

A report of 31 unrecorded bacterial species isolated from freshwater

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Abstract: A total of 31 bacterial strains were isolated from the Geum River basin in the Republic of Korea during our investigation of indigenous prokaryotic species. The isolated bacterial strains had high 16S rRNA gene sequence similarity (>98.7%) with those of validly published bacterial species, which have not been reported in Republic of Korea. The 31 bacterial strains were phylogenetically diverse and assigned to 4 phyla, 8 classes, 18 orders, 21 families, and 27 genera. At the genus level, the unreported species were affiliated with *Kineococcus*, *Pedococcus*, *Rhodoluna*, *Salinibacterium*, *Rhodoluna*, *Arthrobacter*, *Williamsia*, *Nakamurella*, *Nocardioides* of the class *Actinobacteria*, *Patulibacter* of the class *Thermoleophilia*, *Pontibacter*, *Hymenobacter* of the class *Cytophagia*, *Flavobacterium* of the class *Flavobacteriia*, *Geomicrobium* of the class *Bacilli*, *Brevundimonas*, *Gellertiella*, *Rhizobium*, *Paracoccus*, *Taonella*, *Sphingomonas* of the class *Alphaproteobacteria*, *Burkholderia*, *Polaromonas*, *Hydrogenophaga*, *Chitinilyticum*, *Azospira*, *Zoogloea* of the class *Betaproteobacteria*, and *Pseudomonas* of the class *Gammaproteobacteria*. The unreported bacterial species were further characterized by examining their morphological, cultural, physiological, and biochemical properties. The detailed descriptions of the 31 bacterial strains were provided.

Keywords: freshwater, Geum River, unreported bacterial species

INTRODUCTION

Freshwater ecosystems such as rivers, lakes and wetlands are home to a wide variety of species and while they constitute approximately < 1% of the Earth's surface, they are inhabited by 10% of earth's species including a third of all vertebrate species (Strayer and Dudgeon 2010). Threats to freshwater ecosystems include pollution, exploitation, invasive species, land-use and climate change. The cumulative impact in recent decades of these threats has significantly reduced freshwater biodiversity (Dudgeon *et al.*, 2006), though in turn has promoted bacterial diversity (Li 2017; Rodríguez *et al.* 2018; Tanentzap *et al.* 2019). The study of microbial diversity is conducted generally with

next-generation sequencing (NGS). However, isolating microbes, particularly any new species and culturing them, needs to be a priority (Han *et al.* 2020) because the majority of microorganisms remain uncultured and thus poorly characterized (Lewis *et al.* 2021), without much data on their roles and functions, nor their ecological impact and industrial applications.

As a part of the research program 'Survey of freshwater organisms and specimen collection' by the Nakdonggang National Institute of Biological Resources (NNIBR), we collected freshwater samples from the Geum River basin to report the taxonomic information and phenotypic characterization of any previously unrecorded and unidentified bacterial strains.

MATERIALS AND METHODS

Freshwater environmental samples were collected from the Geum River basin in Republic of Korea for bacterial specimens (Table 1). The samples were inoculated on to Reasoner's 2A agar (R2A; BD), tryptic soy agar (TSA; BD), 1/10-diluted TSA, marine agar 2216 (MA; BD), 1/10 diluted MA and nutrient agar (NA) using a standard dilution plating technique and plates were incubated at 25°C for 3 days. The pure cultures of bacterial isolates were preserved at -80°C as a suspension in 20% (w/v) glycerol. The 31 bacterial species designations, isolation sources, medium and incubation conditions are summarized in Table 1.

The genomic DNA of isolates were extracted using the DNeasy Blood and Tissue kit (Qiagen, Germany) and the almost complete 16S rRNA gene was sequenced using the universal primers 27F and 1492R (Weisburg *et al.* 1991) by Macrogen (Republic of Korea). The complete sequence was compared to sequences obtained from the EzBioCloud server (Yoon *et al.* 2017) and a cutoff value of 98.7% sequence similarity was applied for bacterial species determination. Bacterial strains showing 98.7% or higher sequence similarity with type strains unreported in Republic of Korea were identified as unreported bacterial species. Phylogenetic trees based on the neighbor-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) algorithms, with 1,000-replicate bootstrap values were constructed using MEGA11 software (Tamura *et al.* 2021).

Cellular morphology and size were examined by transmission electron microscopy. Gram staining was performed using a Gram-staining kit (bioMérieux, France). Biochemical characteristics were assessed using API 20NE (bioMérieux) according to the manufacturer's instructions. Oxidase activity was measured using oxidase reagent (bioMérieux).

RESULTS AND DISCUSSION

Based on the phylogenetic analyses using the 16S rRNA gene sequences, the 31 strains (NCS-2-32, KCS-47, NSS-12, MYG-2-5, GAP-35, JU-13, KMU-11, MS-33, IBC-2-120, 20LEG-88, KCS-46, MBC-2-58, 20LEG-73, 9S-12, KCS-3, JKS-1, MND-3-64, 20HNW-03, IND-2-6, JU-40, 7J1-06, 7J3-01, JU-31, NSW-11, JJ1S-14, 20CSW-3, 9W-13, YDC-53, 9S-10, 9W-02 and KKMS-29) isolated from Geum River were assigned to the phyla *Actinobacteria* (Fig.

1), *Bacteroidetes* (Fig. 2), *Firmicutes* (Fig. 3) and *Proteobacteria* (Fig. 4). The strains represented 31 unrecorded species in Korea, belonging to 8 classes, 18 orders, 21 families. The taxonomic identification of the isolates is summarized in Table 1. Transmission electron micrographs of the isolates are provided in Fig. 5. The morphological, physiological and biochemical properties of isolated strains are described in detail as below.

Description of *Kineococcus radiotolerans* NCS-2-32

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, opaque, rough and faint orange colored after 2 days on NA at 25°C. Positive for glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease and β -galactosidase, but negative for oxidase, gelatin hydrolysis, nitrate reduction and indole production. 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 NCS-2-32 (= NNIBR2020641BA782) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW367770.

