

## A report of 34 unrecorded bacterial species in Korea, belonging to the *Actinobacteria*

Kwan Su Ko<sup>1</sup>, Chang-Jun Cha<sup>2</sup>, Wan-Taek Im<sup>3</sup>, Seung-Bum Kim<sup>4</sup>, Chi-Nam Seong<sup>5</sup>, Jin-Woo Bae<sup>6</sup>, Kwangyeop Jahng<sup>7</sup>, Jang-Cheon Cho<sup>8</sup>, Ki-seong Joh<sup>9</sup> and Soon Dong Lee<sup>1,\*</sup>

<sup>1</sup>Faculty of Science Education, Jeju National University, Jeju 63243, Republic of Korea

<sup>2</sup>Department of Biotechnology, Chung-Ang University, Anseong 17546, Republic of Korea

<sup>3</sup>Department of Biotechnology, Hankyong National University, Anseong 17579, Republic of Korea

<sup>4</sup>Department of Microbiology and Molecular Biology, Chungnam National University, Daejeon 34134, Republic of Korea

<sup>5</sup>Department of Biology, Sunchon National University, Suncheon 57922, Republic of Korea

<sup>6</sup>Department of Biology, Kyung Hee University, Seoul 02447, Republic of Korea

<sup>7</sup>Department of Life Sciences, Chonbuk National University, Jeonju 54896, Republic of Korea

<sup>8</sup>Department of Biological Sciences, Inha University, Incheon 22212, Republic of Korea

<sup>9</sup>Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies, Gyeonggi 17035, Republic of Korea

\*Correspondent: sdlee@jejunu.ac.kr

As a subset study to discover indigenous prokaryotic species in Korea in 2014, a total of 34 bacterial strains assigned to the phylum *Actinobacteria* were isolated from various environmental samples collected from activate sludge, biotite, freshwater, gut of marine organisms, mud flat, sediment, soil, spent mushroom compost and sea water. On the basis of high 16S rRNA gene sequence similarity and a tight phylogenetic association with the closest species, it was revealed that each strain was assigned to independent and previously described bacterial species, with the exception of one isolate. There is no official report that these 34 species included in the phylum *Actinobacteria* have been described in Korea: 6 species of 5 genera in the order *Corynebacteriales*, 1 species of 1 genus in the order *Frankiales*, 2 species of 2 genera in the *Micromonosporales*, 14 species of 10 genera in *Micrococcales*, 2 species of 2 genera in the *Propionibacteriales*, 1 species of 1 genus in the *Pseudonocardiales*, 4 species of 2 genera in the *Streptomycetales*, 2 species of 2 genera in the *Streptosporangiales* and 1 species of 1 genus in the *Solirubrobacterales*. Gram reaction, cell and colony morphology, pigmentation, physiological characteristics, isolation sources and strain IDs are described in the section of species description.

Keywords: 16S rRNA gene, *Actinobacteria*, indigenous prokaryotic species, unrecorded species

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DOI:10.12651/JSR.2017.6.1.001

### INTRODUCTION

The recently proposed phylum *Actinobacteria* (Ludwig *et al.*, 2012) is composed of aerobic, Gram-staining-positive bacteria that have high-G+C contents in their genomic DNAs. They exhibit a very wide range of morphology and form cocci, rods or fragmented or branched hyphae depending on species. Some of them produce resting structure such as spores and sporangium on permanent, well-developed mycelium (Goodfellow and Cross, 1984; Ensign, 1992).

The phylum *Actinobacteria* is well supported by analyses of the 16S and 23S rRNA, presence of conserved

insertions and deletions in certain proteins, and characteristic gene rearrangements (Goodfellow and Fiedler, 2010) and encompasses six classes *Actinobacteria*, *Acidimicrobiia*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria* and *Thermoleophilia* (Ludwig *et al.*, 2012).

The class *Actinobacteria* is composed of 15 orders *Actinomycetales*, *Actinopolysporales*, *Bifidobacteriales*, *Catenulisporales*, *Corynebacteriales*, *Frankiales*, *Glycomycetales*, *Jiangellales*, *Kineosporiales*, *Micromonosporales*, *Micrococcales*, *Propionibacteriales*, *Pseudonocardiales*, *Streptomycetales* and *Streptosporangiales*. Members of this class have been received considerable

concerns as the source of antibiotics since the discovery of actinomycin from actinomycetes (Waksman and Woodruff, 1940) and widely distributed in natural environment such as soil, fresh or sea water, manure and compost where contribute significantly to turnover of complex biopolymers as the decomposer (Williams *et al.*, 1984).

The class *Thermoleophila* contains two orders *Solirubrobacterales* and *Thermoleophilales*; the former consists of the families *Solirubrobacteraceae*, *Conexibacteraceae* and *Patulibacteraceae*, while the latter comprises the family *Thermoleophilaceae* (Reddy and Garcia-Pichel, 2009; Ludwig *et al.*, 2012).

We collected diverse environmental samples for bacterial isolation and recovered a great number of indigenous bacterial species in Korea by the research program supported by NIBR (National Institute of Biological Resources) in 2014. The aim of present study is to deal with the classification and identification of bacterial strains assigned to the phylum *Actinobacteria* which have not been previously reported in Korea and to describe 34 unrecorded bacterial species belonging to 9 orders of the two classes *Actinobacteria* and *Thermoleophila*.

## MATERIALS AND METHODS

A total of 34 bacterial strains which were assigned to the phylum *Actinobacteria* were isolated from various environmental samples collected from activate sludge, biotite, freshwater, gut of marine organisms, mud flat, sediment, soil, spent mushroom compost and sea water (Table 1). Treatment of environmental samples and bacterial isolation was done independently in several laboratories. The pure cultures of isolated bacteria were grown on diverse 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 and 4 media (Shirling & Gottlieb, 1966) at 15-30°C for 2-7 day, depending on the strains. The designated strain IDs, isolation sources, culture media, and incubation conditions are summarized in Table 1. The pure cultures were maintained as 10-20% glycerol suspensions at -80°C and lyophilized ampoules.

