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A report of 21 unreported bacterial species in Korea, belonging to the *Betaproteobacteria*

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As a subset investigation to discover indigenous prokaryotic species in Korea, a total of 21 bacterial strains assigned to the class *Betaproteobacteria* were isolated from a wide range of environmental samples which collected from fresh water, roots of plants, mineral water and soil from ginseng farm. Phylogenetic analysis based on 16S rRNA gene sequences indicated that 21 isolated strains were most closely related to the class *Betaproteobacteria*, with high 16S rRNA gene sequence similarity (>99.1%) and constructed a robust phylogenetic clade with the closest species in the class *Betaproteobacteria*. These isolated species have no previous report or publication in Korea; therefore 17 species in 14 genera of 6 families in the order *Burkholderiales*, 1 species in the order *Methylophilales*, 2 species in 2 genera of 1 family in the order *Neisseriales* are reported for betaproteobacterial species found in Korea. Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs are also described in the species description section and as an image.

Keywords: *Betaproteobacteria*, unreported bacterial species, bacterial divesity

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INTRODUCTION

Prokaryotic organisms, especially bacteria, are the least well understood part of our ecosystem. It is now known that only less than 4,500 bacterial species have been characterized, leaving most of the amount of them still unexplored (Whitman *et al*., 1998). It is notorious that microbes have an important role in energy conservation, material transformation, nitrogen and carbon cycling. The existence of widespread but uncultivated groups of microorganisms has been suggested by analysis of 16S rRNA gene of the microbial community in natural environments (Sogin *et al*., 2006; Liu *et al*., 2007). In case of human intestinal tract, 80% of the bacterial species which are uncultured or even unculturable (Turnbaugh *et al*., 2007), were identified by NGS techniques.

However, current culture-independent approaches also have defects on the accurate microbial description from environmental samples. These drawbacks are mainly about the depth and resolution of the frequently used molecular clock, the 16S rRNA gene sequence. Sequence-based techniques with insufficient sequencing

depth leads to miss relevant minority populations and generalized 16S rRNA gene sequencing classified closely related bacteria, such as *Bacillus anthracis*, *Bacillus cereus* and related species (Ash *et al*., 1991; Lagier *et al*. 2012). Another major disadvantage of culture-independent approaches is their limitations to provide information regarding the metabolic functions of molecularly-detected bacteria, especially novel bacteria (Shigematsu *et al*., 2009). The attraction of culture-dependent approaches is that a pure culture may be obtained and deposited in a culture collection as reference biomaterial for use by other researchers. A comprehensive understanding of the physiology of these organisms and of their complex biogeochemical processes undoubtedly requires their cultivation, isolation and characterization.

In 2013, we collected diverse environmental samples from Korean Peninsula and isolated hundreds of novel bacterial species including, unreported bacterial species in Korea. The isolated novel bacterial species assigned to the classes/phyla *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*. In this study, we present the report focuses on the description of unreported bacterial species belonging to the class *Betaproteobacteria*. The class Betaproteobacteria is mainly consisted of 7 orders (*Burkholderiales*, *Hydrogenophilales*, *Methylophilales*, *Neisseriales*, *Nitrosomonadales*, "*Procabacteriales*", and *Rhodocyclales*) (Garrity *et al*., 2005). Here we report 21 unreported bacterial species in Korea belonging to the *Betaproteobacteria*.

Materials and Methods

Various environmental samples collected from fresh water, roots of plants (bristle-hair aster, garden cosmos and evening primrose), mineral water and soil from ginseng farm (Table 1). Collected environmental samples were homogenized with shaking in a tube containing glass beads (0.4-0.6 mm diameter) and 1 mL filtered phosphate-buffered saline (PBS) for 1 min using a bead beater. Bacterial isolation from the homogenized samples were achieved by serial dilutions using PBS and separately spreading onto diverse culture media including, R2A, Marine agar 2216 and Tryptic soy agar (BD). The plates were incubated 25-30°C for 2-3 days (Table 1). Single colonies were transferred repeatedly to fresh medium to obtain pure cultures. After primary purification, the isolate was stored as 10-20% glycerol suspension −80°C as well as lyophilized ampoules.