Description of *Pedococcus aerophilus* KCS-47

Cells are Gram-staining-positive, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, entire and white colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis, gelatin hydrolysis and β -galactosidase, but negative for oxidase, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and urease. D-Glucose, D-Mannitol, potassium gluconate and malic acid are utilized. Does not utilize L-Arabinose, D-Mannose, N-acetyl-glucosamine, D-Maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain KCS-47 (= NNIBR2019641BA240) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN782285.

Description of *Pedococcus bigeumensis* NSS-12

Cells are Gram-staining-positive, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, entire and white colored after 3 days on R2A at 25°C. Positive

Table 1. Summary of isolated strains from the Geum River basin and their taxonomic affiliations

| Phylum | Class | Order | Family | Strain ID | NNIBR ID | Accession number | Identification | Similarity (%) | Isolation source (GPS) | Medium |
|-----------------|-----------------|---------------------|-------------------|-----------|-------------------|------------------|-------------------------------------|----------------|--|--------|
| Actinobacteria | Actinobacteria | Kineosporiales | Kineosporiaceae | NCS-2-32 | NNIBR2020641BA782 | MW367770 | <i>Kineococcus radiotolerans</i> | 99.3 | River water (36°20'57.7"N, 127°33'36.6"E) | NA |
| | | | | KCS-47 | NNIBR2019641BA240 | MN782285 | <i>Pedococcus aerophilus</i> | 100.0 | River sediment (36°20'41.7"N, 127°21'07.0"E) | RZA |
| | | | | NSS-12 | NNIBR2020641BA815 | MW293919 | <i>Pedococcus bigeumensis</i> | 99.9 | River sediment (36°14'00.1"N, 127°07'47.3"E) | RZA |
| | | | | MYG-2-5 | NNIBR2020641BA789 | MW367771 | <i>Pedococcus cremeus</i> | 99.8 | River water (36°27'46.9"N, 127°35'16.2"E) | MA |
| | | | | GAP-35 | NNIBR2019641BA25 | MN559417 | <i>Rhodoluna limnophila</i> | 99.1 | River water (36°20'41.7"N, 127°21'7"E) | RZA |
| | | | | JU-13 | NNIBR2019641BA26 | MN559418 | <i>Salinibacterium xinjiangense</i> | 99.5 | River water (36°56'19.2"N, 127°26'13.3"E) | RZA |
| | | | | KMU-11 | NNIBR2020641BA794 | MT893382 | <i>Rhodoluna lacicola</i> | 100.0 | River water (36°01'38.70"N, 127°38'07.57"E) | RZA |
| | | | | MS-33 | NNIBR2020641BA795 | MT893383 | <i>Arthrobacter flavus</i> | 99.7 | River sediment (36°01'38.70"N, 127°38'07.57"E) | RZA |
| | | | | IBC-2-120 | NNIBR2020641BA788 | MW367775 | <i>Williamsia herbipolensis</i> | 100.0 | River water (36°19'03.1"N, 127°44'33.8"E) | RZA |
| | | | | 20LEG-88 | NNIBR2020641BA804 | MW295517 | <i>Nakamurella deserti</i> | 99.1 | River water (36°27'0.34"N, 127°26'32.09"E) | 0.1TSA |
| Mycobacteriales | Mycobacteriales | Gordoniaceae | Gordoniaceae | MS-33 | NNIBR2020641BA795 | MT893383 | <i>Arthrobacter flavus</i> | 99.7 | River sediment (36°01'38.70"N, 127°38'07.57"E) | RZA |
| | | | | IBC-2-120 | NNIBR2020641BA788 | MW367775 | <i>Williamsia herbipolensis</i> | 100.0 | River water (36°19'03.1"N, 127°44'33.8"E) | RZA |
| Bacteroidetes | Bacteroidetes | Nakamurellales | Nakamurellaceae | 20LEG-88 | NNIBR2020641BA804 | MW295517 | <i>Nakamurella deserti</i> | 99.1 | River water (36°27'0.34"N, 127°26'32.09"E) | 0.1TSA |
| | | | | KCS-46 | NNIBR2019641BA239 | MN749884 | <i>Nocardioides zeicaulis</i> | 99.0 | River sediment (36°20'41.7"N, 127°21'07.0"E) | RZA |
| Thermoleophila | Thermoleophila | Solirubrobacterales | Patulibacteraceae | MBC-2-58 | NNIBR2020641BA787 | OP872588 | <i>Nocardioides taihuensis</i> | 100.0 | River water (36°19'03.1"N, 127°44'33.8"E) | MA |
| | | | | 20LEG-73 | NNIBR2020641BA803 | MW295516 | <i>Patulibacter americanus</i> | 99.7 | River water (36°27'0.34"N, 127°26'32.09"E) | TSA |
| Cyanobacteria | Cyanobacteria | Cytophagales | Hymenobacteraceae | 9S-12 | NNIBR2019641BA1 | MN602460 | <i>Pontibacter populi</i> | 99.8 | River sediment (36°39'1.78"N, 127°22'25.63"E) | RZA |
| | | | | KCS-3 | NNIBR2019641BA238 | MN749883 | <i>Hymenobacter perfusus</i> | 99.5 | River sediment (36°20'41.7"N, 127°21'07.0"E) | RZA |
| Firmicutes | Firmicutes | Caryophanales | Bacillaceae | JKS-1 | NNIBR2019641BA244 | MN749893 | <i>Flavobacterium viviphilum</i> | 99.9 | River sediment (35°43'49.96"N, 127°35'03.36"E) | RZA |
| | | | | MIND-3-64 | NNIBR2020641BA791 | MW367772 | <i>Geomicrobium sediminis</i> | 99.8 | River water (36°00'19.6"N, 127°39'44.3"E) | MA |