Cell morphology was observed by either transmission or scanning electron microscopy. Gram staining was performed using a Gram-staining kit according to the instructions of the manufacturer. Colony morphology and pigmentation were observed on agar plates with cells grown to stationary phase. Utilization of carbon sources and some biochemical properties were examined by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification and

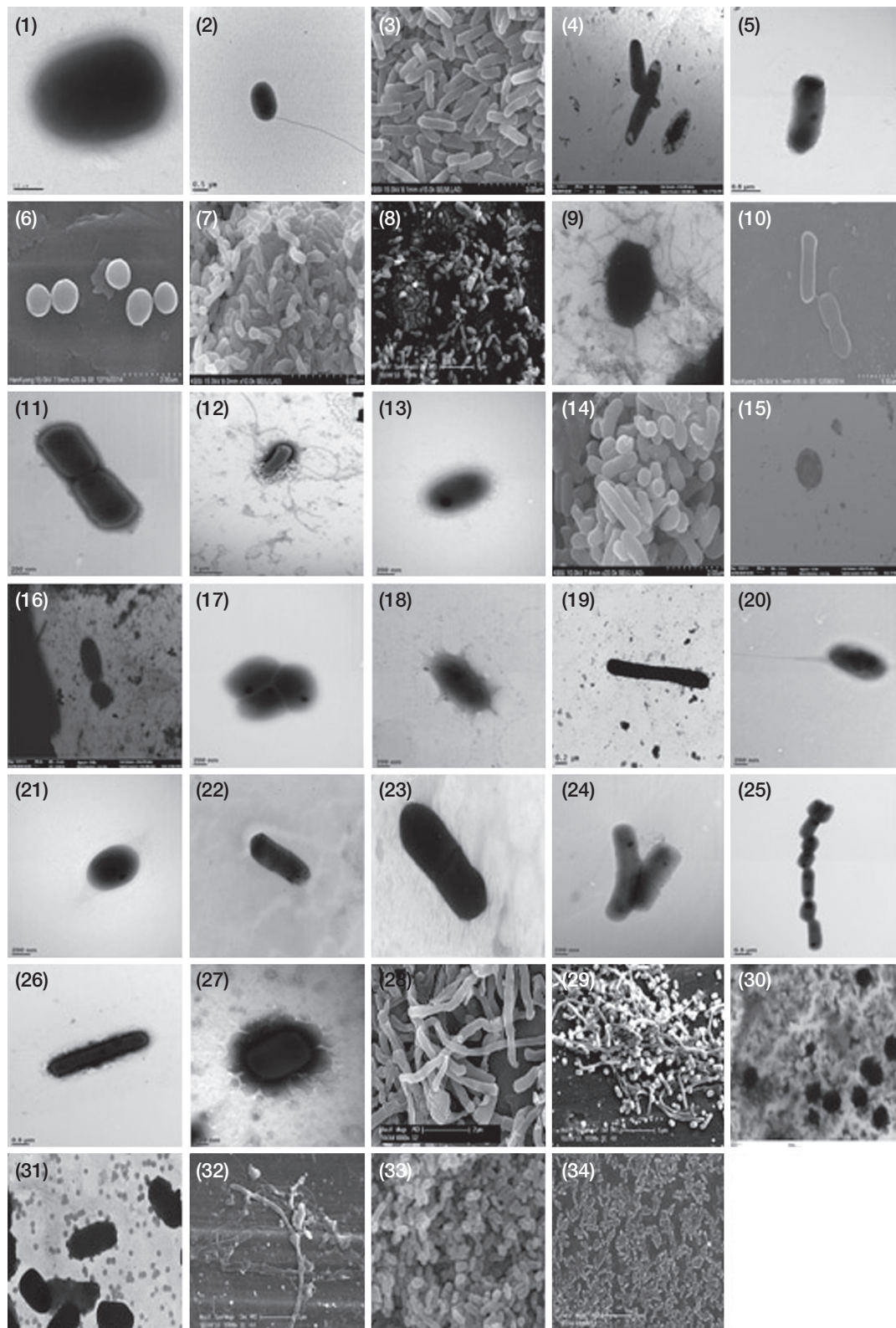
sequencing of the 16S rRNA gene were performed using the standard procedures described elsewhere. The 16S rRNA gene sequences of the strains assigned to the phylum *Actinobacteria* were compared with the corresponding sequences collected from and the EzTaxon-e server (Kim *et al.*, 2012) and public database. Multiple alignments of the sequences were performed using the Clustal\_X program (Thompson *et al.*, 1997) and optimized manually according to the secondary structure of *Escheichia coli*. Evolutionary distances were calculated using the correction of Jukes & Cantor (1969). Phylogenetic analyses were performed by using the neighbor-joining (Saitou and Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) treeing algorithms contained in the PHYLIP package (Felsenstein, 2008). A neighbour-joining tree was drawn with bootstrap values based on 1,000 replications (Felsenstein, 1985).

## RESULTS AND DISCUSSION

The 34 strains were found to belong to the two classes *Actinobacteria* (33 strains) and *Thermoleophila* (1 strains) in the phylum *Actinobacteria*. Among them, 33 strains were distributed in 8 orders of the *Actinobacteria*; 6 strains for the order *Corynebacteriales*, 1 strain for the *Frankiales*, 2 strains for the *Micromonosporales*, 14 strains for the *Micrococcales*, 2 strain for the *Propionibacteriales*, 1 strain for the *Pseudonocardiales*, 5 strains for the *Streptomycetales* and 2 strains for the *Streptosporangiales*. One strain which belonged to the class *Thermoleophila* was assigned to the *Solirubrobacterales* (Table 1). These strains were Gram-staining-positive and chemoheterotrophic and were morphologically characterized by the formation of cocci, rods or mycelia (Fig. 1). The strains of the order *Corynebacteriales* (Fig. 2) were assigned to 5 genera of 3 families: *Dietzia* (1 species) the family *Dietziaceae*, *Mycobacterium* (2 species) of the *Mycobacteriaceae*, *Gordonia* (1 species), *Nocardia* (1 species) and *Rhodococcus* (1 species) of the *Nocardiaceae*. In general, members of the genus *Nocardia* are morphologically characterized by fragmentation of mycelium, in contrast to other genera of the family *Nocardiaceae* having coccoid- or rod-shaped morphology (Goodfellow and Maldonado, 2012). The *Frankiales* strain (Fig. 2) was affiliated to the genus *Geodermatophilus* (1 species) of the family *Geodermatophilaceae*. The 2 *Micromonosporales* strains were distributed to two genera *Actinocatenispora* (1 species) and *Micromonospora* (1 species) of the family *Micromonosporaceae*, which were morphologically characterized by the formation of mycelia (Fig. 1). The phylogenetic relationships between the *Micrococcales* strains and their closest relatives are

