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A report on 22 unrecorded freshwater bacterial species isolated from the Seomjin River, Republic of Korea

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The Seomjin River in South Korea has been recognized for its rich biodiversity, which is attributed to its excellent water quality. Given its potential to harbor diverse oligotrophic bacterial species, a biodiversity monitoring program has been conducted since 2020. This study catalogs 22 previously unreported bacterial species from the Seomjin River basin in South Korea, identified based on 16S rRNA gene sequencing, and further characterized by their Gram staining properties, colony and cellular morphology, biochemical traits, and phylogenetic affiliation. The 22 bacterial species exhibited 16S rRNA gene sequence similarities of at least 98.7% and were classified into 4 phyla, 6 classes, 10 orders, 17 families, and 19 genera. The identified genera were classified into the following classes: *Methylorubrum*, *Aureimonas*, *Bosea*, *Tsuneonella*, *Erythrobacter*, *Sphingomonas*, *Parablastomonas*, and *Asticcacaulis* (*Alphaproteobacteria*); *Rivibacter*, *Sheuella*, and *Duganella* (*Betaproteobacteria*); *Pseudomonas* and *Dickeya* (*Gammaproteobacteria*); *Mycobacterium*, *Nocardia*, and *Brachybacterium* (*Actinomycetes*); *Paenibacillus* and *Neobacillus* (*Bacilli*); and *Chitinophaga* (*Chitinophagia*). This study expands the understanding of bacterial diversity in the Seomjin River ecosystem and contributes to national species conservation and sustainable utilization efforts.

Keywords: freshwater, Seomjin River, unreported bacterial species

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Introduction

The Seomjin River, situated in the southwestern region of South Korea, serves as an important geographical, ecological, cultural, and historical relevance. Originating from the Demi Spring at the foot of the Palgong Mountain and extending to the Korean Strait, this 223 km long river with a 4,911 km^2 basin area encompasses diverse landscapes and a rich biodiversity, potentially housing numerous oligotrophic bacterial species. Celebrated for its immaculate water quality, the river may play a central role in both environmental conservation and regional sustainability (Savio *et al*., 2015). The complexity of freshwater ecosystems could present diverse research opportunities, reflecting broader ecological dynamics and human-environment interactions (Bae *et al*., 2023). Considering the status of the Seomjin River as a habitat with great biodiversity, there is a need to expand biodiversity monitoring of this area and to study the assemblages of aquatic microorganisms connected to the food web and biogeochemical cycles for the functional diversity of freshwater organisms (i.e., plants and animals) (Falkowski *et al*., 2008, Battin *et al*., 2016).

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This study aimed to enhance understanding of bacterial diversity in lotic ecosystems, resulting in the discovery of 22 unrecorded bacterial species from the Seomjin River basin that had not been previously identified in South Korea. The discovered bacterial species belonged to the phyla *Pseudomonadota*, *Actinomycetota*, *Bacillota*, and *Bacteroidota*, respectively. By reporting the unrecorded bacterial species inhabiting the Seomjin River basin, it is expected to contribute to national species conservation efforts and support their sustainable utilization.

Materials and Methods

The isolation and cultivation of 22 freshwater bacterial strains from the Seomjin River were conducted with slight modifications based on a previously reported method (Park *et al*., 2024). To facilitate the isolation of bacterial strains with diverse physiological characteristics, the standard dilution plating method was applied on multiple culture media, including Reasoner's 2A (R2A), International Streptomyces Project medium 4 (ISP4), tryptic soy agar (TSA), marine agar 2211 (MA), and nutrient agar (NA). The plates were incubated at 20 and 25°C for 7 days, which correspond to the water temperatures recorded at the time of sample collection, facilitating the cultivation of bacterial strains adapted to their native conditions. Following isolation, each isolate was purified as a single colony via serial dilution spreading, and the pure cultures were preserved in 20% glycerol suspensions at −80°C.

Genomic DNA was extracted, and the 16S rRNA gene was amplified using the universal primers 27F and 1492R. The resulting sequences were analyzed by comparison with reference bacterial strains available in the EzBioCloud database (Chalita *et al*., 2024), following standard molecular protocols. A similarity threshold of 98.7% was applied, and bacterial strains showing ≥98.7% sequence similarity to known species yet to be recorded in Korea were classified as unreported species. For phylogenetic analysis, 16S rRNA gene sequences from the isolates were aligned with those of type strains using Clustal_W, and the alignments were manually refined with EzEditor (Jeon *et al*., 2014). Phylogenetic trees were constructed using MEGA 7.0 (Kumar *et al*., 2016) with the neighbor-joining method (Saitou and Nei, 1987) and the Kimura 2-parameter evolutionary model (Kimura, 1980). The reliability of the constructed trees was evaluated through bootstrap analysis with 1,000 replicates (Felsenstein, 1985).