Table 1. Continued

| Phylum | Class | Order | Family | Strain ID | NNIBR ID | Accession number | Identification | Similarity (%) | Isolation source (GPS) | Medium | | | | | | | | |
|---------------------|--------------------|-------------------|--------------------|-------------------|-------------------|----------------------------------|------------------------------------|--|---|---|---|---|-------------------|----------|------------------------------|------|--|-----|
| Alphaproteobacteria | Caulobacterales | Caulobacteraceae | 20HNW-03 | NNIBR2020641BA805 | MW295518 | <i>Brevundimonas balnearis</i> | 99.8 | River water (36°26'49.87"N, 127°35'2.28"E) | 0.1MA | | | | | | | | | |
| | | | | | | | | | | Hyphomicrobiales | Rhizobiaceae | IND-2-6 | NNIBR2020641BA790 | MW367774 | <i>Gellertella hungarica</i> | 98.8 | River water (36°00'19.6"N, 127°39'44.3"E) | R2A |
| | | | | | | | | | | | | JU-40 | NNIBR2019641BA29 | MN559421 | <i>Rhizobium ipomoeae</i> | 99.4 | River water (35°56'19.2"N, 127°26'13.3"E) | R2A |
| | Rhodobacterales | Rhodobacteraceae | 7J1-06 | NNIBR2019641BA4 | MN602458 | <i>Paracoccus salipaludis</i> | 99.2 | River water (36°19'35.49"N, 126°51'33.5"E) | R2A | | | | | | | | | |
| | | | Sneathiellales | Sneathiellaceae | 7J3-01 | NNIBR2019641BA5 | MN602464 | <i>Taonella mepensis</i> | 99.9 | River water (36°7'42.77"N, 126°55'36.26"E) | R2A | | | | | | | |
| | Sphingomonadales | Sphingomonadaceae | JU-31 | NNIBR2020641BA793 | MN559425 | <i>Sphingomonas osychrolutea</i> | 99.5 | River water (35°56'19.2"N, 127°26'13.3"E) | R2A | | | | | | | | | |
| | | | Burkholderiales | Burkholderiaceae | NSW-11 | NNIBR2020641BA816 | MW293920 | <i>Burkholderia gladioli</i> | 99.6 | River water (36°14'00.1"N, 127°07'47.3"E) | R2A | | | | | | | |
| | | | | | Burkholderiales | Comamonadaceae | JJ1S-14 | NNIBR2019641BA236 | MN749850 | <i>Polaromonas eurypsychrophila</i> | 98.9 | River sediment (35°50'38.1"N, 127°24'51.8"E) | R2A | | | | | |
| | Betaproteobacteria | Neisseriales | Chromobacteriaceae | 20CSW-3 | NNIBR2020641BA802 | MW295515 | <i>Hydrogenophaga laconesensis</i> | 99.5 | River water (36°20'57.67"N, 127°33'36.57"E) | 0.1TSA | | | | | | | | |
| | | | | Rhodocyclales | Rhodocyclaceae | 9W-13 | NNIBR2019641BA3 | MN602461 | <i>Chitinilyticum litopenaei</i> | 99.7 | River water (36°39'1.78"N, 127°22'25.63"E) | R2A | | | | | | |
| YDC-53 | | | | | | NNIBR2020641BA796 | MT893384 | <i>Azospira oryzae</i> | 99.9 | River water (36°09'12.2"N, 127°44'57.4"E) | R2A | | | | | | | |
| Rhodocyclales | | Zoogloeaceae | 9S-10 | NNIBR2019641BA2 | MN602459 | <i>Zoogloea resiniphila</i> | 99.5 | River sediment (36°39'1.78"N, 127°22'25.63"E) | R2A | | | | | | | | | |
| | | | 9W-02 | NNIBR2019654BA3 | MN696219 | <i>Zoogloea oryzae</i> | 99.9 | River water (36°39'1.78"N, 127°22'25.63"E) | R2A | | | | | | | | | |
| Gammaproteobacteria | | Pseudomonadales | Pseudomonadaceae | KKMS-29 | NNIBR2019641BA237 | MN749851 | <i>Pseudomonas furukawai</i> | 99.2 | River sediment (36°01'19.3"N, 126°45'56.2"E) | TSA | | | | | | | | |

