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 1020% 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*;

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species (Name of type strain)	Similarity (%)	Isolation source	Medium	Incubation con.
Class Actinobacteria									
	Mycobacteriaceae	Mycobacterium Mycobacterium	C1-45 C6-3	NIBRBAC000498661 NIBRBAC000498662	Mycobacterium aubagnense Mycobacterium algericum	98.9 98.8	Natural cave Natural cave	ISP2 ISP2	30°C, 22 days 30°C, 22 days
Corynebacteriales	Nocardiaceae	Gordonia Gordonia Nocardia Nocardia Rhodocorcus	BE5-3 LPB0127 YC6-12 YC7-20 AR23308 YHJ2	NIBRBAC000498659 NIBRBAC000498517 NIBRBAC000498552 NIBRBAC000498653 NIBRBAC000498653 NIBRBAC000498386 NIBRBAC000498431	Gordonia aichiensis Gordonia terrae Nocardia soli Nocardia fluminea Nocardia vermiculata Rhodococcus ruber	100 99.5 99.6 99.8 100	Mud flat Squid Yongcheon Cave Yongcheon Cave Droppings of eagles Sewage	TSA MA ISP2 NA Mueller Hinton R2A	30°C, 5 days 25°C, 1 day 30°C, 5 days 30°C, 5 days 30°C, 5 days 37°C, 2 days 30°C, 2 days
	Demequinaceae Dermabacteraceae	Demequina Brachybacterium Brachybacterium Dermacoccus	JGM01 LPB0135 6228 10038	NIBRBAC000498631 NIBRBAC000498523 NIBRBAC000498570 NIBRBAC000498577 NIBRBAC000498577	Demequina flava Brachybacterium muris Brachybacterium rhannosum Dermacoccus nishinomiyaensis	100 99.8 99.9	Sea water Squid Sediment Sediment	R2A MA R2A R2A	25°C, 3 days 25°C, 1 day 30°C, 2 days 30°C, 2 days
	Intrasporangiaceae	Intrasporangium	Gsoil 608	NIBRBAC000498587	Intrasporangium oryzae Lonoia aninabaiomie	9.89 7.00	Ginseng soil	R2A D7A	25°C, 3 days
Micrococcales	Microbacteriaceae	Agromyces Gryflotatpicola Leifsonia Microbacterium Microbacterium Microbacterium Microbacterium	UL003 UL003 HMF7422 MMS316-CNU056 ATS3307 PTS2402 AMA3210 Gsoil 163 CAH-3 2JSS04	NIBRBAC000498614 NIBRBAC000498614 NIBRBAC000498620 NIBRBAC000498378 NIBRBAC000498381 NIBRBAC000498381 NIBRBAC000498599 NIBRBAC000498595 NIBRBAC000498634 NIBRBAC000498634	Agromyces italicus Agromyces italicus Grylloialpicola reticulitermitis Leifsonia poae Microbacterium estareum Microbacterium aerolatum Microbacterium aerolatum Microbacterium hatanonis	99.5 99.7 99.3 99.2 99.2 99.9	Soil Soil Tree Droppings of eagles Droppings of eagles Ginseng soil Freshwater Freshwater	TSA TSA SCA(pH5) SCA(pH5) TSA TSA MA R2A R2A R2A R2A R2A R2A	30°C, 2 days 30°C, 2 days 30°C, 3 days 30°C, 2 days 37°C, 2 days 37°C, 2 days 25°C, 3 days 25°C, 3 days 25°C, 3 days 25°C, 3 days
	Micrococcaceae	Arthrobacter Arthrobacter Arthrobacter Arthrobacter Arthrobacter Kocurria Micrococcus Nesterenkonia Renibactertium	J52 Gsoil 1513 PMA2201 ZOD2-24 LPD0133 PMA2305 LPB0125 IMCC25640 5187	NIBRBAC000498411 NIBRBAC000498596 NIBRBAC000498399 NIBRBAC0004985480 NIBRBAC000498521 NIBRBAC000498515 NIBRBAC000498515 NIBRBAC000498539 NIBRBAC000498558	Arthrobacter niigatensis Arthrobacter nicotinovorans Arthrobacter nicotianae Arthrobacter rhombi Kocuria carniphila Micrococcus lylae Nesterenkonia lutea Renibacterium salmoninarum	98.8 99.6 99.7 99.3 99.3 99.3 99.3	Sewage Ginseng soil Droppings of eagles Eelgrass Quid Droppings of eagles Squid Mud flat Sediment	R2A R2A MA MA MA MA MA R2A R2A	30°C, 2 days 25°C, 3 days 20°C, 2 days 24°C, 3 days 24°C, 2 days 20°C, 2 days 25°C, 1 day 30°C, 2 days 30°C, 3 days 30°C, 2 days
	Promicromonosporaceae	-		NIBRBAC000498393	Cellulosimicrobium cellulans	6.66	Droppings of spoonbills	MA	37°C, 2 days
Micromonosporales	Sanguibacteraceae Micromonosporaceae	Oerskovia Micromonospora	YC4-25 Gsoil 1174	NIBRBAC000498651 NIBRBAC000498604	Oerskovia turbata Micromonospora echinospora	9.99 9.66	Yongcheon Cave Ginseng soil	NA R2A	30°C, 4 days 25°C, 3 days
Propionibacteriales	Nocardioidaceae	Aeromicrobium	YC3-14	NIBRBAC000498650	Aeromicrobium fastidiosum	98.8	Yongcheon Cave	ISP2	30°C, 5 days
Streptomycetales	Streptomycetaceae	Kitasatospora Streptomyces Streptomyces	MMS16-CNU292 Gsoil 224 UL451 MMS16-CNU183	NIBRBAC000498624 NIBRBAC000498585 NIBRBAC000498617 NIBRBAC000498623	Kitasatospora kifunensis Streptomyces durhamensis Streptomyces kurssanovii Streptomyces blastmyceticus	99 99.3 99.9	Soil Ginseng soil Soil Soil	SCA (pH5) R2A TSA ISP2 (pH5)	30°C, 3 days 25°C, 3 days 30°C, 2 days 30°C, 3 days
Streptosporangiales	Nocardiopsaceae	Nocardiopsis	LPB0126	NIBRBAC000498516	Nocardiopsis symemataformans	7.66	Squid	MA	25°C, 1 day
	Streptosporangiaceae	Nonomuraea	Gsoil 1046	NIBRBAC000498588	NIBRBAC000498588 Nonomuraea harbinensis	9.66	Ginseng soil	K2A	25°C, 3 days

