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# A report on 17 unrecorded bacterial species in Korea isolated from Lakes Soyang and Chungju in 2016

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As a part of the research program 'Survey of freshwater organisms and specimen collection', freshwater samples were collected from Lakes Soyang and Chungju in 2016. Hundreds of bacterial strains were isolated from the samples and were identified based on 16S rRNA gene sequences. Among the bacterial isolates, strains showing higher than 98.7% sequence similarity with validly published bacterial species not reported in Korea were selected as unrecorded bacterial species. Based on 16S rRNA gene sequence similarity, 17 strains were identified as unrecorded bacterial species in Korea. The 17 bacterial strains were phylogenetically diverse and belonged to four phyla, seven classes, 13 orders, 14 families, and 16 genera. At generic level, the unreported species were affiliated with *Caulobacter*, *Paracoccus*, and *Mesorhizobium* of the class *Alphaproteobacteria*, *Deefgea*, *Undibacterium*, *Chitinimonas*, *Inhella*, and *Sphaerotilus* of the class *Betaproteobacteria*, *Vibrio* and *Cellvibrio* of the class *Gammaproteobacteria*, *Sanguibacter* and *Clavibacter* of the phylum *Actinobacteria*, *Lactococcus* of the phylum *Firmicutes*, *Deinococcus* of the class *Deinococci*, and *Chryseobacterium* and *Flavobacterium* of the phylum *Bacteroidetes*. The unreported species were further characterized by examining Gram reaction, colony and cell morphology, biochemical properties, and phylogenetic position. The detailed description of the 17 unreported species are also provided.

Keywords: Freshwater, unreported bacterial species, Lake Soyang, Lake Chungju


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## Introduction

Freshwater comprises approximately 0.6% of the total water on Earth and exists in the form of lakes, rivers, and groundwater (Wetzel, 2001). Although the freshwater environment has less impact on the global carbon cycling than the marine environment, microorganisms present in freshwater are known to act as indicators of global climate change due to their major impact on biological communities in freshwater environments (Williamson *et al*., 2008). In aquatic environments, prokaryotes convert organic matter into inorganic matter through remineralization, transfer carbon and energy to higher trophic levels through microbial food webs, and play an important role in the cycling of phosphorus, nitrogen, sulfur, iron and other nutrients (Azam *et al*., 1983; Cotner and Biddanda, 2002).

Although freshwater microbial community structure is thought to be similar to that of the soil environment due to its presence in the terrestrial ecosystem (Rheinheimer, 1980), 16S rRNA gene sequence analyses using culture-independent approaches revealed that soil and freshwater ecosystems have distinct microbial community structures (Lozupone and Knight, 2007). For example, a comparison of the major groups of *Actinobacteria* found in freshwater, soil, and ocean environments, major bacterial groups found in freshwater, such as the acI and acIV clades, were endemic only in the freshwater environment (Newton *et al*., 2011).

Bacterial communities in the freshwater environment are generally composed of ca. 20 phyla, of which *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Verrucomicrobia* are dominant. The phyla *Acidobacteria*, *Planctomycetes*, *Chlorobi*, *Chloroflexi*, *Fibrobacteres*, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Lentisphaerae*, and *Nitrospira* also exist in freshwater environments, but at low abundance (Newton *et al*., 2011). Among the bacterial groups frequently found in freshwater environments, many groups such as acI, acIV and acSTL of the *Actinobacteria* and

LD12 of the *Alphaproteobacteria* remain uncultivated (Kara *et al*., 2013). Therefore, cultivation of these groups of bacteria is urgently required to improve our understanding of the physiology of these bacteria and their ecological roles in freshwater environments.

As a part of the research program 'Survey of freshwater organisms and specimen collection', freshwater samples were collected from Lakes Soyang and Chungju in 2016 and hundreds of bacterial strains were isolated. Using phylogenetic analyses based on 16S rRNA gene sequences, 17 bacterial species were recognized as unrecorded bacterial species in Korea. Here, we report the phylogenetic information and phenotypic characteristics bacterial species.

