

Report on 34 unrecorded filamentous and sporulating bacterial species isolated from Korea in 2023

Hyosun Lee¹, Jung-Hoon Yoon², Wonyong Kim³, Seung Bum Kim⁴, Wan-Taek Im⁵ and Dong-Uk Kim^{1,*}

¹Department of Biological Science, Sangji University, Wonju 26339, Republic of Korea

²Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon 16419, Republic of Korea

³Department of Microbiology, Chung-Ang University College of Medicine, Seoul 06973, Republic of Korea

⁴Department of Microbiology, Chungnam National University, Daejeon 34134, Republic of Korea

⁵Department of Biotechnology, Hankyong National University, Anseong 17579, Republic of Korea

*Correspondent: dukim@sangji.ac.kr

In 2023, as research of discover native prokaryotic species in Korea, 34 filamentous and sporulating bacterial strains were affiliated to the phylum *Actinomycetota*. The strains formed monophyletic clades with the most closely related species with $\geq 98.7\%$ of the 16S rRNA gene similarity. The 34 filamentous and sporulating *Actinomycetota* strains belonged to the families *Micromonosporaceae*, *Nocardiaceae*, *Pseudonocardiaceae*, *Streptomycetaceae*, and *Streptosporangiaceae* (of the orders *Micromonosporales*, *Mycobacteriales*, *Pseudonocardiales*, *Streptomycetales* and *Streptosporangiales*). 34 species of the phylum *Actinomycetota* have not been reported in Korea, suggesting unrecorded species in Korea. Information on strains such as Gram staining reaction, colony and cell morphology, biochemical characteristics, and isolation sources were provided in the species description.

Keywords: 16S rRNA, *Actinomycetota*, bacterial diversity, unrecorded species



All content in the Journal of Species Research (JSR), published by the National Institute of Biological Resources (NIBR), is Open Access, meaning it is freely accessible online to everyone without fees or the need for authors' permission. Open Access articles are automatically archived in the Korea Institute of Science and Technology Information (KISTI)'s Open Access repository, AccessON. All JSR content is published and distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License (CC BY-NC-ND 4.0). This license permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are properly credited. However, no modifications or derivative works are allowed.

INTRODUCTION

In 2023, 34 unrecorded bacteria were isolated from agricultural soil samples from various regions in Korea and identified as phylum of *Actinomycetes*. The taxonomic information of 34 unrecorded species is based on the nomenclature described in List of Prokaryotic names with Standing in Nomenclature (<https://lpsn.dsmz.de>) and consists of *Actinomycetota*. The phylum *Actinomycetota*, one of the largest phyla within the domain Bacteria, is widely distributed in aquatic and terrestrial environments (Lawson *et al.*, 2018). The phylum is comprised of Gram-stain-positive, non-sporeforming, rod-shaped bacteria with a high G + C content (Gao and Gupta, 2005). The phylum *Actinomycetota* includes multicellular filamentous bacteria

such as *Streptomyces* species (Feeney *et al.*, 2022). These filamentous bacteria have garnered significant attention due to their intricate developmental life cycles, where growth and differentiation frequently coincide with the production of specialized metabolites (Gerwick and Moore, 2012). Among filamentous actinobacteria, the genus *Streptomyces* has been the most extensively studied, primarily because its species are the most prolific producers of specialized metabolites (Hutchings *et al.*, 2019).

In 2023, a total of 192 unrecorded species were isolated from various samples during the research of discover native prokaryotic species in Korea, of which 34 belong to the phylum *Actinomycetota* (filamentous and sporulating species). In this study, we report these species and describe their characteristics.

Table 1. The taxonomic affiliations of isolated strains belonging to the phylum *Actinomycetota*.

