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A report of 10 unrecorded bacterial species of Korea, isolated from agricultural soil in 2022

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Contribution to Environmental Biology

- Since the Nagoya Protocol was issued, the value of biological resources has been increasing.
- In this study, we would like to report a taxonomic aspect of unrecorded species that have been reported abroad but not yet reported in Korea.
- The unrecorded species that were discovered are expected to be valuable for use as biological and genetic resources.

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Abstract: In 2022, research for native prokaryotic species in Korea reported 10 unrecorded bacterial strains affiliated to phyla Actinomycetota, Bacillota, and Pseudomonadota. The strains formed monophyletic clades with the most closely related species (with ≥98.7% sequence similarity) in the 16S rRNA gene sequencing. Among them, four species of the phylum Actinomycetota, two species of the phylum Bacillota, and four species of the phylum Pseudomonadota have not been reported in Korea, suggesting unrecorded species in Korea. Information on strains such as Gram staining reaction, colony and cell morphology, biochemical characteristics, and isolation sources were provided in the species description.

Keywords: 16S rRNA, bacterial diversity, unrecorded species

1. INTRODUCTION

For the discover indigenous prokaryotic species in Korea, various environmental samples are collected (Jang *et al.* 2022). In 2022, 10 unrecorded bacteria were isolated from agricultural soil samples from various regions in Korea and identified as phyla of Actinomycetes, Bacillota and Pseudomonadota. The taxonomic information of 10 unrecorded species is based on the nomenclature described in List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de) and consists of Actinomycetota, Bacillota and Pseudomonadota. The phylum Actinomycetota, one of the largest phyla within the domain Bacteria, is widely distributed in aquatic and terrestrial environments (Lawson 2018). The phylum is comprised of Gram stain positive, non-sporeforming, rod-shaped bacteria with a high G+C content (Gao and Gupta 2005); the members of the phylum Bacillota, which are resistant to desiccation and can survive extreme conditions and distributed in diverse environments, are known as Gram stain positive and low G+C content containing rod/coccus-shaped bacteria (Nahar *et al.* 2018); the phylum Pseudomonadota are known as Gram stain negative bacteria and responsible for nitrogen fixation, which one of the largest phyla within the domain Bacteria (Seong *et al.* 2019).

This report focuses on the description of the charac-

teristics that 10 unrecorded species belonging to the phyla Actinomycetota, Bacillota, and Pseudomonadota, which have not been reported in Korea so far.

2. MATERIALS AND METHODS

A total of 10 bacterial strains assigned to the phyla Actinomycetota, Bacillota and Pseudomonadota were isolated from agricultural soil. The agricultural soil samples collected from each region were independently processed serial dilution and spread onto diverse culture media Reasoner'2A agar (R2A; BD Difco), nutrient agar (NA; BD Difco) and tryptic soy agar (TSA; BD Difco) and incubated at 28°C for 3−7 days (Table 1). All strains were purified as single colonies and stored as 15−17% glycerol suspension at −80°C as well as lyophilized ampoules.

The morphology and cell size of colonies for each strain were determined by using a scanning electron microscopy and transmission electron microscopy. The electron micrographs of each strain are shown in Figure 1. Gram reaction was performed according to the classic Gram procedure described by Doetsch (1981). The biochemical characteristics of the strains were tested using the API test kit (API 20NE, API 32GN, API ZYM) by referring to the bioMérieux company's instructions. After extracting genomic DNA and amplify the 16S rRNA gene using 27mF, 1492R (universal) primers (Han *et al.* 2022), and the sequence database of each strain was submission in NCBI GenBank. The 16S rRNA gene sequences of the related taxa were obtained from EzBioCloud server (Yoon *et al.* 2017). Ten bacterial strains and related taxa (retrieved from the NCBI database) were aligned with SINA (v1.2.12) according to the SILVA seed alignment (http://www.arb-silva.de; Pruesse *et al.* 2012). Evolutionary distances applied during phylogenetic analysis were calculated through a two-parameter model (Kimura 1983). A phylogenetic tree was constructed using bootstrap values (Felsenstein 1985) based on 1,000 replicates using neighbor joining (Saitou and Nei 1987) in the MEGA X program (Kumar *et al.* 2018).

