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A report of 42 unrecorded actinobacterial species in Korea

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During a study to discover indigenous prokaryotic species in Korea in 2016, a total of 42 actinobacterial isolates were recovered from various environmental samples collected from natural cave, squid, sewage, sea water, trees, droppings of birds, freshwater, eelgrass, mud flat, sediment and soil. On the basis of a tight phylogenetic clade with the closest species and high level of 16S rRNA gene sequence similarity, it was shown that each isolate was assigned to independent and previously described bacterial species which were assigned to the phylum *Actinobacteria*. The following 42 species have not been reported in Korea: eight species in two genera n the order *Corynebacteriales*, 26 species of 16 genera in the *Micrococcales*, one species of one genus in the *Micromonosporales*, one species of one genus in the *Propionibacteriales*, four species of two genera in the *Streptomycetales* and two species of two genera in the *Streptosporangiale*. Cell morphology, Gram staining reaction, colony colors and features, the media and conditions of incubation, physiological and biochemical characteristics, origins of isolation and strain IDs of 42 unrecorded actinobacterial species are presented in the species description.

Keywords: 16S rRNA gene, indigenous prokaryotic species, unrecorded actinobacterial species

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Introduction

Actinobacteria are generally described as aerobic, high-G+C-content, Gram-staining-positive bacteria and contain morphologically diverse groups that ranged from coccoids or rods to hyphal forms. The branched, filamentous hyphae may either remain stable or split into smaller fragments (Lechevalier & Lechevalier, 1981). In many mycelium-forming actinobacteria, the hyphae are differentiated into aerial mycelia, as well as vegetative mycelia, that bear spores that may by located on the tips of filaments or inside sporangia (Lechevalier, 1989; Ensign *et al*., 1992; Prescott *et al*., 2002).

The phylum *Actinobacteria* (Ludwig *et al*., 2012) consists of classes *Actinobacteria*, *Acidimicrobiia*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria* and *Thermoleophilia* based on 16S rRNA gene sequences. Among these, the class *Actinobacteria* contains 15 orders: *Actinomycetales*, *Actinopolysporales*, *Bifidobacteriales*, *Catenulisporales*, *Corynebacteriales*, *Frankiales*, *Glycomycetales*, *Jiangellales*, *Kineosporiales*, *Micromonosporales*, *Micrococcales*, *Propionibacteriales*, *Pseudonocardiales*, *Streptomycetales* and *Streptosporangiales*. Members of these bacteria are widely distributed in nature and occur in both terrestrial and marine habitats such as soil, fresh or sea water, plants, insects,

manure and compost. Owing to their saprophytic nature, actinobacteria play an important role as decomposers by degrading a wide range of organic compounds and thus contribute to nutrient cycling in natural environments through the turnover of complex organic materials (Lechevalier & Lechevalier, 1981; Williams *et al*., 1984; Mincer *et al*., 2002; Prescott *et al*., 2002). Actinobacteria are of significant industrial importance as producers of a wide range of secondary metabolites such as antibiotics, anti-tumor agents, immunomodulatory agents and other industrial useful enzymes (Nolan and Cross, 1988; Mincer *et al*., 2002; Prescott *et al*., 2002; Busti *et al*., 2006). Many actinobacteria form symbiotic relationships with plants and insects, and reside as endophytes by providing beneficial secondary metabolites to the host plants through the production of antimicrobial molecules or plant growth promoters (Coombs & Franco, 2003; Hasegawa *et al*., 2006; Zhang *et al*., 2007). Some actinobacteria may also be harmful and cause disease in human, animals and plants (Lechevalier & Lechevalier, 1981; Williams *et al*., 1989; Prescott *et al*., 2002)

During an investigation of bacterial species indigenous to Korea that was performed through a research program supported by the National Institute of Biological Resources (NIBR) in 2016, a large number of unrecorded species in Korea were recovered from diverse environmental samples. In this study, we identified and classified bacterial isolates that belonged to the phylum *Actinobacteria* and describe 42 unrecorded actinobacterial species that have not been previously reported in Korea.

Materials and Methods

A total of 42 bacterial strains in the phylum *Actinobacteria* were isolated from various environmental samples collected from natural cave, lava cave, squid, sewage, sea water, tree, droppings of birds, freshwater, eelgrass, mud flat, sediment and soils (Table 1). For bacterial isolation, the environmental samples were treated independently in several laboratories. Pure cultures were obtained after subcultures were attempted several times and grown at 20-37°C for 1-22 days on varied culture media including R2A agar (BD), marine agar 2216 (MA; BD), tryptic soy agar (TSA; BD), nutrient agar (NA; BD), ISP (International *Streptomyces* Project) 2 agar (Shirling & Gottlieb, 1966), Mueller Hinton medium (BD) and starch-casein agar (SCA; Küster and Williams, 1964), depending on the strains. The strain numbers, designated NIBR numbers, most closely related species, origins of isolation, culture media and incubation conditions of 42 unrecorded actinobacterial species are summarized in Table 1. The pure cultures were stored as 10-20% glycerol suspensions (or including 2% sea salts) at −80°C and as lyophilized ampoules.

Gram staining was performed using a Gram-staining kit (bioMérieux) following the manufacturer's instructions. The morphology and color of colonies were observed on agar plates showing optimal growth. Cell morphology was observed using either transmission or scanning electron microscopy. Utilization of sole carbon and energy sources and biochemical properties and were tested with API 20NE kit (bioMérieux) according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification of the 16S rRNA gene by PCR and its sequencing were performed using the standard procedures described elsewhere. The 42 16S rRNA gene sequences of the actinobacterial isolates were aligned with the corresponding sequences of closely related bacterial species retrieved from the EzTaxon-e server (Kim *et al*., 2012), using the Clustal_X program (Thompson *et al*., 1997). After removal of gaps and manual optimization according to the secondary structure of *Escheichia coli*, the sequences were used for phylogenetic analysis. A neighbor-joining (Saitou and Nei, 1987) tree was drawn using evolutionary distances generated using the model of Jukes & Cantor (1969). Phylogenetic analyses were also performed using maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis (Felsenstein, 1985) was performed for estimating reliability of tree topology, based on 1,000 replications.