Colony morphology was examined on agar plates using a magnifying glass once cultures reached the stationary phase. Cellular morphology and dimensions were further examined by transmission electron microscopy. Gram staining was conducted using a Gram-staining kit (bioMérieux), while biochemical characteristics were evaluated with API 20NE galleries (bioMérieux) following the manufacturer's protocols. The strain identifiers, sources of isolation, culture media used, and incubation conditions are detailed in Table 1.

Results and Discussion

A total of 22 bacterial strains were isolated from the Seomjin River, all representing species not previously recorded in Korea. These strains were classified into 4 phyla, 6 classes, 10 orders, 17 families, and 19 genera. The taxonomic distribution and identification details of these species are summarized in Table 1. At the genus level, the newly identified species belong to *Methylorubrum*, *Aureimonas*, *Bosea*, *Tsuneonella*, *Erythrobacter*, *Sphingomonas*, *Paracoccus*, *Blastomonas*, and *Asticcacaulis* within the class *Alphaproteobacteria*; *Rivibacter*, *Sheuella*, and *Duganella* within the class *Betaproteobacteria*; *Pseudomonas* and *Dickeya* within the class *Gammaproteobacteria*; *Mycobacterium*, *Nocardia*, and *Brachybacterium* within the class *Actinomycetes*; *Paenibacillus* and *Neobacillus* within the class *Bacilli*; and *Chitinophaga* within the class *Chitinophagia*. The phylogenetic relationships between these isolates and their closest relatives are shown in Fig. 1 (*Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*), Fig. 2 (*Actinomycetes*) and Fig. 3 (*Bacilli* and *Chitinophagia*). Transmission electron micrographs of the isolates are presented in Fig. 4, while their morphological, physiological, and biochemical characteristics are described in detail below.

Description of *Methylorubrum rhodinum* 20SJ16W-72

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and bright red colored after 7 days of incubation at 20°C on R2A. Positive for nitrate reduction, urease, esculin hydrolysis and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, gelatin hydrolysis, and β-galactosidase. Potassium gluconate is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 20SJ16W-72 displays the highest 16S rRNA gene sequence similarity with *Methylorubrum rhodinum* JCM 2811^T (99.9%). Strain 20SJ 16W-72 (=NNIBR2021641BA1641) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912 965.

Description of *Aureimonas glaciei* 21SJ13W-57

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are irregular, dry and bright yellow colored after 7 days of incubation at 20°C on ISP4. Posi-

Table 1. Summary of unrecorded freshwater bacterial strains isolated from the Seomjin River and their taxonomic affiliations.

Phylum	Class	Order	Family	Strain ID	GPS	NNIBR ID	Accession number	Most closely related species	Similarity (%)	Medium	Incubation conditions
Pseudomonadota	*Alphaproteobacteria*	*Hyphomicrobiales*	*Methylobacteriaceae*	20SJ16W-72	35°00′4.07″N 127°07′6.20″E	NNIBR2021641BA1641	OL912965	*Methylorubrum rhodinum*	99.9	R2A	20°C, 7 d
			Aurantimonadaceae	21SJ13W-57	35°0′1.65″N 127°7′4.99″E	NNIBR2021641BA1652	OL912976	*Aureimonas glaciei*	99.6	ISP4	20°C, 7 d
			Boseaceae	21SJ14S-33	35°04′16.83″N 127°14′09.42″E	NNIBR2021641BA1589	OK562845	*Bosea psychrotolerans*	99.8	R2A	25°C, 7 d
		Sphingomonadales	*Erythrobacteraceae*	21SJ02S-50	35°39′19.96″N 127°13′30.70″E	NNIBR2021641BA1654	OL677209	*Tsuneonella troitsensis*	99.2	R2A	25°C, 7d
				21SJ10S-S20	35°3′46.92″N 127°44′27.91″E	NNIBR2021641BA1661	OL677216	*Tsuneonella amylolytica*	99.1	R2A	25°C, 7 d
				21SJ02W-27	35°39′19.96″N 127°13′30.70″E	NNIBR2021641BA1656	OL677211	*Erythrobacter ramosus*	98.9	R2A	25°C, 7 d
			Sphingomonadaceae	21SJ08W-1	35°9′57.43″N 127°27′16.10″E	NNIBR2021641BA1648	OL912972	*Sphingomonas aerolata*	98.8	ISP4	20°C, 7 d
				21SJ09W-S21	35°11′58.47″N 127°36′0.88″E	NNIBR2021641BA1660	OL677215	*Sphingomonas fonticola*	99.7	R2A	25°C, 7 d
				21SJ09S-26	35°11′58.47″N 127°36′0.88″E	NNIBR2021641BA1653	OL677208	*Parablastomonas arctica*	99.4	R2A	25°C, 7 d
		Caulobacterales	*Caulobacteraceae*	21SJ04S-1	35°31′12.67″N 127°7′4.86″E	NNIBR2022641BA2257	OP847229	*Asticcacaulis biprosthecium*	99.7	R2A	25°C, 7 d
	Betaproteobacteria	*Burkholderiales*	*Sphaerotilaceae*	21SJ04S-8	35°31′12.67″N 127°7′4.86″E	NNIBR2022641BA2258	OP847230	*Rivibacter subsaxonicus*	99.9	R2A	25°C, 7 d
			Alcaligenaceae	21SJ08W-16	35°9′57.43″N 127°27′16.10″E	NNIBR2021641BA1650	OL912974	*Sheuella amnicola*	98.9	R2A	20°C, 7 d
			Oxalobacteraceae	SJ13W-44	35°0′4.44″N 127°7′1.04″E	NNIBR2022641BA2291	OQ073732	*Duganella margarita*	99.5	ISP4	25°C, 7 d
	Gammaproteobacteria	*Pseudomonadales*	*Pseudomonadaceae*	20SJ16W-91	35°00′4.07″N 127°07′6.20″E	NNIBR2021641BA1642	OL912966	*Pseudomonas otitidis*	99.6	TSA	20°C, 7 d
		Enterobacterales	*Pectobacteriaceae*	20SJ17W-63	35°4′17.55″N 127°14′9.13″E	NNIBR2021641BA1643	OL912967	*Dickeya lacustris*	99.5	MA	20°C, 7 d