3. RESULTS AND DISCUSSION

The 10 strains were distributed into 3 phyla: Actinomycetota, Bacillota, Pseudomonadota (Table 1). All

Table 1. The taxonomic affiliations of isolated strains belonging to the phylum Actinomycetota, Bacillota, and Pseudomonadota

Phylum	Class	Order	Family	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium[a]	Incubation conditions
Actinomycetota	Actinomycetes	Propionibacteriales	Nocardioidaceae	GW31	NIBRBAC000509779	*Nocardioides jensenii*	99.31	Agricultural soil	NA	28°C, 4 d
		Mycobacteriales	Gordoniaceae	R6	NIBRBAC000509780	*Gordonia bronchialis*	100	Agricultural soil	NA	28°C, 4 d
			Corynebacteriaceae	JN1306	NIBRBAC000509785	*Corynebacterium glucuronolyticum*	99.26	Agricultural soil	NA	28°C, 4 d
		Micrococcales	Intrasporangiaceae	SW56	NIBRBAC000509786	*Intrasporangium chromatireducens*	100	Agricultural soil	TSA	28°C, 4 d
Pseudomonadota	Alphaproteobacteria	Hyphomicrobiales	Nitrobacteraceae	TA-E7	NIBRBAC000509781	*Afipia clevelandensis*	100	Agricultural soil	R2A	28°C, 4 d
			Phyllobacteriaceae	SY19	NIBRBAC000509784	*Mesorhizobium thiogangeticum*	98.88	Agricultural soil	NA	28°C, 4 d
				SY31	NIBRBAC000509787	*Aquamicrobium soli*	99.57	Agricultural soil	TSA	28°C, 4 d
			Rhizobiaceae	NOY11-2	NIBRBAC000509782	*Mycoplana dimorpha*	99.79	Agricultural soil	NA	28°C, 4 d
Bacillota	Bacilli	Bacillales	Paenibacillaceae	ROW11-2	NIBRBAC000509783	*Paenibacillus montanisoli*	99.30	Agricultural soil	R2A	28°C, 4 d
			Planococcaceae	TW11	NIBRBAC000509788	*Paenibacillus frigoriresistens*	99.04	Agricultural soil	NA	28°C, 4 d

[a]NA, nutrient agar; R2A, Reasoner'2A agar; TSA, tryptic soy agar

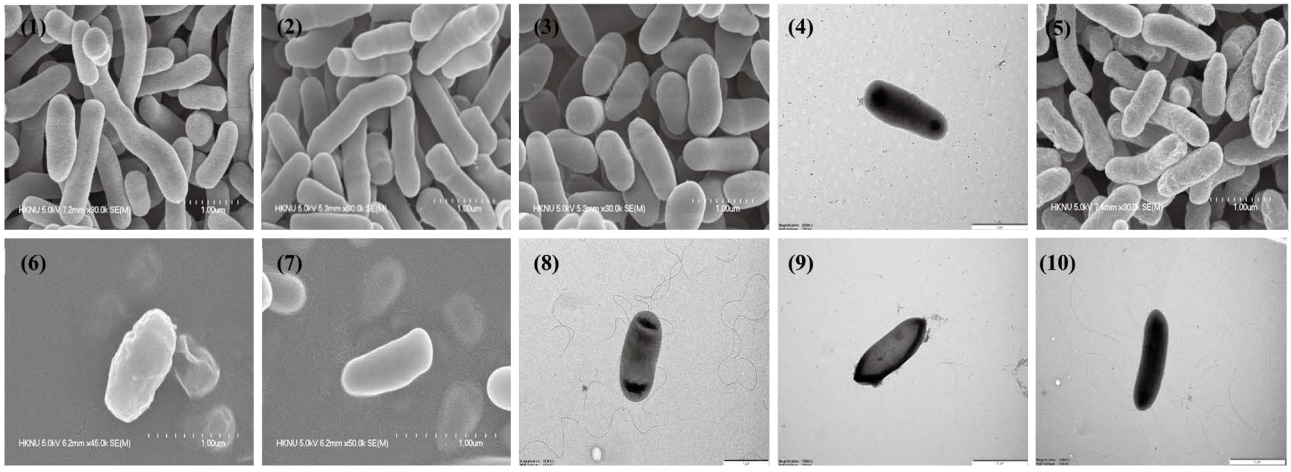

Fig. 1. Scanning electron microscopy and transmission electron microscopy of the cells of the unrecorded strains isolated in this study. The strains were cultured at their optimal growth conditions. 1, GW31; 2, R6; 3, JN1306; 4, SW56; 5, TA-E7; 6, SY19; 7, SY31; 8, NOY11-2; 9, ROW11-2; 10, TW11.

strains were rod-shaped (Fig. 1). Unrecorded bacteria were identified as 10 genera of *Afipia*, *Aquamicrobium*, *Corynebacterium*, *Gordonia*, *Intrasporangium*, *Mesorhizobium*, *Mycoplana*, *Nocardioides* and *Paenibacillus* (Fig. 2). Here we report 10 unrecorded bacterial species in Korea belonging to 5 orders, which were isolated in Korea; 4 strains of the Hyphomicrobiales, 1 strain of the Micrococcales, 2 strains of the Mycobacteriales, 2 strains of the Paenibacillaceae, and 1 strain of the Propionibacteriales.