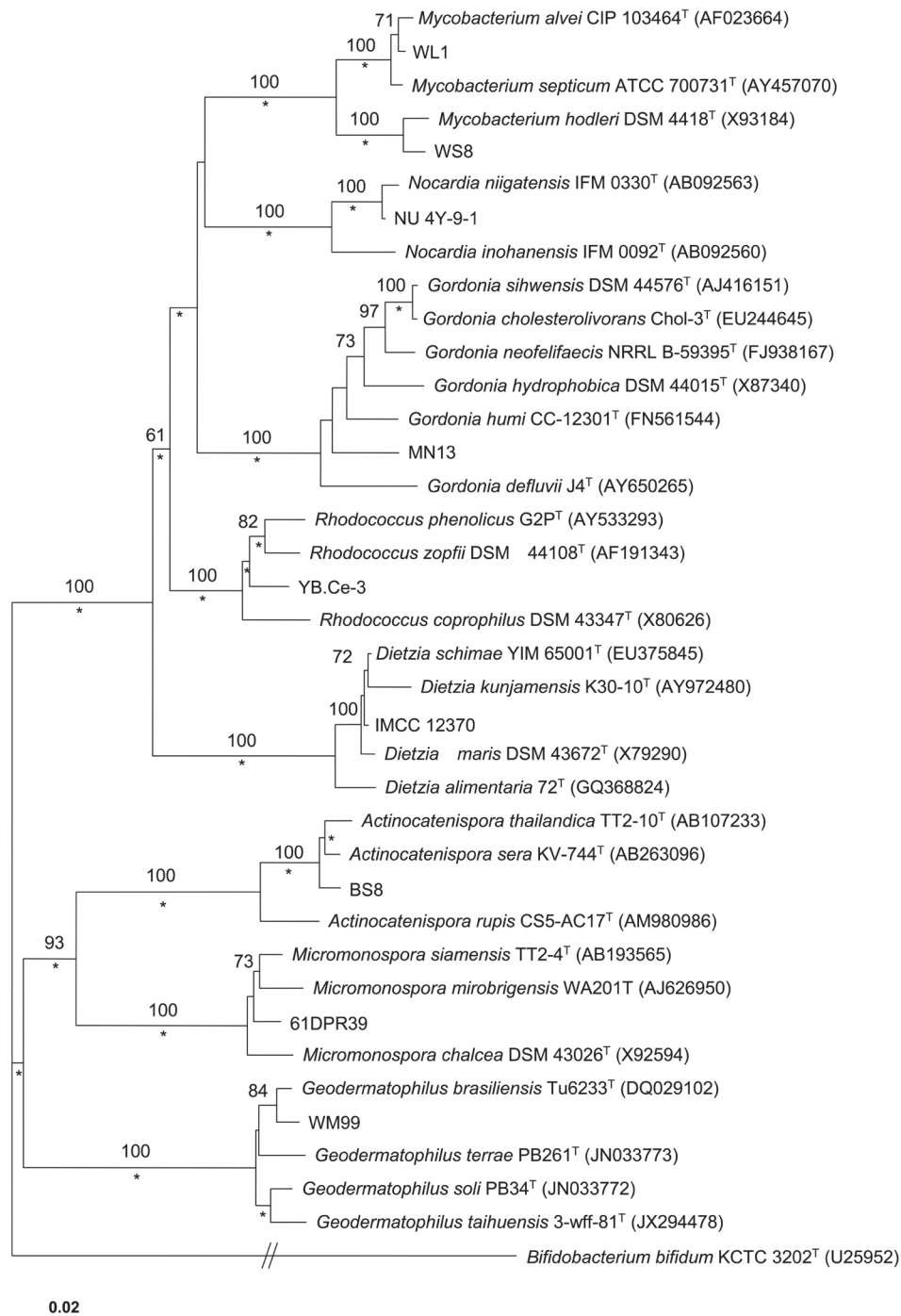
**Table 1.** Summary of the isolated strains belonging to the Actinobacteria and their taxonomic affiliations.

