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A report of 31 unrecorded bacterial species belonging to the phylum *Actinomycetota* in Korea

Byeong Jun Choi[1], Jae Kyeong Lee[1], Jung Hoon Yoon[2], Chang Jun Cha[3], Wonyong Kim[4], Wan Taek Lim[5], Seung Bum Kim[6], Myung Kyum Kim[7], Dong Uk Kim[8], Myung Ji Seo[9] and Che Ok Jeon[1,*]

[1]*Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea*
[2]*Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea*
[3]*Department of Systems Biotechnology, Chung-Ang University, Anseong 17546, Republic of Korea*
[4]*Department of Microbiology, College of Medicine, Chung-Ang University, Seoul 06974, Republic of Korea*
[5]*Department of Biotechnology, Hankyong National University, Anseong 17579, Republic of Korea*
[6]*Department of Microbiology & Molecular Biology, Chung-Nam National University, Daejeon 34134, Republic of Korea*
[7]*Department of Bio & Environmental Technology, Seoul Women's University, Seoul 01797, Republic of Korea*
[8]*Department of Biological Science and Engineering, Sangji University, Wonju 26339, Republic of Korea*
[9]*Department of Department of Biotechnology, Incheon National University, Incheon 22012, Republic of Korea*

**Correspondent: cojeon@cau.ac.kr*

During a comprehensive investigation of indigenous prokaryotic species in Korea, a total of 31 bacterial strains assigned to the phylum *Actinomycetota* were isolated from diverse environmental habitats, including soil, mud, wetland, tidal field, and seawater. Based on their high 16S rRNA gene sequence similarities (>98.7%) and the formation of robust phylogenetic clades with the closest type species, each strain was assigned to an independent and predefined bacterial species. Because there have been no publications or official reports regarding 31 isolates in Korea, these 31 species of 12 families within seven orders of the phylum *Actinomycetota* are reported as unrecorded species in Korea. In addition, Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs are also described in the species description section.

Keywords: 16S rRNA, *Actinomycetota*, bacterial diversity, indigenous prokaryotic species in Korea, taxonomy, unrecorded species

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Introduction

The *Actinomycetota* is the largest bacterial phylum that was renamed from "*Actinobacteria*" in 2021 (Goodfellow, 2012; Oren and Garrity, 2021). Most species of the phylum *Actinomycetota* are found in diverse environments, and they are Gram-positive bacteria. At the time of writing, the taxonomic classification based on 16S rRNA gene sequences showed the phylum *Actinomycetota* includes six classes: *Acidimicrobiia*, *Actinomycetes*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria*, *Thermoleophilia* (Oren and Garrity, 2021). In 2023, we collected the number of environmental samples from diverse habitats in Korea and isolated various novel and unrecorded bacterial species. In this study, we describe 31 unrecorded bacterial species belonging to the phylum *Actinomycetota* that were not previously reported in Korea.

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Table 1. Summary of strains isolated belonging to the phyla *Actinomycetota* and their taxonomic affiliations.