3.1. Description of *Nocardioides jensenii* GW31

Cells are Gram-staining-positive and rod-shape. Cell size is 1.2−2.2 μm. Colonies are circular, smooth, entire and white color after 4 days of incubation at 28°C on NA. In API 20NE, the reactions in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), and utilization of arginine dihydrolase, urease, D-glucose, gluconate, adipate, and malate are positive, but the reaction in reduction of indole production, glucose acidification, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, caprate, and citrate are negative. In API 32GN, the reaction in D-glucose, and utilization of L-fucose, D-sorbitol, valerate, L-histidine, 3-hydroxy-butyrate, L-rhamnose, L-alanine glycogen, and 3-hydroxy-benzoate are positive, but reaction in D-mannitol, salicin, D-melibiose, L-arabinose, propionate, caprate, citrate, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, *N*-acetyl-D-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, acetate, lactate, 5-ketogluconate, and L-serine are negative. Positive reactions for enzymatic activity in alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-glucosidase, and α-mannosidase at API ZYM, but negative reactions for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-fucosidase. Strain GW31 (=NIBRBAC000509779) was isolated an agricultural soil sample, Gyeongsangnam-do, Korea (35°23′43.2″N 128°08′54.3″E).

3.2. Description of *Gordonia bronchialis* R6

Cells are Gram-staining-positive and rod-shape. Cell size is 1.5−2.2 μm. Colonies are circular, smooth, entire and orange color after 4 days of incubation at 28°C on NA. In API 20NE, the reactions in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), and utilization of D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, and malate are positive, but the reaction in indole production, glucose acidification, arginine dihydrolase, protease (gelatin hydrolysis), β-galactosidase (PNPG), L-arabinose, D-maltose, gluconate, caprate, citrate, phenylacetate. In API 32GN, the reaction in D-mannitol, and

