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A report of 31 unrecorded bacterial species in South Korea belonging to the class *Gammaproteobacteria*

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During recent screening to discover indigenous prokaryotic species in South Korea, a total of 31 bacterial strains assigned to the class *Gammaproteobacteria* were isolated from a variety of environmental samples including soil, tidal flat, freshwater, seawater, and plant roots. From the high 16S rRNA gene sequence similarity (>98.7%) and formation of a robust phylogenetic clade with the closest species, it was determined that each strain belonged to each independent and predefined bacterial species. There is no official report that these 31 species have been described in South Korea; therefore 5 species of 3 genera in the order *Alteromonadales*, 11 species of 3 genera in the order *Pseudomonadales*, 8 species of 6 genera in the order *Enterobacteriales*, 2 species of 1 genera in the order *Vibrionales*, 1 species of 1 genera in the order *Oceanospirillales*, 3 species of 3 genera in the order *Xanthomonadales*, and 1 species in the order *Spongiibacter_o* within the *Gammaproteobacteia* are reported for proteobacterial species found in South Korea. Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs are also described in the species description section.

Keywords: 16S rRNA gene, bacterial diversity, *Gammaproteobacteria*, prokaryote, unrecorded species

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Introduction

Microorganisms, including prokaryotes, are estimated to occupy more than approximately 60% of total biomass on earth and are most important organisms to sustain ecological system on earth. They are currently being utilized as the most valuable resources in biotechnology and demands of novel and useful microorganisms are estimated to increase in the futures due to their high economic values. Nevertheless, majority of microorganisms existing in nature has been known to be uncultured in laboratory, because the current cultivation methods can cultivate only small fraction (<0.1%) of microbial cells (Delong *et al*., 1989; Giovannoni *et al*., 1990). Since the value on biodiversity is becoming important increasingly, many attempts have been made to find novel microorganisms that have not been yet discovered (Connon & Giovannoni, 2002; Cho & Giovannoni, 2004; Yoon *et al*., 2011). At a time of writing, approximately 12,400 prokaryotic species with validly published names have been described (Parte, 2014).

In 2013, we collected a variety of environmental samples in South Korea and isolated a number of novel bacterial species and unrecorded bacterial species from them. The identified bacterial species belonged to the

classes/phyla *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria*. Of these bacterial species, the present report focuses on the descriptions of unrecorded species belonging to the *Gammaproteobacteria*. The class *Gammaproteobacteria* is the largest class in the phylum *Proteobacteria* and currently composed of 16 orders. It this study, we report 31 unrecorded bacterial species in South Korea belonging to 10 families of 7 orders in the *Gammaproteobacteria*.

Materials and Methods

A total of 31 bacterial strains assigned to the class *Gammaproteobacteria* were isolated from diverse environmental samples including tidal flat, freshwater, seawater, plant roots, ginseng field and air bladder of fish (Table 1). Each environmental sample was processed separately, spread onto diverse culture media including R2A, Marine Agar 2216 and Tryptic Soy Agar, and incubated at 25-30°C for 2-10 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 as 10-20% glycerol suspension −80°C as well as lyophilized ampoules.

Colony morphology of the strains was observed on agar plates with a magnifying glass after cells grew up to stationary phase. Cellular morphology and cell size were examined by either transmission electron microscopy or scanning electron microscopy. Gram staining was performed using a Gram-staining kit or the standard procedures. Biochemical characteristics were tested by using API 20NE galleries (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 the *Gammaproteobacteria* were compared with the sequences held in GenBank by BLASTN and also analyzed using the EzTaxon-e server (Kim *et al*., 2012). For phylogenetic analyses, alignment of sequences was carried out with CLUSTAL W software (Thompson *et al*., 1994). Gaps at the 5′ and 3′ ends of the alignment were omitted from further analysis. Phylogenetic trees were inferred by using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods implemented within the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated by using the algorithm of Jukes & Cantor (1969) with the program DNADIST. The stability of relationships was assessed by bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

Results and Discussion

Strains assigned to the *Gammaproteobacteria*

On the basis of 16S rRNA gene sequence comparisons and phylogenetic analyses, a total of 31 strains were assigned to the class *Gammaproteobacteria*. The 31 strains were distributed in 7 orders of the *Gammaproteobacteria*; 11 strains for the *Pseudomonadales*, 8 strains for the *Enterobacteriales*, 5 strains for the order *Alteromonadales*, 3 strains for the *Xanthomonadales*, 2 strains for the *Vibrionales*, 1 strains for the *Oceanospirillales*, and 1 strain for the *Spongiibacter_o* (Table 1). These strains were Gram-staining-negative, chemoheterotrophic, and rod-shaped bacteria except for strain WSW-MW5 showing coccoid-shaped (Fig. 1). Colony size, morphology, and physiological characteristics are also shown in the species description section.

