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

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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

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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*, *Procabacteriales* 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 *Betaproteobateria* were isolated from various environmental habitats, including soil, wastewater, wetland, agricultural soil, natural caves, freshwater, and sediment soil. 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 MEGA7 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 *Neisseriale* (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 *Aquitalea*. One strain that was assigned to the family *Oxalobacteraceae* in the order *Burkholderiales* belonged to the genus *Massilia* and another strain that were 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, gelatinase and urease activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain POA39 (=NIBRBAC000498475) 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, gelatinase and urease activities. Does not utilize D-glucose, L-arabinose, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 2PKSH112 (=NIBRBAC000498637) 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-maltose. Strain D8-11 (=NIBRBAC000498666) 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*.

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Neisseriales	*Neisseriaceae*	*Aquitalea*	8312	NIBRBAC000498572	*Aquitalea magnusonii*	99.0	Sediment soil	R2A	30°C, 2d
		Iodobacter	HMF4541	NIBRBAC000498438	*Iodobacter fluviatilis*	98.8	Wetland	R2A	25°C, 3d
Burkholderiales	*Burkholderiaceae*	*Paraburkholderia*	MMS16-CNU072	NIBRBAC000498621	*Paraburkholderia insulsa*	99.3	Soil	SCA	30°C, 3d
			MMS16-CNU376	NIBRBAC000498625	*Paraburkholderia megapolitana*	99.1	Soil	SCA	30°C, 3d
			MMS16-CNU436	NIBRBAC000498626	*Paraburkholderia oxyphila*	98.8	Soil	ISP-2	30°C, 3d
			MMS16-CNU135	NIBRBAC000498622	*Paraburkholderia phenazinium*	99.1	Soil	ISP-2	30°C, 3d
			MMS16-CNU462	NIBRBAC000498627	*Paraburkholderia unamae*	98.8	Soil	ISP-2	30°C, 3d
	Comamonadaceae	*Comamonas*	7227	NIBRBAC000498571	*Comamonas denitrificans*	99.7	Sediment soil	R2A	30°C, 2d
			6191	NIBRBAC000498578	*Comamonas terrigena*	99.0	Sediment soil	R2A	30°C, 2d
		Diaphorobacter	POA39	NIBRBAC000498475	*Diaphorobacter nitroreducens*	99.9	Wastewater	R2A	25°C, 3d
		Hydrogenophaga	SH4	NIBRBAC000498424	*Hydrogenophaga flava*	99.5	Agricultural soil	MA	30°C, 2d
			2PKSH112	NIBRBAC000498637	*Hydrogenophaga taeniospiralis*	98.9	Freshwater	2×R2A	25°C, 3d
		Rhodoferax	HMF4664	NIBRBAC000498444	*Rhodoferax fermentans*	99.2	Wetland	R2A	25°C, 3d
	Oxalobacteraceae	*Massilia*	HMF4544	NIBRBAC000498439	*Massilia aurea*	99.5	Wetland	R2A	25°C, 3d
	*Ralstonia*_f	*Cupriavidus*	D8-11	NIBRBAC000498666	*Cupriavidus oxalaticus*	98.9	Natural cave	R2A	30°C, 4d

