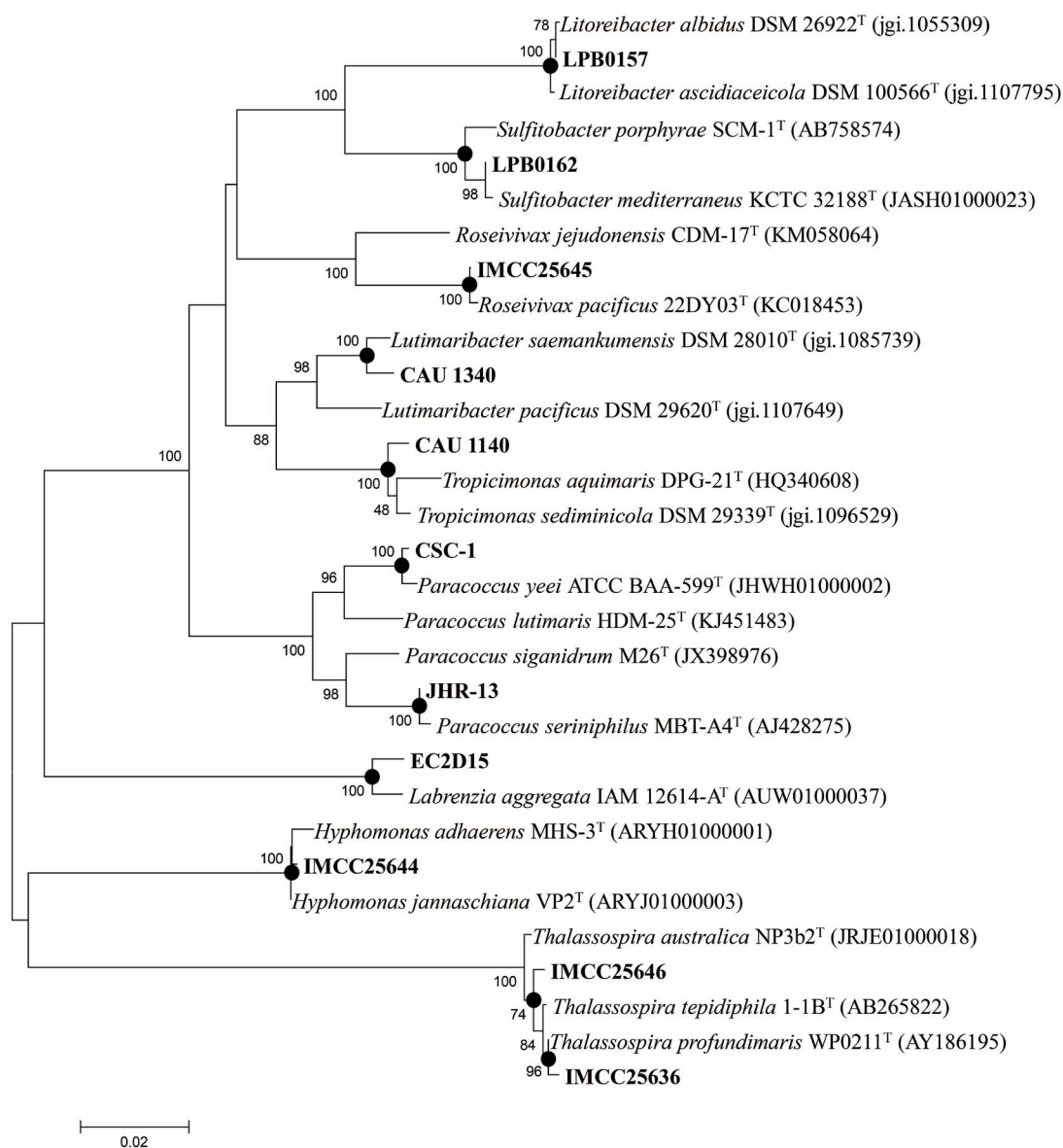


Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Rhodobacterales* in the class *Alphaproteobacteria*. Bootstrap values (>70%) are shown in the neighbor-joining method. Filled circles indicate the nodes recovered by the maximum-likelihood & maximum-parsimony treeing algorithms. Bar, 0.02 substitutions per nucleotide position.