For morphological, physiological, and biochemical characterization, Gram-staining, electron microscopy, API 20NE tests were performed after cells grew up to stationary phase. A Gram-staining kit (bioMérieux, France) was used according to the manufacturer's instructions. Gram staining were observed using a light microscope. Cellular morphology and cell size were examined by either transmission electron microscopy or scanning electron microscopy. The oxidase activity was assessed using 1% (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux). API 20NE test strips (bioMérieux) with API AUX medium (bioMérieux) were used to assess the enzyme activities of the strains, according to the manufacturer's instructions.

For the phylogenetic analysis, amplification of the 16S rRNA gene from strains were performed using a standard PCR method with a PCR pre-mix and two universal bacterial-specific primers: forward primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Lane, 1991). The PCR products were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), according to the manufacturer's instructions. The reaction mixtures were analyzed using an automated DNA analyzer system (PRISM 3730XL DNA Analyzer, Applied Biosystems). The almost complete 16S rRNA gene sequence fragments of strains were assembled and compared with those of type strains of in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; (Kim *et al*., 2012)). The 16S rRNA gene were aligned with those of the most closely related species using the multiple alignment program CLUSTALW (Thompson *et al*., 1994). The aligned sequences manually checked with Bioedit program (Hall, 1999). Phylogenetic correlations were ascertained using a conjoined phylogenetic tree which was constructed with neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Kluge and Farris, 1969), and maximum-likelihood (Felsenstein, 1981) methods with 1000 bootstrap replicates by MEGA6 (Tamura *et al*., 2013).

Results and Discussion

Based on the phylogenetic analysis by 16S rRNA gene sequences comparison, a total 21 species were assigned to the class *Betaproteobacteria*. They were all confirmed as Gram-staining-negative, chemoheterotrophic, and rod or coccoid-shaped bacteria (Fig. 1). Detailed morphological, physiological, and biochemical characteristics are shown in the species description section.

Strain W9-3-1, MS6Y-8-9, W3-2-5, SR2-07, JJ9008, MWS-11, MY2F9, DT6-05, NC3Y-10-3, 2013 Y1, JJ 9012, HME9229, W6-4-1, HME9441, SR4-06, HME 9445, 2013 C24, HME9408 and MY2F10 were most closely related to *Acidovorax delafieldii* ATCC 17505^T (AF078764; 99.59% 16S rRNA gene sequence similarity), *Advenella kashmirensis* CCUG 45225^T (AM944734;

Table 1. Summary of strains isolated and their taxonomic affiliations.

