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 Oceanospirilales, 3 species of 3 genera in the order Xanthomonadales, and 1 species in the order Spongibacter_o within the Gammaproteobacteria 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 at -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 Spongibacter 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 borborym (Vanparys et al., 2006), Pseudomonas deceptionensis (Carrió et al., 2011), Pseudomonas ficuserectae (Goto, 1983), Pseudomonas frederiksebergensis (Andersen et al., 2000), Pseudomonas libanensis (Dabbousi et al., 1999), Pseudomonas mediterranea (Catará et al., 2002), Pseudomonas reinkei (Cámara et al., 2007) and Pseudomonas rhodesiae (Coroller 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.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Family</th>
<th>Genus</th>
<th>Strain ID</th>
<th>NIBR ID</th>
<th>Most closely related species</th>
<th>Similarity (%)</th>
<th>Isolation source</th>
<th>Medium</th>
<th>Incubation conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gammaproteobacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromatiaceae</td>
<td>Rheinheimera</td>
<td>HME9414</td>
<td>NIBRBA00000114409</td>
<td>Rheinheimera tilapiae</td>
<td></td>
<td>99.6</td>
<td>Water</td>
<td>R2A</td>
<td>30°C, 2 days</td>
</tr>
<tr>
<td></td>
<td><em>Marinobacter</em></td>
<td>IMCC2016</td>
<td>NIBRBA00000114261</td>
<td><em>Marinobacter</em> lipolyticus</td>
<td></td>
<td>99.4</td>
<td>Sea water</td>
<td>MA</td>
<td>30°C, 3 days</td>
</tr>
<tr>
<td></td>
<td><em>Marinobacter</em></td>
<td>JW17</td>
<td>NIBRBA00000114374</td>
<td><em>Marinobacter</em> pelagius</td>
<td></td>
<td>99.4</td>
<td>Tidal flat</td>
<td>MA</td>
<td>25°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Marinobacter</em></td>
<td>HME9331</td>
<td>NIBRBA00000114407</td>
<td><em>Marinobacter</em> hydrocarbonoclasticus</td>
<td></td>
<td>99.9</td>
<td>Sea water</td>
<td>MA</td>
<td>30°C, 2 days</td>
</tr>
<tr>
<td>Pseudoalteromonadaceae</td>
<td><em>Pseudoalteromonas</em></td>
<td>IMCC1859</td>
<td>NIBRBA00000114255</td>
<td><em>Pseudoalteromonas</em> issachenkii</td>
<td></td>
<td>99.5</td>
<td>Sea water</td>
<td>MA</td>
<td>25°C, 3 Days</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td><em>Enterobacter</em></td>
<td>SJ3-2</td>
<td>NIBRBA00000114372</td>
<td><em>Enterobacter</em> mair</td>
<td></td>
<td>99.5</td>
<td>Tidal flat</td>
<td>MA</td>
<td>25°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella</em></td>
<td>DT1-05</td>
<td>NIBRBA00000114176</td>
<td><em>Klebsiella</em> michiganensis</td>
<td></td>
<td>99.7</td>
<td>Tall smartweed root</td>
<td>TSA</td>
<td>30°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella</em></td>
<td>DR3-01</td>
<td>NIBRBA00000114190</td>
<td><em>Klebsiella</em> pneumoniae subsp. rhinoscleromatis</td>
<td></td>
<td>99.5</td>
<td>Dandelion root</td>
<td>R2A</td>
<td>30°C, 3 Days</td>
</tr>
<tr>
<td></td>
<td><em>Kosakonia</em></td>
<td>DT1-04</td>
<td>NIBRBA00000114175</td>
<td><em>Kosakonia</em> cowanii</td>
<td></td>
<td>99.8</td>
<td>Tall smartweed root</td>
<td>TSA</td>
<td>30°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Kosakonia</em></td>
<td>SR3-03</td>
<td>NIBRBA00000114196</td>
<td><em>Kosakonia</em> oryzae</td>
<td></td>
<td>99.5</td>
<td>Evening primrose root</td>
<td>R2A</td>
<td>30°C, 3 Days</td>
</tr>
<tr>
<td></td>
<td><em>Plesiomonas</em></td>
<td>MBB2-1</td>
<td>NIBRBA00000114150</td>
<td><em>Plesiomonas</em> shigelloides</td>
<td></td>
<td>99.9</td>
<td>Air(air bladder)</td>
<td>R2A</td>
<td>25°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Rhahnella</em></td>
<td>MDY2F18</td>
<td>NIBRBA00000114149</td>
<td><em>Rhahnella</em> aquaritae</td>
<td></td>
<td>99.7</td>
<td>Fresh water</td>
<td>R2A</td>
<td>25°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Serratia</em></td>
<td>ST6-03</td>
<td>NIBRBA00000114184</td>
<td><em>Serratia</em> nematodiphila</td>
<td></td>
<td>99.5</td>
<td>Canadian horseweed root</td>
<td>TSA</td>
<td>30°C, 2 Days</td>
</tr>
<tr>
<td>Halomonadaceae</td>
<td><em>Salinicola</em></td>
<td>IMCC20105</td>
<td>NIBRBA00000114260</td>
<td><em>Salinicola</em> sakuriae</td>
<td></td>
<td>99.6</td>
<td>Seawater</td>
<td>MA</td>
<td>25°C, 5 Days</td>
</tr>
<tr>
<td>Moraxellaceae</td>
<td><em>Enhydrobacter</em></td>
<td>WS85</td>
<td>NIBRBA00000114430</td>
<td><em>Enhydrobacter</em> aerophilus</td>
<td></td>
<td>99.4</td>
<td>Fresh water</td>
<td>R2A</td>
<td>25°C, 3 Days</td>
</tr>
<tr>
<td></td>
<td><em>Psychro bacter</em></td>
<td>WW126</td>
<td>NIBRBA00000114443</td>
<td><em>Psychro bacter</em> pulmonis</td>
<td></td>
<td>99.8</td>
<td>Fresh water</td>
<td>R2A</td>
<td>25°C, 3 Days</td>
</tr>
<tr>
<td>Pseudomonadaceae</td>
<td><em>Pseudomonas</em></td>
<td>DT1-03</td>
<td>NIBRBA00000114174</td>
<td><em>Pseudomonas</em> frederikssbergensis</td>
<td></td>
<td>99.9</td>
<td>Tall smartweed root</td>
<td>TSA</td>
<td>30°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em></td>
<td>DT5-05</td>
<td>NIBRBA00000114178</td>
<td><em>Pseudomonas</em> reinekei</td>
<td></td>
<td>99.7</td>
<td>Canadian horseweed root</td>
<td>TSA</td>
<td>30°C, 2 Days</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em></td>
<td>SR4-03</td>
<td>NIBRBA00000114198</td>
<td><em>Pseudomonas</em> mediterranea</td>
<td></td>
<td>99.2</td>
<td>Evening primrose root</td>
<td>R2A</td>
<td>30°C, 3 Days</td>
</tr>
</tbody>
</table>
### Table 1. Continued.