Phylum	Order	Family	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
<i>Micromonosporales</i>	<i>Micromonosporaceae</i>		AN1124	NIBRBAC000510621	<i>Micromonospora provocatoris</i>	99.86	Wetland	R2A	28°C, 3 d
			AN003	NIBRBAC000510592	<i>Nocardia aurea</i>	100.00	Wetland	R2A	28°C, 3 d
			SBCC-2246	NIBRBAC000510516	<i>Nocardia higoensis</i>	99.51	Tidal Flat	MA	25°C, 7 d
			AN064	NIBRBAC000510597	<i>Nocardia salmonicida</i> subsp. <i>cummidelens</i>	99.02	Wetland	R2A	28°C, 3 d
			AN043	NIBRBAC000510595	<i>Nocardia salmonicida</i> subsp. <i>salmonicida</i>	99.44	Wetland	R2A	28°C, 3 d
<i>Mycobacteriales</i>	<i>Nocardiaceae</i>		AN051	NIBRBAC000510596	<i>Nocardia singurfluensis</i>	98.96	Wetland	R2A	28°C, 3 d
			MMS23-KA4	NIBRBAC000510822	<i>Anycolatopsis vasitatis</i>	99.71	Soil	AA	30°C, 3 d
			AN625	NIBRBAC000510611	<i>Lentzea pudingi</i>	99.16	Wetland	R2A	28°C, 3 d
			AN040	NIBRBAC000510594	<i>Saccharothrix espanaensis</i>	99.79	Wetland	R2A	28°C, 3 d
			MMS23-KR2	NIBRBAC000510823	<i>Saccharothrix syringae</i>	98.96	Soil	R2A	30°C, 3 d
<i>Pseudonocardiales</i>	<i>Pseudonocardiaceae</i>		AN189	NIBRBAC000510601	<i>Umezawaea tangerina</i>	99.30	Wetland	R2A	28°C, 3 d
			AN696	NIBRBAC000510615	<i>Actinacidiphila paucispora</i>	98.76	Wetland	R2A	28°C, 3 d
			SBCC-2341	NIBRBAC000510527	<i>Streptomyces althioticus</i>	99.86	Tidal Flat	R2A	25–30°C, 7 d
			AN214	NIBRBAC000510603	<i>Streptomyces amrisarensis</i>	100.00	Wetland	R2A	28°C, 3 d
			AN286	NIBRBAC000510604	<i>Streptomyces atroviens</i>	98.75	Wetland	R2A	28°C, 3 d
<i>Actinomycetota</i>			AN001	NIBRBAC000510591	<i>Streptomyces botropensis</i>	100.00	Wetland	R2A	28°C, 3 d
			SBCC-2337	NIBRBAC000510525	<i>Streptomyces caniferus</i>	99.93	Tidal Flat	R2A	25–30°C, 7 d
			MMS23-LS6	NIBRBAC000510813	<i>Streptomyces cerasius</i>	99.13	Soil	SCA	30°C, 7 d
			MMS23-IK53	NIBRBAC000510833	<i>Streptomyces chloris</i>	98.86	Soil	R2A	30°C, 3 d
			AN1078	NIBRBAC000510620	<i>Streptomyces daghestanicus</i>	99.72	Wetland	R2A	28°C, 3 d
<i>Streptomyces</i>	<i>Streptomycetaceae</i>		AN016	NIBRBAC000510593	<i>Streptomyces endophyticus</i>	99.79	Wetland	R2A	28°C, 3 d
			MMS23-CK42	NIBRBAC000510832	<i>Streptomyces erringtonii</i>	98.67	Soil	R2A	30°C, 3 d
			MMS23-KR22	NIBRBAC000510825	<i>Streptomyces fractus</i>	99.79	Soil	R2A	30°C, 3 d
			MMS23-GM15	NIBRBAC000510820	<i>Streptomyces hirsutus</i>	99.86	Soil	R2A	30°C, 3 d
			AN679	NIBRBAC000510614	<i>Streptomyces hyalurocycini</i>	99.24	Wetland	R2A	28°C, 3 d
<i>Streptomyces</i>			MMS23-LE10	NIBRBAC000510814	<i>Streptomyces kronopolitis</i>	99.55	Soil	SCA	30°C, 7 d
			MMS23-CK28	NIBRBAC000510831	<i>Streptomyces lanatus</i>	98.89	Soil	R2A	30°C, 3 d
			CAU 1726	NIBRBAC000510783	<i>Streptomyces naganishii</i>	100	Soil	GYE	30°C, 3 d
			AN211	NIBRBAC000510602	<i>Streptomyces racemochromogenes</i>	99.79	Wetland	R2A	28°C, 3 d
			SBCC-2338	NIBRBAC000510526	<i>Streptomyces sparsus</i>	99.72	Tidal flat	R2A	25–30°C, 7 d
<i>Streptomyces</i>			SBCC-2348	NIBRBAC000510528	<i>Streptomyces sandarbansensis</i>	99.79	Tidal flat	R2A	25–30°C, 7 d
			AN074	NIBRBAC000510599	<i>Streptomyces violaceus</i>	99.59	Wetland	R2A	28°C, 3 d
<i>Streptosporangiales</i>	<i>Streptosporangaceae</i>		MMS23-TB5	NIBRBAC000510818	<i>Nonomuraea rhodomycinica</i>	99.10	Soil	SCA	30°C, 7 d
			AR0	NIBRBAC000510799	<i>Streptosporangium sandarckinum</i>	99.84	Agricultural soil	R2A	28°C, 7 d

MATERIALS AND METHODS

A total of 34 bacterial strains assigned to the phylum *Actinomycetota* were isolated from various samples. The collected samples from each region were independently processed serial dilution and spread onto diverse culture media Reasoner's 2A agar (R2A; BD Difco), marine agar (MA; BD Difco), glucose yeast extract agar (GYE) actinomycete isolation agar (AA) and starch-casein agar (SCA) and incubated at 20–30°C for 3–7 days (Table 1). All strains were purified as single colonies and stored as 15–17% (v/v) glycerol suspension at –80°C as well as lyophilized ampoules.

The morphology and cell size of colonies for each strain were determined by using a scanning electron microscopy and transmission electron microscopy. The electron micrographs of each strain are shown in Fig. 1. Gram reaction was performed according to the classic Gram procedure described by Doetsch (1981). Biochemical characteristics were tested by using API 20NE galleries (bioMérieux) according to the manufacturer's instructions. After extracting genomic DNA and amplify the 16S rRNA gene using (universal) primers (Weisburg *et al.*, 1991), and the sequence database of each strain was submission in NCBI GenBank. The 16S rRNA gene sequences of the related taxa were obtained from EzBioCloud server (Yoon *et al.*, 2017). 34 bacterial strains and related taxa (retrieved from the NCBI database) were aligned with SINA (v1.2.12) according to

the SILVA seed alignment (<http://www.arb-silva.de>; Pruesse *et al.*, 2012). Evolutionary distances applied during phylogenetic analysis were calculated through a two-parameter model (Kimura, 1983). A phylogenetic tree was constructed using bootstrap values (Felsenstein, 1985) based on 1,000 replicates using neighbor joining (Saitou and Nei, 1987) in the MEGA 11 program (Tamura *et al.*, 2021).