Results and Discussion

The 42 isolates were recovered from various environments and were found to belong to the phylum *Actinobacteria*. They were distributed in six orders of the class *Actinobacteria*: eight strains in the order *Corynebacteriales*, 26 strains in the *Micrococcales*; one strain in the *Micromonosporales*; one strain in the *Propionibacteriales*; four strains in the *Streptomycetales*; and two strains in the *Streptosporangiales* (Table 1). All the strains were Gram-staining-positive, non-motile and most were represented by coccoid, oval or rod-shaped morphology (Fig. 1). The neighbor-joining tree (Fig. 2) based on 16S rRNA gene sequences shows the phylogenetic relationships of the strains of the order *Corynebacteriales*: *Mycobacterium* (2 species) of the *Mycobacteriaceae* and *Gordonia* (2 species); and *Nocardia* (3 species) and *Rhodococcus* (1 species) of the *Nocardiaceae*. The strains of the order *Micrococcales* were assigned to 16 genera in eight families: *Demequina* (1 species) of the family *Demequinaceae*; *Brachybacterium* (2 species) and *Demacoccus* (1 species) of the *Dermabacteraceae*;

Table 1. Summary of the isolated strains belonging to the Actinobacteria and their taxonomic affiliations.

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species (Name of type strain)	Similarity (%)	Isolation source	Medium	Incubation con.
Class *Actinobacteria*									
Corynebacteriales	*Mycobacteriaceae*	*Mycobacterium*	C1-45	NIBRBAC000498661	*Mycobacterium aubagnense*	98.9	Natural cave	ISP2	30°C, 22 days
		Mycobacterium	C6-3	NIBRBAC000498662	*Mycobacterium algericum*	98.8	Natural cave	ISP2	30°C, 22 days
	Nocardiaceae	*Gordonia*	BE5-3	NIBRBAC000498659	*Gordonia aichiensis*	100	Mud flat	TSA	30°C, 5 days
		Gordonia	LPB0127	NIBRBAC000498517	*Gordonia terrae*	99.5	Squid	MA	25°C, 1 day
		Nocardia	YC6-12	NIBRBAC000498652	*Nocardia soli*	99	Yongcheon Cave	ISP2	30°C, 5 days
		Nocardia	YC7-20	NIBRBAC000498653	*Nocardia fluminea*	99.6	Yongcheon Cave	NA	30°C, 5 days
		Nocardia	AR23308	NIBRBAC000498386	*Nocardia vermiculata*	99.8	Droppings of eagles	Mueller Hinton	37°C, 2 days
		Rhodococcus	YHJ2	NIBRBAC000498431	*Rhodococcus ruber*	100	Sewage	R2A	30°C, 2 days
Micrococcales	*Demequinaceae*	*Demequina*	JGM01	NIBRBAC000498631	*Demequina flava*	100	Sea water	R2A	25°C, 3 days
	Dermabacteraceae	*Brachybacterium*	LPB0135	NIBRBAC000498523	*Brachybacterium muris*	99.8	Squid	MA	25°C, 1 day
		Brachybacterium	6228	NIBRBAC000498570	*Brachybacterium rhamnosum*	100	Sediment	R2A	30°C, 2 days
		Dermacoccus	10038	NIBRBAC000498577	*Dermacoccus nishinomiyaensis*	99.9	Sediment	R2A	30°C, 2 days
	Intrasporangiaceae	*Intrasporangium*	Gsoil 608	NIBRBAC000498587	*Intrasporangium oryzae*	98.9	Ginseng soil	R2A	25°C, 3 days
	Jonesiaceae	*Jonesia*	5189	NIBRBAC000498569	*Jonesia quinghaiensis*	99.7	Sediment	R2A	30°C, 2 days
	Microbacteriaceae	*Agromyces*	UL003	NIBRBAC000498614	*Agromyces italicus*	99.5	Soil	TSA	30°C, 2 days
		Gryllotalpicola	HMF7422	NIBRBAC000498454	*Gryllotalpicola reticulitermitis*	100	Tree	R2A	30°C, 3 days
		Leifsonia	MMS16-CNU056	NIBRBAC000498620	*Leifsonia poae*	99.7	Soil	SCA (pH5)	30°C, 3 days
		Microbacterium	ATS3307	NIBRBAC000498378	*Microbacterium esteraromaticum*	99.8	Droppings of eagles	TSA	37°C, 2 days
		Microbacterium	PTS2402	NIBRBAC000498381	*Microbacterium testaceum*	99.3	Droppings of spoonbills	TSA	20°C, 2 days
		Microbacterium	AMA3210	NIBRBAC000498394	*Microbacterium resistens*	98.7	Droppings of eagles	MA	37°C, 2 days
		Microbacterium	Gsoil 163	NIBRBAC000498599	*Microbacterium aerolatum*	99.2	Ginseng soil	R2A	25°C, 3 days
		Microbacterium	CAH-3	NIBRBAC000498634	*Microbacterium fluvii*	98.8	Freshwater	R2A	25°C, 3 days
		Microbacterium	2JSS04	NIBRBAC000498635	*Microbacterium hatanonis*	99.9	Freshwater	R2A (2x)	25°C, 3 days
	Micrococcaceae	*Arthrobacter*	J52	NIBRBAC000498411	*Arthrobacter niigatensis*	98.8	Sewage	R2A	30°C, 2 days
		Arthrobacter	Gsoil 1513	NIBRBAC000498596	*Arthrobacter nicotinovorans*	99.6	Ginseng soil	R2A	25°C, 3 days
		Arthrobacter	PMA2201	NIBRBAC000498399	*Arthrobacter nicotianae*	99.1	Droppings of eagles	MA	20°C, 2 days
		Arthrobacter	ZOD2-24	NIBRBAC000498480	*Arthrobacter gandavensis*	99.7	Eelgrass	MA	24°C, 3 days
		Arthrobacter	LPB0133	NIBRBAC000498521	*Arthrobacter rhombi*	99.4	Squid	MA	25°C, 1 day
		Kocuria	PMA2305	NIBRBAC000498398	*Kocuria carniphila*	99.3	Droppings of eagles	MA	20°C, 2 days
		Micrococcus	LPB0125	NIBRBAC000498515	*Micrococcus lylae*	99.3	Squid	MA	25°C, 1 day
		Nesterenkonia	IMCC25640	NIBRBAC000498539	*Nesterenkonia lutea*	99.7	Mud flat	MA	30°C, 3 days
		Renibacterium	5187	NIBRBAC000498568	*Renibacterium salmoninarum*	99.1	Sediment	R2A	30°C, 2 days
	Promicromonosporaceae	*Cellulosimicrobium*	PMA3209	NIBRBAC000498393	*Cellulosimicrobium cellulans*	99.9	Droppings of spoonbills	MA	37°C, 2 days
	Sanguibacteraceae	*Oerskovia*	YC4-25	NIBRBAC000498651	*Oerskovia turbata*	99.9	Yongcheon Cave	NA	30°C, 4 days
Micromonosporales	*Micromonosporaceae*	*Micromonospora*	Gsoil 1174	NIBRBAC000498604	*Micromonospora echinospora*	99.6	Ginseng soil	R2A	25°C, 3 days
Propionibacteriales	*Nocardioidaceae*	*Aeromicrobium*	YC3-14	NIBRBAC000498650	*Aeromicrobium fastidiosum*	98.8	Yongcheon Cave	ISP2	30°C, 5 days
Streptomycetales	*Streptomycetaceae*	*Kitasatospora*	MMS16-CNU292	NIBRBAC000498624	*Kitasatospora kifunensis*	99	Soil	SCA (pH5)	30°C, 3 days
		Streptomyces	Gsoil 224	NIBRBAC000498585	*Streptomyces durhamensis*	99.3	Ginseng soil	R2A	25°C, 3 days
		Streptomyces	UL451	NIBRBAC000498617	*Streptomyces kurssanovii*	99.4	Soil	TSA	30°C, 2 days
		Streptomyces	MMS16-CNU183	NIBRBAC000498623	*Streptomyces blastmyceticus*	99.9	Soil	ISP2 (pH5)	30°C, 3 days
Streptosporangiales	*Nocardiopsaceae*	*Nocardiopsis*	LPB0126	NIBRBAC000498516	*Nocardiopsis synnemataformans*	99.7	Squid	MA	25°C, 1 day
	Streptosporangiaceae	*Nonomuraea*	Gsoil 1046	NIBRBAC000498588	*Nonomuraea harbinensis*	99.6	Ginseng soil	R2A	25°C, 3 days