Phylum	Class	Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Actinomycetota	*Actinomycetia*	*Microbacteriales*	*Microbacteriaceae*	*Microbacterium*	19H1F11	NIBRBAC000510546	*Microbacterium oryzae*	99.79	Soil	R2A	30°C, 2d
					19H7F20	NIBRBAC000510545	*Microbacterium petrolearium*	99.14	Soil	R2A	30°C, 2d
					19H7G11	NIBRBAC000510544	*Microbacterium sediminis*	99.52	Soil	R2A	30°C, 2d
					AN1007	NIBRBAC000510618	*Microbacterium kunmingense*	99.10	Wetland	R2A	28°C, 3d
					WC15	NIBRBAC000510792	*Microbacterium lindanitolerans*	100.00	Soil	R2A	28°C, 4d
				Agreia	B5	NIBRBAC000510738	*Agreia bicolorata*	99.02	Soil	R2A	25°C, 3d
				Leucobacter	17H1P12	NIBRBAC000510547	*Leucobacter iarius*	99.79	Soil	R2A	30°C, 2d
				Microcella	MMS23-DY36	NIBRBAC000510835	*Microcella alkalica*	100.00	Soil	R2A	30°C, 3d
			Nocardiaceae	*Rhodococcus*	17H2F15	NIBRBAC000510554	*Rhodococcus corynebacterioides*	99.65	Soil	R2A	30°C, 2d
					CAU 1749	NIBRBAC000510786	*Rhodococcus gannanensis*	98.81	Mud	GYE	30°C, 3d
				Prescottella	BU40	NIBRBAC000510755	*Prescottella soli*	99.86	Soil	R2A	25°C, 3d
			Mycobacteriaceae	*Mycobacterium*	AN126	NIBRBAC000510600	*Mycobacterium peregrinum*	100.00	Wetland	R2A	28°C, 3d
				Mycolicibacterium	KR3-105	NIBRBAC000510748	*Mycolicibacterium mucogenicum*	99.16	Soil	R2A	25°C, 3d
			Gordoniaceae	*Gordonia*	MBLB3299	NIBRBAC000510675	*Gordonia amicalis*	99.93	Soil	R2A	30°C, 3d
		Propionibacteriales	*Nocardioidaceae*	*Nocardioides*	KR1UV-15	NIBRBAC000510730	*Nocardioides baculatus*	99.30	Soil	R2A	25°C, 3d
					MBLB3548	NIBRBAC000510676	*Nocardioides luteus*	99.24	Soil	R2A	30°C, 3d
					WU27	NIBRBAC000510798	*Nocardioides soli*	98.96	Soil	R2A	28°C, 3d
					YJW22	NIBRBAC000510804	*Nocardioides cavernaquae*	99.72	Soil	R2A	28°C, 3d
				Aeromicrobium	KR3UV-104	NIBRBAC000510741	*Aeromicrobium lacus*	98.80	Soil	R2A	25°C, 3d
				Mumia	MBLB3055	NIBRBAC000510672	*Mumia xiangluensis*	98.83	Soil	MA	30°C, 3d
		Micrococcales	*Micrococcaceae*	*Arthrobacter*	ASW17	NIBRBAC000510800	*Arthrobacter ramosus*	99.63	Soil	R2A	28°C, 3d
					EW49	NIBRBAC000510788	*Arthrobacter woluwensis*	100.00	Soil	PTYG	28°C, 4d
					WP28	NIBRBAC000510796	*Arthrobacter mangrovi*	99.79	Soil	R2A	28°C, 3d
				Citricoccus	MBLB3007	NIBRBAC000510671	*Citricoccus zhacaiensis*	98.99	Soil	MA	30°C, 3d
				Paenarthrobacter	MMS23-GA13	NIBRBAC000510821	*Paenarthrobacter aurescens*	98.75	Soil	AIA	30°C, 3d
		Dermatophilales	*Dermacoccaceae*	*Flexivirga*	DG3-2	NIBRBAC000510733	*Flexivirga alba*	99.31	Soil	R2A	25°C, 3d
			Intrasporangiaceae	*Knoellia*	AN362	NIBRBAC000510605	*Knoellia subterranea*	98.76	Wetland	R2A	28°C, 3d
			Ornithinimicrobiaceae	*Ornithinimicrobium*	WE9	NIBRBAC000510846	*Ornithinimicrobium algicola*	99.22	Mud	MA	28°C, 3d
		Cellulomonadales	*Promicromonosporaceae*	*Isoptericola*	SBCC-2277	NIBRBAC000510510	*Isoptericola cucumis*	99.58	Tidal field	MA	25°C, 3−5d
		Brevibacteriales	*Brevibacteriaceae*	*Brevibacterium*	CAU 1727	NIBRBAC000510784	*Brevibacterium sanguinis*	99.59	Seawater	MA	30°C, 3d
	Thermoleophilia	*Solirubrobacterales*	*Solirubrobacteraceae*	*Solirubrobacter*	AN992	NIBRBAC000510617	*Solirubrobacter phytolaccae*	98.81	Wetland	R2A	28°C, 3d

Abbreviations of the agar medium: R2A, Reasoner's 2A; MA, Marine; AIA, Actinomycetota Isolation; PTYG, Peptone Tryptone Yeast extract Glucose; GYE, Glucose Yeast Extract.