RESULTS AND DISCUSSION

The 34 strains were distributed into 1 phylum: *Actinomycetota* (Table 1). All strains filamentous and sporulating bacteria (Figs. 1, 2). Unrecorded bacteria were identified as 10 genera of *Actinacidiphila*, *Amycolatopsis*, *Lentzea*, *Micromonospora*, *Nocardia*, *Nonomuraea*, *Saccharothrix*, *Streptomyces*, *Streptosporangium* and *Umezawaea* (Figs. 3, 4). Here we report 34 unrecorded bacterial species in Korea belonging to 5 orders, which were isolated in Korea; 1 strain of the *Micromonosporales*, 5 strains of the *Mycobacteriales*, 5 strains of the *Pseudonocardiales*, 21 strains of the *Streptomycetales*, and 2 strains of the *Streptosporangiales*.

Description of *Micromonospora provocatoris* AN1124

Cells are Gram-staining-positive and spherical to oval. Colonies are filamentous, unbonate and orange color after 3 days of incubation at 28°C on R2A. In API 20NE, the

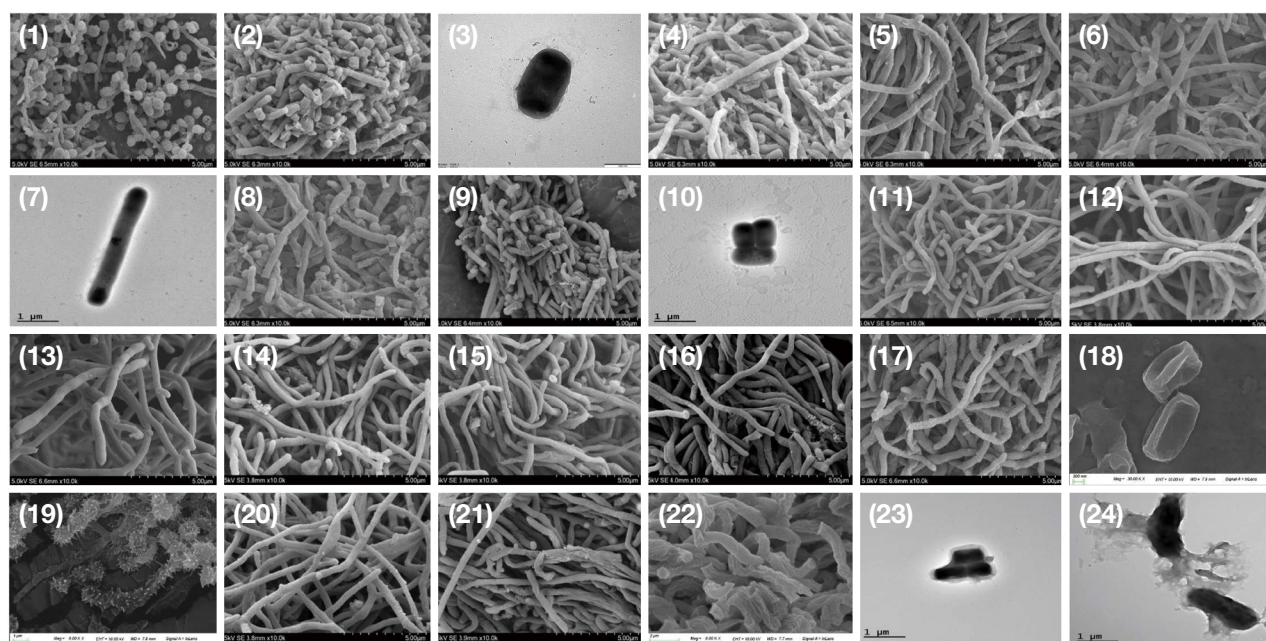


Fig. 1. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strains: 1, AN1124; 2, AN003; 3, SBCC-2246; 4, AN064; 5, AN043; 6, AN051; 7, MMS23-KA4; 8, AN625; 9, AN040; 10, MMS23-KR2; 11, AN189; 12, AN696; 13, SBCC-2341; 14, AN214; 15, AN286; 16, AN001; 17, SBCC-2337; 18, MMS23-LS6; 19, MMS23-IK53; 20, AN1078; 21, AN016; 22, MMS23-CK42; 23, MMS23-KR22; 24, MMS23-GM15.

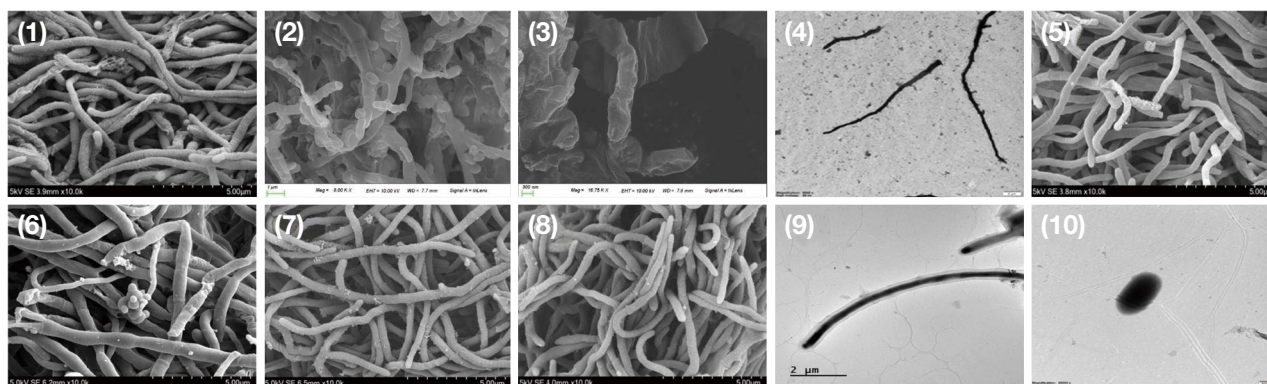


Fig. 2. Transmission electron micrographs or scanning electron micrographs of cells of the strains isolated in this study. The cells were cultured at their optimal growth conditions. Strains: 1, AN679; 2, MMS23-LE10; 3, MMS23-CK28; 4, CAU 1726; 5, AN211; 6, SBCC-2338; 7, SBCC-2348; 8, AN074; 9, MMS23-TB5; 10, AR0.

reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-maltose and adipate are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain AN1124 (=NIBRBAC000510621) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Nocardia aurea* AN003

Cells are Gram-staining-positive and rod shape. Colonies are filamentous, unbonate and pale-yellow color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, potassium gluconate and adipate are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain AN003 (=NIBRBAC000510592) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Nocardia higoensis* SBCC-2246

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat, smooth and pale light pink color after 7 days of incubation at 25°C on MA. In API 20NE, assimilation of potassium gluconate and adipate are positive, but the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -glucosidase (esculin hydrolysis), protease (gelatin hydroly-

sis), β -galactosidase (PNPG), indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain SBCC-2246 (=NIBRBAC000510516) was isolated a tidal flat sample, Chungcheong-do, Korea (36°53'17"N 126°19'39"E).

Description of *Nocardia salmonicida* subsp. *cummidelens* AN064

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale orange color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, assimilation of D-glucose and *N*-acetyl-D-glucosamine are positive and β -glucosidase (esculin hydrolysis), potassium gluconate and adipate are weakly positive but protease (gelatin hydrolysis), β -galactosidase (PNPG), indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, D-mannose, D-maltose, D-mannitol, capric acid, malic acid trisodium citrate and phenylacetic acid are negative. Strain AN064 (=NIBRBAC000510597) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Nocardia salmonicida* subsp. *salmonicida* AN043

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale orange color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, β -glucosidase (esculin hydrolysis), assimilation of D-glucose, D-mannose, *N*-acetyl-D-glucosamine, potassium gluconate, adipate and trisodium citrate are positive and β -galactosidase

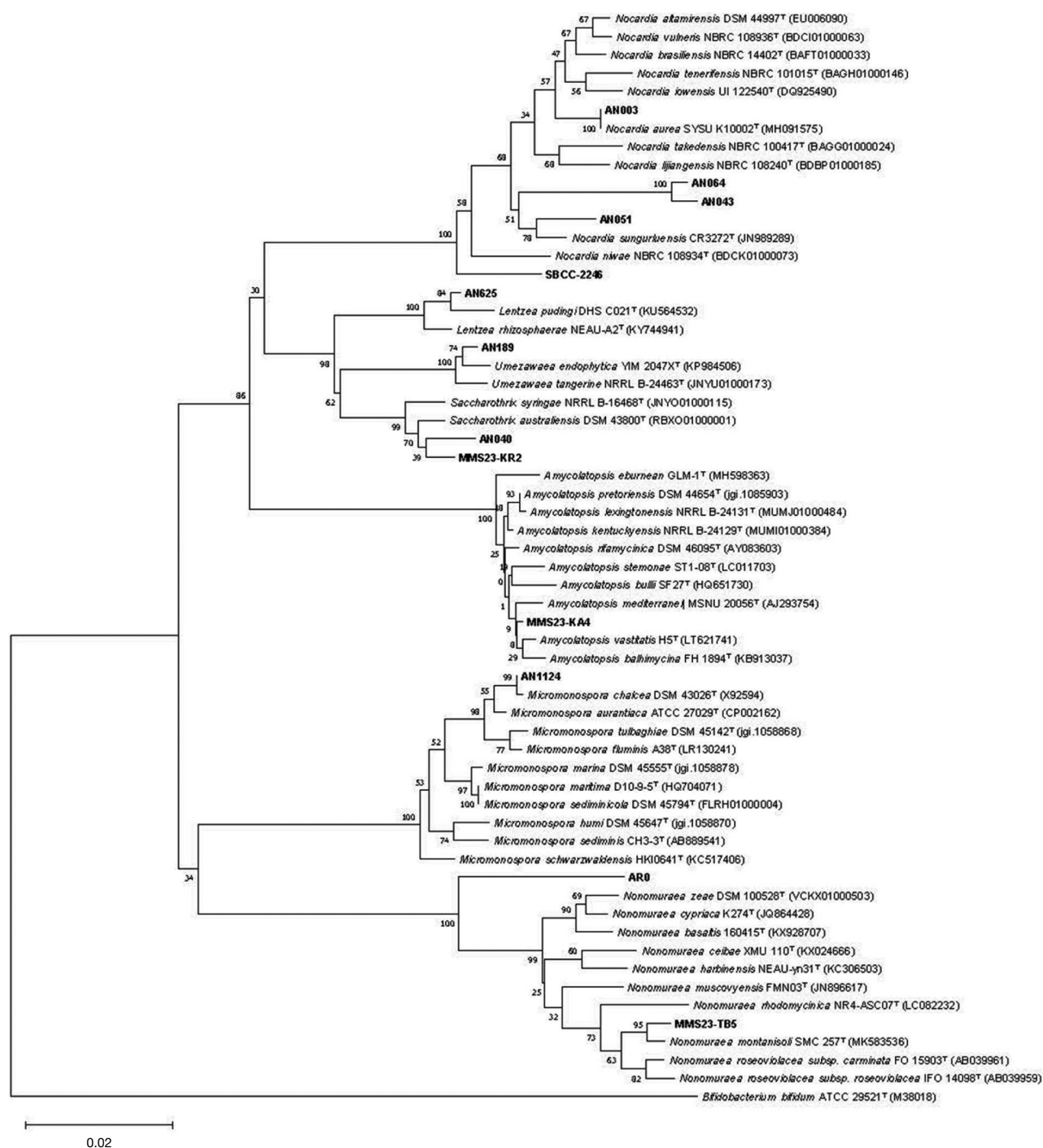


Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family *Micromonosporaceae*, *Nocardiaceae*, *Pseudonocardiaceae* and *Streptosporangiaceae*. Bootstrap values (> 70%) are shown above nodes for the neighbor-joining methods. *Bifidobacterium bifidum* ATCC 29521^T used as an outgroup. Bar: 0.01 substitutions per nucleotide position.

