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Description of 17 unrecorded bacterial species isolated from freshwater showing antibiotic resistance in Korea

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Abstract: As part of the research program "2018 Rapid screening and identification of freshwater microorganisms using MALDI-TOF/MS library" freshwater samples were collected from a branch of the Nakdong River. Almost 300 antibiotic-resistant bacterial strains were isolated from freshwater samples and subsequently identified by 16S rRNA gene sequencing. Seventeen strains among the isolates shared high 16S rRNA gene sequence similarity (>99.0%) with known species that were not previously recorded in Korea, and each of the isolates also formed a robust phylogenetic clade with the closest species. These species were phylogenetically diverse, belonging to four phyla, seven classes, 10 orders, and 13 genera. At the genus and class level, the previously unrecorded species belonged to *Rhodovarius*, *Xanthobacter*, and *Shinella* of the class *Alphaproteobacteria*; *Ottowia*, *Simplicispira*, and *Zoogloea* of *Betaproteobacteria*; *Pseudomonas*, *Acinetobacter*, and *Shewanella* of *Gammaproteobacteria*; *Arcobacter* of *Epsilonproteobacteria*; *Sphingobacterium* of Sphingobacteriia; *Trichococcus* of *Bacilli*; and *Leucobacter* of *Actinobacteria*. The previously unrecorded species were further characterized by examining their gram-staining, colony and cell morphology, biochemical properties, and phylogenetic position.

Keywords: unrecorded antibiotic resistant bacterial species, freshwater

INTRODUCTION

Freshwater streams may have an adverse effect on ecosystems and human water resources, resulting in an increase in antibiotic-resistant bacteria owing to the inflow of wastewater and an increase in residual antibiotics (Kümmerer 2009). Antibiotic-resistant bacteria are bacteria that are not controlled or killed by antibiotics (Engelberg-Kulka *et al.* 2004). They can survive and reproduce even in the presence of antibiotics. In particular, the livestock field is known to be highly dependent on antibiotics. Antibiotic resistance in animals is increasing owing to the misuse of antibiotics rather than regulated medications, mainly via feed containing antibiotics. Bacteria that are resistant to many antibiotics are known as multi-resistant organisms (MRO) (Woodford *et al.* 2011). Livestock products with increased resistance pose a risk because antibiotic-resistant bacteria can multiply and spread to people taking antibiotics (Ji *et al.* 2012). Antibiotic resistance is a serious public health problem; thus, identifying and securing resistant

bacteria is highly important.

As part of the research program "2018 Rapid screening and identification of freshwater microorganisms using MALDI-TOF/MS library", freshwater samples were collected from a branch of the Nakdong River in 2018. We selected the Gwangpyeong Stream, where wastewater is discharged from the national industrial complex in Gumi-si, Gyeongsangbuk-do, and conducted on-site surveys of possible areas with industrial wastewater contamination. Using phylogenetic analyses based on 16S rRNA gene sequencing, 17 bacterial species were identified that had not been previously recorded in Korea. Here, we report the phylogenetic information and phenotypic characteristics of these bacterial species.

MATERIALS AND METHODS

During a screening of bacteria with antibacterial activity, almost 300 bacterial strains were isolated from freshwater in the brand of Nakdong River in 2018, using standard dilution plating method on Reasoner's 2A (R2A), Nutrient agar (NA) and Muller Hinton agar (HMA) media added to antibiotics and subsequently incubated at 20−25°C for 3−7 days. Antibiotics used a total of seven antibiotics, including synergistic antibiotics such as 1% penicillin-streptomycin solution (Sigma-Aldrich, St. Louis, MO), 5% penicillin-streptomycin-neomycin solution (Sigma-Aldrich), 5% ampicillin-gentamicin solution (Sigma-Aldrich), 5% carbenicillin-gentamicin solution (Sigma-Aldrich), and antibiotics with different mechanisms of action such as 1% tetracycline solution (Sigma-Aldrich), 10% lincomycin solution (Sigma-Aldrich) and 5% novobiocin (Sigma-Aldrich) (Standiford *et al.* 1970). The concentration of antibiotics was increased by 10% based on Minimum Inhibitory Concentration value for accurate isolation of resistant bacteria. 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, 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, Marcy I'Etoile, France). 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 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 bacteria-specific primers (Bac 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and Bac 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Lane 1991). The 16S rRNA gene sequences were compared with other bacterial strains with validly published names using the EzBioCloud database (Yoon *et al.* 2017). 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 generated by using a neighbor-joining method (Saitou and Nei 1987) with the Kimura 2-parameter model (Kimura 1980) implemented in MEGA 7.0 software (Kumar *et al.* 2016). The robustness of the inferred phylogenetic trees was evaluated by bootstrap analyses based on 1,000 random re-samplings (Felsenstein 1985).