Materials and Methods

A total of 31 bacterial strains assigned to the phylum *Actinomycetota* were isolated from various environmental habitats, including soil, mud, wetland, tidal field, and seawater (Table 1). All environmental samples were processed independently, serially diluted, and spread onto diverse culture agar media, including Reasoner's 2A agar (R2A, BD, USA), marine agar 2216 (MA; BD), *Actinomycetota* isolation agar (AIA; BD), peptone tryptone yeast extract glucose agar (PTYG; 0.25 g peptone, 0.25 g tryptone, 0.5 g yeast extract, 0.5 g glucose, and 0.03 g $MgSO_4$ in 1 liter) and glucose yeast extract agar (GYE; HIMEDIA, India) and incubated at 25−30°C for 2−5 days (Table 1). The designated strain identifications (IDs), most closely related species, isolation sources, culture media, and incubation conditions of the isolates are described in Table 1. All strains were isolated as pure cultures and stored as 10−20% glycerol suspension at −80°C and as lyophilized ampoules.

The colony morphology of the strains was observed by eye or a magnifying glass after the cells were cultivated to their exponential phases on their culture agar media. Cellular morphologies and cell sizes were examined by using either transmission electron or scanning electron microscopy. Gram staining tests were performed using a Gram-staining kit according to the standard procedures. Biochemical characteristics were evaluated using API 20NE kit (bioMérieux, France), according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures, as described previously (Baek *et al*., 2023). The 16S rRNA gene sequences of the strains assigned to the phylum *Actinomycetota* were compared with those of the type strains of validated bacterial species by using the nucleotide similarity search program in the EzBioCloud server (http://www.ezbiocloud.net/identify) (Yoon *et al*., 2017). For phylogenetic analyses, multiple alignments of the 16S rRNA gene sequences between the isolates and established bacterial species were carried out using the MUSCLE program (Edgar, 2004). Evolutionary distances were calculated using the Kimura two-parameter model and the phylogenetic trees were constructed using the MEGA11 program (Tamura *et al*., 2021), based on the neighbor-joining algorithm with 1,000 replications.

Results and Discussion

Thirty strains of *Actinomycetota* were distributed into two classes: 30 strains in the *Actinomycetia* and one strain in the *Thermoleophilia* (Table 1). One strain of the class *Thermoleophilia* was assigned to the genus *Solirubrobacter* of the family *Solirubrobacteraceae* in the order *Solirubrobacter*ales (Table 1). Thirty strains in the class of *Actinomycetia* belonged to six different orders: *Microbacteriales* (14 strains), *Propionibacteriales* (6 strains), *Micrococcales* (5 strains), *Dermatophilales* (3 strains), *Cellulomonadales* (1 strain), and *Brevibacteriales* (1 strain). Fourteen strains in the order *Microbacteriales* belonged to four different families: *Microbacteriaceae* (8 strains), *Nocardiaceae* (3 strains), *Mycobacteriaceae* (2 strains), and *Gordoniaceae* (1 strain). Eight strains in the family *Microbacteriaceae* belonged to four different genera: *Microbacterium* (5 strains), *Agreia* (1 strain), *Leucobacter* (1 strain), and *Microcella* (1 strain). Three strains in the family *Nocardiaceae* belonged to two different genera: *Rhodococcus* (2 strains) and *Prescottella* (1 strain). Two strains in the family *Mycobacteriaceae* belonged to two different genera: *Mycobacterium* (1 strain) and *Mycolicibacterium* (1 strain). One strain in the family *Gordoniaceae* was assigned to the genus *Gordonia*. Six strains in the order *Propionibacteriales* belonged to the family *Nocardioidaceae* (6 strains), and these 6 strains in the family *Nocardioidaceae* belonged to three different genera: *Nocardioides* (4 strains), *Aeromicrobium* (1 strain), and *Mumia* (1 strain). Five strains in the order *Micrococcales* belonged to the family *Micrococcaceae* (5 strains), and these five strains in the family *Micrococcaceae* belonged to three different genera: *Arthrobacter* (3 strains), *Citricoccus* (1 strain), and *Paenarthrobacter* (1 strain). Two strains of the order *Dermatophilales* belonged to three different families: *Dermacoccaceae* (1 strain), *Intrasporangiaceae* (1 strain), and *Ornithinimicrobiaceae* (1 strain). One strain in the family *Dermacoccaceae* was assigned to the genus *Flexivirga*, one strain in the family *Intrasporangiaceae* was assigned to the genus *Knoellia*, and the other strain in the family *Ornithinimicrobiaceae* was assigned to the genus *Ornithinimicrobium*. One strain in the order *Cellulomonadales* was assigned to the genus *Isoptericola* of the family *Promicromonosporaceae*. One strain in the order *Brevibacteriales* was assigned to the genus *Brevibacterium* of the family *Brevibacteriaceae* (Fig. 2). In conclusion, we report 31 unrecorded bacterial species belonging to the phylum *Actinomycetota* which were isolated in Korea.