(PNPG) are weakly positive but protease (gelatin hydrolysis), indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, D-maltose, D-mannitol, capric acid, malic acid and phenylacetic acid are

negative. Strain AN043 (= NIBRBAC000510595) was isolated a wetland sample, Gyeonggi-do, Korea (37°01' 02.6"N 127°15'48.9"E).

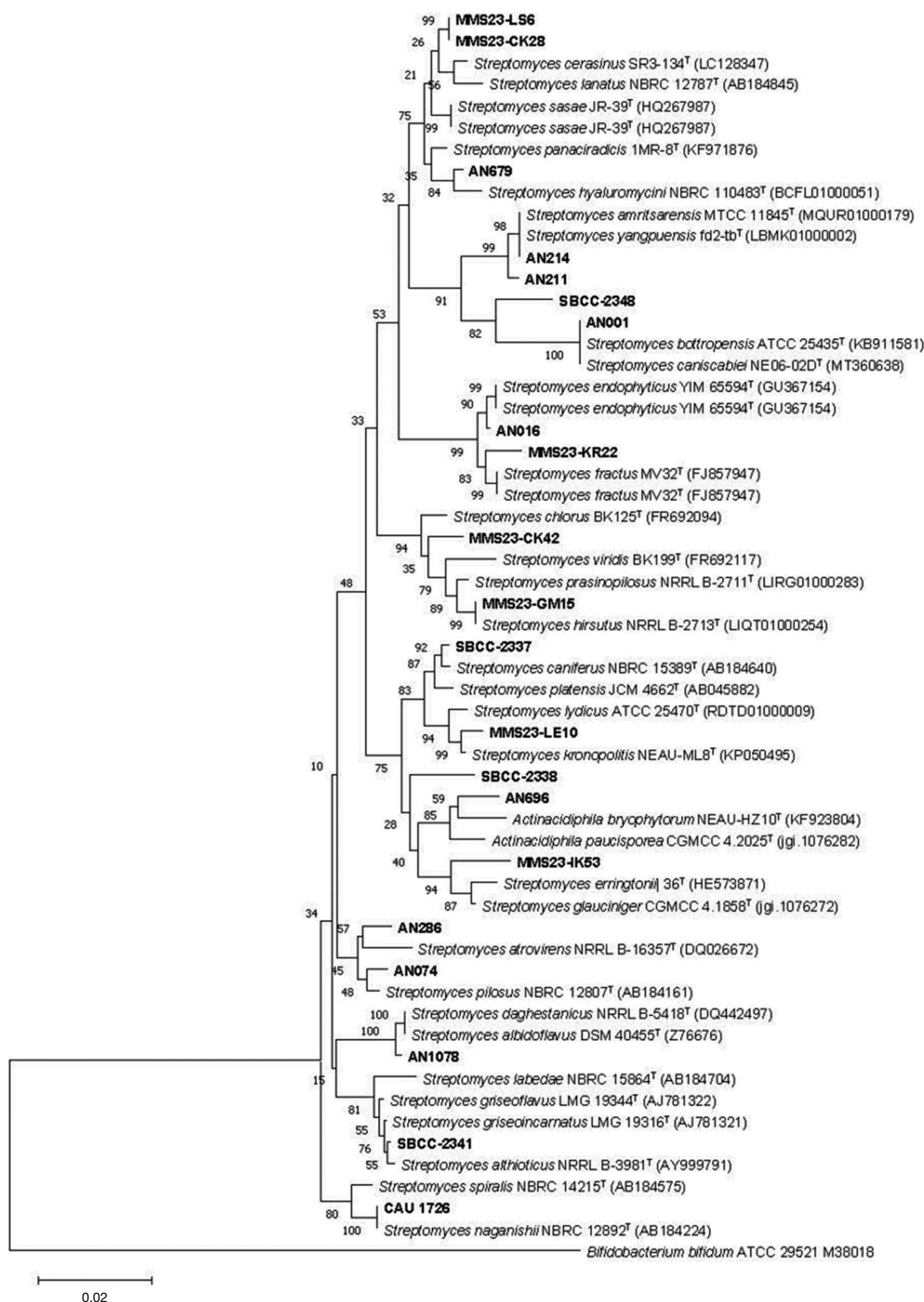


Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the relationship between the strains isolated in this study and their relatives of the family Streptomycetaceae. Bootstrap values (>70%) are shown above nodes for the neighbor-joining methods. *Bifidobacterium bifidum* ATCC 29521^T used as an outgroup. Bar: 0.1 substitutions per nucleotide position.

Description of *Nocardia sungurluensis* AN051

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE, the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), assimilation of L-arabinose, potassium gluconate, adipate and malic acid are positive and assimilation of D-glucose are weakly positive. But protease (gelatin hydrolysis), indole production, glucose fermentation, arginine dihydrolase, assimilation of D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate and phenylacetic acid are negative. Strain AN051 (=NIBRBAC000510595) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Amycolatopsis vastitatis* MMS23-KA4

Cells are Gram-staining-positive and rod shape. Colonies are circular, erose, irregular pale orange color after 3 days of incubation at 30°C on AA. In API 20NE, the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of potassium gluconate are positive. But urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate, adipate, malic acid and phenylacetic acid are negative. Strain MMS23-KA4 (=NIBRBAC000510822) was isolated a soil sample, Gyeongsang-do, Korea (35°50'17"N 129°17'11"E).