RESULTS AND DISCUSSION

A total of 334 bacterial strains with antibacterial activity were isolated from freshwater during a screening of bacteria. Of these strains, the 17 unrecorded species belonging to four phyla, seven classes, ten orders, and thirteen genera were found in Korea. The taxonomic composition and identification results of these species are summarized in Table 1. At the genus level, these unreported species belonged to the genera *Rhodovarius*, *Xanthobacter*, and *Shinella* (two species) of the *Alphaproteobacteria*, *Ottowia*, *Simplicispira*, and *Zoogloea* of the class *Betaproteobacteria*, *Pseudomonas* (two species), *Acinetobacter* (three species) and *Shewanella* of the class *Gammaproteobacteria*, *Arcobacter* of the class *Epsilonproteobacteria*, *Sphingobacterium* of the class *Sphingobacteriia*, *Trichococcus* of the class *Bacilli* and *Leucobacter* of the *Actinobacteria*. Phylogenetic tree of bacterial strains assigned to the classes *Alphaproteobac-*

Table 1. Summary of antibiotic-resistant bacterial strains isolated from freshwater and their taxonomic affiliations

Class	Order	Family	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Medium	Incubation conditions
Alphaproteobacteria	*Rhodospirillales*	*Acetobacteraceae*	KPS-102	NNIBRBA2018141BA29	*Rhodovarius lipocyclicus*	100.0	R2A	25°C, 3 d
	Rhizobiales	*Xanthobacteraceae*	KPS-34	NNIBRBA2018141BA32	*Xanthobacter agilis*	99.0	MHA	25°C, 3 d
		Rhizobiaceae	KPS-58	NNIBRBA2018141BA34	*Shinella curvata*	99.4	NA	25°C, 2 d
			KPS-69	NNIBRBA2018141BA35	*Shinella yambaruensis*	100.0	NA	25°C, 3 d
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	KPS-14	NNIBRBA2018141BA30	*Ottowia beijingensis*	99.9	MHA	25°C, 3 d
			KPS-73	NNIBRBA2018141BA36	*Simplicispira metamorpha*	99.3	NA	25°C, 3 d
	Rhodocyclales	*Zoogloeaceae*	KPW-80	NNIBRBA2018141BA41	*Zoogloea oleivorans*	99.8	R2A	25°C, 3 d
Gammaproteobacteria	*Pseudomonadales*	*Pseudomonadaceae*	4AnSW4-16	NNIBRBA2018141BA25	*Pseudomonas litoralis*	99.6	MHA	25°C, 2 d
			KPS-50	NNIBRBA2018141BA33	*Pseudomonas protegens*	100.0	NA	25°C, 3 d
		Moraxellaceae	KPW-39	NNIBRBA2018141BA38	*Acinetobacter celticus*	99.9	MHA	25°C, 3 d
			KPW-4	NNIBRBA2018141BA39	*Acinetobacter bohemicus*	99.7	MHA	25°C, 3 d
			KPW-8	NNIBRBA2018141BA40	*Acinetobacter harbinensis*	99.9	MHA	25°C, 3 d
	Alteromonadales	*Shewanellaceae*	KPS-21	NNIBRBA2018141BA31	*Shewanella glacialipiscicola*	100.0	MHA	25°C, 2 d
Epsilonproteobacteria	*Campylobacterales*	*Campylobacteraceae*	4KPW53	NNIBRBA2018141BA28	*Arcobacter cibarius*	99.3	R2A	25°C, 4 d
Sphingobacteriia	*Sphingobacteriales*	*Sphingobacteriaceae*	KPW-32	NNIBRBA2018141BA37	*Sphingobacterium kitahiroshimense*	99.7	MHA	25°C, 3 d
Bacilli	*Lactobacillales*	*Carnobacteriaceae*	4KPW16	NNIBRBA2018141BA26	*Trichococcus flocculiformis*	99.9	MHA	25°C, 3 d
Actinobacteria	*Micrococcales*	*Microbacteriaceae*	4KPW17	NNIBRBA2018141BA27	*Leucobacter komagatae*	99.3	MHA	25°C, 2 d