Description of *Microbacterium oryzae* 19H1F11

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, smooth, and yellow-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urase activities. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain 19H1F11

(=NIBRBAC000510546) was isolated from soil in Hanam, Gyeonggi-do, Korea.

Description of *Microbacterium petrolearium* 19H7F20

Cells are Gram-stain-positive, motile, and rod-shaped with flagellum. Colonies are circular and white-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, and potassium gluconate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urase, and gelatinase activities. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 19H7F20 (=NIBRBAC000510545) was isolated from soil in Goyang, Gyeonggi-do, Korea.

Description of *Microbacterium sediminis* 19H7G11

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, convex, and white-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, and malic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urase, and gelatinase activities. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain 19H1F11 (=NIBRBAC000510544) was isolated from soil in Goyang, Gyeonggi-do, Korea.

Description of *Microbacterium kunmingense* AN1007

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, entire, raised, and pale yellow-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis and utilize D-mannose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain AN1007 (=NIBRBAC000510618) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Description of *Microbacterium lindanitolerans* WC15

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, entire, smooth, and yellow-colored after four days of incubation at 28°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activities. Negative for 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 WC15 (=NIBRBAC000510792) was isolated from soil in Chuncheon, Gangwon-do, Korea.

Description of *Flexivirga alba* DG3-2

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, glistening, and cream yellow-colored after three days of incubation at 25°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DG3-2 (=NIBRBAC000510733) was isolated from soil in Daegu, Korea.

Description of *Agreia bicolorata* B5

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, glistening, and cream yellow-colored after three days of incubation at 25°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannitol, and D-maltose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain B5 (=NIBRBAC000510738) was isolated from soil in Seoul, Korea.

Description of *Leucobacter iarius* 17H1P12

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are smooth, convex, opaque, and white-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activity. Utilizes *N*-acetyl-glucosamine, D-maltose, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation arginine dihydrolase, urease, and gelatinase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain 17H1P12 (=NIBRBAC000510547) was isolated from soil in Hanam, Gyeonggi-do, Korea.

Description of *Microcella alkalica* MMS23-DY36

Cells are Gram-stain-negative, rod-shaped without flagellum. Colonies are circular, smooth, glistening, and yellow-

colored after three days of incubation at 30°C on R2A agar. Positive for nitrate reduction, glucose fermentation, and esculin hydrolysis. Negative for indole production, arginine dihydrolase, urease, 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 MMS23-DY36 (=NIBRBAC 000510835) was isolated from soil in Incheon, Korea.

Description of *Rhodococcus corynebacterioides* 17H2F15

Cells are Gram-stain-negative, rod-shaped without flagellum. Colonies are circular, convex, translucent, and light cream-colored after two days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis and β-galactosidase activitiy. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, adipic acid, malic acid, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-mannose, D-maltose, capric acid, and phenylacetic acid. Strain 17H2F15 (=NIBRBAC000510554) was isolated from soil in Guri, Gyeonggi-do, Korea.

Description of *Rhodococcus gannanensis* CAU 1749

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, rought, entire, pulvinate, opaques, and cream-colored after three days of incubation at 30°C on GYE. Positive for nitrate reduction. Negative for indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, 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 CAU 1749 (=NIBRBAC000510786) was isolated from mud in Jeongeup, Jeollabuk-do, Korea.

