A report on 15 unrecorded bacterial species of Korea isolated in 2016, belonging to the class Betaproteobacteria

Dong-Uk Kim1, Chi-Nam Seong2, Kwangyeop Jahng3, Soon Dong Lee4, Chang-Jun Cha5, Kiseong Joh6, Che Ok Jeon7, Seung-Bum Kim8 and Myung Kyum Kim1,*

1Department of Bio & Environmental Technology, College of Natural Science, Seoul Women’s University, Seoul 01797, Republic of Korea
2Department of Biology, Sunchon National University, Suncheon 57922, Republic of Korea
3Department of Life Sciences, Chonbuk National University, Jeonju 54899, Republic of Korea
4Department of Science Education, Jeju National University, Jeju 63243, Republic of Korea
5Department of Biotechnology, Chung-Ang University, Anseong 17546, Republic of Korea
6Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Gyeonggi 17035, Republic of Korea
7Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea
8Department of Microbiology, Chungnam National University, Daejeon 34134, Republic of Korea

*Correspondent: biotech@swu.ac.kr

In 2016, as a subset study to discover indigenous prokaryotic species in Korea, a total of 15 bacterial strains were isolated and assigned to the class Betaproteobacteria. From the high 16S rRNA gene sequence similarity (>98.8%) 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 15 species have been described in Korea; therefore, 1 strain of the Aquitalea, 5 strains of the Paraburkholderia, 2 strains of the Comamonas, 1 strain of the Cupriavidus, 1 strain of the Diaphorobacter, 2 strains of the Hydrogenophaga, 1 strain of the Iodobacter, 1 strain of the Massilia and 1 strain of the Rhodoferax within the Betaproteobacteria are described for unreported bacterial species in Korea. Gram reaction, colony and cell morphology, basic biochemical characteristics, and isolation sources are also described in the species description section.

Keywords: 16S rRNA, bacterial diversity, Betaproteobacteria, unreported species

© 2018 National Institute of Biological Resources
DOI:10.12651/JSR.2018.7.2.097

**INTRODUCTION**

In 2016, 15 unrecorded bacterial species were isolated from various samples collected in Korea and identified as members of the class Betaproteobacteria. The present report focuses on the isolation and description of unrecorded species belonging to the class Betaproteobacteria.

Carl Woese established the Proteobacteria and major phylum of Gram-negative bacteria (Woese, 1987). At the time of writing, the phylum Proteobacteria comprises Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, Zetaproteobacteria and Oligoflexia based on the List of Prokaryotic names with Standing in Nomenclature (LPSN) (http://www.bacterio.net/-classifphyla.html#proteobacteria).

Betaproteobacteria is one of the largest Gram negative bacterial group that include the 7 orders; Burkholderiales, Hydrogenophilales, Methylophilales, Neisseriales, Nitrosomonadales, Procbacteriales and Rhodocyclales (Euzéby, 2016). Betaproteobacteria includes the functionally diverse bacteria like a nitrogen fixing bacteria and biodegrading bacteria (Garrity et al., 2005; Nakatsu et al., 2006; Martin et al., 2012).

In this study, the present report focuses on the description of bacterial species belonging to the Betaproteobacteria that have not officially reported in Korea. Here in the present study we report 15 unreported bacterial species in Korea belonging to 5 families of 2 orders in the Betaproteobacteria.
MATERIALS AND METHODS

A total of 15 bacterial strains assigned to the class Betaproteobacteria were isolated from various environmental habitats, including soil, wastewater, wetland, agricultural soil, natural caves, freshwater, and sediments. All environmental samples were processed independently, serially diluted, spread onto diverse culture agar media and incubated at 25-30°C for 2-4 days (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 and cell size of the strains were observed by using transmission electron microscopy or scanning electron microscopy. Electron micrograph of the strains are shown in Fig. 1. Gram reaction was performed according to the classic Gram procedure described by Doetsch (1981). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer’s instructions. Genomic DNA was extracted and 16S rRNA gene was amplified by PCR with 9F and 1492R universal bacterial primers (Weisburg et al., 1991). The 16S rRNA gene sequences of the related taxa were obtained from EzBioCloud server (Yoon et al., 2017). 15 bacterial strains and related taxa (retrieved from the NCBI database) were aligned with SINA (v1.2.11) according to the SILVA seed alignment (http://www.arb-silva.de; Pruesse et al., 2012). Using the two-parameter model (Kimura, 1983) calculated the evolutionary distances. Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei, 1987) in MEGAN program (Kumar et al., 2016) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