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species (Name of type strain)	Similarity (%)	Isolation source	Medium	Incubation conditions
Class Actinobacteria Corynebacteriales	Dietziaceae	<i>Dietzia</i>	IMCC 12370	NIBRBA0000114860	<i>Dietzia schimae</i>	99.8%	Seawater	MA	15°C, 3 days
	Mycobacteriaceae	<i>Mycobacterium</i>	WL1	NIBRBA0000114765	<i>Mycobacterium septicum</i>	99.7%	Freshwater	R2A	25°C, 2 days
		<i>Mycobacterium</i>	WS80	NIBRBA0000115031	<i>Mycobacterium hodleri</i>	99.2%	Freshwater	R2A	25°C, 3 days
		<i>Gordonia</i>	MN13	NIBRBA0000114947	<i>Gordonia neofelifaecis</i>	98.2%	Gut of <i>Mugil cephalus</i>	MA	15°C, 3 days
Frankiales Micromonosporales	Nocardiaceae	<i>Nocardia</i>	NU 4Y-9-1	NIBRBA0000114874	<i>Nocardia nitgatensis</i>	100%	Ginseng soil	TSA	30°C, 3 days
		<i>Rhodococcus</i>	YB.Ce-3	NIBRBA0000114843	<i>Rhodococcus phenolicus</i>	98.9%	Activated sludge	R2A	30°C, 2 days
	Geodermatophilaceae	<i>Geodermatophilus</i>	WM99	NIBRBA0000115029	<i>Geodermatophilus terrae</i>	98.8%	Freshwater	R2A	25°C, 3 days
	Micromonosporaceae	<i>Actinocatenispora</i>	B58	NIBRBA0000114830	<i>Actinocatenispora sera</i>	99.0%	Spent mushroom compost	R2A	30°C, 2 days
Micrococcales	Brevibacteriaceae	<i>Micromonospora</i>	61DPR39	NIBRBA0000114798	<i>Micromonospora siamensis</i>	99.1%	Freshwater	R2A	25°C, 7 days
		<i>Brevibacterium</i>	Ho-02	NIBRBA0000114825	<i>Brevibacterium casei</i>	100%	Activated sludge	R2A	30°C, 2 days
	Cellulomonadaceae	<i>Cellulomonas</i>	RMD 3Y-15-4	NIBRBA0000114878	<i>Brevibacterium epidermidis</i>	99.1%	Ginseng soil	R2A	30°C, 2 days
		<i>Cellulomonas</i>	HMF2762	NIBRBA0000115002	<i>Cellulomonas cellasea</i>	99.2%	Sediment	R2A	30°C, 5 days
Dermabacteraceae	Cellulomonas	<i>Cellulomonas</i>	NK 4Y-9-3	NIBRBA0000114888	<i>Cellulomonas biozotea</i>	99.9%	Ginseng soil	R2A	30°C, 2 days
		<i>Cellulomonas</i>	WW28	NIBRBA0000115028	<i>Cellulomonas persica</i>	99.0%	Freshwater	R2A	25°C, 3 days
	<i>Brachybacterium</i>	EgT0207	NIBRBA0000114942	<i>Brachybacterium saurashitrense</i>	98.2%	Gut of <i>Fulvia mutica</i>	TSA	25°C, 3 days	
	<i>Ornithinimicrobium</i>	KHS04	NIBRBA0000114950	<i>Ornithinimicrobium murale</i>	96.3%	Gut of <i>Todarodes pacificus</i>	NA	25°C, 3 days	
Micrococcales Micrococcaceae	Arthrobacter	<i>Arthrobacter</i>	NK 6Y-6-4	NIBRBA0000114890	<i>Arthrobacter chlorophenolicus</i>	99.3%	Ginseng soil	R2A	30°C, 2 days
		<i>Arthrobacter</i>	NS 4Y-8-4	NIBRBA0000114873	<i>Arthrobacter pascens</i>	99.3%	Ginseng soil	R2A	30°C, 2 days
	Kocuria	<i>Kocuria</i>	145-10	NIBRBA0000114763	<i>Kocuria rosea</i>	99.8%	Freshwater	TSA	25°C, 2 days
		<i>Micrococcus</i>	AX5	NIBRBA0000114894	<i>Micrococcus antarcticus</i>	99.7%	Mud flat	R2A	30°C, 2 days
Micrococcales Microbacteriaceae	Sinomonas	<i>Sinomonas</i>	NGS 3Y-15-2	NIBRBA0000114885	<i>Sinomonas flava</i>	99.9%	Ginseng soil	R2A	30°C, 2 days
		<i>Diaminobutyricibacter</i>	R1-6	NIBRBA0000114812	<i>Diaminobutyricibacter tongyongensis</i>	99.8%	Soil	R2A	30°C, 7 days
	Promicromonosporaceae	<i>Luteimicrobium</i>	NI-9	NIBRBA0000114811	<i>Luteimicrobium subarcticum</i>	99.2%	Soil	NA	30°C, 7 days
		<i>Kribbella</i>	RK 4Y-2-4	NIBRBA0000114877	<i>Kribbella antibiotica</i>	99.4%	Ginseng soil	R2A	30°C, 2 days
Pseudonocardiales Pseudonocardiaceae	Nocardioideae	<i>Nocardioideae</i>	RS 4Y-2-4	NIBRBA0000114880	<i>Nocardioideae albus</i>	99.5%	Ginseng soil	ISP4	30°C, 2 days
		<i>Kutzneria</i>	RMD 3Y-3-1	NIBRBA0000114879	<i>Kutzneria buriramensis</i>	99.5%	Ginseng soil	ISP4	30°C, 2 days
	Streptomycetales Streptomycetaceae	<i>Kitasatospora</i>	NU 4Y-9-4	NIBRBA0000114875	<i>Kitasatospora paranensis</i>	99.2%	Ginseng soil	ISP4	30°C, 3 days
		<i>Streptomyces</i>	BBT1	NIBRBA0000114837	<i>Streptomyces drozdowiczii</i>	99.0%	Powder of biotite	R2A	30°C, 2 days
Streptosporangiales Streptosporangiaceae	Streptomyces	<i>Streptomyces</i>	BS22	NIBRBA0000114835	<i>Streptomyces thermocoprophilus</i>	98.9%	Spent mushroom compost	R2A	30°C, 2 days
		<i>Streptomyces</i>	II-6	NIBRBA0000114809	<i>Streptomyces olivochromogenes</i>	99.0%	Soil	ISP2	30°C, 7 days
	Actinomadura	<i>Streptomyces</i>	TI-6	NIBRBA0000114816	<i>Streptomyces tsukubensis</i>	99.1%	Soil	TSA	30°C, 7 days
		<i>Streptosporangium</i>	WM35	NIBRBA0000115020	<i>Streptosporangium amethystogenes</i>	99.3%	Freshwater	R2A	25°C, 7 days
Class Thermoleophila Solirubrobacteriales	Thermomonosporaceae	<i>Actinomadura</i>	7C-18	NIBRBA0000114839	<i>Actinomadura bangladeshensis</i>	99.3%	Activated sludge	R2A	30°C, 2 days
	Conexibacteraceae	<i>Conexibacter</i>	BS10	NIBRBA0000114831	<i>Conexibacter arvalis</i>	98.9%	Spent mushroom compost	R2A	30°C, 4 days



**Fig. 1.** Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, IMCC 12370; 2, WL1; 3, WS80; 4, MN13; 5, NU 4Y-9-1; 6, YB.Ce-3; 7, WM99; 8, BS8; 9, 61DPR39; 10, Ho-02; 11, RMD 3Y-15-4; 12, HMF2762; 13, NK 4Y-9-3; 14, WW28; 15, EgT0207; 16, KHS04; 17, NK 6Y-6-4; 18, NS 4Y-8-4; 19, 145-10; 20, AX5; 21, NGS 3Y-15-2; 22, R1-6; 23, N1-9; 24, RK 4Y-2-4; 25, RS 4Y-2-4; 26, RMD 3Y-3-1; 27, NU 4Y-9-4; 28, BBT1; 29, BS22; 30, I1-6; 31, T1-6; 32, 7C-18; 33, WM35; 34, BS10.