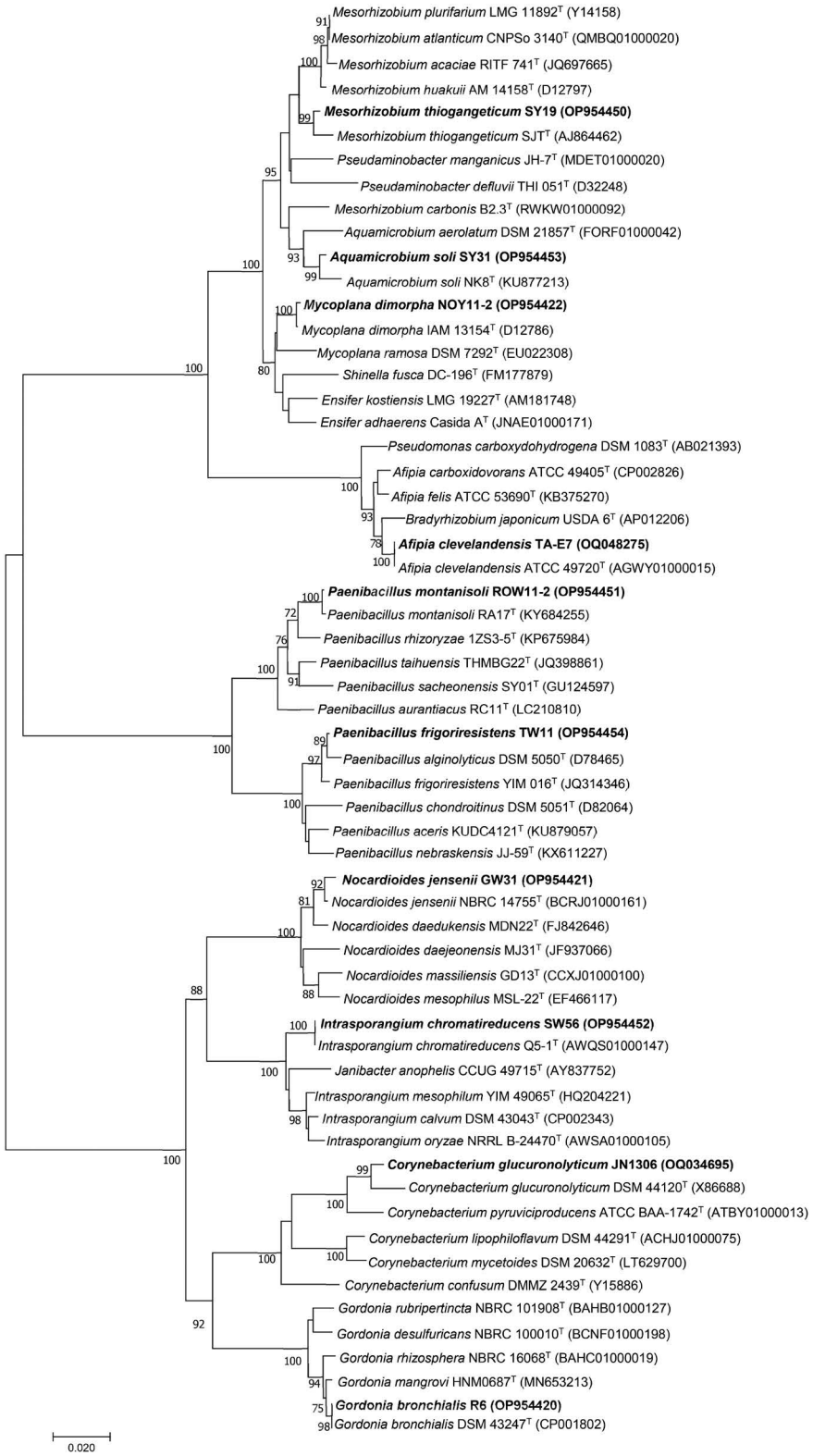

Fig. 2. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. Bar: 0.020 substitutions per nucleotide position.

utilization of D-glucose, propionate, valerate, L-histidine, 3-hydroxy-butyrate, L-proline, L-rhamnose, *N*-acetyl-D-glucosamine, inositol, acetate, and lactate are positive, but reaction in salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, caprate, citrate, 2-ketogluconate, 4-hydroxy-benzoate, D-ribose, D-sucrose, D-maltose, itaconate, suberate, malonate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine are negative. Positive reactions for enzymatic activity in alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-glucosidase, and β-glucosidase at API ZYM, but negative reactions for lipase (C14), valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain R6 (=NIBR BAC000509780) was isolated from an agricultural soil sample, Chungcheongnam-do, Korea (36°53′11.8″N 126°44′34.7″E).

3.3. Description of *Corynebacterium glucuronolyticum* JN1306

Cells are Gram-staining-positive and rod-shape. Cell size is 1.2−1.5 μm. Colonies are circular, smooth, entire and white color after 3 days of incubation at 28°C on NA. In API 20NE, the reactions in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), and utilization of D-glucose are positive, but the reaction in indole production, glucose acidification, arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate, and phenyl-acetate are negative. In API 32GN, the reaction in D-glucose, and utilization of L-fucose, propionate, D-sucrose, and glycogen are positive, but reaction in D-mannitol, salicin, D-melibiose, D-sorbitol, L-arabinose, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, *N*-acetyl-D-glucosamine, D-ribose, inositol, D-maltose, itaconate, suberate, malonate, acetate, lactate, L-alanine, 5-ketogluconate, 3-hydroxy-benzoate, and L-serine are negative. Positive reactions for enzymatic activity in esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, and β-glucuronidase at API ZYM, but negative reactions for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain JN1306 (=NIBRBAC000509785) was isolated from an agricultural sample, Jeollabuk-do, Korea (35°33′02.5″N 127°08′14.6″E).