## Materials and Methods

Freshwater samples were collected from Lakes Soyang and Chungju at depths of 1 m and 40 m in June 2016 using a Niskin sampler. One hundred microliters of the freshwater samples were spread onto R2A agar (BD, USA) and 1/3-diluted R2A agar media and subsequently incubated at 20-25°C for 3-7 days. All strains were purified as single colonies after serial dilution spreading and the pure cultures were stored as 20% glycerol suspension at −80°C and as lyophilized ampoules. The designation of strains, source of isolation, culture medium, and incubation conditions are summarized in Table 1.

Colony morphology of bacterial strains was observed on agar plates with a magnifying glass after the cells were cultivated to their stationary phase. Cellular morphology and cell size were examined by transmission electron microscopy. Gram staining was performed using a Gram-staining kit (bioMérieux). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures. For 16S rRNA gene sequencing, primers 27F and 1492R were used. The 16S rRNA gene sequences were compared with other bacterial strains with validly published names using the EzTaxon-e server (Kim *et al*., 2012). The cutoff value of 98.7% sequence similarity was applied for identification. Bacterial strains showing 98.7% or higher sequence similarity with known bacterial species never reported in Korea were selected as unreported bacterial species. For phylogenetic analyses, multiple sequence alignments between the 16S rRNA gene sequences of the isolates and those of the reference type strains were carried out using ClustalW and manually checked with EzEditor (Jeon *et al*., 2014). Based on the sequences aligned, phylogenetic trees were gen-

**Table 1.** Summary of bacterial strains isolated from Lakes Soyang and Chungju and their taxonomic affiliations.

| Phylum/Class | Order | Family | Strain ID | Most closely related species | Similarity (%) | Isolation source | Medium | Incubation conditions |
|---|---|---|---|---|---|---|---|---|
| *Alphaproteobacteria* | *Caulobacterales* | *Caulobacteraceae* | IMCC25662 (IHBA_13) | *Caulobacter fusiformis* | 99.3 | Lake Soyang | R2A | 20°C, 3 d |
| | *Rhodobacterales* | *Rhodobacteraceae* | IMCC25631 (IHBA_15) | *Paracoccus marinus* | 98.9 | Lake Soyang | R2A | 20°C, 3 d |
| | *Rhizobiales* | *Phyllobacteriaceae* | IMCC25632 (IHBA_16) | *Mesorhizobium amorphae* | 100 | Lake Soyang | R2A | 20°C, 3 d |
| *Betaproteobacteria* | *Neisseriales* | *Chromobacteriaceae* | IMCC25674 (IHBA_12) | *Deefgea chitinilytica* | 99.4 | Lake Chungju | R2A | 20°C, 3 d |
| | *Burkholderiales* | *Oxalobacteraceae* | IMCC25668 (IHBA_6) | *Undibacterium macrobrachii* | 99.5 | Lake Chungju | R2A | 20°C, 3 d |
| | | *Burkholderiaceae* | IMCC25669 (IHBA_7) | *Chitinimonas taiwanensis* | 99.9 | Lake Chungju | 1/3R2A | 20°C, 3 d |
| | | *"Incertae sedis"* | IMCC25675 (IHBA_8) | *Inhella fonticola* | 99.9 | Lake Chungju | R2A | 20°C, 3 d |
| | | | IMCC25671 (IHBA_9) | *Sphaerotilus natans* | 99.9 | Lake Chungju | R2A | 20°C, 3 d |
| *Gammaproteobacteria* | *Vibrionales* | *Vibrionaceae* | IMCC25664 (IHBA_2) | *Vibrio cholera* | 100 | Lake Chungju | R2A | 20°C, 3 d |
| | *Cellvibrionales* | *Cellvibrionaceae* | IMCC25666 (IHBA_4) | *Cellvibrio mixtus* | 99.6 | Lake Chungju | R2A | 20°C, 3 d |
| *Actinobacteria* | *Micrococcales* | *Sanguibacteraceae* | IMCC25661 (IHBA_1) | *Sanguibacter keddieii* | 99.7 | Lake Soyang | 1/3R2A | 20°C, 3 d |
| | | *Microbacteriaceae* | IMCC25665 (IHBA_3) | *Clavibacter michiganensis* | 100 | Lake Soyang | 1/3R2A | 20°C, 3 d |
| *Firmicutes* | *Lactobacillales* | *Streptococcaceae* | IMCC25630 (IHBA_17) | *Lactococcus piscium* | 99.5 | Lake Soyang | R2A | 20°C, 3 d |
| *Deinococci* | *Deinococcales* | *Deinococcaceae* | IMCC25673 (IHBA_11) | *Deinococcus grandis* | 99.2 | Lake Chungju | 1/3R2A | 20°C, 3 d |
| *Bacteroidetes* | *Flavobacteriales* | *Flavobacteriaceae* | IMCC25672 (IHBA_10) | *Chryseobacterium massiliae* | 99.8 | Lake Chungju | R2A | 20°C, 3 d |
| | | | IMCC25663 (IHBA_14) | *Flavobacterium piscis* | 99.1 | Lake Soyang | R2A | 20°C, 3 d |
| | | | IMCC25667 (IHBA_5) | *Flavobacterium qiangtangense* | 99.4 | Lake Soyang | 1/3R2A | 20°C, 3 d |

