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A report of 20 unrecorded bacterial species in Korea, isolated from soils of coastal areas in 2022

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To obtain unrecorded bacterial species in Korea, various soils of coastal areas were collected from the Republic of Korea in 2022. After plating the samples on marine agar and incubating aerobically and anaerobically, approximately 1,700 bacterial strains were isolated and identified using 16S rRNA gene sequences. A total of 20 strains showed ≥98.7% 16S rRNA gene sequence similarity with *v*alidly published bacterial species but not reported in Korea, indicating they are unrecorded bacterial species in Korea. The unrecorded bacterial strains belonged to four phyla, six classes, 15 orders, 16 families, and 19 genera which were assigned to *Blastomonas* and *Sphingomonas* of the class *Alphaproteobacteria*; *Pseudidiomarina*, *Kushneria*, *Salinicola*, and *Salinisphaera* of the class *Gammaproteobacteria*; *Evansella*, *Virgibacillus*, and *Paenibacillus* of the class *Bacilli*; *Cyclobacterium* of the class *Cytophagia*; *Pedobacter* of the class *Sphingobacteriia*; and *Demequina*, *Ornithinimicrobium*, *Blastococcus*, *Jatrophihabitans*, *Kineococcus*, *Glaciihabitans*, *Aeromicrobium* and *Streptomyces* of the class *Actinomycetes*. The details of the 20 unreported species, including Gram reaction, morphology, biochemical characteristics, and phylogenetic position are also provided in the description of the strains.

Keywords: 16S rRNA gene, coastal area, halophyte, tidal-flat sediment, unrecorded bacterial species

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Introduction

Coastal areas are located at the land-sea interface, characterized by strong dynamic interactions between tide and river runoff, and between surface water and groundwater (Yuan *et al*., 2011; Osland *et al*., 2015). Typically, a natural salinity gradient, oligohaline-mesohaline-hypersaline, is formed from tidal freshwater wetlands to salt marsh habitats.

At the cellular level, salinity leads to high internal ion concentrations that can become toxic, e.g., by modifying enzyme activities or even disrupting intracellular homeostasis (Van Horn *et al*., 2014). However, halophytes are generally known to have good immunity to environmental stresses such as salt, dryness, high temperature, and wind (Munns and Tester, 2008; Rodriguez *et al*., 2008; Yang *et al*., 2009). The physiology and metabolism of the halophytes are affected by the plant microbiome which includes plant growth-promoting rhizobacteria, ectomycorrhizal fungi, and pathogenic microorganisms (Smith *et al*., 2015; Upadhyay and Singh, 2015). The rhizosphere of halophytes harbors a variety of microorganisms that can promote plant growth by increasing the availability and uptake of carbon, nitrogen, and minerals from soil (Ruppel *et al*., 2013; Mukhtar *et al*., 2016; Khan *et al*., 2017). Global interest in halophytes having immunity to various environmental stresses and interactions with their microorganisms is increasing, but domestic research on this is insufficient. As one of the studies in the Republic of Korea, the research program of 'The Survey of Korean Indigenous Species,' has been conducted by the National Institute of Biological Resources (NIBR) since 2006. On going to the research program, many unrecorded prokaryotic species or new species have been discovered in diverse marine ecosystems on the Korean Peninsula (Cho *et al*., 2017; Joung *et al*., 2018; Jung *et al*., 2021). The Honam National Institute of Biological Resources (HNIBR) was established for biological surveys from coastal areas and launched a research program focusing on unrecorded species discovered therein. This study reported herein is

a part of the research program supported by HNIBR in 2022. We tried to isolate previously unrecorded bacterial species in various soil samples collected from coastal areas in Korea. Based on the 16S rRNA gene-based phylogenetic analyses herein, 20 bacterial strains assigned to the classes *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Cytophagia*, *Sphingobacteriia*, and *Actinomycetia* were identified as new records for bacterial species in Korea, for which taxonomic information and phenotypic characteristics are reported.

Materials and Methods

Various soils of coastal areas were collected from tidal flats in 2022. Details on the samples are presented in Table 1. Soil samples were suspended in distilled water and serially diluted. Rhizosphere soil samples were 5-g root samples vortexed for 1 min in 95 mL sterile distilled water and serially diluted. Using a spread plating technique on agar media, an aliquot (100 μL) of the soil and root samples was spread onto Marine agar (BD Diagnostics) and incubated at 25°C for 14 days. Bacterial strains were purified as single colonies and the pure cultures were preserved at −80°C in 20% (v/v) glycerol suspension, as well as lyophilized ampoules. Details on the strains are presented in Table 2.

For the determination of colony morphology, bacterial colonies that reached the stationary phase on agar plates were observed. Cellular morphology including cell shape, presence of flagella, and cell size were examined by transmission electron microscopy (CM200; Philips) after staining with 2% (w/v) uranyl acetate and a scanning electron microscope (S-4800; Hitachi). Gram staining was performed using a Gram-staining kit (bioMérieux). Catalase and oxidase activity were examined using 3% hydrogen peroxide and oxidase reagent (bioMérieux), respectively. Biochemical characteristics were tested using API 20E, API 50CH, and API ZYM (bioMérieux) according to the manufacturer's instructions.

