

A report of 32 unrecorded bacterial species belonging to the phylum *Bacillota* in Korea

Hyeryeon Gyeong¹, Jung-Hoon Yoon², Chang-Jun Cha³, Wonyong Kim⁴, Wan-Taek Im⁵, Seung Bum Kim⁶, Myung Kyum Kim⁷, Dong-Uk Kim⁸, Taegun Seo⁹ and Myung-Ji Seo^{1,10,11,*}

¹Division of Bioengineering, Incheon National University, Incheon 22012, Republic of Korea

²Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea

³Department of Systems Biotechnology, Chung-Ang University, Anseong 17546, Republic of Korea

⁴Department of Microbiology, College of Medicine, Chung-Ang University, Seoul 06974, Republic of Korea

⁵Department of Biotechnology, Hankyung National University, Anseong 17579, Republic of Korea

⁶Department of Microbiology and Molecular Biology, Chungnam National University, Daejeon 34134, Republic of Korea

⁷Department of Bio & Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 01797, Republic of Korea

⁸Department of Biological Science, College of Science and Engineering, Sangji University, Wonju 26339, Republic of Korea

⁹Department of Life Science, Dongguk University-Seoul, Goyang 10326, Republic of Korea

¹⁰Department of Bioengineering and Nano-Bioengineering, Incheon National University, Incheon 22012, Republic of Korea

¹¹Research Center for Bio Materials & Process Development, Incheon National University, Incheon 22012, Republic of Korea

*Correspondent: mjseo@inu.ac.kr

As a part of a comprehensive investigation project of indigenous prokaryotic species in Korea, a total of 32 bacterial strains assigned to the phyla *Bacillota* were isolated from various environmental niches, including soil, mud flat, tidal flat, wetland, and seawater. Phylogenetic analysis based on their high 16S rRNA gene sequence similarities (>98.7%) and the formation of robust phylogenetic clades with their closest related reported species, each strain was identified as belonging to a distinct and validly published bacterial species. In the present study, we report 32 species of 22 genera of three families in the phylum *Bacillota*, which have not been reported in Korea. These 32 species were assigned to *Bacillus*, *Cytobacillus*, *Exiguobacterium*, *Gottfriedia*, *Gracilibacillus*, *Halobacillus*, *Jeotgalibacillus*, *Mesobacillus*, *Metabacillus*, *Neobacillus*, *Ornithinibacillus*, *Peribacillus*, *Pontibacillus*, *Pseudoneobacillus*, *Robertmurraya*, *Siminovitchia*, *Sutcliffeiella*, and *Virgibacillus* of the Family *Bacillaceae*; *Brevibacillus*, *Fontibacillus*, and *Paenibacillus* of the Family *Paenibacillaceae*; *Staphylococcus* of the Family *Staphylococcaceae*. In addition, Colony and cell morphology, gram staining, basic biochemical characteristic, isolation source, and strain ID of each strain are also described in the species descriptions.

Keywords: 16S rRNA, *Bacillota*, environmental niches, taxonomy, unrecorded species



All content in the Journal of Species Research (JSR), published by the National Institute of Biological Resources (NIBR), is Open Access, meaning it is freely accessible online to everyone without fees or the need for authors' permission. Open Access articles are automatically archived in the Korea Institute of Science and Technology Information (KISTI)'s Open Access repository, AccessON. All JSR content is published and distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (CC BY-NC-ND 4.0). This license permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are properly credited. However, no modifications or derivative works are allowed.

INTRODUCTION

The phylum *Bacillota* is one of the major phyla in the domain bacteria that was renamed from “*Firmicutes*” in 2021 (Oren and Garrity, 2021) which was first described by Gibbons and Murray (1978). *Bacillota* are ubiquitous in nature, thriving in diverse environments ranging from soil and water to the gastrointestinal tracts

of animals and humans, where they play crucial roles in nutrient cycling, fermentation, and metabolic processes. *Bacillota* species are generally Gram-staining positive, rod/cocci shaped bacteria with a low G + C content (Qin *et al.*, 2010). Cells in this phylum have rigid or semi-rigid cell wall containing peptidoglycan and some species can form endospores and have flagella for motility (Gibbons and Murray, 1978). *Bacillota* includes several important bacterial groups, such as industrially valuable lactic acid-producing bacteria, and medically significant species like *Clostridium* (Dworkin *et al.*, 2006). At the time of writing, based on 16S rRNA gene and genome sequences, the phylum *Bacillota* comprises seven classes: *Bacilli*, *Clostridia*, *Culicoidibacteria*, *Erysipelotrichia*, *Limnochordia*, *Negativicutes*, and *Thermolithobacteria* (Oren and Garrity, 2021). In this study, we collected several environmental samples from diverse habitats including soil, mud flat, tidal flat, wetland, and seawater in Korea and isolated various novel and unrecorded bacterial species. Consequently, we described 32 bacterial species belonging *Bacillota* phyla that have not been previously reported in Korea.