Table 1. Continued.

Phylum	Class	Order	Family	Strain ID	GPS	NNIBR ID	Accession number	Most closely related species	Similarity (%)	Medium	Incubation conditions
Actinomycetota	*Actinomycetes*	*Mycobacteriales*	*Mycobacteriaceae*	21SJ03S-24	35°35'14.90"N 127°6'18.07"E	NNIBR2021641BA1657	OL677212	*Mycobacterium pallens*	98.9	R2A	25°C, 7 d
			Nocardiaceae	21SJ08S-113	35°9'57.43"N 127°27'16.10"E	NNIBR2021641BA1645	OL912969	*Nocardia aobensis*	99.2	MA	20°C, 7 d
		Micrococcales	*Dermabacteraceae*	21SJ08W-46	35°9'57.43"N 127°27'16.10"E	NNIBR2021641BA1651	OL912975	*Brachybacterium tyrofermentans*	99.2	MA	20°C, 7 d
Bacillota	*Bacilli*	*Caryophanales*	*Paenibacillaceae*	21SJ08S-108	35°9'57.43"N 127°27'16.10"E	NNIBR2021641BA1644	OL912968	*Paenibacillus susongensis*	98.8	MA	20°C, 7 d
				21SJ08S-65	35°9'57.43"N 127°27'16.10"E	NNIBR2021641BA1647	OL912971	*Paenibacillus rhizoplanae*	99.7	TSA	20°C, 7 d
			Bacillaceae	21SJ08S-43	35°9'57.43"N 127°27'16.10"E	NNIBR2021641BA1646	OL912970	*Neobacillus bataviensis*	99.4	NA	20°C, 7 d
Bacteroidota	*Chitinophagia*	*Chitinophagales*	*Chitinophagaceae*	21SJ14W-13	35°04'16.8"N 127°14'09.4"E	NNIBR2021641BA1590	OK562846	*Chitinophaga sancti*	99.0	R2A	25°C, 7 d

tive for nitrate reduction, glucose fermentation, urease, esculin hydrolysis and oxidase in API 20NE, but negative for indole production, arginine dihydrolase, gelatin hydrolysis and β-galactosidase. D-glucose, D-mannose and *N*-acetyl-glucosamine 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 21SJ13W-57 displays the highest 16S rRNA gene sequence similarity with *Aureimonas glaciei* B5-2^T (99.6%). Strain 21SJ13W-57 (=NNIBR2021641BA1652) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912976.

Description of *Bosea psychrotolerans* 21SJ14S-33

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex and pale-yellow colored after 7 days of incubation at 25°C on R2A. Positive for arginine dihydrolase, urease, and esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, gelatin hydrolysis, β-galactosidase and oxidase. L-arabinose, D-mannose, adipic acid, malic acid, and trisodium citrate are utilized. Does not utilize D-glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid and phenylacetic acid. Strain 21SJ14S-33 displays the highest 16S rRNA gene sequence similarity with *Bosea psychrotolerans* 1131^T (99.8%). Strain 21SJ14S-33 (=NNIBR2021641BA1589) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OK562845.

Description of *Tsuneonella troitsensis* 21SJ02S-50

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and yellow colored after 7 days of incubation at 25°C on R2A. Positive for β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis and oxidase. Potassium gluconate is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ02S-50 displays the highest 16S rRNA gene sequence similarity with *Tsuneonella troitsensis* JCM 17037^T (99.2%). Strain 21SJ02S-50 (=NNIBR2021641BA1654) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677209.