<table>
<thead>
<tr>
<th>GENUS</th>
<th>STRAIN ID</th>
<th>ISOLATION SOURCE</th>
<th>SIMILARITY (%)</th>
<th>STRAIN ID</th>
<th>ISOLATION SOURCE</th>
<th>SIMILARITY (%)</th>
<th>PHYLUM</th>
<th>FAMILY</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em></td>
<td>MWC117</td>
<td>Fresh water</td>
<td>99.6</td>
<td>NIBRBA00001144144</td>
<td>Fresh water</td>
<td>99.6</td>
<td>Gammaproteobacteria</td>
<td>Vibrionaceae</td>
<td>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-acetylglucosamine, 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.</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>MB101</td>
<td>Sea water</td>
<td>99.5</td>
<td>NIBRBA00001144144</td>
<td>Fresh water</td>
<td>99.6</td>
<td>Gammaproteobacteria</td>
<td>Vibrionaceae</td>
<td>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-acetylglucosamine, 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.</td>
</tr>
</tbody>
</table>

### Description of *Pseudomonas frederiksbergensis* DT1-03

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 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-acetylglucosamine, 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 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-acetylglucosamine, 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.
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, DR3-03; 7, SR3-03; 8, SR4-03; 9, SR6-02; 10, Gsoil852; 11, MBM1; 12, MDY2F18; 13, MBB2-1; 14, JB010; 15, IMCC1859; 16, IMCC20105; 17, IMCC20160; 18, IMCC20164; 19, IMCC20180; 20, MW24; 21, NMWL17; 22, SD19; 23, B-12; 24, SJ3-2; 25, JW17; 26, HME9287; 27, HME9331; 28, HME9414; 29, HME9429; 30, WS85; 31, WW126.
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-α-glucosamine, D-maltose, potassium glutonate, malic acid and trisodium citrate are utilized. Does not utilize capric acid, adipic acid and phenylactic acid. Strain DT1-04 (= KACC 15356) has been isolated from a 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-α-glucosamine, D-maltose, potassium glutonate, malic acid, trisodium citrate and phenylactic acid are utilized. Does not utilize capric acid and adipic acid. Strain DT1-05 (= KACC 15357) has been isolated from a 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 glutonate, capric acid, malic acid, trisodium citrate, and phenylactic acid are utilized. Does not utilize adipic acid. Strain DT5-05 (= KACC 15358) has been isolated from a 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-
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 pneumoniae* 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, malic acid and trisodium citrate are utilized. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain DR3-01 (= NIBRBA0000114190) has been isolated from plant roots sample, 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, 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, umbo-nate, 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 IMCC1859 (= NIBRBA0000114255) has been isolated from a seawater sample, Kosung, the East Sea, 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 nitrate reduction, 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, smooth, and yellowish colored after 2 days on MA 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-mannose, potassium gluconate, capric 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 glutonate, 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-
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, and gelatin hydrolysis in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltoolose, 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-maltoolose, 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.

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**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.
Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and 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 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 hydrolysis, 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 borbri 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 (=NIBRBA000013994) 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 (=NIBRBA000011432) 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, malic acid are utilized. Does not utilize D-mannose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain JW17 (=NIBRBA000014374) 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, urease in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, adipic acid, trisodium citrate, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease in API 20NE. 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 HME9414 (= NIBRBA0000114409) has been isolated from a seawater sample, Yongin, 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-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain WW126 (= NIBRBA0000114443) 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-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain WW126 (= NIBRBA0000114443) has been isolated from a fresh water, Changnyeong, Korea.

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