Description of *Amycolatopsis vastitatis* MMS23-KA4

Cells are Gram-staining-positive and rod shape. Colonies are circular, erose, irregular pale orange color after 3 days of incubation at 30°C on AA. In API 20NE, the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of potassium gluconate are positive. But urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate, adipate, malic acid and phenylacetic acid are negative. Strain MMS23-KA4 (=NIBRBAC000510822) was isolated a soil sample, Gyeongsang-do, Korea (35°50'17"N 129°17'11"E).

Description of *Lentzea pudingi* AN625

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in β -galactosidase (PNPG), assimilation of D-glucose, L-arab-

inose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, potassium gluconate adipate and malic acid are positive and β -glucosidase (esculin hydrolysis) is weakly positive. But the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid, trisodium citrate, phenylacetic acid are negative. Strain AN625 (=NIBRBAC000510611) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Saccharothrix espanaensis* AN040

Cells are Gram-staining-positive and rod shape. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in β -galactosidase (PNPG), assimilation of D-glucose, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid are positive and β -glucosidase (esculin hydrolysis) is weakly positive. But the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid, trisodium citrate, L-arabinose, D-mannose, D-maltose, D-mannitol, adipate, phenylacetic acid are negative. Strain AN040 (=NIBRBAC000510594) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Saccharothrix syringae* MMS23-KR2

Cells are Gram-staining-positive and rod shape. Colonies are circular, convex, entire white color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in β -glucosidase (esculin hydrolysis) and protease (gelatin hydrolysis) are positive. But nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, potassium gluconate, *N*-acetyl-D-glucosamine, D-maltose, D-mannitol, capric acid, trisodium citrate, adipate, malic acid and phenylacetic acid are negative. Strain MMS23-KR2 (=NIBRBAC000510823) was isolated a soil sample, Gyeongsang-do, Korea (35°50'17"N 129°17'11"E).

Description of *Umezawaea tangerine* AN189

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate white color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, adipate, trisodium citrate, and malic acid are positive. But the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of potassium glu-

conate, capric acid, phenylacetic acid are negative. Strain AN189 (=NIBRBAC000510601) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Actinacidiphila paucisporea* AN696

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate brown color after 3 days of incubation at 28°C on R2A. In API 20NE reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), assimilation of potassium gluconate, capric acid, phenylacetic acid, D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-D-glucosamine, adipate, trisodium citrate, and malic acid are negative. Strain AN696 (=NIBRBAC000510615) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces althoticus* SBCC-2341

Cells are Gram-staining-positive and filamentous. Colonies are Circular to irregular, flat, forming aerial and substrate mycelia white to gray color after 7 days of incubation at 25–30°C on R2A. In API 20NE reactions in β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG) (weak), assimilation of D-glucose, L-arabinose (weak), D-mannose (weak), D-mannitol, potassium gluconate and malic acid are positive. But the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-maltose, capric acid, *N*-acetyl-D-glucosamine, adipate, trisodium citrate, phenylacetic acid are negative. Strain SBCC-2341 (=NIBRBAC000510527) was isolated a wetland sample, Jeolla-do, Korea (35°40'45"N 126°31'47"E).

Description of *Streptomyces amritsarensis* AN214

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale purple to white color after 3 days of incubation at 28°C on R2A. In API 20NE assimilation of D-glucose, D-mannose (weak), *N*-acetyl-D-glucosamine, D-maltose (weak), potassium gluconate, malic acid and trisodium citrate are positive. But reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), assimilation of capric acid, phenylacetic acid, D-glucose, L-arabinose, D-mannose, D-mannitol, and adipate are negative. Strain AN214 (=NIBRBAC000510603) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces atrovirens* AN286

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate gray color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate and malic acid are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, urease, assimilation of capric acid, trisodium citrate and phenylacetic acid are negative. Strain AN286 (=NIBRBAC000510604) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces bottropensis* AN001

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate gray color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate (weak) and malic acid are positive, but the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid, trisodium citrate and phenylacetic acid are negative. Strain AN001 (=NIBRBAC000510591) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces caniferus* SBCC-2337

Cells are Gram-staining-positive and filamentous. Colonies are Circular to irregular, umbonate, forming aerial and substrate mycelia white to dark gray color after 7 days of incubation at 25–30°C on R2A. In API 20NE reactions in β -galactosidase (PNPG), assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate are positive. But the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, capric acid, adipate, phenylacetic acid are negative. Strain SBCC-2337 (=NIBRBAC000510525) was isolated a tidal flat sample, Jeolla-do, Korea (35°40'45"N 126°31'47"E).

Description of *Streptomyces cerasinus* MMS23-LS6

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat to slightly convex brown color after

7 days of incubation at 30°C on SCA. In API 20NE, the reactions in β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid are positive. But nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-LS6 (= NIBRBAC000510813) was isolated a soil sample, Gyeongsang-do, Korea (36°19'41"N 127°21'44"E).

Description of *Streptomyces cerasinus* MMS23-IK53

Cells are Gram-staining-positive and filamentous. Colonies are irregular, Convex green color after 7 days of incubation at 30°C on R2A. In API 20NE, the reactions in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose and potassium gluconate are positive. But β -glucosidase (esculin hydrolysis), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of malic acid capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-IK53 (= NIBRBAC000510833) was isolated a soil sample, Chungcheong-do, Korea (36°03'25"N 127°35'59"E).