Description of *Tsuneonella amylolytica* 21SJ10S-S20

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

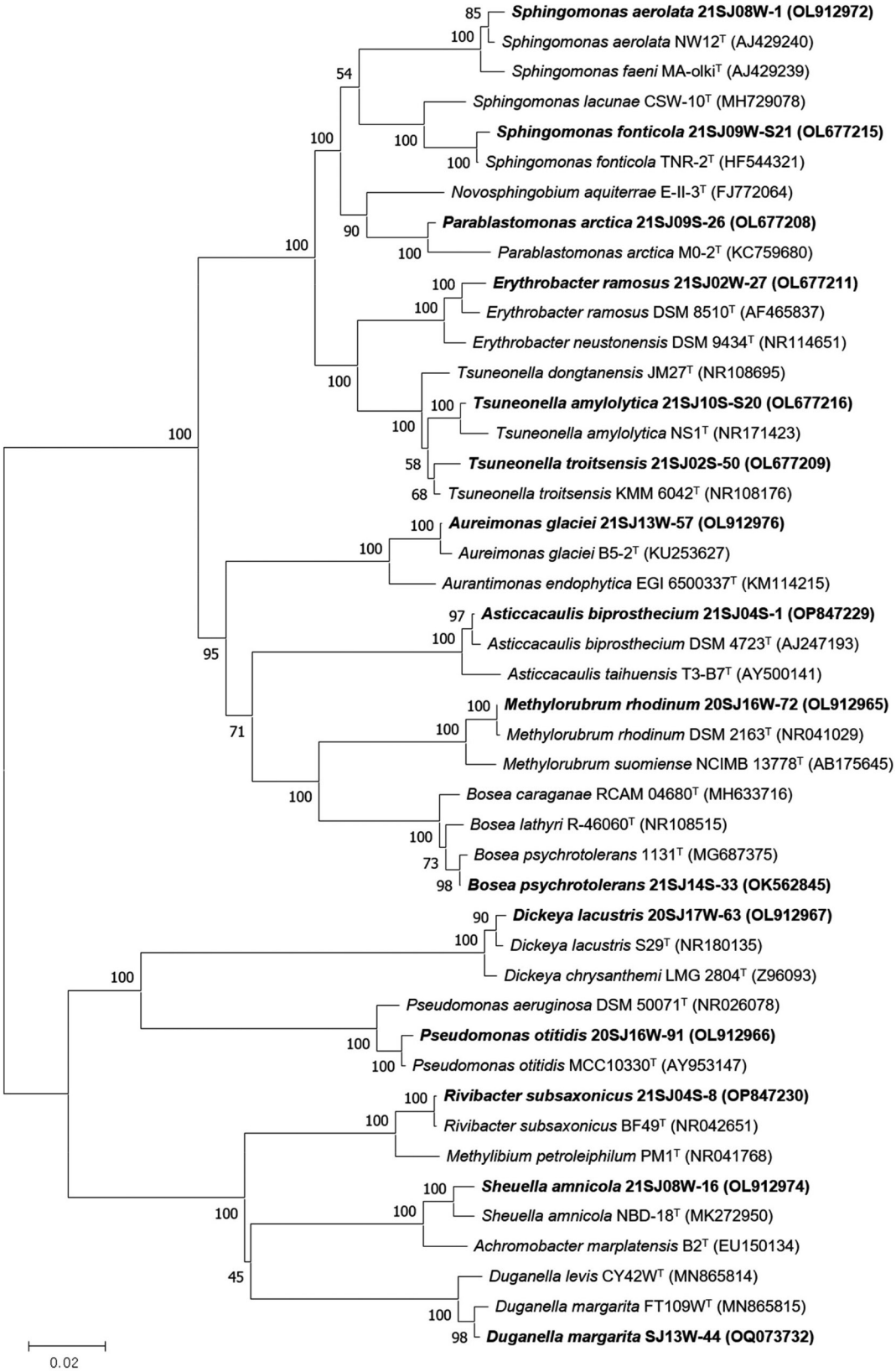

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains and their relatives in the classes *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*. Bootstrap values (>45%) are shown at nodes. Bar, 0.02 substitutions per nucleotide position.