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

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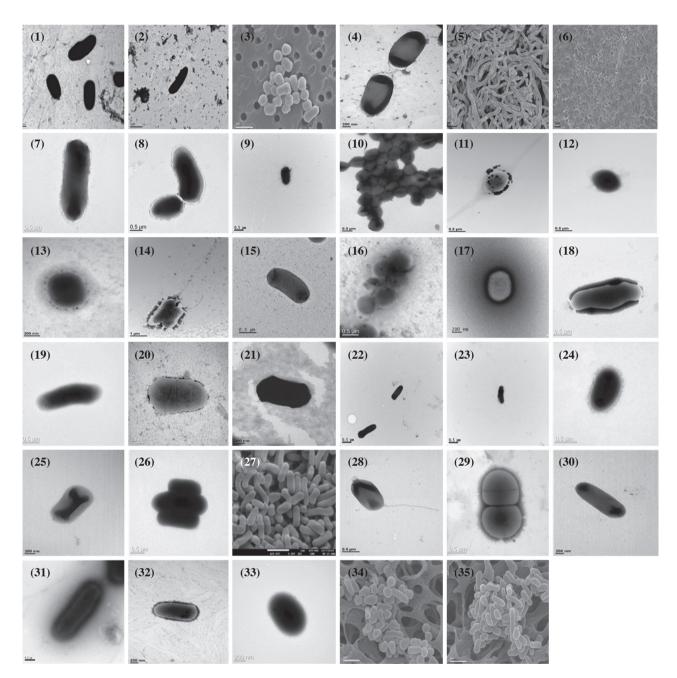


Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, C1-45; 2, C6-3; 3, BE5-3; 4, LPB0127; 5, YC6-12; 6, YC7-20; 7, AR23308; 8, YHJ2; 9, JGM01; 10, LPB0135; 11, 6228; 12, 10038; 13, Gsoil 608; 14, 5189; 15, UL003; 16, HMF7422; 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; Cellu*losimicrobium* (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-*