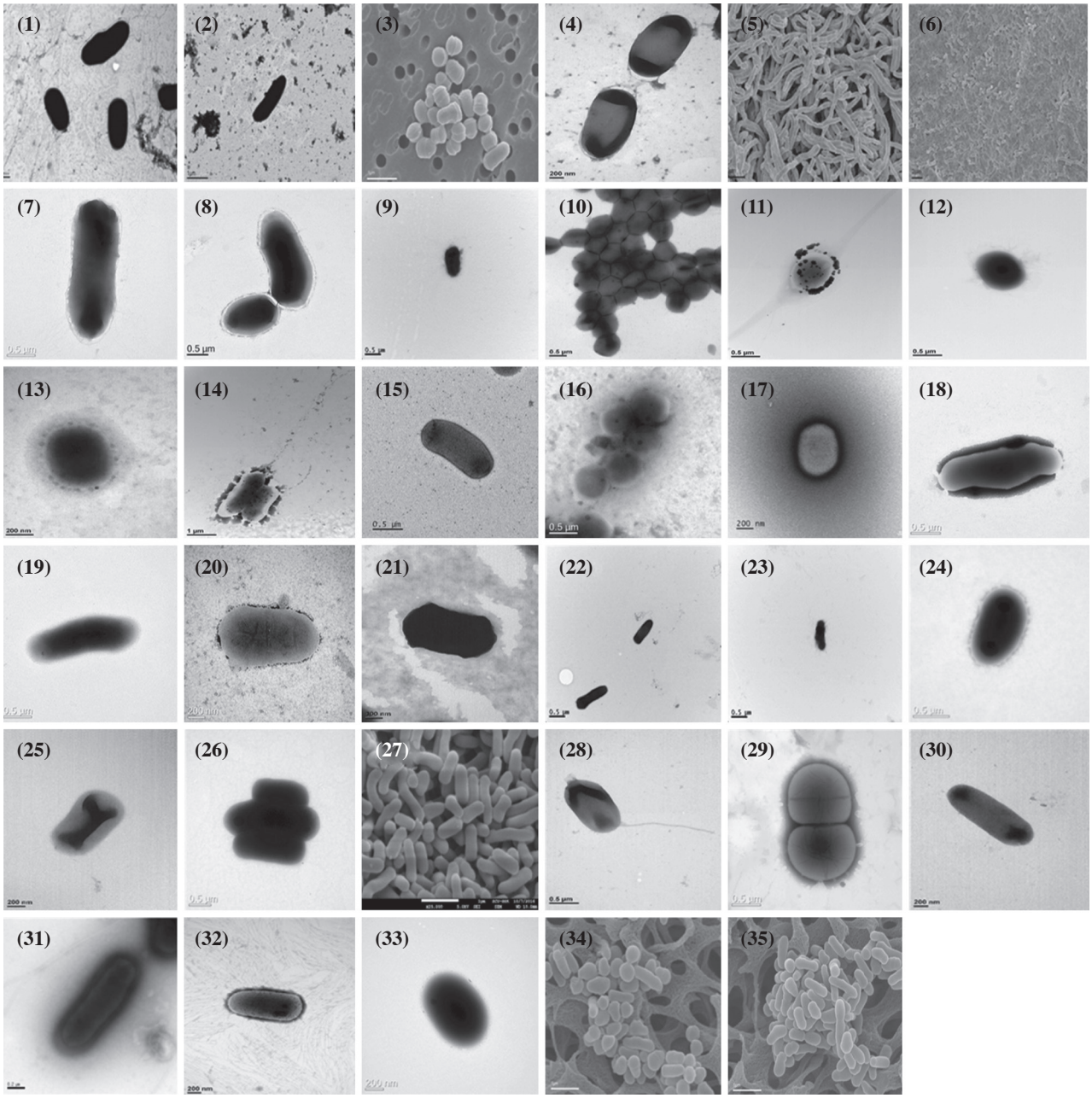

Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, C1-45; 2, C6-3; 3, BE5-3; 4, LPB0127; 5, YC6-12; 6, YC7-20; 7, AR23308; 8, YHJ2; 9, JGM01; 10, LPB0135; 11, 6228; 12, 10038; 13, Gsoil 608; 14, 5189; 15, UL003; 16, HMF7422; 17, MMS16-CNU056; 18, ATS3307; 19, PTS2402; 20, AMA3210; 21, Gsoil 163; 22, CAH-3; 23, 2JSS04; 24, J52; 25, Gsoil 1513; 26, PMA2201; 27, ZOD2-24; 28, LPB0133; 29, PMA2305; 30, LPB0125; 31, IMCC25640; 32, 5187; 33, PMA3209; 34, YC4-25; 35, YC3-14.