Description of *Paracoccus aminovorans* YH6C

Cells are Gram-staining-negative, non-flagellated, non-pigmented and coccoid-rod shaped. Colonies are punctiform, smooth and red orange colored after 3 days of incubation on R2A agar medium at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β -galactosidase; but negative for urease and indole production. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate,

malic acid, trisodium citrate and phenylacetic acid. Does not utilize capric acid and adipic acid. Strain YH6C (= NIBRBAC000498429) was isolated from sewage treatment plant, Busan, Korea.

Description of *Brevundimonas bullata* YHD2

Cells are Gram-reaction-negative, non-flagellated, non-pigmented and rod-shaped. Colonies grown on R2A agar plates are ivory, circular, raised and entire after 3 days of incubation at 30°C. In API 20NE, positive for nitrate

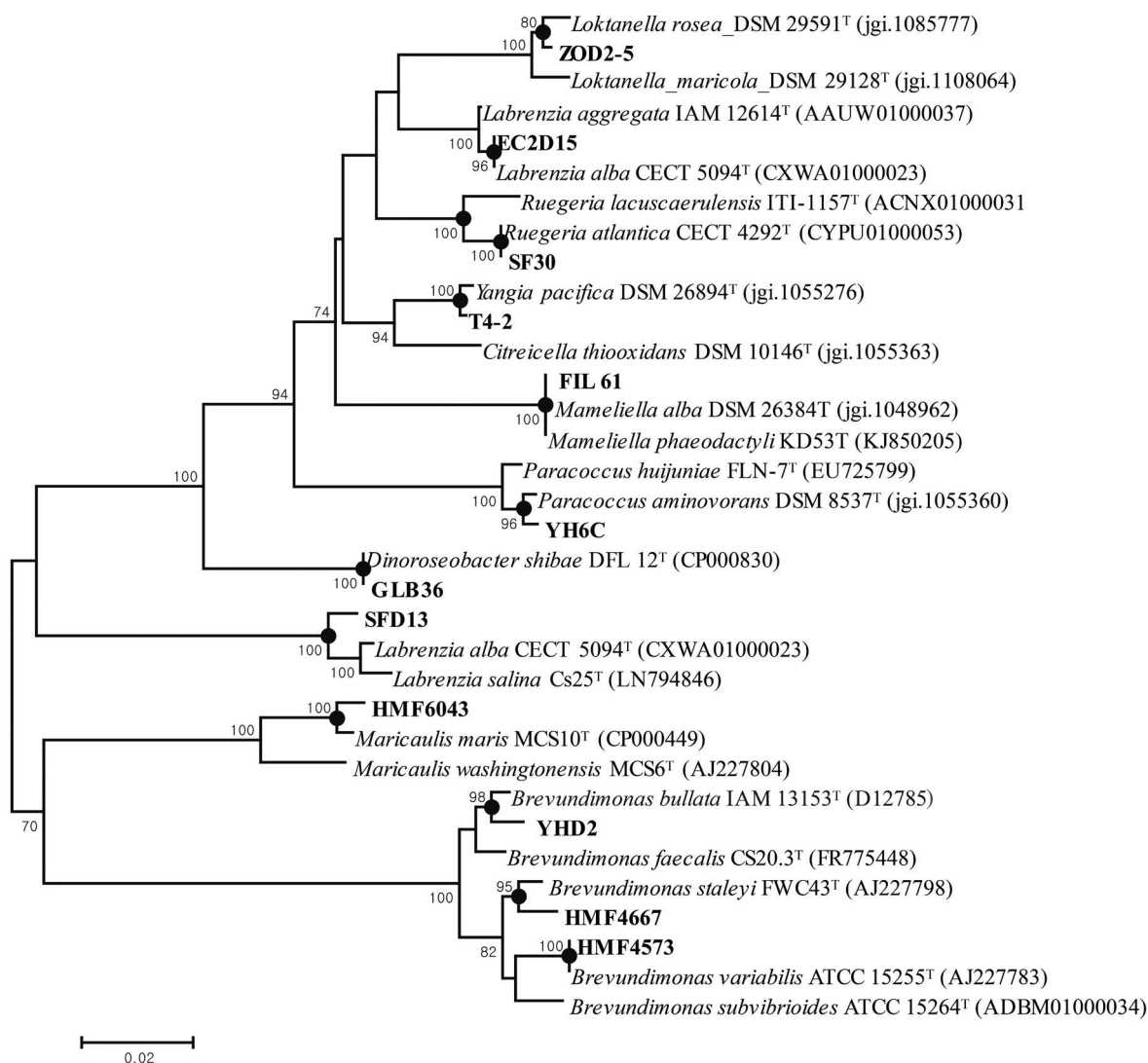


Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Rhizobiales*, *Caulobacterales* and *Rhodobacterales* in the class *Alphaproteobacteria*. Bootstrap values are greater than 70% are shown in the neighbor-joining tree method. Filled circles indicate the nodes recovered by the maximum-likelihood & maximum-parsimony treeing methods. Bar, 0.02 substitutions per nucleotide position.

reduction and urease; but negative for gelatinase, esculin hydrolysis, glucose fermentation, β -galactosidase, indole production and arginine dihydrolase. Does not utilize D-glucose, D-mannitol, *N*-acetyl-glucosamine, capric acid, malic acid, adipic acid, L-arabinose, D-maltose and trisodium citrate. Utilizes potassium gluconate and phenylacetic acid. Strain YDH2 (= NIBRBAC000498430) was isolated from sewage treatment plant, Busan, Korea.

Description of *Brevundimonas variabilis* HMF4573

Cells are Gram-staining-negative, non-spore-forming, flagellated and non-pigmented rods. Colonies are circular,

raised, entire and orange colored on R2A agar medium after 3 days of incubation at 25°C. In API 20NE, positive for esculin and β -galactosidase; but negative for nitrate reduction, urease, gelatinase, arginine dihydrolase, indole production and glucose fermentation. Utilize D-glucose, D-maltose, capric acid, adipic acid, malic acid and phenylacetic acid. Utilizes L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-Mannitol, trisodium citrate and potassium gluconate. Strain HMF4573 (= NIBRBAC000498442) was isolated from wetland/marsh, Yongin, Korea.