Description of *Prescottella soli* BU40

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, mucoid, and pale orange-colored after three days of incubation at 25°C on R2A agar. Positive esculin hydrolysis and utilizes D-glucose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize 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 BU40 (=NIBRBAC000510755) was isolated from soil in Seoul, Korea.

Description of *Mycobacterium peregrinum* AN126

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, entire, flat, and white-colored after three days of incubation at 28°C on R2A agar. Positive for nitrate reduction and utilizes D-glucose, D-mannose, D-mannitol, potassium gluconate, and malic acid. Negative for indole production, glucose fermentation, esculin hydrolysis, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize L-arabinose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain AN126 (=NIBR BAC000510600) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Description of *Mycolicibacterium mucogenicum* KR3-105

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, and pale orange-colored after three days of incubation at 25°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, urease, gelatinase, and β-galactosidase activities. Negative for indole production, glucose fermentation, and arginine dihydrolase 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 KR3-105 (=NIBRBAC000510748) was isolated from soil in Gangneung, Gangwon-do, Korea.

Description of *Gordonia amicalis* MBLB3299

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are irregular, convex, translucent, and orange-colored after three days of incubation at 30°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, urease, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannitol, and D-maltose. Negative for indole production, glucose fermentation, arginine dihydrolase, and gelatinase activity. Does not utilize D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MBLB3299 (=NIBRBAC000510675) was isolated from soil in Incheon, Korea.

Description of *Nocardioides baculatus* KR1UV-15

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, glistening, and cream-colored after three days of incubation at 25°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, gelatinase, and β-galactosidase activities. Negative for 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 KR1UV-15 (=NIBRBAC000510730) was isolated from soil in Gangneung, Gangwon-do, Korea.

Description of *Nocardioides luteus* MBLB3548

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, flat, opaque, and cream-colored after three days of incubation at 30°C on R2A agar. Positive for esculin hydrolysis, urease, and β-galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, 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 MBLB 3548 (=NIBRBAC000510676) was isolated from soil in Incheon, Korea.

Description of *Nocardioides soli* WU27

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and cream-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-mannitol and D-maltose. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain WU27 (=NIBRBAC000510798) was isolated from soil in Sunchang, Jeollabuk-do, Korea.

Description of *Nocardioides cavernaquae* YJW22

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and white-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, 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 YJW22 (=NIBRBAC000510804) was isolated from soil in Yeongwol, Gangwon-do, Korea.

Description of *Aeromicrobium lacus* KR3UV-104

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, convex, glistening, and cream-colored after three days of incubation at 25°C on R2A agar. Positive for indole production, esculin hydrolysis, and gelatinase activity. Utilizes D-glucose, D-mannitol, and malic acid. Negative for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase activities. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain KR3UV-104 (=NIBRBAC000510741) was isolated from soil in Gangneung, Gangwon-do, Korea.

Description of *Mumia xiangluensis* MBLB3055

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire margine, raised, and cream-colored after three days of incubation at 30°C on MA. 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 MBLB 3055 (=NIBRBAC000510672) was isolated from soil in Ulleung, Gyeongsangbuk-do, Korea.

Description of *Arthrobacter ramosus* ASW17

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and white-colored after three days of incubation at 28°C on R2A agar. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize capric acid and adipic acid. Strain ASW17 (=NIBRBAC000510800) was isolated from soil in Suncheon, Jeollanam-do, Korea.

Description of *Arthrobacter woluwensis* EW49

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are irregular, undulate, umbonate, smooth, and white-colored after four days of incubation at 28°C on PTYG. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize L-arabinose, capric acid, and adipic acid. Strain EW49 (=NIBRBAC000510788) was isolated from soil in Chuncheon, Gangwon-do, Korea.