MATERIALS AND METHODS

Thirty-two bacterial species belonging to *Bacillota* were isolated from various environment niches including soil, tidal flat, mud, sediment, seawater, and wetland (Table 1). Treatment of environmental samples and bacterial isolation were handled independently in several laboratories. Environmental samples were transported to the laboratory and stored at 4°C until inoculation. All environmental samples were subsequently diluted, and spread on various culture media including R2A agar (BD; USA), marine agar 2216 (MA; BD), tryptic soy agar (TSA; BD) and incubated at 25–37°C for 2–5 days (Table 1). We used appropriate media for the condition of each environmental sample. Additionally, we further used marine agar media for some wetland and agricultural soil samples to isolate salt-tolerant bacterial strains. The designated strain identifications (IDs), most closely related species, isolation sources, culture media, and incubation conditions are described in Table 1. All pure culture strains were maintained as 10–20% glycerol suspension at –80°C and as lyophilized ampoules. The 16S rRNA gene sequences of the strains were aligned with those of the related type strains of validly published bacterial species collected from the EzBioCloud (<https://www.ezbiocloud.net/>) (Yoon *et al.*, 2017). Multiple alignments of the 16S rRNA gene sequences of the strains and those of the related type strains were performed using the CLUSTALX program (Larkin *et al.*, 2007) for the phylogenetic analysis. Phylogenetic trees were reconstructed by neighbour-joining

(NJ), maximum-parsimony (MP), and maximum-likelihood (ML) method (Fitch, 1971; Felsenstein, 1981; Saitou and Nei, 1987) using the MEGA X program (Kumar *et al.*, 2018). The Jukes-Cantor model for NJ trees, Minimum heuristic algorithm for MP trees, and Tamura-Nei model for ML trees were used to reconstruct phylogenetic trees (Jukes and Cantor, 1969; Tamura and Nei, 1993; Nei, 2000). Tree topologies were evaluated on the basis of bootstrap analysis of 1000 replicates (Felsenstein, 1985). Colony morphology of the strains was observed by eye or a magnifying glass after the isolates were cultivated to their exponential growth phases on their culture agar media. Cellular morphologies and sizes were examined by using either transmission electron or scanning electron microscopy. Gram staining was performed using a Gram-staining kit according to the manufacturer's standard procedures. Biochemical characteristics were evaluated using the API 20NE kit (bioMérieux, France), according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Thirty-two strains belonging to the phylum *Bacillota* were classified in an order; 32 strains in the *Bacillales*. In the phylum of *Bacillota*, one strain was assigned to the genus *Staphylococcus* of the family *Staphylococcaceae* and twenty one strains in the family *Bacillaceae* belonged to 18 different Genera: *Bacillus* (2 strains), *Cytobacillus* (1 strain), *Exiguobacterium* (1 strain), *Gottfriedia* (1 strain), *Gracilibacillus* (1 strain), *Halobacillus* (1 strain), *Jeotgalibacillus* (1 strain), *Mesobacillus* (1 strain), *Metabacillus* (1 strain), *Neobacillus* (1 strain), *Ornithinibacillus* (1 strain), *Peribacillus* (1 strain), *Pontibacillus* (1 strain), *Pseudoneobacillus* (1 strain), *Robertmurraya* (1 strain), *Siminovitchia* (2 strains), *Sutcliffeiella* (2 strains), and *Virgibacillus* (1 strain). Ten strains in the family *Paenibacillaceae* were separated into three genera: *Brevibacillus* (1 strain), *Fontibacillus* (1 strain), and *Paenibacillus* (8 strains) (Fig. 2). The 32 strains belonging to *Bacillota* were isolated from various environmental habitats, including tidal flat, mud, sediment, seawater, and wetland, which can be attributed to their diverse physiological and biochemical characteristics. All strains were gram-staining negative, and cells were rods or cocci-shaped (Fig. 1). Other biochemical and physiological characteristics are described below. In conclusion, in this study, we reported 32 unrecorded bacterial species belonging to bacterial phyla *Bacillota* which was isolated in Korea.

Description of *Bacillus pumilus* SBCC-2286

Cells are Gram-staining positive and rods with flagella. Colonies are circular to irregular, flat cream colored after

Table 1. Summary of strains isolated belonging to the phylum *Bacillota* and their taxonomic affiliations.