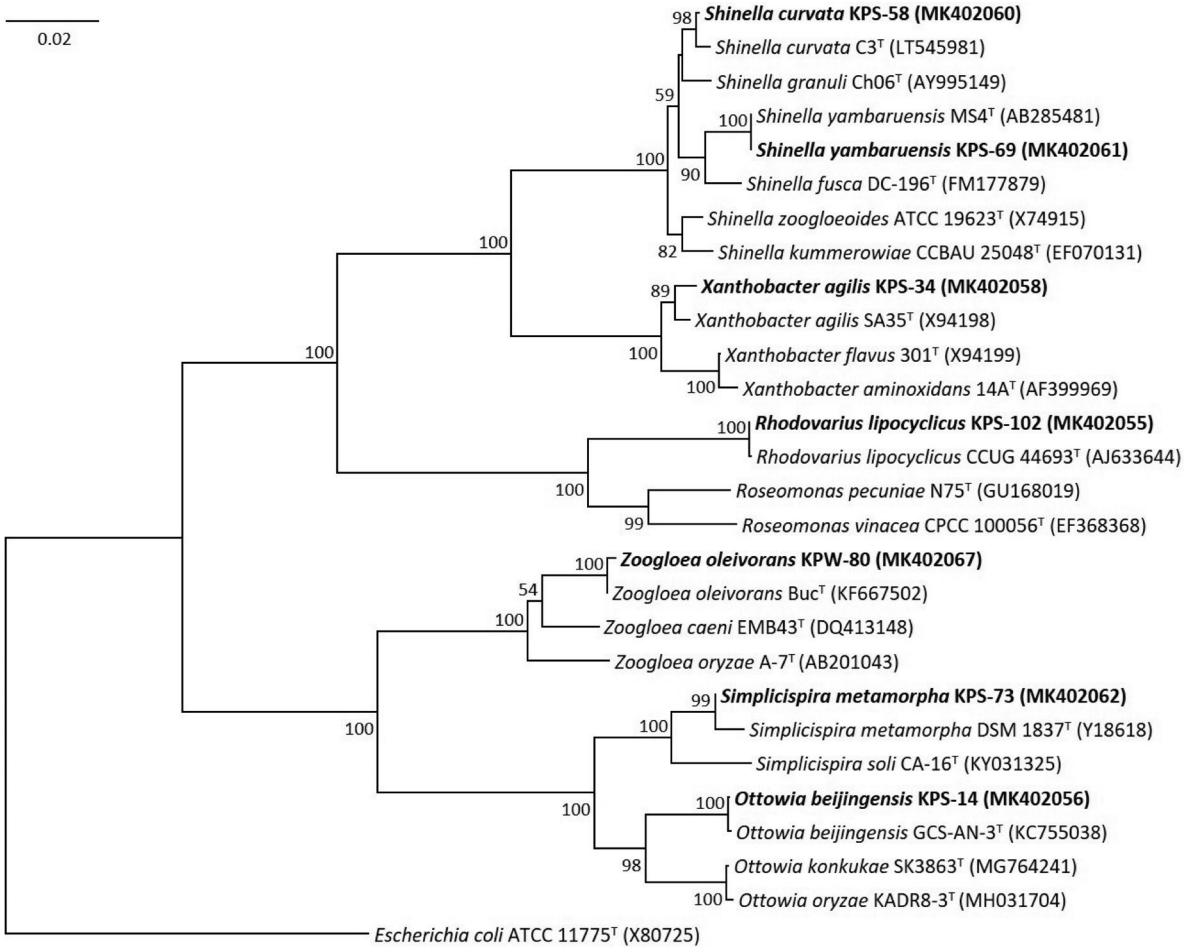

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the classes *Alphaproteobacteria* and *Betaproteobacteria*. Bootstrap values (>50%) are shown at the nodes. Bar, 0.02 substitutions per nucleotide position.

teria and *Betaproteobacteria* is shown in Fig. 1, to the class *Gammaproteobacteria* is shown in Fig. 2 and to the four of classes *Epsilonproteobacteria*, *Sphingobacteriia*, *Bacilli* and *Actinobacteria* is shown in Fig. 3, respectively. As expected from the high 16S rRNA gene sequence similarities of the ten strains with their closest relatives, each strain formed a robust phylogenetic clade with the most closely related species in the trees. The 17 unrecorded antibiotic resistant bacterial species were Gram-stain-negative or positive, rod or short-rod or oval-shaped bacteria (Fig. 4). Other detailed morphological and physiological characteristics are given in the strain descriptions.

In summary, in this study, 17 bacterial species were identified as unrecorded bacterial species that have not previously been reported from Korean ecosystems. Although 17 unrecorded antibiotic resistant bacterial species had some physiological characteristics different from the previously known species, but their morphology and phylogenetic analysis showed that they belonged to those species. Therefore, here we describe the characteristics of these unreported bacterial species.

1. Description of *Rhodovarius lipocyclicus* KPS-102

Cells are Gram-stain-negative, non-flagellated and short rod-shaped. Colonies grown on R2A are circular, convex and pink colored after incubation for 3 days at 25°C. Positive for urease in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose,