Description of *Brevundimonas staley* HMF4667

Cells are Gram-staining-negative, non-spore-forming,

flagellated, pigmented and rod-shaped. Colonies are circular, convex, entire and yellow colored after 3 days of incubation on R2A agar medium at 25°C. In API 20NE, positive for esculin hydrolysis; but negative for nitrate reduction, urease, indole production, glucose fermentation, arginine dihydrolase, gelatinase and β -galactosidase. Does not utilize L-arabinose, capric acid, trisodium citrate, potassium gluconate, D-mannose and *N*-acetyl-glucosamine but D-glucose, D-maltose, adipic acid, D-mannitol and phenyl-acetic acid are utilized. Strain HMF4667 (= NIBRBAC000498446) was isolated from wetland/marsh, Yongin, Korea.

Description of *Maricaulis maris* HMF6043

Cells are Gram-staining-negative, flagellated and rod or fusiform-shaped. Colonies are circular, drop-like, entire and beige colored after 3 days of incubation on MA medium at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and β -galactosidase; but positive for esculin hydrolysis and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, citric acid, *N*-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate, D-mannitol, potassium gluconate, capric acid, adipic acid and phenyl-acetic acid. Strain HMF6043 (= NIBRBAC000498449) was isolated from seawater, Boseong-gun, Jeollanam-do, Korea.

Description of *Labrenzia alba* SFD13

Cells are Gram-staining-negative, non-flagellated, pigmented and rod-shaped. Colonies are opaque, circular, smooth, convex and pinkish-beige colored after 5 days of incubation on R2A at 25°C. In API 20NE positive for arginine dihydrolase; weakly positive for esculin hydrolysis; and negative for nitrate reduction, urease, indole production, glucose fermentation, gelatinase, β -galactosidase, D-glucose, L-arabinose, D-maltose, D-mannitol, D-mannose, potassium gluconate, malic acid, trisodium citrate, *N*-acetyl-glucosamine, capric acid, adipic acid and phenyl-acetic acid. Strain SFD13 (= NIBRBAC000498470) was isolated from a gulfweed sample, Jeju Island, Korea.