Phylum	Order	Family	Genus	Strain ID	GenBank accession number	NIRB ID / GenBank accession number	Most closely related species	Similarity (%)	Isolation source	Medium [†]	Incubation conditions
<i>Bacillota</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	SBCC-2286	NIBRBAC000510511 / PV156053	NIBRBAC000510511 / PV156053	<i>Bacillus pumilus</i>	100.00	Tidal flat	MA	25°C, 3-5d
				US27	NIBRBAC000510636 / OR251357	NIBRBAC000510636 / OR251357	<i>Bacillus sonorensis</i>	99.86	Wetland	R2A	30°C, 2d
			<i>Cytobacillus</i>	AN479	NIBRBAC000510607 / OR527143	NIBRBAC000510607 / OR527143	<i>Cytobacillus horneckiae</i>	99.86	Wetland	R2A	28°C, 3d
			<i>Exiguobacterium</i>	US12	NIBRBAC000510635 / OR251353	NIBRBAC000510635 / OR251353	<i>Exiguobacterium undae</i>	99.60	Wetland	R2A	30°C, 3d
			<i>Gottfriedia</i>	GM6	NIBRBAC000510645 / OR251348	NIBRBAC000510645 / OR251348	<i>Gottfriedia acidiceris</i>	99.79	Soil	R2A	30°C, 3d
			<i>Gracilibacillus</i>	CAU 1725	NIBRBAC000510782 / OR708690	NIBRBAC000510782 / OR708690	<i>Gracilibacillus halotolerans</i>	98.79	Mud	MA	30°C, 3d
			<i>Halobacillus</i>	CAU 1720	NIBRBAC000510777 / OR708684	NIBRBAC000510777 / OR708684	<i>Halobacillus andaensis</i>	99.65	Mud flat	MA	30°C, 3d
			<i>Jeotgalibacillus</i>	MMS23-YR12	NIBRBAC000510826 / OR787593	NIBRBAC000510826 / OR787593	<i>Jeotgalibacillus alkaliphilus</i>	99.86	Soil	R2A	30°C, 3d
			<i>Mesobacillus</i>	CAU 1751	NIBRBAC000510787 / OR770161	NIBRBAC000510787 / OR770161	<i>Mesobacillus harenae</i>	100.00	Mud flat	MA	30°C, 3d
			<i>Metabacillus</i>	SBCC-1704	NIBRBAC000510513 / PV156054	NIBRBAC000510513 / PV156054	<i>Metabacillus sediminilitoris</i>	99.86	Tidal flat	MA	25°C, 3d
			<i>Neobacillus</i>	DHW12	NIBRBAC000510806 / OR751824	NIBRBAC000510806 / OR751824	<i>Neobacillus massiliamazoniensis</i>	98.85	Soil	R2A	28°C, 3d
			<i>Ornithinibacillus</i>	MBLB2969	NIBRBAC000510680 / PQ496898	NIBRBAC000510680 / PQ496898	<i>Ornithinibacillus bavariensis</i>	99.42	Mud flat	TSA	30°C, 3d
			<i>Peribacillus</i>	SBCC-2316	NIBRBAC000510512 / PV156057	NIBRBAC000510512 / PV156057	<i>Peribacillus huizhouensis</i>	99.86	Tidal flat	MA	25°C, 3d
			<i>Pontibacillus</i>	CAU 1722	NIBRBAC000510779 / OR708687	NIBRBAC000510779 / OR708687	<i>Pontibacillus littoralis</i>	99.79	Mud	MA	30°C, 3d
			<i>Pseudoneobacillus</i>	SBCC-1576	NIBRBAC000510517	NIBRBAC000510517	<i>Pseudoneobacillus rhizosphaerae</i>	99.39	Tidal flat	MA	25°C, 5d
			<i>Robertmurraya</i>	MBLB3707	NIBRBAC000510683 / PQ510306	NIBRBAC000510683 / PQ510306	<i>Robertmurraya siralis</i>	99.63	Soil	MA	30°C, 3d
			<i>Siminovitchia</i>	CAU 1715	NIBRBAC000510774 / OR708679	NIBRBAC000510774 / OR708679	<i>Siminovitchia acidifaciens</i>	100.00	Mud flat	MA	30°C, 3d
				CAU 1724	NIBRBAC000510781 / OR708688	NIBRBAC000510781 / OR708688	<i>Siminovitchia composti</i>	100.00	Mud	MA	30°C, 3d
			<i>Sutcliffeella</i>	CAU 1717	NIBRBAC000510775 / OR708682	NIBRBAC000510775 / OR708682	<i>Sutcliffeella cohnii</i>	99.46	Mud flat	MA	30°C, 3d
				CAU 1748	NIBRBAC000510785 / OR770157	NIBRBAC000510785 / OR770157	<i>Sutcliffeella rhizosphaerae</i>	99.52	Wetland	MA	30°C, 3d
<i>Bacillota</i>	<i>Bacillales</i>	<i>Paenibacillaceae</i>	<i>Virgibacillus</i>	DCW25	NIBRBAC000510843 / OR751822	NIBRBAC000510843 / OR751822	<i>Virgibacillus indicus</i>	99.79	Mud flat	MA	28°C, 3d
			<i>Brevibacillus</i>	MBLB3826	NIBRBAC000510682 / PQ499573	NIBRBAC000510682 / PQ499573	<i>Brevibacillus agri</i>	99.55	Soil	MA	30°C, 3d
			<i>Fontibacillus</i>	J2W1	NIBRBAC000510848 / OR636890	NIBRBAC000510848 / OR636890	<i>Fontibacillus aquaticus</i>	98.84	Soil	R2A	28°C, 3d
				SBCC-1747	NIBRBAC000510515 / PV156733	NIBRBAC000510515 / PV156733	<i>Paenibacillus piscarius</i>	99.30	Tidal flat	MA	25°C, 5-7d
				16S3E7	NIBRBAC000510541 / OR717470	NIBRBAC000510541 / OR717470	<i>Paenibacillus agaridevorans</i>	98.78	Sediment	R2A	30°C, 2d
				AN637	NIBRBAC000510612 / OR527148	NIBRBAC000510612 / OR527148	<i>Paenibacillus rhizoryzae</i>	99.66	Wetland	R2A	28°C, 3d
				SU15	NIBRBAC000510754 / AB818664	NIBRBAC000510754 / AB818664	<i>Paenibacillus radialis</i>	98.91	Soil	MA	25°C, 3d
				AHW26	NIBRBAC000510803 / OR636871	NIBRBAC000510803 / OR636871	<i>Paenibacillus phytotum</i>	99.24	Soil	R2A	28°C, 3d
				MMS23-KR7	NIBRBAC000510824 / OR787591	NIBRBAC000510824 / OR787591	<i>Paenibacillus plantarum</i>	99.16	Soil	R2A	30°C, 3d
				DSW28	NIBRBAC000510839 / OR751825	NIBRBAC000510839 / OR751825	<i>Paenibacillus planticola</i>	98.74	Soil	R2A	28°C, 3d
<i>Bacillota</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Staphylococcus</i>	DCW4	NIBRBAC000510845 / OR751676	NIBRBAC000510845 / OR751676	<i>Paenibacillus aquistagni</i>	99.86	Mud flat	MA	28°C, 3d
				SBCC-2353	NIBRBAC000510524 / PV156056	NIBRBAC000510524 / PV156056	<i>Staphylococcus shiitii</i>	99.93	Tidal flat	MA	25°C, 3d