erated by using a neighbor-joining method (Saitou and Nei, 1987) with the Kimura 2-parameter model (Kimura, 193) implemented in MEGA 6.0 software (Tamura *et al*., 2013). The robustness of the inferred phylogenetic trees was evaluated by bootstrap analyses based on 1,000 random re-samplings (Felsenstein, 1985).

## Results and Discussion

After obtaining 16S rRNA gene sequences of approximately 700 bacterial strains and subsequent comparative sequence analyses, a number of previously unreported bacterial species were isolated. Based on 16S rRNA gene sequence similarity, 17 strains were identified as unrecorded bacterial species that have never been officially reported in Korea. The taxonomic composition and identification results of these species are summarized in Table 1. The 17 unrecorded bacterial species were assigned to three species of *Alphaproteobacteria*, five species of *Betaproteobacteria*, two species of *Gammaproteobacteria*, two species of *Actinobacteria*, one species of *Firmicutes*, one species of *Deinococci*, and three species of *Bacteroidetes*. The 17 bacterial strains were phylogenetically diverse and belonged to four phyla, seven classes, 13 orders, 14 families, and 16 genera. At the generic level, these unreported species belonged to the genera *Caulobacter*, *Paracoccus*, and *Mesorhizobium* of the class *Alphaproteobacteria*, the genera *Deefgea*, *Undibacterium*, *Chitinimonas*, *Inhella*, and *Sphaerotilus* of the class *Betaproteobacteria*, the genera *Vibrio* and *Cellvibrio* of the class *Gammaproteobacteria*, the genera *Sanguibacter* and *Clavibacter* of the phylum *Actinobacteria*, the genus *Lactococcus* of the phylum *Firmicutes*, the genus *Deinococcus* of the class *Deinococci*, and the genera *Chryseobacterium* and *Flavobacterium* (2 species) of the phylum *Bacteroidetes*. Phylogenetic tree of bacterial strains assigned to the phylum *Proteobacteria* is shown in Fig. 1 and the tree of *Actinobacteria*, *Firmicutes*, *Deinococcus-Thermus* and *Bacteroidetes* is shown in Fig. 2. The delineation of the species based on sequence similarity was confirmed by the phylogenetic trees, which showed that each bacterial strain formed a robust clade with the most closely related species (Fig. 1 and Fig. 2). The 17 unrecorded bacterial species were Gram-staining-negative or positive, rod- or coccoid-shaped bacteria (Fig. 3). Other detailed morphological and physiological characteristics are given in the strain descriptions.