Order	Family	Strain ID	NIBR ID	Most closely related species	16S similarity (%)	Isolation source	Medium	Incubation conditions
Burkholderiales	*Oxalobacteraceae*	HME9229	NIBRBA0000114393	*Massilia aurea*	99.6	*Lagoon*	R2A	30°C, 2 days
	Burkholderiales Genera incertae sedis	HME9408	NIBRBA0000114408	*Rubrivivax gelatinosus*	99.9	*Fresh water*	R2A	30°C, 2 days
	Burkholderiales Genera incertae sedis	HME9445	NIBRBA0000114413	*Roseateles terrae*	99.8	*Fresh water*	R2A	30°C, 2 days
	Burkholderiaceae	DT6-05	NIBRBA0000114180	*Cupriavidus pampae*	99.72	*Root of Bristle-hair aster (Aster meyendorfii)*	TSA	30°C, 2 days
	Burkholderiaceae	SR2-07	NIBRBA0000114195	*Burkholderia metallica*	99.72	*Root of garden cosmos (Cosmos bipinnatus)*	R2A	30°C, 3 days
	Ralstoniaceae	SR4-06	NIBRBA0000114199	*Ralstonia solanacearum*	99.58	*Root of Evening primrose (Oenothera biennis)*	R2A	25°C, 2 days
	Comamonadaceae	W9-3-1	NIBRBA0000114212	*Acidovorax delafieldii*	99.59	*Mineral water*	R2A	25°C, 2 days
	Burkholderiales Genera incertae sedis	W3-2-5	NIBRBA0000114213	*Aquabacterium parvum*	100	*Mineral water*	R2A	25°C, 2 days
	Burkholderiales Genera incertae sedis	W6-4-1	NIBRBA0000114232	*Methylibium petroleiphilum*	99.93	*Mineral water*	R2A	25°C, 2 days
	Comamonadaceae	MY2F10	NIBRBA0000114147	*Variovorax paradoxus*	99.43	*Fresh water*	R2A	25°C, 2 days
	Burkholderiaceae	MY2F9	NIBRBA0000114148	*Cupriavidus basilensis*	100	*Fresh water*	R2A	25°C, 2 days
	Comamonadaceae	JJ9012	NIBRBA0000114163	*Limnohabitans planktonicus*	99.64	*Fresh water*	R2A	25°C, 2 days
	Burkholderiales Genera incertae sedis	2013 C24	NIBRBA0000114167	*Rubrivivax benzoatilyticus*	99.93	*Fresh water*	R2A	25°C, 2 days
	Comamonadaceae	2013 Y1	NIBRBA0000114171	*Hydrogenophaga palleronii*	99.12	*Fresh water*	R2A	25°C, 2 days
	Burkholderiaceae	NC3Y-10-3	NIBRBA0000114280	*Cupriavidus pauculus*	99.93	*Soil from ginseng farm*	MA	30°C, 3 days
	Alcaligenaceae	MS6Y-8-9	NIBRBA0000114293	*Advenella kashmirensis*	100	*Soil from ginseng farm*	MA	30°C, 3 days
	Oxalobacteraceae	MWS-11	NIBRBA0000114320	*Collimonas pratensis*	99.6	*Fresh water*	R2A	25°C, 2 days
Methylophilales	*Methylophilaceae*	HME9441	NIBRBA0000114411	*Methylophilus methylotrophus*	99.6	*Fresh water*	R2A	30°C, 3 days
Neisseriales	*Neisseriaceae*	HME9289	NIBRBA0000114398	*Vogesella perlucida*	99.8	*Fresh water*	R2A	30°C, 3 days
	Neisseriaceae	JJ9008	NIBRBA0000114159	*Chromobacterium haemolyticum*	99.14	*Fresh water*	R2A	25°C, 2 days
	Neisseriaceae	2013 C3	NIBRBA0000114164	*Vogesella perlucida*	99.35	*Fresh water*	R2A	25°C, 2 days