The 15 strains were distributed into 2 orders of the class Betaproteobacteria: 13 strains in the Burkholderiales, 2 strains in the Neisseriales (Table 1). These strains were Gram-staining-negative or positive and rod-shaped bacteria (Fig. 1). Five strains that were assigned to the family Burkholderiaceae in the order Burkholderiales within the genera Paraburkholderia (Fig. 2). 6 strains in the order Burkholderiales belonged to 4 genera of family Comamonadaceae; Rhodoferax (1 species), Comamonas (2 species), Diaphorobacter (1 species) and Hydrogenophaga (2 species) (Fig. 3). Two strains that were assigned to the family Neisseriaceae of the order Neisseriales belonged to the genera Iodobacter and Aqutalea. One strain that was assigned to the family Oxalobacteraceae in the order Burkholderiales belonged to the genus Massilia and another strain that was assigned to the family Burkholderiaceae in the order Burkholderiales belonged to the genus Cupriavidus (Fig. 4). Here we report 15 unrecorded bacterial species belonging to 5 families of 2 orders in the Betaproteobacteria, which were isolated in Korea; 1 strain of the Aquitalea, 5 strains of the Paraburkholderia, 2 strains of the Comamonas, 1 strain of the Cupriavidus, 1 strain of the Diaphorobacter, 2 strains of the Hydrogenophaga, 1 strain of the Iodobacter, 1 strain of the Massilia and 1 strain of the Rhodoferax.

Description of Diaphorobacter nitroreducens POA39

Cells are gram-staining-negative, non-flagellated and rod-shaped. Colonies are transparent, circular, smooth, entire and white colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction. Negative for β-galactosidase activity, esculin hydrolysis, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-malate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain POA39 (= NIBRBC000498475) was isolated from a wastewater sample, Gwangyang, Korea.

Description of Hydrogenophaga taeniospiralis 2PKSH112

Cells are gram-staining-positive, flagellated and rod-shaped. Colonies are circular, convex, and yellow colored after 3 days of incubation at 25°C on 2× R2A. Positive for nitrate reduction and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, and urease activities. Does not utilize D-glucose, L-arabinose, N-acetylglucosamine, D-malate, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 2PKSH112 (= NIBRBC000498637) was isolated from a freshwater sample, Kunsan, Korea.

Description of Cupriavidus oxalaticus D8-11

Cells are gram-staining-negative, flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and cream colored after 4 days of incubation at 30°C on R2A. Positive for nitrate reduction, and urease activities (weak). Negative for indole production, glucose fermentation, and arginine dihydrolase, and esculin hydrolysis. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, and D-malate. Strain D8-11 (= NIBRBC000498666) was isolated from a natural cave sample, Jeju, Korea.

Description of Comamonas denitrificans 7227

Cells are gram-staining-negative, flagellated and rod-
Table 1. The taxonomic affiliations of isolated strains belonging to the class Betaproteobacteria.