In this study a total of 17 bacterial species were identified as unrecorded bacterial species that have not previously been reported from Korean ecosystems. Therefore, here we describe the characteristics of these unreported bacterial species.

### Description of *Caulobacter fusiformis* IMCC25662

Cells are Gram-staining-negative, rod, stalk-presenting, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow-pigmented. Positive for nitrate reduction, urease, esculin hydrolysis, and $\beta$-galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, and gelatinase. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, and D-maltose are utilized. Does not utilize D-mannitol, D-maltose, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25662 displays the highest 16S rRNA gene sequence similarity with *Caulobacter fusiformis* ATCC 15257$^T$ (99.3%). Strain IMCC25662 (=IHBA_13) was isolated from a freshwater sample of Lake Soyang. The GenBank accession number of 16S rRNA gene sequence is KY053186.

### Description of *Paracoccus marinus* IMCC25631

Cells are Gram-staining-negative, rod-shaped, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow-pigmented. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and $\beta$-galactosidase in API 20NE. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized. Strain IMCC25631 displays the highest 16S rRNA gene sequence similarity with *Paracoccus marinus* KKL-A5$^T$ (98.9%). Strain IMCC25631 (=IHBA_15) was isolated from a freshwater sample of Lake Soyang. The GenBank accession number of 16S rRNA gene sequence is KY053157.

### Description of *Mesorhizobium amorphae* IMCC25632

Cells are Gram-staining-negative, short rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and white. Positive for esculin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and D-maltose are utilized. Does not utilize L-arabinose, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25632 displays the highest 16S rRNA gene sequence similarity with *Mesorhizobium amorphae* ACCC 19665$^T$ (100%). Strain IMCC25632 (=IHBA_16) was isolated from a freshwater sample of Lake Soyang. The GenBank accession number of 16S rRNA gene sequence is KY053155.