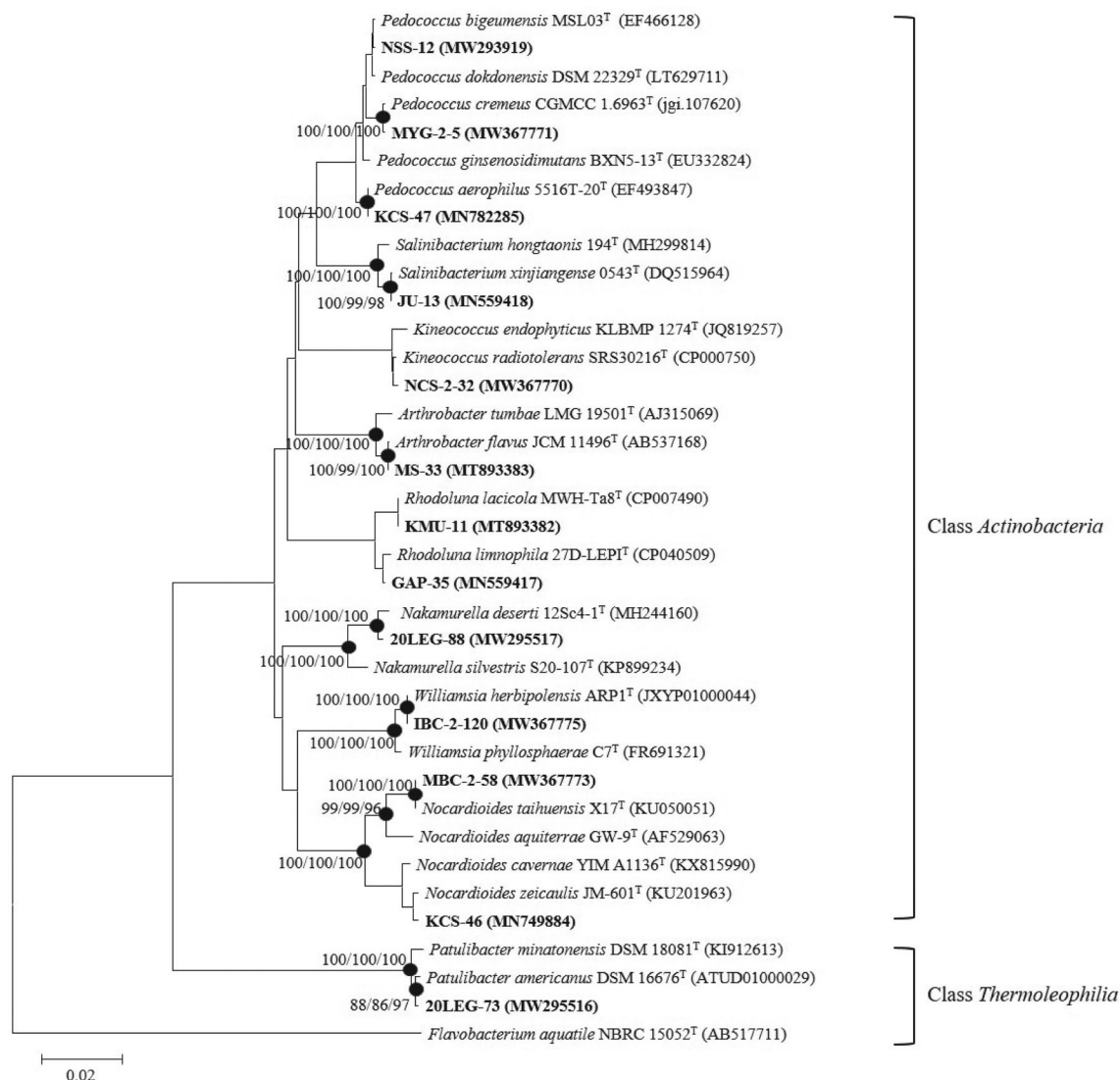


Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest relatives of the class *Actinobacteria* and *Thermoleophilia*. Bootstrap values over 70% are shown at nodes for neighbor-joining, maximum likelihood and maximum parsimony methods, respectively. Filled circles indicate nodes recovered by the maximum likelihood and maximum parsimony algorithms. Bar, 0.02 substitutions per nucleotide position.

for oxidase, nitrate reduction, esculin hydrolysis, gelatin hydrolysis and β -galactosidase, but negative for glucose fermentation, indole production and urease. D-Glucose, D-Mannitol, D-Maltose and potassium gluconate are utilized. Does not utilize L-Arabinose, D-Mannose, *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain NSS-12 (= NNI-BR2020641BA815) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW293919.

Description of *Pedococcus cremeus* MYG-2-5

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, smooth and cream colored after 3 days on MA at 25°C. Positive for oxidase, esculin hydrolysis, glucose fermentation and β -galactosidase, but negative for nitrate reduction, gelatin hydrolysis, indole production, arginine dihydrolase and urease. D-Glucose and potassium gluconate are utilized. Does not utilize L-Arabinose, D-Mannose, D-Mannitol, *N*-acetyl-glucosamine, D-Maltose, capric acid, adipic acid, malic