A total of 11 strains were assigned to the order *Pseudomonadales*: 9 strains for the family *Pseudomonadaceae* and 2 strains for the *Moraxellaceae* (Fig. 2, Table 1). All strains assigned to the family *Pseudomonadaceae* belonged to the genus *Pseudomonas* and were isolated mainly from terrestrial ecosystem. Phylogenetic analyses based on 16S rRNA gene sequences showed that 9 strains are members of the following species of the genus *Pseudomonas*: *Pseudomonas alcaligenes* (Monias, 1928), *Pseudomonas borbori* (Vanparys *et al*., 2006), *Pseudomonas deceptionensis* (Carrión *et al*., 2011), *Pseudomonas ficuserectae* (Goto, 1983), *Pseudomonas frederiksbergensis* (Andersen *et al*., 2000), *Pseudomonas libanensis* (Dabboussi *et al*., 1999), *Pseudomonas mediterranea* (Catara *et al*., 2002), *Pseudomonas reinekei* (Cámara *et al*., 2007) and *Pseudomonas rhodesiae* (Coroler *et al*., 1996). Two strains of the family *Moraxellaceae* belonged to *Enhydrobacter aerosaccus* (Staley *et al*., 1987) and *Psychrobacter pulmonis* (Vela *et al*., 2003).

The strains in the order *Enterobacteriales* (Fig. 3) isolated from this study were isolated mainly from terrestrial ecosystem (Table 1). Based on the phylogenetic analyses showing robust clades (Fig. 3) and comparative sequence analyses representing high 16S rRNA gene sequence similarities with the closest relatives (Table 1), it was found that the strains in the order *Enterobacteriales* belonged to 8 separate species: *Enterobacter mori* (Zhu *et al*., 2011), *Kosakonia cowanii* and *Kosakonia oryzae* (Brady *et al*., 2013), *Klebsiella michiganensis* (Saha *et*

Table 1. Summary of strains isolated belonging to the class *Gammaproteobacteria* and their taxonomic affiliations.

Phylum	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Gammaproteobacteria	*Chromatiaceae*	*Rheinheimera*	HME9414	NIBRBA0000114409	*Rheinheimera tilapiae*	99.6	Water	R2A	30°C, 2 days
	Marinobacter_f	*Marinobacter*	IMCC2016	NIBRBA0000114261	*Marinobacter lipolyticus*	99.4	Sea water	MA	30°C, 3 days
		Marinobacter	JW17	NIBRBA0000114374	*Marinobacter pelagius*	99.4	Tidal flat	MA	25°C, 2 Days
		Marinobacter	HME9331	NIBRBA0000114407	*Marinobacter hydrocarbonoclasticus*	99.9	Sea water	MA	30°C, 2 days
	Pseudoalteromonadaceae	*Pseudoalteromonas*	IMCC1859	NIBRBA0000114255	*Pseudoalteromonas issachenkonii*	99.5	Sea water	MA	25°C, 3 Days
	Enterobacteriaceae	*Enterobacter*	SJ3-2	NIBRBA0000114372	*Enterobacter mori*	99.5	Tidal flat	MA	25°C, 2 Days
		Klebsiella	DT1-05	NIBRBA0000114176	*Klebsiella michiganensis*	99.7	Tall smartweed root	TSA	30°C, 2 Days
		Klebsiella	DR3-01	NIBRBA0000114190	*Klebsiella pnuemoniae* subsp. *rhinoscleromatis*	99.5	Dandelion root	R2A	30°C, 3 Days
		Kosakonia	DT1-04	NIBRBA0000114175	*Kosakonia cowanii*	99.8	Tall smartweed root	TSA	30°C, 2 Days
		Kosakonia	SR3-03	NIBRBA0000114196	*Kosakonia oryzae*	99.5	Evening primrose root	R2A	30°C, 3 Days
		Plesiomonas	MBB2-1	NIBRBA0000114150	*Plesiomonas shigelloides*	99.9	Air (air bladder)	R2A	25°C, 2 Days
		Rahnella	MDY2F18	NIBRBA0000114149	*Rahnella aquatilis*	99.7	Fresh water	R2A	25°C, 2 Days
		Serratia	ST6-03	NIBRBA0000114184	*Serratia nematodiphila*	99.5	Canadian horseweed root	TSA	30°C, 2 Days
	Halomonadaceae	*Salinicola*	IMCC20105	NIBRBA0000114260	*Salinicola salarius*	99.6	Seawater	MA	25°C, 5 Days
	Moraxellaceae	*Enhydrobacter*	WS85	NIBRBA0000114430	*Enhydrobacter aerosaccus*	99.4	Fresh water	R2A	25°C, 3 Days
		Psychrobacter	WW126	NIBRBA0000114443	*Psychrobacter pulmonis*	99.8	Fresh water	R2A	25°C, 3 Days
	Pseudomonadaceae	*Pseudomonas*	DT1-03	NIBRBA0000114174	*Pseudomonas frederiksbergensis*	99.9	Tall smartweed root	TSA	30°C, 2 Days
		Pseudomonas	DT5-05	NIBRBA0000114178	*Pseudomonas reinekei*	99.7	Canadian horseweed root	TSA	30°C, 2 Days
		Pseudomonas	SR4-03	NIBRBA0000114198	*Pseudomonas mediterranea*	99.2	Evening primrose root	R2A	30°C, 3 Days

Table 1. Continued.