Intrasporangium (1 species) of the *Intrasporangiaceae*; *Jonesia* (1 species) of the *Jonesiaceae*; *Agromyces* (1 species), *Gryllotalpicola* (1 species), *Leifsonia* (1 species) and *Microbacterium* (6 species) of the *Microbacteriaceae*; *Arthrobacter* (5 species), *Kocuria* (1 species), *Micrococcus* (1 species), *Nesterenkonia* (1 species) and *Renibacterium* (1 species) of the *Micrococcaceae*; *Cellulosimicrobium* (1 species) of the *Promicromonosporaceae*; and *Oerskovia* (1 species) of the *Sanguibacteraceae* and their phylogenetic relationships were shown in Fig. 3. The order *Streptomycetales* consisted of four isolates that were distributed in the genera *Kitasatospora* (1 species) and *Streptomyces* (3 species) of the family *Streptomycataceae*. The orders *Micromonosporales* and *Pro-*

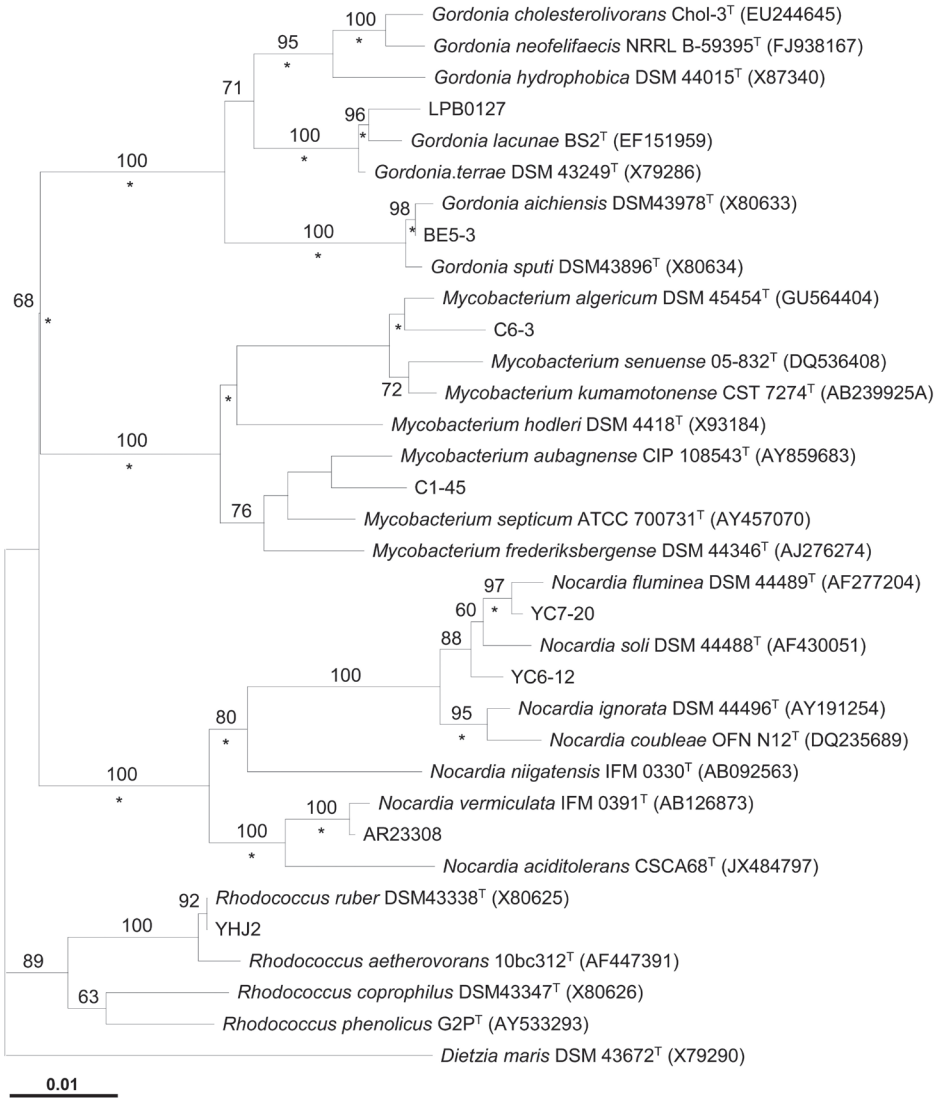

Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the orders *Corynebacteriales* in the class *Actinobacteria*. Asterisks indicate that the corresponding branches were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bootstrap values (>60%) are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

pionibacteriales contained only one isolate that was affiliated to the genus *Micromonospora* of the family *Micromonosporaceae* and to the genus *Nocardioidaceae*, respectively. The order *Streptosporangiales* comprised two isolates, each of which was assigned to the genus *Nocardiopsis* of the family *Nocardiopsaceae* and the genus *Nonomuraea* of the family *Stereptosporangiaceae*, respectively. The tree given in Fig. 4 reveals the phylogenetic relationships between the isolates of the above four orders and their closest relatives. Most strains of these orders formed well-developed, branched aerial and substrate hypha. Among members of these orders, most produce vegetative and aerial mycelia depending on the species, with exception of the order *Propionibacteriales*, whose members form coccoids or rods. Some mycelium-forming species showed further differentiated features such as the formation of spores on the ends of aerial mycelia (Lechevalier, 1989; Prescott *et al*., 2002).