For the determination of the phylogenetic position of the strains isolated herein, bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures as previously described (Yang and Cho, 2008). The 16S rRNA gene sequences were obtained using the primers 518F and 805R. The resultant 16S rRNA gene sequences were initially compared with those of other bacterial strains with validly published names using the EzBioCloud (Yoon *et al*., 2017) and NIBR database. The 16S rRNA gene sequence similarity of 98.7% was used as the cut-off value for bacterial species demarcation (Chun *et al*., 2018). Therefore, the bacterial strains exhibiting ≥98.7% 16S rRNA gene sequence similarities with validly published species, but never reported in Korea were determined as unreported bacterial species. For determining phylogenetic position, multiple sequence alignments between the 16S rRNA gene sequences of 30 strains and those of the unreported species were performed using Clustal W implemented in MEGA 11 (Kumar *et al*., 2021). Using the unambiguously aligned 16S rRNA gene sequences, phylogenetic trees based on the neighbor-joining method were reconstructed. The robustness of the inferred phylogenetic trees was evaluated by bootstrap analyses based on 1,000 random resamplings (Felsenstein, 1985).

Results and Discussion

The 16S rRNA gene sequence analyses using approximately 1,700 bacterial strains obtained herein revealed that many strains belonged to novel species or previously unreported species from Korea. Of these, a total of 20 strains showed ≥98.7% 16S rRNA gene sequence sim-

Table 1. Sampling site and sample type.

NO.	Sample name (scientific name)	Sampling site	Latitude and longitude
1	Rhizosphere soils of halophyte (*Salicornia europaea*)	Goha-do	34°45′50.69″N, 126°22′14.52″E
2	Rhizosphere soils of halophyte (*Salicornia europaea*)	Goha-do	34°45′39.60″N, 126°21′47.27″E
3	Tidal flat	Naenaro-do	34°31′35.80″N, 127°26′29.62″E
4	Tidal flat	Amtae-do	34°51′36.14″N, 126°8′38.62″E
5	Tidal flat	Amtae-do	34°51′32.08″N, 126°8′1.03″E
6	Rhizosphere soils of halophyte (*Calystegia soldanella*)	Anmyeon-do	36°33′38.25″N, 126°19′23.05″E
7	Rhizosphere soils of halophyte (*Argusia sibirica*)	Anmyeon-do	36°33′36.71″N, 126°19′23.87″E
8	Soils of saltern	Anmyeon-do	36°29′37.42″N, 126°23′12.65″E
9	Soils of saltern	Anmyeon-do	36°29′41.43″N, 126°23′11.47″E
10	Rhizosphere soils of halophyte (*Zoysia sinica* Hance)	Wando-gun	34°22′10.71″N, 126°38′39.22″E
11	Rhizosphere soils of halophyte (*Limonium tetragonum*)	Wando-gun	34°23′50.86″N, 126°41′38.29″E

Table 2. Summary of strains isolated and their taxonomic affiliations.

Class	Order	Family	Strain ID	Accession number	Closest species	Similarity (%)	Source
Alphaproteobacteria	*Sphingomonadales*	*Sphingomonadaceae*	HNIBRBA4475	OP601696	*Blastomonas marina*	98.89	Rhizosphere soil of halophyte
	Sphingomonadales	*Sphingomonadaceae*	HNIBRBA4483	OP602333	*Sphingomonas crusticola*	98.89	Rhizosphere soil of halophyte
Gammaproteobacteria	*Alteromonadales*	*Idiomarinaceae*	HNIBRBA4493	OP604082	*Pseudidiomarina atlantica*	99.38	Rhizosphere soil of halophyte
	Oceanospirillales	*Halomonadaceae*	HNIBRBA4476	OP601845	*Kushneria phyllosphaerae*	99.71	Rhizosphere soil of halophyte
	Oceanospirillales	*Halomonadaceae*	HNIBRBA4492	OP602342	*Salinicola endophyticus*	99.66	Rhizosphere soil of halophyte
	Salinisphaerales	*Salinisphaeraceae*	HNIBRBA4491	OP602341	*Salinisphaera shabanensis*	99.18	Rhizosphere soil of halophyte
Bacilli	*Bacillales*	*Bacillaceae*	HNIBRBA4480	OP602330	*Evansella clarkii*	99.87	Tidal flat
	Bacillales	*Bacillaceae*	HNIBRBA4479	OP602329	*Virgibacillus chiguensis*	99.86	Tidal flat
	Bacillales	*Paenibacillaceae*	HNIBRBA4487	OP602337	*Paenibacillus macquariensis*	98.74	Solar saltern soil
Cytophagia	*Cytophagales*	*Cyclobacteriaceae*	HNIBRBA4478	OP602328	*Cyclobacterium marinum*	100	Tidal flat
Sphingobacteriia	*Sphingobacteriales*	*Sphingobacteriaceae*	HNIBRBA4485	OP602335	*Pedobacter lithocola*	98.9	Rhizosphere soil of halophyte
Actinomycetia	*Demequinales*	*Demequinaceae*	HNIBRBA4490	OP602340	*Demequina salsinemoris*	99.31	Rhizosphere soil of halophyte
	Dermatophilales	*Ornithinimicrobiaceae*	HNIBRBA4488	OP602338	*Ornithinimicrobium cerasi*	98.75	Solar saltern soil
	Geodermatophilales	*Geodermatophilaceae*	HNIBRBA4486	OP602336	*Blastococcus saxobsidens*	98.98	Solar saltern soil
	Jatrophihabitantales	*Jatrophihabitantaceae*	HNIBRBA4482	OP602332	*Jatrophihabitans huperziae*	98.8	Rhizosphere soil of halophyte
	Kineosporiales	*Kineosporiaceae*	HNIBRBA4477	OP602327	*Kineococcus mangrovi*	99.2	Rhizosphere soil of halophyte
	Microbacteriales	*Microbacteriaceae*	HNIBRBA4481	OP602331	*Glaciihabitans tibetensis*	98.78	Rhizosphere soil of halophyte
	Microbacteriales	*Microbacteriaceae*	HNIBRBA4484	OP602334	*Glaciihabitans arcticus*	98.74	Rhizosphere soil of halophyte
	Propionibacteriales	*Nocardioidaceae*	HNIBRBA4489	OP602339	*Aeromicrobium piscarium*	99.21	Rhizosphere soil of halophyte
	Streptomycetales	*Streptomycetaceae*	HNIBRBA4494	OP602343	*Streptomyces somaliensis*	98.82	Rhizosphere soil of halophyte