[†]Abbreviations: MA, marine agar; R2A, Reasoner's 2A

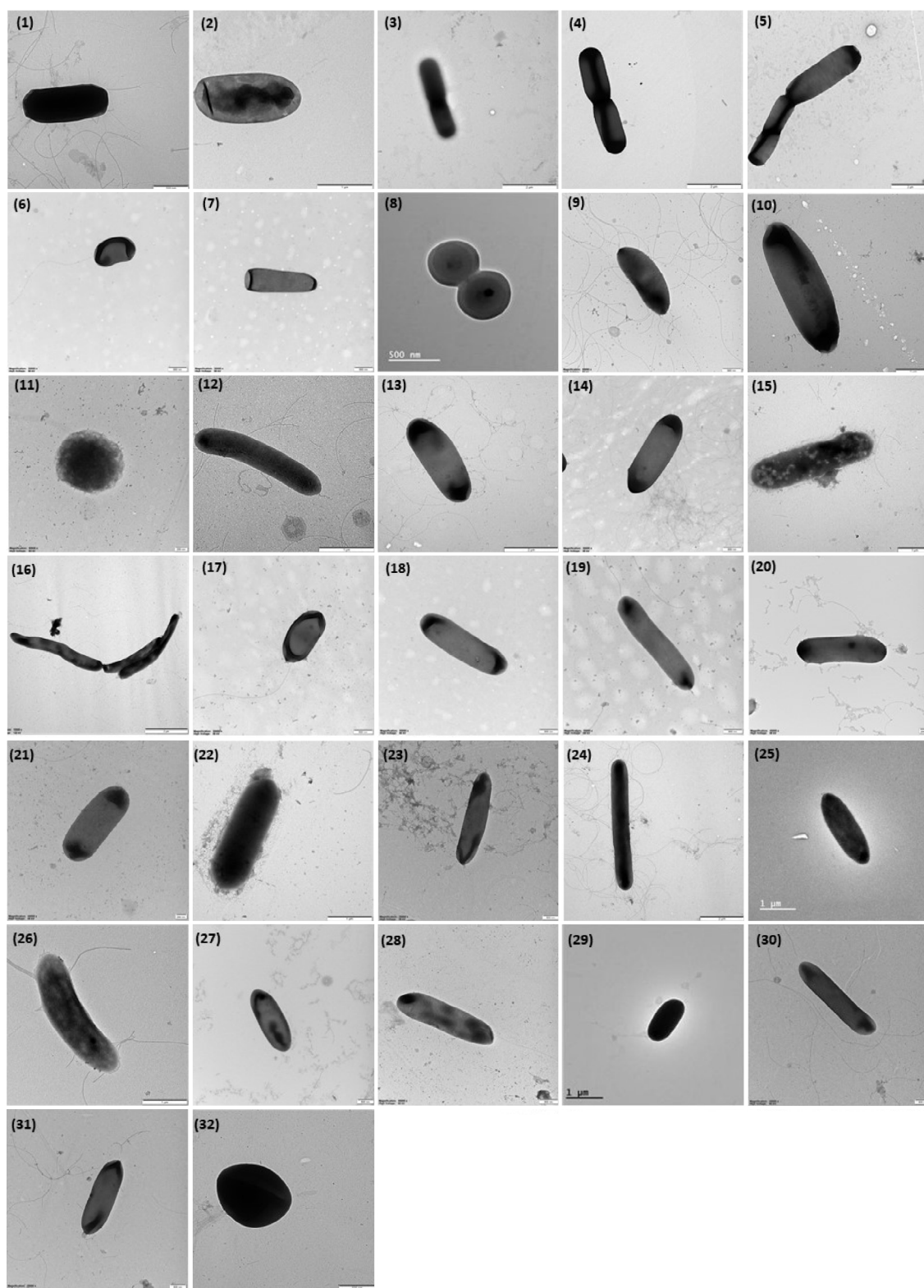


Fig. 1. Transmission electron micrographs showing the cell morphologies of bacterial strains isolated in this study. The strains were cultured at their optimal growth conditions. Strains: 1, SBCC-2286; 2, US27; 3, AN479; 4, US12; 5, GM6; 6, CAU 1725; 7, CAU 1720; 8, MMS23-YR12; 9, CAU 1751; 10, SBCC-1704; 11, DHW12; 12, MBLB2969; 13, SBCC-2316; 14, CAU 1722; 15, SBCC-1576; 16, MBLB3707; 17, CAU 1715; 18, CAU 1724; 19, CAU 1717; 20, CAU 1748; 21, DCW25; 22, MBLB3826; 23, J2W1; 24, SBCC-1747; 25, 16S3E7; 26, AN637; 27, SU15; 28, AHW26; 29, MMS23-KR7; 30, DSW28; 31, DCW4; 32, SBCC-2353.