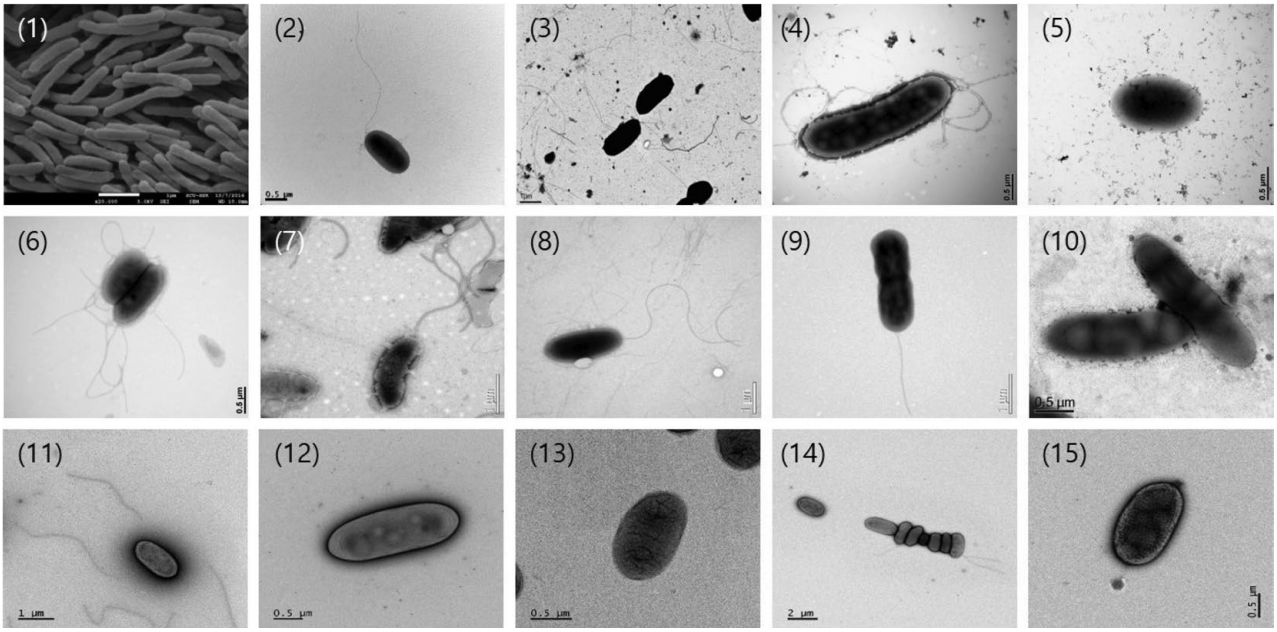

Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strains: 1, POA39; 2, 2PKSH112; 3, D8-11; 4, 7227; 5, 8312; 6, 6191; 7, HMF4664; 8, HMF4541; 9, HMF4544; 10, SH4; 11, MMS16-CNU072; 12, MMS16-CNU135; 13, MMS16-CNU376; 14, MMS16-CNU436; 15, MMS16-CNU462.

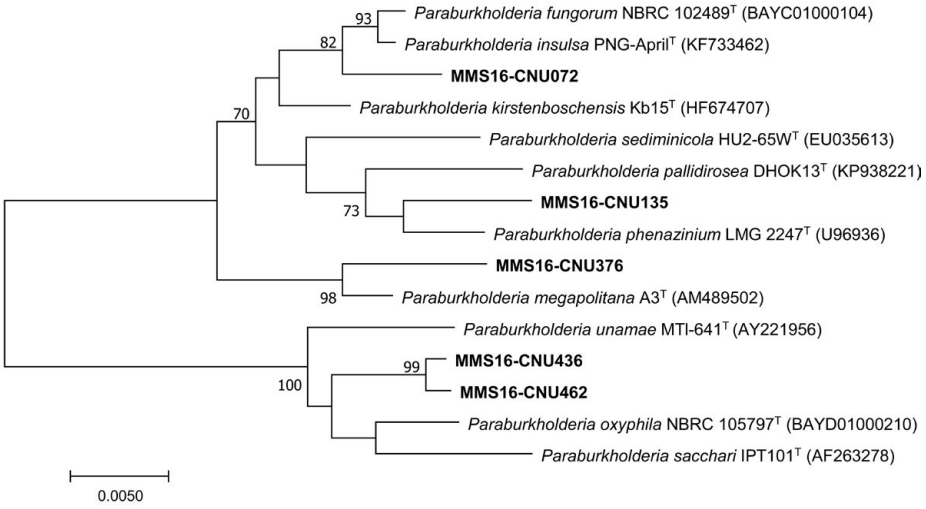

Fig. 2. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the genus *Paraburkholderia*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.005 substitutions per nucleotide position.

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 magnusonii* 8312

Cells are gram-staining-negative, non-flagellated and

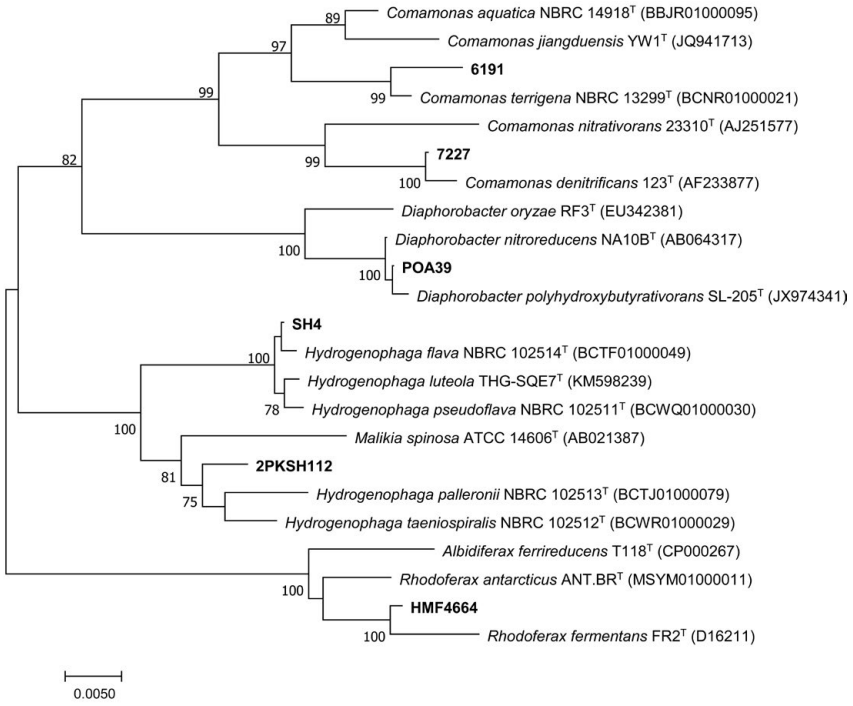

Fig. 3. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family *Comamonadaceae*. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.005 substitutions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