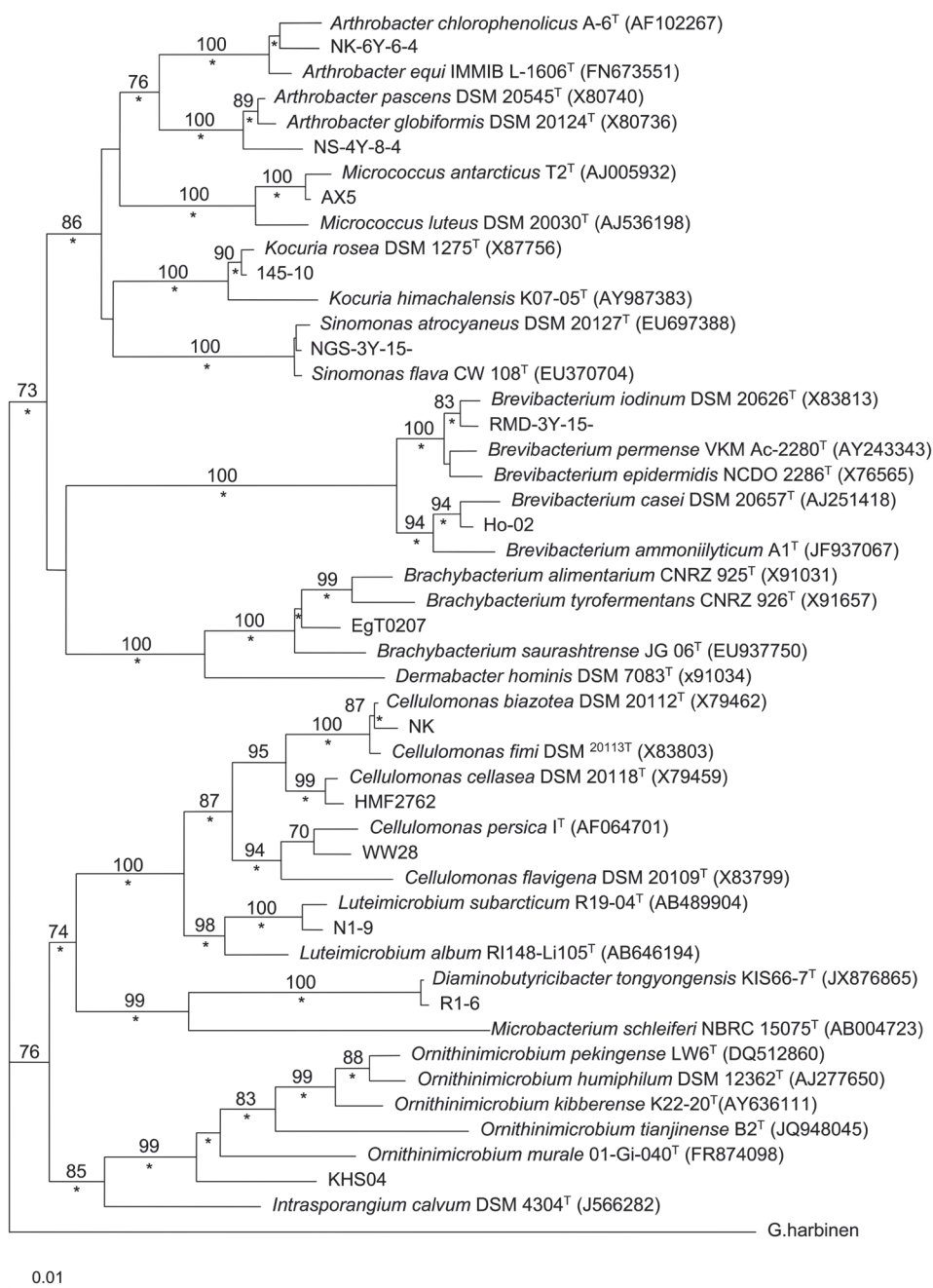




**Fig. 2.** Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between the strains isolated in this study and their relatives of the orders *Corynebacteriales*, *Frankiales* and *Micromonosporales* in the class *Actinobacteria*. Bootstrap values (>70%) are shown at the branching points. Asterisks indicate that the corresponding branches were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.

given in Fig. 3. The 14 strains belonged to the 9 genera of 7 families: the genus *Brevibacterium* (2 species) of the family *Brevibacteriaceae*, *Cellulomonas* (3 species) of the *Cellulomonadaceae*, *Brachybacterium* (1 species) of

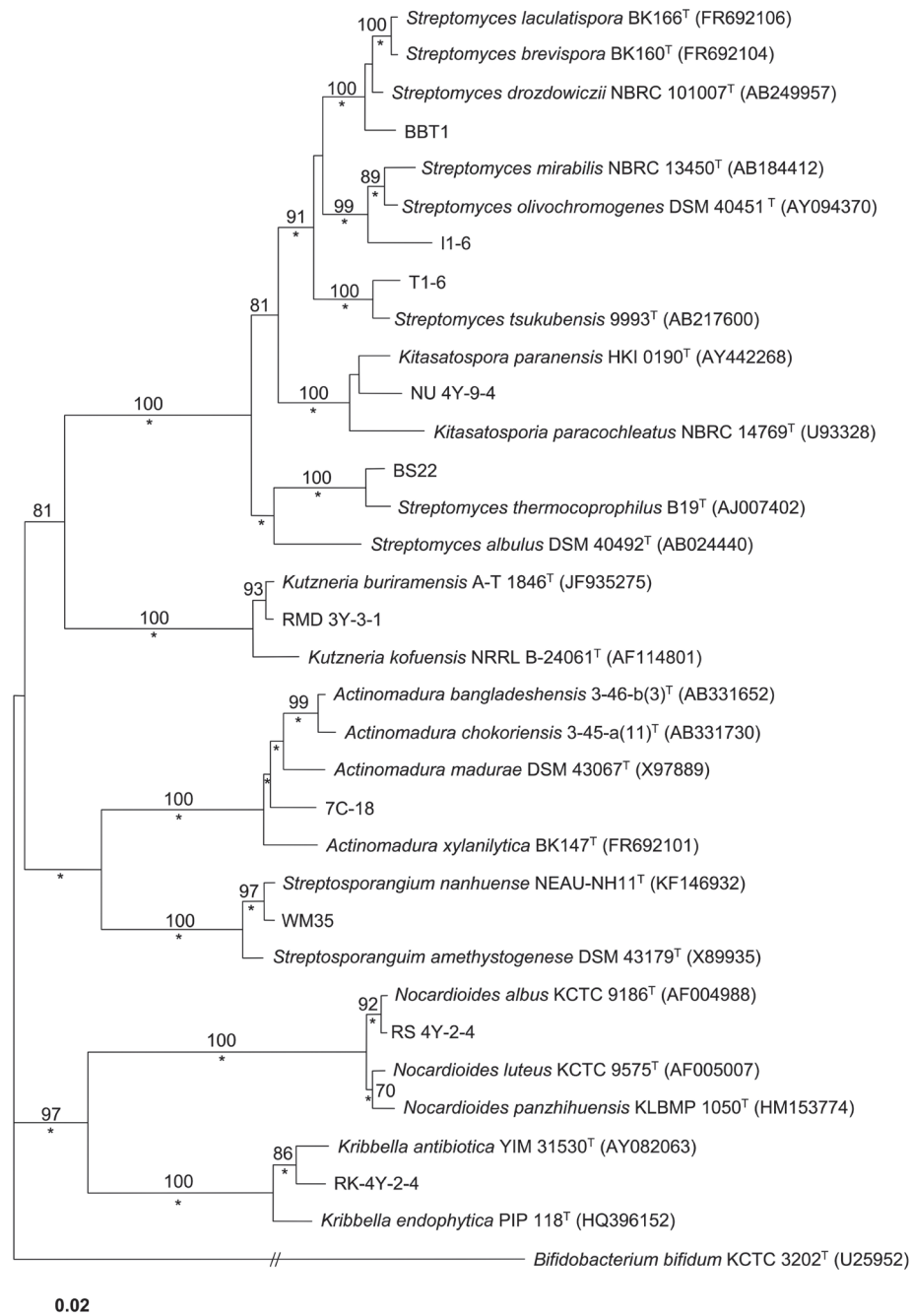
the *Dermabacteraceae*, *Ornithinimicrobium* (1 species) of the *Intrasporangiaceae*, *Arthrobacter* (2 species), *Kocuria* (1 species), *Micrococcus* (1 species) and *Sinomonas* (1 species) of the *Micrococcaceae*, *Diaminobutyricibacter*



**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*. Bootstrap values (>70%) are shown at the branching points. Asterisks indicate that the corresponding branches were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.