3.4. Description of *Intrasporangium chromatireducens* SW56

Cells are Gram-staining-positive and rod-shape. Cell size is 1.0−1.7 μm. Colonies are circular, smooth, entire and white color after 4 days of incubation at 28°C on TSA. In API 20NE, the reactions in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), and utilization of urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), D-glucose, D-mannose, D-maltose, and gluconate are positive, but the reaction in indole production, glucose acidification, arginine dihydrolase, β-galactosidase (PNPG), L-arabinose, D-mannitol, *N*-acetyl-D-glucosamine, caprate, adipate, malate, citrate, and phenyl-acetate are negative. In API 32GN, the reaction in D-glucose, and utilization of propionate, valerate, L-histidine, 3-hydroxy-butyrate, L-proline, D-ribose, D-sucrose, D-maltose, acetate, L-alanine, 3-hydroxy-benzoate, and L-serine are positive, but reaction in D-mannitol, salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, caprate, citrate, 2-ketogluconate, 4-hydroxy-benzoate, L-rhamnose, *N*-acetyl-D-glucosamine, inositol, itaconate, suberate, malonate, lactate, 5-ketogluconate, and glycogen are positive. Positive reactions for enzymatic activity in esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, β-glucuronidase, and α-glucosidase at API ZYM, but negative reactions for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain SW56 (=NIBRBAC000509786) was isolated from an agricultural soil sample, Chungcheongbuk-do, Korea (36°47′30.5″N 127°29′47.1″E).

3.5. Description of *Afipia clevelandensis* TA-E7

Cells are Gram-staining-negative and rod-shape. Cell size is 1.1−1.6 μm. Colonies are circular, smooth, entire and white color after 4 days of incubation at 28°C on

R2A. In API 20NE, the reactions in reduction of nitrate reduction ($NO_3^- \rightarrow NO_2^-$), and utilization of arginine dihydrolase, urease, gluconate, adipate, and malate are positive, but the reaction in indole production, glucose acidification, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, caprate, citrate, and phenyl-acetate are negative. In API 32GN, the reaction in propionate, and utilization of valerate, 3-hydroxy-butyrate, itaconate, suberate, acetate, and glycogen are positive, but reaction in D-mannitol, D-glucose, salicin, D-melibiose, L-fucose, D-sorbitol, L-arabinose, caprate, citrate, L-histidine, 2-ketogluconate, 4-hydroxy-benzoate, L-proline, L-rhamnose, *N*-acetyl-D-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, malonate, lactate, L-alanine, 5-ketogluconate, 3-hydroxy-benzoate, and L-serine are negative. Positive reactions for enzymatic activity in alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, and naphtol-AS-BI-phosphohydrolase at API ZYM, but negative reactions for lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain TA-E7 (=NIBRBAC000509781) was isolated from an agricultural soil sample, Gyeonggi-do, Korea (37°06′53.9″N 126°58′48.1″E).

3.6. Description of *Mesorhizobium thiogangeticum* SY19

Cells are Gram-staining-negative and rod-shape. Cell size is 0.8−1.2 μm. Colonies are circular, smooth, entire and yellow color after 4 days of incubation at 28°C on NA. In API 20NE, the reactions in glucose acidification, and utilization of arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), D-glucose, L-arabinose, D-mannitol, *N*-acetyl-D-glucosamine, and gluconate are positive, but the reaction in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), reduction of nitrates to nitrogen, indole production, protease (gelatin hydrolysis), β-galactosidase (PNPG), D-maltose, caprate, adipate, malate, citrate, and phenyl-acetate are negative. In API 32GN, the reaction in D-mannitol, and utilization of D-glucose, L-fucose, D-sorbitol, L-arabinose, propionate, valerate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, *N*-acetyl-D-glucosamine, acetate, lactate, L-alanine, and L-serine are positive, but reaction in salicin, D-melibiose, caprate, citrate, L-rhamnose, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, 5-ketogluconate, and 3-hydroxy-benzoate are negative. Positive reactions for enzymatic activity in alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, and naphtol-AS-BI-phosphohydrolase at API ZYM, but negative reactions for lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain SY19 (=NIBRBAC000509784) was isolated from an agricultural soil sample, Jeollabuk-do, Korea (35°45′12.3″N 127°08′42.5″E).

3.7. Description of *Aquamicrobium soli* SY31

Cells are Gram-staining-negative and rod-shape. Cell size is 0.7−1.1 μm. Colonies are circular, smooth, entire and yellow color after 4 days of incubation at 28°C on TSA. In API 20NE, the reactions in D-glucose, and utilization of L-arabinose, D-mannitol, *N*-acetyl-D-glucosamine, and gluconate are positive, but the reaction in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), reduction of nitrates to nitrogen, indole production, arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), D-mannose, D-maltose, caprate, adipate, malate, citrate, and phenyl-acetate are negative. In API 32GN, the reaction in D-mannitol, and utilization of D-glucose, L-fucose, D-sorbitol, L-arabinose, propionate, valerate, L-histidine, 3-hydroxy-butyrate, L-proline, L-rhamnose, *N*-acetyl-D-glucosamine, D-ribose, acetate, lactate, and L-alanine are positive, but reaction in salicin, D-melibiose, caprate, citrate, 2-ketogluconate, 4-hydroxy-benzoate, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine are negative. Positive reactions for enzymatic activity in esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, and α-glucosidase at API ZYM alkaline phosphatase, esterase (C4), leucine arylamidase, trypsin, acid phosphatase, and α-glucosidase at API ZYM, but negative reactions for lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-

β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain SY31 (=NIBRBAC000509787) was isolated from an agricultural soil sample, Jeollanam-do, Korea (35°02′19.3″N 127°02′23.5″E).