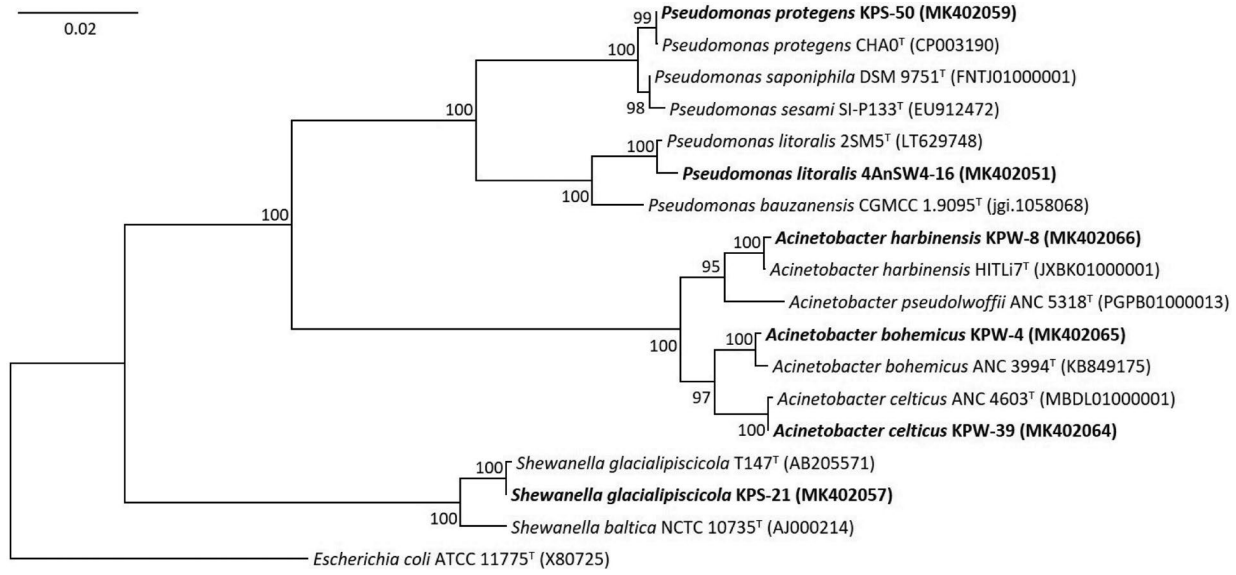

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the class *Gammaproteobacteria*. Bootstrap values (>50%) are shown at the nodes. Bar, 0.02 substitutions per nucleotide position.

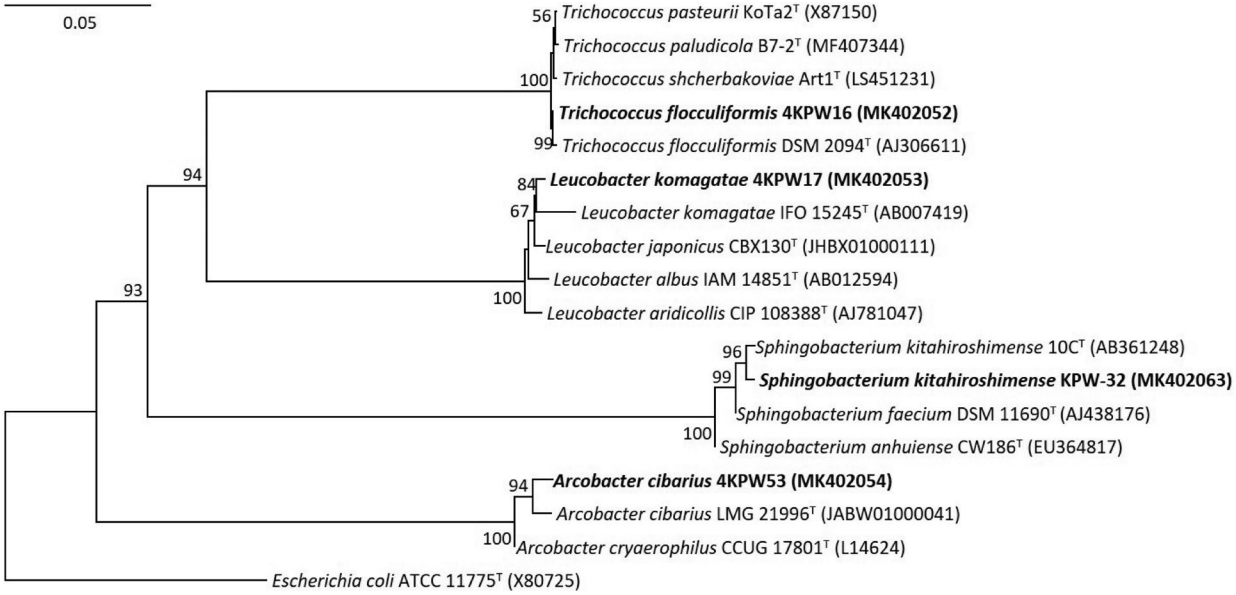

Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the classes *Epsilonproteobacteria*, *Sphingobacteriia*, *Bacilli*, and *Actinobacteria*. Bootstrap values (>50%) are shown at the nodes. Bar, 0.05 substitutions per nucleotide position.

D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KPS-102 displays the highest 16S rRNA gene sequence similarity with *Rhodovarius lipocyclicus* CCUG 44693^{T} (100.0%). Strain KPS-102 (=NNIBRBA2018141BA29) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402055.