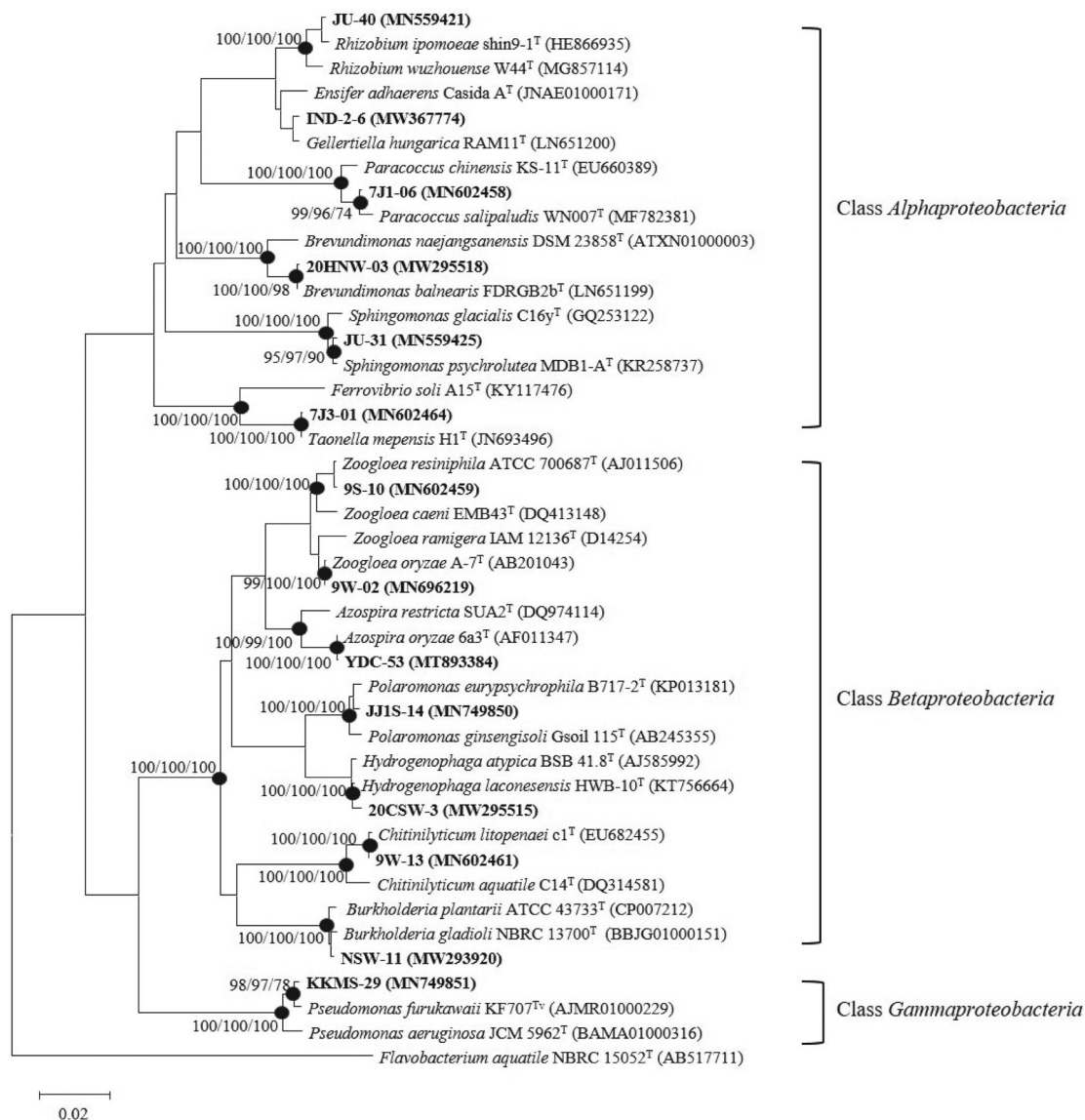


Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest relatives of the class Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria. Bootstrap values over 70% are shown at nodes for neighbor-joining, maximum likelihood and maximum parsimony methods, respectively. Filled circles indicate nodes recovered by the maximum likelihood and maximum parsimony algorithms. Bar, 0.02 substitutions per nucleotide position.

acid, trisodium citrate and phenylacetic acid. Strain MYG-2-5 (= NNIBR2020641BA789) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW367771.

Description of *Rhodoluna limnophila* GAP-35

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex with

entire edge and orange colored after 3 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis and β -galactosidase, but negative for oxidase, gelatin, glucose fermentation, indole production, arginine dihydrolase and urease. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, malic acid and trisodium citrate, D-Maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid are not utilized. GAP-35 (= NNIBR2019641BA25) was isolated from freshwater of the

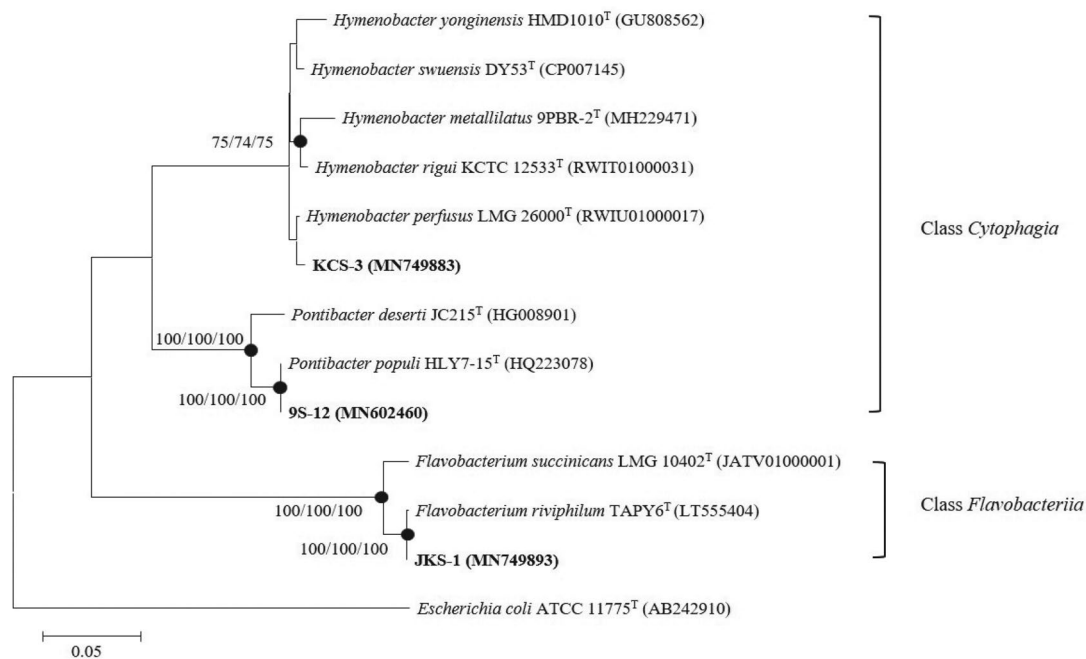


Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest relatives of the class *Cytophagia* and *Flavobacteriia*. Bootstrap values over 70% are shown at nodes for neighbor-joining, maximum likelihood and maximum parsimony methods, respectively. Filled circles indicate nodes recovered by the maximum likelihood and maximum parsimony algorithms. Bar, 0.05 substitutions per nucleotide position.

Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN559417.

Description of *Salinibacterium xinjiangense* JU-13

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, are circular, convex with entire edge and yellow colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and β -galactosidase, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and urease. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol and D-Maltose are utilized. Does not utilize *N*-acetyl-glucosamine, malic acid, trisodium citrate, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain JU-13 (= NNIBR2019641BA26) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN559418.

Description of *Rhodoluna laticola* KMU-11

Cells are Gram-staining-negative, non-flagellated, fila-

ment-shaped and aerobic. Colonies are circular, convex, smooth and red colored after 3 days on R2A at 25°C. Positive for oxidase and esculin hydrolysis, but negative for gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, β -galactosidase, arginine dihydrolase and urease. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, *N*-acetyl-glucosamine, malic acid, trisodium citrate, D-Maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid are not utilized. Strain KMU-11 (= NNIBR2020641BA794) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MT893382.