Description of *Ruegeria atlantica* SF30

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are circular and beige colored after 3 days on MA at 25°C. Positive for nitrate reduction, esculin hydrolysis and β -galactosidase in API 20NE. Negative for indole production, arginine dihydrolase, urease, gelatinase and glucose fermentation. Potassium gluconate and malic acid are utilized. Does not utilize L-arabinose *N*-acetyl-glucosamine, citrate, capric acid, adipic acid, malic acid, D-glucose, D-mannose, D-maltose, D-mannitol, trisodium citrate and phenylacetic acid.

Strain SF30 (= NIBRBAC000498476) was isolated from a gulfweed sample, Jeju Island, Korea.

Description of *Loktanella rosea* ZOD2-5

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are pink colored, opaque, circular, entire, convex, and smooth after 3 days of incubation on MA at 25°C. Positive for esculin hydrolysis and β -galactosidase in API 20NE; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose and phenyl-acetic acid. Strain ZOD2-5 (= NIBRBAC000498479) was isolated from seagrass, Seosan, Chungnam, Korea.

Description of *Phaeobacter inhibens* EC2

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are opaque, circular, smooth, convex and brown colored after 3 days of incubation on MA medium at 25°C. Positive for esculin hydrolysis; but negative for nitrate reduction, indole production, urease, glucose fermentation, arginine dihydrolase, gelatinase and β -galactosidase. Does not utilize D-glucose, capric acid, *N*-acetyl-glucosamine, L-arabinose, D-mannose, D-mannitol, D-maltose, D-maltose, potassium gluconate, trisodium citrate, adipic acid and phenyl acetic acid. Strain EC2 (= NIBRBAC000498481) was isolated from seaweed Jeonnam, Yeosu, Korea.

Description of *Dinoroseobacter shibae* GLB36

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are opaque, circular, smooth, convex and wine-red colored after 5 days of incubation on MA at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, β -galactosidase, D-glucose and D-mannose. Negative for glucose fermentation, arginine dihydrolase, urease, gelatinase, indole production, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain GLB36 (= NIBRBAC000498483) was isolated from a grilled scallop sample, Sokcho, Gangwon-do, Korea.

Description of *Labrenzia aggregata* EC2D15

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are opaque, circular, smooth, flat, and beige colored after 3 days on MA at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis and β -galactosidase; but negative for indole

production, arginine dihydrolase, urease, glucose fermentation and gelatinase. Does not utilize D-glucose, D-mannose, L-arabinose and *N*-acetyl-glucosamine. D-Mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, D-maltose, adipic acid and phenylacetic acid are utilized. Strain EC2D15 (= NIBRBAC000498485) was isolated from a seaweed (Gamuta) sample, Jeonnam, Yeosu, Korea.

Description of *Tropicimonas sediminicola* CAU 1140

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex and cream colored after 3 days of incubation on MA at 30°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, urease and β -galactosidase; but negative for arginine dihydrolase, gelatinase, glucose fermentation and indole production. Utilizes L-arabinose, D-maltose and D-mannitol. Does not utilize D-glucose, D-mannose, malic acid, trisodium citrate, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain CAU 1140 (= NIBRBAC000498503) was isolated from reclaimed soil, Incheon, Korea.

Description of *Lutimaribacter saemankumensis* CAU 1340

Cells are Gram-staining-negative, non-flagellated and non-pigmented rods. Colonies grown on MA agar medium are circular, raised, entire and cream colored after 3 days at 35°C. In API 20NE, negative for β -galactosidase, esculin, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase; but positive for nitrate reduction. Utilizes D-glucose, potassium gluconate and adipic acid. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, capric acid, phenylacetic acid, trisodium citrate, D-maltose and malic acid. Strain CAU 1340 (= NIBRBAC000498508) was isolated from a sea soil sample, Incheon, Korea.

Description of *Litoreibacter albidus* LPB0157

Cells are Gram-staining-negative, non-flagellated and long rods. Colonies are circular, convex, entire and beige colored after 1 day of incubation on MA at 25°C. In API 20NE, only positive for nitrate reduction; but negative for esculin hydrolysis, β -galactosidase, indole production, gelatinase, glucose fermentation, arginine dihydrolase, urease, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate, phenylacetic acid, D-glucose, adipic acid, D-maltose and malic acid. Strain LPB0157 (= NIBRBAC000498528) was isolated from seashore sand, Jebu Island, Korea.