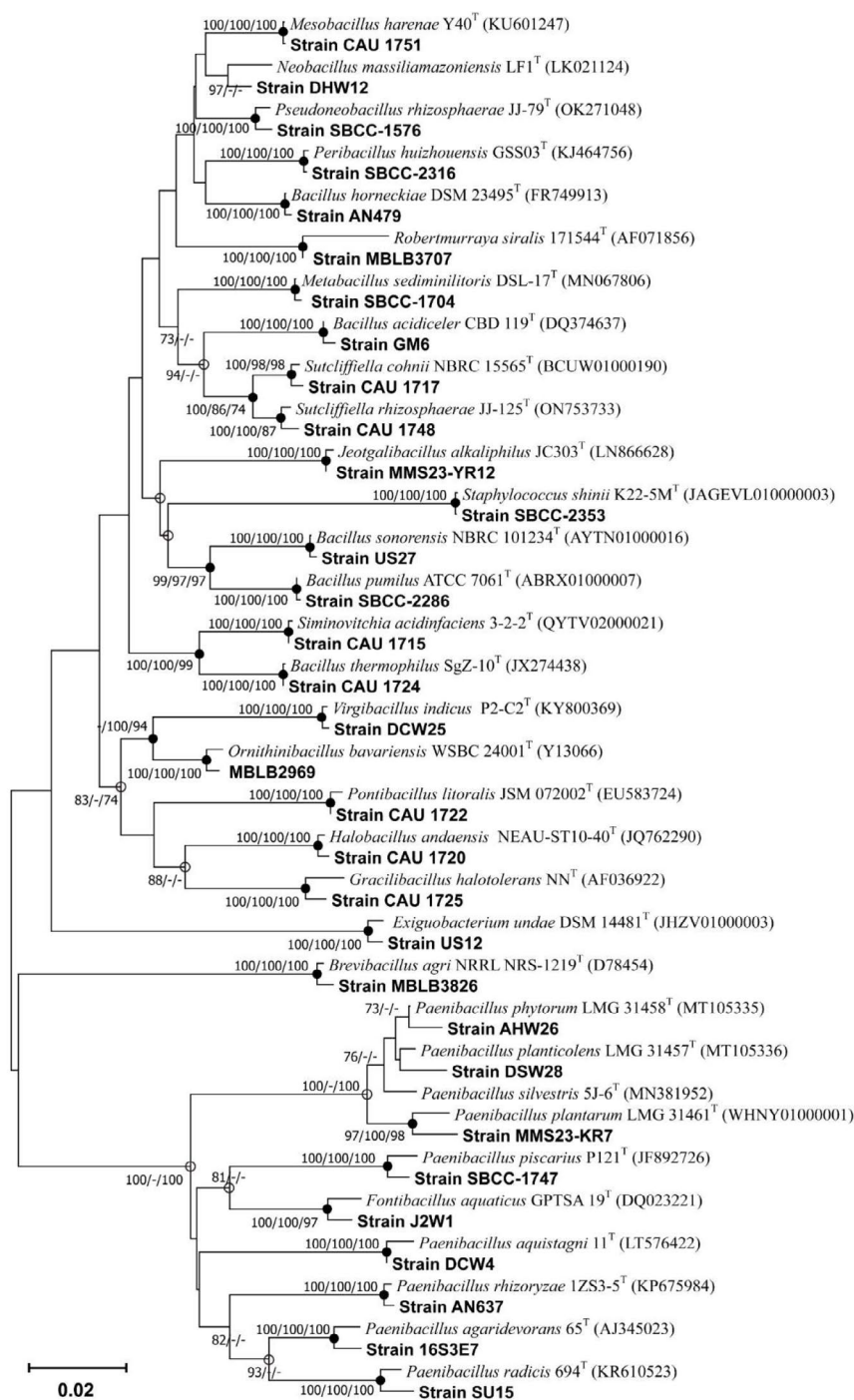


Fig. 2. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between bacterial strains isolated in this study and their relatives in the class *Bacilli* of the phylum *Bacillota*. Filled circles indicate that both maximum-likelihood and maximum-parsimony trees obtained the same node arrangement, while empty circles indicate either only maximum-likelihood or maximum-parsimony obtained the same corresponding nodes. Bootstrap values (> 70%) are shown on the nodes. Scale bar: 0.02 changes per nucleotide.