<table>
<thead>
<tr>
<th>Order</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>Neisserales</td>
<td>Neisseriaceae</td>
<td>Aquitalea</td>
<td>8312</td>
<td>NIBRBAC000498572</td>
<td>Aquitalea magnusoni</td>
<td>99.0</td>
<td>Sediment soil</td>
<td>R2A</td>
<td>30°C, 2d</td>
</tr>
<tr>
<td></td>
<td>Iodobacter</td>
<td>HMF4541</td>
<td></td>
<td>NIBRBC000498438</td>
<td>Iodobacter fluviatilis</td>
<td>98.8</td>
<td>Wetland</td>
<td>R2A</td>
<td>25°C, 3d</td>
</tr>
<tr>
<td>Burkholderiales</td>
<td>Paraburkholderia</td>
<td>MMS16-CNU072</td>
<td></td>
<td>NIBRBAC000498621</td>
<td>Paraburkholderia insulsa</td>
<td>99.3</td>
<td>Soil</td>
<td>SCA</td>
<td>30°C, 3d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMS16-CNU376</td>
<td></td>
<td>NIBRBAC000498625</td>
<td>Paraburkholderia megapolitana</td>
<td>99.1</td>
<td>Soil</td>
<td>SCA</td>
<td>30°C, 3d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMS16-CNU436</td>
<td></td>
<td>NIBRBAC000498626</td>
<td>Paraburkholderia oxypila</td>
<td>98.8</td>
<td>Soil</td>
<td>ISP-2</td>
<td>30°C, 3d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMS16-CNU135</td>
<td></td>
<td>NIBRBAC000498622</td>
<td>Paraburkholderia phenazinum</td>
<td>99.1</td>
<td>Soil</td>
<td>ISP-2</td>
<td>30°C, 3d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMS16-CNU462</td>
<td></td>
<td>NIBRBAC000498627</td>
<td>Paraburkholderia unamae</td>
<td>98.8</td>
<td>Soil</td>
<td>ISP-2</td>
<td>30°C, 3d</td>
</tr>
<tr>
<td>Burkholderiales</td>
<td>Comamonadaceae</td>
<td>Comamonas</td>
<td>7227</td>
<td>NIBRBC000498571</td>
<td>Comamonas denitrificans</td>
<td>99.7</td>
<td>Sediment soil</td>
<td>R2A</td>
<td>30°C, 2d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6191</td>
<td></td>
<td>NIBRBC000498578</td>
<td>Comamonas terrigena</td>
<td>99.0</td>
<td>Sediment soil</td>
<td>R2A</td>
<td>30°C, 2d</td>
</tr>
<tr>
<td></td>
<td>Diaphorobacter</td>
<td>POA39</td>
<td></td>
<td>NIBRBC000498475</td>
<td>Diaphorobacter nitroreducens</td>
<td>99.9</td>
<td>Wastewater</td>
<td>R2A</td>
<td>25°C, 3d</td>
</tr>
<tr>
<td></td>
<td>Hydrogenophaga</td>
<td>SH4</td>
<td></td>
<td>NIBRBC000498424</td>
<td>Hydrogenophaga flava</td>
<td>99.5</td>
<td>Agricultural soil</td>
<td>MA</td>
<td>30°C, 2d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2PKSH112</td>
<td></td>
<td>NIBRBC000498637</td>
<td>Hydrogenophaga taeniospiralis</td>
<td>98.9</td>
<td>Freshwater</td>
<td>2 × R2A</td>
<td>25°C, 3d</td>
</tr>
<tr>
<td></td>
<td>Rhodoferax</td>
<td>HMF4664</td>
<td></td>
<td>NIBRBC000498444</td>
<td>Rhodoferax fermentans</td>
<td>99.2</td>
<td>Wetland</td>
<td>R2A</td>
<td>25°C, 3d</td>
</tr>
<tr>
<td>Oxalobacteraceae</td>
<td>Massilia</td>
<td>HMF4544</td>
<td></td>
<td>NIBRBC000498439</td>
<td>Massilia aurea</td>
<td>99.5</td>
<td>Wetland</td>
<td>R2A</td>
<td>25°C, 3d</td>
</tr>
<tr>
<td></td>
<td>Ralstonia_f</td>
<td>D8-11</td>
<td></td>
<td>NIBRBC000498666</td>
<td>Cupriavidus oxalaticus</td>
<td>98.9</td>
<td>Natural cave</td>
<td>R2A</td>
<td>30°C, 4d</td>
</tr>
</tbody>
</table>
shaped. Colonies are circular, entire, smooth, raised and white colored after 2 days of incubation at 30°C on R2A. Positive for nitrate reduction. Negative for indole production, glucose fermentation, and arginine dihydrolase, urease activities, esculin hydrolysis, gelatinase and β-galactosidase activity. Does not utilize D-mannitol, N-acetylglucosamine, D-maltose, capric acid, adipic acid and trisodium citrate. Strain 7227 ( = NIBR BAC000498571) was isolated from a sediment soil sample, Han River, Korea.

**Description of Aquitalea magnusoni**i 8312

Cells are gram-staining-negative, non-flagellated and
rod-shaped. Colonies are circular, entire, smooth, raised and white colored after 2 days of incubation at 30°C on R2A. Positive for nitrate reduction and arginine dihydrolase. Negative for indole production, glucose fermentation, urease activities, esculin hydrolysis, gelatinase and β-galactosidase activity. Does not utilize L-arabinose, D-mannose, D-maltose and phenylacetic acid. Strain 8312 (= NIBRBAC000498572) was isolated from a sed-

Fig. 3. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family Comamonadaceae. Bootstrap values (> 70%) are shown above nodes for the neighbor-joining methods. Bar: 0.005 substitutions per nucleotide position.