Description of *Mycobacterium aubagnense* C1-45

Cells are Gram-staining-positive, flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and light brown-colored after 22 days on ISP2 at 30°C. Positive for nitrate reduction in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Does not utilize D-glucose,

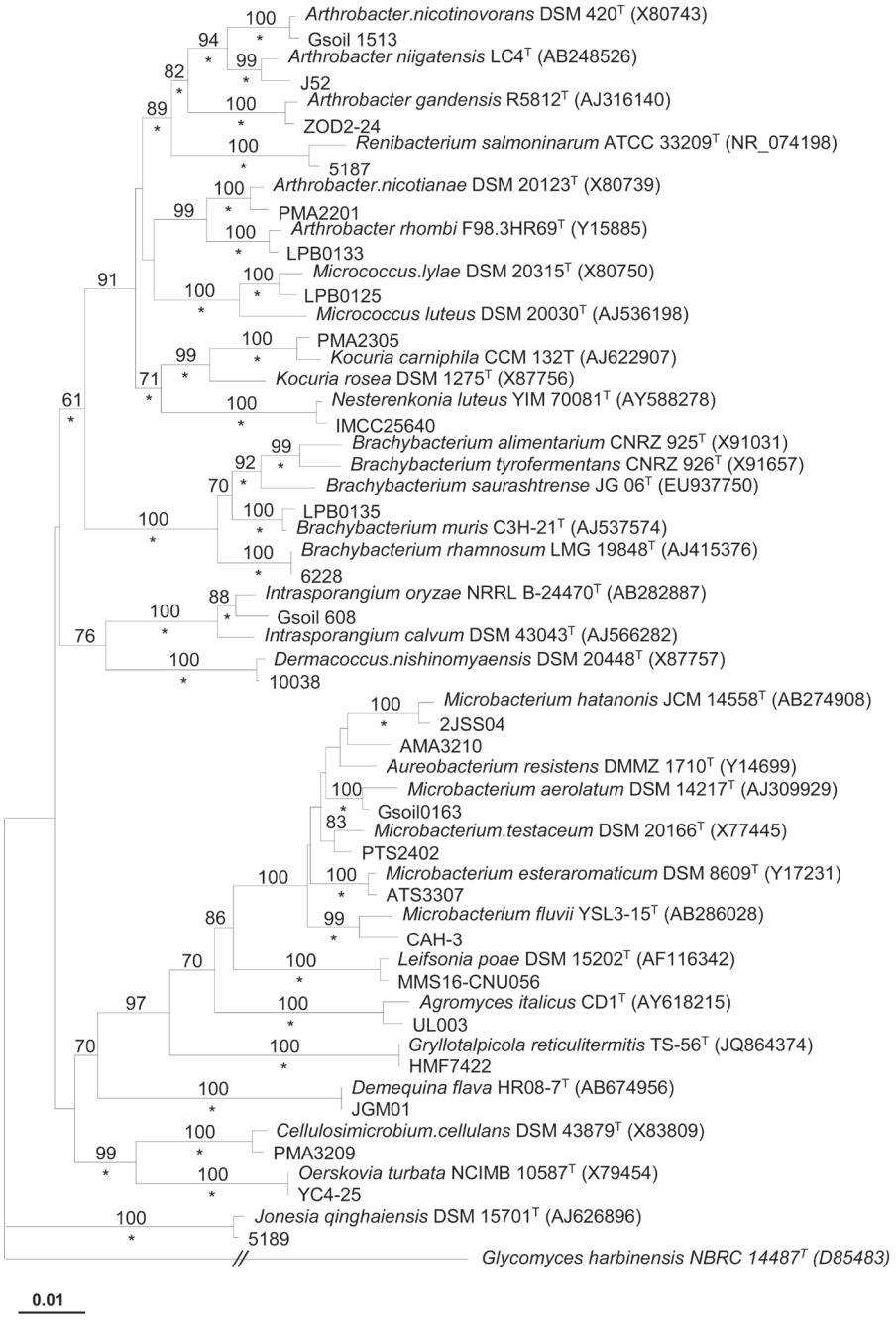

Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Micrococcales* in the class *Actinobacteria*. Asterisks indicate that the corresponding branches were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bootstrap values (>60%) are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain C1-45 (=NIBRBA000498661) has been isolated from natural cave, Jeju, Korea.

Description of *Mycobacterium algericum* C6-3

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and light brown-colored after 22 days on ISP2 at 30°C. Positive for nitrate reduction, esculin