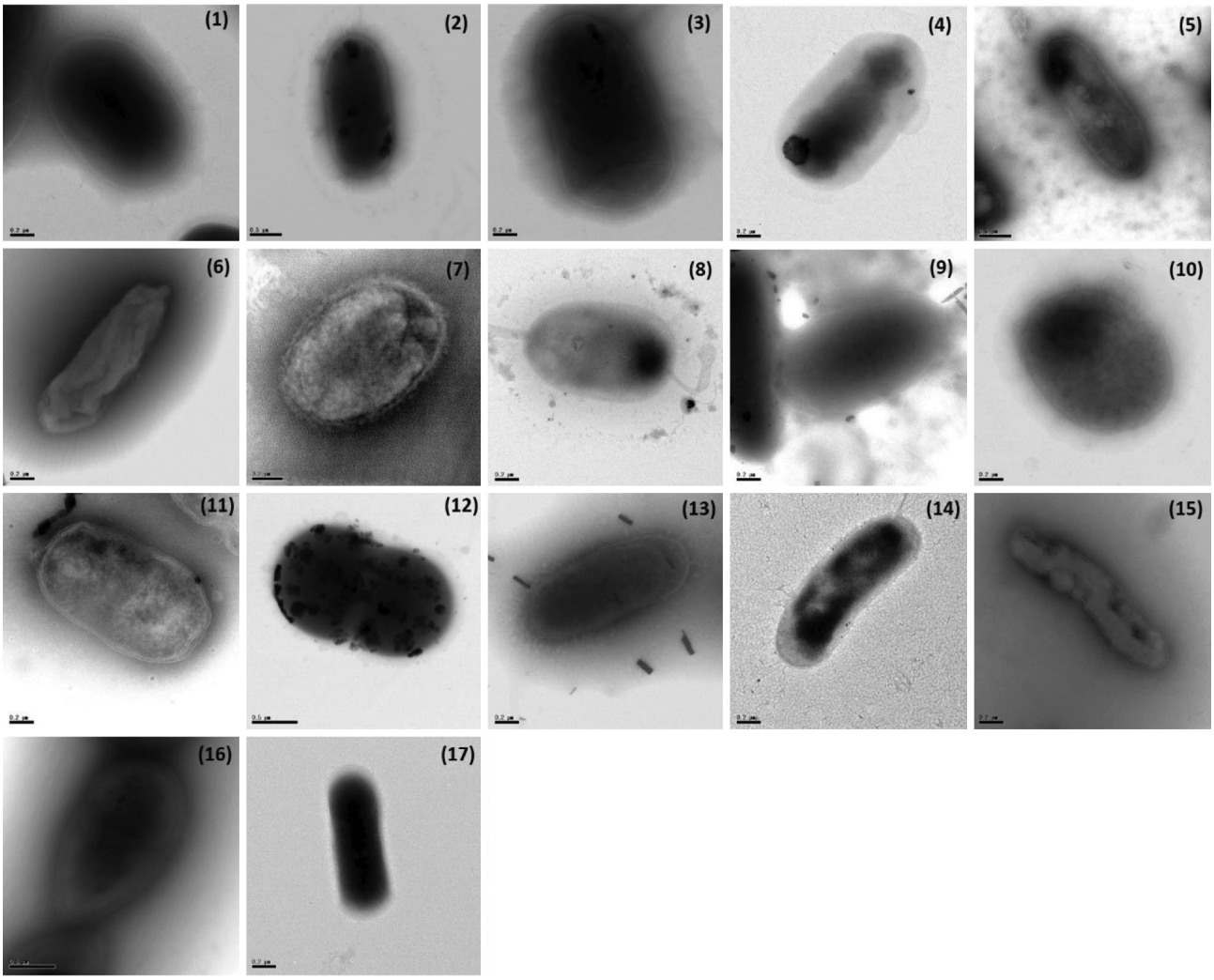

Fig. 4. Transmission electron micrographs of cells of the strains isolated in this study. Strains: 1, KPS-102; 2, KPS-34; 3, KPS-58; 4, KPS-69; 5, KPS-14; 6, KPS-73; 7, KPW-80; 8, 4AnSW4-16; 9, KPS-50; 10, KPW-39; 11, KPW-4; 12, KPW-8; 13, KPS-21; 14, 4KPW53; 15, KPW-32; 16, 4KPW16; 17, 4KPW17.