(1 species) of the *Microbacteriaceae* and *Luteimicrobium* (1 species) of the *Promicromonosporaceae*. Among them, the strain belonging to the family *Intrasporangiaceae* showed low 16S rRNA gene sequence similarity (96.20%) to the closest species *Ornithinimicrobium murale* (Table 1), suggesting that this isolate represents a novel taxon at the taxonomic ranks of species and genus.

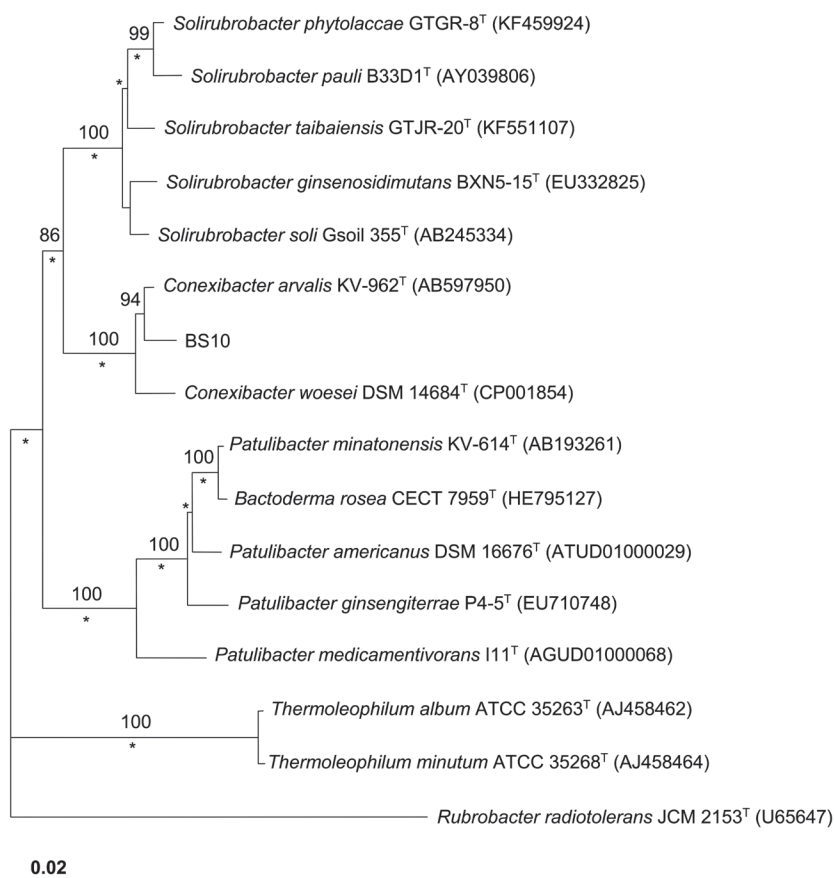
Fig. 4 shows phylogenetic affiliations of the 10 strains of the orders *Propionibacteriales*, *Pseudonocardiales*, *Streptomycetales* and *Streptosporangiales*. The 2 strains belonged to the orders *Propionibacteriales*; each strain was assigned to the genera *Kribbella* and *Nocardioides* of the family *Nocardioidaceae*, respectively. The order *Pseudonocardiales* contained only 1 strain assigned to the



**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 *Propionibacteriales*, *Pseudonocardiales*, *Streptomycetales* and *Streptosporangiales* in the class *Actinobacteria*. Bootstrap values (>70%) are shown at the branching points. Asterisks indicate that the corresponding branches were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.

genus *Kutzneria* of the family *Pseudonocardiaceae*. The order *Streptomycetales* consisted of 5 isolates that were affiliated to the genera *Kitasatospora* (1 species) and *Streptomyces* (3 species) of the family *Streptomycataceae*, some of which formed branched mycelia (Fig. 1). Members of the family *Streptomycataceae* are morphologi-

cally characterized by the formation of well-developed, branched hypha. Most of them further produced the chains of spores with the various surface ornamentations borne on the tips of branched mycelia (Kämpfer, 2012a; 2012b). The 2 strains of the order *Streptosporangiales* were assigned to the genus *Actinomadura* of the family



**Fig. 5.** 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 *Solirubrobacterales* in the class *Thermoleophilia*. Bootstrap values (>70%) are shown at the branching points. Asterisks indicate that the corresponding branches were also recovered in both the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.

*Thermomonosporaceae* and the genus *Streptosporangium* of the family *Stereosporangiaceae*, respectively. These strains were also characterized by the formation of branched mycelium (Fig. 1). Members of the genera *Actinoadura* and *Streptosporangium* are further characterized by the formation of short chains of spores and globose sporangia, respectively (Trujillo and Goodfellow, 2012; Quintana and Goodfellow, 2012). Lastly, 1 strain were found to belong to the class *Thermoleophilia* and further assigned to the genus *Conexibacter* of the *Conexibacteraceae* in the order *Solirubrobacterales* (Fig. 5).

#### Description of *Dietzia schimae* IMCC 12370

Cells are Gram-staining-positive, non-flagellated, non-pigmented and short rod-shaped. Colonies are circular, convex, smooth and white-colored after 3 days of incubation on MA at 15°C. Positive for nitrate reduction in API 20NE but negative for indole production, glu-

cose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and  $\beta$ -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 IMCC 12370 (=NIBRBA0000114860) has been isolated from seawater, Sokcho, Korea.

#### Description of *Mycobacterium septicum* WL1

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, raised, entire and white-colored after 2 days on R2A at 25°C. Positive for nitrate reduction, glucose fermentation, urease and  $\beta$ -galactosidase in API 20NE but negative for indole production, arginine dihydrolase, esculin hydrolysis 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 and adipate. Strain WL1 (=NIBRBA0000114765) has



been isolated from freshwater, Jeonju, Korea.

#### **Description of *Mycobacterium hodleri* WS80**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, smooth and orange-colored after 3 days on R2A at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase in API 20NE. Gluconate is utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, caprate, adipate, malate, citrate and phenylacetate. Strain WS80 (= NIBRBA0000115031) has been isolated from freshwater, Changnyeong, Korea.

#### **Description of *Gordonia neofelifaecis* MN13**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and cream-colored after 3 days on MA at 15°C. Positive for  $\beta$ -galactosidase in API 20NE but negative for indole production, arginine dihydrolase, esculin hydrolysis and gelatinase. D-Glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine, gluconate and adipate are utilized. Does not utilize D-mannose, D-maltose, caprate, malate, citrate and phenylacetate. Strain MN13 (= NIBRBA0000114947) has been isolated from gut of mugil cephalus, Korea.