Description of *Arthrobacter mangrovi* WP28

Cells are Gram-stain-positive, coccus-shaped without flagellum. Colonies are circular, entire, convex, smooth, and white-colored after three days of incubation at 28°C on R2A agar. Positive for nitrate reduction and esculin hydrolysis. Utilizes D-glucose, D-mannitol, malic acid, and phenylacetic acid. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, and trisodium citrate. Strain WP28 (=NIBRBAC000510796) was isolated from soil in Pyeongtaek, Gyeonggi-do, Korea.

Description of *Citricoccus zhacaiensis* MBLB3007

Cells are Gram-stain-positive, coccus-shaped without flagellum. Colonies are irregular, glistening, translucent, and yellow-colored after three days of incubation at 30°C on MA. Positive for nitrate reduction and esculin hydrolysis. Utilizes malic acid, trisodium citrate, and phenylacetic acid. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, 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, and adipic acid. Strain MBLB3007 (=NIBRBAC000510671) was isolated from soil in Ulleung, Gyeongsangbuk-do, Korea.

Description of *Paenarthrobacter aurescens* MMS23-GA13

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, convex, entire, and pale yellow-colored after three days of incubation at 30°C on AIA. Positive for indole production, glucose fermentation, esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, arginine dihydrolase, and urease activities. Does not utilize capric acid and adipic acid. Strain MMS23-GA13 (=NIBRBAC000510821) was isolated from soil in Muju, Jeollabuk-do, Korea.

Description of *Knoellia subterranea* AN362

Cells are Gram-stain-positive, oval-shaped without flagellum. Colonies are round, entire, raised, and yellow-colored after three days of incubation at 28°C on R2A agar. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activities. Utilizes D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, and potassium gluconate. Negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize L-arabinose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain AN362 (=NIBRBAC000510605) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Description of *Ornithinimicrobium algicola* WE9

Cells are Gram-stain-negative, rod-shaped without flagellum. Colonies are circular, entire, convex, smooth, and yellow-colored after three days of incubation at 28°C on MA. Positive for nitrate reduction, esculin hydrolysis, and gelatinase activity. Negative for 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 WE9 (=NIBRBAC000510846) was isolated from mud in Incheon, Korea.

Description of *Isoptericola cucumis* SBCC-2277

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, slightly convex, smooth, glistening, and cream-colored after 3−5 days of incubation at 25°C on MA. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, malic acid, and trisodium citrate. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activities. Does not utilize D-maltose, capric acid, adipic acid, and phenylacetic acid. Strain SBCC-2277 (=NIBRBAC000510510) was isolated from tidal field in Taean, Chungcheongnam-do, Korea.

Description of *Brevibacterium sanguinis* CAU 1727

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are circular, smooth, entire, convex, opaques, and cream-colored after three days of incubation at 30°C on MA. Positive for esculin hydrolysis, arginine dihydrolase, and gelatinase activity. Utilizes D-glucose, *N*-acetylglucosamine, D-maltose, potassium gluconate, malic acid, trisodium citrate, and phenylacetic acid. Negative for nitrate reduction, indole production, glucose fermentation, urease, and β-galactosidase activities. Does not utilize L-arabinose, D-mannose, D-mannitol, capric acid, and adipic acid. Strain CAU 1727 (=NIBRBAC000510784) was isolated from seawater in Busan, Korea.

Description of *Solirubrobacter phytolaccae* AN992

Cells are Gram-stain-positive, rod-shaped without flagellum. Colonies are round, entire, raised, and white-colored after three days of incubation at 28°C on R2A agar. Posi-