2. Description of *Xanthobacter agilis* KPS-34

Cells are Gram-stain-positive, non-flagellated and rod-shaped. Colonies grown on MHA are circular, convex and ivory colored after incubation for 3 days at 25°C. Positive for nitrate reduction and urease in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. Adipic acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Strain KPS-34 displays the highest 16S rRNA gene sequence similarity with *Xanthobacter agilis* SA35^{T} (99.0%). Strain KPS-34 (= NNIBRBA2018141BA32) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402058.

3. Description of *Shinella curvata* KPS-58

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on NA are circular, convex and white colored after incubation for 2 days at 25°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and malic acid are uti-

lized. Does not utilize potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain KPS-58 displays the highest 16S rRNA gene sequence similarity with *Shinella curvata* C3^T (99.4%). Strain KPS-58 (=NNIBRBA2018141BA34) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402060.

4. Description of *Shinella yambaruensis* KPS-69

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on NA are circular, convex and white colored after incubation for 3 days at 25°C. Positive for gelatinase and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and esculin hydrolysis. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid and phenylacetic acid are utilized. Does not utilize potassium gluconate, capric acid, adipic acid and trisodium citrate. Strain KPS-69 displays the highest 16S rRNA gene sequence similarity with *Shinella yambaruensis* MS4^T (100.0%). Strain KPS-69 (=NNIBRBA2018141BA35) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402061.

5. Description of *Ottowia beijingensis* KPS-14

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on MHA are circular, convex and ivory colored after incubation for 3 days at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. Adipic acid is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain KPS-14 displays the highest 16S rRNA gene sequence similarity with *Ottowia beijingensis* GCS-AN-3^T (99.9%). Strain KPS-14 (=NNIBRBA2018141BA30) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402056.

6. Description of *Simplicispira metamorpha* KPS-73

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on NA are circular, convex with entire edge and ivory colored after incubation for 3 days at 25°C. Positive for gelatinase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and β-galactosidase. Phenylacetic acid is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. Strain KPS-73 displays the highest 16S rRNA gene sequence similarity with *Simplicispira metamorpha* DSM 1837^T (99.3%). Strain KPS-73 (=NNIBRBA2018141BA36) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402062.

7. Description of *Zoogloea oleivorans* KPW-80

Cells are Gram-stain-negative, non-flagellated and short rod-shaped. Colonies grown on R2A are circular, convex with entire edge and white colored after incubation for 3 days at 25°C. Positive for urease in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. Does not utilize 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. Strain KPW-80 displays the highest 16S rRNA gene sequence similarity with *Zoogloea oleivorans* CCUG 44693^T (100.0 %). Strain KPW-80 (=NNIBRBA2018141BA41) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402067.

8. Description of *Pseudomonas litoralis* 4AnSW4-16

Cells are Gram-stain-positive, non-flagellated and rod-shaped. Colonies grown on MHA are circular, convex, smooth and ivory colored after incubation for 2 days at 25°C. Positive for glucose fermentation in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Adipic acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Strain 4AnSW4-16 displays the highest 16S rRNA gene sequence similarity with *Pseudomonas litoralis* 2SM5^T (99.6%). Strain 4AnSW4-16 (=NNIBRBA2018141BA25) was isolated from freshwater. The GenBank acces-

sion number of 16S rRNA gene sequence is MK402051.

9. Description of *Pseudomonas protegens* KPS-50

Cells are Gram-stain-negative, non-flagellated and short rod-shaped. Colonies grown on NA are circular, convex, smooth and ivory colored after incubation for 3 days at 25°C. Positive for nitrate reduction, glucose fermentation, arginine dihydrolase and gelatinase in API 20NE, but negative for indole production, urease, esculin hydrolysis and β-galactosidase. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Dose not utilize L-arabinose and D-maltose. Strain KPS-50 displays the highest 16S rRNA gene sequence similarity with *Pseudomonas protegens* CHA0^T (100.0%). Strain KPS-50 (=NNIBRBA2018141BA33) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402059.