3–5 days of incubation at 25°C on MA. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activities. Positive for esculin hydrolysis, gelatinase, and β -galactosidase activity. Utilizes

D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid, and trisodium citrate. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain SBCC-2286 (NIBRBAC000510511)

was isolated from tidal flat from Taean, Chungcheongnam-do, Korea (36°53'17"N 126°19'39"E).

Description of *Bacillus sonorensis* US27

Cells are Gram-staining positive and short-rods without flagella. Colonies are pale yellow, irregular, raised after 2 days of incubation on R2A at 30°C. Positive for nitrate reduction, esculin hydrolysis, arginine dihydrolase, esculin hydrolysis and urease, gelatinase, and β -galactosidase activities. Negative for indole production, glucose fermentation, urease activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Does not utilize capric acid, adipic acid and phenylacetic acid. Strain US27 (NIBRBAC000510636) was isolated from wetland of Paju, Gyeonggi-do, Korea (37°44'29.6"N, 126°47'35.2"E).

Description of *Cytobacillus horneckiae* AN479

Cells are Gram-staining positive and rods without flagella. Colonies are cream, Round, entire, and raised after 2 days of incubation on R2A at 28°C. Positive for nitrate reduction and gelatinase activity. Negative indole production, glucose fermentation, arginine dihydrolase, urease activities, esculin hydrolysis, and β -galactosidase activity. Utilizes D-maltose, potassium gluconate, adipic acid, malic acid, trisodium, and citrate acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, capric acid, and phenylacetic acid. Strain AN479 (NIBRBAC000510607) was isolated from wetland of Anseong, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Exiguobacterium undae* US12

Cells are Gram-staining positive and rod-shaped with 1.1–1.2 μ m in diameter. Colonies are ivory, circular and raised after 3 days of incubation on R2A at 30°C. Positive for glucose fermentation, esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, and urease activities. Utilizes D-maltose only and 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 US12 (NIBRBAC000510635) was isolated from wetland of Paju, Gyeonggi-do, Korea (37°44'29.6"N, 126°47'35.2"E).

Description of *Gottfriedia acidiceris* GM6

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are yellow, circular, convex, and transparent after 3 days of incubation on R2A at 30°C.

Positive for esculin hydrolysis and gelatinase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β -galactosidase activity. 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 GM6 (NIBRBAC000510645) was isolated from soil of Paju, Gyeonggi-do, Korea (37°14'29.4"N 131°52'00.9"E).

Description of *Gracilibacillus halotolerans* CAU 1725

Cells are Gram-staining positive and short-rod with flagella. Colonies are cream, circular, smooth, entire, convex and translucent after 3 days of incubation on MA at 30°C. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. 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 CAU 1725 (NIBRBAC000510782) was isolated from wetland of Ansan, Gyeonggi-do, Korea (37°17'19.8"N 126°34'57.1"E).

Description of *Halobacillus andaensis* CAU 1720

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are cream, circular, smooth, entire, convex and opaques after 3 days of incubation on MA at 30°C. Positive for esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activity. 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 CAU 1720 (NIBRBAC000510777) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°14'31.0"N 126°33'38.4"E).

Description of *Jeotgalibacillus alkaliphilus* MMS23-YR12

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are ivory, circular, convex and entire after 3 days of incubation on R2A at 30°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, and β -galactosidase activities. Negative for indole production, arginine dihydrolase, and urease activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and phenylacetic acid. Does not utilize capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain MMS23-YR12 (NIBRBAC000510826) was

isolated from soil of Gangneung, Gangwon-do, Korea (37°47'53"N 128°53'49"E).

Description of *Mesobacillus harenae* CAU 1751

Cells are Gram-staining positive and short-rod shaped with flagella. Colonies are cream, circular smooth, convex, entire and opaques after 3 days of incubation on MA at 30°C. Positive for nitrate reduction. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, gelatinase, and β -galactosidase activities. Utilizes *N*-acetyl-glucosamine. 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 CAU 1751 (NIBRBAC000510787) was isolated from soil of Ansan, Gyeonggi-do, Korea (37°14'31.0"N 126°33'38.4"E).

Description of *Metabacillus sediminilitoris* SBCC-1704

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are light yellow, Irregular, flat, and glistening after 3 days of incubation on MA at 25°C. Positive for esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. 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 SBCC-1704 (NIBRBAC000510513) was isolated from tidal flat of Ansan, Gyeonggi-do, Korea (37°12'24"N 126°38'39"E).

Description of *Neobacillus massiliamazoniensis* DHW12

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are white, circular, entire convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for nitrate reduction, esculin hydrolysis, and β -galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activity, gelatinase, activities. 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 DHW12 (NIBRBAC000510806) was isolated from soil of Jeonbuk-do, Korea (35°41'29.4"N 126°32'48.8"E).