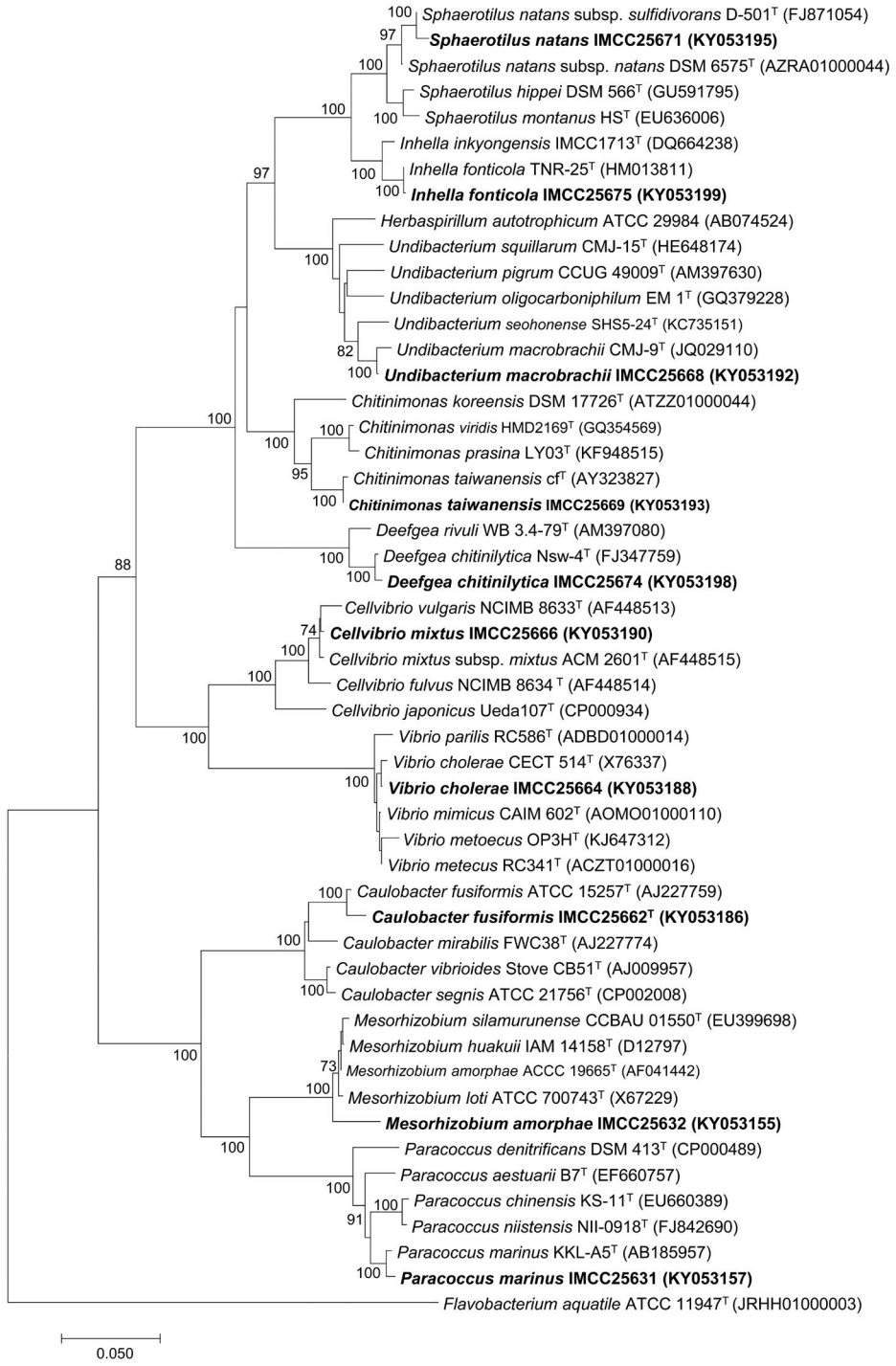


**Fig. 1.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. Bootstrap values (>70%) are shown at nodes. Bar, 0.05 substitutions per nucleotide position.

## Description of *Deefgea chitinilytica* IMCC25674

Cells are Gram-staining-negative, rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and white. Positive for glucose fermentation in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and $\beta$-galactosidase. D-Glucose, D-mannose, *N*-acetyl-glucosamine, and gluconic acid are utilized. Does