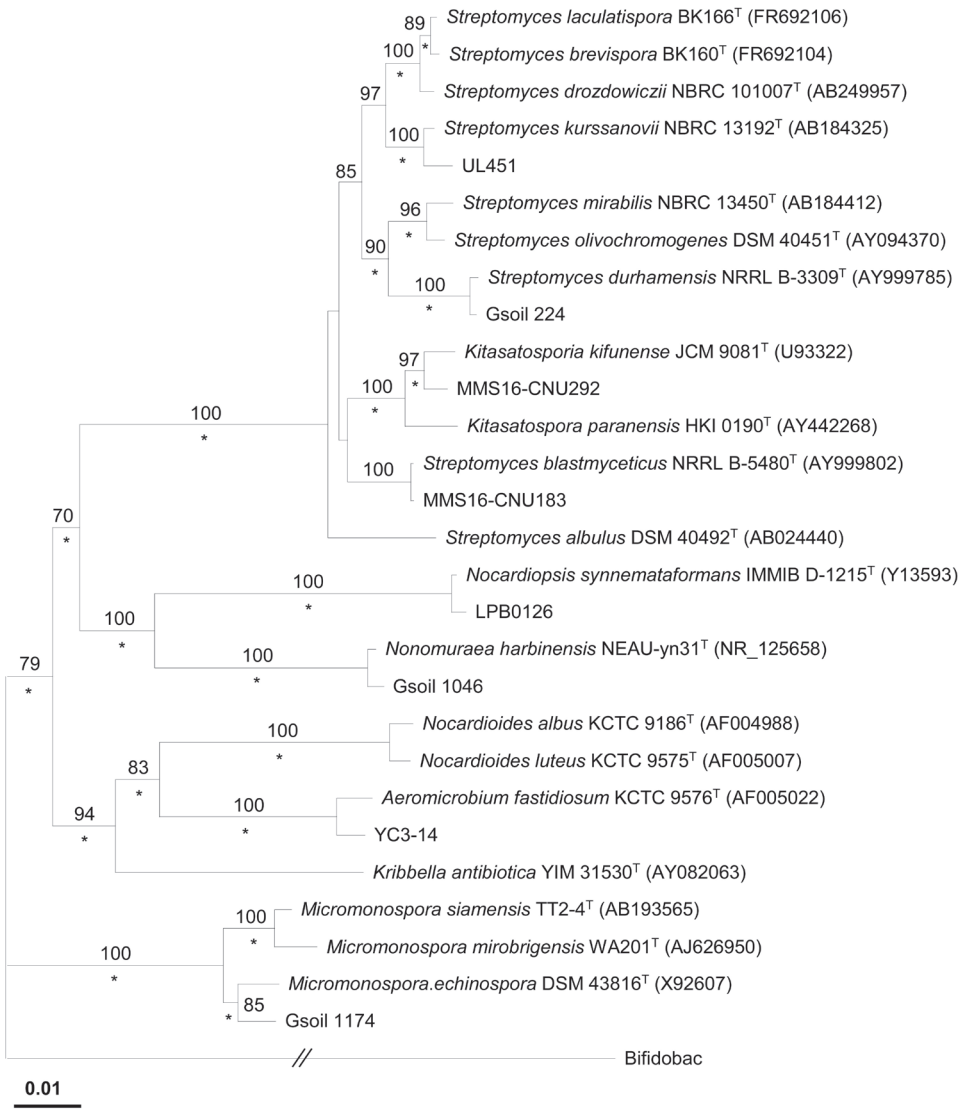

Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the orders *Micromonosporales*, *Propionibacteriales*, *Streptomycetales* and *Streptosporangiales* in the class *Actinobacteria*. Asterisks indicate that the corresponding branches were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bootstrap values (>60%) are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

hydrolysis and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain C6-3 (=NIBRBA000498662) has been isolated from natural cave, Jeju, Korea.

Description of *Gordonia aichiensis* BE5-3

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and orange yellow-colored after 5 days on TSA at 30°C. Positive for nitrate reduction and urease in API 20NE, weakly positive for esculin hydrolysis but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase and β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain BE5-3 (=NIBRBA000498659) has been isolated from mud flat, Eulsukdo, Busan, Korea.

Description of *Gordonia terrae* LPB0127

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular,

convex, entire and pink-colored after 1 day on MA at 25°C. Positive for urease in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. D-Mannos and malate are utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, citrate and phenylacetate. Strain LPB0127 (=NIBRBAC000498517) has been isolated from squid, Jumunjin, Gangwon-do, Korea.

Description of *Nocardia soli* YC6-12

Cells are Gram-staining-positive, non-flagellated, non-pigmented and fragmented mycelium. Colonies are circular, convex, entire and whitish pink-colored after 5 days on IPS2 at 30°C. Esculin hydrolysis is weakly positive in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase and β-galactosidase. N-Acetyl-glucosamine and gluconate are weakly utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, D-maltose, caprate, adipate, malate, citrate and phenylacetate. Strain YC6-12 (=NIBRBA000498652) has been isolated from Yongcheon Cave, Jeju, Korea.

Description of *Nocardia fluminea* YC7-20

Cells are Gram-staining-positive, non-flagellated, non-pigmented and fragmented mycelium. Colonies are circular, convex, entire and whitish pink-colored after 5 days on NA at 30°C. Esculin hydrolysis is weakly positive in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and β-galactosidase. N-Acetyl-glucosamine is weakly utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain YC7-20 (=NIBRBA000498653) has been isolated from Yongcheon Cave, Jeju, Korea.

Description of *Nocardia vermiculata* AR23308

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, hard and cream-colored after 2 days on Mueller Hinton at 37°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Maltose, gluconate, adipate, malate is utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, caprate, citrate and phenylacetate. Strain AR23308 (=NIBRBAC000498386) has been isolated from droppings of eagles, Seoul Grand Park, Gwacheon, Gyeonggi, Korea.

Description of *Rhodococcus ruber* YHJ2

Cells are Gram-staining-positive, non-flagellated and rod/oval-shaped. Colonies are Irregular, raised, entire and orange-colored after 2 days on R2A at 30°C. Positive for nitrate reduction in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. D-Glucose, D-mannose and malate are utilized. Does not utilize L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, citrate and phenylacetate. Strain YHJ2 (=NIBRBAC000498431) has been isolated from sewage, Busan, Korea.

Description of *Demequina flava* JGM01

Cells are Gram-staining-positive, non-flagellated and oval-shaped. Colonies are circular, convex and yellow-colored after 3 days on R2A at 25°C. Strain JGM01 (=NIBRBA000498631) has been isolated from sea water, Mokji island, Jeju, Korea.

Description of *Brachybacterium muris* LPB0135

Cells are Gram-staining-positive, non-flagellated, non-pigmented and coccus-shaped. Colonies are circular, convex, entire and yellow-colored after 1 day on MA at 25°C. Positive for urease and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannos, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain LPB0135 (=NIBRBAC000498523) has been isolated from squid, Jumunjin, Gangwon-do, Korea.

Description of *Brachybacterium rhamnosum* 6228

Cells are Gram-staining-positive, non-flagellated and coccus-shaped. Colonies are circular, entire, smooth, raised and pale-yellow-colored after 2 days on R2A at 30°C. Positive for glucose fermentation, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, arginine dihydrolase and urease. D-Glucose, D-mannitol, D-mannose, D-maltose, gluconate are utilized. Does not utilize L-arabinose, N-acetyl-glucosamine, caprate, adipate, malate, citrate and phenylacetate. Strain 6228 (=NIBRBAC000498570) has been isolated from sediment, Han River, Korea.

Description of *Dermacoccus nishinomiyaensis* 10038

Cells are Gram-staining-positive, flagellated and coccus-shaped. Colonies are circular, entire, smooth, raised and yellow-colored after 2 days on R2A at 30°C. Positive for gelatinase and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and esculin hydrolysis. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, adipate, malate, citrate and phenylacetate are utilized. Does not utilize caprate. Strain 10038 (=NIBRBAC000498577) has been isolated from sediment, Han River, Korea.

Description of *Intrasporangium oryzae* Gsoil 608

Cells are Gram-staining-positive, non-flagellated and coccus-shaped. Colonies are round, convex, circular and yellow-colored after 3 days on R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, D-mannitol, D-maltose, gluconate, malate are utilized. Does not utilize L-arabinose, D-mannose, N-acetyl-glucosamine, caprate, adipate, citrate and phenylacetate. Strain Gsoil 608 (=NIBRBAC000498587) has been isolated from ginseng soil, Anseong, Korea.