Description of *Arthrobacter flavus* MS-33

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth and yellow colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis, β -galactosidase and urease, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, *N*-acetyl-glucosamine, malic acid, trisodium citrate, D-Maltose, potassium gluconate, capric acid, adipic acid

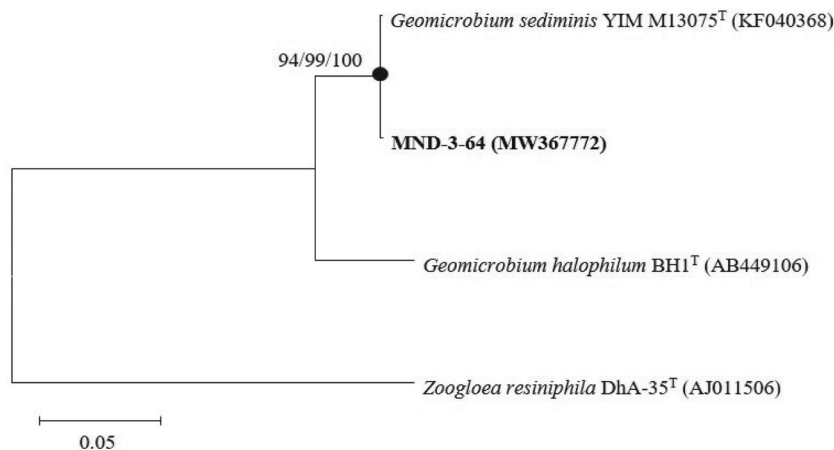


Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest relatives of the class *Bacilli*. Bootstrap values over 70% are shown at nodes for neighbor-joining, maximum likelihood and maximum parsimony methods, respectively. Filled circles indicate nodes recovered by the maximum likelihood and maximum parsimony algorithms. Bar, 0.05 substitutions per nucleotide position.

and phenylacetic acid are not utilized. Strain MS-33 (= NNIBR2020641BA795) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MT893383.

Description of *Williamsia herbipolensis* IBC-2-120

Cells are Gram-staining-positive, non-flagellated, coccid or rod-shaped and aerobic. Colonies are circular, convex with entire edge, opaque, smooth and light orange colored after 3 days on R2A at 25°C. Positive for glucose fermentation, urease and β -galactosidase (week) but negative for oxidase, esculin hydrolysis, gelatin hydrolysis, nitrate reduction, indole production and arginine dihydrolase. D-Glucose, D-Mannose, D-Mannitol, malic acid and trisodium citrate are utilized. Does not utilize L-Arabinose, *N*-acetyl-glucosamine, D-Maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain IBC-2-120 (= NNIBR2020641BA788) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW367775.

Description of *Nakamurella deserti* 20LEG-88

Cells are Gram-staining-positive, non-flagellated, coccus-shaped and aerobic. Colonies are irregular, dry, undulate and ivory colored after 3 days on 0.1TSA at 25°C. Positive for esculin hydrolysis, urease and β -galactosidase, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production and arginine

dihydrolase. D-Glucose, L-Arabinose, D-Mannitol, *N*-acetyl-glucosamine, D-Maltose, potassium gluconate and malic acid are utilized. Does not utilize D-Mannose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain 20LEG-88 (= NNIBR2020641BA804) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW295517.

Description of *Nocardioides zeicaulis* KCS-46

Cells are Gram-staining-positive, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, entire and yellow colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and β -galactosidase, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and urease. D-Glucose and D-Mannitol are utilized. Does not utilize L-Arabinose, D-Mannose, *N*-acetyl-glucosamine, D-Maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KCS-46 (= NNIBR2019641BA239) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN749884.

Description of *Nocardioides taihuensis* MBC-2-58

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are convex, opaque with entire edge and white colored after 4 days on MA at 25°C.