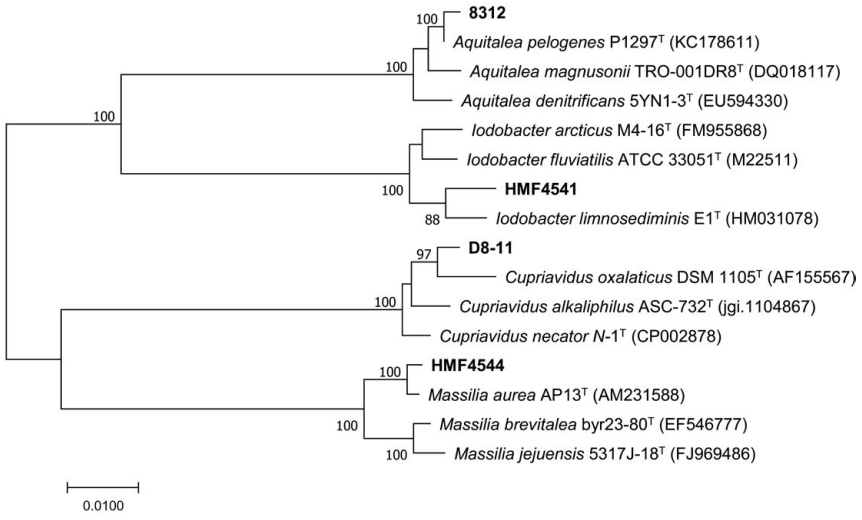

Fig. 4. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family *Neisseriaceae*, *Oxalobacteraceae* and *Ralstonia*_f. Bootstrap values (>70%) are shown above nodes for the neighbor-joining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

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-

iment soil sample, Han River, Korea.

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, adipic acid, malic 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 irregular, smooth, and pale yellow colored after 2 days of incubation at 30°C on MA. Positive for nitrate reduction and urease activities. Negative for indole production, 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 an agricultural 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.

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References

Doetsch, R.N. 1981. Determinative methods of light microscopy. Manual of Methods for General Bacteriology, pp. 21-33. In: P. Gerhardt, R.G.E. Murray, R.N. Costilow, E.W. Nester, W.A. Wood, N.R. Krieg and G.H. Phillips (eds.), American Society for Microbiology. Washington, DC, USA.

Euzéby, J.P. 2016. List of Prokaryotic Names with Standing in Nomenclature, as of February 2016 (www.bacterio.net).

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Garrity, G.M., J.A. Bell and T. Lilburn. 2005. Class II. *Betaproteobacteria* class. nov. In: D.J. Brenner, N.R. Krieg, J.T. Staley and G.M. Garrity (eds.), Bergey'sManual of Systematic Bacteriology, second edition, vol. 2 (The *Proteobacteria*), part C (The Alpha-, Beta-, Delta-, and *Epsilonproteobacteria*), Springer, New York, 2005, p. 575.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge University Press.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874.

Martin, F., S. Torelli, D. Le Paslier, A. Barbance, F. Martin-Laurent, D. Bru, R. Geremia, G. Blake and Y. Jouanneau (2012). *Betaproteobacteria* dominance and diversity shifts in the bacterial community of a PAH-contaminated soil exposed to phenanthrene. Environmental Pollution 162: 345-353.

Nakatsu, C.H., K. Hristova, S. Hanada, X.Y. Meng, J.R. Hanson, K.M. Scow and Y. Kamagata. 2006. *Methylibium petroleiphilum* gen. nov., sp. nov., a novel methyl tert-butyl ether-degrading methylotroph of the *Betaproteobacteria*. International Journal of Systematic and Evolutionary Microbiology 56(5):983-989.

Pruesse, E., J. Peplies and F.O. Glockner. 2012. SINA: accurate highthroughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823-1829

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173:697-703.

Woese, C.R. 1987. Bacterial evolution. Microbiological reviews 51:221-271.

Yoon, S.H., S.M. Ha, S. Kwon, J. Lim, Y. Kim, H. Seo and J. Chun. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. International Journal of Systematic and Evolutionary Microbiology 67:1613-1617.

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