Description of *Streptomyces daghestanicus* AN1078

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate ivory color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in urease, β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate, malic acid and trisodium citrate (weak) are positive. But the reaction in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), reduction of indole production, glucose fermentation, arginine dihydrolase, assimilation of capric acid, and phenylacetic acid are negative. Strain AN1078 (= NIBRBAC000510620) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces endophyticus* AN016

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate gray color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), urease, β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-malt-

ose (weak), potassium gluconate, adipate (weak) and malic acid are positive. But the reaction in reduction of indole production, glucose fermentation, arginine dihydrolase, assimilation of capric acid, and trisodium citrate and phenylacetic acid are negative. Strain AN016 (= NIBRBAC000510593) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces cerasinus* MMS23-CK42

Cells are Gram-staining-positive and filamentous. Colonies are irregular, convex white to gray color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis) and assimilation of D-glucose are positive. But β -galactosidase (PNPG), urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose and potassium gluconate malic acid capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-CK42 (= NIBRBAC000510832) was isolated a soil sample, Chungcheong-do, Korea (36°03'25"N 127°35'59"E).

Description of *Streptomyces fractus* MMS23-KR22

Cells are Gram-staining-positive and rod shape. Colonies are circular, convex, entire ivory color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), arginine dihydrolase, urease, β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-mannose, *N*-acetyl-D-glucosamine and potassium gluconate are positive. But indole production, glucose fermentation, assimilation of D-glucose, L-arabinose, D-mannitol, D-maltose, malic acid, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-KR22 (= NIBRBAC000510825) was isolated a soil sample, Gyeongsang-do, Korea (35°50'17"N 129°17'11"E).

Description of *Streptomyces hirsutus* MMS23-GM15

Cells are Gram-staining-positive and rod shape. Colonies are circular, convex, entire light brown color after 3 days of incubation at 30°C on MA. In API 20NE, the reactions in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis) and β -galactosidase (PNPG), are positive. But arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of D-mannose, *N*-acetyl-D-glucosamine, potassium gluconate D-glucose, L-arabinose, D-mannitol, D-maltose, malic acid, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-GM15 (= NIBRBAC000510820) was isolated a soil sample, Jeolla-do, Korea (35°54'31"N 127°46'48"E).

Description of *Streptomyces hyaluromycini* AN679

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate pale brown color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate, malic acid and trisodium citrate are positive. But the reaction in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of capric acid and phenylacetic acid are negative. Strain AN679 (=NIBRBAC000510614) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces kronopolitis* MMS23-LE10

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat to slightly convex brown color after 7 days of incubation at 30°C on SCA. In API 20NE, the reactions in arginine dihydrolase, urease, β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid are positive. But nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), β -galactosidase (PNPG), indole production, glucose fermentation, assimilation of L-arabinose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-LE10 (=NIBRBAC000510814) was isolated a soil sample, Gyeongsang-do, Korea (36°19'41"N 127°21'44"E).

Description of *Streptomyces lanatus* MMS23-CK28

Cells are Gram-staining-positive and filamentous. Colonies are circular, umbonate brown color after 3 days of incubation at 30°C on R2A. In API 20NE, the reactions in β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid are positive. But nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of D-maltose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-CK28 (=NIBRBAC000510831) was isolated a soil sample, Chungcheong-do, Korea (36°03'25"N 127°35'59"E).

Description of *Streptomyces naganishii* CAU 1726

Cells are Gram-staining-positive and filamentous. Colonies are circular, entire, pulvinate, opaques cream color after 3 days of incubation at 30°C on GYE agar. In API 20NE, the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$),

arginine dihydrolase, protease (gelatin hydrolysis), assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate, malic acid, trisodium citrate and phenylacetic acid are positive. But urease, β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), indole production, glucose fermentation and assimilation of capric acid are negative. Strain CAU 1726 (=NIBRBAC000510783) was isolated a soil sample, Gyeongsang-do, Korea (35°10'03.2"N 129°05'42.7"E).

Description of *Streptomyces racemochromogenes* AN211

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate orange to white color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), assimilation of D-glucose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate (weak), malic acid and trisodium citrate are positive. But the reaction in β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of L-arabinose, D-mannitol, capric acid, adipate and phenylacetic acid are negative. Strain AN211 (=NIBRBAC000510602) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Streptomyces sparsus* SBCC-2338

Cells are Gram-staining-positive and filamentous. Colonies are circular to slightly irregular, flat, forming substrate mycelium dark greyish yellow color after 7 days of incubation at 25–30°C on R2A. In API 20NE, the reactions in β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG), assimilation of D-glucose, L-arabinose (weak), D-mannose (weak), D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipate (weak), malic acid and trisodium citrate are positive. But nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}_2^-$), protease (gelatin hydrolysis), arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of capric acid and phenylacetic acid are negative. Strain SBCC-2338 (=NIBRBAC000510526) was isolated a tidal flat sample, Jeolla-do, Korea (35°40'45"N 126°31'47"E).

Description of *Streptomyces sundarbansensis* SBCC-2348

Cells are Gram-staining-positive and filamentous. Colonies are circular to irregular, umbonate, forming aerial and substrate mycelia yellowish whitecolor after 7 days of incubation at 25–30°C on R2A. In API 20NE, the reactions in urease (weak), β -glucosidase (esculin hydrolysis), β -galactosidase (PNPG) (weak), assimilation of D-glu-

cose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose (weak), potassium gluconate, adipate (weak), malic acid and trisodium citrate are positive. But nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), protease (gelatin hydrolysis), arginine dihydrolase, indole production, glucose fermentation, assimilation of L-arabinose, capric acid and phenylacetic acid are negative. Strain SBCC-2348 (=NIBRBAC000510528) was isolated a tidal flat sample, Chungcheong-do, Korea (36°35'51"N 126°22'38"E).