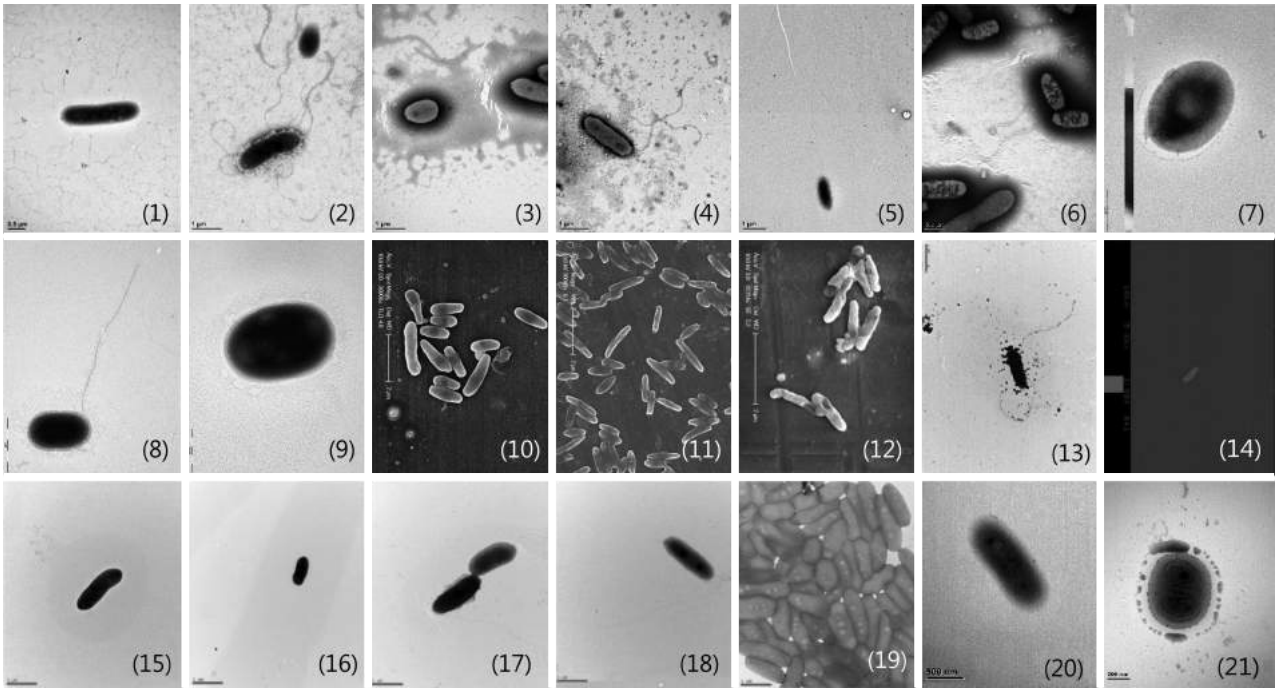

Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, MWS-11; 2, HME9229; 3, HME9289; 4, HME94085; 5, HME9441; 6, HME9445; 7, DT6-05; 8, SR2-07; 9, SR4-06; 10, W9-3-1; 11, W3-2-5; 12, W6-4-1; 13, MY2F10; 14, MY2F9; 15, JJ9008; 16, JJ9012; 17, 2013 C3; 18, 2013 C24; 19, 2013 Y1; 20, NC3Y-10-3; 21, MS6Y-8-9.

100%), *Aquabacterium parvum* B6^T (AF035052; 100%), *Burkholderia metallica* R-16017^T (AM747632; 99.72 %), *Chromobacterium haemolyticum* MDA0585^T (DQ 785104; 99.14%), *Collimonas pratensis* Ter91^T (AY28 1137; 99.60%), *Cupriavidus basilensis* RK1^T (AJ0023 02; 100%), *Cupriavidus pampae* CPDB6^T (FN430567; 99.72%), *Cupriavidus pauculus* LMG 3413^T (AF085226; 99.93%), *Hydrogenophaga palleronii* DSM 63^T (AF019 073; 99.12%), *Limnohabitans planktonicus* II-D5^T (FM 165535; 99.64%), *Massilia aurea* AP13^T (AM231588; 99.60%), *Methylibium petroleiphilum* PM1^T (CP000555; 99.93%), *Methylophilus methylotrophus* NCIMB 10515^T (AB193724; 99.60%), *Ralstonia solanacearum* GMI 1000^T (AL646052; 99.58%), *Roseateles terrae* CCUG 52222^T (AM501445; 99.80%), *Rubrivivax benzoatilyticus* JA2^T (AEWG01000018; 99.93%), *Rubrivivax gelatinosus* ATCC 17011^T (D16213; 99.90%) and *Variovorax paradoxus* IAM 12373^T (D88006; 99.43%), respectively. Strain HME9408 and 2013 C3 were most closely related to *Vogesella perlucida* DS-28^T (EF626691) with 99.80% and 99.35 16S rRNA gene sequence similarity, respectively (Table 1). In the conjoined, phylogenetic consensus tree, isolated strains constructed a robust phylogenetic clade with the most closely related species in the class *Betaproteobacteria*, as expected from high 16S rRNA gene sequence similarities (Fig. 2). Strain MS6Y-8-9, SR2-07, MY2F9, DT6-05, W3-2-5, W6-4-1, HME9445, 2013 C24, HME9408, W9-3-1, 2013 Y1, JJ9012, MY2 F10, HME9229, SR4-06 and MWS-11 were identified as the member of the order *Burkholderiales*, strain HME 9441 was identified as the member of the order *Methylophilales* and strain JJ9008, HME9289 and 2013 C3 were identified as the member of the order *Neisseriales*, respectively. At present, there has been no official report that these 28 species have been isolated in Korea; therefore 17 species in 14 genera of 6 families in the order *Burkholderiales*, 1 species in the order *Methylophilales*, 2 species in 2 genera of 1 family in the order *Neisseriales* are reported for betaproteobacterial species found in Korea.