Description of *Sulfitobacter mediterraneus* LPB0162

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and beige colored after 1 day of incubation on MA at 25°C. Positive for nitrate reduction and arginine dihydrolase in API 20NE. Negative for esculin hydrolysis, indole production, β -galactosidase, glucose fermentation, urease and gelatinase. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, trisodium citrate, phenylacetic acid, D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose and malic acid. Strain LBP0162 (= NIBRBAC000498532) was isolated from seashore sand, Jebu Island, Korea.

Description of *Thalassospira profundimaris* IMCC25636

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white colored after 3 days on MA at 20°C. In API 20NE, negative for all kind of substrates such as arginine dihydrolase, urease, esculin hydrolysis, nitrate reduction, indole production, glucose fermentation, gelatinase, β -galactosidase, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate, malic acid, phenylacetic acid, D-glucose, D-maltose and adipic acid. Strain IMCC25636 (= NIBRBAC000498535) was isolated from plant roots, Incheon, Korea.

Description of *Hyphomonas jannaschiana* IMCC25644

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white colored after 3 days of incubation on MA at 20°C. Strain IMCC25644 is negative for nitrate reduction, β -galactosidase, glucose fermentation, arginine dihydrolase, esculin hydrolysis, indole production, urease, gelatinase, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, phenylacetic acid, capric acid, adipic acid, D-glucose and L-arabinose in API 20NE. Strain IMCC25644 (= NIBRBAC000498543) was isolated from plant roots, Incheon, Korea.

Description of *Roseivivax pacificus* IMCC25645

Cells are Gram-staining-negative, non-flagellated, diffusible pigmented and rod-shaped. Colonies are circular, convex, entire and white colored after 3 days on MA at 20°C. In API 20NE, negative for nitrate reduction, indole production, gelatinase, urease and β -galactosidase; but positive for esculin hydrolysis, arginine dihydrolase and glucose fermentation. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potas-

sium gluconate, capric acid, malic acid, phenylacetic acid, adipic acid and trisodium citrate are not utilized. Strain IMCC25645 (= NIBRBAC000498544) was isolated from plant root, Incheon, Korea.

Description of *Thalassospira tepidiphila* IMCC25646

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies grown on MA agar medium are circular, convex, entire and white colored after 3 days of incubation at 20°C. Negative for glucose fermentation, arginine dihydrolase, urease, β -galactosidase, indole production and gelatinase but positive for nitrate reduction and esculin hydrolysis in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, adipic acid and malic acid, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Strain IMCC25646 (= NIBRBAC000498545) was isolated from fresh water, Incheon, Korea.

Description of *Paracoccus seriniphilus* JHR-13

Cells are Gram-staining-negative, non-flagellated and rod or oval-shaped. Colonies are punctiform, convex, entire and white colored after 3 days of incubation on R2A at 25°C. Positive for nitrate reduction, glucose fermentation and β -galactosidase; but negative for esculin hydrolysis, arginine dihydrolase, urease, indole production and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate. D-Mannose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Strain JHR-13 (= NIBRBAC000498633) was isolated from seawater, Jeju Island, Korea.

Description of *Paracoccus yeei* CSC-1

Cells are Gram-staining-positive, non-flagellated and circular-shaped. Colonies are irregular and beige colored after 3 days of incubation on R2A at 25°C. In API 20NE, positive for nitrate reduction and urease; but negative for indole production, glucose fermentation, β -galactosidase, arginine dihydrolase, gelatinase, and esculin hydrolysis. Does not utilize capric acid and phenylacetic acid but D-glucose, D-maltose, malic acid, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid and trisodium citrate are utilized. Strain CSC-1 (= NIBRBAC000498638) was isolated from freshwater, Jeonju, Jeollabuk-do, Korea.

ACKNOWLEDGEMENTS

This study was supported by the research grant “The Survey of Korean Indigenous Species” from the National

Institute of Biological Resources of the Ministry of Environment in Korea.