Phylum	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Gammaproteobacteria	*Pseudomonadaceae*	*Pseudomonas*	SR6-02	NIBRBA0000114202	*Pseudomonas rhodesiae*	99.7	Canadian horseweed root	R2A	30°C, 3 Days
		Pseudomonas	MWS24	NIBRBA0000114321	*Pseudomonas deceptionensis*	99.5	Fresh water	R2A	25°C, 2 Days
		Pseudomonas	NMWL17	NIBRBA0000114322	*Pseudomonas libanensis*	99.7	Fresh water	R2A	25°C, 2 Days
		Pseudomonas	SD19	NIBRBA0000114360	*Pseudomonas borbori*	99.3	Tidal flat	MA	25°C, 2 Days
		Pseudomonas	B-12	NIBRBA0000114367	*Pseudomonas alcaligenes*	99.4	Tidal flat	MA	25°C, 2 Days
		Pseudomonas	HME9429	NIBRBA0000114410	*Pseudomonas ficuserectae*	99.7	Water	R2A	30°C, 2 Days
	Vibrionaceae	*Vibrio*	MBM1	NIBRBA0000114144	*Vibrio lentus*	99.6	Sea water	MA	25°C, 2 Days
		Vibrio	IMCC20164	NIBRBA0000114262	*Vibrio shilonii*	99.5	Sea water	MA	30°C, 3 Days
	Xanthomonadaceae	*Dyella*	Gsoil 852	NIBRBA0000114223	*Dyella japonica*	99	Ginseng cultivating filed	R2A	25°C, 2 Days
		Lysobacter	HME9287	NIBRBA0000114397	*Lysobacter brunescens*	100	Water	R2A	30°C, 3 Days
		Stenotrophomonas	JJ9010	NIBRBA0000114161	*Stenotrophomonas maltophilia*	99.4	Freshwater	R2A	25°C, 2 Days
	Spongiibacter_f	*Zhongshania*	IMCC20180	NIBRBA0000114263	*Zhongshania guokunii*	99.6	Sea water	MA	25°C, 10 Days

al., 2013), *Klebsiella pneumoniae* subsp. *rhinoscleromatis* (Ørskov, 1984), *Plesiomonas shigelloides* (Habs & Schubert, 1962), *Rahnella aquatilis* (Izard *et al*., 1979) and *Serratia nematodiphila* (Zhang *et al*., 2009).

Fig. 4 shows phylogenetic assignment of 12 strains into 12 species of the orders *Alteromonadales*, *Oceanospirillales*, *Vibrionales*, *Xanthomonadales*, and *Spongiibacter_o*. These strains belonged to *Rheinheimera tilapiae* (Chen *et al*., 2013) of the family *Chromatiaceae*, *Marinobacter hydrocarbonoclasticus* (Gauthier *et al*., 1992), *Marinobacter lipolyticus* (Martín *et al*., 2003) and *Marinobacter pelagius* (Xu *et al*., 2008) of the family *Marinobacter_f*, *Pseudoalteromonas issachenkonii* (Ivanova *et al*., 2002) of the family *Pseudoalteromonadaceae*, *Dyella japonica* (Xie & Yokota, 2005), *Lysobacter brunescens* (Christensen & Cook, 1978) and *Stenotrophomonas maltophilia* (Palleroni & Bradbury, 1993) of the family *Xanthomonadaceae*, *Vibrio lentus* (Macián *et al*., 2001) and *Vibrio shilonii* (Kushmaro *et al*., 2001) of the family *Vibrionaceae*, *Salinicola salaries* (de la Haba *et al*., 2010) of the family *Halomonadaceae*, and *Zhongshania guokunii* (Li *et al*., 2011) of the family *Spongiibacter_f*.

There is no official report that these 31 species have been isolated in South Korea; therefore 11 species in 3 genera of 2 families in the order *Pseudomonadales*, 8 species in 6 genera in the order *Enterobacteriales*, 5 species in 3 genera of 3 families in the order *Alteromonadales*, 3 species in 3 genera in the order *Xanthomonadales*, 2 species in the order *Vibrionales*, 1 species in the order *Oceanospirillales* and 1 species in the order *Spongiibacter_o* are reported for gammaproteobacterial species found in South Korea.