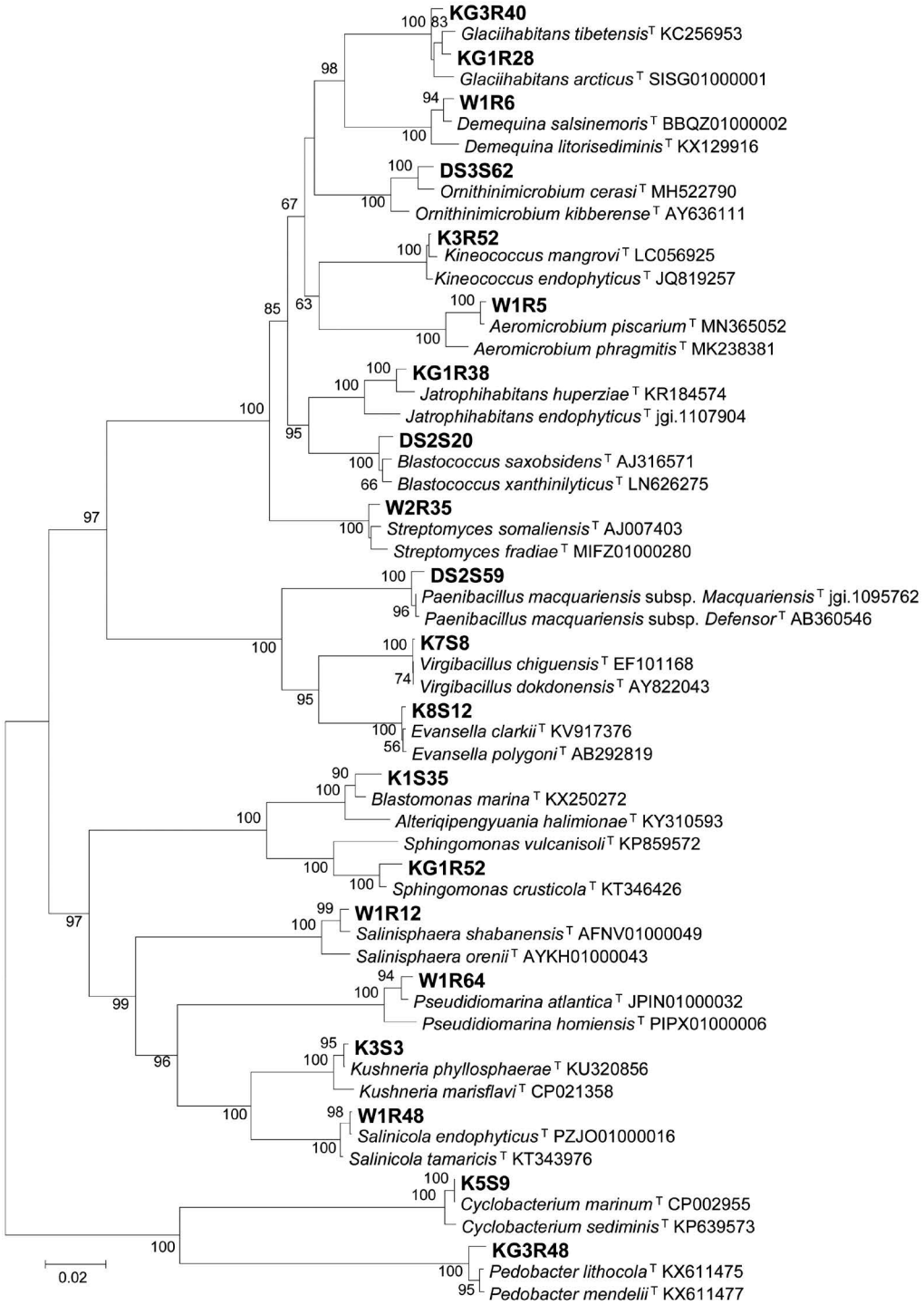


Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their closest bacterial species. Bootstrap values over 70% are shown. Scale bar = 0.02 substitutions per nucleotide position.