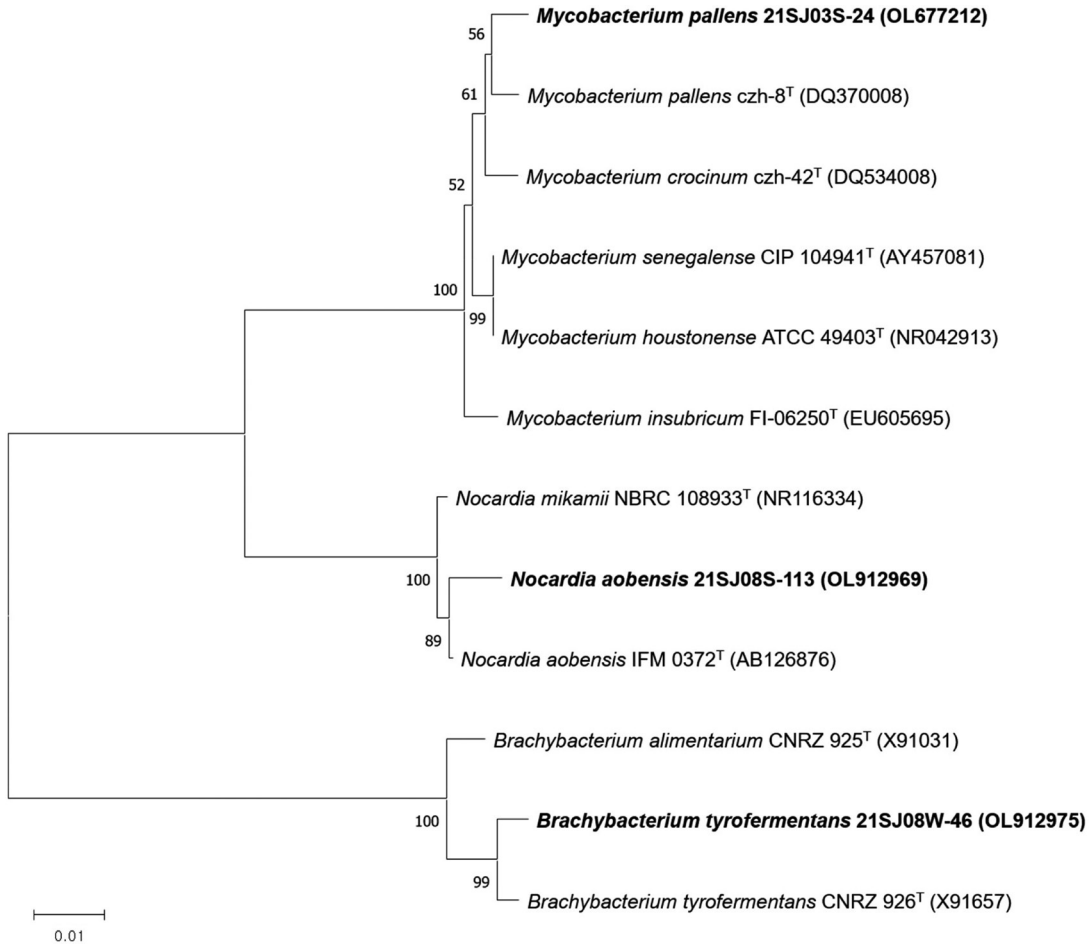

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains and their relatives in the classes *Actinomycetes*. Bootstrap values (>50%) are indicated at nodes. Bar, 0.01 substitutions per nucleotide position.

low colored after 7 days of incubation at 25°C on R2A. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase and oxidase. D-glucose and D-maltose are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ10S-S20 displays the highest 16S rRNA gene sequence similarity with *Tsuneonella amylolytica* NS1^T (99.1%). Strain 21SJ10S-S20 (=NNIBR 2021641BA1661) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677216.

Description of *Erythrobacter ramosus* 21SJ02W-27

Cells are Gram-staining-negative, non-flagellated and short, rod-shaped. Colonies are convex, circular, smooth and red colored after 7 days of incubation at 25°C on R2A. Positive for esculin hydrolysis, gelatin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and oxidase. D-glucose, D-maltose and adipic acid are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 21SJ02W-27 displays the highest 16S rRNA gene sequence similarity with *Erythrobacter ramosus* DSM 8510^T (98.9%). Strain 21SJ02W-27 (=NNIBR 2021641BA1656) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677211.

Description of *Sphingomonas aerolata* 21SJ08W-1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and orange colored after 7 days of incubation at 20°C on ISP4. Positive for esculin hydrolysis, and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease,