Description of *Pseudomonas frederiksbergensis* DT1-03

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, glistening, moist, and beige-colored after 2 days of incubation on TSA at 30°C. Positive for nitrate reduction, arginine dehydrolase, esculin hydrolysis and gelatin hydrolysis, but negative for indole production, glucose fermentation, urease, and β-galactosidase in API 20NE. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and trisodium citrate are utilized. Strain DT1-03 (= NIBRBA0000114174) has been isolated from plant roots sample, Daecheongho-lake, Daejeon, Korea.

Description of *Enterobacter cowanii* DT1-04

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, glistening, moist, and yellow colored after 2 days of incubation

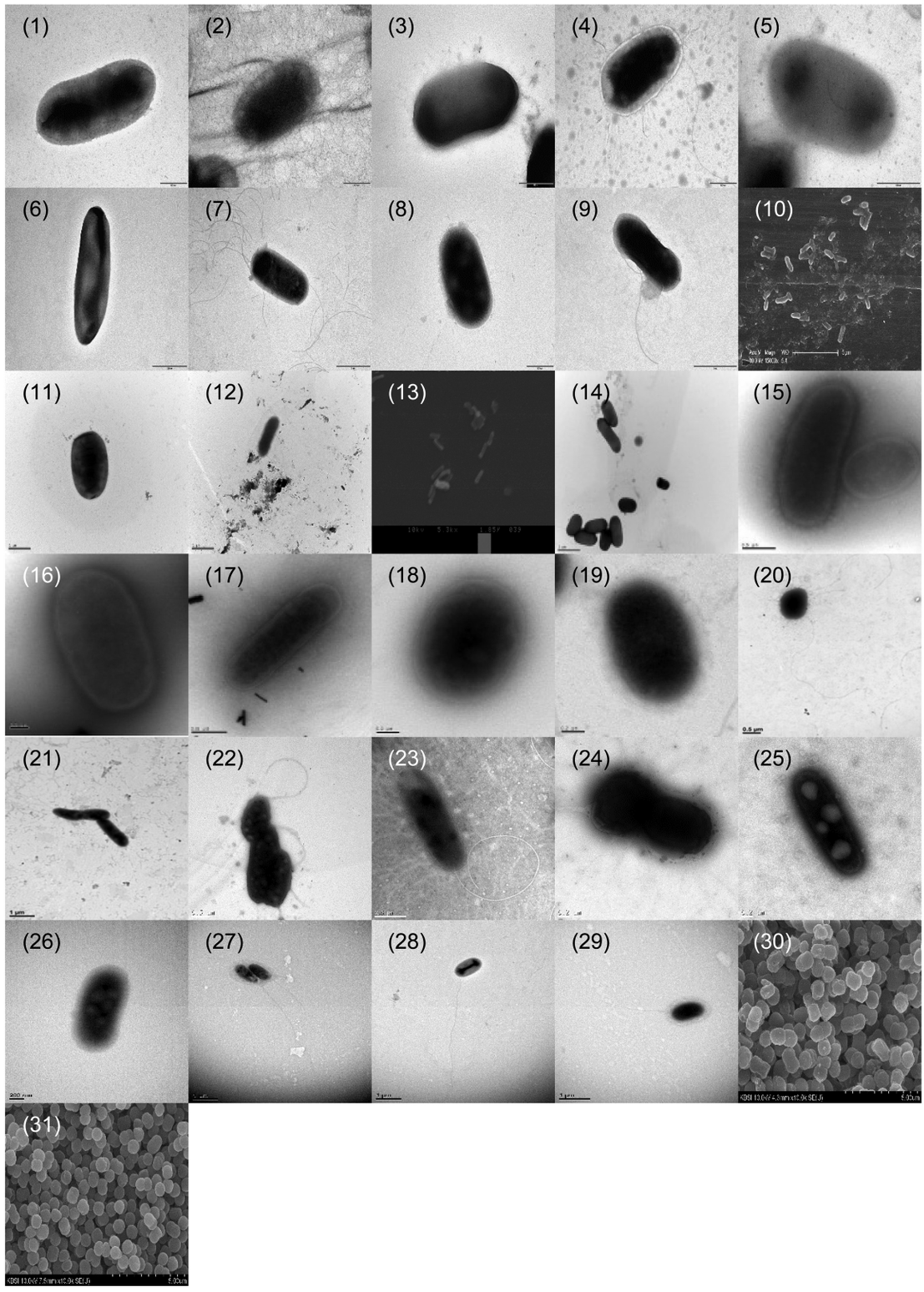


Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, DT1-03; 2, DT1-04; 3, DT1-05; 4, DT5-05; 5, ST6-03; 6, DR3-01; 7, SR3-03; 8, SR4-03; 9, SR6-02; 10, Gsoil 852; 11, MBM1; 12, MDY2F18; 13, MBB2-1; 14, JJ9010; 15, IMCC1859; 16, IMCC20105; 17, IMCC20160; 18, IMCC20164; 19, IMCC20180; 20, MWS24; 21, NMWL17; 22, SD19; 23, B-12; 24, SJ3-2; 25, JW17; 26, HME9287; 27, HME9331; 28, HME9414; 29, HME9429; 30, WS85; 31, WW126.