Description of *Jonesia quinghaiensis* 5189

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, entire, smooth, raised and pale-yellow-colored after 2 days on R2A at 30°C. Positive for nitrate reduction, esculin hydrolysis, β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose, D-maltose, gluconate are utilized. Does not utilize N-acetyl-glucosamine, caprate, adipate, malate, citrate and phenylacetate. Strain 5189 (=NIBRBAC000498569) has been isolated from sediment, Han River, Korea.

Description of *Agromyces italicus* UL003

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and cream-colored after 2 days on TSA at 30°C. Positive for nitrate reduction and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. D-Glucose, D-mannitol, D-mannose, N-acetyl-glucosamine and D-maltose are utilized. Does not utilize L-arabinose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain UL003 (=NIBRBAC000498614) has been isolated from soil, Ulleung-gun, Gyeongsangbuk-do, Korea.

Description of *Gryllotalpicola reticulitermitis* HMF7422

Cells are Gram-staining-positive, non-flagellated and irregular oval-shaped. Colonies are Circular, convex, entire and white-colored after 3 days on R2A at 30°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. D-Glucose, D-mannitol and gluconate are utilized. Does not utilize L-arabinose, D-mannose, N-acetyl-glucosamine, D-maltose, caprate, adipate, malate, citrate and phenylacetate. Strain HMF7422 (=NIBRBAC000498454) has been isolated from tree, Yongin, Gyeonggi, Korea.

Description of *Leifsonia poae* MMS16-CNU056

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, glistering, moist and white-colored after 3 days on SCA (pH 5) at 30°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine and D-maltose are utilized. Does not utilize D-mannose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain MMS16-CNU056 (=NIBRBAC000498620) has been isolated from soil, Daejeon, Korea.

Description of *Microbacterium esteraromaticum* ATS3307

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are Circular, slightly convex, smooth, glistening and pale yellow-colored after 2 days of incubation on TSA at 37°C. Positive for esculin hydrolysis in API 20NE, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and β-galactosidase. D-Glucose, L-arabinose, D-mannitol, D-maltose are utilized. Does not utilize, D-mannose, N-acetyl-glucosamine, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain ATS3307 (=NIBRBAC000498378) has been isolated from droppings of eagles, Seoul Grand Park, Gwacheon, Gyeonggi, Korea.

Description of *Microbacterium testaceum* PTS2402

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, glistening and cream-colored after 2 days on TSA at 20°C. Positive

for esculin hydrolysis and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, urease, arginine dihydrolase and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose and D-maltose are utilized. Does not utilize N-acetyl-glucosamine, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain PTS2402 (=NIBRBA000498381) has been isolated from droppings of spoonbills, Seoul Grand Park, Gwacheon, Gyeonggi, Korea.

Description of *Microbacterium resistens* AMA3210

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and yellow-colored after 2 days on MA at 37°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-Arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain AMA3210 (=NIBRBAC000498394) has been isolated from droppings of eagles, Seoul Grand Park, Gwacheon, Gyeonggi, Korea.

Description of *Microbacterium aerolatum* Gsoil 163

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are convex, smooth and ivory-colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, malate, citrate and phenylacetate are utilized. Does not utilize caprate, adipate. Strain Gsoil 163 (=NIBRBAC000498599) has been isolated from ginseng soil, Anseong, Korea.

Description of *Microbacterium fluvii* CAH-3

Cells are Gram-staining-positive, non-flagellated and rod and oval-shaped. Colonies are circular, convex, entire and yellow-colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, D-mannose and D-maltose and are utilized. Does not utilize L-arabinose, D-mannitol, N-acetyl-glucosamine, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain CAH-3 (=NIBRBA000498634) has been isolated from freshwater, Jeonju, Korea.

Description of *Microbacterium hatanonis* 2JSS04

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, slightly convex, smooth and yellow-colored after 3-4 days on 2x R2A at 25°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Mannose is utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain 2JSS04 (=NIBRBA000498635) has been isolated from freshwater, Jeonju, Korea.

Description of *Arthrobacter niigatensis* J52

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, raised, entire and white-colored after 2 days on R2A at 30°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE but negative nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, D-mannitol, D-mannose, D-maltose, gluconatem and malate are utilized. Does not utilize L-arabinose, N-acetyl-glucosamine, caprate, adipate, citrate and phenylacetate. Strain J52 (=NIBRBAC000498411) has been isolated from sewage, Busan, Korea.

Description of *Arthrobacter nicotinovorans* Gsoil 1513

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are convex, circular, smooth and pale-yellow-colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, malate, citrate and phenylacetate are utilized. Does not utilize caprate, adipate. Strain Gsoil 1513 (=NIBRBAC000498596) has been isolated from ginseng soil, Anseong, Korea.

Description of *Arthrobacter nicotianae* PMA2201

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and cream-colored after 2 days on MA at 20°C. Positive for nitrate reduction, urease, gelatinase and β-galactosidase but negative for indole production, glucose fermentation, arginine dihydrolase and esculin hydrolysis. D-Glucose, L-arabinose, D-mannose, N-acetyl-glucosamine, D-maltose, D-mannitol, gluconate, caprate, adipate, malate, citrate and phenylacetate are utilized. Strain PMA2201 (=NI-

BRBAC000498399) has been isolated from droppings of eagles, Seoul Grand Park, Gwacheon, Gyeonggi, Korea.

Description of *Arthrobacter gandavensis* ZOD2-24

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are opaque, circular, smooth, low convex and pale-yellow-colored after 3 days on MA at 24°C. Positive for nitrate reduction and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate are not utilized. Strain ZOD2-24 (=NIBRBAC000498480) has been isolated from eelgrass, Seosan, Chungcheongnam-do, Korea.

Description of *Arthrobacter rhombi* LPB0133

Cells are Gram-staining-positive, flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and yellow-colored after 1 day on MA at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannos, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain LPB0133 (=NIBRBAC000498521) has been isolated from squid, Jumunjin, Gangwon-do, Korea.

Description of *Kocuria carniphila* PMA2305

Cells are Gram-staining-positive, non-flagellated and coccoid shaped. Colonies are circular and cream-colored after 2 days on MA at 20°C. Positive for nitrate reduction and urease in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase and β-galactosidase. D-Glucose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, adipate, malate, citrate and phenylacetate are utilized. Does not utilize L-arabinose and caprate. Strain PMA2305 (=NIBRBAC000498398) has been isolated from droppings of eagles, Seoul Grand Park, Gwacheon, Gyeonggi, Korea.