Description of *Streptomyces violaceus* AN074

Cells are Gram-staining-positive and filamentous. Colonies are filamentous, unbonate yellow color after 3 days of incubation at 28°C on R2A. In API 20NE the reactions in β -glucosidase (esculin hydrolysis) (weak), β -galactosidase (PNPG), assimilation of D-glucose, *N*-acetyl-D-glucosamine, D-maltose (weak), potassium gluconate (weak), adipate, malic acid, trisodium citrate and phenylacetic acid are positive. But the reaction in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), urease, indole production, glucose fermentation, arginine dihydrolase, protease (gelatin hydrolysis), assimilation of D-mannose, L-arabinose, D-mannitol and capric acid are negative. Strain AN074 (=NIBRBAC000510599) was isolated a wetland sample, Gyeonggi-do, Korea (37°01'02.6"N 127°15'48.9"E).

Description of *Nonomuraea rhodomycinica* MMS23-TB5

Cells are Gram-staining-positive and rod shape. Colonies are circular, flat to slightly convex pink color after 7 days of incubation at 30°C on SCA. In API 20NE, the reactions in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), β -glucosidase (esculin hydrolysis) and β -galactosidase (PNPG) are positive. But protease (gelatin hydrolysis), arginine dihydrolase, urease, indole production, glucose fermentation, assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate and malic acid, D-maltose, capric acid, trisodium citrate, adipate and phenylacetic acid are negative. Strain MMS23-TB5 (=NIBRBAC000510818) was isolated a soil sample, Chungcheong-do, Korea (36°17'32"N 127°21'00"E).

Description of *Streptosporangium sandarakinum* AR0

Cells are Gram-staining-positive and rod shape. Colonies are circular, entire, convex, rough red color after 7 days of incubation at 28°C on R2A. In API 20NE the reactions in nitrate reduction ($\text{NO}^{3-} \rightarrow \text{NO}^{2-}$), β -glucosidase (esculin hydrolysis), protease (gelatin hydrolysis) and β -galactosidase (PNPG), are positive. But the reaction in urease, indole production, glucose fermentation, arginine dihydrolase, assimilation of D-mannose, L-arabinose, D-mannitol D-glucose, *N*-acetyl-D-glucosamine, D-maltose, potassium glu-

conate, adipate, malic acid, trisodium citrate, phenylacetic acid and capric acid are negative. Strain AR0 (=NIBRBAC000510799) was isolated a agricultural soil sample, Gyeonggi-do, Korea (37°44'05"N 126°42'67"E).

CONFLICTS OF INTEREST

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

ACKNOWLEDGEMENTS

This work was supported by the National Institute of Biological Resources funded by the Ministry of Environment (No. NIBR202304204).

REFERENCES

- Doetsch, R.N. 1981. Determinative methods of light microscopy. In: P. Gerhardt, R.G.E. Murray, R.N. Costilow, E.W. Nester, W.A. Wood and N.R. Krieg (eds.), *Manual of Methods for General Bacteriology*, American Society for Microbiology, Washington DC. pp. 21-33.
- Feeney, M.A., J.T. Newitt, E. Addington, L. Algora-Gallardo, C. Allan, L. Balis, A.S. Birke, L. Castaño-Espriu, L.K. Charkoudian, R. Devine, D. Gayard, J. Hamilton, O. Hennrich, P.A. Hoskisson, M. Keith-Baker, J.G. Klein, W. Kruasuwat, D.R. Mark, Y. Mast, R.E. McHugh, T.C. McLean, E. Mohit, J.T. Munnoch, J. Murray, K. Noble, H. Otani, J. Parra, C.F. Pereira, L. Perry, L. Pintor-Escobar, L. Pritchard, S.M.M. Prudence, A.H. Russell, J.K. Schniete, R.F. Seipke, N. Sélem-Mojica, A. Undabarrena, K. Vind, G.P. van Wezel, B. Wilkinson, S.F. Worsley, K.R. Duncan, L.T. Fernández-Martínez and M.I. Hutchings. 2022. ActinoBase: tools and protocols for researchers working on *Streptomyces* and other filamentous actinobacteria. *Microbial Genomics* 8(7): 000824.
- Felsenstein, J. 1985. Confidence limits in phylogenies: an approach using the bootstrap. *Evolution* 39:783-791.
- Gao, B. and R.S. Gupta. 2005. Conserved indels in protein sequences that are characteristic of the phylum *Actinobacteria*. *International Journal of Systematic and Evolutionary Microbiology* 55(6):2401-2412.
- Gerwick, W.H. and Moore B.S. 2012. Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. *Chemistry & Biology* 19:85-98.
- Hutchings, M.I., A.W. Truman and B. Wilkinson. 2019. Antibiotics: past, present and future. *Current Opinion in Microbiology* 51:72-80.
- Kimura, M. 1983. The neutral theory of molecular evolution.

- Cambridge University Press.
- Lawson, P.A. 2018. The phylum actinobacteria. In: P. Mattarelli, B. Biavati, W.H. Holzapfel and B.J.B. Wood (eds.), *The Bifidobacteria and Related Organisms*. Academic Press. pp. 1-8.
- Pruesse, E., J. Peplies and F.O. Glockner. 2012. SINA: accurate highthroughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28:1823-1829
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.
- Tamura, K., G. Stecher and S. Kumar. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38(7):3022-3027.
- Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology* 173:697-703.
- Yoon, S.H., S.M. Ha, S. Kwon, J. Lim, Y. Kim, H. Seo and J. Chun. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. *International Journal of Systematic and Evolutionary Microbiology* 67:1613-1617.

Submitted: November 12, 2024

Revised: September 25, 2025

Accepted: November 4, 2025