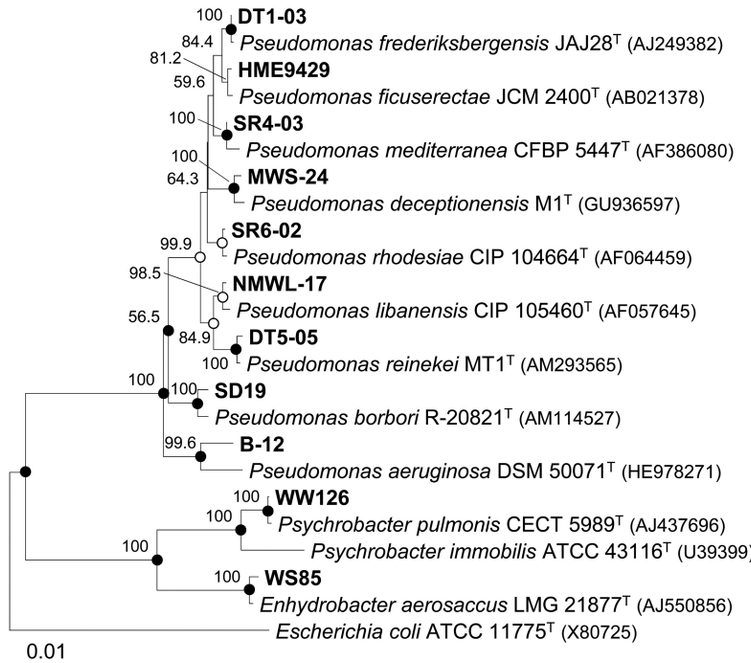


Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Pseudomonadales* in the class *Gammaproteobacteria*. Bootstrap values (>50%) are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms, while open circles indicate that the corresponding nodes were also recovered in the tree generated with one of these algorithms. Bar, 0.01 substitutions per nucleotide position.

on TSA at 30°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for indole production, arginine dihydrolase and urease in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-aglucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize captic acid, adipic acid and phenylacetic acid. Strain DT1-04 (=NIBRBA0000114175) has been isolated from from plant roots sample, Daecheongho-lake, Daejeon, Korea.

Description of *Klebsiella michiganensis* DT1-05

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, glistening, moist and beige colored after 2 days of incubation on TSA at 25°C. Positive for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for arginine dihydrolase and urease in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate and phenylacetic acid are utilized. Does not utilize capric acid and adipic acid. Strain DT1-05 (=NIBRBA0000114176) has been isolated from plant roots sample, Daecheongho-lake, Daejeon, Korea.

Description of *Pseudomonas reinekei* DT5-05

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are irregular, glistening, viscous, and beige colored after 2 days of incubation on TSA at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Does not utilize adipic acid. Strain DT5-05 (=NIBRBA0000114178) has been isolated from plant roots sample, Daecheongho-lake, Daejeon, Korea.

Description of *Chromobacterium aquaticum* ST6-03

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, glistening, moist, and beige colored after 2 days of incubation on TSA at 30°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for indole production, arginine dihydrolase and urease in API 20NE. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-malt-