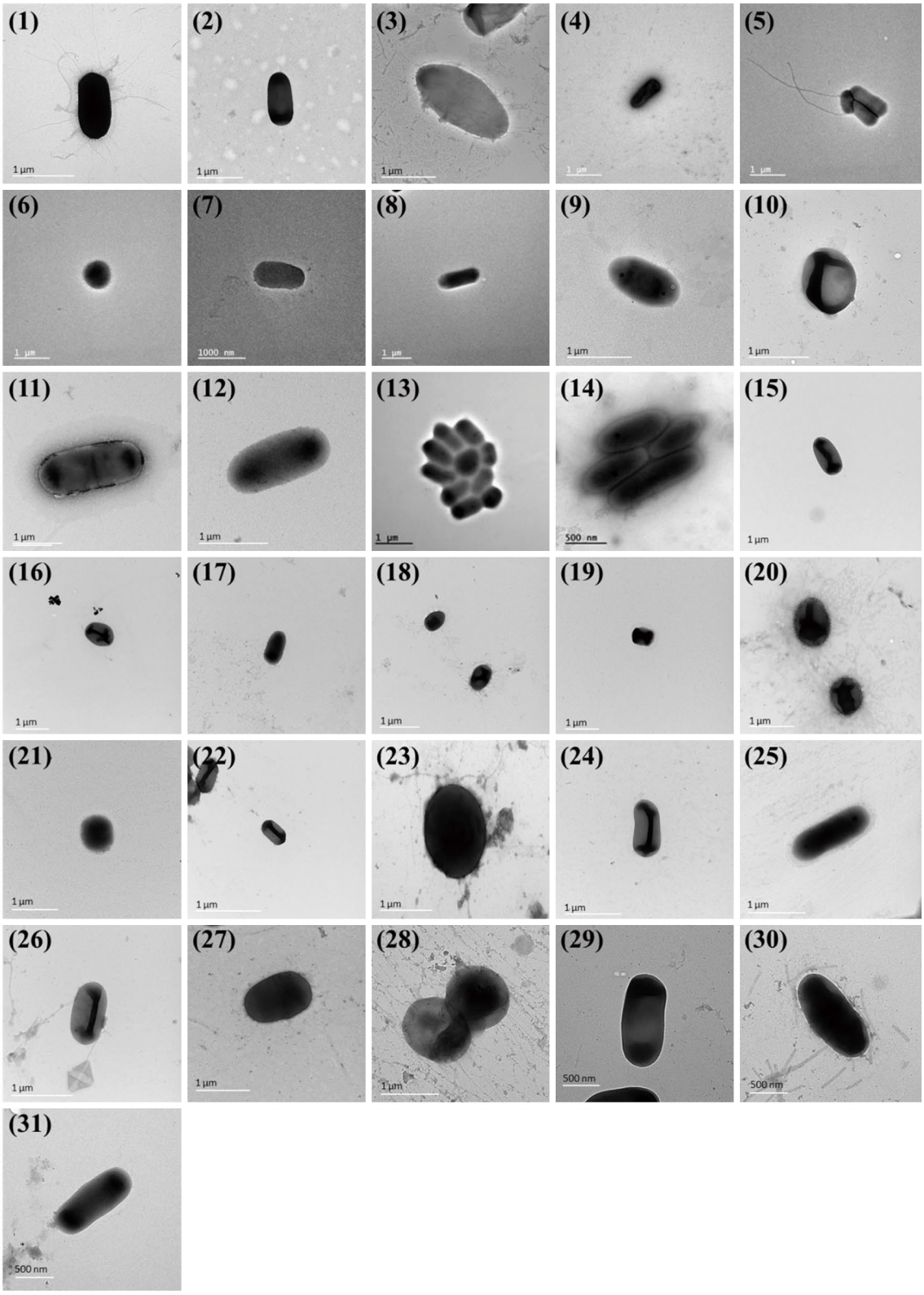

Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strain: 1, SBCC-2277; 2, CAU 1727; 3, CAU 1749; 4, 19H7G11; 5, 19H7F20; 6, 19H1F11; 7, 17H1P12; 8, 17H2F15; 9, AN126; 10, AN362; 11, AN992; 12, AN1007; 13, MMS23-GA13; 14, MMS23-DY36; 15, KR1UV-15; 16, DG3-2; 17, B5; 18, KR3UV-104; 19, KR3-105; 20, BU40; 21, EW49; 22, WC15; 23, WP28; 24, WU27; 25, ASW17; 26, YJW22; 27, WE9; 28, MBLB3007; 29, MBLB3055; 30, MBLB3299; 31, MBLB3548.