#### **Description of *Nocardia niigatensis* NU 4Y-9-1**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are punctiform dry, flat and orange-colored after 3 days on TSA at 30°C. Positive for esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. L-Arabinose, N-acetyl-glucosamine, gluconate and malate are utilized. Does not utilize D-glucose, D-mannitol, D-mannose, D-maltose, caprate, adipate, citrate and phenylacetate. Strain NU 4Y-9-1 (= NIBRBA0000114874) has been isolated from ginseng soil, Anseong, Korea.

#### **Description of *Rhodococcus phenolicus* YB.Ce-3**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and coccus or coccoid-rod-shaped. Colonies are circular, raised, entire and pale-yellow-colored after 2 days on R2A at 30°C. Positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative for indole production, glucose fermentation and gelatinase. N-Acetyl-glucosamine and adipate are utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, D-maltose, gluconate, caprate, malate, citrate and phenyl-

acetate. Strain YB.Ce-3 (= NIBRBA0000114843) has been isolated from activated sludge, Daejeon, Korea.

#### **Description of *Geodermatophilus terrae* WM99**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, smooth and pink-colored after 3 days on R2A at 25°C. Positive for esculin hydrolysis and nitrate reduction (weak) but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannose, D-maltose and malate are utilized. Does not utilize D-mannitol, N-acetyl-glucosamine, gluconate, caprate, adipate, citrate and phenylacetate. Strain WM99 (= NIBRBA0000115029) has been isolated from freshwater, Changnyeong, Korea.

#### **Description of *Actinocatenispora sera* BS8**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and branched mycelium-forming. Colonies are lenticular, raised, entire and white-colored after 2 days on R2A at 30°C. Positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative indole production, glucose fermentation and gelatinase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, adipate, malate and citrate are utilized. Does not utilize caprate and phenylacetate. Strain BS8 (= NIBRBA0000114830) has been isolated from spent mushroom compost, Yesan, Korea.

#### **Description of *Micromonospora siamensis* 61DPR39**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are punctiform, digging and orange-colored on R2A at 25°C. Positive for esculin hydrolysis, gelatinase and  $\beta$ -galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, D-mannitol, D-mannose, D-maltose and malate are utilized. Does not utilize L-arabinose, N-acetyl-glucosamine, gluconate, caprate, adipate, citrate and phenylacetate. Strain 61DPR39 (= NIBRBA0000114798) has been isolated from freshwater, Daejeon, Korea.

#### **Description of *Brevibacterium casei* Ho-02**

Cells are Gram-staining-negative, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, raised, undulate and white-yellow-colored after 2 days on R2A at 30°C. Positive for gelatinase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and  $\beta$ -galactosidase. D-Glucose, D-mannitol,

N-acetyl-glucosamine, D-maltose, gluconate, adipate, malate, citrate and phenylacetate are utilized. Does not utilize L-arabinose, D-mannose and caprate. Strain HO-02 (= NIBRBA0000114825) has been isolated from activated sludge, Daejeon, Korea.

#### **Description of *Brevibacterium epidermidis* RMD 3Y-15-4**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, entire, convex and pale-yellow-colored after 2 days on R2A at 30°C. Positive for glucose fermentation and gelatinase in API 20NE but negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis and  $\beta$ -galactosidase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate, phenylacetate are utilized. Strain RMD 3Y-15-4 (= NIBRBA0000114878) has been isolated from ginseng soil, Anseong, Korea.

#### **Description of *Cellulomonas cellasea* HMF2762**

Cells are Gram-staining-positive, flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and yellow-colored after 5 days on R2A at 30°C. Positive for nitrate reduction and esculin hydrolysis in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and  $\beta$ -galactosidase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate are not utilized. Strain HMF2762 (= NIBRBA0000115002) has been isolated from sediment, Taebaek, Korea.

#### **Description of *Cellulomonas biazotea* NK 4Y-9-3**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, entire, raised and yellow-colored after 2 days on R2A at 30°C. Positive for esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, L-arabinose, D-mannitol, N-acetyl-glucosamine, D-maltose and gluconate are utilized. Does not utilize D-mannose, caprate, adipate, malate, citrate and phenylacetate. Strain NK 4Y-9-3 (= NIBRBA0000114888) has been isolated from ginseng soil, Anseong, Korea.

#### **Description of *Cellulomonas persica* WW28**

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

light-yellow-colored after 3 days on R2A at 25°C. Positive for nitrate reduction and esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease 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 WW28 (= NIBRBA0000115028) has been isolated from freshwater, Changnyeong, Korea.

#### **Description of *Brachybacterium saurashtrense* EgT0207**

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular and cream-colored after 3 days on TSA at 25°C. Positive for nitrate reduction, esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease and 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 EgT0207 (= NIBRBA0000114942) has been isolated from gut of *fulvia mutica*, Korea.

#### **Description of *Ornithinimicrobium murale* KHS04**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and cream-colored after 3 days on NA at 25°C. Positive for esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, D-mannitol, D-mannose, D-maltose and citrate are utilized. Does not utilize L-arabinose, N-acetyl-glucosamine, gluconate, caprate, adipate, malate and phenylacetate. Strain KHS04 (= NIBRBA0000114950) has been isolated from gut of *Todarodes pacificus*, Korea.

#### **Description of *Arthrobacter chlorophenolicus* NK 6Y-6-4**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, entire, raised and white-colored after 3 days on NA at 30°C. Positive for esculin hydrolysis and  $\beta$ -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 and adipate. Strain NK 6Y-6-4 (= NIBRBA0000114890) has been isolated from ginseng soil, Anseong, Korea.

**Description of *Arthrobacter pascens* NS 4Y-8-4**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, entire, convex and white-colored after 2 days on R2A at 30°C. Positive for esculin hydrolysis and  $\beta$ -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 and citrate are utilized. Does not utilize caprate, adipate and phenylacetate. Strain NS 4Y-8-4 (=NIBRBA 0000114873) has been isolated from ginseng soil, Anseong, Korea.

**Description of *Kocuria rosea* 145-10**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and yellow-colored after 2 days on TSA at 25°C. Positive for nitrate reduction and  $\beta$ -galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. D-Glucose, D-maltose and malate are utilized. Does not utilize L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, gluconate, caprate, adipate, citrate and phenylacetate. Strain 145-10 (=NIBRBA 0000114763) has been isolated from freshwater, Cheongsong, Korea.

**Description of *Micrococcus antarcticus* AX5**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and pale-yellow-colored after 2 days on R2A at 30°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase in API 20NE. D-Glucose, N-acetyl-glucosamine, D-maltose and malate are utilized. Does not utilize L-arabinose, D-mannitol, D-mannose, gluconate, caprate, adipate, citrate and phenylacetate. Strain AX5 (=NIBRBA0000114894) has been isolated from mud flat, Incheon, Korea.