ilarities with unrecorded bacterial species in Korea. The strain information, identification, taxonomic assignment from species to classes, isolation source, and sequence accession numbers including HNIBR and GenBank are listed in Table 2. Phylogenetic position of the strains to established bacterial species based on 16S rRNA gene sequence similarity was confirmed by phylogenetic tree (Fig. 1). All strains identified as unrecorded species formed a robust clade with the type strains of each corresponding species with high bootstrap values.

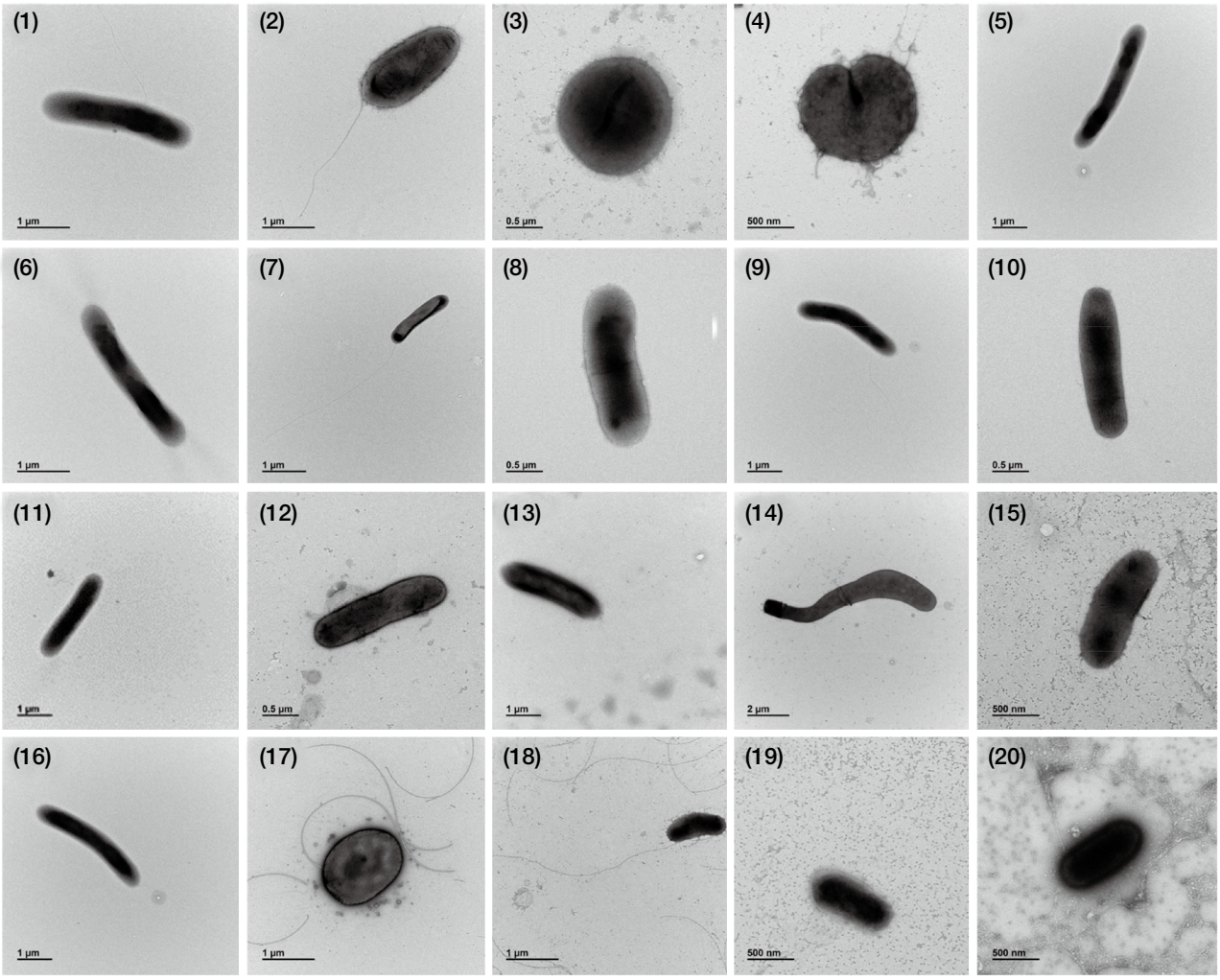


Fig. 2. Transmission electron micrographs of cells of the strain isolated in this study. Strains: 1, HNIBRBA4475; 2, HNIBRBA4476; 3, HNIBRBA4477; 4, HNIBRBA4478; 5, HNIBRBA4479; 6, HNIBRBA4480; 7, HNIBRBA4481; 8, HNIBRBA4482; 9, HNIBRBA4483; 10, HNIBRBA4484; 11, HNIBRBA4485; 12, HNIBRBA4486; 13, HNIBRBA4487; 14, HNIBRBA4488; 15, HNIBRBA4489; 16, HNIBRBA4490; 17, HNIBRBA4491; 18, HNIBRBA4492; 19, HNIBRBA4493; 20, HNIBRBA4494.