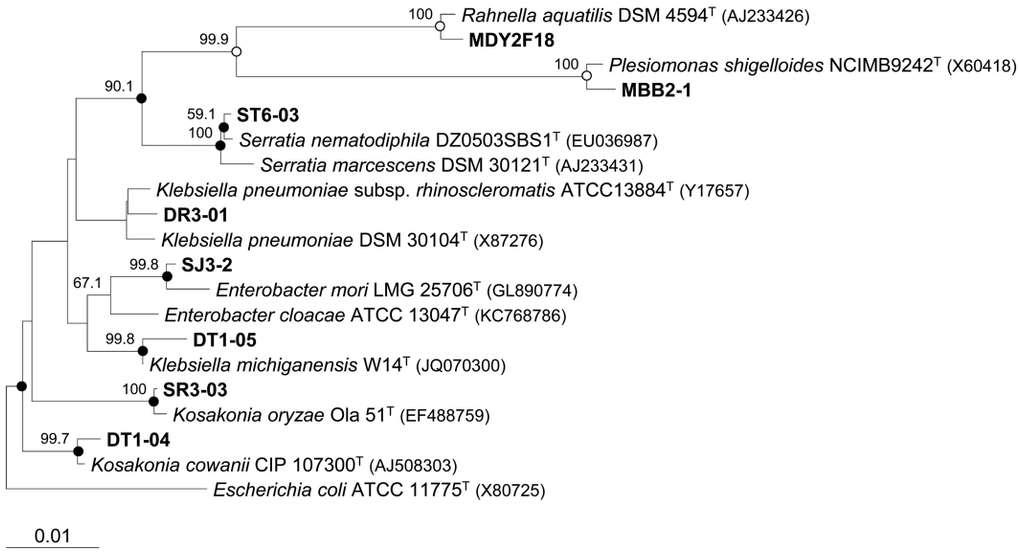


Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Enterobacteriales* in the class *Gammaproteobacteria*. Bootstrap values (>50%) are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms, while open circles indicate that the corresponding nodes were also recovered in the tree generated with one of these algorithms. Bar, 0.01 substitutions per nucleotide position.

ose, potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Does not utilize L-arabinose and adipic acid. Strain ST6-03 (=NIBRBA0000114184) has been isolated from plant roots sample, Daejeon, Korea.

Description of *Klebsiella pnuemoniae* subsp. *rhinoscleromatis* DR3-01

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and short-rod-shaped. Colonies are circular, undulate, pulvinate, and white colored after 3 days on R2A at 30°C. Positive for nitrate reduction, indole production, glucose fermentation, urease, esculin hydrolysis and β-galactosidase, but negative for arginine dihydrolase and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Does not utilize adipic acid. Strain DR3-01 (=NIBRBA0000114190) has been isolated from plant roots sample, Daecheongho-lake, Daejeon, Korea.

Description of *Enterobacter oryzae* SR3-03

Cells are Gram-staining-negative, flagellated, non-pigmented, and short-rod-shaped. Colonies are circular, entire, smooth, and pale yellow colored after 3 days on R2A at 30°C. Positive for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for urease in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain SR3-03 (=NIBRBA0000114196) has been isolated from plant roots sample, Daejeon, Korea.

Description of *Pseudomonas mediterranea* SR4-03

Cells are Gram-staining-positive, non-flagellated, non-pigmented, and short-rod-shaped. Colonies are circular, entire, smooth and dark yellow colored after 3 days on R2A at 30°C. Positive for gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase in API 20NE. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and trisodium citrate are utilized. Does not utilize D-maltose, adipic acid, and phenylacetic acid. Strain SR4-03 (=NIBRBA0000114198) has been isolated from plant roots sample, Daejeon, Korea.

Description of *Pseudomonas rhodesiae* SR6-02

Cells are Gram-staining-negative, non-flagellated, pigmented, and rod-shaped. Colonies are circular, entire, umbonate, and ivory colored after 3 days on R2A at 30°C.

Positive for nitrate reduction, arginine dihydrolase, esculin hydrolysis and gelatin hydrolysis, but negative for indole production, glucose fermentation, urease, and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and trisodium citrate is utilized. Does not utilize D-maltose, adipic acid, and phenylacetic acid. Strain SR6-02 (=NIBRBA0000114202) has been isolated from plant roots sample, Daejeon, Korea.

Description of *Dyella japonica* Gsoil 852

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire, and white colored after 2 days on R2A at 25°C. Positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for indole production, and glucose fermentation in API 20NE. 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 Gsoil 852 (=NIBRBA0000114223) has been isolated from soil sample, ginseng cultivating filed, Pocheon, Korea.

Description of *Vibrio lentus* MBM1

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire and yellow colored after 2 days on MA at 25°C. Positive for esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, D-Mannitol *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MBM1 (=NIBRBA0000114144) has been isolated from a sea water sample, Busan, Korea.

Description of *Rahnella aquatilis* MDY2F18

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire, and white colored after 2 days on R2A at 25°C. Positive for glucose fermentation, esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain MDY2F18 (=NIBRBA0000114149) has been isolated from a fresh water sample, Deogyusan, Korea.

Description of *Plesiomonas shigelloides* MBB2-1

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, raised, entire, and white colored after 2 days on R2A at 25°C. Positive for indole production, glucose fermentation, arginine dihydrolase, and β-galactosidase, but negative for nitrate reduction, urease, esculin hydrolysis, and gelatin hydrolysis in API 20NE. D-Glucose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, and malic acid are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, adipic acid, trisodium citrate, and phenylacetic acid. Strain MBB2-1 (=NIBRBA0000114150) has been isolated from air sample, air bladder of carp.