REFERENCES

- Bazylnski, D.A., T.J. Williams, C.T. Lefèvre, R.J. Berg, C.L. Zhang, S.S. Bowser, A.J. Dean and T.J. Beveridge. 2012. *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* 63:801-808.
- Brenner, D.J., N.R. Krieg and J.T. Staley. 2005. George M. Garrity (ed.), *The Proteobacteria*. *Bergey's Manual of Systematic Bacteriology 2C* (2nd ed.) p. 1388. ISBN 978-0-387-24145-6. New York: Springer British Library no. GBA561951.
- DeLong, E.F., C.M. Preston, T. Mincer, V. Rich, S.J. Hallam, N.U. Frigaard, A. Martinez, M.B. Sullivan, R. Edwards, B.R. Brito, S.W. Chisholm and D.M. Karl. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311:496-503.
- Esser, C., N. Ahmadinejad, C. Wiegand, C. Rotte, F. Sebastiani, G. Gelius-Dietrich, K. Henze, E. Kretschmann, E. Richly, D. Leister, D. Bryant, M.A. Steel, P.J. Lockhart, D. Penny and W. Martin. 2004. A genome phylogeny for mitochondria among *alpha-proteobacteria* and a predominantly eubacterial ancestry of yeast nuclear genes. *Molecular Biology and Evolution* 21:1643-1660.
- Euzéby, J.P. 2011. “*Alphaproteobacteria*”. List of Prokaryotic names with Standing in Nomenclature (LPSN).
- Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* 39:783-791.
- Ferla, M.P., J.C. Thrash, S.J. Giovannoni and W.M. Patrick. 2013. New rRNA gene-based phylogenies of the *Alphaproteobacteria* provide perspective on major groups, mitochondrial ancestry and phylogenetic instability. *PLoS One* 8(12):e83383.
- Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20:406-416.
- Fitzpatrick, D.A., C.J. Creevey and J.O. McInerney. 2006. Genome phylogenies indicate a meaningful alpha-proteobacterial phylogeny and support a grouping of the mitochondria with the *Rickettsiales*. *Molecular Biology and Evolution* 23:74-85.
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.
- Kimura, M. 1983. *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press, Cambridge, New York.

- Krom, M.D., N. Kress, S. Brenner and L.I. Gordon. 1991. Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnology and Oceanography* 36:424-432.
- Oren, A. and G.M. Garrity. 2014. Then and now: a systematic review of the systematics of prokaryotes in the last 80 years. *Antonie van Leeuwenhoek* 106(1):43-56.
- Parte, A.C. 2014. LPSN - list of prokaryotic names with standing in nomenclature. *Nucleic Acids Research* 42(Database issue):D613-616.
- Pitta, P., N. Stambler, T. Tanaka, T. Zohary, A. Tselepidis and F. Rassoulzadegan. 2005. Biological response to P addition in the Eastern Mediterranean Sea. The microbial race against time. *Deep Sea Research. Part II: Topical Studies in Oceanography* 52:2961-2974.
- 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.
- Tamura, K., G. Stecher, D. Peterson, A. Filipinski and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725-2729.
- 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.
- Thompson, L.R., C. Field, T. Romanuk, D.K. Ngugi, R. Siam, H.E. Dorry and U. Stingl. 2013. Patterns of ecological specialization among microbial populations in the Red Sea and diverse oligotrophic marine environments. *Ecology and Evolution* 3(6):1780-1797.
- Viollier, P.H. and L. Shapiro. 2004. Spatial complexity of mechanisms controlling a bacterial cell cycle. *Current Opinion in Microbiology* 7:572-578.
- Williams, K.P., B.W. Sobral and A.W. Dickerman. 2007. A robust species tree for the *Alphaproteobacteria*. *Journal of Bacteriology* 189(13): 4578-4586.
- Woese, C.R. 1987. Bacterial Evolution. *Microbiological Review* 51(2):221-271.
- Wu, M., L.V. Sun, J. Vamathevan, M. Riegler, R. Deboy, J.C. Brownlie, E.A. McGraw, W. Martin, C. Esser, N. Ahmadinejad, C. Wiegand, R. Madupu and others. 2004. Phylogenomics of the Reproductive Parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. *PLoS Biology* 2:E69.
- 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* 67:1613-1617.

Submitted: January 8, 2018

Revised: May 14, 2018

Accepted: May 15 2018