The 20 unrecorded bacterial species were phylogenetically diverse, belonging to four phyla, six classes, 15 orders, 16 families, and 19 genera (Table 2). At the generic level, the unreported species were assigned to *Blastomonas* and *Sphingomonas* of the class *Alphaproteobacteria*; *Pseudidiomarina*, *Kushneria*, *Salinicola*, and *Salinisphaera* of the class *Gammaproteobacteria*; *Evansella*, *Virgibacillus*, and *Paenibacillus* of the class *Bacilli*; *Cyclobacterium* of the class *Cytophagia*; *Pedobacter* of the class *Sphingobacteriia*; and *Demequina*, *Ornithinimicrobium*, *Blastococcus*, *Jatrophihabitans*, *Kineococcus*, *Glaciihabitans*, *Aeromicrobium* and *Streptomyces* of the class *Actinomycetia*.

The 20 unrecorded bacterial species identified in this study were Gram-staining-negative or positive, flagellated or non-flagellated, short- or straight-rod or coccoid- or spirillum-shaped bacteria (Fig. 2). Detailed morphological, physiological, and biochemical characteristics of the unrecorded bacterial species are given in the following strain descriptions.

Description of *Blastomonas marina* K1S35

Cells are Gram-stain-negative, aerobic, flagellated, and rod shaped. Colonies are circular, convex, entire, and yellow colored after incubation for 2 weeks on MA at 25°C. Positive for β-galactosidase, arginine dihydrolase, citrate utilization, indole production, and gelatinase; but negative for lysine decarboxylase, ornithine decarboxylase, H_2S production, urease, tryptophane deaminase, and acetoin production in API 20E. D-Glucose, L-rhamnose, D-melibiose, amygdalin, and L-arabinose are utilized for fermentation/oxidation; but not D-mannitol, inositol, D-sorbitol,

and D-sucrose. In the API 50CH test, positive for methyl-α D-mannopyranoside, esculine, xylitol, and potassium 5-ketogluconate. The API ZYM test is positive for alkaline phosphatase, esterase, lipase esterase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphtol-as-bi-phosphohydrolase, and *N*-acetyl-β-glucosaminidase. Strain K1S35 (=HNIBRBA4475) was isolated from rhizosphere soils of a halophyte (*Salicornia europaea*) collected from Goha-do (34°45′50.69″N, 126°22′14.52″E), Mokpo, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain K1S35 is OP601696.

Description of *Kushneria phyllosphaerae* K3S3

Cells are Gram-stain-negative, aerobic, flagellated, and rod shaped. Colonies are circular, raised, entire, and orange colored after incubation for 3 days on MA at 25°C. Positive for arginine dihydrolase and citrate utilization; but negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urease, tryptophane deaminase, indole production, acetoin production, and gelatinase in API 20E. D-Glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are not utilized for fermentation/oxidation. In the API 50CH test, positive for erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, *N*-acetylglucosamine, amygdalin, esculine, D-cellobiose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, glycogen, D-lyxose, D-fucose, and potassium 5-ketogluconate. The API ZYM test is positive for alkaline phosphatase, leucine arylamidase, α-chymotrypsin, acid phosphatase, naphtol-as-bi-phosphohydrolase, and β-glucosidase. Strain K3S3 (=HNIBRBA4476) was isolated from rhizosphere soils of a halophyte (*Salicornia europaea*) collected from Goha-do (34°45′39.6″N, 126°21′47.27″E), Mokpo, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain K3S3 is OP601845.

Description of *Kineococcus mangrovi* K3R52

Cells are Gram-stain-positive, aerobic, non-flagellated, and coccus shaped. Colonies are irregular, crateriform, undulate, and yellow colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase, arginine dihydrolase, and acetoin production; but negative for lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, indole production, and gelatinase in API 20E. L-Arabinose is utilized for fermentation/oxidation; but not D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, and amygdalin. In API 50CH test, positive for D-ribose, D-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, D-mannitol, arbutin, esculine, salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose, D-melezitose, glycogen, and potassium 5-ketogluconate. The API ZYM test is positive for alkaline phosphatase, esterase, leucine arylamidase, naphtol-as-bi-phosphohydrolase, β-glucuronidase, and β-glucosidase. Strain K3R52 (=HNIBRBA4477) was isolated from rhizosphere soils of a halophyte (*Salicornia europaea*) collected from Goha-do (34°45′39.60″N, 126°21′47.27″E), Mokpo, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain K3R52 is OP602327.