Description of *Collimonas pratensis* MWS11

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are irregular, smooth, convex, glistening and yellowish white-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase, nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase, but negative for indole production and glucose fermentation in API 20NE test. D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, malic acid and trisodium citrate are utilized. Strain MWS11 (2013-2-027) (=NIBRBA) has

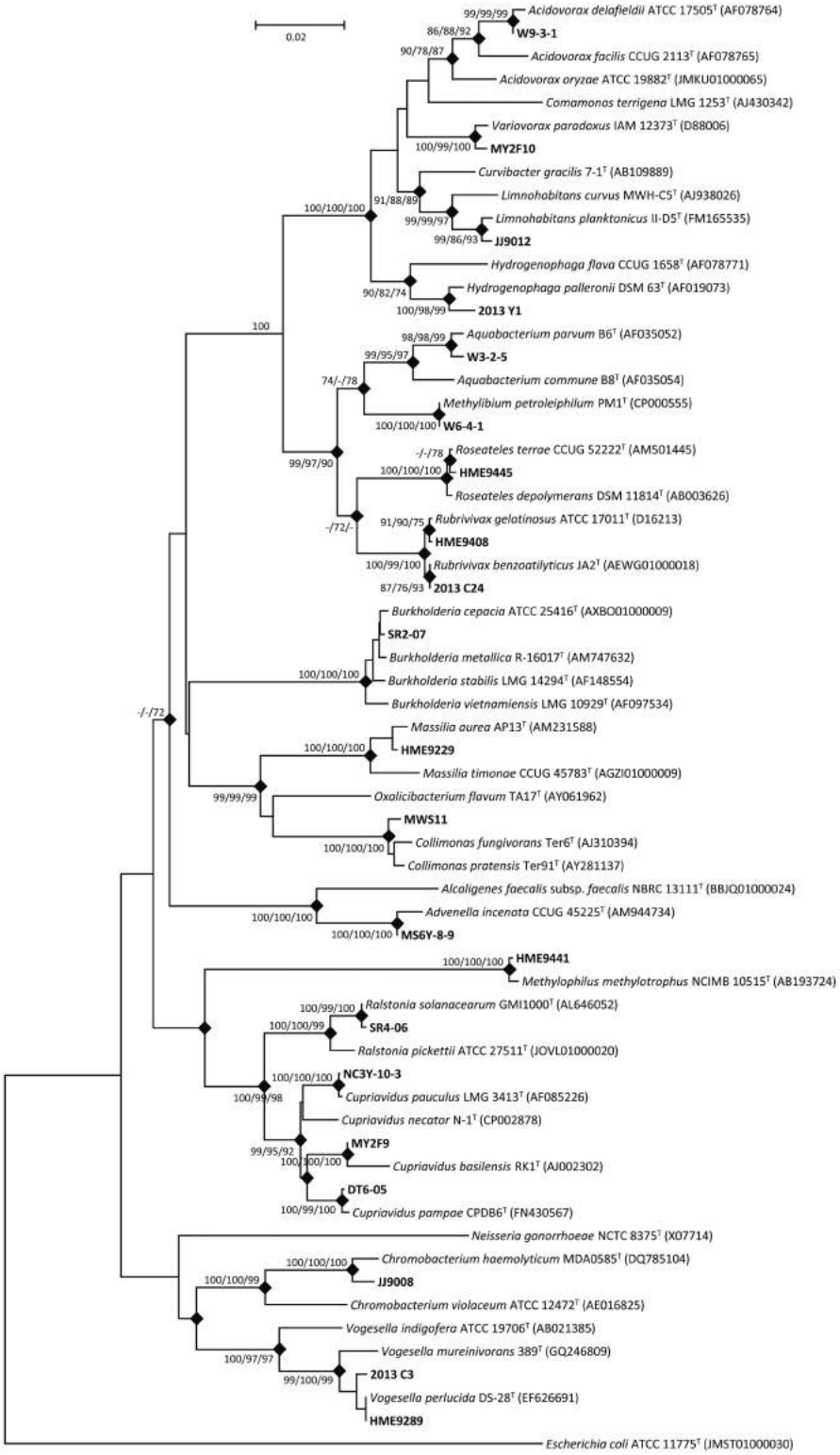

Fig 2. Phylogenetic tree based on 16S rRNA gene sequence comparisons, showing the relationship between the isolated 21 strains in this study and the notable type species from class *Betaproteobacteria*. The tree was mainly reconstructed using the neighbour-joining algorithm (NJ). Maximum-likelihood (ML) and maximum-parsimony (MP) algorithms were applied for additional comparison. Filled diamonds indicate branches present in the phylogenetic trees generated using the three different methods. Numbers on the nodes (>70%) represent bootstrap values as percentages of 1000 replicates (NJ/ML/MP). *Escherichia coli* ATCC 11775^{T} (JMST01000030) was used as an outgroup. Values <70% are not shown at the branch points. Bar, 0.02 accumulated changes per nucleotide.

been isolated from freshwater sample, Miryang, Gyeongsangnam-do, Korea.

Description of *Massilia aurea* HME9229

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, translucent and yellow-colored after 2 days of incubation on R2A at 30°C. Positive for cytochrome oxidase, esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase and glucose fermentation in API 20NE test. D-glucose, L-arabinose, D-mannose, D-maltose, adipic acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Strain HME9229 (2013-2-100) (=NIBRBA0000114393) has been isolated from lagoon, Gangneung, Gangwon-do, Korea.