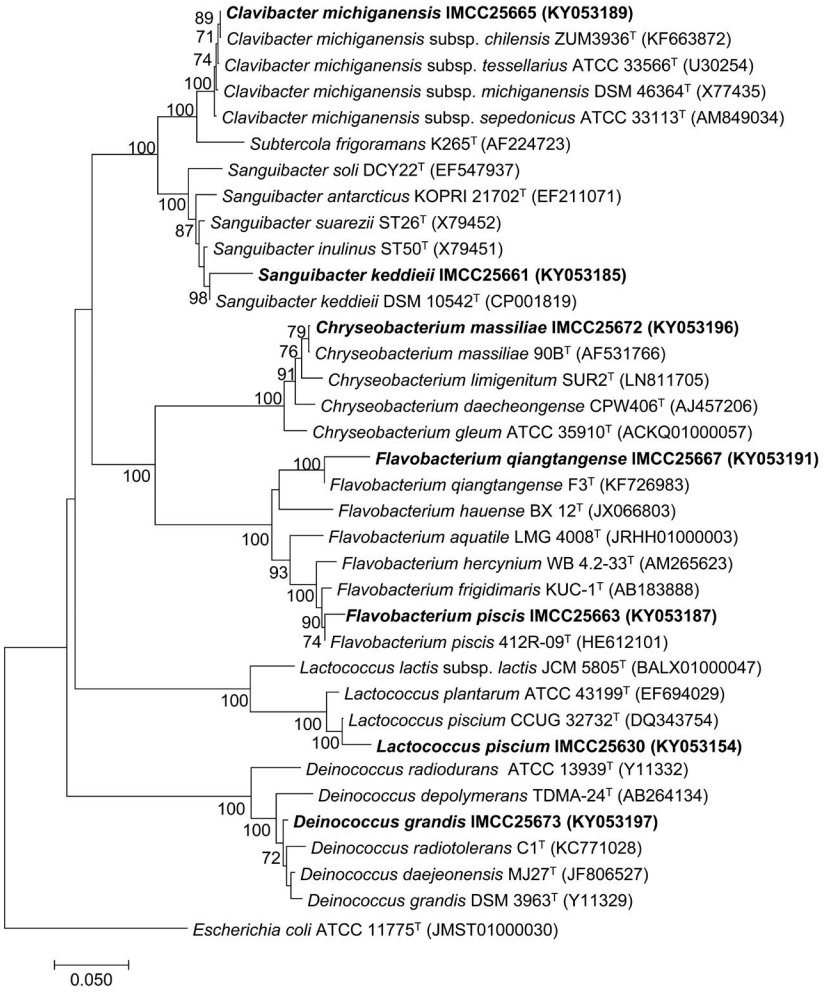


**Fig. 2.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the phyla *Actinobacteria*, *Firmicutes*, *Deinococcus-Thermus*, and *Bacteroidetes*. Bootstrap values (>70%) are shown at nodes. Bar, 0.05 substitutions per nucleotide position.

not utilize L-arabinose, D-mannitol, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25674 displays the highest 16S rRNA gene sequence similarity with *Deefgea chitinilytica* Nsw-4$^T$ (99.4%). Strain IMCC25674 (=IHBA_12) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053198.

## Description of *Undibacterium macrobrachii* IMCC25668

Cells are Gram-staining-negative, rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and whitish-yellow pigmented. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and $\beta$-galactosidase in API 20NE. Gluconic acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25668 displays the highest 16S rRNA gene sequence similarity with *Undibacterium macrobrachii* CMJ-9$^T$ (99.5%). Strain IMCC25668 (=IHBA_6) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053192.

## Description of *Chitinimonas taiwanensis* IMCC25669

Cells are Gram-staining-negative, rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and white. Positive for glucose fermentation and gelatinase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, and $\beta$-galactosidase. D-Glucose, D-man-