Description of *Cyclobacterium marinum* K5S9

Cells are Gram-stain-negative, aerobic, non-flagellated, and coccus shaped. Colonies are circular, convex, entire, and orange colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase and acetoin production; but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, indole production, and gelatinase in API 20E. D-Glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, and amygdalin, L-arabinose are utilized for fermentation/oxidation. In API 50CH test, positive for *N*-acetylGlucosamine, arbutin, esculine, D-saccharose, D-melezitose, D-raffinose, xylitol, D-lyxose, D-tagatose, D-fucose, and L-arabitol. The API ZYM test is positive for alkaline phosphatase, esterase, lipase esterase, leucine arylamidase, valine arylamidase, acid phosphatase, naphtol-as-bi-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, and α-mannosidase. Strain K5S9 (=HNIBR BA4478) was isolated from a tidal flat sample collected from Naenaro-do (34°31′35.80″N, 127°26′29.62″E), Goheung-gun, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain K5S9 is OP602328.

Description of *Virgibacillus chiguensis* K7S8

Cells are Gram-stain-positive, aerobic, flagellated, and rod shaped. Colonies are irregular, flat, undulate, and white colored after incubation for 3 days on MA at 25°C. Positive for citrate utilization, tryptophane deaminase, and gelatinase; but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urease, indole production, and acetoin production in API 20E. D-Glucose, D-mannitol, inositol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are utilized for fermentation/oxidation; but not D-sorbitol. Strain K7S8 (=HNIBRBA4479) was isolated from a tidal flat sample collected from Amtae-do (34°51′36.14″N, 126°8′38.62″E), Shinan, Jeollanam-do,

Korea. The GenBank accession number of the 16S rRNA gene sequence of strain K7S8 is OP602329.

Description of *Evansella clarkii* K8S12

Cells are Gram-stain-positive, aerobic, flagellated, and rod shaped. Colonies are circular, raised, entire, and white colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase, tryptophane deaminase, and acetoin production; but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, indole production, and gelatinase in API 20E. D-Glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are utilized for fermentation/oxidation. In the API 50CH test, positive for L-xylose, D-arabitol, L-arabitol, and potassium 5-ketogluconate. The API ZYM test is positive for alkaline phosphatase, esterase, lipase esterase, leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase, naphtol-as-bi-phosphohydrolase, and β-glucuronidase. Strain K8S12 (=HNIBRBA4480) was isolated from a tidal flat sample collected from Amtae-do (34°51′32.08″N, 126°8′1.03″E), Shinan, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain K8S12 is OP602330.

Description of *Glaciihabitans tibetensis* KG1R28

Cells are Gram-stain-positive, aerobic, flagellated, and rod shaped. Colonies are circular, raised, entire, and yellow colored after incubation for 3 days on MA at 25°C. Positive for arginine dihydrolase, acetoin production, and gelatinase; but negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, and indole production in API 20E. D-Glucose and L-arabinose are utilized for fermentation/oxidation; but not D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, and amygdalin. In the API 50CH test, positive for D-lactose and potassium 5-ketogluconate. In API ZYM, positive for esterase, lipase esterase, leucine arylamidase, naphtol-as-bi-phosphohydrolase, and α-glucosidase. Strain KG1R28 (=HNIBRBA4481) was isolated from rhizosphere soils of a halophyte (*Calystegia soldanella*) collected from Anmyeon-do (36°33′38.25″N, 126°19′23.05″E), Taean, Chungcheongnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain KG1R28 is OP602331.

Description of *Jatrophihabitans huperziae* KG1R38

Cells are Gram-stain-positive, aerobic, non-flagellated, and rod shaped. Colonies are circular, raised, entire, and brown colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase, arginine dihydrolase, acetoin production, and gelatinase; but negative for lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, and indole production in API 20E. D-Glucose, inositol, L-rhamnose, D-melibiose, and L-arabinose are utilized for fermentation/oxidation; but not D-mannitol, D-sorbitol, D-sucrose, and amygdalin. In the API 50CH test, positive for L-arabinose, methyl-α D-glucopyranoside, esculine, glycogen, and potassium 5-ketogluconate. The API ZYM test is positive for alkaline phosphatase, esterase, lipase esterase, leucine arylamidase, valine arylamidase, crystine arylamidase, acid phosphatase, naphtol-as-bi-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and α-mannosidase. Strain KG1R38 (=HNIBRBA4482) was isolated from rhizosphere soils of a halophyte (*Calystegia soldanella*) collected from Anmyeon-do (36°33′38.25″N, 126°19′23.05″E), Taean, Chungcheongnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain KG1R38 is OP602332.