Description of *Vogesella perlucida* HME9289

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire, transparent and colorless after 3 days of incubation on R2A at 30°C. Positive for cytochrome oxidase, indole production and gelatinase, but negative for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, and glucose fermentation in API 20NE test. D-glucose, N-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid are utilized. Strain HME9289 (2013-2-105) (=NIBRBA0000114398) has been isolated from freshwater sample, Yongin, Gyeonggi-do, Korea.

Description of *Rubrivivax gelatinosus* HME9408

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex, entire and Orange-brown-colored after 2 days of incubation on R2A at 30°C. Positive for cytochrome oxidase, indole production and urease, but negative for nitrate reduction, and glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE test. D-glucose, D-maltose, potassium gluconate and trisodium citrate are utilized. Strain HME9408 (2013-2-115) (=NIBRBA0000114408) has been isolated from freshwater sample, Yongin, Gyeonggi-do, Korea.

Description of *Methylophilus methylotrophus* HME9441

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are convex, translucent and white-colored after 3 days of incubation on R2A at 30°C. Positive for cytochrome oxidase, arginine dihydrolase, urease, but negative for nitrate reduction, indole production, glucose fermentation esculin hydrolysis, gelatinase and β-galactosidase in API 20NE test. D-glucose, D-mannose, D-maltose and phenylacetic acid are utilized. Strain HME9441 (2013-2-118) (=NIBRBA0000114411) has been isolated from freshwater sample, Yongin, Gyeonggi-do, Korea