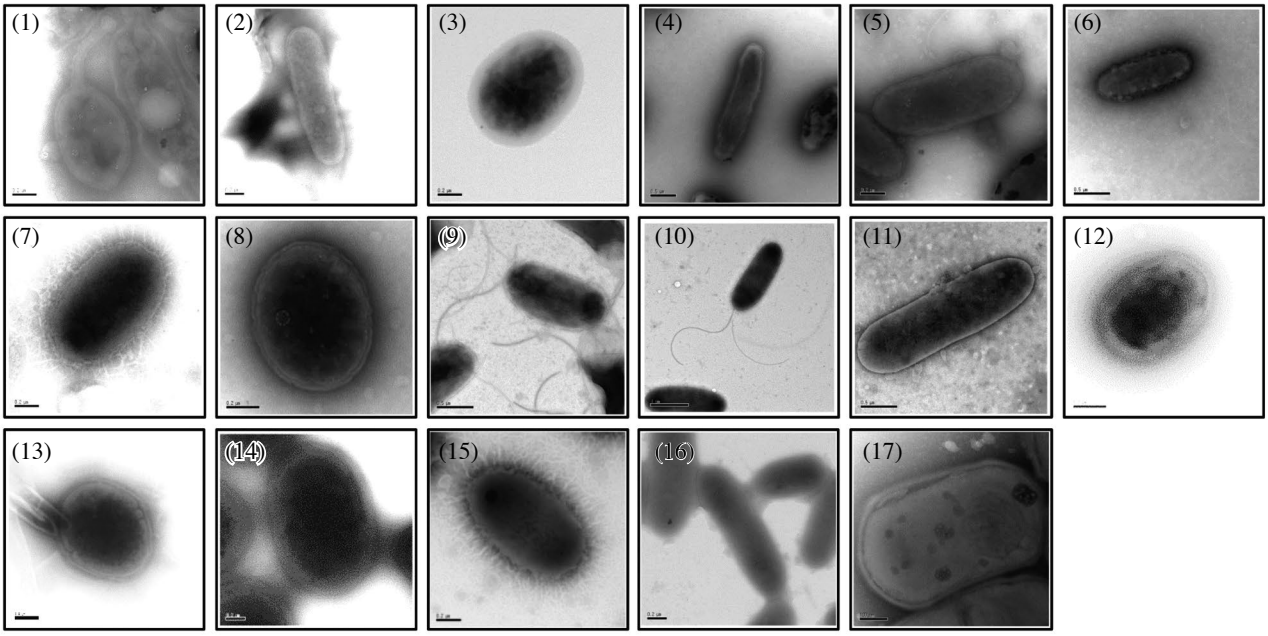


**Fig. 3.** Transmission electron micrographs of cells of the strains isolated in this study. Strains: 1, IMCC25662; 2, IMCC25631; 3, IMCC25632; 4, IMCC25674; 5, IMCC25668; 6, IMCC25669; 7, IMCC25675; 8, IMCC25671; 9, IMCC25664; 10, IMCC25666; 11, IMCC25661; 12, IMCC25665; 13, IMCC25630; 14, IMCC25673; 15, IMCC25672; 16, IMCC25663; 17, IMCC25667.

nose, D-maltose, gluconic acid, malic acid, and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannitol, *N*-acetyl-glucosamine, capric acid, adipic acid, and phenylacetic acid. Strain IMCC25669 displays the highest 16S rRNA gene sequence similarity with *Chitinimonas taiwanensis* cf$^{T}$ (99.8%). Strain IMCC 25669 (=IHBA_7) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053193.

### Description of *Inhella fonticola* IMCC25675

Cells are Gram-staining-negative, rod-shaped, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow-pigmented. Positive for nitrate reduction, urease, esculin hydrolysis, and $\beta$-galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, and gelatinase. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, and D-maltose are utilized. Does not utilize D-mannitol, D-maltose, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25675 displays the highest 16S rRNA gene sequence similarity with *Inhella fonticola* DSM 6976$^{T}$ (99.9%). Strain IMCC25675 (=IHBA_8) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053199.

### Description of *Sphaerotilus natans* IMCC25671

Cells are Gram-staining-negative, short rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow-pigmented. Positive for $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatinase. D-Glucose, D-mannose, D-mannitol, and D-maltose are utilized. Does not utilize L-arabinose, *N*-acetyl-glucosamine, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25671 (=IHBA_9) displays the highest 16S rRNA gene sequence similarity with *Sphaerotilus natans* subsp. *sulfidivorans* D-501$^{T}$ (99.9%). Strain IMCC25671 (=IHBA_9) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053195.

### Description of *Vibrio cholerae* IMCC25664

Cells are Gram-staining-negative, rod, flagellated, and aerobic. Colonies grown on R2A are circular, convex, entire, and white-yellow pigmented. Positive for esculin hydrolysis, gelatinase, and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. D-Glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, and malic acidare utilized. Does not utilize

L-arabinose, D-mannose, gluconic acid, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25664 displays the highest 16S rRNA gene sequence similarity with *Vibrio cholerae* CECT 514$^T$ (100%). Strain IMCC25664 (=IHBA_2) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053188.

**Description of *Cellvibrio mixtus* IMCC25666**

Cells are Gram-staining-negative, rod, flagellated, and aerobic. Colonies grown on R2A are circular, convex, entire, and white. Positive for esculin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, *N*-acetyl-glucosamine, and D-maltose are utilized. Does not utilize D-mannose, D-mannitol, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25666 displays the highest 16S rRNA gene sequence similarity with *Cellvibrio mixtus* subsp. *mixtus* ACM 2601$^T$ (99.6%). Strain IMCC25666 (=IHBA_4) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053190.