Description of *Micrococcus lylae* LPB0125

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and yellow-colored after 1 day on MA at 25°C. Positive for gelatinase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and β-galactosidase. gluconate, adipate, malate, citrate are utilized. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, caprate and phenylacetate are not utilized. Strain LPB0125 (=NIBRBAC000498515) has been isolated from squid, Jumunjin, Gangwon-do, Korea.

Description of *Nesterenkonia lutea* IMCC25640

Cells are Gram-staining-positive, non- flagellated and rod-shaped. Colonies are circular, convex, entire and yellow-colored after 3 days on MA at 20°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. D-Glucose is utilized. Does not utilize L-arabinose, D-mannitol, D-mannos, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain IMCC25640 (=NIBRBAC000498539) has been isolated from mud flat, Yeongjongdo island, Incheon, Korea.

Description of *Renibacterium salmoninarum* 5187

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, entire, dry, flat and white-colored after 2 days on R2A at 30°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose is utilized. Does not utilize L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain 5187 (=NIBRBAC000498568) has been isolated from sediment, Han river, Korea.

Description of *Cellulosimicrobium cellulans* PMA3209

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and yellow-colored after 2 days on MA at 37°C. Positive for nitrate reduction, glucose fermentation, gelatinase and esculin hydrolysis in API 20NE but negative for indole production, arginine dihydrolase, and β-galactosidase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, gluconate, D-maltose, adipate, malate, citrate and phenylacetate are utilized. Does not utilize caprate, Strain PMA3209 (=NIBRBAC000498393) has been isolated from droppings of spoonbills, Seoul Grand Park, Gwacheon, Gyeonggi, Korea.

Description of *Oerskovia turbata* YC4-25

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod to coccoid-shaped. Colonies are circular, convex, entire and light yellow-colored after

4 days on NA at 30°C. Positive for nitrate reduction, esculin hydrolysis and β-galactosidase in API 20NE, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, D-mannitol and N-acetyl-glucosamine are weakly utilized. L-arabinose, D-maltose and gluconate are utilized. Does not utilize D-mannose, caprate, adipate, malate, citrate and phenylacetate. Strain YC4-25 (=NIBRBA000498651) has been isolated from Yongcheon Cave, Jeju, Koraea.

Description of *Micromonospora echinospora* Gsoil 1174

Cells are Gram-staining-positive, non-flagellated and substrate mycelium-forming. Colonies are convex, irregulaer and brown-colored after 3 days on R2A at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. L-Arabinose, D-mannitol, gluconate, malate are utilized. Does not utilize D-glucose, D-mannose, N-acetyl-glucosamine, D-maltose, caprate, adipate, citrate and phenylacetate. Strain Gsoil 1174 (=NIBRBAC000498604) has been isolated from ginseng soil, Anseong, Korea.

Description of *Aeromicrobium fastidiosum* YC3-14

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, filamentous and cream-colored after 5 days on ISP2 at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase in API 20NE. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain YC3-14 (=NIBRBA000498650) has been isolated from Yongcheon Cave, Jeju, Korea.

Description of *Kitasatospora kifunensis* MMS16-CNU292

Cells are Gram-staining-positive, non-flagellated and produce well-developed, branched aerial and vegetative mycelia. Colonies are circular, dry, rough, opaque and beige-colored after 3 days on SCA (pH 5) at 30°C. Positive for esculin hydrolysis and gelatinase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase, urease and β-galactosidase. D-Glucose is utilized. Does not utilize L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain MMS16-CNU292 (=NIBRBAC000498624) has been isolated from soil, Daejeon, Korea.

Description of *Streptomyces durhamensis* Gsoil 224

Cells are Gram-staining-positive, non-flagellated and produce well-developed, branched aerial and vegetative mycelia. Colonies are round, convex, circular, entire and gray-colored after 3 days on R2A at 25°C. Positive for nitrate reduction and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, adipate, malate, citrate and phenylacetate are utilized. Does not utilize caprate. Strain Gsoil 224 (=NIBRBAC000498585) has been isolated from ginseng soil, Anseong, Korea.

Description of *Streptomyces kurssanovii* UL451

Cells are Gram-staining-positive, non-flagellated, non-pigmented and produce well-developed, branched aerial and vegetative mycelia. Colonies are circular, convex, rough, entire and mud yellow-colored after 3 days on TSA at 30°C. Positive for esculin hydrolysis and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate and malate are utilized. Does not utilize caprate, adipate, citrate and phenylacetate. Strain UL451 (=NIBRBAC000498617) has been isolated from soil, Ulleung-gun, Gyeongsangbuk-do, Korea.

Description of *Streptomyces blastmyceticus* MMS16-CNU183

Cells are Gram-staining-positive, non-flagellated, non-pigmented and produce well-developed, branched aerial and vegetative mycelia. Colonies are circular, convex, rough, entire and mud yellow-colored after 3 days on ISP2 (pH 5) at 30°C. Positive for urease, gelatinase, esculin hydrolysis and β-galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain MMS16-CNU183 (=NIBRBAC000498623) has been isolated from soil, Daejeon, Korea.

Description of *Nocardiopsis synnemataformans* LPB0126

Cells are Gram-staining-positive, non-flagellated, non-pigmented and produce branched aerial and vegetative mycelia. Colonies are circular, convex, entire

and white-colored after 1 day on MA at 25°C. Positive for nitrate reduction and β-galactosidase in API 20NE but negative indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. D-Mannos and malate are utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, citrate and phenylacetate. Strain LPB0126 (=NIBRBAC000498516) has been isolated from squid, Jumunjin, Gangwon-do, Korea.

Description of *Nonomuraea harbinensis* Gsoil 1046

Cells are Gram-staining-positive, non-flagellated and mycelium-forming. Colonies are convex, circular and white-colored after 3 days on R2A at 25°C. Positive for nitrate reduction and β-galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, gluconate are utilized. Does not utilize D-maltose, caprate, adipate, malate, citrate and phenylacetate. Strain Gsoil 1046 (=NIBRBAC 000498588) has been isolated from ginseng soil, Anseong, Korea.

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