Fig. 4. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family Neisseriaceae, Oxalobacteraceae and Ralstonia f. Bootstrap values (> 70%) are shown above nodes for the neighbor-joining methods. Bar: 0.01 substitutions per nucleotide position.
Description of *Comamonas terrigena* 6191

Cells are gram-staining-negative, flagellated and rod-shaped. Colonies are circular, entire, smooth, raised and white colored after 2 days of incubation at 30°C on R2A. Positive for nitrate reduction and gelatinase. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activities, esculin hydrolysis and β-galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, trisodium citrate and phenylacetic acid. Strain 6191 (= NIBRBAC000498578) was isolated from a sediment soil sample, Han River, Korea.

Description of *Rhodoferax fermentans* HMF4664

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, convex, entire and pale pink colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction, glucose fermentation and urease activities. Negative for indole production, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase activity. Does not utilize D-mannitol, capric acid, trisodium citrate and phenylacetic acid. Strain HMF4664 (= NIBRBAC000498444) was isolated from a wetland sample, Yongin, Korea.

Description of *Iodobacter fluviatilis* HMF4541

Cells are gram-staining-negative, flagellated, and rod-shaped. Colonies are circular, convex, entire and white colored after 3 days of incubation at 25°C on R2A. Positive for nitrate reduction, glucose fermentation and gelatinase. Negative for indole production, arginine dihydrolase, urease activities, esculin hydrolysis, gelatinase and β-galactosidase activity. Does not utilize D-mannitol, capric acid, trisodium citrate and phenylacetic acid. Strain HMF4541 (= NIBRBAC000498438) was isolated from a wetland sample, Yongin, Korea.

Description of *Massilia aurea* HMF4544

Cells are gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and yellow colored after 3 days of incubation at 25°C on R2A. Positive for esculin hydrolysis, gelatinase and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase and urease activities. Does not utilize D-mannitol, N-acetylglucosamine, potassium gluconate and capric acid. Strain HMF4544 (= NIBRBAC000498439) was isolated from a wetland sample, Yongin, Korea.

Description of *Hydrogenophaga flava* SH4

Cells are gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, smooth, and pale yellow colored after 2 days of incubation at 30°C on MA. Positive for nitrate reduction and urease activities. Negative for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase activity. Does not utilize D-mannose, N-acetylglucosamine, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain SH4 (= NIBRBAC000498424) was isolated from a soil sample, Changnyeong, Korea.

Description of *Paraburkholderia insulsa* MMS16-CNU072

Cells are gram-staining-negative, flagellated and rod-shaped. Colonies are circular, glistering, moist and white colored after 3 days of incubation at 30°C on pH 5, SCA. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activities and gelatinase. Strain MMS16-CNU072 (= NIBRBAC000498621) was isolated from a soil sample, Daejeon, Korea.

Description of *Paraburkholderia phenazinium* MMS16-CNU135

Cells are gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, smooth, entire and pale yellow colored after 3 days of incubation at 30°C on pH 5, ISP-2. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activities and gelatinase. Does not utilize D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain MMS16-CNU135 (= NIBRBAC000498622) was isolated from a soil sample, Daejeon, Korea.

Description of *Paraburkholderia megapolitana* MMS16-CNU376

Cells are gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, glistering, moist and paled beige colored after 3 days of incubation at 30°C on pH 5, SCA. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activities and gelatinase. Strain MMS16-CNU376 (= NIBRBAC000498625) was isolated from a soil sample, Daejeon, Korea.
Description of *Paraburkholderia oxyphila* MMS16-CNU436

Cells are gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire and creamy and beige colored after 3 days of incubation at 30°C on pH 5, ISP-2 agar. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activities and gelatinase. Does not utilize L-arabinose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS16-CNU436 (= NIBRBAC000498626) was isolated from a soil sample, Daejeon, Korea.

Description of *Paraburkholderia unamae* MMS16-CNU462

Cells are gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire and creamy and beige colored after 3 days of incubation at 30°C on pH 5, ISP-2 agar. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activities and gelatinase. Does not utilize L-arabinose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS16-CNU462 (= NIBRBAC000498627) was isolated from a soil sample, Daejeon, Korea.

Acknowledgements

This study was supported by the research grant, “Survey of Korean Indigenous Species” sponsored by the National Institute of Biological Resources (NIBR) of the Ministry of Environment, Korea.

References