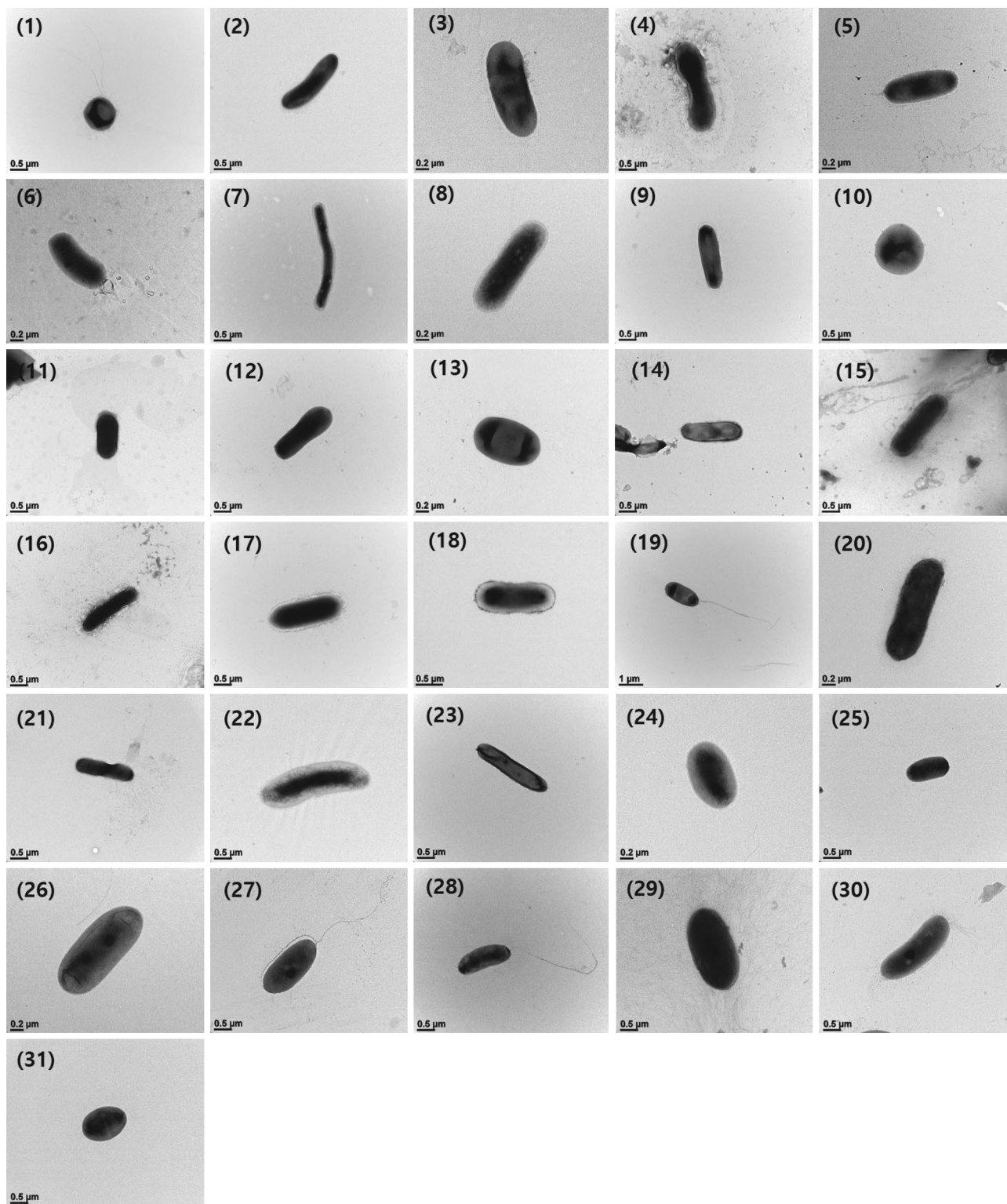


Fig. 5. Transmission electron micrographs of the isolated strains. Stains: 1, NCS-2-32; 2, KCS-47; 3, NSS-12; 4, MYG-2-5; 5, GAP-35; 6, JU-13; 7, KMU-11; 8, MS-33; 9, IBC-2-120; 10, 20LEG-88; 11, KCS-46; 12, MBC-2-58; 13, 20LEG-73; 14, 9S-12; 15, KCS-3; 16, JKS-1; 17, MND-3-64; 18, 20HNW-03; 19, IND-2-6; 20, JU-40; 21, 7J1-06; 22, 7J3-01; 23, JU-31; 24, NSW-11; 25, JJ1S-14; 26, 20CSW-3; 27, 9W-13; 28, YDC-53; 29, 9S-10; 30, 9W-02; 31, KKMS-29.

Positive for glucose fermentation and β -galactosidase, but negative for oxidase, esculin hydrolysis and gelatin hydrolysis, nitrate reduction, indole production, arginine dihydrolase and urease. D-Glucose, L-Arabinose, adipic acid are utilized. Does not utilize D-Mannose, D-Mannitol, *N*-acetyl-glucosamine, D-Maltose, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain MBC-2-58 (= NNIBR2020641BA787) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW367773.

Description of *Patulibacter americanus* 20LEG-73

Cells are Gram-staining-positive, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth and yellowish ivory colored after 3 days on TSA at 25°C. Positive for oxidase, esculin hydrolysis, gelatin hydrolysis and arginine dihydrolase, but negative for nitrate reduction, glucose fermentation, indole production, urease and β -galactosidase. D-Glucose and malic acid are utilized. Does not utilize L-Arabinose, D-Mannose, D-Mannitol, *N*-acetyl-glucosamine, D-Maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain 20LEG-73 (= NNIBR2020641BA803) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW295516.

Description of *Pontibacter populi* 9S-12

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, shiny and pink colored after 3 days on R2A at 25°C. Positive for oxidase, but negative for esculin hydrolysis, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, β -galactosidase and urease. D-Glucose, D-Mannose, *N*-acetyl-glucosamine, D-Maltose (weakly) and potassium gluconate are utilized. Does not utilize L-Arabinose, D-Mannitol, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 9S-10 (= NNIBR2019641BA1) was isolated from sediment of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN602460.

Description of *Hymenobacter perfusus* KCS-3

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, entire

and red colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and gelatin hydrolysis, but negative for oxidase, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, urease and β -galactosidase. D-Mannose, D-Mannitol and trisodium citrate are utilized. Does not utilize D-Glucose, L-Arabinose, *N*-acetyl-glucosamine, D-Maltose, potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid. Strain KCS-3 (= NNIBR2019641BA238) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN749883.

Description of *Flavobacterium riviphilum* JKS-1

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, entire and yellow colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, urease and β -galactosidase. D-Maltose are utilized. Does not utilize D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain JKS-1 (= NNIBR2019641BA244) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN749893.

Description of *Geomicrobium sediminis* MND-3-64

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex with entire edge, smooth and beige colored after 2 days on MA at 25°C. Positive for oxidase, esculin hydrolysis, gelatin hydrolysis arginine dihydrolase and urease, but negative for nitrate reduction, glucose fermentation, indole production and β -galactosidase. 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 are not utilized. Strain MND-3-64 (= NNIBR2020641BA791) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW367772.

Description of *Brevundimonas balnearis* 20HNW-03

Cells are Gram-staining-negative, non-flagellated, rods-

shaped and aerobic. Colonies are circular, convex, smooth and yellow colored after 3 days on 0.1 MA at 25°C. Positive for esculin hydrolysis and β -galactosidase, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and urease. 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 are not utilized. Strain 20HNW-03 (= NNIBR2020641BA805) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW295518.

Description of *Gellertiella hungarica* IND-2-6

Cells are Gram-staining-negative, flagellated, rod-shaped and aerobic. Colonies are mucoid, irregular, opaque, convex and ivory colored after 2 days on R2A at 25°C. Positive for oxidase, esculin hydrolysis, β -galactosidase, arginine dihydrolase and urease, but negative for gelatin hydrolysis, nitrate reduction, glucose fermentation and indole production. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine and D-Maltose are utilized. Does not utilize potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain IND-2-6 (= NNIBR2020641BA790) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW367774.