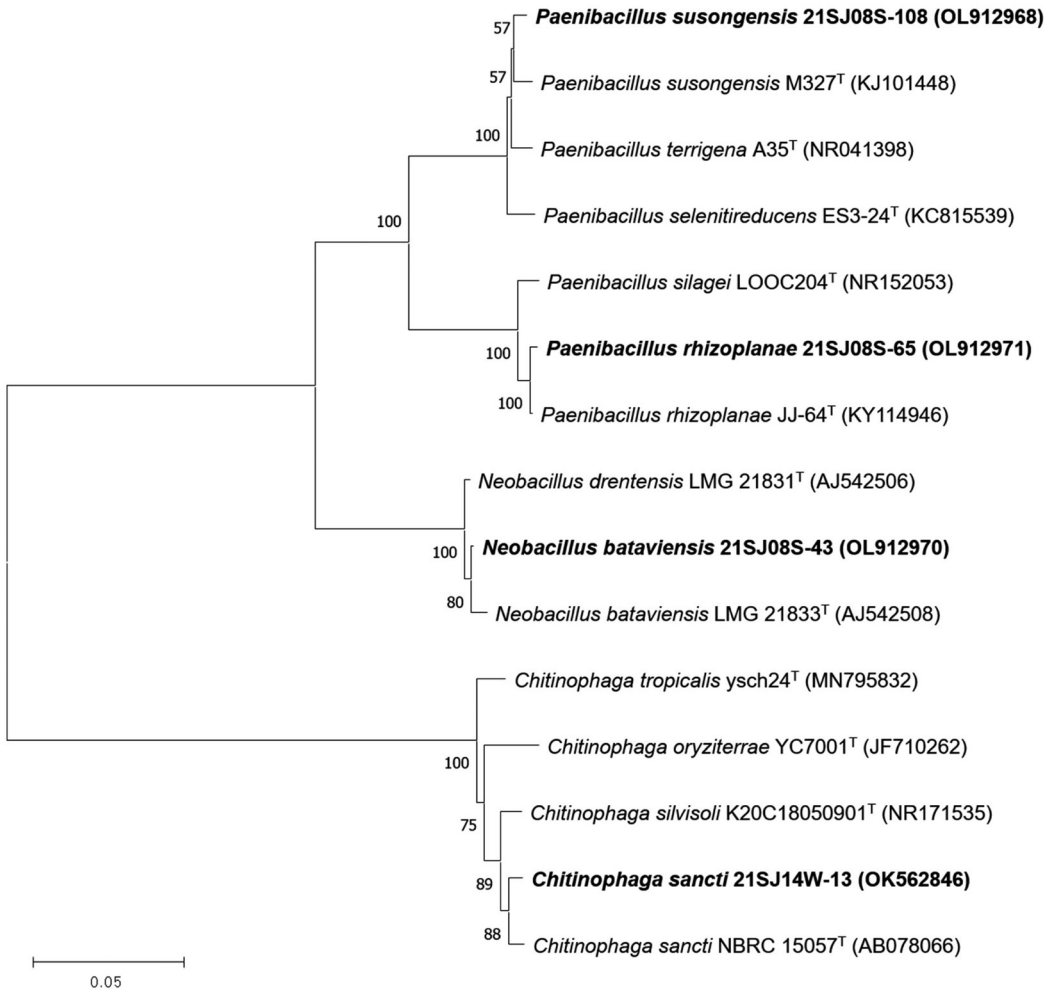

Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains and their relatives in the classes *Bacilli* and *Chitinophagia*. Bootstrap values (>50%) are shown at nodes. Bar, 0.05 substitutions per nucleotide position.

gelatin hydrolysis and oxidase. D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize D-mannitol, capric acid, adipic acid and phenylacetic acid. Strain 21SJ08W-1 displays the highest 16S rRNA gene sequence similarity with *Sphingomonas aerolata* NW12^T (98.8%). Strain 21SJ08W-1 (=NNIBR 2021641BA1648) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912972.

Description of *Sphingomonas fonticola* 21SJ09W-S21

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow colored after 7 days of incubation at 25°C on R2A. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and oxidase. D-maltose is 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 21SJ09W-S21 displays the highest 16S rRNA gene sequence similarity with *Sphingomonas fonticola* TNR-2^T (99.7%). Strain 21SJ09W-S21 (=NNIBR 2021641BA1660) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677215.

Description of *Parablastomonas arctica* 21SJ09S-26

Cells are Gram-staining-negative, non-flagellated and spherical-shaped. Colonies are circular, convex and white colored after 7 days of incubation at 25°C on R2A. Positive for β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydro-