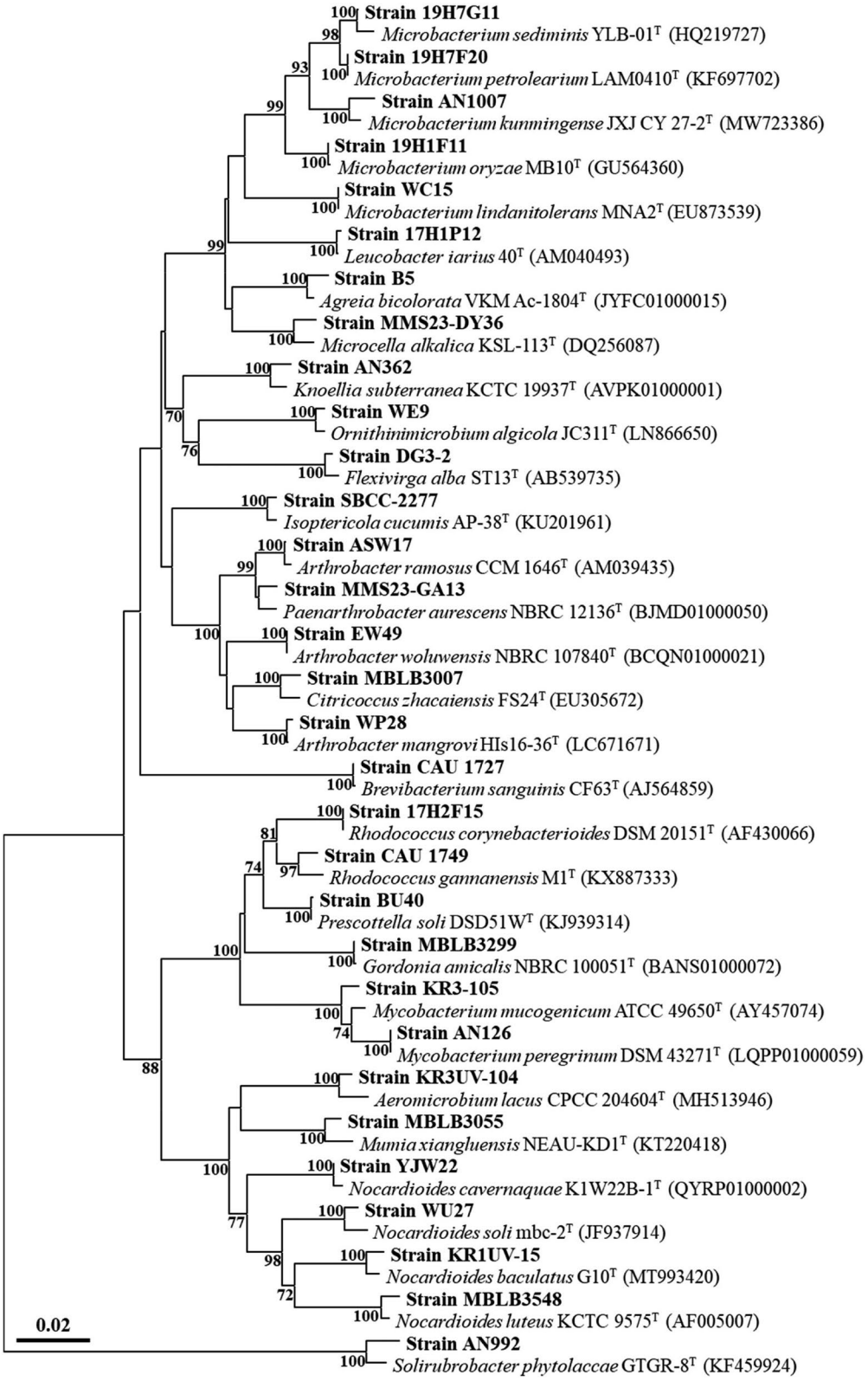

Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic relationship between the strains isolated in this study and their relatives in the phyla *Actinomycetota*. Bootstrap values (>70%) are shown above nodes. Scale bar: 0.02 changes per nucleotide.

tive for esculin hydrolysis. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, and malic acid. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase activities. Does not utilize D-mannose, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain AN992 (=NIBR BAC000510617) was isolated from wetland in Anseong, Gyeonggi-do, Korea.

Conflicts of Interest

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

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