10. Description of *Acinetobacter celticus* KPW-39

Cells are Gram-stain-positive, non-flagellated and oval-shaped. Colonies grown on MHA are circular, convex and ivory colored after incubation for 3 days at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. Capric acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain KPW-39 displays the highest 16S rRNA gene sequence similarity with *Acinetobacter celticus* ANC 4603^T (99.9%). Strain KPW-39 (=NNIBRBA2018141BA38) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402064.

11. Description of *Acinetobacter bohemicus* KPW-4

Cells are Gram-stain-positive, non-flagellated and oval-shaped. Colonies grown on MHA are circular, convex, slightly and ivory colored after incubation for 3 days at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. Capric acid and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain KPW-39 displays the highest 16S rRNA gene sequence similarity with *Acinetobacter bohemicus* ANC 3994^T (99.7%). Strain KPW-4 (=NNIBRBA2018141BA39) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402065.

12. Description of *Acinetobacter harbinensis* KPW-8

Cells are Gram-stain-positive, non-flagellated and oval-shaped. Colonies grown on MHA are circular, convex, slightly and ivory colored after incubation for 3 days at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. Capric acid is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KPW-8 displays the highest 16S rRNA gene sequence similarity with *Acinetobacter harbinensis* HITLi 7^T (99.9%). Strain KPW-8 (=NNIBRBA2018141BA40) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402066.

13. Description of *Shewanella glacialipiscicola* KPS-21

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on MHA are circular, convex with entire edge and ivory colored after incubation for 2 days at 25°C. Positive for nitrate reduction, esculin hydrolysis and gelatinase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and β-galactosidase. *N*-Acetyl-glucosamine, potassium gluconate and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain KPS-21 displays the highest 16S rRNA gene sequence similarity with *Shewanella glacialipiscicola* T147^T (100.0%). Strain KPS-21 (=NNIBRBA2018141BA31) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402057.

14. Description of *Arcobacter cibarius* 4KPW53

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on R2A are circular, convex and pink colored after incubation for 4 days at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. Does not utilize 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. Strain 4KPW53 displays the highest 16S rRNA gene sequence similarity with *Arcobacter cibarius* LMG 21996^T (99.3%). Strain 4KPW53 (=NNIBRBA2018141 BA28) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402054.

15. Description of *Sphingobacterium kitahiroshimense* KPW-32

Cells are Gram-stain-negative, non-flagellated and rod-shaped. Colonies grown on MHA are circular, convex with entire edge and ivory colored after incubation for 3 days at 25°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine and D-maltose are utilized. Does not utilize D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain KPW-32 displays the highest 16S rRNA gene sequence similarity with *Sphingobacterium kitahiroshimense* 10C^T (99.7%). Strain KPW-32 (=NNIBRBA2018141 BA37) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402063.

16. Description of *Trichococcus flocculiformis* 4KPW16

Cells are Gram-stain-positive, non-flagellated and rod-shaped. Colonies grown on MHA are circular, convex and white colored after incubation for 3 days at 25°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize 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. Strain 4KPW16 displays the highest 16S rRNA gene sequence similarity with *Trichococcus flocculiformis* DSM 2094^T (99.9%). Strain 4KPW16 (=NNIBRBA2018141BA26) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402052.

17. Description of *Leucobacter komagatae* 4KPW17

Cells are Gram-stain-positive, non-flagellated and rod-shaped. Colonies grown on MHA are circular, convex, smooth and ivory colored after incubation for 2 days at 25°C. Positive for gelatinase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and β-galactosidase. Does not utilize 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. Strain 4KPW17 displays the highest 16S rRNA gene sequence similarity with *Leucobacter komagatae* IFO 15245^T (99.3%). Strain 4KPW17 (=NNIBRBA2018141BA27) was isolated from freshwater. The GenBank accession number of 16S rRNA gene sequence is MK402053.

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