Description of *Stenotrophomonas maltophilia* JJ9010

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, and beige colored after 2 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, and urease in API 20NE. D-Glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, malic acid, and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, and phenylacetic acid. Strain JJ9010 (=NIBRBA0000114161) has been isolated from a fresh water, Juwangsan, Korea.

Description of *Pseudoalteromonas issachenkonii* IMCC1859

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, regular, convex, smooth, and yellowish colored after 3 days on MA at 25°C. Positive for esculin hydrolysis, gelatinase, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC1859 (=NIBRBA0000114255) has been isolated from a seawater sample, Kosung, the East Sea, Korea.

Description of *Salinicola salarius* IMCC20105

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are convex, smooth, and cream colored after 5 days on MA at 25°C. Positive for nitrate reduction and urease, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-malt-

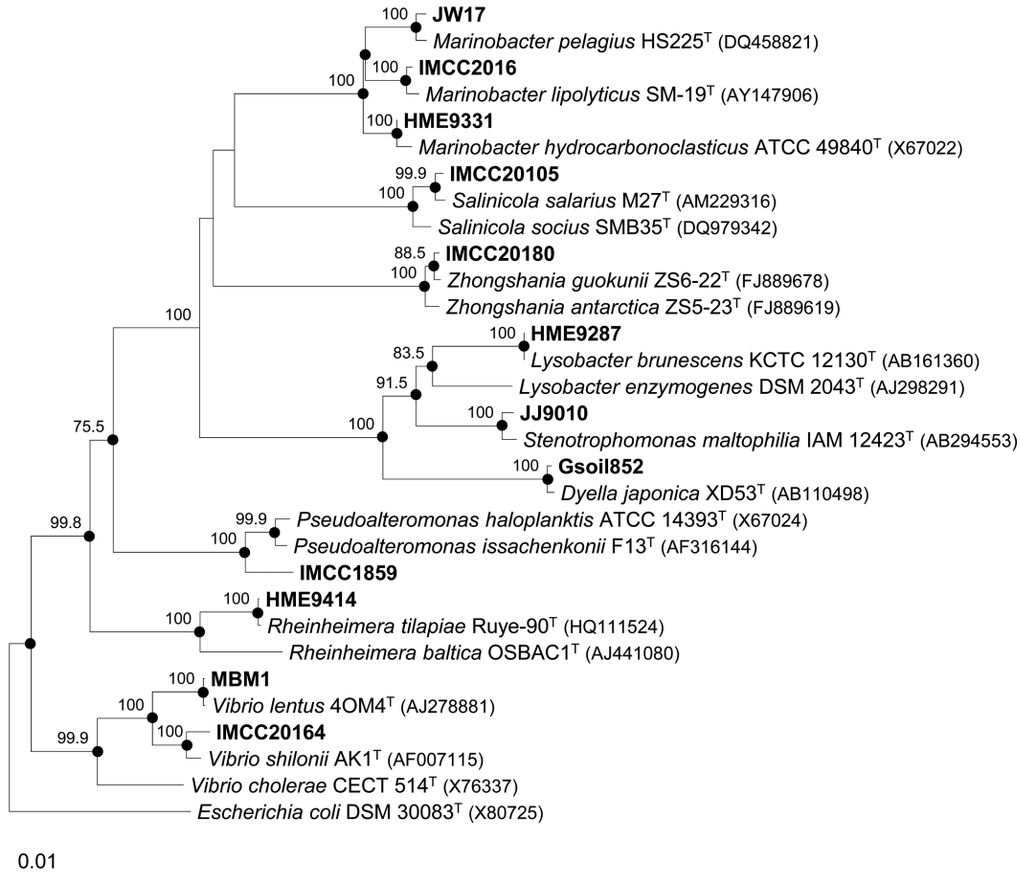


Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the class *Gammaproteobacteria*. Bootstrap values (>50%) are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

ose, adipic acid, and malic acid are utilized. Does not utilize *N*-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Strain IMCC20105 (=NIBRBA0000114260) has been isolated from a seawater sample, the East Sea, Korea.

Description of *Marinobacter lipolyticus* IMCC20160

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are convex, circular and cream colored after 3 days on MA at 30°C. Positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain IMCC20160 (=NIBRBA0000114261) has been isolated from a seawater sample, the East Sea, Korea.

Description of *Vibrio shilonii* IMCC20164

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, smooth, convex, and pale-yellow colored after 3 days on MA at 30°C. Positive for esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatin hydrolysis in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain IMCC20164 (=NIBRBA0000114262) has been isolated from a seawater sample, the East Sea, Korea.

Description of *Zhongshania guokunii* IMCC20180

Cells are Gram-staining-negative, non-flagellated, and rod-shaped. Colonies are circular, convex, smooth surface, and white colored after 10 days on MA at 25°C.

Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatin hydrolysis and β-galactosidase in API 20NE. Dose not utilized D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC20180 (=NIBRBA0000114263) has been isolated from a seawater sample, the East Sea, Korea.

Description of *Pseudomonas mendocina* MWS24

Cells are Gram-staining-negative, flagellated, non-pigmented and coccoid- or rod-shaped. Colonies are circular, smooth, convex, glistening, and yellowish white colored after 2 days on R2A at 25°C. Positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatin hydrolysis, but negative for indole production, and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and trisodium citrate are utilized. Does not utilize D-maltose, adipic acid, and phenylacetic acid. Strain MWS24 (=NIBRBA0000114321) has been isolated from a fresh water sample, Miryang, Korea.

Description of *Pseudomonas nitroreducens* NMWL17

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, smooth, convex, glistening, and yellowish white colored after 2 days on R2A at 25°C. Positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatin hydrolsis, but negative for indole production, and β-galactosidase in API 20NE. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Does not utilize L-arabinose, and D-maltose. Strain NMWL17 (=NIBRBA0000114322) has been isolated from a fresh water sample, Miryang, Korea.

Description of *Pseudomonas borbori* SD19

Cells are Gram-staining-negative, flagellated, non-pigmented and rod-shaped. Colonies are irregular, smooth, and yellow colored after 2 days on MA at 25°C. Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate are utilized. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain SD19 (=NIBRBA0000114360) has been isolated from a tidal flat sample, Taean, Korea.

Description of *Pseudomonas alcaligenes* B-12

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire, and pale-yellow colored after 2 days on MA at 25°C. Positive for esculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase in API 20NE. Capric acid, malic acid, and trisodium citrate is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and phenylacetic acid. Strain B-12 (=NIBRBA0000113994) has been isolated from a tidal flat sample, Taean, Korea.

Description of *Enterobacter mori* SJ3-2

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire, and pale-yellow colored after 2 days on MA at 25°C. Positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and β-galactosidase, but negative for indole production, and gelatin hydrolysis in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid is utilized. Does not utilize capric acid, and adipic acid. Strain SJ3-2 (=NIBRBA0000114372) has been isolated from a tidal flat sample, Taean, Korea.

Description of *Marinobacter pelagius* JW17

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire, and yellow colored after 2 days on MA at 25°C. Positive for glucose fermentation, arginine dihydrolase, esculin hydrolysis, and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, urease, and gelatin hydrolysis. D-Glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and malic acid are utilized. Does not utilize D-mannose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain JW17 (=NIBRBA0000114374) has been isolated from a tidal flat sample, Taean, Korea.

Description of *Lysobacter brunescens* HME9287

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire, and yellow colored after 3 days on R2A at 30°C. Positive for urease esculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and

β-galactosidase, in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain HME9287 (=NIBRBA0000114397) has been isolated from a water sample, Yongin, Korea.

Description of *Marinobacter hydrocarbonoclasticus* HME9331

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire, and beige colored after 2 days on MA at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis and β-galactosidase in API 20NE. Malic acid is utilized. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid trisodium citrate, and phenylacetic acid. Strain HME9331 (=NIBRBA0000114407) has been isolated from a seawater sample, Sinan, Korea.

Description of *Rheinheimera tilapiae* HME9414

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are raised, round, entire, and yellow colored after 2 days on R2A at 30°C. Positive for esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease in API 20NE. D-Mannose is utilized. Dose not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain HME9414 (=NIBRBA0000114409) has been isolated from a water sample, Yongin, Korea.

Description of *Pseudomonas ficuserectae* HME9429

Cells are Gram-staining-negative, flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, transparent, and white colored after 2 days on R2A at 30°C. Positive for arginine dihydrolase and esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, urease, gelatin hydrolysis, and β-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. Does not utilize *N*-acetyl-glucosamine, D-maltose, and phenylacetic acid. Strain HME9429 (=NIBRBA0000114410) has been isolated from a water sample, Yongin, Korea.

Description of *Enhydrobacter aerosaccus* WS85

Cells are Gram-staining-negative, non-flagellated, and coccoid to rod-shaped. Colonies are entire margin, convex, and white colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease in API 20NE. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, and D-maltose are utilized. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain WS85 (=NIBRBA0000114430) has been isolated from a fresh water, Changnyeong, Korea.

Description of *Psychrobacter pulmonis* WW126

Cells are Gram-staining-negative, non-flagellated, and coccoid-shaped. Colonies are opaque, round, smooth, convex, and cream colored after 3 days on R2A at 25°C. Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β-galactosidase in API 20NE. Capric acid is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain WW126 (=NIBRBA0000114443) has been isolated from a fresh water, Changnyeong, Korea.

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Kosakonia gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. Syst. Appl. Microbiol. 36:309-319.

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