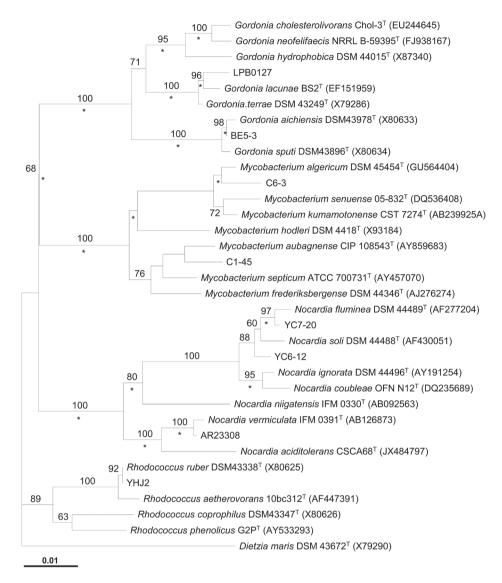


Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the orders *Corynebacteriales* in the class *Actinobacteria*. Asterisks indicate that the corresponding branches were also recovered 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 *Propionibac*- *teriales*, 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,

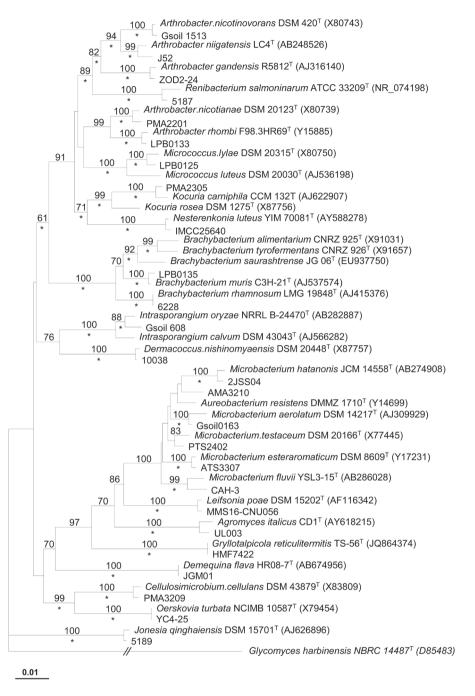


Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the order *Micrococcales* in the class *Actinobacteria*. Asterisks indicate that the corresponding branches were also recovered in 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 (=NI-BRBA000498661) 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

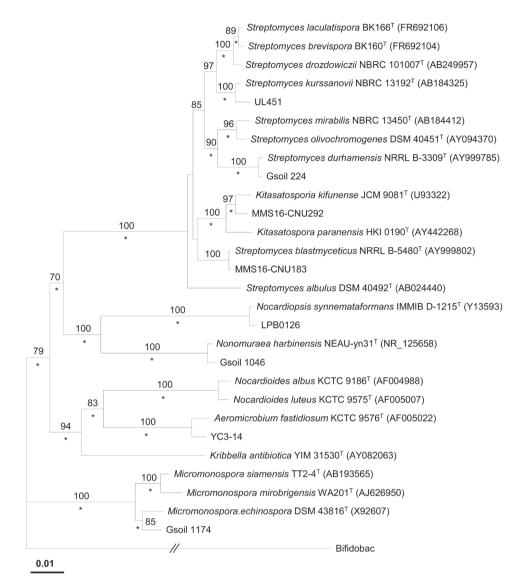


Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the orders *Micromonosporales*, *Propionibacteriales*, *Streptomycetales* and *Streptosporangiales* in the class *Actinobacteria*. 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 (=NI-BRBA000498653) 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 (=NI-BRBAC000498431) 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 (=NI-BRBAC000498577) 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 (=NI-BRBAC000498587) 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 (=NI-BRBAC000498569) 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 (=NI-BRBA000498381) 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 (=NI-BRBAC000498599) 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 (=NI-BRBA000498635) 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 (=NI-BRBAC000498480) 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 (=NI-BRBA000498651) 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 (= NI-BRBAC000498585) 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 (=NI-BRBAC000498516) 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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