**Description of *Sanguibacter keddieii* IMCC25661**

Cells are Gram-staining-positive, rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and white. Positive for esculin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, and D-maltose are utilized. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25661 displays the highest 16S rRNA gene sequence similarity with *Sanguibacter keddieii* DSM 10542$^T$ (99.7%). Strain IMCC25661 (=IHBA_1) was isolated from a freshwater sample of Lake Soyang. The GenBank accession number of 16S rRNA gene sequence is KY053185.

**Description of *Clavibacter michiganensis* IMCC25665**

Cells are Gram-staining-positive, short rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow pigmented. Positive for esculin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, gluconic acid, and malic acid are utilized. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25665 displays the highest 16S rRNA gene sequence similarity with *Clavibacter michiganensis* subsp. *phaseoli* LPPA 982$^T$ (100%). Strain IMCC25665 (=IHBA_3) was isolated from a freshwater sample of Lake Soyang. The GenBank accession number of 16S rRNA gene sequence is KY053189.

**Description of *Lactococcus piscium* IMCC25630**

Cells are Gram-staining-positive, coccoid-shaped, and aerobic. Colonies grown on R2A are circular, convex, entire, and white pigmented. Positive for esculin hydrolysis, gelatinase and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized. Strain IMCC25630 displays the highest 16S rRNA gene sequence similarity with *Lactococcus piscium* CCUG 32732$^T$ (99.5%) (Liu *et al*., 2011). Strain IMCC25630 (=IHBA_17) was isolated from a freshwater sample of Lake Soyang.

The GenBank accession number of 16S rRNA gene sequence is KY053154.

**Description of *Deinococcus grandis* IMCC25673**

Cells are Gram-staining-negative, short rod-shaped, and aerobic. Colonies grown on R2A are circular, convex, entire, and red pigmented. Positive for urease, esculin hydrolysis, gelatinase, and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase. D-Glucose, D-mannose, D-mannitol, D-maltose and malic acid are utilized. Does not utilize L-arabinose, *N*-acetyl-glucosamine, gluconic acid, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25673 displays the highest 16S rRNA gene sequence similarity with *Deinococcus grandis* DSM 3963$^T$ (99.2%). Strain IMCC25673 (=IHBA_11) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053197.

**Description of *Chryseobacterium massiliae* IMCC25672**

Cells are Gram-staining-negative, rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow-pigmented. Positive for esculin hydrolysis and gelatinase in API 20NE, but negative for nitrate reduc-

tion, indole production, glucose fermentation, arginine dihydrolase, urease, and $\beta$-galactosidase. D-Glucose is utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain IMCC25672 displays the highest 16S rRNA gene sequence similarity with *Chryseobacterium massiliae* 90B$^T$ (99.8%). Strain IMCC25672 (=IHBA_10) was isolated from a freshwater sample of Lake Chungju. The GenBank accession number of 16S rRNA gene sequence is KY053196.

**Description of *Flavobacterium piscis* IMCC25663**

Cells are Gram-staining-negative, rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow-pigmented. Positive for $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized. Strain IMCC25663 displays the highest 16S rRNA gene sequence similarity with *Flavobacterium piscis* 412R-09$^T$ (99.1%). Strain IMCC25663 (=IHBA_14) was isolated from a freshwater sample of Lake Soyang. The GenBank accession number of 16S rRNA gene sequence is KY053187.

**Description of *Flavobacterium qiangtangense* IMCC25667**

Cells are Gram-staining-negative, rod, and aerobic. Colonies grown on R2A are circular, convex, entire, and yellow-pigmented. Positive for esculin hydrolysis and $\beta$-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltos, gluconic acid, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are not utilized. Strain IMCC25667 displays the highest 16S rRNA gene sequence similarity with *Flavobacterium qiangtangense* F3$^T$ (99.4%). Strain IMCC25667 (=IHBA_5) was isolated from a freshwater sample of Lake Soyang. The GenBank accession number of 16S rRNA gene sequence is KY053191.

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