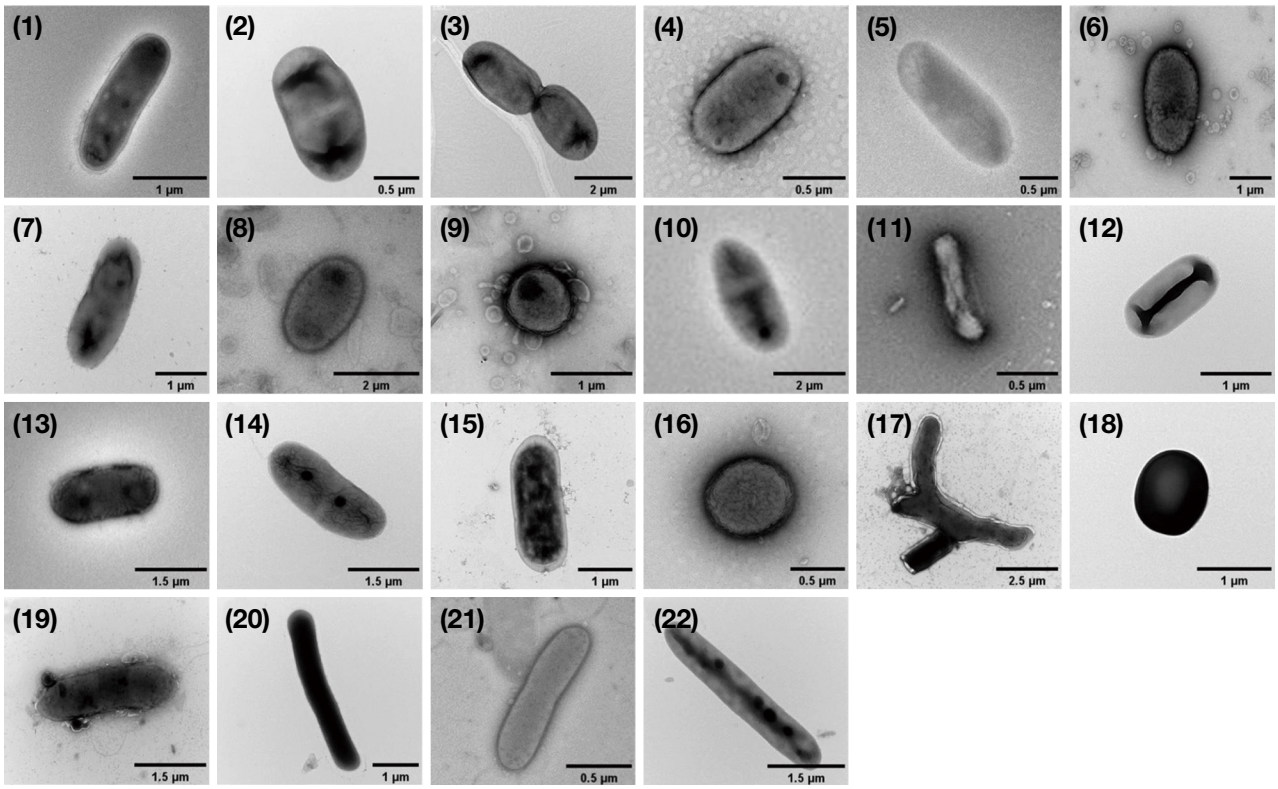

Fig. 4. Transmission electron micrographs of cells of the strains. Strains: 1, 20SJ16W-72; 2, 21SJ13W-57; 3, 21SJ14S-33; 4, 21SJ02S-50; 5, 21SJ10S-S20; 6, 21SJ02W-27; 7, 21SJ08W-1; 8, 21SJ09W-S21; 9, 21SJ09S-26; 10, 21SJ04S-1; 11, 21SJ04S-8; 12, 21SJ08W-16; 13, SJ13W-44; 14, 20SJ16W-91; 15, 20SJ17W-63; 16, 21SJ03S-24; 17, 21SJ08S-113; 18, 21SJ08W-46; 19, 21SJ08S-108; 20, 21SJ08S-65; 21, 21SJ08S-43; 22, 21SJ14W-13.

lysis and oxidase. 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 21SJ 09S-26 displays the highest 16S rRNA gene sequence similarity with *Parablastomonas arctica* M0-2^T (99.4%). Strain 21SJ09S-26 (=NNIBR2021641BA1653) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677208.

Description of *Asticcacaulis biprosthecium* 21SJ04S-1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and white colored after 7 days of incubation at 25°C on R2A. Positive for esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and oxidase. D-mannitol is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ04S-1 displays the highest 16S rRNA gene sequence similarity with *Asticcacaulis biprosthecium* DSM 4723^T (99.7%). Strain 21SJ04S-1 (=NNIBR2022 641BA2257) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OP847229.

Description of *Rivibacter subsaxonicus* 21SJ04S-8

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and white colored after 7 days of incubation at 25°C on R2A. Positive for nitrate reduction in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase and oxidase. 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 21SJ04S-8 displays the highest 16S rRNA gene sequence similarity with *Rivibacter subsaxonicus* DSM 19570^T (99.9%). Strain 21SJ04S-8 (=NNIBR2022641BA2258) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OP847230.

Description of *Sheuella amnicola* 21SJ08W-16

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and dark-beige colored after 7 days of incubation at 20°C on R2A. Positive for nitrate reduction, urease and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatin 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 21SJ08W-16 displays the highest 16S rRNA gene sequence similarity with *Sheuella amnicola* NBD-18^T (98.9%). Strain 21SJ08W-16 (=NNIBR2021641BA1650) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912974.

Description of *Duganella margarita* SJ13W-44

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex and pale-yellow colored after 7 days of incubation at 25°C on ISP4. Positive for nitrate reduction, urease, esculin hydrolysis, β-galactosidase and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, and gelatin hydrolysis. *N*-acetyl-glucosamine is utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SJ13W-44 displays the highest 16S rRNA gene sequence similarity with *Duganella margarita* FT109W^T (99.5%). Strain SJ13W-44 (=NNIBR2022641BA2291) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OQ073732.

Description of *Pseudomonas otitidis* 20SJ16W-91

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and ivory colored after 7 days of incubation at 20°C on TSA. Positive for nitrate reduction, esculin hydrolysis and oxidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and β-galactosidase. D-glucose, L-arabinose, D-mannitol, capric acid, malic acid, trisodium citrate and phenylacetic acid are utilized. Does not utilize D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and adipic acid. Strain 20SJ16W-91 displays the highest 16S rRNA gene sequence similarity with *Pseudomonas otitidis* MCC 10330^T (99.6%). Strain 20SJ16W-91 (=NNIBR2021641 BA1642) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912966.