Description of *Roseateles terrae* HME9445

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex with an undulate transparent margin and beige-colored after 2 days of incubation on R2A at 30°C. Positive for cytochrome oxidase, esculin hydrolysis, gelatinase and β-galactosidase, but negative for nitrate reduction, arginine dihydrolase, urease, indole production and glucose fermentation in API 20NE test. D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and capric acid are utilized. Strain HME9445 (2013-2-120) (=NIBRBA0000114413) has been isolated from freshwater sample, Yongin, Gyeonggi-do, Korea

Description of *Cupriavidus pampae* DT6-05

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 30°C. Positive for cytochrome oxidase, but negative for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, indole production β-galactosidase, and glucose fermentation in API 20NE test. Potassium gluconate, capric acid, adipic acid and malic acid are utilized. Strain DT6-05 (=NIBRBA0000114180) has been isolated from root of bristle-hair aster (*Aster meyendorfii*), Daejeon, Korea.

Description of *Burkholderia metallica* SR2-07

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are circular, entire, convex and white-colored after 3 days of incubation on R2A at 30°C. Positive for cytochrome oxidase, esculin hydrolysis, gelatinase and β-galactosidase, but negative for nitrate reduction, arginine dihydrolase, urease, indole production and glucose fermentation in API 20NE test. D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Strain SR2-07 (=NIBRBA0000 114195) has been isolated from root of garden cosmos (*Cosmos bipinnatus*), Daejeon, Korea.

Description of *Ralstonia solanacearum* SR4-06

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and coccoid-shaped. Colonies are circular, entire, convex and pale yellow-colored after 3 days of incu-

bation on R2A at 30°C. Positive for nitrate reduction, glucose fermentation and urease but negative for cytochrome oxidase, arginine dihydrolase, indole production esculin hydrolysis, gelatinase and β-galactosidase in API 20NE test. D-glucose and capric acid are utilized. Strain SR4-06 (=NIBRBA0000114199) has been isolated from root of evening primrose (Oenothera biennis), Daejeon, Korea.

Description of *Acidovorax delafieldii* W9-3-1

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire and cream-colored after 3 days of incubation on R2A at 30°C. Positive for cytochrome oxidase and nitrate reduction, but negative for arginine dihydrolase, urease, indole production, esculin hydrolysis, gelatinase and glucose fermentation and β-galactosidase in API 20NE test. D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, adipic acid and malic acid are utilized. Strain W9-3-1 (=NIBRBA0000114212) has been isolated from mineral water, Daejeon, Korea.

Description of *Aquabacterium parvum* W3-2-5

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire and white-colored after 3 days of incubation on R2A at 30°C. Positive for cytochrome oxidase and nitrate reduction, but negative for esculin hydrolysis, gelatinase, β-galactosidase, arginine dihydrolase, urease, indole production and glucose fermentation in API 20NE test. Adipic acid and malic acid are utilized. Strain W3-2-5 (=NIBRBA0000114213) has been isolated from mineral water, Daejeon, Korea.

Description of *Methylibium petroleiphilum* W6-4-1

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire and cream-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase and urease, but negative for nitrate reduction, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase, indole production and glucose fermentation in API 20NE test. Strain SR2-07 (=NIBRBA0000114195) has been isolated from root of garden cosmos (*Cosmos bipinnatus*), Daejeon, Korea.

Description of *Variovorax paradoxus* MY2F10

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire and yellow-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase and urease, but negative for nitrate reduction, arginine dihydrolase, esculin hydrolysis, gelatinase, indole production, β-galactosidase and glucose fermentation in API 20NE test. D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Strain MY2F10 (=NIBRBA0000114147) has been isolated from freshwater, Imsil, Jeollabuk-do, Korea.

Description of *Cupriavidus basilensis* MY2F9

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, raised, entire and yellow-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase, but negative for nitrate reduction, arginine dihydrolase, esculin hydrolysis, gelatinase, urease, indole production, β-galactosidase and glucose fermentation in API 20NE test. Potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Strain MY2F9 (=NIBRBA0000114148) has been isolated from freshwater, Imsil, Jeollabuk-do, Korea.