Description of *Sphingomonas crusticola* KG1R52

Cells are Gram-stain-negative, aerobic, flagellated, and rod shaped. Colonies are circular, convex, entire, and yellow colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase and acetoin production; but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, indole production, and gelatinase in API 20E. D-Glucose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are utilized for fermentation/oxidation; but not D-mannitol, inositol, D-sorbitol, and L-rhamnose. In the API 50CH test, positive for L-arabinose, D-xylose, methyl-α D-glucopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, *N*-acetylglucosamine, amygdalin, arbutin, esculine, salicin, D-cellobiose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-melezitose, glycogen, xylitol, gentiobiose, D-lyxose, D-fucose, and potassium 5-ketogluconate. The API ZYM test is positive for alkaline phosphatase, esterase, lipase esterase, leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase, naphtol-as-bi-phosphohydrolase, α-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosaminidase. Strain KG1R52 (=HNIBRBA4483) was isolated from rhizosphere soils of a halophyte (*Calystegia soldanella*) collected from Anmyeon-do (36°33′38.25″N, 126°19′23.05″E), Taean, Chungcheongnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain KG1R52 is OP602333.

Description of *Glaciihabitans arcticus* KG3R40

Cells are Gram-stain-positive, aerobic, non-flagellated,

and rod shaped. Colonies are circular, flat, entire, and yellow colored after incubation for 3 days on MA at 25°C. Positive for tryptophane deaminase, indole production, acetoin production, and gelatinase; but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, and urease in API 20E. D-Glucose, D-mannitol, L-rhamnose, D-sucrose, amygdalin, and L-arabinose are utilized for fermentation/oxidation; but not inositol, D-sorbitol, and D-melibiose. In API 50CH test, positive for glycerol, L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-mannitol, esculine, salicin, D-cellobiose, D-maltose, D-saccharose, D-trehalose, amidon (starch), glycogen, D-turanose, D-lyxose, D-tagatose, potassium gluconate, and potassium 5-ketogluconate. The API ZYM test is positive for esterase, lipase esterase, leucine arylamidase, valine arylamidase, crystine arylamidase, naphtol-as-bi-phosphohydrolase, β-glucuronidase, α-glucosidase, and β-glucosidase. Strain KG3R40 (=HNIBRBA4484) was isolated from rhizosphere soils of a halophyte (*Argusia sibirica*) collected from Anmyeon-do (36°33′36.71″N, 126°19′23.87″E), Taean, Chungcheongnam-do, Korea. The Gen Bank accession number of the 16S rRNA gene sequence of strain KG3R40 is OP602334.

Description of *Pedobacter lithocola* KG3R48

Cells are Gram-stain-negative, aerobic, flagellated, and rod shaped. Colonies are circular, umbonate, entire, and orange colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase; but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, indole production, acetoin production, and gelatinase in API 20E. D-Glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are not utilized for fermentation/oxidation. Strain KG3R48 (=HNIBRBA4485) was isolated from rhizosphere soils of a halophyte (*Argusia sibirica*) collected from Anmyeon-do (36°33′36.71″N, 126°19′23.87″E), Taean, Chungcheongnam-do, Korea. The Gen Bank accession number of the 16S rRNA gene sequence of strain KG3R48 is OP602335.

Description of *Blastococcus saxobsidens* DS2S20

Cells are Gram-stain-positive, aerobic, non-flagellated, and rod shaped. Colonies are circular, umbonate, entire, and orange colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase, arginine dihydrolase, tryptophane deaminase, indole production, acetoin production, and gelatinase; but negative for lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, and urease in API 20E. D-Glucose, inositol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are utilized for fermentation/oxidation; but not D-mannitol, and D-sorbitol. In the API 50CH test, positive for esculine. The API ZYM test is positive for esterase, lipase esterase, leucine arylamidase, valine arylamidase, crystine arylamidase, naphtol-as-bi-phosphohydrolase, β-glucuronidase, α-glucosidase, and β-glucosidase. Strain DS2S20 (=HNIBRBA4486) was isolated from soils of a saltern collected from Anmyeon-do (36°29′37.42″N, 126°23′12.65″E), Taean, Chungcheongnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain DS2S20 is OP602336.

Description of *Paenibacillus macquariensis* DS2S59

Cells are Gram-stain-positive, aerobic, flagellated, and rod shaped. Colonies are circular, raised, entire, and yellow colored after incubation for 3 days on MA at 25°C. Positive for tryptophane deaminase and acetoin production; but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, indole production, and gelatinase in API 20E. D-Glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are not utilized for fermentation/oxidation. In API 50CH test, positive for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, D-adonitol, methyl-β D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, duloitol, inositol, D-mannitol, D-sorbitol, methyl-α D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, esculine, salicin, D-cellobiose, D-maltose, D-lactose, D-melibose, D-saccharose, inulin, D-melezitose, D-raffinose, amidon (starch), glycogen, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, and potassium 5-ketogluconate. The API ZYM test is positive for naphtol-as-bi-phosphohydrolase. Strain DS2S59 (=HNIBRBA4487) was isolated from soils of a saltern collected from Anmyeon-do (36°29′37.42″N, 126°23′12.65″E), Taean, Chungcheongnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain DS2S59 is OP602337.