**Description of *Sinomonas flava* NGS 3Y-15-2**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and pale-yellow-colored after 2 days on R2A at 30°C. Positive for nitrate reduction, esculin hydrolysis and  $\beta$ -galactosidase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. D-Glucose, D-mannitol, D-mannose, D-maltose, gluconate, malate, citrate and phenylacetate are utilized. Does not utilize L-arabinose, N-acetyl-glu-

cosamine, caprate and adipate. Strain NGS 3Y-15-2 (=NIBRBA0000114885) has been isolated from Ginseng soil, Anseong, Korea.

**Description of *Diaminobutyricibacter tongyongensis* R1-6**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are circular, convex, entire and yellow-colored on R2A at 30°C. Positive for esculin hydrolysis and  $\beta$ -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, caprate, adipate, malate, citrate and phenylacetate are not utilized. Strain R1-6 (=NIBRBA0000114812) has been isolated from soil, Daejeon, Korea.

**Description of *Luteimicrobium subarcticum* N1-9**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-coccus-shaped. Colonies are circular, convex, entire and white-colored on NA at 30°C. Positive for esculin hydrolysis and  $\beta$ -galactosidase in API 20NE, weakly positive for nitrate reduction and glucose fermentation but negative for indole production, arginine dihydrolase, urease 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 N1-9 (=NIBRBA0000114811) has been isolated from soil, Daejeon, Korea.

**Description of *Kribbella antibiotica* RK 4Y-2-4**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are punctiform, dry, flat and white-colored after 2 days on R2A at 30°C. Positive for urease, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase. L-Arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine and D-maltose are utilized. Does not utilize D-glucose, gluconate, caprate, adipate, malate, citrate and phenylacetate. Strain RK 4Y-2-4 (=NIBRBA0000114877) has been isolated from ginseng soil, Anseong, Korea.

**Description of *Nocardioides albus* RS 4Y-2-4**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, raised and white-colored after 2 days on ISP4 at 30°C. Positive for esculin hydrolysis, gelatinase and  $\beta$ -galactosidase in API 20NE but negative for nitrate reduction, indole pro-

duction, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, gluconate, adipate and malate, citrate are utilized. Does not utilize D-maltose, caprate and phenylacetate. Strain RS 4Y-2-4 (= NIBRBA 0000114880) has been isolated from ginseng soil, Anseong, Korea.

#### **Description of *Kutzneria buriramensis* RMD 3Y-3-1**

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are undulate, dry, flat and white-colored after 2 days on ISP4 at 30°C. Positive for  $\beta$ -galactosidase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. L-Arabinose and malate are utilized. Does not utilize D-glucose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, citrate and phenylacetate. Strain RMD 3Y-3-1 (= NIBRBA 0000114879) has been isolated from ginseng soil, Anseong, Korea.

#### **Description of *Kitasatospora paranensis* NU 4Y-9-4**

Cells are Gram-staining-positive, non-flagellated and coccus-shaped. Colonies are circular, dry, flat and brown-colored after 3 days on ISP4 at 30°C. Positive for gelatinase and  $\beta$ -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 NU 4Y-9-4 (= NIBRBA0000114875) has been isolated from ginseng soil, Anseong, Korea.

#### **Description of *Streptomyces drozdowiczii* BBT1**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and branched mycelium-forming. Colonies are lenticular, raised, entire and white-colored after 2 days on R2A at 30°C. Positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatinase,  $\beta$ -galactosidase in API 20NE but negative for indole production and glucose fermentation. 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 BBT1 (= NIBRBA0000114837) has been isolated from powder of biotite, Yesan, Korea.

#### **Description of *Streptomyces thermocophilus* BS22**

Cells are Gram-staining-positive, non-flagellated, non-

pigmented and branched mycelium-forming. Colonies are lenticular, raised, entire and white-colored after 2 days on R2A at 30°C. Positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatinase,  $\beta$ -galactosidase in API 20NE but negative for indole production and glucose fermentation. 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 BS22 (= NIBRBA0000114835) has been isolated from spent mushroom compost, Yesan, Korea.

#### **Description of *Streptomyces olivochromogenes* I1-6**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and branched mycelium-forming. Colonies are rhizoid, penetrate and light brownish-colored on ISP2 at 30°C. Positive for esculin hydrolysis in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and  $\beta$ -galactosidase. L-Arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, adipate, malate, citrate and phenylacetate are utilized. Does not utilize D-glucose and caprate. Strain I1-6 (= NIBRBA 0000114809) has been isolated from soil, Daejeon, Korea.

#### **Description of *Streptomyces tsukubensis* T1-6**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and rod-shaped. Colonies are rhizoid, penetrate and white to creamy-colored on TSA at 30°C. Positive for nitrate reduction arginine dihydrolase and urease in API 20NE but negative for indole production, glucose fermentation, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase. D-Glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate and phenylacetate are not utilized. Strain T1-6 (= NIBRBA0000114816) has been isolated from soil, Daejeon, Korea.

#### **Description of *Actinomadura bangladeshensis* 7C-18**

Cells are Gram-staining-positive, non-flagellated, non-pigmented and branched mycelium-forming. Colonies are lenticular, raised, entire and white-colored after 2 days on R2A at 30°C. Positive for arginine dihydrolase and urease in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatinase and  $\beta$ -galactosidase. D-Glucose, D-mannose, D-maltose and gluconate are utilized. Does not utilize L-arabinose, D-mannitol, N-acetyl-glucosamine, caprate, adipate, malate, citrate and phenylacetate. Strain 7C-18 (= NIBRBA0000114839) has been isolated from activated sludge, Daejeon, Korea.



### Description of *Streptosporangium amethystogenes* WM35

Cells are Gram-staining-positive, non-flagellated, non-pigmented and branched mycelium-forming. Colonies are round, circular, convex and pale red-colored after 7 days on R2A at 25°C. Positive for nitrate reduction and gelatinase in API 20NE but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and  $\beta$ -galactosidase. D-Maltose and citrate are utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, D-mannose, N-acetyl-glucosamine, gluconate, caprate, adipate, malate and phenylacetate. Strain WM35 (=NIBRBA0000115020) has been isolated from freshwater, Changnyeong, Korea.

### Description of *Conexibacter arvalis* BS10

Cells are Gram-staining-positive, non-pigmented and rod-shaped. Colonies are circular, raised, entire and white-colored after 4 days on R2A at 30°C. Positive for urease and gelatinase in API 20NE but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis and  $\beta$ -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 BS10 (=NIBRBA0000114831) has been isolated from spent mushroom compost, Yesan, Korea.

## ACKNOWLEDGEMENTS

This study was supported by the research grant “The Survey of Korean Indigenous Species” from the National Institute of Biological Resources of the Ministry of Environment in Korea.

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Submitted: February 26, 2016

Revised: August 25, 2016

Accepted: February 7, 2017