Description of *Rhizobium ipomoeae* JU-40

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex with entire edge and ivory colored after 3 days on R2A at 25°C. Positive for oxidase, esculin hydrolysis, nitrate reduction and β -galactosidase, but negative for gelatin hydrolysis, glucose fermentation, indole production, arginine dihydrolase and urease. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, D-Maltose and trisodium citrate are utilized. Does not utilize potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid. Strain JU-40 (= NNIBR2019641BA29) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN559421.

Description of *Paracoccus salipaludis* 7J1-06

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, smooth, flat and yellow-brown colored after 3 days on R2A at 25°C. Posi-

tive for oxidase, but negative for esculin hydrolysis, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, β -galactosidase and urease. L-Arabinose are utilized. Does not utilize D-Glucose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, D-Maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 7J1-06 (= NNIBR2019641BA4) was isolated from water of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN602458.

Description of *Taonella mepensis* 7J3-01

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth and cream white colored after 3 days on R2A at 25°C. Positive for oxidase, gelatin hydrolysis, nitrate reduction, arginine dihydrolase and urease, but negative for esculin hydrolysis, glucose fermentation, indole production and β -galactosidase. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, D-Maltose, potassium gluconate, malic acid are utilized. Does not utilize capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain 7J3-01 (= NNIBR2019641BA5) was isolated from water of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN602464.

Description of *Sphingomonas psychrolutea* JU-31

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth and yellow colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and β -galactosidase, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and urease. D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, malic acid, trisodium citrate, D-Maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid are not utilized. Strain JU-31 (= NNIBR2020641BA793) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN559425.

Description of *Burkholderia gladioli* NSW-11

Cells are Gram-staining-negative, non-flagellated, short rod-shaped and aerobic. Colonies are circular, convex, entire and white colored after 3 days on R2A at 25°C. Positive for oxidase, esculin hydrolysis, gelatin hydrolysis, nitrate

reduction and β -galactosidase, but negative for glucose fermentation, indole production, arginine dihydrolase and urease. 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 NSW-11 (= NNIBR2020641BA816) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW293920.

Description of *Polaromonas eurypsychophila* JJ1S-14

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth and white colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and nitrate reduction, but negative for oxidase, gelatin hydrolysis, glucose fermentation, indole production, arginine dihydrolase, urease and β -galactosidase. D-Maltose and adipic acid are utilized. Does not utilize D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain JJ1S-14 (= NNIBR2019641BA236) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN749850.

Description of *Hydrogenophaga laconesensis* 20CSW-3

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth, translucent and red colored after 3 days on 0.1 TSA at 25°C. Positive for esculin hydrolysis, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, urease and β -galactosidase. 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 are not utilized. Strain 20CSW-3 (= NNIBR2020641BA802) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MW295515.

Description of *Chitinilyticum litopenaei* 9W-13

Cells are Gram-staining-positive, flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth and

cream white colored after 3 days on R2A at 25°C. Positive for oxidase, nitrate reduction and glucose fermentation, but negative for esculin hydrolysis, gelatin hydrolysis, indole production, arginine dihydrolase, β -galactosidase and urease. D-Glucose, D-Mannose, N-acetyl-glucosamine and potassium gluconate are utilized. Does not utilize L-Arabinose, D-Mannitol, D-Maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 9W-13 (= NNIBR2019641BA3) was isolated from water of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN602461.

Description of *Azospira oryzae* YDC-53

Cells are Gram-staining-negative, single-flagellated, rod-shaped and aerobic. Colonies are circular, convex, opaque and cream colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and β -galactosidase, but negative for oxidase, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase and urease. Malic acid are utilized. Does not utilize D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, trisodium citrate, D-Maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain YDC-53 (= NNIBR2020641BA796) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MT893384.

Description of *Zoogloea resiniphila* 9S-10

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are circular, convex, smooth and cream white colored after 3 days on R2A at 25°C. Positive for oxidase, but negative for esculin hydrolysis, gelatin hydrolysis, nitrate reduction, glucose fermentation, indole production, arginine dihydrolase, β -galactosidase and urease. N-acetyl-glucosamine and malic acid are utilized. Does not utilize D-Glucose, L-Arabinose, D-Mannose, D-Mannitol, D-Maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain 9S-10 (= NNIBR2019641BA2) was isolated from sediment of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN602459.

Description of *Zoogloea oryzae* 9W-02

Cells are Gram-staining-negative, non-flagellated, rod-shaped and aerobic. Colonies are translucent, convex and

bright yellow colored after 3 days on R2W at 25°C. Positive for oxidase, gelatin hydrolysis, nitrate reduction and urease, but negative for esculin hydrolysis, glucose fermentation, indole production, arginine dihydrolase and β -galactosidase. D-Glucose, D-Mannitol and D-Maltose are utilized. Does not utilize L-Arabinose, D-Mannose, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 9W-02 (= NNIBR2019654BA3) was isolated from water of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN696219.

Description of *Pseudomonas furukawaii* KKMS-29

Cells are Gram-staining-negative, non-flagellated, short rod-shaped and aerobic. Colonies are circular, convex, entire and white colored after 3 days on R2A at 25°C. Positive for arginine dihydrolase, but negative for oxidase, esculin hydrolysis, gelatin hydrolysis, nitrate reduction, indole production, glucose fermentation, β -galactosidase and urease. D-Glucose, capric acid and phenylacetic acid are utilized. Does not utilize L-Arabinose, D-Mannose, D-Mannitol, N-acetyl-glucosamine, D-Maltose, potassium gluconate, adipic acid, malic acid and trisodium citrate. Strain KKMS-29 (= NNIBR2019641BA237) was isolated from freshwater of the Geum River, Korea. The GenBank accession number for the 16S rRNA gene sequence is MN749851.

CRedit authorship contribution statement

H Kim: Writing, Reviewing and Editing. S Park: Data curation. KJ Yim: Data curation. JY Cho: Data curation. E Kim: Data curation.

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