Description of *Ornithinimicrobium cerasi* DS3S62

Cells are Gram-stain-positive, aerobic, non-flagellated, and spirillum shaped. Colonies are circular, convex, entire, and yellow colored after incubation for 10 days on MA at 25°C. Negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, indole production, acetoin production, and gelatinase in API 20E. Amygdalin is utilized for fermentation/oxidation; but not D-glucose, D-mannitol, inositol, D-

sorbitol, L-rhamnose, D-sucrose, D-melibiose, and L-arabinose. Strain DS3S62 (=HNIBRBA4488) was isolated from soils of a saltern collected from Anmyeon-do (36°29′41.43″N, 126°23′11.47″E), Taean, Chungcheongnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain DS3S62 is OP602338.

Description of *Aeromicrobium piscarium* W1R5

Cells are Gram-stain-positive, aerobic, non-flagellated, and rod shaped. Colonies are circular, convex, entire, and white colored after incubation for 3 days on MA at 25°C. Positive for acetoin production and gelatinase; but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, and indole production in API 20E. D-Glucose and D-sucrose are utilized for fermentation/oxidation; but not D-mannitol, inositol, D-sorbitol, L-rhamnose, D-melibiose, amygdalin, and L-arabinose. Strain W1R5 (=HNIBRBA4489) was isolated from rhizosphere soils of a halophyte (*Zoysia sinica* Hance) collected from Wando-gun (34°22′10.71″N, 126°38′39.22″E), Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain W1R5 is OP602339.

Description of *Demequina salsinemoris* W1R6

Cells are Gram-stain-positive, aerobic, flagellated, and rod shaped. Colonies are circular, umbonate, entire, and yellow colored after incubation for 3 days on MA at 25°C. Positive for β-galactosidase, arginine dihydrolase, indole production, and acetoin production; but negative for lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, and gelatinase in API 20E. D-Glucose, D-mannitol, D-sucrose, D-melibiose, amygdalin, and L-arabinose are utilized for fermentation/oxidation; but not inositol, D-sorbitol, and L-rhamnose. Strain W1R6 (=HNIBRBA4490) was isolated from rhizosphere soils of a halophyte (*Zoysia sinica* Hance) collected from Wando-gun (34°22′10.71″N, 126°38′39.22″E), Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain W1R6 is OP602340.

Description of *Salinisphaera shabanensis* W1R12

Cells are Gram-stain-negative, aerobic, flagellated, and coccus shaped. Colonies are circular, convex, entire, and white colored after incubation for 3 days on MA at 25°C. Positive for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, and tryptophane deaminase; but negative for β-galactosidase, H_2S production, urease, indole production, acetoin production, and gelatinase in API 20E. D-Glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are not utilized for fermentation/oxidation. Strain W1R12 (=HNIBRBA4491) was isolated from rhizosphere soils of a halophyte (*Zoysia sinica* Hance) collected from Wando-gun (34°22′10.71″N, 126°38′39.22″E), Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain W1R12 is OP602341.

Description of *Salinicola endophyticus* W1R48

Cells are Gram-stain-positive, aerobic, flagellated, and rod shaped. Colonies are circular, convex, entire, and brown colored after incubation for 3 days on MA at 25°C. Positive for arginine dihydrolase, ornithine decarboxylase, citrate utilization, tryptophane deaminase, indole production, and acetoin production; but negative for β-galactosidase, lysine decarboxylase, H_2S production, urease, and gelatinase in API 20E. D-Glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, amygdalin, and L-arabinose are utilized for fermentation/oxidation. Strain W1R48 (=HNIBRBA4492) was isolated from rhizosphere soils of a halophyte (*Zoysia sinica* Hance) collected from Wando-gun (34°22′10.71″N, 126°38′39.22″E), Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain W1R48 is OP602342.

Description of *Pseudidiomarina atlantica* W1R64

Cells are Gram-stain-negative, aerobic, non-flagellated, and rod shaped. Colonies are circular, convex, entire, and white colored after incubation for 3 days on MA at 25°C. Positive for gelatinase; but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophane deaminase, indole production, and acetoin production in API 20E. D-Mannitol and amygdalin are utilized for fermentation/oxidation; but not D-glucose, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, and L-arabinose. Strain W1R64 (=HNIBRBA4493) was isolated from rhizosphere soils of a halophyte (*Zoysia sinica* Hance) collected from Wando-gun (34°22′10.71″N, 126°38′39.22″E), Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain W1R64 is OP604082.

Description of *Streptomyces somaliensis* W2R35

Cells are Gram-stain-positive, aerobic, non-flagellated, and rod shaped. Colonies are circular, umbonate, entire, and brown colored after incubation for 3 days on MA at 25°C. Positive for citrate utilization; but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urease, trypto-

phane deaminase, indole production, acetoin production, and gelatinase in API 20E. L-Arabinose is utilized for fermentation/oxidation; but not D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-sucrose, D-melibiose, and amygdalin. Strain W2R35 (=HNIBRBA4494) was isolated from rhizosphere soils of a halophyte (*Limonium tetragonum*) collected from Wando-gun (34°23′50.86″N, 126°41′38.29″E), Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence of strain W2R35 is OP602343.

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