3.8. Description of *Mycoplana dimorpha* NOY11-2

Cells are Gram-staining-negative and rod-shape. Cell size is 1.5−2.4 μm. Colonies are irregular, smooth, entire and yellow color after 4 days of incubation at 28°C on NA. In API 20NE, the reactions in reduction of D-glucose, and utilization of L-arabinose, D-mannose, D-mannitol, and *N*-acetyl-D-glucosamine are positive, but the reaction in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), reduction of nitrates to nitrogen, indole production, glucose acidification, arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), D-maltose, gluconate, caprate, adipate, malate, citrate, and phenyl-acetate are negative. In API 32GN, the reaction in D-mannitol, and utilization of D-glucose, L-arabinose, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, L-proline, *N*-acetyl-D-glucosamine, acetate, lactate, L-alanine, and L-serine ware positive, but reaction in salicin, D-melibiose, L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, 4-hydroxy-benzoate, L-rhamnose, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, 5-ketogluconate, glycogen, and 3-hydroxy-benzoate are negative. Positive reactions for enzymatic activity in alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, and α-glucosidase at API ZYM, but negative reactions for esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain NOY11-2 (=NIBRBAC000509782) was isolated from an agricultural soil sample, Gyeonggi-do, Korea (37°09′07.3″N 126°58′46.8″E).

3.9. Description of *Paenibacillus montanisoli* ROW11-2

Cells are Gram-staining-negative and rod-shape. Cell size is 4.1−4.8 μm. Colonies are circular, smooth, entire and white color after 4 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), and utilization of L-arabinose, D-mannose, and phenyl-acetate are positive, but the reaction in indole production, glucose acidification, arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β-galactosidase (PNPG), D-glucose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, gluconate, caprate, adipate, malate, and citrate are positive. In API 32GN, the reaction in L-arabinose are positive, but reaction in D-mannitol, D-glucose, salicin, D-melibiose, L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, *N*-acetyl-D-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, acetate, lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine are negative. Enzymatic activity positive reaction was observed alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, and naphtol-AS-BI-phosphohydrolase at API ZYM, but negative reactions for lipase (C14), trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain ROW11-2 (=NIBRBAC000509783) was isolated from an agricultural soil sample, Gyeongsangnam-do, Korea (35°19′59.8″N 128°12′34.7″E).

3.10. Description of *Paenibacillus frigoriresistens* TW11

Cells are Gram-staining-positive and rod-shape. Cell size is 3.8−4.5 μm. Colonies are circular, smooth, entire and yellow color after 4 days of incubation at 28°C on NA. In API 20NE, the reactions in nitrate reduction ($NO_3^- \rightarrow NO_2^-$), and utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, and gluconate are positive, but the reaction in indole production, glucose acidification, arginine dihydrolase, urease, β-glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), caprate, adipate, malate, citrate, and phenyl-acetate are negative. In API 32GN, the reaction in D-mannitol, and utilization of D-glucose, salicin, D-melibiose, L-arabinose, L-histidine, L-rhamnose, *N*-acetyl-D-glucosamine, D-ribose, D-sucrose, D-maltose, and glycogen are positive, but reaction in L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, inositol, itaconate, su-

berate, malonate, acetate, lactate, L-alanine, 5-ketogluconate, 3-hydroxy-benzoate, and L-serine are negative. Positive reactions for enzymatic activity in esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, and α-glucosidase at API ZYM, but negative reactions for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. Strain TW11 (=NIBRBAC000509788) was isolated from an agricultural soil sample, Gyeonggi-do, Korea (37°08′16.5″N 126°58′12.9″E).

CRediT authorship contribution statement

OB Lim: Writing and Inverstigation. JS Lee: Inverstigation. H Lee: Conceptualization and Supervision. KE Lee: Data curation. IT Cha: Project administration. WJ Chi: Project administration. D-U Kim: Supervison, Funding acquisition, Resources, Writing-Reviewing and editing.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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