Description of *Ornithinibacillus bavariensis* MBLB2969

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, round, smooth, and flat after

3 days of incubation on TSA at 30°C. Positive for esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. 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 MBLB2969 (NIBRBAC000510680) was isolated from mud flat of Gyeongsangbuk-do, Korea (36°37'01.3"N 129°41'36.8"E).

Description of *Peribacillus huizhouensis* SBCC-2316

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circular to irregular, flat, smooth and glistening after 3 days of incubation on MA at 25°C. Positive for urease activity, esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and gelatinase activity. Utilize D-glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and trisodium citrate. Does not utilize L-arabinose, D-mannose, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain SBCC-2316 (NIBRBAC000510512) was isolated from tidal flat of Taean Chungchungnam-do, Korea (36°53'17"N 126°19'39"E).

Description of *Pontibacillus litoralis* CAU 1722

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, Circular, smooth, entire, convex, and opaques after 3 days of incubation on MA at 25°C. Positive for esculin hydrolysis and gelatinase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β -galactosidase activities. 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 CAU 1722 (NIBRBAC000510779) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°17'18.9"N 126°34'56.1"E).

Description of *Pseudoneobacillus rhizosphaerae* SBCC-1576

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circular, flat, smooth, and glistening after 5 days of incubation on MA at 25°C. Positive for nitrate reduction. Negative indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, gelatinase, and β -galactosidase activities. 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 SBCC-1576 (NI-

BRBAC000510517) was isolated from tidal flat of Taean Chungchungnam-do, Korea (36°50'29"N 126°11'24"E).

Description of *Robertmurraya siralis* MBLB3707

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circle, flat, and entire opaque after 3 days of incubation on MA at 30°C. Positive for urease activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and β -galactosidase activities. 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 MBLB 3707 (NIBRBAC000510683) was isolated from soil of Gangneung, Gangwon-do, Korea (37°48'14.9"N 128°54'23.4"E).

Description of *Siminovitchia acidinfaciens* CAU 1715

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, convex, and translucent after 3 days of incubation on MA at 30°C. Positive for urease activity, esculin hydrolysis, and gelatinase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and β -galactosidase activities. Utilizes D-glucose, L-arabinose, D-maltose, malic acid, and phenylacetic acid. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, and trisodium citrate. Strain CAU 1715 (NIBRBAC000510774) was isolated from soil of Gangneung, Gangwon-do, Korea (37°48'14.9"N 128°54'23.4"E).

Description of *Siminovitchia composti* CAU 1724

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, convex, and translucent after 3 days of incubation on MA at 30°C. Positive for nitrate reduction, esculin hydrolysis, and gelatinase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease and β -galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid. Does not utilize capric acid, trisodium citrate, and phenylacetic acid. Strain CAU 1724 (NIBRBAC000510781) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°17'19.8"N 126°34'57.1"E).

Description of *Sutcliffiella cohnii* CAU 1717

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, con-

vex, and translucent after 3 days of incubation on MA at 30°C. Positive for glucose fermentation, esculin hydrolysis, gelatinase, and β -galactosidase activities. Negative for nitrate reduction, indole production, arginine dihydrolase, urease activity. Utilizes *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain CAU 1717 (NIBRBAC000510775) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°14'31.3"N 126°33'38.2"E).

Description of *Sutcliffiella rhizosphaerae* CAU 1748

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, convex, and translucent after 3 days of incubation on MA at 30°C. Positive for esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activity. Utilizes D-glucose, D-mannitol, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, capric acid, adipic acid, and phenylacetic acid. Strain CAU 1748 (NIBRBAC000510785) was isolated from wetland of Jeongeup, Jeollabuk-do Korea (35°31'29.2"N 126°52'37.5"E).

Description of *Virgibacillus indicus* DCW25

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, entire convex, and smooth after 3 days of incubation on MA at 28°C. Positive for esculin hydrolysis, gelatinase, and β -galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activity. 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 DCW25 (NIBRBAC000510843) was isolated from mud flat of Ganghwa, Incheon, Korea (37°40'08.8"N 126°20'07.8"E).

Description of *Brevibacillus agri* MBLB3826

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, and flat after 3 days of incubation on MA at 30°C. Positive for nitrate reduction, and urease activity. Negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and β -galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid. Does not utilize D-mannose, capric acid, trisodium citrate, and phenylacetic acid. Strain MBLB

3826 (NIBRBAC000510682) was isolated from soil of Gangneung, Gangwon-do Korea (37°48'14.9"N 128°54'23.4"E).

Description of *Fontibacillus aquaticus* J2W1

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circle, entire convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activities. 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 J2W1 (NIBRBAC000510848) was isolated from soil of Yeongwol, Gangwon-do Korea (37°19'08.0"N 128°11'01.7"E).

Description of *Paenibacillus piscarius* SBCC-1747

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, flat, smooth, and glistening after 5–7 days of incubation on MA at 25°C. Positive for esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activities. Utilizes L-arabinose, D-mannose, *N*-acetyl-glucosamine, and potassium gluconate. Does not utilize D-glucose, D-mannitol, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain SBCC-1747 (NIBRBAC000510515) was isolated from tidal flat of Seocheon, Chungcheongnam-do, Korea (36°01'45"N 126°39'57"E).