Description of *Chromobacterium haemolyticum* JJ9008

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex and beige-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase, nitrate reduction, glucose fermentation, arginine dihydrolase and gelatinase, but negative for urease, esculin hydrolysis, indole production and β-galactosidase in API 20NE test. D-glucose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and trisodium citrate are utilized. Strain JJ9008 (=NIBRBA0000114159) has been isolated from freshwater, Cheongsong, Gyeongsangbuk-do, Korea.

Description of *Limnohabitans planktonicus* JJ9012

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular, convex and white-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase, but negative for nitrate reduction, glucose fermentation, arginine dihydrolase urease, esculin hydrolysis, gelatinase, indole production and β-galactosidase in API 20NE test. Strain JJ9012 (=NIBRBA0000114163) has been isolated from freshwater, Cheongsong, Gyeongsangbuk-do, Korea.

Description of *Vogesella perlucida* 2013 C3

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are circular and transparent after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase, nitrate reduction and indole production, but negative for glucose fermentation,

arginine dihydrolase, gelatinase, urease, esculin hydrolysis, and β-galactosidase in API 20NE test. D-glucose, D-mannitol, N-acetyl-glucosamine, D-maltose and malic acid are utilized. Strain 2013 C3 (=NIBRBA0000114164) has been isolated from freshwater, Yeongcheon, Gyeongsangbuk-do, Korea.

Description of *Rubrivivax benzoatilyticus* 2013 C24

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are round, convex and brown-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase, indole production, glucose fermentation, arginine dihydrolase and urease, but negative for nitrate reduction, gelatinase, esculin hydrolysis, and β-galactosidase in API 20NE test. D-glucose and potassium are utilized. Strain 2013 C24 (=NIBRBA 0000114167) has been isolated from freshwater, Yeongcheon, Gyeongsangbuk-do, Korea.

Description of *Hydrogenophaga palleronii* 2013 Y1

Cells are Gram-staining-negative, non-flagellated, non-pigmented, and rod-shaped. Colonies are round and yellow-colored after 2 days of incubation on R2A at 25°C. Positive for cytochrome oxidase, glucose fermentation, arginine dihydrolase and urease, but negative for nitrate reduction, indole production, gelatinase, esculin hydrolysis, and β-galactosidase in API 20NE test. D-mannitol and potassium are utilized. Strain 2013 Y1 (=NIBRBA 0000114171) has been isolated from freshwater, Jinan, Jeollabuk-do, Korea.

Description of *Cupriavidus pauculus* NC3Y-10-3

Cells are Gram-staining-negative, flagellated, non-pigmented, and rod-shaped. Colonies are punctiform, entire, smooth, flat and white-colored after 3 days of incubation on MA at 30°C. Positive for urease, but negative for cytochrome oxidase, esculin hydrolysis, gelatinase, nitrate reduction, arginine dihydrolase, β-galactosidase, indole production and glucose fermentation in API 20NE test. Potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate are utilized. Strain NC3Y-10-3 (=NIBRBA0000114280) has been isolated from ginseng farm, Anseong, Gyeonggi-do, Korea.

Description of *Advenella kashmirensis* MS6Y-8-9

Cells are Gram-staining-negative, flagellated, non-pigmented, and coccoid-shaped. Colonies are irregular, entire, smooth, raised and pale white-colored after 3 days of incubation on MA at 30°C. Negative for cytochrome oxidase, esculin hydrolysis, gelatinase, nitrate reduction, urease, arginine dihydrolase, β-galactosidase, indole production and glucose fermentation in API 20NE test. D-glucose, L-arabinose, potassium gluconate, adipic acid, malic acid and trisodium citrate are utilized. Strain MS6Y-8-9 (=NIBRBA0000114293) has been isolated from ginseng farm, Anseong, Gyeonggi-do, Korea.

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