Description of *Dickeya lacustris* 20SJ17W-63

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are convex, smooth and ivory colored after 7 days of incubation at 20°C on MA. Positive for nitrate reduction, indole production, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE, but negative for glucose fermentation, arginine dihydrolase, urease and oxidase. D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize D-maltose, capric acid, adipic acid and phenylacetic acid. Strain 20SJ17W-63 displays the highest 16S rRNA gene sequence similarity with *Dickeya lacustris* S29^T (99.5%). Strain 20SJ17W-63 (=NNIBR2021641BA1643) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912967.

Description of *Mycobacterium pallens* 21SJ03S-24

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and yellow colored after 7 days of incubation at 25°C on R2A. Positive for nitrate reduction in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase and oxidase. D-mannitol and potassium gluconate are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ03S-24 displays the highest 16S rRNA gene sequence similarity with *Mycobacterium pallens* czh-8^T (98.9%). Strain 21SJ03S-24 (=NNIBR2021641 BA1657) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL677212.

Description of *Nocardia aobensis* 21SJ08S-113

Cells are Gram-staining-positive, non-flagellated and hyphae-shaped. Colonies are irregular, dry and bright yellow colored after 7 days of incubation at 20°C on MA. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase and oxidase. D-glucose, capric acid, malic acid and trisodium citrate are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and phenylacetic acid. Strain 21SJ08S-113 displays the highest 16S rRNA gene sequence similarity with *Nocardia aobensis* IFM 0372^T (99.2%). Strain 21SJ08S-113 (=NNIBR20216 41BA1645) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912969.

Description of *Brachybacterium tyrofermentans* 21SJ08W-46

Cells are Gram-staining-positive, non-flagellated and spherical-shaped. Colonies are convex, smooth and bright yellow colored after 7 days of incubation at 20°C on MA. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, β-galactosidase and oxidase in API 20NE, but negative for indole production, arginine dihydrolase, urease and gelatin hydrolysis. D-glucose, L-arabinose, D-mannose, D-maltose, potassium gluconate, capric acid, and trisodium citrate are utilized. Does not utilize D-mannitol, *N*-acetyl-glucosamine, adipic acid, malic acid and phenylacetic acid. Strain 21SJ08W-46 displays the highest 16S rRNA gene sequence similarity with *Brachybacterium tyrofermentans* CNRZ 926^T (99.2%). Strain 21SJ08W-46 (=NNIBR2021641BA1651) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912975.

Description of *Paenibacillus susongensis* 21SJ08S-108

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, smooth and ivory colored after 7 days of incubation at 20°C on MA. Positive for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, β-galactosidase and oxidase in API 20NE, but negative for indole production and urease. L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are utilized. Does not utilize D-glucose, capric acid, adipic acid and phenylacetic acid. Strain 21SJ08S-108 displays the highest 16S rRNA gene sequence similarity with *Paenibacillus susongensis* M327^T (98.8%). Strain 21SJ08S-108 (=NNIBR2021641BA1644) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912968.

Description of *Paenibacillus rhizoplanae* 21SJ08S-65

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and ivory colored after 7 days of incubation at 20°C on TSA. Positive for esculin hydrolysis and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis and oxidase. D-mannose and D-maltose are utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain 21SJ08S-65 displays the highest 16S rRNA gene sequence similarity with *Paenibacillus rhizoplanae* JJ-64^T (99.7%). Strain 21SJ08S-65 (=NNIBR 2021641BA1647) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912971.

Description of *Neobacillus bataviensis* 21SJ08S-43

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular, convex, smooth and ivory colored after 7 days of incubation at 20°C on NA. Positive for glucose fermentation, esculin hydrolysis, gelatin hydrolysis, β-galactosidase and oxidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase and urease. 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 21SJ08S-43 displays the highest 16S rRNA gene sequence similarity with *Neobacillus bataviensis* LMG 21833^T (99.4%). Strain 21SJ08S-43 (=NNIBR2021 641BA1646) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OL912970.

Description of *Chitinophaga sancti* 21SJ14W-13

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex and pale-yellow colored after 7 days of incubation at 25°C on R2A. Positive for glucose fermentation, urease, esculin hydrolysis, gelatin hydrolysis, and β-galactosidase in API 20NE, but negative for nitrate reduction, indole production, arginine dihydrolase and oxidase. 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 21SJ14W-13 displays the highest 16S rRNA gene sequence similarity with *Chitinophaga sancti* NBRC 15057^T (99.0%). Strain 21SJ14W-13 (=NNIBR 2021641BA1590) was isolated from a freshwater sample of the Seomjin River. The GenBank accession number of 16S rRNA gene sequence is OK562846.

Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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