Description of *Paenibacillus agaridevorans* 16S3E7

Cells are Gram-staining negative and rod-shaped without flagella. Colonies are white, Round with entire margins after 2 days of incubation on R2A at 30°C. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activities. Utilizes D-mannitol, *N*-acetyl-glucosamine, D-maltose, adipic acid, and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, potassium gluconate, capric acid, malic acid, and phenylacetic acid. Strain 16S3E7 (NIBRBAC000510541) was isolated from soil of Yeosu, Gyeonggi-do, Korea (37°17'23.51" N 127°39'5.92"E).

Description of *Paenibacillus rhizoryzae* AN637

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, Round, entire and convex after 3 days of incubation on R2A at 28°C. Positive for

esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activity. Utilizes D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain AN637 (NIBRBAC000510612) was isolated from wetland of Anseong, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Paenibacillus radialis* SU15

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle slightly convex, and glistening after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase and urease activity. Utilizes L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and D-maltose. Does not utilize D-glucose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain SU15 (NIBRBAC000510754) was isolated from soil of Nowon, Seoul, Korea (37°38'20.4"N 127°02'53.3"E).

Description of *Paenibacillus phytorum* AHW26

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circle undulate, convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for nitrate reduction, esculin hydrolysis, and β -galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase and urease activity. Utilizes D-glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and malic acid. Does not utilize L-arabinose, D-mannose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain AHW26 (NIBRBAC000510803) was isolated from soil of Pyeongchang, Gangwon-do, Korea (37°22'18.5"N 128°29'07.4"E).

Description of *Paenibacillus plantarum* MMS23-KR7

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are transparent, circular, convex, and entire after 3 days of incubation on R2A at 30°C. Positive for nitrate reduction, indole production, esculin hydrolysis, and β -galactosidase activity. Negative for glucose fermentation, arginine dihydrolase, gelatinase, and urease activity. Utilizes D-glucose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MMS23-KR7 (NIBRBAC000510824) was isolated from

soil of Gyeongju, Gyeongsangbuk-do, Korea (35°50'17"N 129°17'11"E).

Description of *Paenibacillus planticola* DSW28

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circular, entire convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for nitrate reduction, esculin hydrolysis, and β -galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activity. Utilizes D-glucose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DSW28 (NIBRBAC000510839) was isolated from soil of Jeju-do, Korea (33°31'25.3"N 126°37'10.6"E).

Description of *Paenibacillus aquistagni* DCW4

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circular, entire convex, and smooth after 3 days of incubation on MA at 28°C. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activity. Utilizes D-glucose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DCW4 (NIBRBAC000510845) was isolated from mud flat of Ganghwa, Incheon, Korea (37°40'08.8"N 126°20'07.8"E).

Description of *Staphylococcus shinii* SBCC-2353

Cells are Gram-staining positive and coccus-shaped without flagella. Colonies are milky white, Circular to slightly irregular, slightly convex, and smooth after 3 days of incubation on MA at 25°C. Positive for nitrate reduction, urease activity, and β -galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, and gelatinase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain SBCC-2353 (NIBRBAC000510524) was isolated from tidal flat of Seocheon, Chungcheongnam-do, Korea (36°01'45"N 126°39'57"E).

CONFLICTS OF INTEREST

These authors of this paper have no affiliation with

any interests and is solely responsible for the paper.

ACKNOWLEDGEMENTS

This work was supported by the National Institute of Biological Resources funded by the Ministry of Environment (No. NIBR202402203).

REFERENCES

- Dworkin, M., S. Falkow, E. Rosenberg, K.-H. Schleifer and E. Stackebrandt (eds). 2006. The prokaryotes: a handbook on the biology of bacteria (Vol. 1), 3rd edn. Springer-verlag, New York.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17:368-376.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39(4):783-791.
- Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Biology* 20(4):406-416.
- Gibbons, N. and R. Murray. 1978. Proposals concerning the higher taxa of bacteria. *International Journal of Systematic and Evolutionary Microbiology* 28(1):1-6.
- Jukes, T.H. and C.R. Cantor. 1969. Evolution of protein molecules. In: H.N. Munro (ed.), *Mammalian Protein Metabolism* (Vol. 3), Academic Press, New York. p. 21-132.
- Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6):1547-1549.
- Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23(21):2947-2948.
- Nei, M. 2000. *Molecular evolution and phylogenetics* (Vol. 333). Oxford University Press.
- Oren, A. and G.M. Garrity. 2021. Valid publication of the names of forty-two phyla of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology* 71(10).
- Qin, J., R. Li, J. Raes, M. Arumugam, K.S. Burgdorf, C. Manichanh, T. Nielsen, N. Pons, F. Levenez and T. Yamada. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464(7285):59-65.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4(4):406-425.
- Tamura, K. and M. Nei. 1993. Estimation of the number of

nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10(3):512-526.

Yoon, S.-H., S.-M. Ha, S. Kwon, J. Lim, Y. Kim, H. Seo and J. Chun. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and

whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology* 67(5):1613-1617.

Submitted: November 9, 2024

Revised: February 24, 2025

Accepted: March 12, 2025